Selective Binding and Cleavage of Peptides: A Combinatorial Approach

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- M. Nold, H. Wennemers “Peptide Damage under Fenton conditions is sequence-dependent”, *Chem. Comm.* **2004**, 16, 1800 – 1801. ("RSC hot article ")
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Abbreviations

AA	amino acid
Ac	acetyl
Ac_2O	acetic acid anhydride
Ala	alanine
Alloc	allyloxycarbonyl
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Boc	tert-butyloxycarbonyl
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one
DIC	diisopropylcarbodiimide
DIPEA	diisopropyl ethylamine
DMAP	4-N,N-dimethylaminopyridine
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
εAhx	ε-Amino hexanoic acid
EDC	3-dimethylaminopropyl)-3-ethylcarbodiimide
ESI	electron spray ionisation
FAB	fast atom bombardment
Fmoc	9-fluorenylmethoxycarbonyl
Gln	glutamine
Glu	glutamic acid
Gly	glycine
h	hours
HATU	(N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridino-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide)
HEPES	N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic acid

His	histidine
hν	light
HOBt	Hydroxybenzotriazol
HPLC	high performance liquid chromatography
IR	infrared
Lac	lactate
Leu	leucine
Lys	lysine
MES	(2-(4-morpholino)-ethane sulfonic acid)
NEt_3	triethyl amine
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect
Phe	phenylalanine
ppm	parts per million
Pr	propyl
Pro	proline
quant.	quantitative
r.t.	room temperature
Rf	retention factor
Ser	serine
TAEA	tris(2-aminoethyl)amine
TBTU	O-(benzotriazol-1-yl)-N,N,N',N',-tetramethyluronium
TFA	trifluoroacetic acid
THF	tetrahydrofurane
Thr	threonin
TIS	triisopropyl silane
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)aminomethane
Tyr	tyrosine
UV	ultra violet
Val	valine

General Section

1. Introduction

1. 1. Peptide binding and cleavage

Proteins and peptides consist of 20 amino acid with different side chain functional groups, thus their possibilities to form diverse structures are immense.[1] Because of this large structural variety, proteins fulfil a wide range of functions in nature. Examples are the use of proteins as structural material or for the storage of nitrogen. Probably the most important function of proteins is the catalysis of chemical reactions as enzymes.

Selective interactions between proteins are crucial not only for enzyme function but also for signal transduction. The principles that govern such intermolecular interactions are still not fully understood because of the complexity of protein structures. Studies on small model systems might lead to a better understanding of these complex interactions.

Therefore, the development of small receptors for peptides has attracted much attention in recent years.[2-5] Apart from serving as a model for the study of complex protein-protein interactions, such receptors have many potential applications. These include the use as stationary phases for the separation of peptides[6], the development of selective sensors[7] as well as the use as pharmaceutical drugs.

Although selective receptors for single amino acids have been rationally designed,[8] the binding of small peptides is a much more challenging task. The reason for this lies in the conformational flexibility of peptides, that complicates the design of a suitable receptor.

An example that demonstrates the difficulty to predict selective peptide binding is the antibiotic vancomycin.[9] It is a macrotricyclic glycopeptide that binds with high affinity

to the *C*-terminal peptide motif D-Ala-D-Ala. This sequence plays an important role in the cell wall crosslinking of gram positive bacteria. When this peptide is blocked, the bacteria are not able to build up their cell wall and cannot survive.

Resistant bacteria appeared that use D-Ala-D-Lac instead of D-Ala-D-Ala.[10] This small modification (just one atom is changed) results in a 1000 fold decrease of the binding affinity.[11] The reason is a repulsive electrostatic interaction of the oxygen instead of a hydrogen bond in case of D-Ala-D-Ala (figure 1.1.). This clarifies what big consequences a minute structural change can have on the selectivity and strength of intermolecular binding.

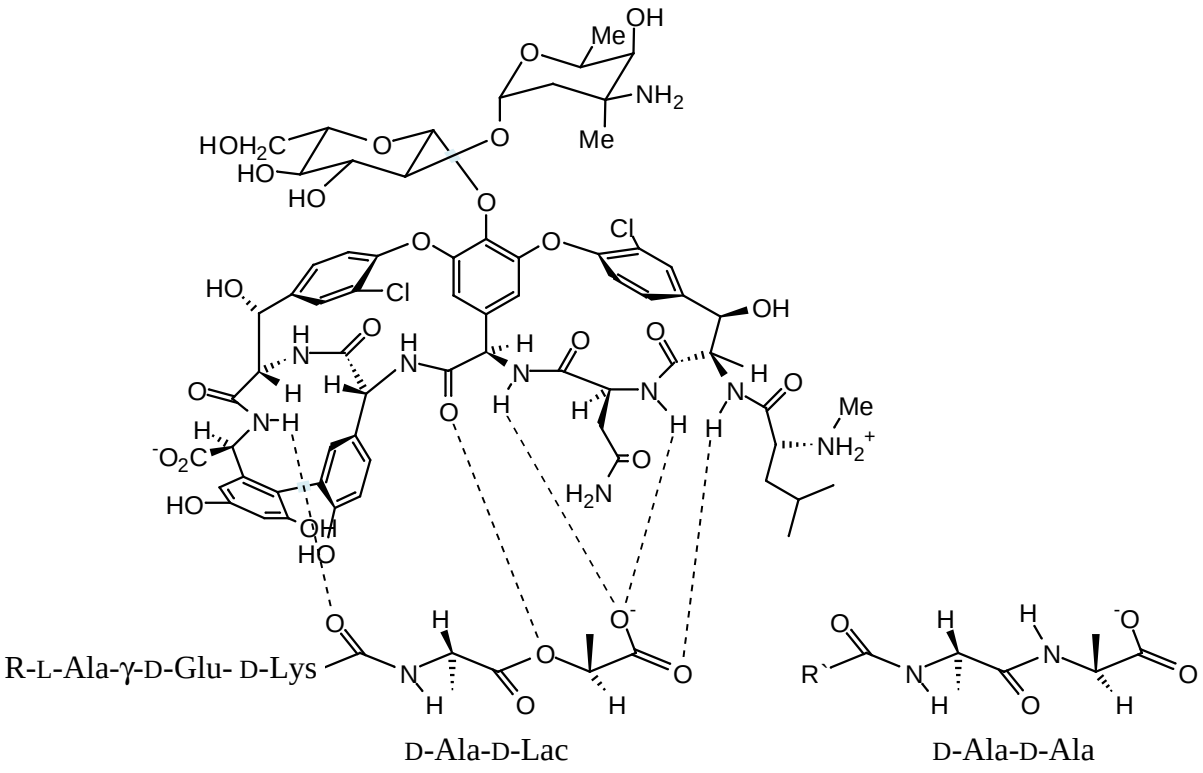


Figure 1.1.: Intermolecular interactions between vancomycin and D-Ala-D-Lac

In analogy, the rational design of a receptor for a given peptide is very difficult. At this point, where rational design reaches its limits, an empirical approach using combinatorial chemistry proves to be helpful. This concept relies on random mutations and selection of the best binding partner out of a big pool of different compounds.

Using combinatorial chemistry, Ellman and co-workers found selective receptors for D-Ala-D-Lac varying a vancomycin fragment combinatorially.[12] A more general concept for peptide receptors is given by tweezer like receptors.[13-17] It involves two arms with possible sites for interactions that are bound to a structure defining backbone.

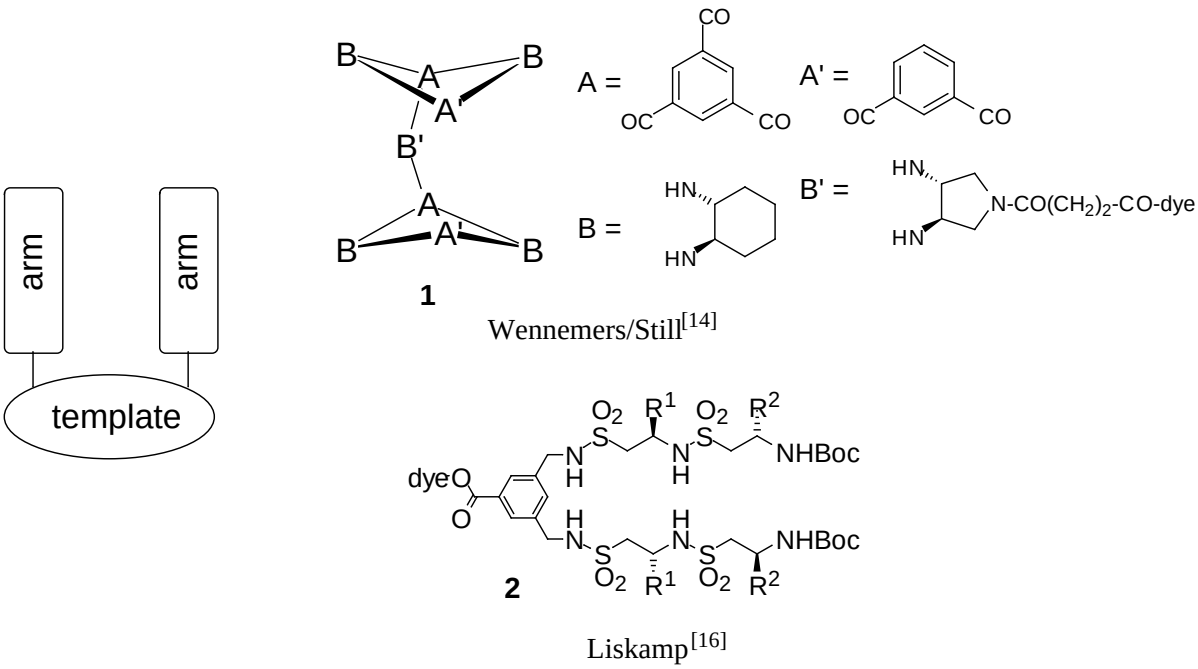


Figure 1.2.: Scematic structure of tweezer like receptors and two examples

There are several examples of tweezer-like receptors that bind to peptides selectively (figure 1. 2.).[13-17] However, the existing tweezer-like peptide receptors bear certain drawbacks, such as the lack of possibility to introduce molecular diversity (**1**) or difficult

synthesis (**2**). Thus, we were interested in the development of a novel class of tweezer like receptors.

An even larger challenge than the selective binding is the selective cleavage of peptides since amide bonds are very stable. In nature, this is accomplished by proteases.[1] They lower the activation barrier for amide hydrolysis and also assure that peptide bonds are cleaved specifically. While proteases provide for the controled cleavage of peptide bonds, also undesired peptide cleavage occurs in nature, for example by radical mediated damage catalyzed by metals.[18]

One example is the Fenton reaction. Although first described already in 1894,[19] it was about 40 years later, that its mechanism was studied thoroughly by Haber and Weiss among others. They were able to show that the iron(II) dependent formation of hydroxyl radicals from hydrogen peroxide is only the first step in a complex cascade of radical formation.[20, 21]

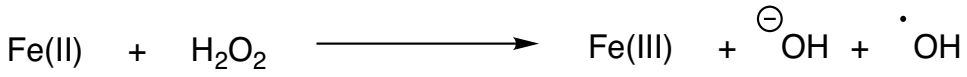


Figure 1.3.: The Fenton reaction

Since iron is a very important metal in nature for example as part of the active centre of many enzymes, the Fenton reaction occurs also in living organisms. The highly reactive radicals produced can then attack biomolecules such as lipids, DNA or proteins and cause damage.[22] Protein damage initiated by hydroxyl radicals involves side chain modifications as well as backbone cleavage. This damage is believed to play an important role in several diseases like diabetes, athereosclerosis, neurodegenerative diseases and

ageing.[23] For that reason, the effect of the Fenton reaction on biomolecules has been studied intensely for many years. The reaction of single amino acids with radicals generated by the Fenton reaction has been studied[24] as well as the mechanistic pathways of protein oxidation.[25] However, almost no studies have addressed the influence of the peptide sequence on the extent of the oxidative damage. Davies and coworkers studied oxidative damage of collagen by a Fenton like reaction using copper focussing on site specificity of the radical attack caused by binding of the metal to the protein.[26] However, so far there exist to the best of our knowledge no systematic studies of the sequence dependence of the Fenton reaction.

1. 2. Research project

The task of this thesis is on one hand to develop a class of receptors that are able to bind peptides selectively and on the other hand to examine whether peptides are cleaved selectively under Fenton conditions.

In the first part the class of diketopiperazine receptors is presented. Their binding properties are studied by combinatorial on-bead screenings against a tripeptide library in organic solvents as well as in water. Furthermore, the importance of the central diketopiperazine as a template for two-armed receptors is emphasised. Structural prerequisites of two armed receptors are studied by exchanging the central diketopiperazine against other templates as well as by conformational analysis of the diketopiperazine template.

In the second part the sequence dependence of peptide cleavage under Fenton conditions is examined by combinatorial screenings of a peptide library where each peptide is flanked by a fluorophore and a quencher.

2. Combinatorial Chemistry

The advent of combinatorial chemistry in the early 1990s has revolutionised the discovery process of new therapeutics.[27] While it was first applied mainly in bioorganic and medicinal chemistry it has since spread to other fields like material science[28] and catalyst development.[29, 30]

The principle of combinatorial chemistry is to synthesise a large number of different molecules ("library"), screen them simultaneously and select the active members. This can be viewed as an attempt to imitate natures' evolution principles of random mutation and survival of the fittest. Combinatorial chemistry is an excellent tool where rational prediction reaches its limits. The success of a combinatorial method relies on two factors, firstly on the capacity to produce a large variety of molecules and on the other hand on the existence of effective screening methods to identify active members of the library. A very simple method to create molecular diversity is the "split-and-mix synthesis" that was presented by Furka and Lam in 1991.[31-34] Although many other methods have been introduced[35, 36] split-and-mix synthesis is still one of the most elegant methods.

2. 1. Split-and-mix synthesis

Split-and-mix synthesis allows for the synthesis of a huge number of different molecules on solid phase with relatively few reaction steps as shown in figure 2.1. It starts with a pool of solid phase resin that is split into several equal portions (In the example three). Then, in each individual reaction vessel a different reaction (A, B or C) is performed.

Afterwards, the resin is recombined leading to a mixture of three different types of beads. The resin is split up again for the second cycle of the synthesis, reactions D, E and F are performed and the beads are mixed again. In our simple example with just three different reactions per cycle we end up with a library of 9 compounds but this number increases exponentially with an increasing number of reaction cycles. After x reaction cycles with n different reactions performed in each cycle the maximal number of compounds equals n^x. It must be pointed out that each single bead carries just one compound ("one bead one compound").

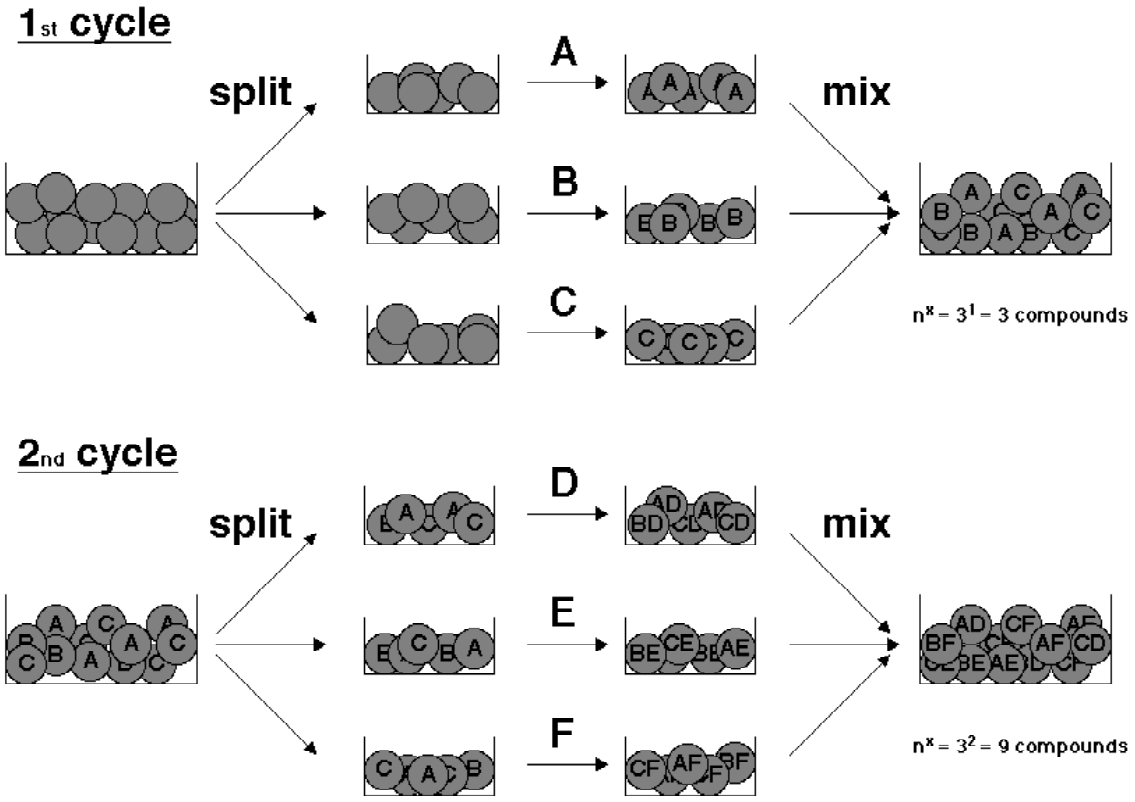


Figure 2.1.: split-and-mix synthesis

The analysis is limited by the amount of compound on a single bead, which is usually around 100 pmol. This allows for the analysis by Edman degradation but this method is restricted to linear peptides. Mass spectrometry[37-39] as well as ^{13}C-NMR and ^{1}H-NMR[40, 41] (only with especially large beads) are possible but the distinction of compounds with identical mass is rather difficult if not impossible by these methods. An alternative to the direct analysis is the use of encoding.[42]

2. 2. Encoded split-and-mix synthesis

The concept of encoding relies on the attachment of tags to the beads in the course of the synthesis. These tags can later be analysed easily and unequivocally. Each reaction step is thus encoded and the tags can later tell the "history" of the corresponding bead. A further advantage of encoding is the fact that the analysis can even be performed when the compound of interest has been modified during the screening.

The tag molecules should ideally 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.

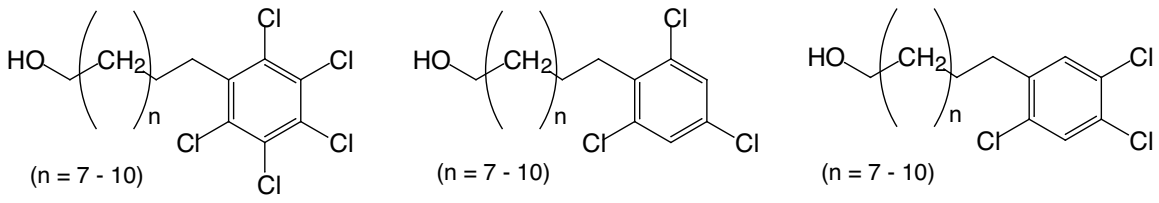


Figure 2.2.: Tag alcohols used for library encoding

All these requirements are fulfilled by the polyhalogenated aromatic alcohols (figure 2.2.) introduced by Still et al.[43, 44] These molecules can be analysed on a 1 pM scale by gas chromatography using electron capture detection (ECGC) since this detection method is very sensitive to halogenated aromates. The varying halogenation patterns as well as the differing chainlengths result in different retention times in the GC. The tags can either be attached to the solid support via a photocleavable[43] or an oxidatively cleavable linker.[44]

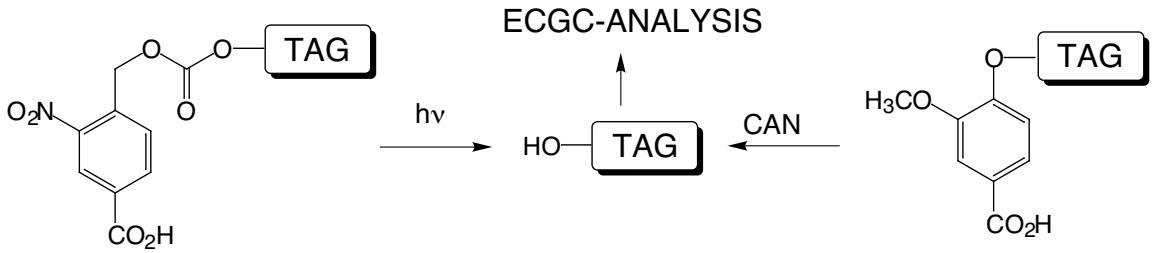


Figure 2.3.: Linkers to attach the tags to the solid support

The tags are coupled either via a carbonate bond to 3-nitro-4-hydroxymethyl-benzoic acid or via an ether bond to vanillic acid. Via the acid functionality, the linkers can be attached to the solid support via amide or ester bonds. Irradiation with UV-light in case of the nitrobenzoic acid or oxidation with ceric ammonium nitrate (CAN) releases the tag alcohols that are then analysed by ECGC after silylation of the alcohol function.

The detection sensitivity (1 pmol) of these tags is so high, that 1-2% of tags in respect to the bead loading is sufficient for analysis. Therefore, in peptide libraries the tags can normally be attached to the free *N*-terminus before the amino acid is coupled. The

resulting capping of a few percent of the peptides can usually be afforded. However, by attaching the tags at a parallel chain, this capping and the resulting inhomogenity of the product peptides can be avoided.

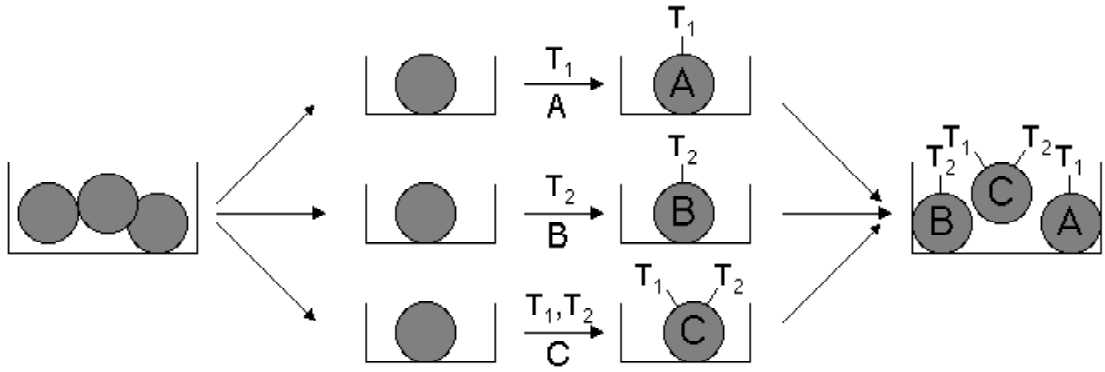


Figure 2.4.: Encoded split synthesis

For encoding of the reactions, a binary code is used: Each reaction is encoded by an array of tags rather than by one single tag. In our example (figure 1.8.), tag 1 encodes for reaction A and tag 2 for reaction B while the combination of tag 1 and tag 2 can be used to encode for reaction C (rather than a third tag). Thus, binary encoding allows to encode 2^N-1 reactions with N different tags.

2. 3. Screening of combinatorial libraries

The real power of combinatorial chemistry lies within the possibility to screen thousands of compounds simultaneously in "on bead screenings" rather than testing each compound individually. An experiment is needed to identify the active compounds in a readily detectable way. Here, two screening methods that are relevant for this work are presented.

2. 3. 1. Screening for selective binding

To visualise selective binding of a receptor to a substrate, the receptor is marked with a dye, a fluorophore[45] or with radioactivity.[46] A solution of this (for example) dye-marked

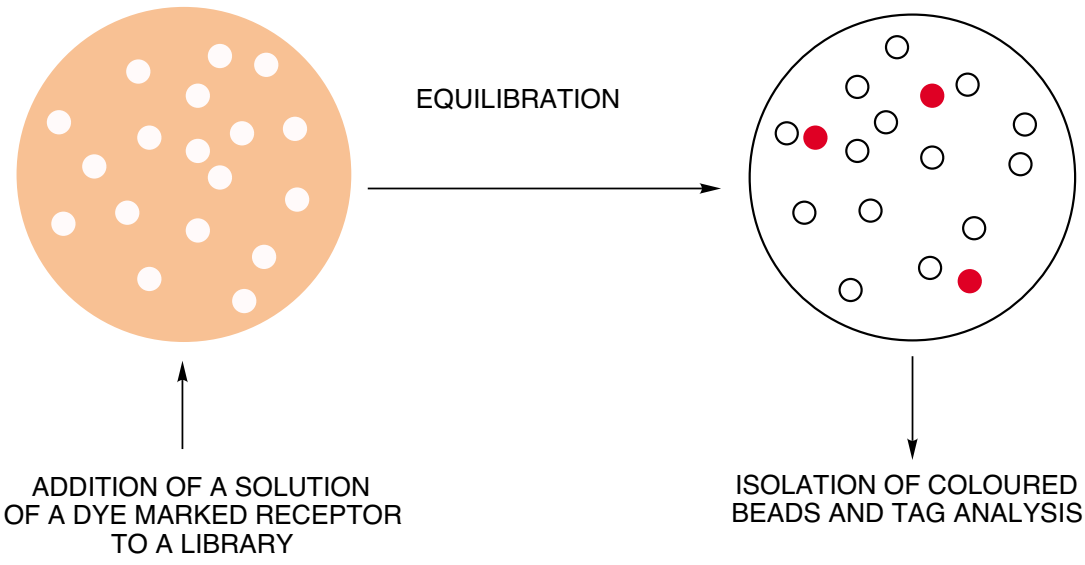


Figure 2.5.: On bead screening of a dye marked receptor against an encoded library

receptor is then equilibrated with an on-bead library. When a library member interacts with the receptor, the receptor and therefore the dye accumulates on the corresponding bead. In case of selective binding, only few beads will pick up the colour. For analysis, the red beads are then isolated and the tags are cleaved and analysed.

2. 3. 2. Screening for Selective Cleavage

On bead detection of selective peptide cleavage can be achieved by a fluorophore quencher library: A fluorescent molecule is attached to a solid support and an adequate quencher is linked to it over a combinatorially varied peptide chain. Fluorescence will not occur as long as the peptide is unharmed. Cleavage of the peptide will lead to removal of the quencher. This, in turn will make the bead appear fluorescent.[47]

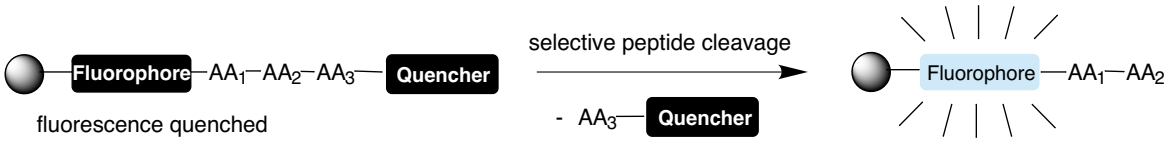


Figure 2.6.: Screening for selective peptide cleavage

3. Selective Binding of Peptides by Diketopiperazine Receptors

3. 1. Receptor design

In order to find a selective receptor for any desired peptide sequence we need a receptor type that can be easily varied combinatorially at sites where such variations have considerable effects on the binding selectivity. A library of such a receptor family should contain receptors for any peptide. As demonstrated in previous studies on synthetic receptors,[13-17] a suitable receptor type has to fulfil certain prerequisites:

-The receptor should possess a certain rigidity
-It should contain functionalities that allow for non covalent interactions with a substrate
-These functionalities should be easily variable

Furthermore, a rather straightforward synthesis that can be transferred to the solid phase is also desirable with respect to the generation of an on-bead library.
Two-armed peptidic molecules with a general structure **3** as shown in Figure 3.1. fulfil all these requirements. The central diproline diketopiperazine restricts the flexibility of the molecule and directs the peptidic arms in positions that should allow for intermolecular interactions with a peptidic guest. The amino acid sidechains of the peptidic arms provide for functionalities that can interact with a peptidic substrate. Since peptide synthesis is well established, these functionalities can be varied combinatorially.
Also the synthesis of the diketopiperazine template should be rather straightforward,

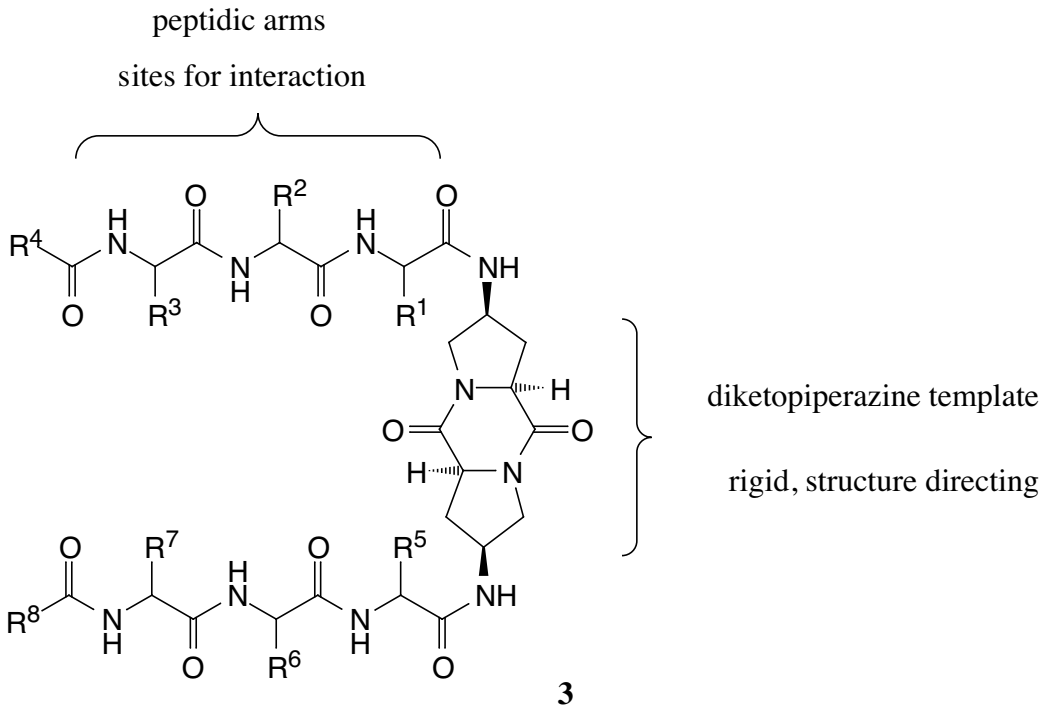


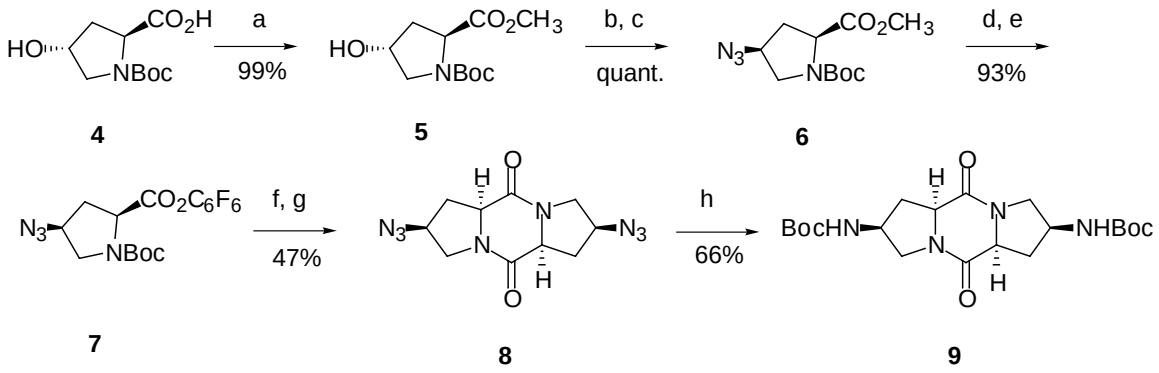
*Figure 3.1.: General structure **3** of the cis-diketopiperazine receptors*

since diketopiperazines are known to form easily. Because the proline diketopiperazine is the essential structural element we termed these molecules "diketopiperazine receptors". The denotation of the template can be further specified in terms of the stereochemistry of Cγ of the proline (the C atom carrying the substituents and thus the arms): This work focuses on diketopiperazines with substituents at Cα and Cγ that are on the same side; these receptors were termed "*cis*-diketopiperazine receptors" or short "*cis*-receptors". This is in contrast to the corresponding *trans*-receptors with inverse stereochemistry on Cγ that will be introduced later in this chapter.

In order to study the binding properties of this receptor type towards peptides we first synthesised several dye-marked *cis*-receptor prototypes and screened them against a tripeptide library.

3. 2. Synthesis of the *cis*-diketopiperazine template

The synthesis of the template starts with commercially available N-Boc protected trans-γ-hydroxy-L-proline **4**. After esterification of the acid group using caesium carbonate and methyl iodide, the hydroxyl functionality of **5** was activated with mesyl chloride. The mesylate was then substituted by an azide under inversion of the configuration at Cγ. The methyl ester **6** was then hydrolysed with sodium hydroxide and the acid was activated as a pentafluorophenyl ester **7**.



a: i. 0.55 eq $CsCO_3$, MeOH/H2O (5:1), r.t., 1 h; ii. 2 eq MeI, DMF, r.t., 1.5 h; b: 1.2 eq MsCl, 1.2 eq NEt_3, CH_2Cl_2, 0°C, 0.5 h; c: 5 eq NaN_3, DMF, 80°C, 3 h; d: 1.2 eq NaOH in H_2O, MeOH/THF (1:1), r.t., 1.5 h; e: 1.1 eq C_6F_5OH, 1.1 eq EDC, CH_2Cl_2, r.t., 1h; f: TFA/CH_2Cl_2(1:3), r.t., 1.5 h ; g: 2 eq iPr_2NEt, THF, r.t., 60 h; h: 10 % Pd/C, H_2, 3 eq Boc_2O, MeOH, r.t., 2 h.

*Figure 3.2.: Synthesis of the bis-Boc-protected diketopiperyzine template **9***

After deprotection of the Boc group by TFA, the cyclisation to the diketopiperazine **8** was performed in CH_2Cl_2 in the presence of Hünigs base at a concentration of 0.3 M.

Reduction of the azide groups with in situ Boc protection of the amino groups gave the well storable *N*-Boc protected diketopiperazine **9** in an overall yield of 29%.

3. 3. Synthesis of five dye-marked receptor prototypes

To study the binding properties of two-armed receptors based on the *cis*-diketopiperazine template, we synthesised a series of dye-marked receptor prototypes in order to screen them against an on-bead peptide library.

For the prototypes, the peptidic arms were chosen to be symmetrical in order to simplify the synthesis. For all prototypes, either D- or L-tyrosine was attached to the template as first amino acids of the arms. Phenylalanine and either trityl protected asparagine or glutamine was chosen as amino acids for positions 2 and 3. We chose tyrosine because a dye, that is required for the combinatorial screening, can be easily attached to its phenolic hydroxyl group. As a dye Disperse red 1 was chosen that could be attached to the tyrosine via an ether linkage. It is known, that Disperse red 1 does not bind to peptides.[48]

We were particularly interested how the binding properties are affected by small changes in the sequences of the peptidic arms. Therefore, the sequences of the receptor arms of the prototypes were chosen to be very similar with only slight differences:

Namely the prototypes **10** and **11** differ only in the configuration of the first amino acid tyrosine. The order of amino acids at positions 2 and 3 are changed in receptors **11** and **12**. Receptor **13** differs from receptor **10** by the exchange of asparagine by the one methylene group longer glutamine. The same is the case for receptors **12** and **14**.

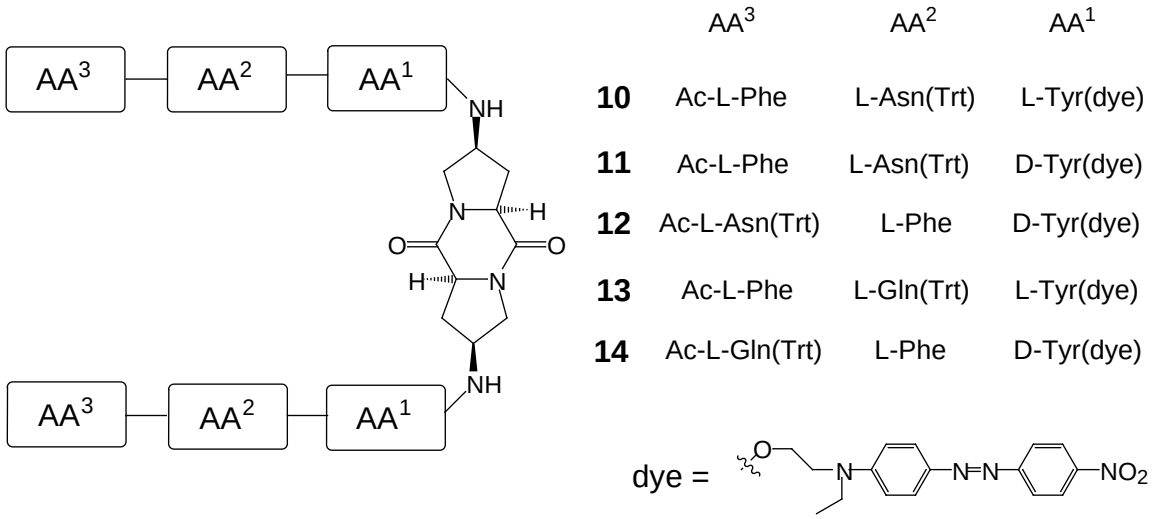
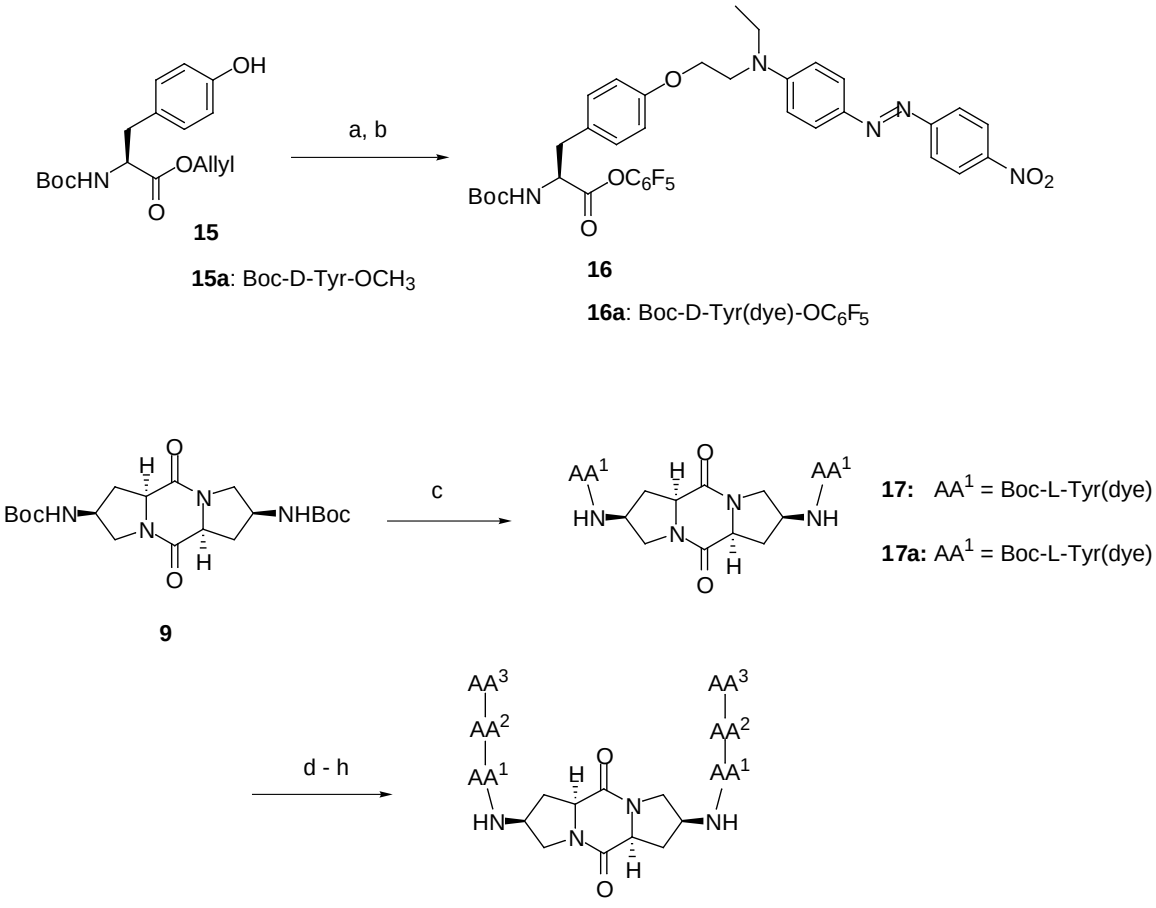


*Figure 3.3.: Structures of prototypes **10 - 14***

The prototypes were synthesised by removal of the Boc groups of **9** and coupling of the dye-marked pentafluorophenylester of *N*-Boc-D- or L-tyrosine **16** or **16a** to yield **17** or **17a** respectively. **16** and **16a** were prepared by coupling Disperse red 1 to the phenolic group of *N*-Boc-protected tyrosine allyl ester **15** or **15a** via a Mitsunobu reaction with subsequent activation as a pentafluorophenylester. After Boc-deprotection of **17, 17a** respectively, the two other amino acids of the arms were assembled by standard couplings of FMOC-amino acids using EDC as coupling reagent and TAEA for FMOC-deprotections.[49, 50] After the removal of the FMOC group of the third amino acid, the aminogroups were acetylated with Ac_2O in the presence of NEt_3 to yield receptor prototypes **10 - 14**. The binding selectivities of these receptor prototypes towards peptides were now determined by combinatorial on-bead screenings.



a: 1 eq Disperse Red 1 (dye), 1 eq PPh_3, 1 eq DEAD, toluene, r.t., 16 h, %; b: i. 1.2 eq NaOH in H_2O, MeOH/THF (1:1), r.t., 1.5 h; ii. 1.1 eq C_6F_5OH, 1.1 eq EDC, CH_2Cl_2, r.t., 1h, %; c: i. TFA/CH_2Cl_2 (1:3), r.t., 1h; ii. 1 eq 16 or 16a, 4 eq iPr_2NEt, CH_2Cl_2, r.t., 16h; d: i. 4M HCl in dioxan, MeOH, r.t., 1 h; ii. 3 eq iPr_2NEt, CH_2Cl_2, r.t.; e: 3 eq FMOC-amino acid, 3 eq EDC, CH_2Cl_2, r.t. 0.5 h; f: TAEA, CH_2Cl_2, r.t. 1 h; repetition of (e) and (f); h: 5 eq Ac_2O, 5 eq NEt_3, CH_2Cl_2, r.t., 1 h.

*Figure 3.4.: Synthesis of the receptor prototypes **10 - 14***

3. 4. Binding properties of the receptor prototypes

In order to test the binding properties of the receptors, we screened them against an encoded tripeptide library.[43] The library we used (**18**) consisted of 29 different side chain deprotected D- and L- amino acids in each position leading to a maximal variety of 29^3 = 24389 different peptides. The library had been synthesised on polystyrene resin and the *N* terminus was acetylated. The general structure is shown in figure 3.5.

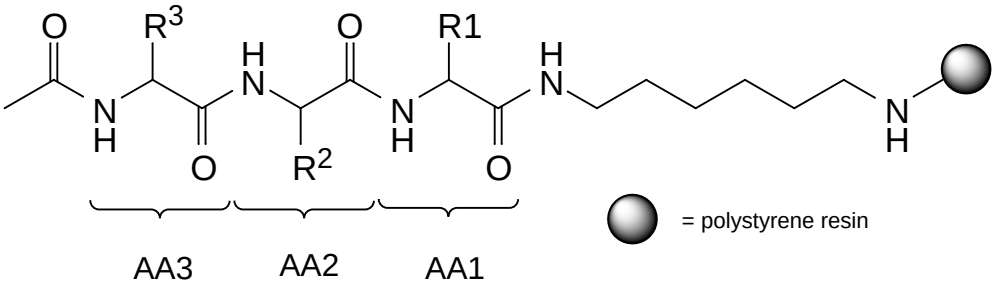


*Figure 3.5.: General structure of the tripeptide library **18**, AA1 - AA3 = Gly, L-Ala, D-Ala, L-Leu, D-Leu, L-Val, D-Val, L-Pro, D-Pro, L-Phe, D-Phe, L-Ser, D-Ser, L-Thr, D-Thr, L-Asn, D-Asn, L-Gln, D-Gln, L-His, D-His, L-Asp, D-Asp, L-Glu, D-Glu, L-Lys, D-Lys, L-Arg, D-Arg.*

The screenings were performed in chloroform (filtered over aluminium oxide) at a receptor concentration of ~30 μM. For the screenings, five theoretical copies of the library were used to ensure that every sequence was present at least once in every assay.[51, 52] After two days of equilibration, in the assays of **10** and **13**, about one bead out of 1500 picked up the red colour of the receptor (figure 3.6.). In contrast, no red beads were observed in the screenings of **11, 12** and **14** even when the concentration was augmented up to 500 μM indicating that these two-armed molecules don't bind to any of

the 24389 different peptides of the library.[53] Remarkably, both prototypes with an L-Tyr in the first position of the arms bind to peptides with high selectivity, all the prototypes with a D-Tyr as first amino acid do not bind to any of the 24389 peptides within the library. Thus, the amino acid that is directly attached to the template has the largest influence on the binding properties. This phenomenon will further be investigated in chapter 4.9.

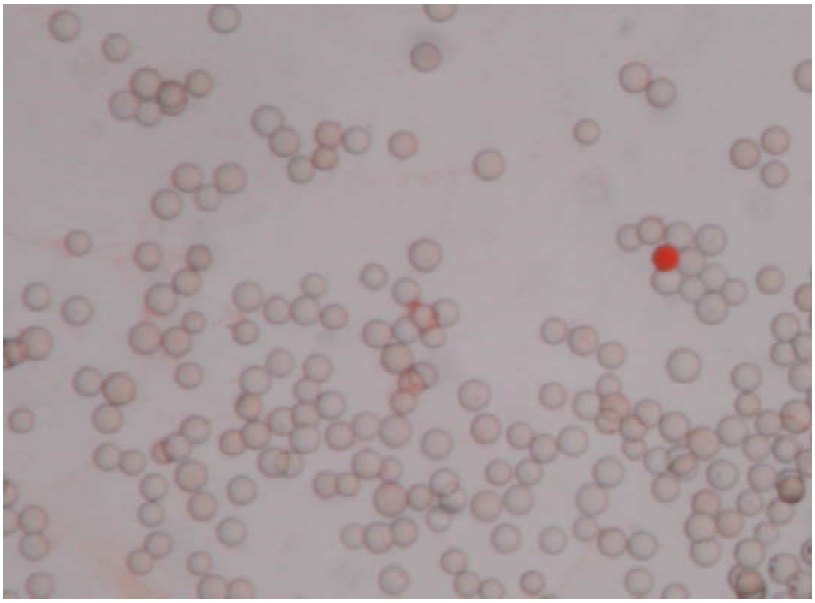


*Figure 3.6.: Screening of receptor **10** against the tripeptidelibrary **18** in chloroform*

The sequences of the peptides bound to **10** and **13** were elucidated by separating the red beads manually, cleaving and analysing the encoding tags on these beads. Table 3.1. shows the sequence on the red beads of the assays of **10** and **13**.

Both receptors preferentially select peptides with a D-histidine in position AA1 followed by two hydrophobic D-amino acids. Furthermore the two receptors although differing only in a single methylene group show certain differences in the binding selectivities.

*Table 3.1.: Binding specifities of the receptors **10** and **13** against the tripeptide library*

	AA3	AA2	AA3	Frequeny found[a] [%]	Frequency expected[b] [%]
10	D-Hph[c]	D-Hph[c]	D-His	59	0.10
	D-Ala	L-Asn	D/L-Hph[c]	26	0.04
	D-Asn	D-Hph[c]	D-Val/Gly	9	0.04
13	D-Val	D-Hph[c]	D-His	56	0.02
	D-Phe/D-Ala	D-Phe/D-Ala	D-His	19	0.02
	D-Gln	D-Val/D-Phe	D-Val/D-Leu	19	0.02

[a] The frequency found column lists the percentage of the corresponding peptide sequence among the selected beads [b] The frequency expected column lists the percentage of the corresponding peptide sequence among all the library beads. Comparison between the frequency found and the frequency expected gives a measure for the selectivity of the receptor [c] Hph = hydrophobic amino acid. Can either be Gly, Ala, Val, Leu or Phe

Receptor **10** also selects for peptides with an L-Asn in position AA2 or AA3 together with hydrophobic D-amino acids while receptor **13** shows a further preference for peptides with a D-Gln at the *N*-terminal position in combination with two hydrophobic D-amino acids in the other two positions. Most importantly, diketopiperazine-receptors are indeed able to bind to peptides selectively and small differences in the arms can alter the binding selectivities.

To get an idea about the nature as well as the strength of these interactions we attempted to perform NMR binding studies with receptor **13** and a representative peptide with the D-Hph-D-Hph-D-His sequence. We chose receptor **13** rather than receptor **10** because of

its better solubility in chloroform. However, the peptides Ac-D-Val-D-Val-D-His-$NH(CH_2)_7CH_3$ as well as Ac-D-Phe-D-Phe-D-His-$NH(CH_2)_7CH_3$ were insoluble in chloroform, thus preventing binding studies with ^{1}H-NMR spectroscopy.

Because of these solubility problems the binding affinities were measured by solid phase binding assays.[54] In table 3.2. the ΔG of receptors **10** and **13** against the peptides Ac-D-Val-D-Val-D-His-resin and Ac-D-Phe-D-Phe-D-His-resin are shown. The affinities lie in the range of ~ 5 kcal/mol which is a reasonable strong affinity for a small receptor.

Table 3.2. Binding affinities ($\Delta G = -RTlnK_a$)

Receptor	Ac-D-Val-D-Val-D-His-resin	Ac-D-Phe-D-Phe-D-His-resin
10	- 5.6	- 5.3
13	- 4.9	- 4.9

Each measurement was repeated multiple times to ascertain the accuracy of the binding affinities within errors of ±0.2 kcal/mol.. Resin = polystyrene (loading 28 μmol/g)

3. 5. How does the length of the peptidic arms influence the binding properties?

To investigate the influence of changes in the length of the receptor arms, two variations of receptor **10** were synthesised with one amino acid more or less in each arm. The arms of prototype **19** consist of one amino acid less than **10**, in prototype **20** the arms are elongated by one asparagine. These two prototypes were screened against the tripeptide library **18** in chloroform. While two-armed molecule **19** didn't show any selective binding, several beads turned red in the assay with receptor **20**. In Table 3 the binding

selectivities of **20** are shown. It is apparent that the bound sequences differ substantially from the peptides bound by receptor **10**. Although **20** binds to sequences containing asparagine combined with hydrophobic amino acids that were also bound by **10**, the most abundant sequence found in the assay of **10**, the D-Hph-D-Hph-D-His motif, is not recognized by receptor **20**.

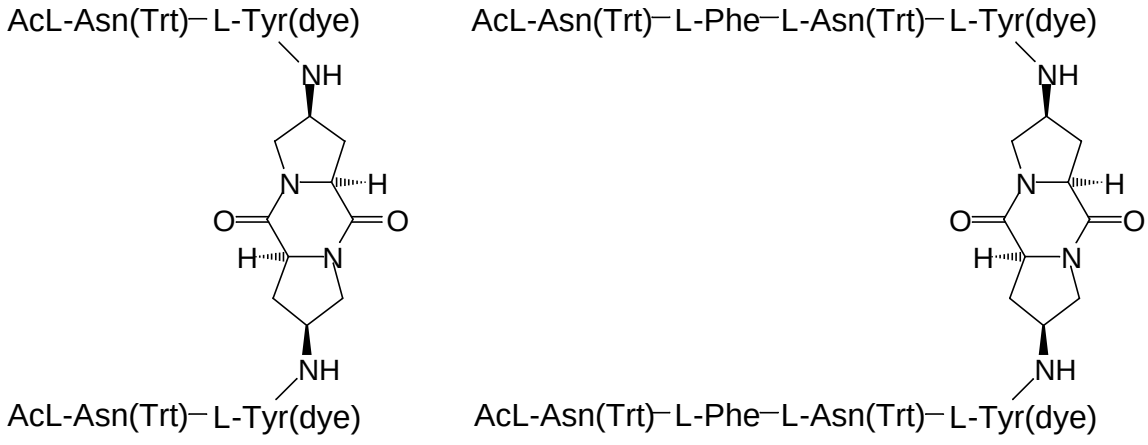


Figure 3.7.: Variation of the number of arm amino acids

These results indicate that three amino acids in the arm are minimally necessary to observe selective binding to peptides. By adding one amino acid, the binding selectivity changes.

So far, we have concentrated on the effect of changes in the receptor arms. In the following, we will examine the influence of the backbone by replacing the diketopiperazine with other diamines.

*Table 3.3.: Sequences bound by receptor **20***

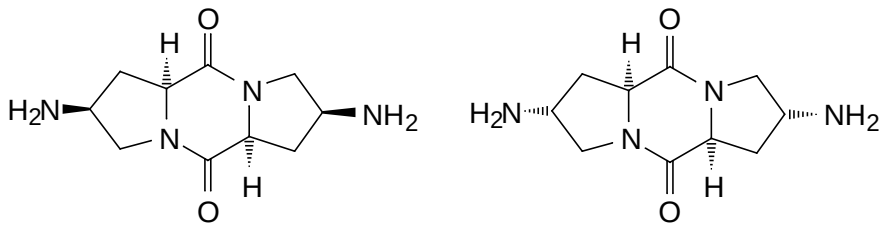
	AA3	AA2	AA1	Frequeny found[a] [%]	Frequency expected[b] [%]
20	L-Asn	L-Hph	L-Asn	55	0.02
	D-Hph	D-Asn	D-Hph/D-Asn	18	0.13
	D-Asn/Gly	L-Hph	L-Asn	18	0.04

[a] The frequency found column lists the percentage of the corresponding peptide sequence among the selected beads [b] The frequency expected column lists the percentage of the corresponding peptide sequence among all the library beads. Comparison between the frequency found and the frequency expected gives a measure for the selectivity of the receptor [c] Hph = hydrophobic amino acid. Can either be Gly, Ala, Val, Leu or Phe

3. 6. Changing the configuration of the template from *cis* to *trans*

First we wanted to investigate the effect of a relatively small change in the template on the binding properties of the corresponding receptors. We were therefore interested in the diastereomeric *trans*-diketopiperazine with inversed stereochemistry at Cγ.

Based on the *trans*-template, receptor prototypes **10' - 14'** analogue to **10 - 14** were synthesised by attaching the same sequences as receptor arms.[55] The *trans*-receptors **10'** and **13'** bound to similar peptide sequences as the corresponding *cis*-receptors **10** and **13**. More surprisingly also receptors **11'**, **12'** and **13'** showed highly selective binding towards peptides in contrast to their *cis*-diastereomeric analogues **11**, **12** and **13** that don't



cis-template *trans*-template

Figure 3.8.: The two diastereomeric diketopiperazine templates

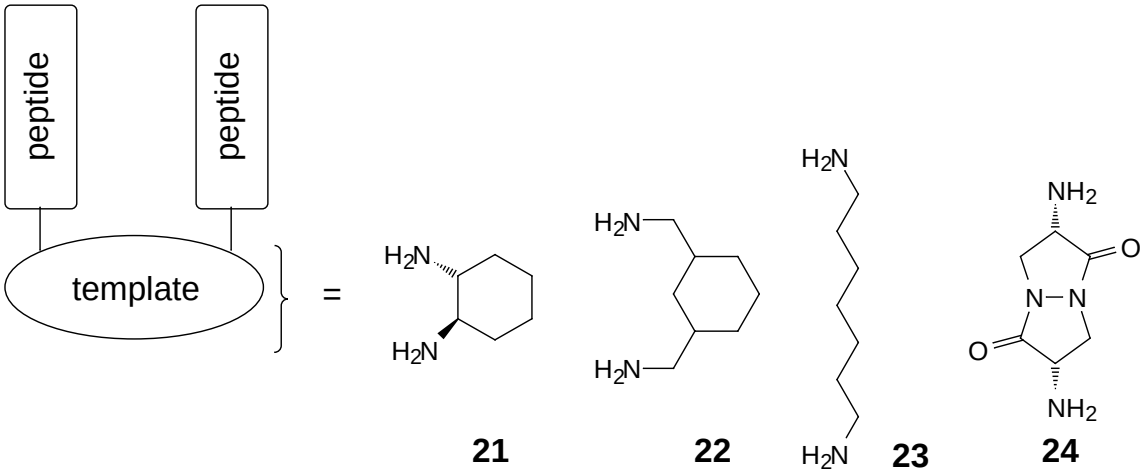
bind towards peptides. Thus, the change from L- to D- in the configuration of the first tyrosine residue has not the same dramatic effect on the binding ability for the *trans*-receptors as it has on the *cis*-receptors.

We thus showed that representatives of two-armed molecules based on both the *cis*-diketopiperazine template and the *trans* diketopiperazine template are able to bind to similar peptides selectively. This gives rise to the question if any template that can anchor two peptidic arms is good enough to serve as a backbone for two-armed receptors.

3. 7. Can a simpler diamino template replace the diketopiperazine?

To address this question we synthesised two-armed molecules based on several diamines. In addition to the commercially available diaminocyclohexane **21**, bis-aminomethylcyclohexane **22**[56] and 1,7 diaminoheptan **23**, diketodiazabicyclooctan **24**[57] was used.

The templates **21 - 24** differ not only in the distance of the two amino groups that serve as anchors for the arms but also in their rigidity. While the templates **21** and **24** are rigid, the direction of the amino groups is not fixed in **22**. The diaminoheptane **23** is completely flexible and was chosen because the distance between the two amino groups is the same as in the diketopiperazine.



	diaminotemplates			
receptor arms	**21**	**22**	**23**	**24**
Ac-L-Phe- L-Asn(Trt)- L-Tyr(dye)-	**25**	**26**	**27**	**28**
Ac-L-Phe- L-Asn(Trt)- L-Tyr(dye)-	**29**	**30**	**31**	**32**

*Figure 3.9.: Diamines **21 - 24** used as templates for two-armed molecules and the corresponding two-armed molecules **25 - 32** [58]*

As arms we used the peptide from receptor **10**, (Ac-L-Phe-L-Asn(Trt)-L-Tyr(dye)) as well as the arm of **11** (Ac-L-Phe-L-Asn(Trt)-D-Tyr(dye)). We thus chose one representative that bound to the peptides with both the *cis*- and the *trans*- template as well as its diastereomer with changed configuration on the tyrosine that binds to peptides with the

trans-diketopiperazine as backbone but not with the *cis*-diketopiperazine. In figure 3.9., the four templates and the resulting eight two-armed molecules are listed.

Unlike the two-armed molecules based on the diketopiperazine templates, **25 - 32** were not synthesised step by step but the peptidic arms were synthesised separately and then coupled to the diamines using either HATU or TBTU as coupling reagents (chapter 6.5.). Screening against the deprotected tripeptide library **18** at concentrations of up to 500μM revealed that all eight potential receptors showed no binding against any of the 24389 peptides within the library.

Thus the template is crucial for selective binding of peptides and exchanging the diketopiperazine template by completely different diamines results in a complete loss of binding ability.

However, already the relatively small change from the *trans* to the *cis*-template has a big effect on the binding properties. The most dramatic difference between the *trans*- and *cis*-receptors is the fact that only receptors based on the *trans*-template are binding to peptides when the first amino acid of the arm has D-configuration. In order to understand this phenomenon, conformational studies of the diketopiperazine templates were performed.

3. 8. Structural studies on the diketopiperazine receptors

In order to explain the binding results of the *cis*- and *trans*-diketopiperazine receptors we now needed to answer the two following questions:

Firstly, is it possible to find a reasonable explanation for the worse binding properties of the receptors based on the *cis*-diketopiperazine in comparison to the corresponding *trans*-diketopiperazine diastereomers by analysing the structure of the two diastereomeric templates?

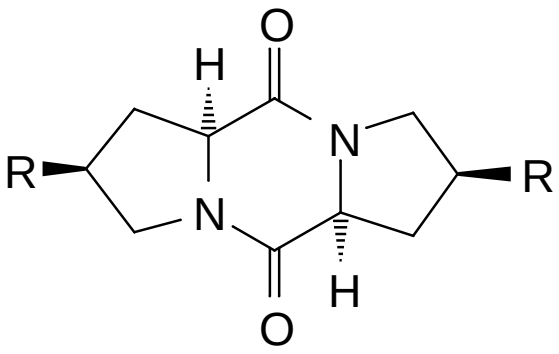
Secondly, why are two armed molecules based on the *cis*-diketopiperazine template only able to bind to peptides when the first amino acid has L-configuration?

Structural studies on the diketopiperazine template were thus performed in the solid state as well as in solution by ^{1}H-NMR spectroscopy.[52]

3. 8. 1. Structure of the template

The receptor prototypes are rather complex molecules which complicates the conformational studies using ^{1}H-NMR by overlapping signals. Furthermore, all attempts to crystallise the receptor prototypes were unsuccessful. Because of these difficulties, we decided to base our structural studies of the template on simpler model compounds. We thus synthesised a couple of *cis*-diketopiperazine derivatives with different substituents on Cγ. The preparation of the bis-azide **8** as well as the *N*-Boc-protected diamino-diketopiperazine **9** has already been presented in chapter 3.2. The bis-acetyl

diketopiperazine **33** was synthesised by reducing azide **8** in the presence of acetic anhydride.



8: R = N_3

9: R = NHBoc

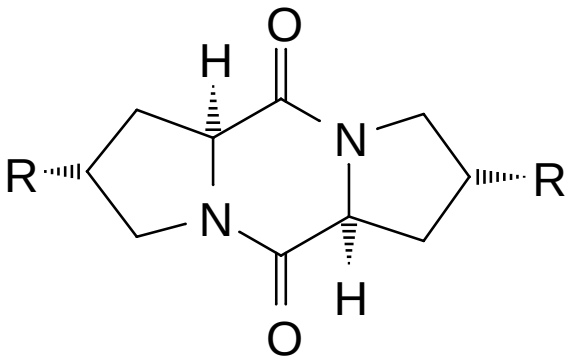
33: R = NHAc

34: R = NH_2*HCl

35: R = $NHCO(CH_2)_5\ CH_3$

Figure 3.10.: cis-diketopiperazine model compounds

Ammoniumsalt **34** was prepared by the deprotection of **9** with HCl. Model compound **35** was synthesised by removing the Boc groups of bisBoc diketopiperazine **9** and reacted with heptanoic acid anhydride. The corresponding model compounds **8', 9'** and **33' - 35'** based on the *trans*-diketopiperazine (figure 3.11.) were obtained in an analogous way.[59]



8': R = N_3

9': R = NHBoc

33': R = NHAc

34': R = NH_2*HCl

35': R = $NHCO(CH_2)_5CH_3$

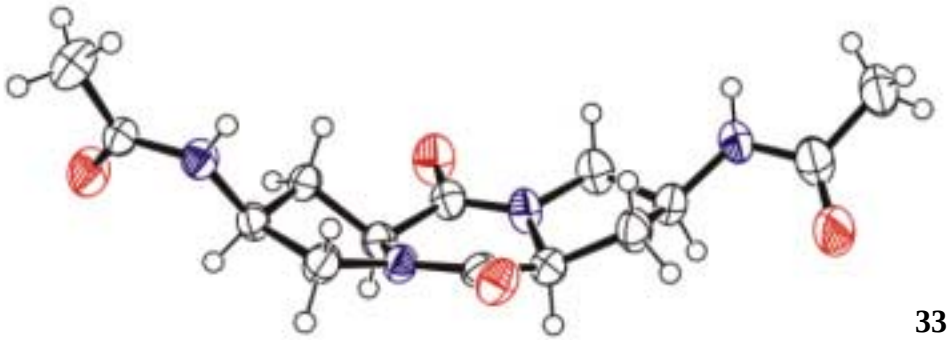
Figure 3.11.: trans-diketopiperazine model compounds

3. 8. 1. 1. Crystal structure of the bis-acetylated diketopiperazine templates

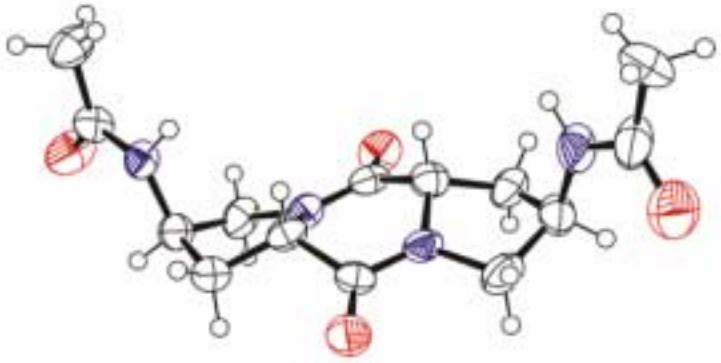
Crystals were obtained from the bis-acetylated derivatives **33** and **33'** of both diketopiperazine diastereomers. Figure 3.12. shows the crystal structures of the two molecules.[60]

The conformation of the tricyclic skeleton of the two diastereomers is nearly identical. The central six-membered ring of the diketopiperazine adopts boat conformation. The pyrrolidine rings possess envelope ($E_{C\beta}$) conformations that are slightly perturbed to twisted ($^{C\gamma}T_{C\beta}$) conformations in both compounds. However, the two structures differ significantly in the orientation of the *N*-acetyl groups. In the *cis*-compound **33** they occupy the pseudo-equatorial positions at Cγ, in the *trans*-compound **33'** the pseudo-axial positions. As a result, the distances between the two N- atoms and also the angle formed between the two side chains and the tricyclic skeleton are considerably different in the two diastereomers. While the distance between the N-atoms of the two N-acetyl groups is 8.7 Å in the crystal structure of the *cis*-template, this distance is almost 1 Å smaller in the crystal structure of the *trans*-template, namely 7.8 Å. As a result, the angle between the two Cγ-N bonds is much narrower in the *trans*-template. The *trans*-diketopiperazine adopts a turn-like conformation, while the *cis*-diketopiperazine is a rather linear structural element. This observation gives a first hint for the explanation of the different binding properties of the *cis*- and *trans*-receptor prototypes: The *trans*-diketopiperazine directs the two receptor arms into the same direction, thereby favouring an interaction with the peptide. However, the unit cell of both crystal structures contains two nearly identical molecules that are connected by two hydrogen bonds that stabilise these conformations.

The structure in solution might therefore differ from the one observed in the crystal structure. Thus conformational studies by ^{1}H-NMR in solution were performed.



33



33'

*Figure 3.12.: Crystal structures of bis acetyl cis-diketopiperazine **33** and bis acetyl trans-diketopiperazine **33'***

3. 8. 1. 2. Conformation of the *cis*-template in solution

The structure in solution was investigated using one and two dimensional ^{1}H-NMR spectroscopy experiments. In all ^{1}H-NMR spectra, only six spin systems were observed,

indicating that all compounds possess C_2-symmetry on the average time scale of the NMR measurement. The unambiguous assignment of the proton signals was achieved using a combination of COrrelated SpectroscopY (COSY) and either Nuclear Overhauser Effect SpectroscopY (NOESY) or Rotational Overhauser Effect SpectroscopY (ROESY). The Karplus curve[61] allows for a comparison between the ^{1}H-^{1}H coupling constants and the expected torsion angles.

*Table 3.4.: ^{1}H-^{1}H-coupling constants (Hz, ±0.1Hz) observed for cis-model compounds **8, 9** and **33 - 35** compared to the coupling constants that would be expected for the torsion angles found in the crystal structure of **33***

Torsion angle[a]	NMR, $^3J_{(H,H)}$ **8**	NMR, $^3J_{(H,H)}$ **9**	NMR, $^3J_{(H,H)}$ **33**	NMR, $^3J_{(H,H)}$ **34**	NMR, $^3J_{(H,H)}$ **35**	X-ray **33**[b]	$^3J_{(H,H)}$ exp.[c] **33**
Hα-Cα-Cβ-Hβ	8.8	7.5	nd[d]	nd[e]	7.7	30±8	6.5-8.5
Hα-Cα-Cβ-Hβ´	5.7	7.5	nd[d]	nd[e]	7.7	152±9	9.0-11.5
Hβ-Cβ-Cγ-Hγ	5.6	7.5	nd[d]	nd[e]	6.9	30±8	6.5-8.5
Hβ´-Cβ-Cγ-Hγ	4.5	7.5	nd[d]	nd[e]	7.4	152±9	9.0-11.5
Hγ-Cγ-Cδ-Hδ	5.6	6.7	5.9	nd[e]	6.9	17±7	7.5-9.5
Hγ-Cγ-Cδ-Hδ´	3.8	6.0	3.7	nd[e]	6.0	139±8	6.5-9.5

*[a] Hα, Hβ, Hγ, Hδ and Hβ´, Hδ´ respectively are on opposite faces of the pyrrolidine ring. [b] Average over all torsional angles within the pyrrolidine rings of the two molecules in the asymmetric unit of the crystal structure. [c] vicinal coupling constants expected for the conformation observed in the crystal structure of **33**. [d] The coupling constants could not be determined unambiguously due to overlapping signals of Hα and Hγ as well as Hβ and Hβ´. [e] **34** is not soluble in chloroform*

The coupling constants of the *trans*-diketopiperazine model compounds correspond very well with the expected values from the crystal structure. Furthermore, neither the

substituent on Cγ nor the solvent has a considerable influence on the structure. Thus, the *trans*-diketopiperazine template is conformationally well defined and adopts the same single conformation in solution as in the crystal.

In contrast to the *trans*-diketopiperazine, the configuration of the *cis* model compounds do not match with the expected values from the cristal structure. Table 3.4. shows that none of the coupling constants of the four model compounds soluble in chloroform lie fully within the range that would be expected from the crystal structure of **33**.

Particularly the azide **33** shows the biggest deviation. Compounds **9** and **35**, whose coupling constants are likewise, look more like the crystal structure although some of the coupling constants still lie outside the expected range. Thus, the crystal structure is not identical with the template structure in chloroform solution. As shown in figure 3.13. two main conformations are conceivable for the *cis*-diketopiperazine: a conformation with the substituents at Cγ in the pseudo equatorial positions ("pseudo-equatorial conformation") as seen in the crystal structure and another one with pseudo-axial-positioned substituents and ring flipped pyrrolidine moieties.

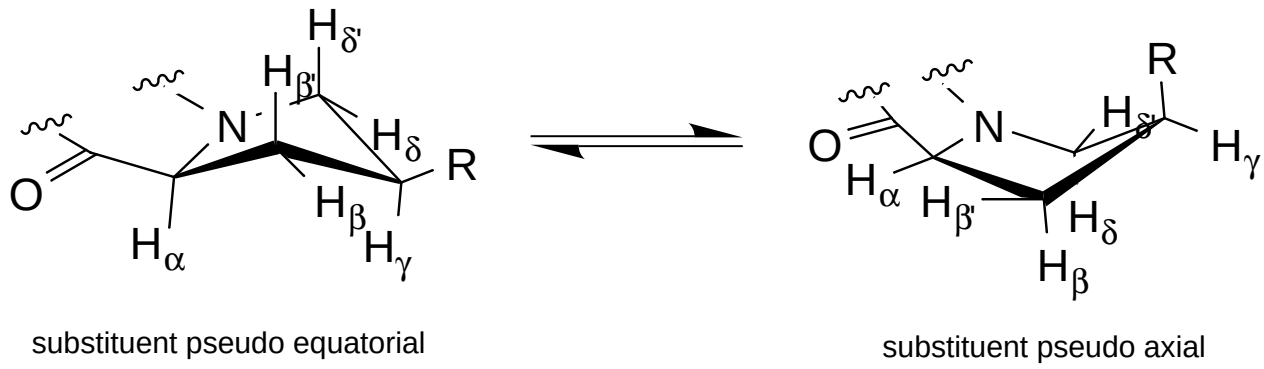


*Figure 3.13.: Simplified conformational equilibr*35*um of the cis-diketopiperazine*

In table 3.5., the expected coupling constants for these two conformations as estimated by the Karplus curve are listed. The most obvious differences exist for the coupling constants between Hα and Hβ', Hγ and Hβ' as well as for Hγ and Hδ'. The torsion angle for all three proton pairs changes from an angle of almost 180° at the pseudo equatorial conformation to a nearly 90° angle at pseudo-axial conformation. This leads to a difference in the coupling constants from >9 down to ≈ 0.

Table 3.5.: Expected ^1H coupling constants for the two possible conformations of the cis-diketopiperazine

Torsion angle[a]	$^3J_{(H,H)}$ expected pseudo equatorial	$^3J_{(H,H)}$ exp. pseudo axial
Hα-Cα-Cβ-Hβ	6.5-8.5	5.5-8.5
Hα-Cα-Cβ-Hβ´	9.0-11.5	≤ 2
Hβ-Cβ-Cγ-Hγ	6.5-8.5	4.0-7.0
Hβ´-Cβ-Cγ-Hγ	9.0-11.5	≤ 2
Hγ-Cγ-Cδ-Hδ	7.5-9.5	4.5-7.5
Hγ-Cγ-Cδ-Hδ´	6.5-9.5	≤ 2

When the expected values from table 3.5. are compared to the experimental coupling constants in chloroform, we clearly see that none of the two conformations can be used to describe the situation in solution. For all four model compounds, the measured values are approximately an average of the expected values for the two conformations.

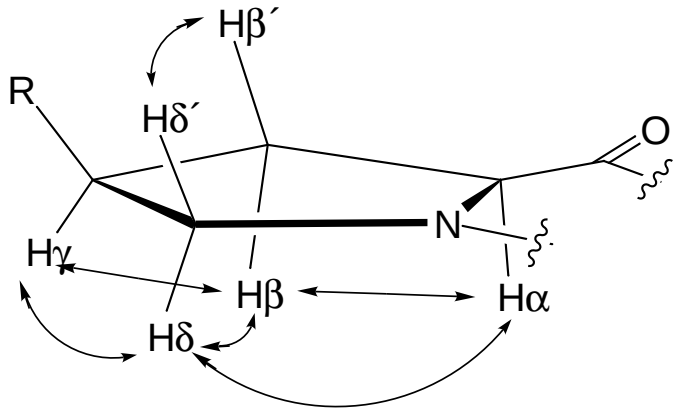


Figure 3.14.: NOE's observed for the cis-diketopiperazine model compounds ***8, 9, 33 - 35.***

The different values found for the model compounds indicate that the position of the equilibrium depends on the substituents. While for all four compounds roughly a 1 : 1 equilibrium between the two conformers is observed the equilibrium lies slightly more on the pseudo equatorial side for **9** and **35** compared to **8** and **33**.

In order to investigate the influence of the solvent on the equilibrium all model compounds were also measured in the more polar solvents d_4-methanol and d_8-DMSO. In these solvents, the bis-amine **34** was also measured. In table 3.6. the coupling constants of all model compounds in all three solvents are listed including the ones in chloroform. For all compounds, the coupling constants measured in the two polar solvents methanol and DMSO are almost identical. A significant difference to the values obtained in chloroform is only observed for bis-acetyl diketopiperazine **33**. The equilibrium for that compound is shifted towards the pseudo equatorial conformation.

*Table 3.8.: ^{1}H-^{1}H-coupling constants (Hz, ±0.1Hz) observed for model compounds **8, 9, 33, 34** and **35** in the solvents $CDCl_3$ (A), CD_3OD (B) and d_8-DMSO (C).*

	8			**9**			**33**			**34**			**35**		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Hα-Cα-Cβ-Hβ	8.8	9.1	9.1	7.5	6.7	7.0	nd[d]	6.9	7.3	nd[e]	8.2	8.2	7.7	9.6	9.8
Hα-Cα-Cβ-Hβ´	5.7	4.9	4.9	7.5	9.7	10.2	nd[d]	9.7	9.5	nd[e]	8.2	8.2	7.7	7.4	7.5
Hβ-Cβ-Cγ-Hγ	5.6	5.4	5.3	7.5	6.7	6.2	nd[d]	6.9	6.9	nd[e]	7.2	7.0	6.9	9.8	9.8
Hβ´-Cβ-Cγ-Hγ	4.5	3.9	3.6	7.5	9.7	10.2	nd[d]	9.7	9.7	nd[e]	8.0	7.0	7.4	6.9	6.8
Hγ-Cγ-Cδ-Hδ	5.6	5.3	5.2	6.7	7.8	8.2	5.9	7.9	7.8	nd[e]	7.2	7.2	6.9	7.6	7.6
Hγ-Cγ-Cδ-Hδ´	3.8	3.1	2.8	6.0	7.8	8.5	3.7	7.7	7.6	nd[e]	5.7	5.1	6.0	7.9	7.8

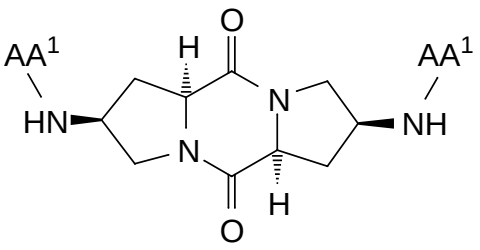
*[d] The coupling constants could not be determined unambiguously due to overlapping signals of Hα and Hγ as well as Hβ and Hβ´. [e] **34** is not soluble in chloroform*

In conclusion, the structural studies on the *cis*- and *trans*- diketopiperazines revealed that the *trans*-diketopiperazine is conformationally defined and adopts a turn-like structure with pseudo-axial standing substituents. For receptors built upon the *trans*-diketopiperazine, this means, that the receptor arms are directed into the same direction by the template. In contrast, the *cis*-diketopiperazine is flexible and the pseudo axial conformation coexists with a conformation with pseudo equatorial standing substituents. Thus, the observed different binding properties of *cis*- and *trans*-diketopiperazine based receptor prototypes were rationalised based on structural studies on the two templates. However there's still no explanation why we don't see any binding for the *cis*-diketopiperazine based molecules when a D-tyrosine is the first amino acid while the receptors have almost the same binding properties as the *trans*-receptors when L-tyrosine

is the first amino acid. We thus analysed why the configuration of the first amino acid of the arms has such a big influence on the binding properties of the receptors.

3. 8. 2. How does the configuration of the first amino acid affect the structure of the *cis*- template?

To find an explanation for the different binding properties of *cis*-diketopiperazine receptors depending on the configuration of the first amino acid, conformational studies in solution were performed. We decided to take a look at simple model compounds to avoid solubility problems and to minimise overlapping of proton signals in the NMR-spectra.



36: AA^1 = Ac-D-Tyr(OMe)
37: AA^1 = Ac-L-Tyr(OMe)

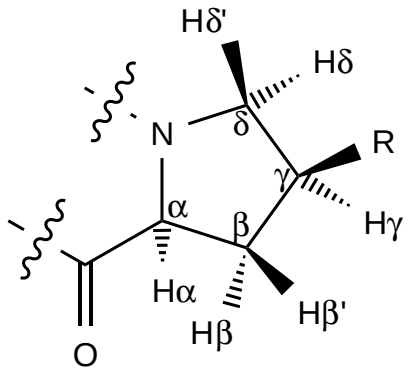
*Figure 3.15.: Model compounds **36** and **37***

The investigations were started with the diastereomeric pair **36** and **37** with either a Ac-L-Tyr(OMe) or Ac-D-Tyr(OMe) respectively coupled to the *cis*-diketopiperazine. By comparing the ^{1}H-NMR spectra of **36** and **37** in chloroform, we observed a remarkable difference in ppm for the proton signals Hβ' and Hδ'. In **36** these protons appear 0.52

ppm or 0.23 ppm, respectively, further downfield compared to **37** (table 3.7.). The trend is reverse for the other signals. These chemical shift differences indicate that the conformation of the *cis*-diketopiperazine is influenced to a significant extent by the configuration of the amino acid that is directly coupled to the diketopiperazine.

*Table 3.7: ^{1}H chemical shifts (δ in ppm) for the pyrrolidine ring protons of compounds **36** and **37** in chloroform. (**36**: R = NH-D-Tyr(OMe)Ac; **37**: R = NH-L-Tyr(OMe)Ac)*

Proton	**36**	**37**
α	4.26	4.14
β	2.42	2.22
β'	2.42	**3.14**
γ	4.35	4.09
δ	3.43	3.21
δ'	3.74	**3.97**



In table 3.8. the mutual coupling constants between the pyrrolidine ring protons for compounds **36** and **37** are listed together with the expected values for either the conformation with pseudo equatorial or pseudo axial substituents. In addition the coupling constants of the bis-azide **8** are listed.

These data show, that the conformation of **36** (D-Tyr(OMe) resembles the conformation of *bis*-azide **8**. The ^{1}H-^{1}H coupling constants of compound **37** however fit perfectly with the values that would be expected for the pseudo-axial conformation. The coupling of close to ~ 0 Hz for the couplings between Hγ and Hβ', Hγ and Hδ' as well as Hα and Hβ'

indicate torsion angles of nearly 90°. This is only possible for the pseudo axial conformation.

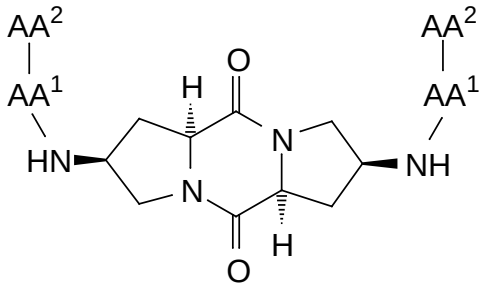
*Table 3.8.: ^{1}H-^{1}H coupling constants for the pyrrolidine ring protons of compounds **36** and **37** and **8** in chloroform as well as the estimated values for the pseudo equatorial and the pseudo axial conformation.*

Torsion angle[a]	NMR, $^{3}J_{(H,H)}$ **36** (D-Tyr)	NMR, $^{3}J_{(H,H)}$ **37** (L-Tyr)	NMR, $^{3}J_{(H,H)}$ **8** (N_3)	$^{3}J_{(H,H)}$ exp. pseudo equatorial	$^{3}J_{(H,H)}$exp. pseudo axial
Hα-Cα-Cβ-Hβ	8.7	10.1	8.8	6.5-8.5	5.5-8.5
Hα-Cα-Cβ-Hβ´	5.0	1.5	5.7	9.0-11.5	≤ 2
Hβ-Cβ-Cγ-Hγ	n.d.[b]	9.0	5.6	6.5-8.5	4.0-7.0
Hβ´-Cβ-Cγ-Hγ	n.d.[b]	1.0	4.5	9.0-11.5	≤ 2
Hγ-Cγ-Cδ-Hδ	5.9	9.0	5.6	7.5-9.5	4.5-7.5
Hγ-Cγ-Cδ-Hδ´	3.3	1.0	3.8	6.5-9.5	≤ 2

[a] Hα, Hβ, Hγ, Hδ and Hβ´, Hδ´ respectively are on opposite faces of the pyrrolidine ring. [b] The coupling constants could not be determined unambiguously due to overlapping signals of Hα and Hγ as well as Hβ and Hβ´.

NOESY measurements confirmed this result by showing crosspeaks between Hβ and Hδ as well as between Hβ' and Hδ' for **36**. For **37**, only a crosspeak between Hβ and Hδ is observed but no crosspeak between Hβ' and Hδ'. Thus model compound **37** is conformationally much better defined than **36** and the pseudo axial conformation is preferred.

It was now interesting to see, if this stabilisation can also be observed for model compounds with longer "arms". We thus synthesised two diastereomeric pairs of two-armed molecules with two amino acids per arm (Figure 3.16.).



	AA^2	AA^1
38:	Ac-D-Ser(O*t*Bu)	D-Tyr(OMe)
39:	Ac-L-Ser(O*t*Bu)	L-Tyr(OMe)
40:	Ac-D-Ser(O*t*Bu)	D-Leu
41:	Ac-L-Ser(O*t*Bu)	L-Leu

*Figure 3.16.: Model compounds **38 - 41***

In **38** and **39**, D-Ser(O*t*-Bu) or L-Ser(O*t*-Bu) respectively is coupled as a second amino acid after D-/L-tyrosine. To check, if the stabilisation effect can also be observed with a different amino acid than tyrosine as first amino acid, D- or L-Tyr was substituted by D- or L-Leu to yield **40** and **41**. The NMR-spectra of **40** and **41** were measured in $CDCl_3$ while for **38** and **39** 5% d_4-methanol had to be added because they were insoluble in pure chloroform. A look at the chemical shifts of the proline signals of compounds **38 - 41** (table 3.9.) reveals a similar effect as for compounds **40** and **41**. Proton signals Hβ' and Hδ' appear more downfield in **39** than in **38** while the other protons appear more highfield.

When the tyrosine is replaced by leucin the same trends are observed except for Hδ. Unfortunately, not all the coupling constants of **38 - 41** could be determined due to overlapping signals or unresolved couplings. In the NOESY spectra we observed a

crosspeak between Hβ' and Hδ' not only for **38** but also for **39**. The crosspeaks between Hβ' and Hδ' as well as between Hβ and Hδ for **38** and **39** were integrated. Comparison of the ratios revealed slightly higher population of the pseudo axial conformation for **39**.

All these results indicate a stabilisation of the pseudo-axial conformation when the first amino acid is L-configurated. This means, that the two-armed molecules based on the *cis*-diketopiperazine look very much alike the *trans*-receptors with parallel receptor arms. This would be a very reasonable explanation for the different binding behaviour of the *cis*-receptors, depending on the configuration of the first amino acid.

*Table 3.9.: ^{1}H chemical shifts (δ in ppm) for the pyrrolidine ring protons of compounds **38** and **39** in chloroform. with 5% d4-MeOH and of compounds **40** and **41** in chloroform*

Proton	**38** (D-Ser-D-Tyr)	**39** (L-Ser-L-Tyr)	**40** (D-Ser-D-Leu)	**41** (L-Ser-L-Leu)
α	4.26	4.17	4.35	4.22
β	2.44	2.32	2.60	2.38
β'	2.44	2.76	2.71	2.95
γ	4.30	4.15	4.30	4.23
δ	3.36	3.28	3.22	3.32
δ'	3.74	3.99	4.03	4.38

By measuring ^{1}H-NMR at low temperature with model compounds **36 - 41**, another interesting effect was observed: By cooling down to 225 K, the proton signals of **36** (D-Tyr(OMe) started broadening while the signals of diastereomer **37** (L-Tyr(OMe)

remained sharp showing the expected temperature shift. The signals of **36** had lost any coupling pattern at this temperature while the coupling in **37** was still visible.

When the two-armed model compounds with two amino acids per arm **38 - 41** were measured in chloroform at low temperature, even a stronger effect was observed (**38** and **39** were measured in chloroform with 1% DMSO). The signals of the two compounds with D-configurated amino acids **38** and **40** were broadening by cooling down to 265 K. By further cooling down, the signals sharpened again, giving rise to two signal sets. One set resembles the NMR-spectrum observed at room temperature with only three amide protons as would be expected for a C_2-symmetrical conformation. The second signal set shows doubled peaks as would be expected for an asymmetric conformation. In contrast to this dramatic temperature effect for the compounds with arms containing of D-amino acids, no such effect was observed for **39** and **41** with arms containing L-amino acids. In Figure 3.17., this different behaviour is illustrated by means of the amide NMR signals of **40** and **41** at different temperatures.

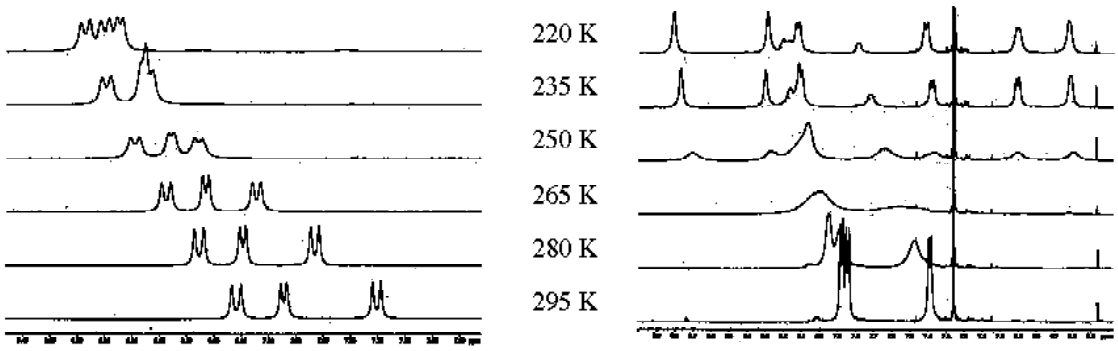


*Figure 3.17.: ^{1}H-NMR-spectra of **41** (left) and **40** (right) amide protons at different temperatures*

By measuring ^{1}H-NMR-spectra at different concentrations it was found, that the ratio between the asymmetric and the symmetric signal set depends on the concentration. The higher the concentration, the more dominant is the asymmetric signal set. On the other hand, dilution of the sample down to a concentration of ≈ 1mM results in total disappearance of the asymmetric signal set. This indicates that an intermolecular interaction occurs.

Thus, the pseudo axial conformation is especially stabilised when an L-amino acid is attached to the template rather than a D-amino acid. Furthermore, dimerisation of model compounds with D-amino acid in the arms was observed at low temperature but not with two-armed molecules with L-amino acids.

3. 9. Selective binding in water

The excellent binding selectivities of representatives of *cis*-receptors in chloroform is very encouraging. Even more challenging is the binding of peptides in water. Unfortunately none of the receptor prototypes **10 - 14** presented in chapter 3.3. is water soluble, even after the hydrophobic trityl protecting groups of the asparagin or glutamine residues were removed.

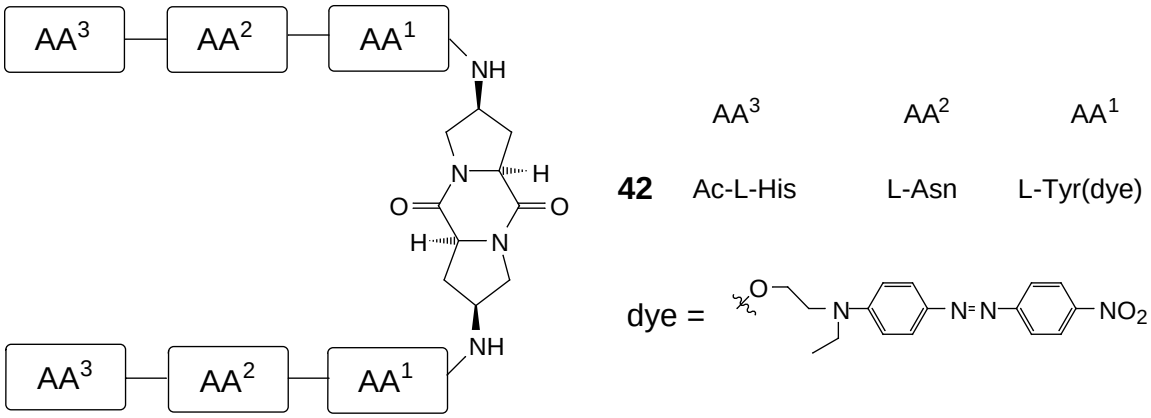


*Figure 3.18.: Receptor prototype **42***

We exchanged the two phenylalanines of receptor **10** by more hydrophilic histidine moieties in order to compensate the hydrophobicity of the dye. The resulting receptor **42** thus still possesses an aromatic side chain in the same position as **10** but the two imidazole residues should render the molecule water-soluble. Indeed the deprotected molecule **42** dissolved in water.[62] To investigate the binding properties of **42** in water, polystyrene-based library **16** could not be used, since polystyrene beads don't swell in water. Instead, a TentaGel[63] based peptide library with three combinatorially varied

positions was used. For each varied position, 31 different D- and L- amino acids were used leading to a variety of 3^{31} = 29791 different peptides. (For more details see chapter 3.1.)

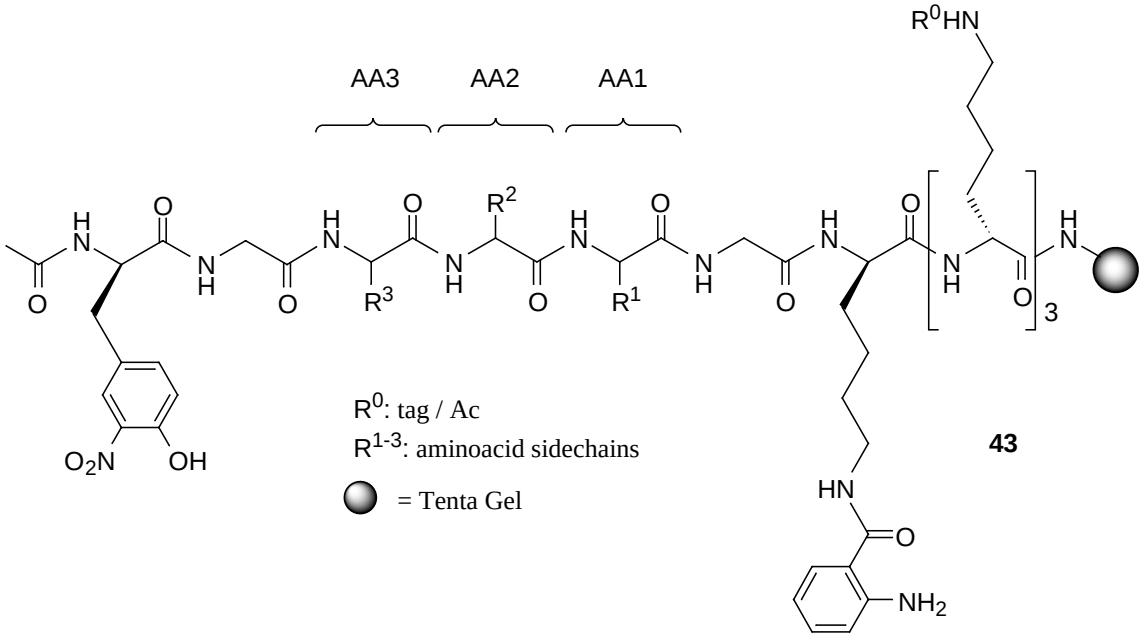


*Figure 3.19.: General structure of the tripeptide library **43** on TentaGel [63] (AA1 - AA3 = Gly, L-Ala, D-Ala, L-Leu, D-Leu, L-Val, D-Val, L-Pro, D-Pro, L-Phe, D-Phe, L-Ser, D-Ser, L-Thr, D-Thr, L-Asn, D-Asn, L-Gln, D-Gln, L-His, D-His, L-Asp, D-Asp, L-Glu, D-Glu, L-Lys, D-Lys, L-Arg, D-Arg, L-Cys, D-Cys)*

When **42** (0.10 μM) was screened against library **43** in acetate buffer at pH 4.0, several beads (ca. 10 %) turned feebly red indicating a selective binding of receptor **42** to peptides.

Analysis of the peptides of 17 of the red beads revealed sequences either containing two acidic amino acids (Glu or Asp) or an acidic amino acid in combination with a primary amide (Gln or Asn). The selectivity observed is rather moderate and the observed selectivities are not surprising, since the basic imidazole moiety of the receptor would be expected to interact with acids.

However, it could be shown, that the peptide binding ability of *cis*-diketopiperazine receptors is not restricted to organic solvents. Also in water peptides are bound.

4. Detection of Selective Peptide Damage by the Fenton Reaction

4. 1. Synthesis of a fluorophore quencher library

To monitor the sequence dependence of the peptide damage caused by the Fenton reaction we needed an appropriate detection method for selective peptide cleavage. Since rational predictions of the cleavage selectivity are difficult, we wanted to address this question by combinatorial chemistry. We used a library where the peptide is flanked by a fluorophore on the side of the solid support and a quencher on the terminal side. Peptide cleavage will thus lead to fluorescing beads (chapter 2. 3. 2.). For such a library, a potent fluorophore-quencher system is needed, which works efficiently up to a distance of at least 15 chemical bonds. A suitable system is the anthranilic acid nitrotyrosine pair (figure 4.1.). The fluorophore anthranilic acid emits light at a maximum of 440nm when irradiated at 320nm. The efficiency of nitrotyrosine to quench this fluorescence over a distance of up to 10 amino acids has been demonstrated by Meldal *et al*. who used a

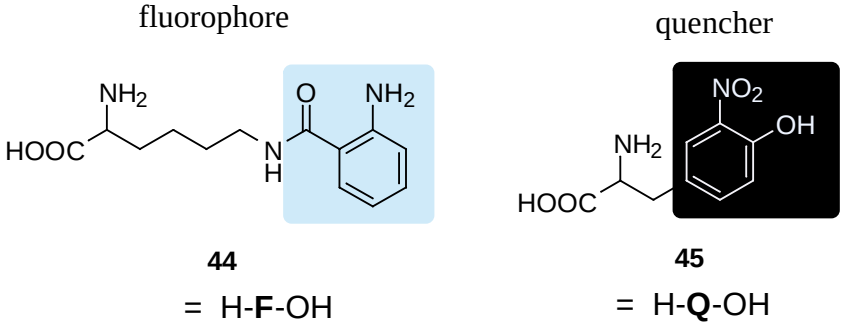


Figure 4.1.: The fluorophore and the quencher used for the library

similar library to investigate the sequence selectivity of proteases.[47, 64] The anthranilic acid can be coupled to the side chain amino group of lysine and is thus easily introduced into a linear peptide.

The synthesis of the building blocks **48** and **49** (the Nα-FMOC protected forms of **44** and **45**) that were used for the solid phase synthesis is shown in figure 4.2. Anthranilic acid **46** is first N-Boc protected. The acid group of **47** is then activated as a pentafluorophenyl ester and coupled via an amide bond to the ε-amino group of Nα-FMOC protected lysine. The quencher building block **50** was obtained in one step from nitrotyrosine using FMOC-Cl.

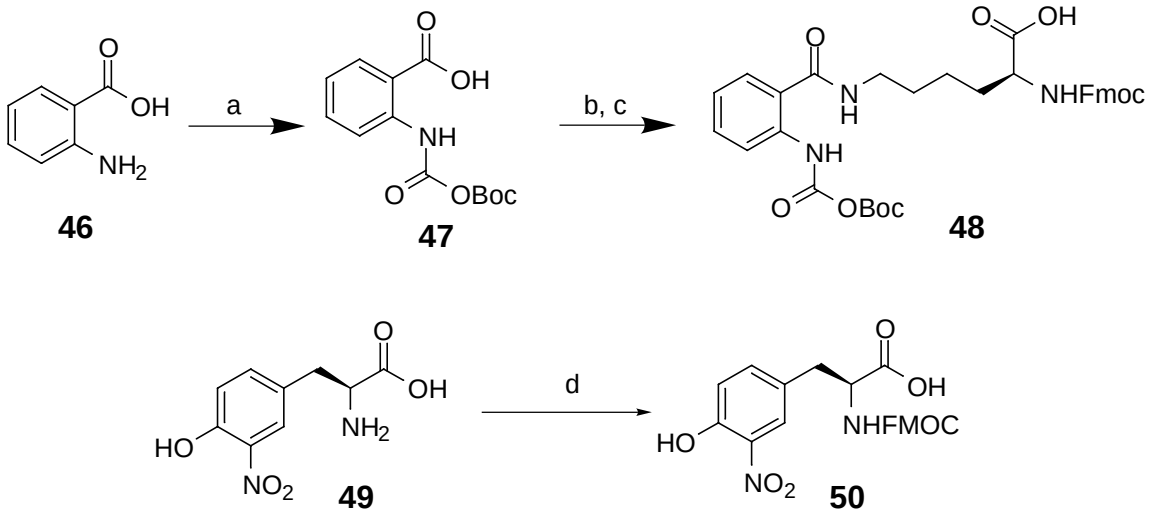


*Figure 4.2.: synthesis of the building blocks **48** and **50***

In figure 4.3. the general structure of library **43** is shown. As solid phase resin we chose amino functionalized TentaGel since it swells in water.[63]

Edman degradation is ruled out for the analysis of peptides on fluorescing beads, since the sequences of interest are not present anymore at the time of selection. Therefore chemical tags were used for encoding of the library (chapter 2.2).[44]

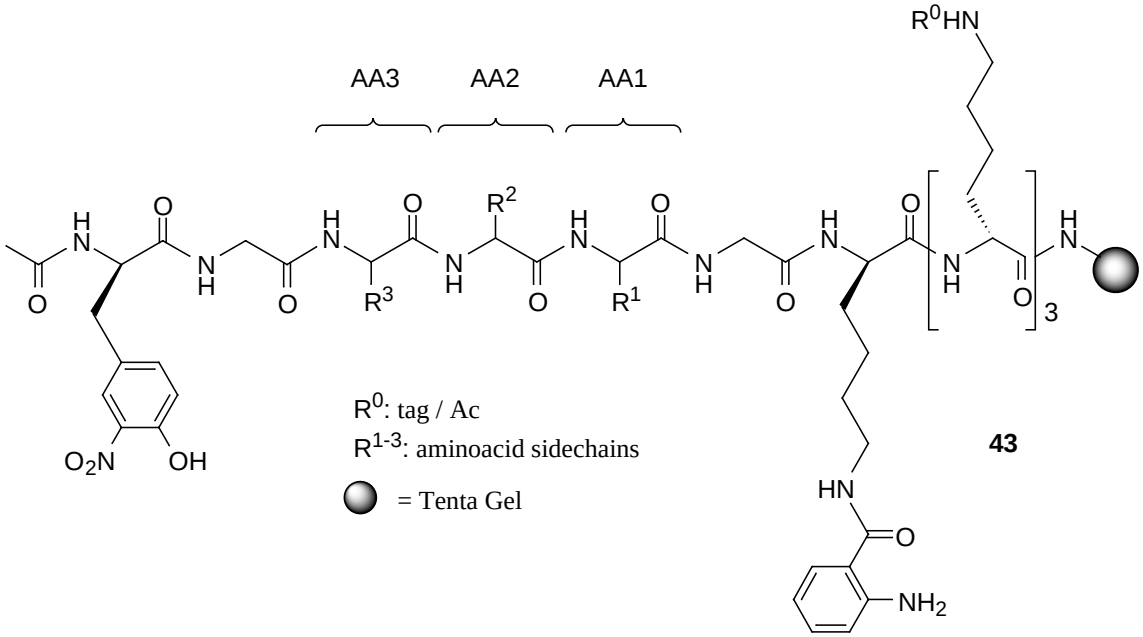


*Figure 4.3.: General structure of the fluorophore quencher library **43** (AA1 - AA3 = Gly, L-Ala, D-Ala, L-Leu, D-Leu, L-Val, D-Val, L-Pro, D-Pro, L-Phe, D-Phe, L-Ser, D-Ser, L-Thr, D-Thr, L-Asn, D-Asn, L-Gln, D-Gln, L-His, D-His, L-Asp, D-Asp, L-Glu, D-Glu, L-Lys, D-Lys, L-Arg, D-Arg, L-Cys, D-Cys)*

As attachment site for the tags we could not use the peptide backbone because this would have resulted in capping of a few percent of the peptides on each bead and therebye have lead to ineffective quenching. Furthermore cleavage of the peptide would also result in loss of the tags. As alternative attachment sites for the tags, we therefore coupled three side-chain Alloc- protected lysines to the bead before the fluorophore was coupled. The entire library synthesis was performed using the standard FMOC protocol. Following the

fluorophore building block **48**, a glycine moiety was coupled as a spacer. Starting from precursor **51**, we synthesised a library of combinatorially varied tripeptides.

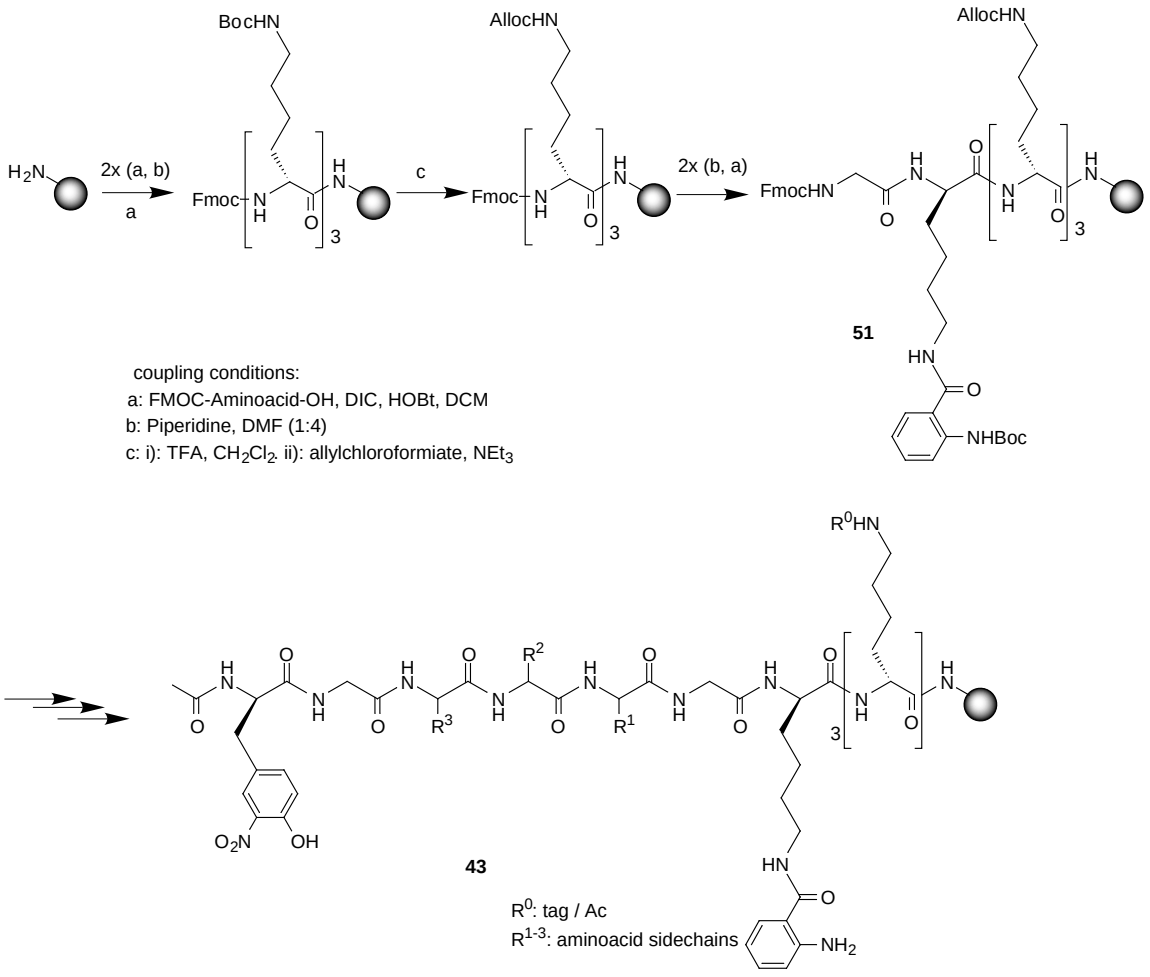


Figure 4.4.: Synthesis of the fluorophore-quencher library ***43***

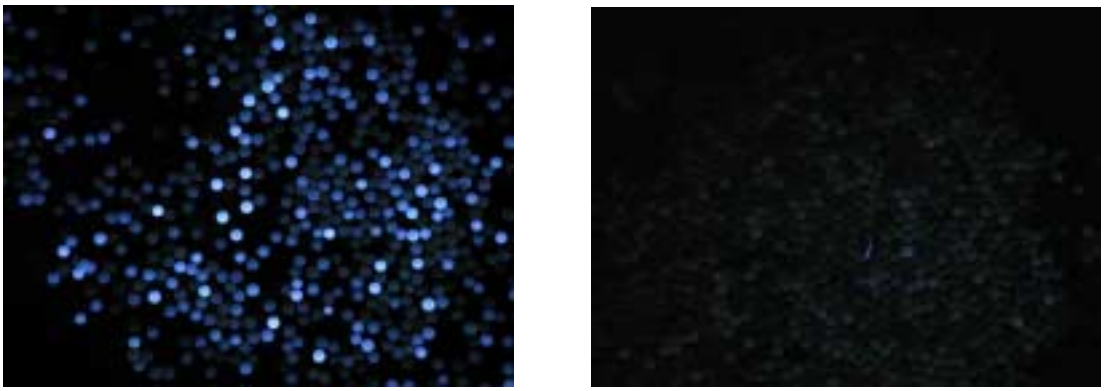
For each of the three varied positions, 31 different FMOC-protected D- and L-aminoacids were used. This leads to a combinatorial variety of maximally 31^3=29791 peptides.

All proteinogenic aminoacids except for methionine, isoleucine, tyrosine and tryptophane were included in the library. Before quencher building block **50** was coupled, another glycine spacer was introduced. The quencher was then acetylated as well as the

remaining amino groups of the tag-carrying lysines. Finally all of the sidechain protecting groups were cleaved with TFA to complete library **43**.

4. 2. Sequence selectivity of peptide damage under Fenton conditions

To test the sequence selectivity of peptide cvleavage under Fenton conditions we treated the fluorophore quencher library **43** with iron(III), ascorbic acid and hydrogen peroxide. Complexing of the library with $FeCl_3$ (0.3M) followed by washing with H_2O (5x) and treatment with ascorbic acid (0.05M) and hydrogen peroxide (0.5M) resulted, after quenching of the reaction by washing with water after 20 minutes, in several fluorescing beads (Figure 4.5.).



a b

Figure 4.5.: a: iron treated library after addition of ascorbic acid (0.05 M) and hydrogen peroxide (0.5 M) b: library without treatment

The reaction was then performed in different buffer systems. In table 1, the applied reaction conditions are summarised together with the resulting contrast observed between fluorescing and non fluorescing beads. As shown in table 4.1., the observed effect is

highly pH dependent. At pH 5 or below, the contrast between fluorescing and non fluorescing beads is biggest, while at higher pH's the effect becomes smaller until at pH > 6 almost no fluorescence is observed.

Table 4.1.: Effect of the pH / buffer system on the damage caused by the Fenton reaction.

Buffer	**pH**	**Contrast**
no buffer[a]	3.0	**High**
no buffer with 20µl HCl (1M)	4.5	**High**
acetate	5.0	**High**
MES	5.0	**High**
MES	5.9	Small
no buffer	6.0	Very small
HEPES	7.0	Very small
Tris	7.3	Very small
Tris	8.5	Very small

Procedure: Library beads were equilibrated with 0.3M $FeCl_3$ solution and washed five times with water. Then 650µl water was added followed by a solution of sodium ascorbate (0.1M) in 500µl of a specified buffer and 100µl of 1M hydrogen peroxide. After 20 minutes, the reaction was quenched by washing with water. [a]: Ascorbic acid was used instead of sodium ascorbate.

Control experiments showed that all three components are required to observe fluorescent beads (table 4.2.). When either the ascorbate or the hydrogen peroxide is left out, no fluorecent beads are observed. The same is the case for library beads that are treated by ascorbate and hydrogen peroxide without previous equilibration with iron(III). No selectivity is observed when the beads are not washed after the equilibration with iron.

.

Table 4.2.: Control experiments

Ascorbic Acid (0.05M)	Hydrogen Peroxide (0.5M)	Iron(III)	Fluorescing beads?
YES	YES	YES	YES
YES	YES	NO	NO
YES	NO	YES	NO
NO	YES	YES	NO

Analysis of the sequences of 70 of the brightest fluorescing beads (table 4.3.) revealed that almost all of the peptides on the selected beads contain two acidic amino acids (glutamic acid and ascorbic acid). In 80% of the cases, the two acidic amino acids are in successive positions, while in about 15% of the sequences, they are separated by another random amino-acid.

Table 4.3.: Sequences on fluorescing beads (X = random amino acid)

AA3	**AA2**	**AA1**	**Freq. Found**
L/D-Asp/Glu	L/D-Asp/Glu	X	34 %
X	L/D-Asp/Glu	L/D-Asp/Glu	47 %
L/D-Asp/Glu	X	L/D-Asp/Glu	13 %

In order to explain these observations we hypothesised the following:

Peptides with two subsequent acidic amino acids are able to coordinate to iron as a chelate complex. Thus, after washing the iron treated beads with water the iron concentration on those peptides will be considerably higher than on peptides without this motif. By the following addition of ascorbate and hydrogen peroxide, radicals are

generated near these peptides. Therefore the extent of cleavage of these acid-rich peptides should be higher.

In order to test this hypothesis, we first studied the complexation behaviour of model peptides containing two subsequent acidic amino acids. These experiments are described in chapter 3.3. while in chapter 3.4. quantitative studies on the cleavage selectivity are presented.

4. 3. Iron complexation of peptides with two consequent acidic amino acids

To study the complex stability of peptides containing two subsequent acidic amino acids with iron we performed experiments on solid phase and in solution.

4. 3. 1. Experiments on the solid phase

First we performed a complexation experiment with library **43**. The library beads were equilibrated with a 0.3 M iron(III)chloride solution and then washed five times with water. To visualise the iron on the beads we then added a 1M KSCN solution to the beads.

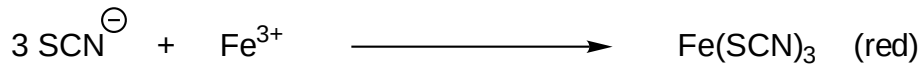


Figure 4.7.: Formation of the red iron rhodanide complex

Thiocyanate anions form red $Fe(SCN)_3$ complexes with iron which was observed on about 5 - 10 % of the beads (figure 4.8.). Analysis of the peptides on these red beads revealed similar sequences (two subsequent acidic amino acids in most cases) as for the fluorescing beads.

To quantify the complex stability we analysed how much Fe^{3+} remained on the beads after complexation with iron. We thus synthesised two sequences on TentaGel following a hexanoic acid spacer. In addition to Ac-L-Glu-L-Glu **52** the negative control Ac-Gly-Gly **53** was synthesised.

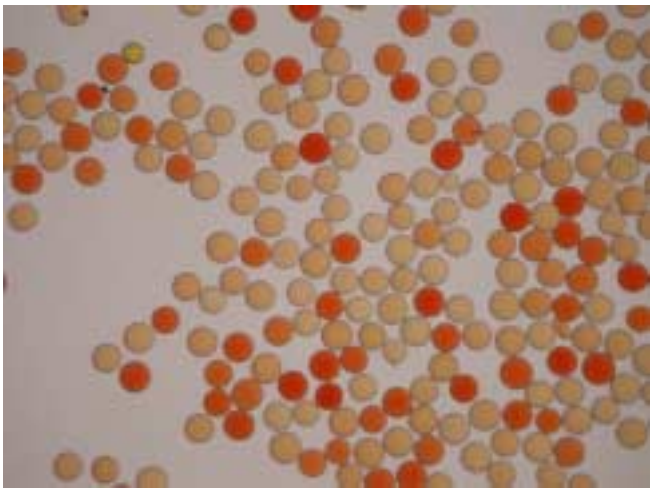


*Figure 4.8.: library **43** after treatment with iron(III), washing with water and addition of KSCN.*

Both of these immobilised peptides were treated with a 0.3 M iron (III) solution. The beads were then washed five times with water. The complex between the iron and the peptide was broken by washing five times with 0.1 M HCl. The iron in the collected acidic washing phases was then quantified by adding KSCN (1M) and measuring the UV absorbtion of the red iron rhodanide complex at 480 nm. This allowed a estimation of the iron content (table 4.4.).

In the case of the negative control peptide **53**, only about 7% of iron was found compared to the loading of the beads. On the other hand Ac-L-Glu-L-Glu **53** complexed iron to an approximately equimolar extent.

*Table 4.4.: Iron complexed by the peptides **52** and **53** by treating with excess iron and washing with water. The values are given in % relative to the amount of peptides.*

Ac-L-Glu-L-Glu **52**	Ac-Gly-Gly **53**
105 (± 5) %	7(± 5) %

Thus, a clear difference was found between the peptide containing two acidic amino acids and the negative control.

To get an idea about the dimension of the complexation constant we equilibrated resins **52** and **53** with an equimolar amount of a 0.6 mM iron(III)chloride solution at pH 5 using acetate buffer. After 30 hours of equilibration time, the iron remaining in solution was measured as well as the iron on the bead (after washing with HCl).

From this data, the complex stability constant was calculated by:

$$K = \frac{[complex]}{[peptide][iron]}$$

where [iron] is the iron concentration measured in solution after equilibration and [complex] is the concentration of the iron washed from the bead with HCl. The concentration of the free peptide [peptide] is given by: [peptide^0] -[complex] where [peptide^0] is calculated from the weighed sample of resin. We found a value in the range of $3x10^3$ for the Glu-Glu peptide **52** while the complex stability constant of the negative control **53** was about two orders of magnitude smaller.

*Table 4.5.: K values for **52** and **53** obtained by equilibration experiments on solid phase (30 h equilibration of resin bound peptide with 1 eq iron(III)chloride (0.6 mM) in 0.5 M acetate buffer pH 5. Values averaged over three experiments).*

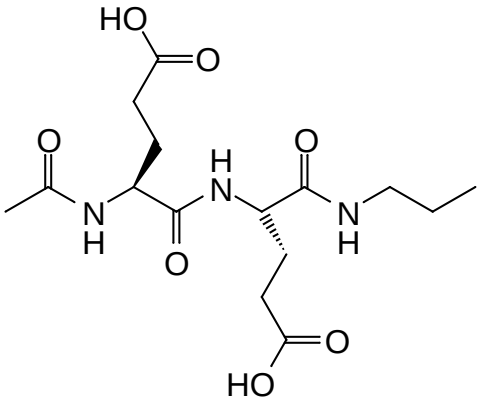
	Complex stability constant K[a]
52 (L-Glu-L-Glu)	2900 (+/- 300)
53 (Gly-Gly)	55 (+/- 30)

[a] complex concentrtion and free iron concentration were obtained by measuring the iron concentrations in solution and on the bead after equilibration (30 h).

These results give us an idea about the dimension of the stability of the iron peptide complex. The values are however not absolute since in the calculation no interference from the buffer or the resin has been considered. Therefore these values can only be compared when obtained under exactly the same conditions. A more accurate way to determine the complex stability would be a pH-titration of the peptide in solution in the presence of iron.

4. 3. 2. Complexation experiments in solution

For solution phase complexation experiments we synthesised peptide Ac-L-Glu-L-Glu-NHPr **54**.



54

*Figure 4.10.: Peptide **54** synthesised for solution phase studies of the iron complexation*

By titration of **54** (2mM) with NaOH first in the absence of iron the pK_a values were determined to be 3.95 and 4.70 respectively, by using the program titfit.[65] The titration

yielded also the distribution of the species depending on the pH (figure 4.9.). This shows, that at pH 5 the predominant form is the doubly deprotonated species with approximately 65 %, followed by the species with one deprotonated acid group (35 %), while the fully protonated peptide is almost non-existent at that pH.

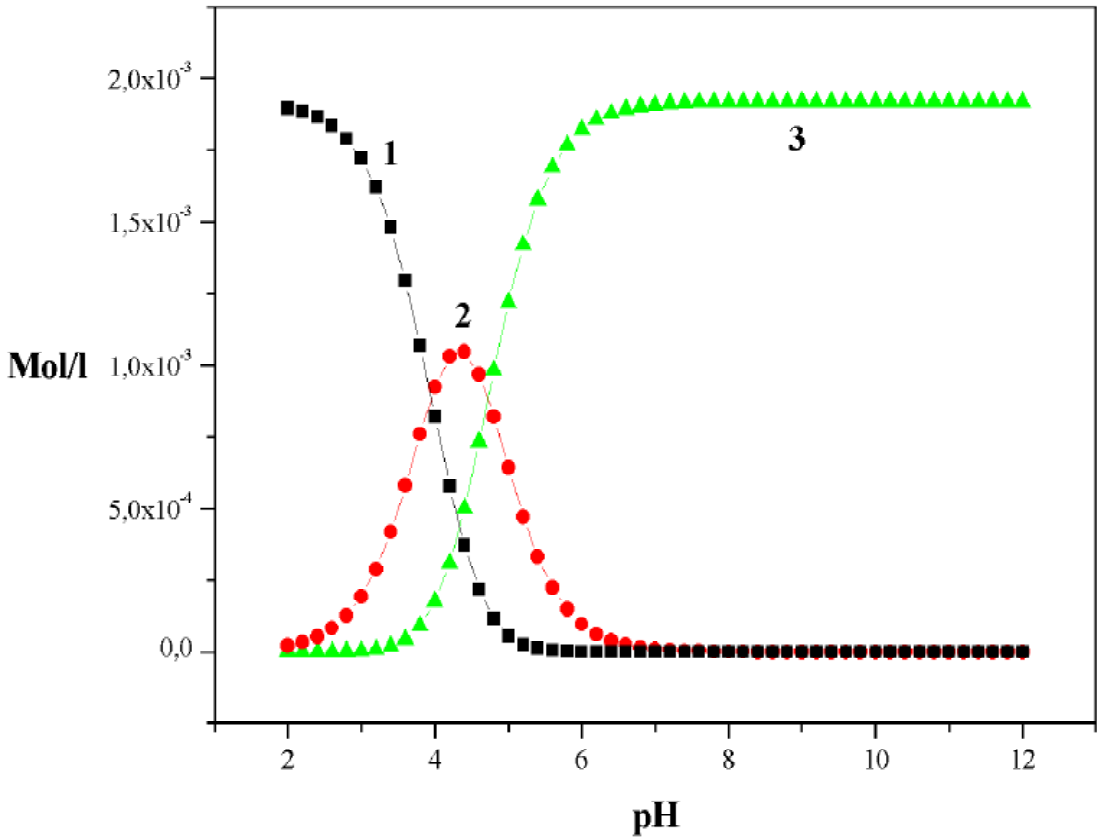


*Figure 4.9.: Particle distribution of **54** depending on the pH as derived from titration with NaOH. ■ (1) = fully protonated, ● (2) = once deprotoneted, ▲ (3) = fully deprotonated.*

Subsequently, we attempted to determine the complexation constant in solution by titration of **54** in the presence of 0.9 eq $FeCl_3$. However, precipitation of $Fe(OH)_3$ at pH 4 in the course of the titration prevented the measurement with Fe^{3+}. Instead, the titration

was performed with $FeCl_2$. Here, precipitation of $Fe(OH)_2$ occurred only at pH 8 and allowed for an estimation of the complexation constant in the range of 10^4. Typically, the complexation constant is higher for iron(III) than for iron(II) by a factor of 10.[66]

We then measured the effect of iron on the peptide with ^{1}H-NMR measurements by titrating iron(III)chloride to an sample of **54**. It is known[67, 68] that the relaxation time is shortened for protons close to a paramagnetic metal center. Indeed we observed different changes of the relaxation time for different protons indicating iron complexation. However, the observed effect is too small to be analysed quantitatively.

The studies demonstrate that peptides with two subsequent acidic amino acids are able to coordinate to iron stronger than peptides without this motif.

4. 4. Quantitative studies on the cleavage selectivity

To quantify the damage of Asp/Glu containing peptides compared to a negative control we performed studies with the peptides bound to solid support and in solution.

For the solid phase studies we terminated the peptides with the fluorophore anthranilic acid. Upon cleavage, the fluorophore is released into solution and can be quantified in order to get relative cleavage and damage activities.

4. 4. 1. Experiments on the solid phase

The Glu-Glu containing peptide **55** and a negative control peptide **56** where the glutamic acid residues are replaced by glycines were synthesised on TentaGel.[63] and terminated with the fluorophore anthranililc acid coupled to the side chain amino group of a lysine.

Ac-∠-Lys(F)-Gly-Gly-Gly-**L-Glu-L-Glu**-εAhx-NH- **55**

Ac-∠-Lys(F)-Gly-Gly-Gly-Gly-Gly-εAhx-NH- **56**

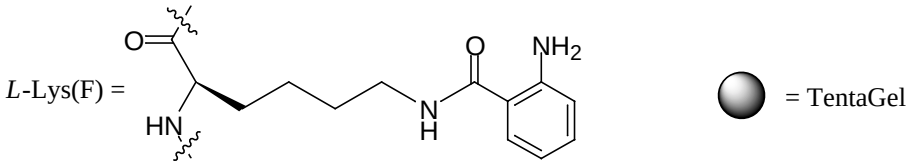


*Figure 4.10.: Resin bound peptides **55** and **56** used for fluorospectrometric detection of the peptide cleavage*

To minimise quenching effects of the fluorescence by ascorbic acid or iron the fluorescence measurement was performed at pH 4 in acetate buffer where quenching is minimal.

Peptides **55** and **56** were both complexed with $FeCl_3$ and washed thoroughly, followed by addition of ascorbic acid (0.05 M pH 3.0) and hydrogen peroxide (0.5 M). After 20 minutes, the reaction was quenched by the addition of MnO_2. Fluorescence of the

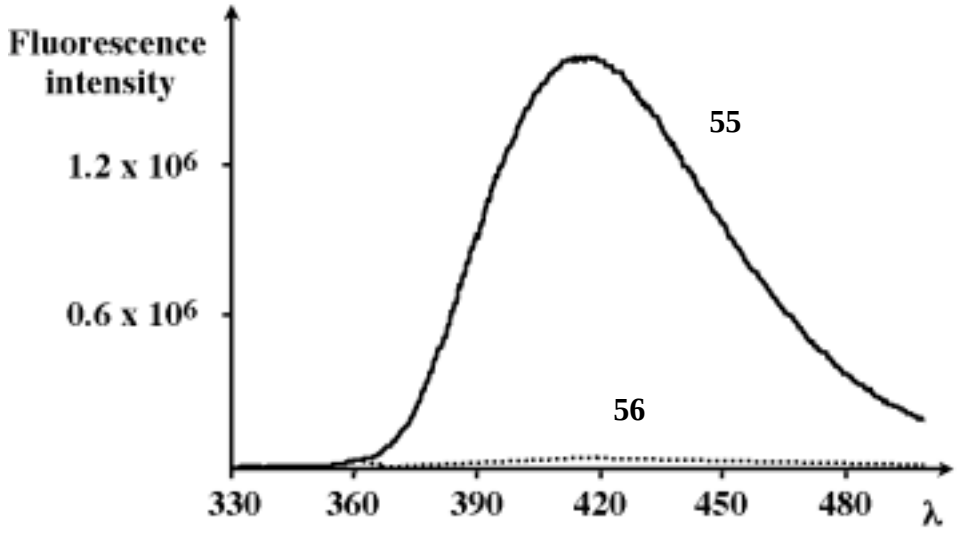


*Figure 4.11.: Fluorescence spectrum of the reaction mixtures of **55 (---)** and the negative control **56 (- -)**. Irradiation wavelength 320 nm).*

solution was measured after removal of the resin by filtration. In figure 4.11. the fluorescence spectra of the reaction mixture of resin **55** as well as of the negative control **56** are shown.

Clearly treatment of resin **55** bearing the Glu-Glu peptide releases much more of the fluorophore into the solution than resin **56** containing two glycins instead. In fact, more than 50 times more of the fluorophore is measured in the solution in case of **55** compared to the negative control peptide **56**. The experiment was also performed using 1 equivalent of iron without washing between the complexation step and the addition of the reagents. Under these more solution-like conditions, **55** still leads to an approximately five times higher fluorescence of the resulting solution than **56**.

This demonstrates, that the cleavage extent is much higher for peptides with two acidic amino acids.

3. 4. 2. Experiments in solution

For the solution phase cleavage studies the fluorophore-marked peptides were prepared on Wang resin and removed from the resin as propylamides **57** and **58**.

Ac-∠-Lys(F)-Gly-Gly-Gly-**L-Glu-L-Glu**-εAhx-NH-Pr **57**

Ac-∠-Lys(F)-Gly-Gly-Gly-Gly-Gly-εAhx-NH-Pr **58**

*Figure 4.12.: Peptides **57** and **58** for solution phase studies*

The peptides were complexed with 3 eq of $FeCl_3$ (30 mM), diluted with MES buffer pH 5 to a concentration of 1mM before sodium ascorbate and H_2O_2 were added. After 20 minutes, excess H_2O_2 was quenched with MnO_2. After filtration, the reaction mixtures were analysed by HPLC using a fluorescence detector. The HPLC analysis did not show any new peaks with neither fluorescence nor UV detection (320 nm) but only a decrease of the peaks corresponding to the peptides **57** and **58** respectively. Thus, the peptides are damaged but no defined cleavage product was observed. This suggests, that the damaged peptides are broken up in pieces too small to allow for their analysis by HPLC. As judged by the decrease of the integrals of the signals detected at 320 nm (UV) the L-Glu-L-Glu containing peptide **57** was damaged to an extent of approximately 35 % while the negative control peptide **58** only was reduced by less than 10 %. Figure 4.13. shows the averaged data depicted as columns. In figure 4.14. the HPLC chromatograms for the L-Glu-L-Glu peptide **57** and negative control **58** untreated and treated with the Fenton conditions are shown as an example.

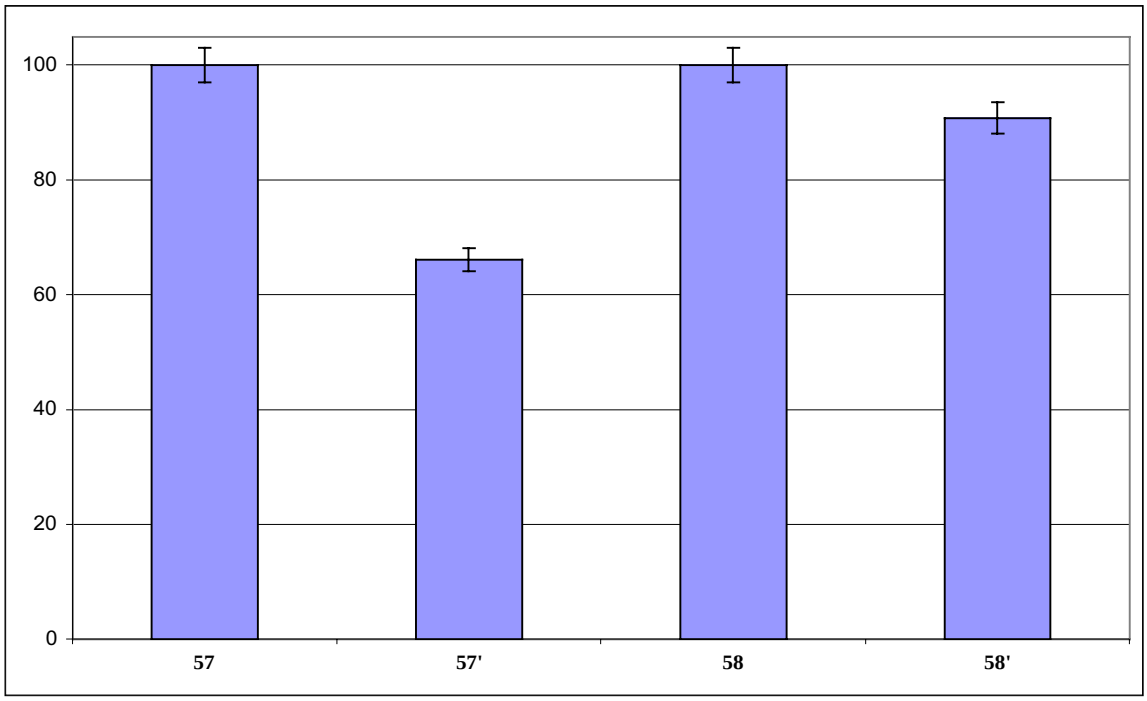


Figure 4.13.: The relative extent of peptide damage by the Fenton reaction in solution as determined by HPLC with UV detection (320 nm). ***57*** *and* ***58*** *represent the untreated,* ***57'*** *and* ***58'*** *the treated peptides. The percentages were calculated by averaging integrals of the peptide peaks out of three experiments. The averages of* ***57*** *and* ***58*** *(untreated) are set to 100% each.*

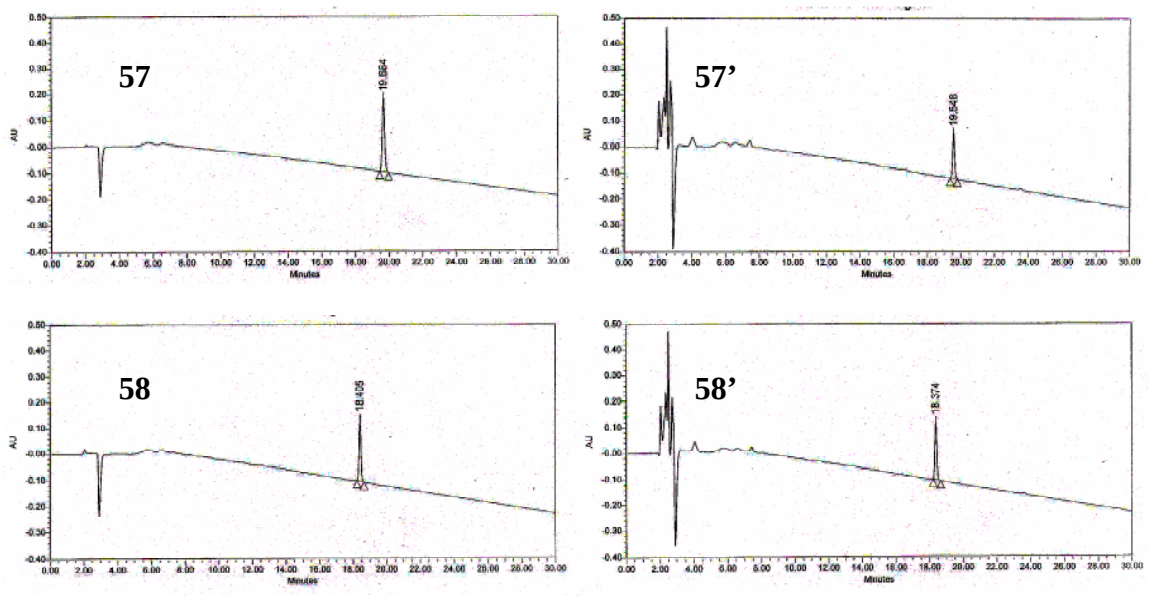


Figure 4.14.: HPLC chromatograms (210 nm) of ***57*** *untreated (upper left),* ***57'*** *treated (upper right),* ***58*** *untreated (lower left),* ***58'*** *treated (lower right).*

To take the results together: We have shown that peptides with two subsequent acidic amino acids are damaged more than other peptides under Fenton conditions. The relative cleavage yield is higher when the peptides are bound to the solid support than in solution. We also demonstrated, that these peptides coordinate stronger to iron than peptides without this motif. This can be interpreted that peptides are cleaved to a higher extent if iron is in the vicinity of the peptide backbone.

5. Overall Conclusions and Outlook

This work presents a novel class of two-armed receptors that bind to peptides with high sequence selectivity. These receptors are composed of a diketopiperazine derived from hydroxyproline as a template and two peptidic arms. The excellent binding properties have been rationalised by the analysis of the influence of the template structure on the binding properties. Exchange of the diketopiperazine against other templates resulted in an entire loss of intermolecular binding ability. Analysis of the diketopiperazine conformation by X-ray crystal structure analysis and by ^{1}H-NMR-spectroscopy revealed, that diketopiperazine receptors with pseudo-axial standing substituents are excellent templates for two armed receptors. These studies demonstrated that the template has the largest impact on the binding properties. The second largest effect comes from the first amino acid that is coupled to the template. The receptors are currently applied for the development of sensors[69] and liquid crystallin materials[70] and might be used for the development of diagnostics.

The second part shows by the use of a combinatorial fluorophore-quencher library, that peptides containing two acidic amino acids are preferably cleaved under Fenton conditions. Such peptides were shown to coordinate to iron more strongly than other peptides. Due to the iron coordination, upon the addition of ascorbic acid and H_2O_2, radicals are generated in the vicinity of the peptide. These peptides are therefore cleaved more than peptides that are not able to coordinate to iron.

The presented results are designed to serve as a starting point for the development of selective proteolytic receptors. By coupling a proteolytically active metal complex (e.g. Fe-EDTA)[71] to, for example a diketopiperazine receptor, peptides will not only be bound, but, in a second step cleaved selectively (as shown in figure 5.)

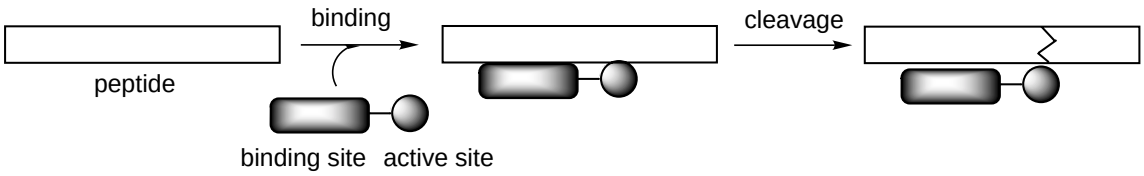


Figure 5.: sequence selective cleavage of peptides as a two step process

Experimental Section

6. Experimental part

6. 1. General aspects

6. 1. 1. Solvents and reagents

All materials and reagents were of the highest commercially available grade and used without further purification as received from *Fluka AG* (Buchs, Switzerland), *Aldrich* (Buchs, Switzerland), *Bachem* (Bubendorf, Switzerland) and *NovaBiochem / Merck Biosciences* (Läufelfingen, Switzerland).
Solvents for chromatography and extractions were distilled prior to use. Further solvents used for reactions correspond to the quality *puriss, p. a., abs.* For analytical and preparative HPLC, HPLC-grade solvents were used. The water used for reactions and HPLC was filtered over *Barnstead* ultrapure water system.

6. 1. 2. Materials and Instruments

Solvents were removed with a *Büchi* (Switzerland) rotary evaporator. For weighing compounds and reagents *Mettler* (Switzerland) balances were used.
Finnigan MAT LCQ and TSQ 700 instruments were used for electrospray ionisation (ESI) mass spectrometry.

6. 1. 3. Chromatographic methods

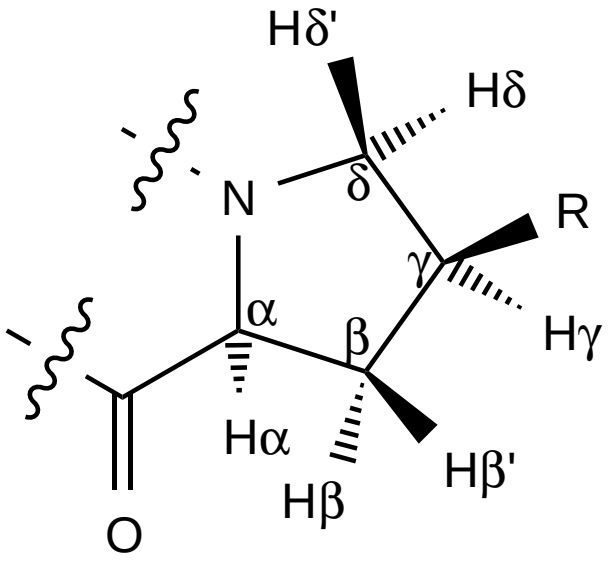
Analytical thin layer chromatography (TLC) was performed on *Merck* silica gel 60 F_{254} plates. Compounds were detected (if not visible by eye) at 254 nm (UV) or visualised with ceric ammonium molybdate (CAM) or ninhydrin.

For normal phase column chromatography sililca gel 60 from *Merck* (40 - 60 µm particle size) was used and for eluting the compounds pressure (0.3 - 0.5 bar) was applied (flash chromatography).
Analytical reversed phase HPLC (RP-HPLC) was performed on LiChrospher® 100 RP - 18 silica gel from *Merck* (5 µm particle size, 4x250 mm column) on a *Waters alliance* 2690 with a 996 photodiode array detector (PDA).
Gas chromatography of the tags was performed on a *Hewlett packard* HP 6890 GC system.

6. 1. 4. Spectroscopic methods

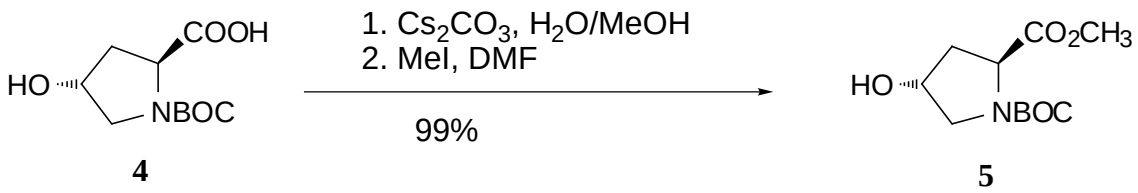
Ultra violet - visible absorption spectra were recorded on a *Perkin Elmer* Lambda Bio 40 UV/VIS spectrometer using *Hellma* quartz cuvettes (10 mm light path). Fluorescence spectra were recorded on a *ISA Jobin Yvon-Spex* FluoroMax-2 spectrometer using 10 mm path length quartz cuvettes (*Hellma*).
Infrared spectra (IR) were measured on a *Perkin Elmer* 1600 Series FTIR in KBr (1% w/w) or neat between NaCl-plates.
^{1}H-Nuclear magnetic resonance spectroscopy (^{1}H-NMR) was performed using either a Varian Gemini (300 MHz), Bruker DPX-NMR (400 MHz), Bruker DRX-500 (500 MHz) or a Bruker DRX-600 (600 MHz) spectrometer. Solvents for NMR were obtained from Dr. Glaser AG (Basel, Switzerland) and Cambridge Isotope Laboratories (Andover, MA, USA). If necessary for the interpretation correlated spectra like COSY, TOCSY, NOESY and ROESY were recorded also.
Description: ^{1}H-NMR (frequency, solvent, temperature): δ_H in ppm relative to residual solvent peaks or trimethylsilyane (peak multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sext = sextet, m = multiplet, br = broad; coupling constants *J* in Hertz)
^{13}C- Nuclear magnetic resonance spectroscopy (^{13}C -NMR) were 1H-decoupled and recorded on a Varian Gemini 300 (75 MHz), Bruker DPX-NMR (100 MHz) or a Bruker DRX-500 (125 MHz).

6. 1. 5. Signature of the Proline Derivates



6. 2. Synthesis of the *cis*-diketopiperazine template

6. 2. 1. N-Boc-*trans*-γ-hydroxy-L-proline methyl ester 5



4 **5**

10.54 g (45.6 mmol, 1 eq; CAS-Nr. 233663-27-9) of *N*-Boc-*trans*-γ-hydroxy-L-proline **4** was dissolved in 120 ml of methanol and a solution of 8.92 g (27.4 mmol, 0.6 eq) Cs_2CO_3 in 10 ml water was added. After stirring for 1 h at RT the methanol was removed in vacuo. After it was coevaporated with toluol three times, the residue was suspendet in 30 ml DMF. Then after addition of 5.7 ml (91 mmol, 2 eq) methyliodide it was stirred one hour at RT and 100 ml water and the same amount of Et_2O was added to the mixture. The waterphase was separated and extracted 7 times with 80 ml of Et_2O. The organic phases were washed with 50 ml of a saturated NaCl solution, dried over $MgSO_4$ filtred and the solvent was removed at reduced pressure to yield 11.00g (99%) of *N*-Boc-*trans*–*γ*–hydroxy-L-proline methyl ester **5** as a yellowish oil. (^{1}H NMR- and ^{13}C NMR-spectra show a double set of peaks (≈ 2 : 1) due to the s-*cis* and s-*trans* conformers around the tertiary carbamate.)

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

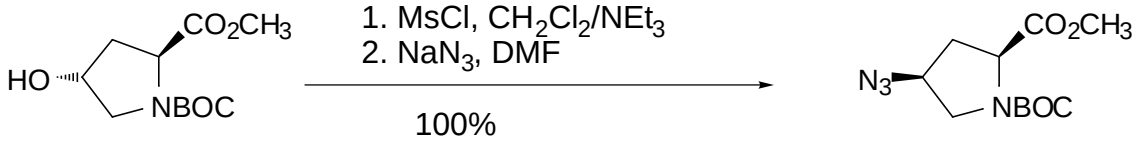
TLC: Pentane/EtOAc (2:1); Rf: 0.17 (Ninhydrin)

^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ (ppm) = 4.49 (s, br, 1H; Hγ), 4.40 (dd, *J* = 8.9 Hz, 1H; Hα), 3.73 (s, 3H; OCH_3), 3.64 (dd, *J* = 5.7, 8.4 Hz, 1H; Hδ'), 3.50 (m, 1H; Hδ), 2.25-2.20 (m, 1H; Hβ'), 2.13-2.00 (m, 1H; Hβ), 1.48/1.37 (2s (1:2), 9H, *t*-Bu);

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ (ppm) = 175.3, 175.1, 154.4, 153.6, 80.3, 71.0, 70.0, 57.8, 55.7, 55.1, 52.6, 52.3, 38.48, 35.3, 28.3, 28.1;

ESI-MS: *m/z*: calcd for $C_{11}H_{19}NO_5$ $[M+Na]^+$ 268; found 268.

6. 2. 2. N-Boc-*cis*-γ-azido-L-Proline-methylester 6



5 **6**

To a solution of 7.44 g (30.4 mmol, 1 eq) *N*-Boc-*trans*–γ–hydroxy-L-proline methyl ester **5** in 100 ml of CH_2Cl_2 5.1 ml (36 mmol, 1.2 eq) of NEt_3 was added and the mixture was cooled to 0°C. 2.9 ml (36 mmol, 1,2 eq) of mesylchloride was added, it was stirred for one hour and then washed with 50 ml saturated NaHCO3. The aquouos phase was extracted twice with 50 ml CH_2Cl_2. The organic phases were dried over satureted NaCl and MgSO4. After filtration and removal of the solvent, the mesylester was dissolved in 100 ml of DMF and 19.8 g (304 mmol, 10 eq) of NaN_3 was added. After stirring for 4 h at 80°C water and Et_2O (80 ml of each) was added. The water phase was separated and extracted three times with 80 ml Et_2O. The organic phases were washed with sat. NaCl solution and dried over $MgSO_4$. After removing of the solvent at reduced pressure, the crude product (7.28 g) was purified by flash chromatography over silica gel (gradient of CH_2Cl_2 / MeOH from 0 to 4% of methanol). This yielded 6.48 g (100%) of the N-Boc-*cis*-γ-azido-L-Proline-methylester **6**. (^{1}H NMR- and ^{13}C NMR- spectra show a double set of peaks (≈ 2 : 1) due to the s-*cis* and s-*trans* conformers around the tertiary carbamate.)

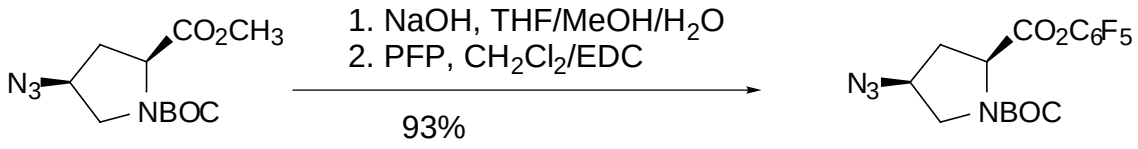
$C_{11}H_{18}N_4O_4$: 270.13

TLC: CH_2Cl_2/MeOH (25:1); Rf: 0.68 (Ninhydrin)

^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ (ppm) = 4.43 / 4.32 (2dd, *J* = 4.4 Hz, 8.8 Hz, 1H; Hγ), 4.18-4.12 (m, 1H; Hα), 3.76 (s, 3H; OCH_3), 3.78-3.68 (m, 1H; Hδ), 3.48 (ddd, *J* = 3.5 Hz, 11.0 Hz, 15.7 Hz, 1H; Hδ), 2.52-2.40 (m, 1H; Hβ), 2.21-2.14 (m, 1H; Hβ), 1.48 / 1.42 (2s (1:1.5), 9H; *t*-Bu);

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ (ppm) = 172.2, 153.9, 153.4, 80.5, 59.2, 58.2, 57.7, 57.27, 51.2, 50.7, 36.0, 35.0, 28.2.

6. 2. 3. N-Boc-*cis*-γ-azido-L-Proline-pentafluorophenylester 7



6 **7**

2.00 g *N*-Boc-*cis*-4-azido-L-Proline-methylester **6** (7.40 mmol, 1 eq) was dissolved in a 1:1 mixture of THF and MeOH (50 ml). After the addition of 0.44 g NaOH (11.04 mmol, 1.5 eq) dissolved in water (1 ml) the mixture was stirred for 1.5 h at room temperature and then carefully acidified with 1M HCl to pH 4. EtOAc (100 ml) and water (50 ml) were added and the mixture was extracted with additional 1M HCl (20 ml). The aqueous layer was extracted with EtOAc (50 ml), the organic layers were washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent at reduced pressure yielded a colorless oil which was dissolved in CH_2Cl_2 (10 ml). Addition of pentafluorophenol (1.43 g, 7.77 mmol) and EDC (2.13 g, 11.11 mmol) yielded a solution which was stirred for 1 h at room temperature and then extracted with water (50 ml) and EtOAc (100 ml). The

aqueous layer was extracted again with EtOAc (50 ml) and the organic layers were washed with brine and dried over $MgSO_4$. Filtration and removal of all volatiles at reduced pressure yielded the pentafluorophenylester **7** (2.90 g, 6.87 mmol, 93%) as a colorless oil. (^{1}H NMR- and ^{13}C NMR- spectra show a double set of peaks (≈ 2 : 1) due to the s-*cis* and s-*trans* conformers around the tertiary carbamate.)

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

TLC: Pentane/EtOAc (5:1); Rf: 0.27 (Ninhydrin)

^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ (ppm) = 4.76 and 4.70 (2dd (1:2), *J* = 9.2 Hz, 3.2 Hz and 9.3 Hz, 2.8 Hz, 1H; Hα), 4.29 (m, 1H; Hγ), 3.76 (m, *J* = 11.9, 1H; Hδ), 3.63 and 3.54 (2dd (2:1), *J* = 11.8 Hz, 2.5 Hz and 11.3 Hz, 3.0 Hz, 1H; Hδ´), 2.68 - 2.37 (m, 2H; Hβ, Hβ´), 1.49 und 1.46 (2s (1:2), 9H; *t*-Bu);

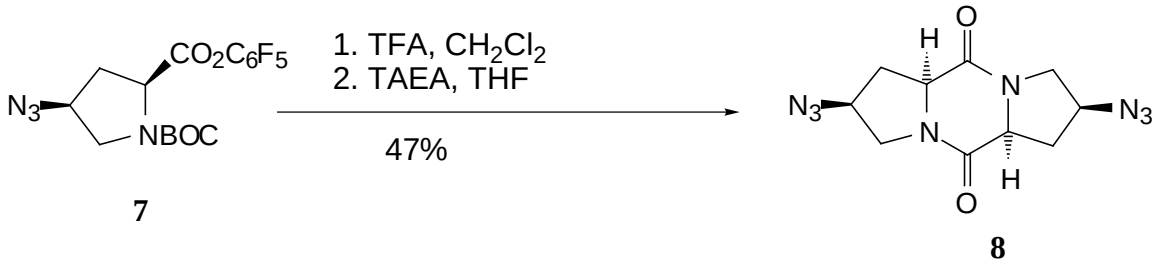
^{13}C-NMR (100.5 MHz, $CDCl_3$, 25°C): δ (ppm) = 168.3, 168.0, 153.9, 153.6, 143.5, 138.8, 81.9, 81.6, 60.8, 59.8, 57.7, 57.5, 51.7, 51.5, 36.9, 35.8, 28.7, 28.5;

FT-IR (NaCl, ν/cm^{-1}): 2980, 2118, 1798, 1713, 1518, 1260;

ESI-MS: *m/z*: calcd for $C_{16}H_{15}F_5N_4O_4$ $[M+H]^+$ 423; found 423.

Elemental analysis: Calcd (%) for $C_{16}H_{15}F_5N_4O_4$ (422.3):
C 45.51, H 3.58, N 13.27; found C 45.27, H 3.56, N 13.14.

6. 2. 4. Cyclo-(*cis*-γ-N_3-L-Pro)$_2$ 8



7 **8**

N-Boc-cis-γ-azido-L-Proline-pentafluorophenylester **7** (1.21 g, 2.87 mmol) was dissolved in a 1 : 3 mixture of TFA and CH_2Cl_2 (10 ml) and allowed to stir at room temperature for 1.5 h. After removal of all volatiles at reduced pressure the oily residue was triturated with Et_2O (20 ml) 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 (10 ml), Hünig`s base (2.00 ml, 11.48 mmol) was added and the mixture was stirred at room temperature for 60 h. After removal of all volatiles at reduced pressure, flash chromatography on silica gel (gradient of CH_2Cl_2 : MeOH from 100 : 0 to 100 : 6) afforded the diketopiperazine **8** (0.19 g, 0.69 mmol, 47 %) as a colorless oil.

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

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.32 (Ninhydrin)

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) = 4.28 (dd, *J* = 8.8 Hz, 5.7 Hz, 2H; Hα), 4.20 (ψqd, *J* = 5.5 Hz, 4.0 Hz, 2H; Hγ), 3.81 (ddd, *J* = 12.4 Hz, 3.9 Hz, 1.2 Hz, 2H; Hδ´), 3.55 (dd, *J* = 12.4 Hz, 5.6 Hz, 2H; Hδ), 2.69 (dddd, *J* = 13.8 Hz, 5.7 Hz, 4.9 Hz, 1.2 Hz, 2H; Hβ´), 2.50 (ddd, *J* = 13.8 Hz, 8.8 Hz, 5.7 Hz, 2H; Hβ).

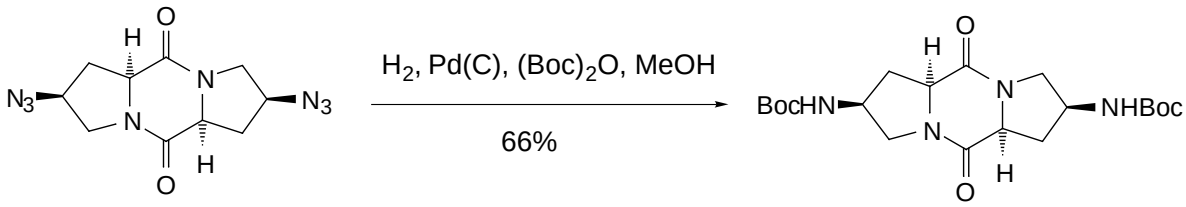
^{13}C NMR (75.5 MHz, $CDCl_3$, 25°C):): δ (ppm) = 165.5, 58.2, 58.0, 50.3, 32.4;

FT-IR (NaCl, ν/cm^{-1}): 2953, 2103, 1680, 1422, 1269.

FAB-MS (NBA): *m/z* (%): $[M+H]^+$ 277.

Elemental analysis calcd (%) for $C_{10}H_{12}N_8O_2$ (276.3): C 43.48, H 4.38, N 40.56; found C 43.33, H 4.54, N 40.26.

6. 2. 5. Cyclo-(*cis*-γ-NHBoc-L-Pro)$_2$ 9



8 **9**

Palladium on carbon (10%, 20 mg) was added to the solution of 0.25 g of the diazide **8** (0.91 mmol, 1 eq) and 0.79 g Boc_2O (3.61 mmol, 4 eq) in MeOH (10 ml). The black suspension was evacuated, flushed with hydrogen and allowed to stir for 4 h at room temperature. After filtration over celite and removal of the solvent at reduced pressure, the residue was purified by flash chromatography over silica gel (gradient of CH_2Cl_2 : MeOH from 100 : 0 to 100 : 6) to afford the bis-*N*-Boc-protected diketopiperazine **9** (0.25 g, 0.59 mmol, 66 %) as a white solid.

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

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.18 (Ninhydrin)

^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ (ppm) = 5.30 (s (broad), 2H; NH), 4.27 (ψt, *J* = 7.8 Hz, 4H; Hα, Hγ), 3.73 (dd, *J* = 12.0 Hz, 7.0 Hz, 2H; Hδ), 3.43 (dd, *J* = 12.0 Hz, 6.5

Hz, 2H; Hδ´), 2.55 (m, *J* = 13.6 Hz, 7.2 Hz, 2H; Hβ, 2.22 (dt, *J* = 13.5 Hz, 7.2 Hz, 2H; Hβ´), 1.44 (s, 18H; *t*-Bu);

^{13}C NMR (100.5 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ (ppm) = 166.7, 155.9, 80.4, 59.3, 51.0, 48.9, 33.4, 28.7;

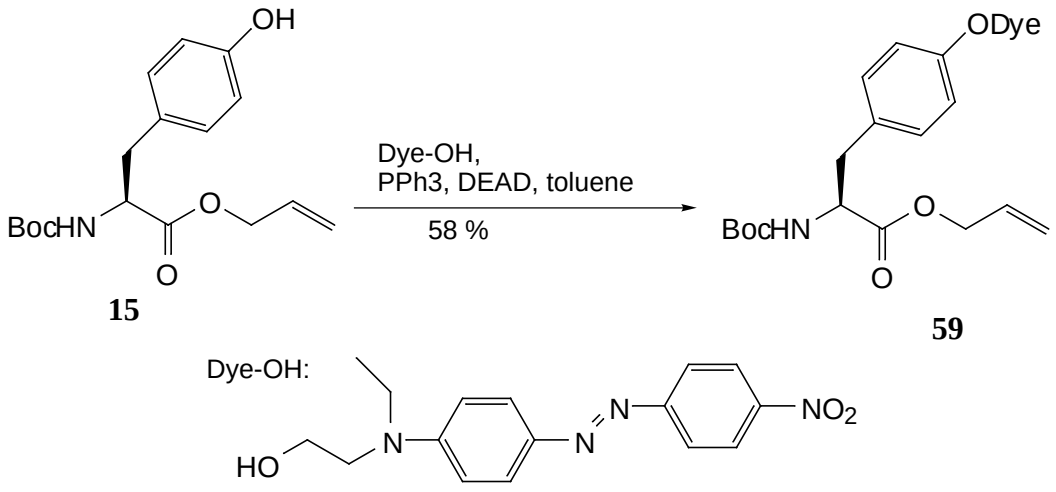
FT-IR (KBr): ν = 3341, 2981, 1728, 1688, 1532, 1166.

ESI-MS: *m/z*: calcd for $C_{20}H_{32}N_4O_5Na$ $[M+Na]^+$ 447; found 447.

Elemental analysis calcd (%) for $C_{20}H_{32}N_4O_6 + H_2O$ (442.5): C 54.28, H 7.74, N 12.66; found C 54.65, H 7.78, N 12.37.

6. 3. Synthesis of receptor precursor 17

6. 3. 1. Boc-Tyrosine(ODye)-Allylester 59



1.0 g (3.10 mmol, 1 eq) of BOC-D-Tyrosine-Allylester **15** was dissolved in 100 ml of toluene and 0.98 g (3.10 mmol, 1 eq) of disperse red 1, 0.81 g of triphenyl phosphine and

0.56 ml (3.10 mmol, 1 eq) of DEAD was added. After stirring for 24 hours at room temperature, another 405 mg (0.5 eq) of triphenylphosphine and 0.3 ml (0.5 eq) of DEAD was and the mixture was stirred for another 6 hours. Then, the toluene was removed under reduced pressure and it was coevaporated twice with DCM. Purification of the crude product by flash chromatography over silica gel (gradient: pentane:EtOAc from 3:1 to 2:1) yielded 1.1 g (58%) of **59**.

$C_{33}H_{39}N_5O_7$: 617.69

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.71

^{1}H-NMR: (300MHz, $CDCl_3$): δ= 8.33 (d, *J* = 8.5 Hz, 2H, ar-DR(*o*-NO_2)), 7.92 (d, *J* = 8.7 Hz, 2H; ar-DR(*m*-NO_2)), 7.91 (d, *J* = 9.0 Hz, 2H; ar-DR(*m*-amino)), 7.04 (d, *J* = 8.3 Hz, 2H; Tyr-Hδ), 6.81 (d, *J* = 9.1 Hz, 2H; Tyr-Hε), 6.81 (d, *J* = 9.1 Hz, 2H; ar-DR(*o*-amino)), 5.93-5.82 (m, 1H; $CH_2C\underline{H}=CH_2$), 5.31 (dd, *J* = 17.4, 1.0 Hz, 1H; $CH_2CH=C\underline{H}_2$), 5.24 (dd, *J* = 10.2, 1.0 Hz, 1H; $CH_2CH=C\underline{H}_2$), 4.94 (d, *J* = 7.0 Hz, 1H; NH), 4.60 (d, *J* = 5.7 Hz, 2H; $C\underline{H}_2CH=CH_2$), 4.58-4.52 (m, 1H; Tyr-Hα) 4.16 (t, *J* = 6.1 Hz, 2H; $OC\underline{H}_2CH_2N$), 3.85 (t, *J* = 5.9 Hz, 2H; $OCH_2C\underline{H}_2N$), 3.61 (q, *J* = 7.0 Hz, 2H; $NC\underline{H}_2CH_3$), 3.10-3.00 (m, 2H; Hβ), 1.41 (s, 9H; boc), 1.29 (t, *J* = 7.0 Hz, 3H; $NCH_2C\underline{H}_3$);

ESI-MS: *m/z*: calcd for $C_{33}H_{39}N_5O_7Na$ $[M+Na]^+$ 640; found 640.

6. 3. 2. Boc-D-Tyrosine(ODye)-Allylester 59a

(**59a** was obtained analogue to the procedure described for **59** using BOC-D-Tyrosine-Allylester **15a**)

$C_{33}H_{39}N_5O_7$: 617.69

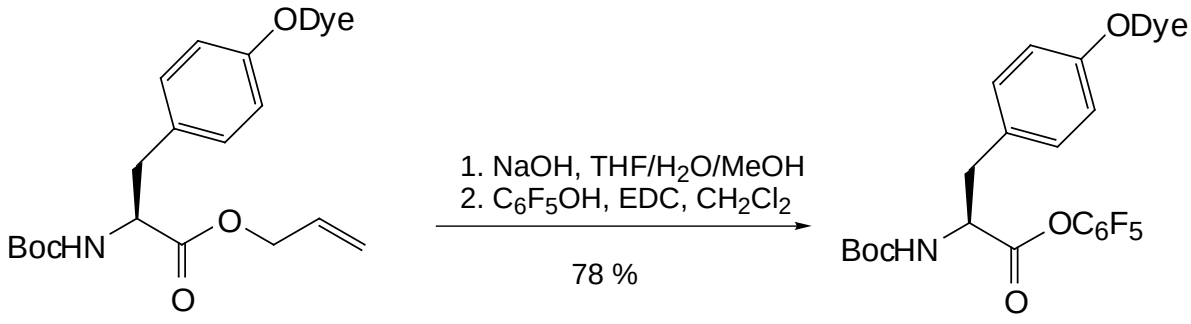
TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.75

^{1}H-NMR: (300MHz, $CDCl_3$): δ= 8.32 (d, *J* = 9.0 Hz, 2H; ar-DR(*o*-NO_2)), 7.93 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-NO_2)), 7.93 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-amino)), 7.04 (d, *J* = 8.7 Hz, 2H; Tyr-Hδ), 6.82 (d, *J* = 9.4 Hz, 2H; Tyr-Hε), 6.81 (d, *J* = 8.7 Hz, 2H; ar-DR(*o*-amino)), 5.94-5.80 (m, 1H; $CH_2C\underline{H}=CH_2$), 5.31 (dd, *J* = 1.4, 17.2 Hz, 1H; $CH_2CH=C\underline{H}_2$), 5.24 (dd, *J* = 1.2, 10.4, 1H; $CH_2CH=C\underline{H}_2$), 4.94 (d, *J* = 7.0 Hz, 1H; NH), 4.60 (d, *J* = 5.8 Hz, 2H; $C\underline{H}_2CH=CH_2$), 4.58-4.52 (m, 1H; Tyr-Hα), 4.16 (t, *J* = 5.9 Hz, 2H; $OC\underline{H}_2CH_2N$), 3.85 (t, *J* = 5.8 Hz, 2H; $OCH_2C\underline{H}_2N$), 3.62 (q, *J* = 7.1 Hz, 2H; $NC\underline{H}_2CH_3$), 3.12- 2.95 (m, 2H; Hβ), 1.41 (s, 9H; boc), 1.29 (t, *J* = 6.9 Hz, 3H; $NCH_2C\underline{H}_3$);

^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ (ppm) = 171.6, 157.5, 156.4, 151.5, 147.4, 143.6, 131.6, 130.5, 128.6, 126.7, 124.7, 122.5, 119.0, 114.5, 111.6, 79.9, 65.9, 65.3, 54.6, 50.0, 46.3, 37.5, 28.31, 12.4;

ESI-MS: *m/z*: calcd for $C_{33}H_{39}N_5O_7Na$ $[M+Na]^+$ 640; found 640.

6. 3. 3. Boc-Tyrosine(ODye)-Pentafluorophenylester 16



59 **16**

280mg (0.47 mmol, 1 eq) of BOC-L-Tyrosine(ODye)-Allylester **59** was suspended in a 1:1 mixture of THF and methanol and a solution of 28 mg (0.71 mmol, 1.5 eq) NaOH in 0.1 ml of water was added. The mixture was stirred at room temperature for 4 hours, then 20 ml of EtOAc was added. After extraction with 20 ml of 1M HCl the aquous phase was

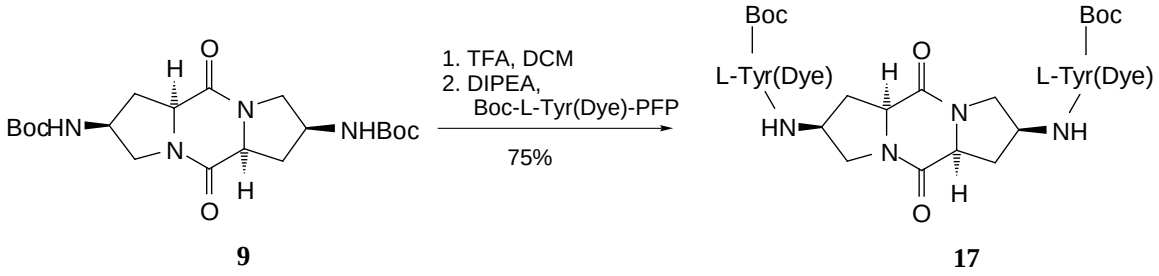
extracted twice with 20 ml of EtOAc. The organic layers were washed with brine and dried over Na_2SO_4 and the solvent was removed at reduced pressure. The residue was dissolved in 1 ml of DCM and 95 mg (0.52 mmol, 1.1 eq) of pentafluorophenol and 135 mg (0.71 mmol, 1.5 eq) of EDC was added. After 1 hour stirring at room temperature, the solution was extracted with water (50 ml) and DCM (3*50 ml). The organic layers were washed with brine and dried over Na_2SO_4. The volatiles were removed and the product was purified over silica gel by flash chromatography (DCM) yielding 273 mg (78 %) of pentafluorophenylester **16**.

$C_{36}H_{34}F_5N_5O_7$: 743.24

^{1}H-NMR: (400MHz, $CDCl_3$): δ= 8.31 (d, *J* = 8.5 Hz, 2H; ar-DR(*o*-NO_2)), 7.91 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-NO_2)), 7.90 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-amino)), 7.14 (d, *J* = 8.6 Hz, 2H; Tyr-Hδ), 6.87 (d, *J* = 8.6 Hz, 2H; Tyr-Hε), 6.81 (d, *J* = 9.8 Hz, 2H; ar-DR(*o*-amino)), 4.95 (d, *J* = 8.6 Hz, 1H; NH), 4.86 (td, *J* = 7.5, 13.6 Hz, 1H; Tyr-Hα), 4.17 (t, *J* = 6.3 Hz, 2H; $OC\underline{H}_2CH_2N$), 3.86 (t, *J* = 6.3 Hz, 2H; $OCH_2C\underline{H}_2N$), 3.61 (q, *J* = 7.1 Hz, 2H; $NC\underline{H}_2CH_3$), 3.23 (dd, *J* =6.1, 14.1 Hz, 1H; Hβ), 3.16 (dd, *J* =6.1, 14.2 Hz, 1H; Hβ), 1.43 (s, 9H; boc), 1.29 (t, *J* = 7.1 Hz, 3H; $NCH_2C\underline{H}_3$);

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ (ppm) = 168.4, 157.8, 156.7, 151.2, 147.4, 143.7, 130.4, 127.3, 126.2, 124.6, 122.6, 114.7, 111.4, 80.6, 65.3, 54.4, 49.8, 46.2, 367.0, 28.2, 12.3;

6. 3. 4. Receptor precursor 17



9 **17**

To remove the Boc groups, 39 mg (92.6 µmol, 1 eq) of the bis-*N*-Boc-protected diketopiperazine **9** were stirred at room temperature in 2 ml of a 1:3 mixture of TFA and DCM. After 30 minutes, the volatile components were removed at reduced pressure and the residue was tritureted with diethyl ether. Then 0.5 ml of DCM was added as well as 60µl (0.37 mmol, 4 eq) of DIPEA followed by 150 mg (0.23 mmol, 2.5 eq) of the pentafluoro ester **16**. After 3 hours the reaction mixture was filtered over silica gel (gradient of CH_2Cl_2 : MeOH from 100 : 0 to 100 : 5). This yielded 92 mg (75 %) of **17**.

$C_{70}H_{82}N_{14}O_{14}$: 1342.61

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.40

^{1}H-NMR: (400MHz, $CDCl_3$): δ= 8.30 (d, *J* = 9.2 Hz, 2H, ar-DR(*o*-NO_2)), 7.89 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-NO_2)), 7.87 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-amino)), 7.03 (d, *J* = 8.5 Hz, 2H; Tyr-Hδ), 6.78 (d, *J* = 9.5 Hz, 4H; Tyr-Hε, ar-DR(*o*-amino)), 6.50 - 6.43 (m, 1H; NH DKP), 5.27 - 5.20 (m, 1H; NH Boc), 4.43 - 4.30 (m, 1H; Tyr-Hα), 4.28 - 4.10 (m, 4H; DKP-Hα, DKP-Hγ, OC$\underline{H}_2$$CH_2$N), 3.81 (t, *J* = 5.4 Hz, 2H; $OCH_2C\underline{H}_2$N), 3.58 (q, *J* = 7.1 Hz, 2H; N$C\underline{H}_2CH_3$), 3.60 - 3.50 (m, 1H; DKP-Hδ), 3.37 (dd, *J* = 3.1, 11.2 Hz, 1H; DKP-Hδ), 2.94 (dd, *J* = 6.9, 13.6 Hz, 1H; Tyr-Hβ), 2.87 (dd, *J* =6.7, 13.8 Hz, 1H; Tyr-Hβ), 2.50 - 2.30 (m, 2H; DKP-Hβ), 1.37 (s, 9H; boc), 1.26 (t, *J* = 7.0 Hz, 3H; $NCH_2C\underline{H}_3$);

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ (ppm) = 171.7, 166.1, 159.5, 157.3, 156.7, 151.2, 147.3, 143.7, 130.4, 128.2, 126.2, 124.7, 122.6, 114.6, 111.4, 78.9, 65.3, 58.7, 56.5, 50.9, 50.1, 49.7, 46.1, 37.4, 30.9, 28.3, 12.3;

ESI-MS: *m/z*: calcd for $C_{33}H_{39}N_5O_7Na$ $[M+Li]^+$ 1349; found 1349.

6. 4. Synthesis of dye marked *cis*-diketopiperazine receptor prototypes

6. 4. 1. A general protocol for the synthesis of two armed molecules

6. 4. 1. 1. Boc-deprotection of precursor 17

To 117 mg (89 mmol, 1 eq) of **17** 2 ml of HCl (4M) in dioxane was added followed by 0.1 ml of methanol. After stirring 1 hour at r.t. all volatiles were removed at reduced pressure and the residue was triturated twice with diethyl ether. The red solid was then dried in vacuo and dissolved in DCM with 50 ml DIPEA (267 mmol, 3eq). This solution was then used for the next amino acid coupling.

6. 4. 1. 2. Coupling of N-α-Fmoc protected amino acids

The desired N-α-Fmoc Protected Amino Acid (3 eq) was added together with 3 eq of EDC to a concetrated solution of the corresponding N-α deprotected educt (1 eq) in as little DCM as possible (usually about 300 - 400 µl for 100 mg educt). The mixture was stirred at r.t. until the reaction was complete (usually about 20 - 30 minutes). Then it was extracted with DCM and 1M HCl. The aqueous layer was extracted twice more with DCM and the organic phases were washed with brine and dried over Na_2SO_4. After removal of the solvent at reduced pressure, the product was purified over silica gel (gradient of DCM/MeOH from 100:0 to 90:10). This afforded the N-α-FMOC-protected receptor precursor which was used without further purification.

6. 4. 1. 3. FMOC-deprotection

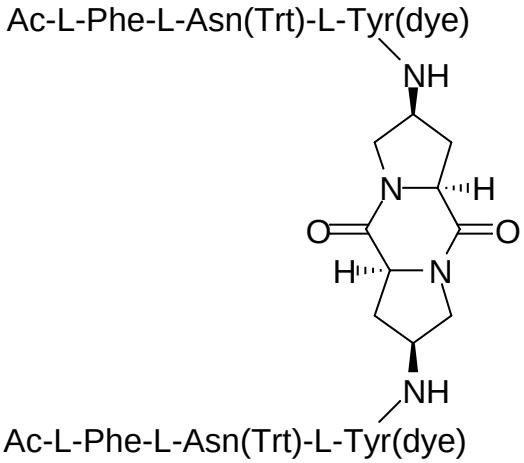
The N-α-FMOC-protected receptor precursor (1 eq) was dissolved in DCM and 50 eq of TAEA were added. After 10 - 20 minutes the mixture was extracted three times with brine and three times with phosphate buffer pH 5.5. The aqueous phases were extracted

twice more with DCM and the organic layers were dried over Na_2SO_4. Removal of the volatiles at reduced pressure yielded the corresponding free amine that was now introduced to a further amino acid coupling or was acetylated. The coupling and FMOC-deprotection cycles was repeated until the desired peptidic arms were assembled.

6. 4. 1. 4. Acetylation of the free amines

The corresponding free amine (1 eq) was dissolved in 1 ml of DCM and 3 eq of each NEt_3 and Ac_2O was added. After stirring of the mixture for 30 minutes the acetylated two armed molecule was purified by flash chromatography and gel filtration (LH 20, DCM/MeOH 90:10). The two armed molecules **10 - 14, 19** and **20** were isolated in amounts of 20 - 40 mg (40 - 55 % from **9**).

6. 4. 2. Receptor 10:



$C_{128}H_{128}N_{20}O_{18}$: 2234.51

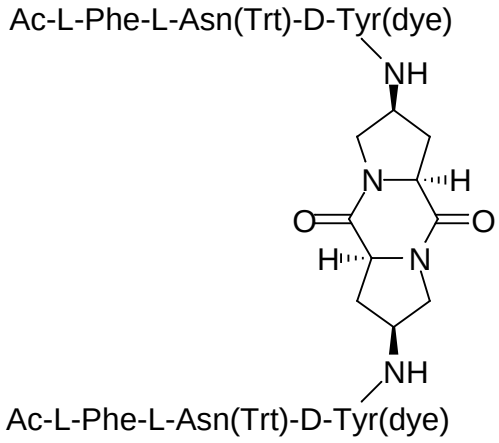
TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.37

^{1}H NMR (500 MHz, 2% CD_3OD in $CDCl_3$, 25°C): δ = 8.32 (d, *J* = 9.0 Hz, 4H; dye), 7.99 (s, 2H; NH), 7.91 (d, *J* = 9.0 Hz, 4H; dye), 7.88 (d, *J* = 9.3 Hz, 4H; dye), 7.36 (d, *J* = 6.7 Hz, 2H; NH), 7.27 - 7.16 (m, 40H; trityl, Phe, NH), 7.09 (d, *J* = 7.0 Hz, 4H; Phe), 7.03 (d, *J* = 8.6 Hz, 4H; Tyr), 6.92 (d, *J* = 6.5 Hz, 2H; NH), 6.77 (d, *J* = 9.3 Hz, 4H; dye), 6.74 (d, *J* = 8.6 Hz, 4H; Tyr), 4.48 (m, 2H; Tyr-Hα), 4.36 (m, 6H; Pro-Hγ, Asn-Hα, Phe-Hα), 4.17 (ψt, *J* = 8.0 Hz, 2H; Pro-Hα), 4.03 (t, *J* = 5.7 Hz, 4H; OCH_2CH_2N), 3.74 (t, *J* = 5.7 Hz, 4H; OCH_2CH_2N), 3.61 (m, 2H; Pro-Hδ), 3.55 (q, *J* = 7.1 Hz, 4H; CH_2CH_3), 3.23 (m, 2H; Pro-Hδ´), 3.06 (dd, *J* = 14.1 Hz, 4.9 Hz, 2H; Tyr-Hβ), 2.90 (m, 4H; Tyr-Hβ´, Phe-Hβ), 2.78 (dd, *J* = 14.9 Hz, 5.7 Hz, 2H; Asn-Hβ), 2.65 (dd, *J* = 14.0 Hz, 9.9 Hz, 2H; Phe-Hβ´), 2.56 (dd, *J* = 14.9 Hz, 6.9 Hz, 2H; Asn-Hβ´), 2.38 (m, 2H; Pro-Hβ), 2.15 (m, 2H; Pro-Hβ´), 1.78 (s, 6H; $COCH_3$), 1.25 (t, *J* = 7.1 Hz, 6H; CH_2CH_3);

^{13}C NMR (125.6 MHz, 2% CD_3OD in $CDCl_3$, 25°C): δ = 172.4, 171.6, 171.4, 170.7, 170.2, 166.0, 157.3, 156.8, 151.3, 147.3, 144.1, 143.4, 136.3, 130.3, 129.4, 129.0, 128.7, 127.8, 127.0, 126.3, 124.7, 122.6, 114.4, 111.4, 70.6, 65.2, 58.9, 55.2, 54.9, 51.1, 49.8, 49.2, 47.5, 46.0, 37.4, 36.9, 35.8, 32.3, 22.5, 12.2;

HRMS (ESI): *m/z*: calcd for $C_{128}H_{128}N_{20}O_{18}$ $[M+2H]^{2+}$ 1117.4931; found 1117.4933.

6. 4. 3. Two armed molecule 11:



$C_{128}H_{128}N_{20}O_{18}$: 2234.51

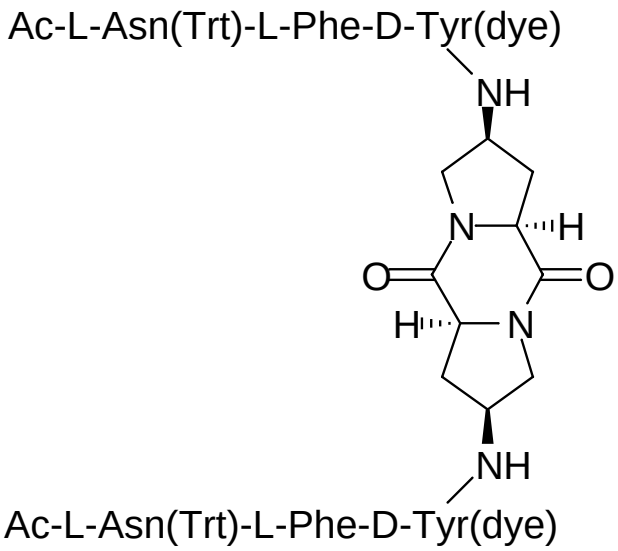
TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.19

^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 8.31 (d, *J* = 9.1 Hz, 4H; dye), 7.90 (d, *J* = 9.1 Hz, 4H; dye), 7.85 (d, *J* = 9.2 Hz, 4H; dye), 7.26 - 7.12 (m, 36H; trityl, Phe-6H,), 7.08 (d, *J* = 8.4 Hz, 8H; Tyr, Phe), 6.78 (d, *J* = 8.6 Hz, 4H; Tyr), 6.73 (d, *J* = 9.2 Hz, 4H; dye), 4.37 (m, 2H; Tyr-Hα), 4.27 (m, 4H; Asn-Hα, Phe-Hα), 4.07 (m, *J* = 6.0 Hz, 8H; OCH_2CH_2N, Pro-Hα, Pro-Hγ), 3.73 (t, *J* = 6.0 Hz, 4H; OCH_2CH_2N), 3.52 (q, *J* = 7.1 Hz, 4H; CH_2CH_3), 3.36 (d (broad), *J* = 9.7 Hz, 2H; Pro-Hδ), 3.18 (dd, *J* = 13.6 Hz, 6.7 Hz, 2H; Asn-Hβ), 3.10 (dd, *J* = 14.3 Hz, 4.2 Hz, 4H; Phe-Hβ, Tyr-Hβ), 2.92 (dd, *J* = 13.6 Hz, 7.8 Hz, 2H; Asn-Hβ´), 2.75 (dd, *J* = 14.3 Hz, 9.7 Hz, 2H; Phe-Hβ´, Pro-Hδ´), 2.64 (dd, *J* = 14.9 Hz, 5.9 Hz, 2H; Tyr-Hβ´), 2.27 (m, 4H; Pro-Hβ, Pro-Hβ´), 1.60 (s, 6H; $COCH_3$), 1.21 (t, *J* = 7.1 Hz, 6H; CH_2CH_3);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 172.1, 171.3, 171.1, 170.9, 170.7, 165.0, 157.1, 156.8, 151.3, 147.3, 144.2, 143.7, 136.0, 130.6, 129.8, 128.9, 128.9, 128.8, 127.9, 127.3, 127.0, 126.3, 124.7, 122.6, 114.4, 111.4, 70.8, 65.1, 58.2, 55.9, 50.9, 50.6, 49.8, 47.5, 46.0, 36.7, 35.4, 32.4, 22.9, 12.3;

HRMS (ESI): *m/z*: calcd for $C_{128}H_{128}N_{20}O_{18}$ $[M+2H]^{2+}$ 1117.4931; found 1117.4937.

6. 4. 4. Two-armed molecule 12:



$C_{128}H_{128}N_{20}O_{18}$: 2234.51

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.46

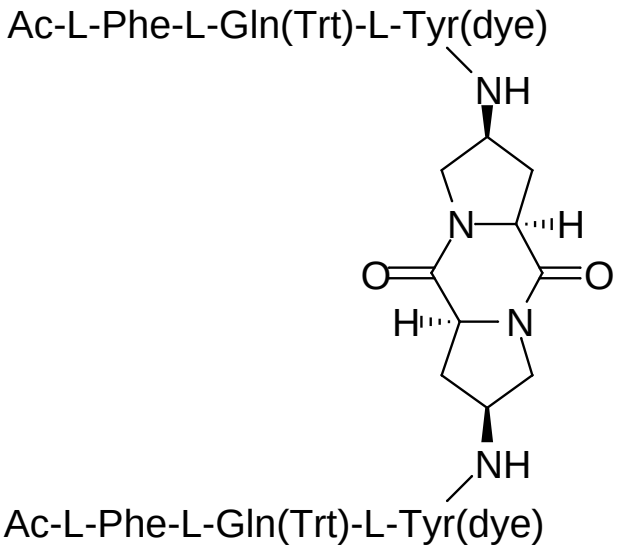
^{1}H NMR (500 MHz, 5% CDOD in $CDCl_3$, 25°C): δ = 8.31 (d, *J* = 9.1 Hz, 4H; dye), 7.91 (d, *J* = 9.1 Hz, 4H; dye), 7.88 (d, *J* = 9.2 Hz, 4H; dye), 7.23 - 7.15 (m, 36H; trityl, Phe-6H), 7.05 (d, *J* = 6.9 Hz, 4H; Phe), 6.78 (ψd, *J* = 9.1 Hz, 8H; dye, Tyr), 6.69 (d, *J* = 8.8 Hz, 4H; Tyr), 4.50 (ψt, *J* = 5.9, 2H; Asn-Hα), 4.39 (ψt, *J* = 7.3 Hz, 2H; Pro-Hγ), 4.32 (t, *J* = 6.6 Hz, 2H; Tyr-Hα), 4.13 (t, *J* = 8.4 Hz, 2H; Pro-Hα), 4.07 (t, *J* = 6.0 Hz, 4H; O*C*$H_2$$CH_2$N), 4.06 (m, 2H; Phe-Hα), 3.78 (t, *J* = 6.0 Hz, 4H; OCH_2CH_2N), 3.61 (dd, *J* = 12.0 Hz, 7.6 Hz, 2H; Pro-Hδ), 3.56 (q, *J* = 7.0 Hz, 4H; C$H_2$$CH_3$), 3.30 (dd, *J* = 12.0 Hz, 7.6 Hz, 2H; Pro-Hδ´), 2.95 (dd, *J* = 13.4 Hz, 6.7 Hz, 2H; Phe-Hβ), 2.80 (dd, *J* = 13.4 Hz, 8.0 Hz, 2H; Phe-Hβ´), 2.74 (m, 8H; Tyr-Hβ, Tyr-Hβ´, Asn-Hβ, Asn-Hβ´), 2.42 (m, 2H; Pro-Hβ), 2.27 (m, 2H; Pro-Hβ´), 1.77 (s, 6H; CO*C*H_3), 1.25 (t, *J* = 7.0 Hz, 6H; CH_2CH_3);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 171.8, 171.4, 171.1, 171.0, 170.3, 165.8, 157.3, 156.7, 151.2, 147.3, 144.1, 143.6, 136.6, 130.2, 129.3, 129.0, 128.6,

128.5, 127.7, 126.9, 126.2, 124.6, 122.5, 114.2, 111.3, 70.5, 65.2, 58.6, 55.9, 54.5, 53.4, 50.2, 49.9, 49.7, 47.0, 46.0, 37.5, 36.3, 35.3, 32.6, 22.5, 12.1;

HRMS (ESI): *m/z*: calcd for $C_{128}H_{128}N_{20}O_{18}$ $[M+2H]^{2+}$ 1117.4931; found 1117.4933.

6. 4. 5. Receptor 13:



$C_{128}H_{128}N_{20}O_{18}$: 2262.56

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.49

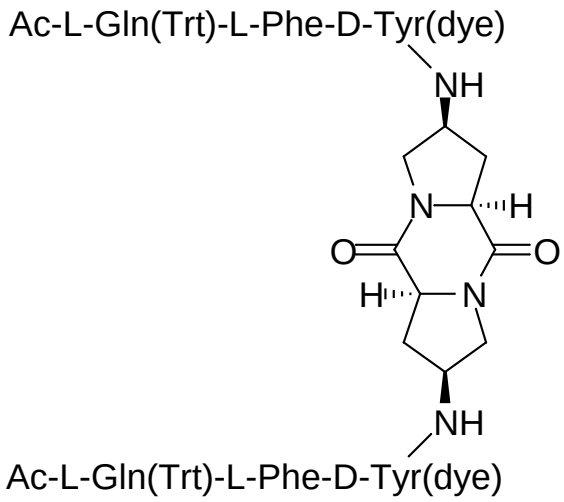
^{1}H NMR (500 MHz, $CDCl_3$, 43°C): $\delta = 8.53$ (s (broad), 2H, NH), 8.30 (d, J = 9.1 Hz, 4H; dye), 7.88 (d, J = 9.1 Hz, 4H; dye), 7.84 (d, J = 9.2 Hz, 4H; dye), 7.27 - 7.09 (m, 48H; trityl, Phe, Tyr-4H, NH-4H), 6.98 (s (broad), 2H, NH), 6.73 (ψd, J = 9.2 Hz, 8H; Tyr, dye), 5.73 (s (broad), 2H, NH), 4.56 (m, 2H; Tyr-Hα), 4.41 (m, 2H; Pro-Hγ), 4.32 (m, 2H; Phe-Hα), 4.13 (m, 2H; Pro-Hα), 4.03 (m, 6H; OCH_2CH_2N, Gln-Hα), 3.73 (t, J = 5.8 Hz, 4H; OCH_2CH_2N), 3.71 (m, 2H; Pro-Hδ), 3.52 (q, J = 7.0 Hz, 4H; CH_2CH_3), 3.35 (dd, J = 11.3 Hz, 7.4 Hz, 2H; Pro-Hδ´), 3.25 (dd, J = 14.1 Hz, 3.7 Hz, 2H; Tyr-Hβ), 3.05

(dd, *J* = 14.1 Hz, 4.5 Hz, 2H; Phe-Hβ), 2.83 (dd, *J* = 14.1 Hz, 10.1 Hz, 2H; Tyr-Hβ´), 2.67 (dd, *J* = 14.0 Hz, 9.9 Hz, 2H; Phe-Hβ´), 2.42 (m, 2H; Pro-Hβ), 2.34 (m, 2H; Pro-Hβ´), 2.08 (m, 4H; Gln-Hγ, Gln-Hγ´), 1.87 (m, 2H; Gln-Hβ), 1.75 (m, 2H; Gln-Hβ´), 1.62 (s, 6H; $COCH_3$), 1.22 (t, *J* = 7.1 Hz, 6H; CH_2CH_3);

^{13}C NMR (125.6 MHz, $CDCl_3$, 25°C): δ = 173.3, 172.9, 172.0, 171.3, 170.9, 165.8, 157.2, 156.8, 151.3, 147.4, 144.3, 143.7, 136.3, 130.4, 129.0, 128.8, 128.7, 128.0, 127.3, 127.1, 126.3, 124.7, 122.6, 114.3, 111.4, 70.8, 65.4, 58.9, 56.0, 54.8, 49.8, 49.4, 47.3, 46.1, 37.0, 36.1, 32.9, 25.8, 23.0, 12.3;

HRMS (ESI): *m/z*: calcd for $C_{130}H_{132}N_{20}O_{18}$ $[M+2H]^{2+}$ 1131.5087; found 1131.5069.

6. 4. 6. Two armed molecule 14:



$C_{128}H_{128}N_{20}O_{18}$: 2262.56

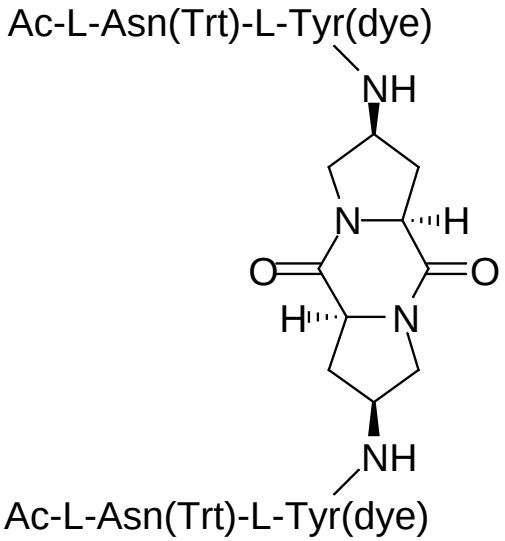
TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.38

^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 8.31 (d, *J* = 9.1 Hz, 4H; dye), 7.91 (d, *J* = 9.1 Hz, 4H; dye), 7.88 (d, *J* = 9.2 Hz, 4H; dye), 7.29 - 7.15 (m, 36H; trityl, Phe-6H), 7.05 (d, *J* = 7.1 Hz, 4H; Phe), 6.77 (d, *J* = 9.2 Hz, 4H; dye), 6.76 (d, *J* = 8.7 Hz, 4H; Tyr), 6.71 (d, *J* = 8.8 Hz, 4H; Tyr), 4.36 (m, 2H, Tyr-Hα), 4.32 (m, 2H, Pro-Hγ), 4.19 (ψt, *J* = 8.4 Hz, 2H; Pro-Hα), 4.09 (m, *J* = 5.8 Hz, 8H; Phe-Hα, OCH_2CH_2N, Gln-Hα), 3.79 (t, *J* = 5.8 Hz, 4H; OCH_2CH_2N), 3.61 (m, 2H; Pro-Hδ), 3.56 (q, *J* = 7.1 Hz, 4H; CH_2CH_3), 3.47 (m, 2H, Pro-Hδ´), 2.97 – 2.90 (m, 4H, Phe-Hβ, Tyr-Hβ), 2.79 (dd, *J* = 13.8 Hz, 8.2 Hz, 2H; Phe-Hβ´), 2.70 (dd, *J* = 13.8 Hz, 5.6 Hz, 2H; Tyr-Hβ´), 2.48 (m, 2H; Pro-Hβ), 2.20 (m, 6H; Pro-Hβ´, Gln-Hγ, Gln-Hγ´), 1.86 (s, 6H; $COCH_3$), 1.80 (m, 4H, Gln-Hβ, Gln-Hβ´), 1.25 (t, *J* = 7.1 Hz, 6H; CH_2CH_3);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 172.7, 172.2, 171.7, 171.6, 171.1, 165.7, 157.3, 156.7, 151.3, 147.3, 144.3, 143.6, 136.8, 130.2, 129.0, 129.0, 128.6, 128.5, 127.8, 126.9, 126.8, 126.2, 124.6, 122.5, 114.4, 111.3, 70.5, 65.2, 58.6, 55.7, 54.4, 52.9, 49.5, 49.3, 47.1, 46.0, 36.2, 35.3, 32.8, 32.3, 26.7, 22.5, 12.2;

HRMS (ESI): *m/z*: calcd for $C_{130}H_{132}N_{20}O_{18}$ $[M+2H]^{2+}$ 1131.5087; found 1131.5101.

6. 4. 7. Two-armed molecule **19:**



$C_{160}H_{152}N_{24}O_{18}$: 2697.17

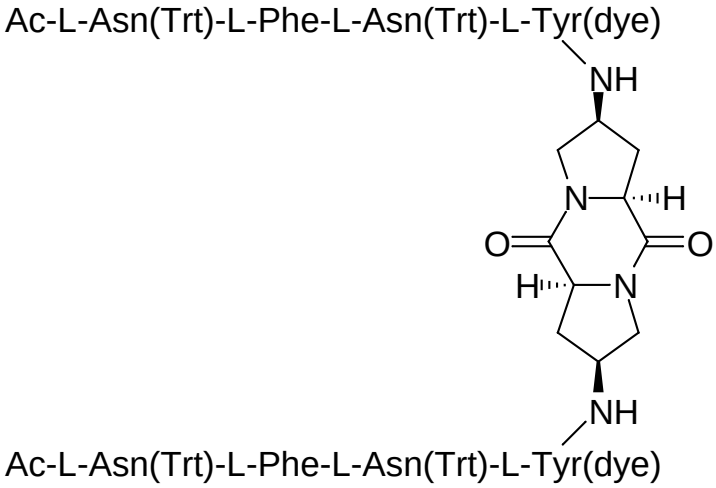
TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.13

^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 8.33 (d, *J* = 9.1 Hz, 4H; dye), 7.92 (d, *J* = 9.1 Hz, 4H; dye), 7.90 (d, *J* = 9.2 Hz, 4H; dye), 7.71 (d, *J* = 6.9 Hz, 2H; NH Asn), 7.29 - 7.16 (m, 30H, Trityl), 6.95 (d, *J* = 9.0 Hz, 2H; NH Tyr), 6.91 (d, *J* = 5.4 Hz, 2H; NH DKP), 6.80 (d, *J* = 8.5 Hz, 4H; Tyr), 6.79 (d, *J* = 9.2 Hz, 4H; dye), 6.63 (d, *J* = 8.7 Hz, 4H; Tyr), 4.93 - 4.88 (m, 2H; Tyr-Hα), 4.84 - 4.80 (m, 2H; Asn-Hα), 4.26 (dd, *J* = 2.0, 8.8 Hz, 2H; Pro-Hα), 4.19 - 4.15 (m 2H; Pro-Hγ), 4.05 (t, *J* = 6.2 Hz, 4H; OCH_2CH_2N), 3.78 (t, *J* = 6.2 Hz, 4H; OCH_2CH_2N), 3.76 (d, *J* = 12.6 Hz, 2H; Pro-Hδ'), 3.57 (q, *J* = 6.9 Hz, 4H; CH_2CH_3), 3.32 (dd, *J* = 4.8, 12.4 Hz, 2H; Pro-Hδ), 3.01 (dd, *J* = 3.8, 15.0 Hz, 2H; Asn-Hβ), 2.64 (dd, *J* = 5.8, 15.1 Hz, 2H; Asn-Hβ), 2.57 (d, *J* = 14.1 Hz, 2H; Pro-Hβ'), 2.55 - 2.50 (m, 4H; Tyr-Hβ), 2.20 (ddd, *J* = 3.2, 5.5, 8.7 Hz, 2H; Pro-Hβ), 1.77 (s, 6H; $COCH_3$), 1.26 (t, *J* = 7.0 Hz, 6H; CH_2CH_3);

^{13}C NMR (125.6 MHz, 5% CD $_3$OD in $CDCl_3$, 25 °C): δ = 172.1, 172.0, 170.9, 170.8, 168.0, 157.1, 156.8, 151.3, 147.3, 144.3, 143.7, 130.1, 129.0, 128.8, 128.7, 127.9, 126.90, 126.3, 124.7, 122.6, 114.2, 111.4, 70.6, 65.1, 59.5, 53.4, 51.5, 50.6, 49.7, 48.6, 46.1, 35.8, 35.5, 30.7, 22.9, 12.3;

ESI-MS: *m/z*: calcd for $C_{110}H_{108}N_{18}O_{16}$ $[M]^+$ 1938.8; found 1938.9.

6. 4. 8. Receptor 20:



$C_{174}H_{168}N_{24}O_{22}$: 2647.28

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.35

^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 8.31 (d, *J* = 9.0 Hz, 4H; dye), 7.90 (d, *J* = 9.0 Hz, 4H; dye), 7.87 (d, *J* = 9.2 Hz, 4H; dye), 7.71 (s, 2H; NH(Trt)), 7.61 (s, 2H; NH(Trt)), 7.23 - 7.10 (m, 66H; Trityl, Phe-6H), 7.07 (d, *J* = 6.7 Hz, 4H; Phe-Hδ), 7.02 (d, *J* = 8.6 Hz, 4H; Tyr-Hδ), 6.75 (d, *J* = 9.3 Hz, 4H; dye), 6.74 (d, *J* = 8.5 Hz, 4H; Tyr-Hε), 4.46 (t, *J* = 6.6 Hz, 2H; Tyr-Hα), 4.38 (t, *J* = 6.2 Hz, 2H; Asn-Hα), 4.34 (t, *J* = 5.6 Hz, 2H; Asn-Hα), 4.32 - 4.25 (m, 4H; Pro-Hγ, Phe-Hα), 4.08 (t, *J* = 8.0 Hz, 2H; Pro-

Hα), 4.02 (t, *J* = 5.8 Hz, 4H; OC*H*$_2$CH$_2$N), 3.72 (t, *J* = 5.8 Hz, 4H; OCH$_2$C*H*$_2$N), 3.53 (q, *J* = 7.1 Hz, 4H; C*H*$_2$CH$_3$), 3.53 - 3.49 (m, 2H, Pro-Hδ), 3.17 (dd, *J* = 8.4, 11.5 Hz, 2H; Pro-Hδ), 3.09 (dd, *J* = 4.2, 14.2 Hz, 2H; Phe-Hβ), 2.94 (dd, *J* = 6.2, 14.3 Hz, 2H; Tyr-Hβ), 2.87 (dd, *J* = 7.2, 14.1 Hz, 2H; Tyr-Hβ), 2.70 (dd, *J* = 10.0, 14.1 Hz, 2H; Tyr-Hβ), 2.60 - 2.30 (m, 10H; Pro-2Hβ, Asn-8Hβ), 2.16 - 2.08 (m, 2H; Pro-Hβ), 1.76 (s, 6H, Ac), 1.23 (t, *J* = 7.0 Hz, 6H; CH$_2$C*H*$_3$);

^{13}C NMR (125.6 MHz, 5% CD$_3$OD in CDCl3, 25°C): δ = 171.7, 171.5, 171.3, 171.1, 170.8, 170.4, 170.1, 165.8, 157.3, 156.7, 151.3, 147.2, 144.1, 143.6, 136.5, 130.3, 129.1, 129.0, 128.6, 128.6, 128.5, 127.8, 127.8, 126.9, 126.8, 126.2, 124.6, 122.5, 114.3, 111.3, 70.5, 70.4, 65.1, 58.8, 54.8, 51.1, 50.5, 49.4, 49.1, 47.3, 45.9, 37.2, 36.9, 36.2, 35.9, 32.4, 22.6, 12.1;

ESI-MS: *m/z*: calcd for $C_{174}H_{168}N_{24}O_{22}$ $[M + Na]^+$ 2968.3; found 2968.5.

6. 5. Synthesis of the two armed molecules *25 - 27 and 29 - 31* based on different diamino templates[58]

6. 5. 1. Synthesis of the tripeptidic arms

The tripeptidic arms **60, 60', 61** and **61'** were assembled starting from the corresponding Nα-Boc-protected tyrosineesters **59, 59a, 59',** and **59'a** respectively following the FMOC-amino acid coupling procedure as described for the receptor prototypes in chapter

6. 5. 1. 1. Tripeptidic arm Ac-L-Phe-L-Asn(Trt)-L-Tyr(dye)OAllyl 60:

$C_{62}H_{62}N_8O_9$: 1063.20

TLC: CH_2Cl_2:MeOH (25:1); Rf**:** 0.37

^{1}H-NMR: (400MHz, $CDCl_3$): δ = 8.32 (d, *J* = 9.2 Hz, 2H; ar-DR(*o*-NO_2)), 7.92 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-NO_2)), 7.89 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-Amino)), 7.45 (d, *J* = 7.0 Hz, 1H; NH(Tyr)), 7.33 (d, *J* = 7.0 Hz, 1H; NH(Asn)), 7.30 - 7.18 (m, 18H; Trt-ar, 3H-Phe,), 7.06 (d, *J* = 6.5 Hz, 2H; Phe-Hδ), 6.96 (d, *J* = 8.5 Hz, 2H; Tyr-Hδ), 6.78 (d, *J* = 9.7 Hz, 2H; ar-DR(*o*-Amino)), 6.73 (d, *J* = 8.6 Hz, 2H; Tyr-Hε), 5.87 (tdd, *J* = 5.5, 11.7, 16.1 Hz, 1H; $CH_2C\underline{H}=CH_2$), 5.87 (s br, 1H; NH(Phe)), 5.27 (dd, *J* = 1.5, 17.1 Hz, 1H; $CH_2CH=C\underline{H}_2$), 5.21 (dd, *J* = 1.1, 10.8 Hz, 1H; $CH_2CH=C\underline{H}_2$), 4.66 - 4.57 (m, 3H; Tyr-Hα, Asn-Hα, Phe-Hα), 4.05 (t, *J* = 5.9 Hz, 2H; $OC\underline{H}_2CH_2N$), 3.77 (t, *J* = 6.1 Hz, 2H; $OCH_2C\underline{H}_2N$), 3.58 (q, *J* = 7.1 Hz, 2H; $NC\underline{H}_2CH_3$), 3.05 - 2.97 (m, 3H; Asn-Hβ, Phe-Hβ, Tyr-Hβ), 2.87 (dd, *J* = 7.5, 13.1 Hz, 2H; Asn-Hβ', Phe-Hβ'), 2.54 (dd, *J* = 7.1, 15.5 Hz, 1H; Tyr-Hβ'), 1.79 (s, 3H; Acetyl), 1.27 (t, *J* = 7.0 Hz, 3H; $NCH_2C\underline{H}_3$);

^{13}C-NMR: (125.8 MHz, $CDCl_3$): δ = 171.1, 170.7, 170.5, 170.2, 170.1, 156.8, 156.3, 151.3, 145.0, 144.3, 143.8, 136.0, 131.6, 130.4, 129.1, 128.5, 127.9, 127.1, 128.7, 126.3,

124.5, 122.6, 118.6, 114.5, 111.4, 70.9, 65.9, 62.3, 54.2, 54.2, 53.4, 49.80, 46.1, 37.7, 36.8, 36.4, 23.0, 12.3;

6. 5. 1. 2. Tripeptidic arm Ac-L-Phe-L-Asn(Trt)-D-Tyr(dye)OAllyl 60':

$C_{62}H_{62}N_8O_9$: 1063.20

TLC: CH_2Cl_2:MeOH (25:1); Rf**:** 0.39

^{1}H-NMR: (300MHz, $CDCl_3$): δ = 8.32 (d, *J* = 8.9 Hz, 2H; ar-DR(*o*-NO_2)), 7.92 (d, *J* = 9.0 Hz, 2H; ar-DR(*m*-NO_2)), 7.89 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-Amino)), 7.51 (d, *J* = 7.7 Hz, 1H; NH(Tyr)), 7.30 - 6.93 (m, 22H; Trt-ar, Phe, Tyr-Hδ), 6.80 (d, *J* = 9.0 Hz, 2H; ar-DR(*o*-Amino)), 6.77 (d, *J* = 8.3 Hz, 2H; Tyr-Hε), 5.88 - 5.73 (m, 1H; $CH_2C\underline{H}=CH_2$), 5.25 (dd, *J* = 1.4, 17.2 Hz, 1H; $CH_2CH=C\underline{H}_2$), 5.16 (dd, *J* = 1.1, 10.3 Hz, 1H; $CH_2CH=C\underline{H}_2$), 4.68 - 4.48 (m, 3H; Phe-Hα, Asn-Hα, Tyr-Hα), 4.11 (t, *J* = 5.8 Hz, 2H; $OC\underline{H}_2CH_2N$), 3.81 (t, *J* = 5.7 Hz, 2H; $OCH_2C\underline{H}_2N$), 3.59 (q, *J* = 7.2 Hz, 2H; $NC\underline{H}_2CH_3$), 3.07 (dd, *J* = 5.5, 14.0 Hz, 2H; Asn-Hβ, Phe-Hβ), 2.97 - 2.82 (m, 3H; Asn-Hβ, Phe-Hβ, Tyr-Hβ), 2.46 (dd, *J* = 6.3, 15.3 Hz, 1H; Tyr-Hβ), 1.80 (s, 3H; Acetyl), 1.28 (t, *J* = 6.9 Hz, 3H; $NCH_2C\underline{H}_3$);

^{13}C-NMR: (125.8 MHz, $CDCl_3$): δ = 171.6, 171.1, 170.9, 170.6, 170.4, 157.8, 157.2, 156.7, 151.7, 147.8, 144.6, 144.2, 136.5, 132.0, 130.9, 129.5, 129.2, 129.1, 128.4, 127.6, 127.5, 126.7, 125.1, 123.0, 119.2, 114.8, 111.8, 71.2, 66.3, 65.6, 61.8, 60.8, 55.2, 54.6, 50.3, 50.1, 46.5, 37.9, 37.4, 23.4, 12.7;

ESI-MS: *m/z*: calcd for $C_{62}H_{62}N_8O_9Na$ $[M+Na]^+$ 1086; found 1086.

6. 5. 1. 3. Tripeptidic arm Ac-L-Phe-L-Asn(Trt)-L-Tyr(dye)OMe 61:

$C_{60}H_{60}N_8O_9$: 1037.17

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.49

^{1}H-NMR: (500MHz, $CDCl_3$): δ = 8.32 (d, *J* = 9.1, 2H; ar-DR(*o*-NO_2)), 7.92 (d, *J* = 9.1, 2H; ar-DR(*m*-NO_2)), 7.89 (d, *J* = 9.2, 2H; ar-DR(*m*-Amino)), 7.49 (d, *J* = 7.3, 1H; NH(Tyr)), 7.31 (d, *J* = 7.2, 1H; NH), 7.28-7.13 (m, 18H; ar H), 7.07 (d, *J* = 6.7, 2H; ar *o*-Phe), 6.98 (s, 1H; NH(Trt)), 6.94 (d, *J* = 8.7, 2H; TyrHδ), 6.78 (d, *J* = 9.3, 2H; ar-DR(*o*-Amino)), 6.74 (d, *J* = 6.7, 2H; Tyr-Hε), 4.66 (td, *J* = 3.4, 6.8, 1H; Tyr-Hα), 4.61-4.55 (m, 2H; Phe-Hα/ Asn-Hα), 4.04 (t, *J* = 5.8, 2H; $OC\underline{H}_2CH_2N$), 3.77 (t, *J* = 5.8, 2H; $OCH_2C\underline{H}_2N$), 3.67 (s, 3H; OCH_3), 3.57 (q, *J* = 7.1, 2H; $NC\underline{H}_2CH_3$), 3.02 (dd, *J* = 3.5, 15.5, 1H; Tyr-Hβ), 3.00-2.95 (m, 2H; Asn-Hβ, Phe-Hβ), 2.89-2.84 (m, 2H; Asn-Hβ, Phe-Hβ), 2.54 (dd, *J* = 6.5, 15.5, 1H; Tyr-Hβ), 1.78 (s, 3H; Acetyl), 1.27 (t, *J* = 7.1Hz, 3H; $NCH_2C\underline{H}_3$);

^{13}C-NMR: (125.8 MHz, $CDCl_3$): δ = 171.4, 171.2, 170.5, 170.2, 170.1, 157.4, 156.8, 151.3, 147.3, 144.2, 143.7, 136.0, 130.3, 129.1, 128.7, 128.0, 127.1, 128.4, 126.3, 124.7, 122.6, 114.4, 111.4, 70.8, 65.1, 54.2, 54.1, 52.3, 49.8, 49.5, 46.1, 37.6, 37.5, 36.5, 23.0, 12.3;

ESI-MS: *m/z*: calcd for $C_{33}H_{39}N_5O_7Na$ $[M+Na]^+$ 1060; found 1060;

6. 5. 1. 4. Tripeptidic arm Ac-L-Phe-L-Asn(Trt)-D-Tyr(dye)OMe 61':

$C_{60}H_{60}N_8O_9$: 1037.17

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.63

^{1}H-NMR: (500MHz, $CDCl_3$): δ = 8.32 (d, *J* = 9.1 Hz, 2H; ar-DR(*o*-NO_2)), 7.92 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-NO_2)), 7.90 (d, *J* = 9.1 Hz, 2H; ar-DR(*m*-Amino)), 7.51 (d, *J* = 7.7 Hz, 1H; NH(Tyr)), 7.30 - 7.10 (m, 21H; Trt-ar, Phe, NH(Asn)), 7.03 (d, *J* = 8.7 Hz, 2H; Tyr), 6.79 (d, *J* = 9.3 Hz, 2H; ar-DR(*o*-Amino)), 6.77 (d, *J* = 8.6 Hz, 2H; Tyr-Hε), 5.90 (d, *J* = 6.8 Hz, 1H; NH(Phe)), 4.66 (ddd, *J* = 3.8, 6.2, 7.6 Hz, 1H; Tyr-Hα), 4.62 (ddd, *J* = 2.1, 5.7, 7.9 Hz, 1H; Asn-Hα), 4.54 (ddd, *J* = 3.9, 5.5, 6.8 Hz, 1H; Phe-Hα), 4.12 (t, *J* = 5.9 Hz, 2H; $OC\underline{H}_2CH_2N$), 3.81 (t, *J* = 5.9 Hz, 2H; $OCH_2C\underline{H}_2N$), 3.61 (s, 3H; OCH_3), 3.59 (q, *J* = 7.1 Hz, 2H; $NC\underline{H}_2CH_3$), 3.08 (dd, *J* = 5.5, 10.1 Hz, 1H; Phe-Hβ), 3.05 (dd, *J* = 5.6, 10.1 Hz, 1H; Asn-Hβ), 2.91 (dd, *J* = 3.8, 15.3, 1H; Tyr-Hβ), 2.89 (dd, *J* = 2.8, 10.1 Hz, 1H; Phe-Hβ'), 2.87 (dd, *J* = 2.1, 10.1 Hz, 1H; Asn-Hβ'), 2.46 (dd, *J* = 6.3, 15.3 Hz, 1H; Tyr-Hβ'), 1.80 (s, 3H; Acetyl), 1.27 (t, *J* = 7.0 Hz, 3H; $NCH_2C\underline{H}_3$);

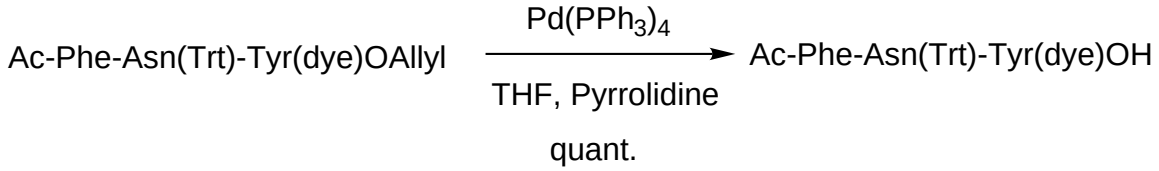
^{13}C-NMR: (125.8 MHz, $CDCl_3$): δ = 171.3, 171.2, 170.7, 170.2, 170.0, 157.4, 156.8, 151.3, 147.3, 144.2, 143.7, 136.1, 130.4, 129.0, 128.8, 127.9, 127.0, 128.6, 126.3, 124.7, 122.6, 114.4, 111.4, 70.7, 65.1, 54.8, 54.2, 52.2, 49.8, 49.7, 46.1, 37.51, 36.9, 22.9, 12.3;

ESI-MS: *m/z*: calcd for $C_{33}H_{39}N_5O_7Na$ $[M+Na]^+$ 1060; found 1060;

6. 5. 2. Ester cleavage of the arms and coupling to the diaminotemplates

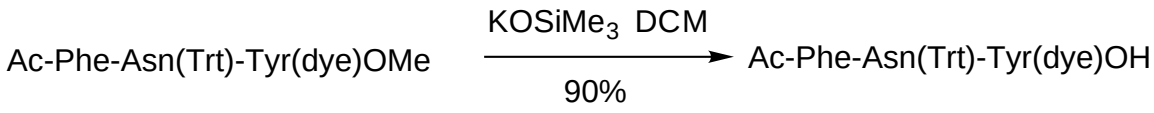
For the synthesis of the two armed molecules **25 - 32**, the methylester as well as the allyester of the tripeptide were tried as starting material. The allylester can be cleaved under milder conditions than the methylester and the amide coupling with the tripeptide-acid arising from the allylester gave slightly purer products. However for some of the two armed molecules purification by preparative HPLC was necessary, even after optimisation of the coupling reaction.

6. 5. 2. 1. General procedure for the ester cleavage of the tripeptide-allylester



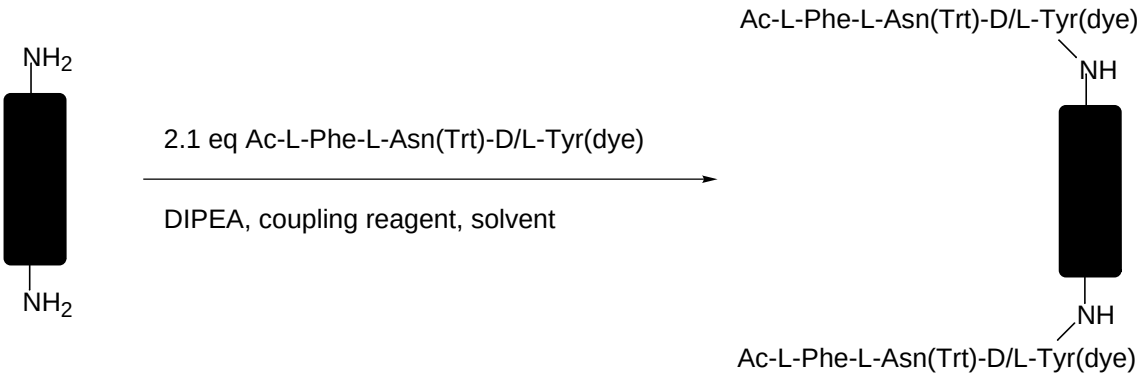
The Tripeptide-allylester was dissolved in 4 ml of dry THF (ca. 4 ml for 100 mg of the ester) together with 2.5 eq of pyrrolidine. The mixture was flushed for 20 minutes with N_2 and 0.01 eq of $Pd(PPh_3)_4$ was added. After stirring at r.t. for 30 minutes it was extracted with DCM and 0.5 M H_3PO_4. After drying over Na_2SO_4 the solvent was removed at reduced pressure to give the free acid in quantitative yield.

6. 5. 2. 2. General procedure for the ester cleavage of the tripeptide-methylester



1 eq of the tripeptide methylester was added to a solution of 1.8 eq $KOSiMe_3$ in DCM (ca. 5 ml per 100 mg tripeptide methylester). After three hours of stirring at room temperature, it was washed twice with 30 ml of 1M HCl and filtered over silica gel (gradient of DCM/MeOH from 95:5 to 85:15).

6. 5. 2. 3. Coupling of the tripeptide arm acid to the diamines



-Determination of the optimal coupling conditions

Several conditions were tested for the coupling of the free acid of the tripeptidic arm to the different diaminotemplates. To determine the best solvent and the best coupling reagent preliminary test reactions were performed using ca. 1 mg of the tripeptide acid. The coupling conditions leading to the least side products as detected by TLC were then used for the synthesis of the corresponding two armed molecule. We tested the coupling reagents DEPBT, EDC, TBTU, HATU as well as the solvents acetonitrile, DCM, THF, toluene and acetone. In table 6.1., the best conditions found for each of the two armed molecules are listed.

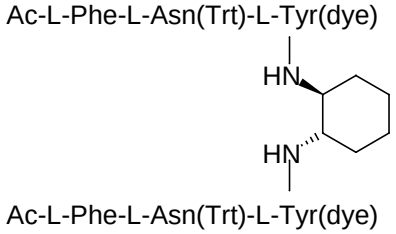
*Table 6. 1.: Optimal coupling conditions for the two armed molecules **25 - 27** and **29 - 31***

Two armed molecule	**coupling reagent**	**solvent**
23	**TBTU**	**DCM**
24	**TBTU**	**DCM**
25	**HATU**	**DCM**
27	**HATU**	**Acetonitrile**
28	**HATU**	**THF**
29	**HATU**	**THF**

-General procedure of the coupling

1 eq of the tripeptide acid was dissolved in ca. 200 µl of the solvent (see table 6.1.) per 20 mg tripeptide. 0.45 eq of the diamine was added, followed by 1.1 eq of DIPEA and 1.1 eq of the coupling reagent (see table 6.1.). After completion of the reaction (typically ca. 20 - 30 minutes) it was extracted with DCM, 1M HCl and brine. After drying over Na_2SO_4 the crude product was purified by flash chromatography over silica gel (gradient of DCM/MeOH from 100:0 to 95:5) and gel filtration (LH 20, DCM/MeOH 90:10). When required the products were further purified by preparative HPLC. Two armed molecules ***25 - 27 and 29 - 31*** were obtained in quantities of 15 - 30 mg with yields in the range of 70 - 90 %.

6. 5. 3. Two-armed molecule 25:



$C_{124}H_{126}N_{18}O_{16}$: 2124.44

TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.20

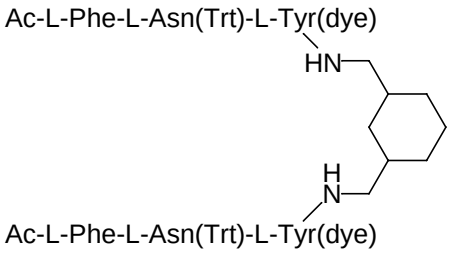
^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 8.31 (d, *J* = 9.1 Hz, 4H; dye), 7.90 (d, *J* = 9.1 Hz, 4H; dye), 7.87 (d, *J* = 9.1 Hz, 4H; dye), 7.23 - 7.11 (m, 42H; trityl, Phe, 6 NH's), 7.10 – 6.98 (m, 8H; Phe, Tyr), 6.73 (d, *J* = 8.7 Hz, 4H; Tyr), 6.72 – 6.67 (m, 4H; dye), 6.60 – 6.40 (m, 2H; NH Tyr), 4.76 – 4.60 (m, 4H; Asn-Hα, Phe-Hα), 4.45 – 4.30 (m, 2H; Tyr-Hα), 4.05 – 3.90 (m, 4H; OCH_2CH_2N), 3.75 – 3.65 (m, 4H; OCH_2CH_2N), 3.60 – 3.50 (m, 4H; CH_2CH_3), 3.50 – 3.40 (m, 2H, cHx), 3.05 – 2.92 (m,

4H; Tyr-Hβ), 2.90 – 2.73 (m, 6H; Phe-Hβ, Asn-Hβ), 2.70 (dd, *J* = 14.3 Hz, 6.2 Hz, 2H; Asn-Hβ´), 1.70 – 1.50 (m, 8H; cHx, COC*H*$_3$), 1.73 (s, 6H; COC*H*$_3$), 1.50 – 1.32 (m, 2H; cHx), 1.22 (t, *J* = 6.9 Hz, 6H; CH_2CH_3), 1.00 – 0.68 (m, 4H; cHx);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 171.2, 170.9, 170.6, 170.4, 170.1, 157.1, 156.8, 151.2, 147.3, 144.4, 143.7, 136.3, 130.7, 130.4, 130.2, 129.6, 129.5, 129.4, 129.2, 128.8, 128.7, 128.5, 128.3, 127.9, 126.9, 126.2, 124.7, 122.6, 114.3, 111.4, 70.8, 65.1, 54.8, 54.6, 54.3, 53.3, 49.7, 46.0, 38.9, 37.9, 36.5, 31.2, 24.3, 22.6, 12.2;

ESI-MS: *m/z*: calcd for $C_{124}H_{126}N_{18}O_{16}Na$ $[M+Na]^+$ 2147; found 2147.

6. 5. 4 Two-armed molecule 26:



$C_{126}H_{130}N_{18}O_{16}$: 2152.49

TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.23

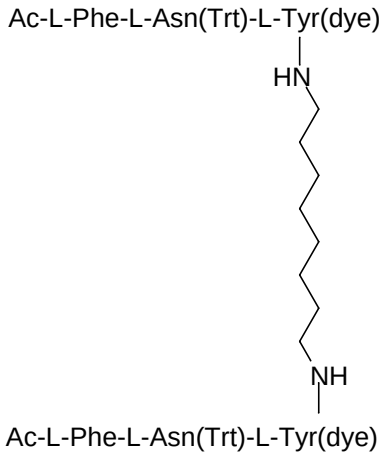
^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 8.61 (s, 2H, NH), 8.32 (d, *J* = 9.1 Hz, 4H; dye), 7.89 (d, *J* = 9.1 Hz, 4H; dye), 7.81 (d, *J* = 9.2 Hz, 4H; dye), 7.23 - 7.04 (m, 44H; 30 trityl, 10 Phe, 4 Tyr), 7.04 (d, *J* = 8.4 Hz, 4H; Tyr), 6.88 (d, *J* = 9.3 Hz, 4H; dye), 6.72 (d, *J* = 8.4 Hz, 4H; Tyr), 4.53 - 4.43 (m, 4H, Tyr-Hα, Asn-Hα), 4.36 – 4.30 (m, 2H, Phe-H), 4.06 - 3.97 (m, 4H; OC*H*$_2$CH$_2$N), 3.79 – 3.75 (m, 4H; OCH$_2$C*H*$_2$N), 3.56 – 3.50 (m, 4H; C*H*$_2$CH$_3$), 2.96 – 2.87 (m, 4H; Phe-Hβ, Tyr-Hβ), 2.87 – 2.78 (m, 4H;

NHCH_2(cyclohexyl)), 2.74 – 2.65 (m, 4H; Phe-Hβ, Tyr-Hβ), 2.63 – 2.52 (m, 2H; Asn-Hβ), 2.47 – 2.40 (m, 2H; Asn-Hβ), 1.72 (s, 6H; COCH_3), 1.56 - 1.48 (m, 2H; cyclohexyl), 1.48 - 1.42 (m, 2H; cyclohexyl), 1.27 - 1.16 (m, 2H; cyclohexyl), 1.13 (t, J = 8.4 Hz, 6H; CH_2CH_3), 1.00 - 0.91 (m, 1H; cyclohexyl), 0.58 - 0.51 (m, 2H; cyclohexyl), 0.34 - 0.29 (m, 1H; cyclohexyl);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 172.2, 170.9, 170.8, 170.0, 169.5, 157.3, 156.7, 152.0, 147.2, 145.1, 143.1, 138.2, 130.8, 130.3, 129.6, 128.9, 128.3, 127.8, 126.8, 126.5, 125.4, 122.9, 114.3, 112.1, 69.8, 65.7, 54.9, 54.4, 50.4, 49.6, 45.8, 43.1, 39.1, 37.9, 37.5, 30.4, 25.3, 22.9, 20.7, 12.4;

ESI-MS: *m/z*: calcd for $C_{126}H_{130}N_{18}O_{16}Na$ $[M+Na]^+$ 2175; found 2175.

6. 5. 5. Two-armed molecule 27:



$C_{125}H_{130}N_{18}O_{16}$: 2140.48

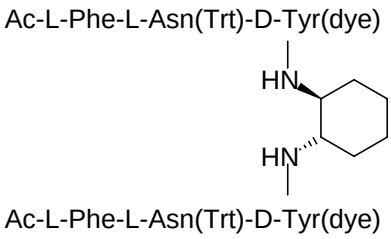
TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.33

^{1}H NMR (500 MHz, DMSO, 25°C): δ = 8.83 – 8.79 (m, 2H; Tyr-NH), 8.40 (d, *J* = 4.7 Hz, 2H; Asn-NH), 8.37 (d, *J* = 9.1 Hz, 4H; dye), 8.04 (m, 2H; Phe-NH), 7.94 (d, *J* = 9.1 Hz, 4H; dye), 7.83 (d, *J* = 9.2 Hz, 4H; dye), 7.72 (t, *J* = 5.9 Hz, 2H; Tyr-NH), 7.62 (t, *J* = 5.1 Hz, 2H; NH), 7.27 - 7.12 (m, 40H; trityl, Phe), 7.08 (d, *J* = 8.6 Hz, 4H; Tyr), 6.86 (d, *J* = 9.4 Hz, 4H; dye), 6.74 (d, *J* = 8.7 Hz, 4H; Tyr), 4.56 – 4.48 (m, 4H; Phe-Hα, Asn-Hα), 4.32 (td, *J* = 7.1 Hz, 4.5 Hz, 2H; Tyr-Hα), 3.95 (t, *J* = 6.6 Hz, 4H; OC*H*$_2$CH$_2$N), 3.69 (t, *J* = 5.1 Hz, 4H; OCH$_2$C*H*$_2$N), 3.50 (q, *J* = 6.6 Hz, 4H; C*H*$_2$CH$_3$), 3.06 (dd, *J* = 5.8 Hz, 5.4 Hz, 4H; heptyl), 3.01 (dd, *J* = 12.4 Hz, 2.1 Hz, 2H; Phe-Hβ), 2.88 – 2.74 (m, 4H; Asn-Hβ, Tyr-Hβ), 2.71 – 2.55 (m, 6H; Asn-Hβ´, Phe-Hβ´, Tyr-Hβ´), 1.71 (s, 6H; COC*H*$_3$), 1.49 (quint, *J* = 6.9 Hz, 4H; heptyl), 1.24 – 1.15 (m, 4H; heptyl), 1.13 (t, *J* = 7.0 Hz, 6H; CH$_2$C*H*$_3$), 1.10 – 1.02 (m, 2H; heptyl);

^{13}C NMR (125.6 MHz, 5% DMSO in CDCl$_3$, 25°C): δ = 171.5, 170.5, 170.1, 169.7, 169.1, 156.7, 156.2, 151.6, 146.9, 144.7, 142.8, 138.1, 130.0, 129.2, 128.6, 128.0, 127.5, 126.4, 126.2, 126.1, 125.0, 122.5, 114.0, 111.7, 69.4, 65.2, 54.4, 53.8, 49.7, 49.1, 45.3, 44.4, 38.4, 37.9, 36.2, 29.2, 28.7, 26.1, 26.0, 22.4, 12.0;

ESI-MS: *m/z*: calcd for $C_{125}H_{130}N_{18}O_{16}Na$ $[M+Na]^+$ 2162; found 2162.

6. 5. 6. Two-armed molecule 29:



$C_{124}H_{126}N_{18}O_{16}$: 2124.44

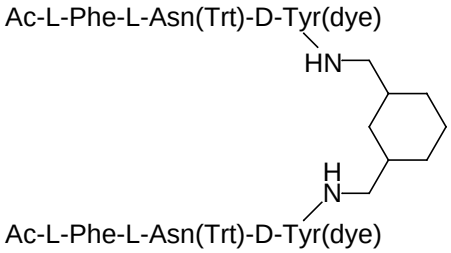
TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.20

^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 8.32 (d, *J* = 9.1 Hz, 4H; dye), 7.91 (d, *J* = 9.1 Hz, 4H; dye), 7.89 (d, *J* = 9.3 Hz, 4H; dye), 7.24 - 7.10 (m, 44H; trityl, Phe, Tyr), 6.79 (d, *J* = 9.3 Hz, 4H; dye), 6.74 (d, *J* = 8.5 Hz, 4H; Tyr), 4.57 (t, *J* = 6.0 Hz, 2H; Asn-Hα), 4.50 (dd, *J* = 9.6 Hz, 4.2 Hz, 2H; Phe-Hα), 4.45 (dd, *J* = 8.7 Hz, 5.4 Hz, 2H; Tyr-Hα), 4.07 (t, *J* = 5.8 Hz, 4H; OC*H*$_2$CH$_2$N), 3.79 (t, *J* = 5.8 Hz, 4H; OCH$_2$C*H*$_2$N), 3.58 (q, *J* = 7.1 Hz, 4H; C*H*$_2$CH$_3$), 3.41 (m, 2H, cHx), 3.10 (m, 4H; Phe-Hβ, Tyr-Hβ), 2.95 (dd, *J* = 13.1 Hz, 8.7 Hz, 2H; Tyr-Hβ´), 2.75 (dd, *J* = 14.0 Hz, 10.0 Hz, 2H; Phe-Hβ´), 2.73 (dd, *J* = 14.4 Hz, 5.7 Hz, 2H; Asn-Hβ), 2.64 (dd, *J* = 14.5 Hz, 6.0 Hz, 2H; Asn-Hβ´), 1.75 (m, 2H; cHx), 1.73 (s, 6H; COC*H*$_3$), 1.56 (m, 4H; cHx), 1.26 (t, *J* = 7.1 Hz, 6H; CH$_2$C*H*$_3$), 1.06 (m, 2H; cHx);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 171.9, 171.5, 170.8, 170.5, 170.0, 157.3, 156.8, 151.4, 147.3, 144.3, 143.7, 136.7, 130.4, 129.7, 129.0, 128.7, 128.6, 127.8, 126.9, 126.8, 126.3, 124.7, 122.6, 114.5, 111.4, 70.4, 65.2, 55.5, 54.9, 52.9, 50.3, 49.8, 46.1, 45.1, 37.8, 37.2, 36.4, 31.7, 29.7, 24.4, 22.7, 12.2;

HRMS (ESI): *m/z*: calcd for $C_{124}H_{126}N_{18}O_{16}$ $[M+2H]^{2+}$ 1062.4872; found 1062.4882.

6. 5. 7. Two-armed molecule 30:



$C_{126}H_{130}N_{18}O_{16}$: 2152.49

TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.19

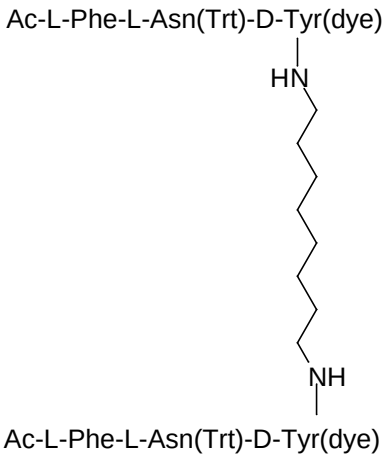
Not all the NMR - signals of the isomeric mixture could be assigned unambigously

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 8.35 (2d, *J* = 9.2 Hz, 4H; dye), 7.91 (2d, *J* = 9.1 Hz, 4H; dye), 7.88 (2d, *J* = 9.3 Hz, 4H; dye), 7.27 - 7.13 (m, 44H; 30 trityl, 10 Phe, 4 Tyr), 6.76 (2d, *J* = 9.9 Hz, 4H; dye), 6.72 (d, *J* = 8.6 Hz, 4H; Tyr), 4.57 - 4.40 (m, 6H, Phe-Hα, Tyr-Hα, Asn-Hα), 4.06 - 3.97 (m, 4H; OC*H*$_2$CH$_2$N), 3.77/3.74 (2t, *J* = 5.6 Hz, 4H; OCH$_2$C*H*$_2$N), 3.56/3.55 (2q, *J* = 7.4 Hz, 4H; C*H*$_2$CH$_3$), 3.28 - 3.14 (m, 2H, Tyr-H), 3.12 - 2.97 (m, 4H, NHC*H*$_2$(cyclohexyl), Phe-Hβ), 2.97 - 2.81 (m, 4H, Phe-Hβ, Tyr-Hβ), 2.69 (s br, 2H, Asn-Hβ), 2.60 - 2.50 (m, 4H, Asn-Hβ, NHC*H*$_2$(cyclohexyl)), 1.75/1.74 (2s, 6H, COC*H*$_3$), 1.68 - 1.55 (m, 2H, cyclohexyl), 1.49 - 1.40 (m, 2H, cyclohexyl), 1.33 - 1.15 (m, 2H, cyclohexyl), 1.28 - 1.23 (m, 6H; CH$_2$C*H*$_3$), 1.05 - 0.95 (m, 1H, cyclohexyl), 0.71 - 0.55 (m, 2H, cyclohexyl), 0.20 - 0.27 (m, 1H, cyclohexyl);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 174.0, 172.0, 171.9, 171.8, 171.0, 170.9, 170.8, 170.3, 170.2, 157.2, 157.1, 156.8, 151.3, 147.3, 144.7, 144.6, 143.7, 136.2, 130.3, 130.0, 129.0, 128.7, 127.7, 127.1, 126.3, 124.7, 122.7, 114.3, 111.4, 70.6, 65.1, 55.5, 50.4, 50.3, 50.2, 49.8, 46.1, 45.7, 37.9, 36.9, 36.4, 30.5, 30.2, 29.3, 25.2, 23.3, 22.6, 22.5, 21.0, 12.2.

ESI-MS: *m/z*: calcd for $C_{126}H_{130}N_{18}O_{16}Na$ $[M+Na]^+$ 2175; found 2175.

6. 5. 8. Two-armed molecule 31:



$C_{125}H_{130}N_{18}O_{16}$: 2140.48

TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.17

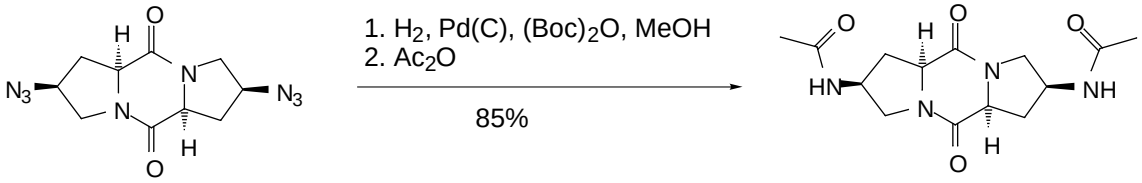
^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 8.64 (s, 2H; Tr-NH), 8.41 (d, *J* = 7.9 Hz, 2H; Asn-NH), 8.35 (d, *J* = 9.1 Hz, 4H; dye), 8.13 (d, *J* = 7.9 Hz, 2H; Phe-NH), 7.93 (d, *J* = 9.1 Hz, 4H; dye), 7.84 (d, *J* = 9.3 Hz, 4H; dye), 7.79 (d, *J* = 8.3 Hz, 2H; Tyr-NH), 7.72 (t, *J* = 5.5 Hz, 2H; NH), 7.25 - 7.13 (m, 40H; trityl, Phe), 7.07 (d, *J* = 8.7 Hz, 4H; Tyr), 6.91 (d, *J* = 9.3 Hz, 4H; dye), 6.75 (d, *J* = 8.7 Hz, 4H; Tyr), 4.52 (m, 2H; Phe-Hα), 4.47 (m, 2H; Asn-Hα), 4.32 (m, 2H; Tyr-Hα), 4.07 (t, *J* = 5.2 Hz, 4H; OCH_2CH_2N), 3.81 (t, *J* = 5.2 Hz, 4H; OCH_2CH_2N), 3.56 (q, *J* = 7.0 Hz, 4H; CH_2CH_3), 2.93 (m, 8H; Phe-Hβ, Tyr-Hβ, heptyl), 2.72 (dd, *J* = 9.7 Hz, 3.9 Hz, 2H; Phe-Hβ´), 2.68 (dd, *J* = 12.9 Hz, 3.7 Hz, 2H; Tyr-Hβ´) 2.60 (m, 2H; Asn-Hβ), 2.49 (m, 2H; Asn-Hβ´), 1.74 (s, 6H; $COCH_3$), 1.24 (m, 6H; heptyl), 1.16 (t, *J* = 7.0 Hz, 6H; CH_2CH_3), 1.11 (m, 4H; heptyl);

^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ = 172.8, 171.3, 171.1, 170.3, 169.8, 157.0, 156.7, 151.6, 147.8, 145.3, 143.2, 138.7, 130.8, 130.1, 129.4, 128.5, 128.4, 127.9, 127.4, 126.2, 124.9, 122.4, 113.8, 111.6, 70.5, 65.1, 54.4, 54.0, 51.1, 50.1, 49.1, 45.1, 38.6, 38.5, 37.3, 28.8, 28.6, 26.2, 11.9;

HRMS (ESI): *m/z*: calcd for $C_{125}H_{130}N_{18}O_{16}$ $[M+2H]^{2+}$ 1070.5029; found 1070.5034.

6. 6. Synthesis of the diketopiperazinemodels 33 - 35

6. 6. 1. Synthesis of bis-acetyl diketopiperazine 33



In 1 ml MeOH, 61 mg (221 μmol, 1 eq) of bis-azido diketopiperazine **8** was dissolved and 5 mg of palladium on carbon was added. The black suspension was evacuated, flushed with hydrogen and allowed to stir for 1.5 h at room temperature. Then, 207 ml (2.2 mmol, 10 eq) of acetic anhydride was added and stirred for another 30 minutes. After filtration over celite and removal of the solvent at reduced pressure, the product was purified by precipitation with Et_2O (4 times). This afforded 58 mg (85%) bisacetylated diketopiperazine **33** as a white solid. Crystals were obtained by diffusion of Et_2O into a solution of **33** in EtOAc and a trace of CH_2Cl_2.

$C_{14}H_{20}N_4O_4$: 308.33

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.35 (Ninhydrin)

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 6.08 (d, *J* = 6.1 Hz, 2H; NH), 4.33 (ψτ, *J* = 6.8 Hz, 2H; Hα), 4.41 (ψtq, *J* = 5.9 Hz, 3.8 Hz, 2H; Hγ), 3.78 (dd, *J* = 12.3 Hz, 3.7 Hz, 2H; Hδ´), 3.57 (dd, *J* = 12.3 Hz, 5.9 Hz, 2H; Hδ), 2.52 – 2.49 (m, 4H; Hβ´, Hβ), 1.91 (s, 6H; CH_3);

^{13}C NMR (100.5 MHz, $CDCl_3$, 25°C): δ = 170.2, 167.1, 59.2, 51.4, 48.3, 32.2, 23.1;

FT-IR (KBr, ν/cm^{-1}): 3446, 3292, 3071, 1661, 1549, 1446.

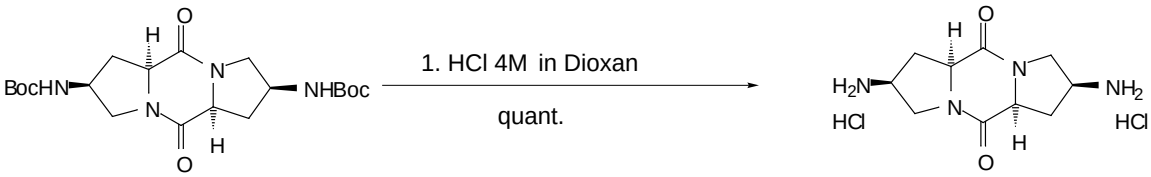
FAB-MS (NBA): *m/z*: (%): calcd for $C_{20}H_{33}N_4O_5$ $[M+H]^+$ 309; found 309.

Elemental analysis: Calcd (%) for $C_{20}H_{32}N_4O_6 + H_2O$ (442.5): C 54.28, H 7.74, N 12.66; found C 54.42, H 7.70, N 12.57.

^{1}H NMR (500 MHz, CD_3OD, 25°C): $\delta = 4.45$ (ψt, J = 7.6 Hz, 2H; Hα), 4.48 (m, J = 9.6 Hz, 7.7 Hz, 2H; Hγ), 3.81 (dd, J = 11.8 Hz, 7.9 Hz, 2H; Hδ), 3.27 (dd, J = 11.8 Hz, 7.7 Hz, 2H; Hδ´), 2.53 (dt, J = 12.7 Hz, 6.9 Hz, 2H; Hβ), 2.10 (dt, J = 12.7 Hz, 9.7 Hz, 2H; Hβ´), 1.94 (s, 6H; CH_3);

^{1}H NMR (500 MHz, d_6-DMSO, 25°C): $\delta = 8.08$ (d, J = 6.8 Hz, 2H; NH), 4.37 (dd, J = 9.3 Hz, 7.5 Hz, 2H; Hα), 4.30 (dtt, J = 9.6 Hz, 7.7 Hz, 6.8 Hz, 2H; Hγ), 3.60 (dd, J = 11.4 Hz, 7.8 Hz, 2H; Hδ), 3.12 (dd, J = 11.4 Hz, 7.6 Hz, 2H; Hδ´), 2.34 (dt, J = 12.4 Hz, 6.9 Hz, 2H; Hβ), 2.05 (dt, J = 12.4 Hz, 9.8 Hz, 2H; Hβ´), 1.80 (s, 6H; CH_3);

6. 6. 2. Synthesis of bis-ammoniumsalt 34



The Boc-protected diamine **6** (24 mg, 56mmol, 1 eq) was dissolved in 4 M HCl in dioxane (1 ml) and allowed to stir at room temperature for 15 min. After removal of all volatiles at reduced pressure the solid residue was triturated with Et_2O (2 ml) to yield a white solid which was isolated by decantation followed by removal of all residual volatiles in vacuo.

$C_{10}H_{18}Cl_2N_4O_2$: 297.18

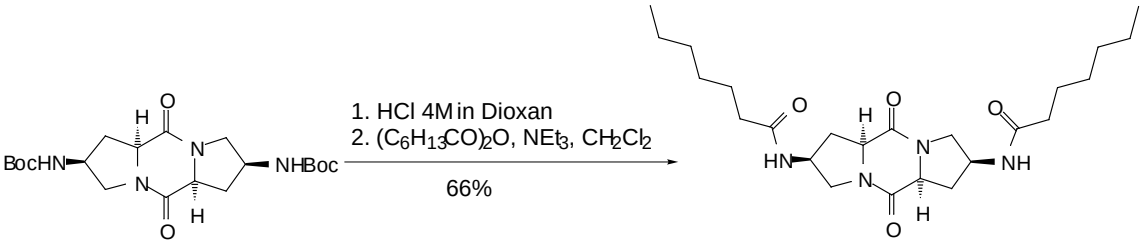
^{1}H NMR (500 MHz, CD_3OD, 25°C): δ = 4.57 (ψt, *J* = 8.2 Hz, 2H; Hα), 4.02 (dq, *J* = 7.2 Hz, ≈6 Hz, 2H; Hγ), 3.81 (dd, *J* = 12.5 Hz, 7.2 Hz, 2H; Hδ), 3.74 (dd, *J* = 12.5 Hz, 5.7 Hz, 2H; Hδ´), 2.73 (dt, *J* = 13.6 Hz, 7.4 Hz, 2H; Hβ), 2.42 (dt, *J* = 13.6 Hz, 7.9 Hz, 2H; Hβ´).

^{1}H NMR (500 MHz, d_6-DMSO, 25°C): δ = 8.51 (s (broad), 4H; NH), 4.45 (ψt, *J* = 8.2 Hz, 2H; Hα), 3.85 (m (broad), 2H; Hγ), 3.61 (dd, *J* = 12.2 Hz, 5.1 Hz, 2H; Hδ´), 3.58 (dd, *J* = 12.2 Hz, 7.2 Hz, 2H; Hδ), 2.53 (dt, *J* = 12.8 Hz, 7.4 Hz, 2H; Hβ), 2.26 (dt, *J* = 12.8 Hz, 7.7 Hz, 2H; Hβ´);

^{13}C NMR (100.5 MHz, d_6-DMSO, 25°C): δ = 165.4, 66.3, 57.9, 48.7, 48.1, 31.3.

ESI-MS: *m/z*: calcd for $C_{10}H_{16}N_4O_2$ $[M+H]^+$ 225; found 225;

6. 6. 3. Synthesis of bis-octyl diketopiperazine 35



58 mg (136 μmol, 1 eq) of *bis*-Boc protected diketopiperazine **9** was dissolved in 2 ml of 4M HCl in dioxane and 0.4 ml of methanol. After stirring 30 minutes at r.t. the solvent was evaporated and the residue was dissolved in 1.5 ml of CH_2Cl_2. After the addition of 132 mg (547 mmol, 4 eq) of oenanth acid anhydride and 0.11 ml (821 mmol, 6 eq) of

NEt_3 the solution was stirred at r.t. for 30 minutes. Then it was extracted twice by 30 ml of AcOEt and 15 ml of 1M NaOH. The organic layers were washed over 15 ml of sat. NaCl, dried over Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by flash chromatography over silica gel (gradient of CH_2Cl_2 / MeOH from 0 to 10% of methanol) to yield 40 mg (66%) of Cyclo-(trans-γ-$NHCOC_6H_{13}$-L-Pro) **35**.

$C_{24}H_{38}N_4O_4$: 448.60

TLC: CH_2Cl_2/MeOH (20:1); Rf: 0.22 (Ninhydrin)

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 6.48 (d, *J* = 6.9 Hz, 2H; NH), 4.54 (m, *J* = 6.8 Hz, 2H; Hγ), 4.33 (t, *J* = 7.7 Hz, Hα), 3.71 (dd, *J* = 12.0 Hz, 6.9 Hz, 2H; Hδ), 3.50 (dd, *J* = 12.0 Hz, 6.0 Hz, 2H; Hδ´), 2.51 (ddd, *J* = 13.5 Hz, 7.7 Hz, 6.9 Hz, 2H; Hβ), 2.29 (dt, *J* = 13.4 Hz, 7.4 Hz, 2H; Hβ´), 2.14 (ψt, *J* = 7.4 Hz, 4H; CH_2), 1.57 (m, 4H; CH_2), 1.29 (m**,** 12H; CH_2), 0.88 (t, *J* = 7.0 Hz, 6H; CH_3);
^{1}H NMR (500 MHz, CD_3OD, 25°C): δ = 4.48 (m, *J* = 7.7 Hz, 2H; Hγ), 4.46 (ψt, *J* = 7.4 Hz, Hα), 3.81 (dd, *J* = 11.7 Hz, 7.9 Hz, 2H; Hδ), 3.27 (dd, *J* = 11.7 Hz, 7.7 Hz, 2H; Hδ´), 2.53 (dt, *J* = 12.7 Hz, 6.8 Hz, 2H; Hβ), 2.17 (ψt, *J* = 7.5 Hz, 4H; CH_2), 2.10 (dt, *J* = 12.7 Hz, 9.7 Hz, 2H; Hβ´), 1.59 (m, 4H; CH_2), 1.31 (m**,** 12H; CH_2), 0.90 (t, *J* = 7.1 Hz, 6H; CH_3);
^{1}H NMR (500 MHz, d_6-DMSO, 25°C): δ = 8.00 (d, *J* = 6.8 Hz, 2H; NH), 4.37 (ψt, *J* = 8.4 Hz, Hα), 4.29 (m, *J* = 9.1 Hz, 7.7 Hz, 2H; Hγ), 3.60 (dd, *J* = 11.4 Hz, 7.8 Hz, 2H; Hδ), 3.11 (dd, *J* = 11.4 Hz, 7.6 Hz, 2H; Hδ´), 2.33 (dt, *J* = 12.4 Hz, 6.8 Hz, 2H; Hβ), 2.04 (ψt, *J* = 7.4 Hz, 4H; CH_2), 1.93 (δt, *J* = 12.4 Hz, 9.7 Hz, 2H; Hβ´), 1.47 (m, 4H; CH_2), 1.23 (m**,** 12H; CH_2), 0.86 (t, *J* = 7.0 Hz, 6H; CH_3);

^{13}C NMR (100.5 MHz, $CDCl_3$, 25°C): δ = 173.5, 166.6, 59.1, 50.7, 47.5, 36.4, 32.7, 31.5, 28.9, 25.5, 22.4, 14.0;

ESI-MS: *m/z*: calcd for $C_{24}H_{39}N_4O_4$ $[M+H]^+$ 449; found 449.

6. 7. Synthesis of two armed model compounds 36 - 41

Two armed molecules **36 - 41** were obtained following the general procedure described in chapter 6. 4. 1. starting with bis-Boc protected diketopiperazine **9**.

6. 7. 1. Two armed molecule 36

$C_{34}H_{42}N_6O_8$: 662.73

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.15 (UV)

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 7.06 (d, *J* = 8.5 Hz, 4H; Tyr-Hδ), 6.98 (d, *J* = 6.2 Hz, 2H; NH-DKP), 6.80 (d, *J* = 8.6 Hz, 4H; Tyr-Hδ), 6.25 (d, *J* = 7.7 Hz, 2H; NH-Tyr), 4.48 (q, *J* = 7.4 Hz, 2H; Tyr-Hα), 6.58 (q, *J* = 3.0 Hz, 2H; Pro-Hγ), 4.26 (dd, *J* = 5.0, 8.7 Hz, 2H; Pro-Hα), 3.74 (dd, *J* = 1.5, 12.0 Hz, 2H; Pro-Hδ), 3.76 (s, 3H; OCH_3), 3.43 (dd, *J* = 5.6, 12.3 Hz, 2H; Pro-Hδ), 3.02 (dd, *J* = 6.8, 14.1 Hz, 2H; Tyr-Hβ), 2.94 (dd, *J* = 7.3, 14.0 Hz, 2H; Tyr-Hβ'), 2.45 - 2.39 (m, 4H; Pro-Hβ), 1.93 (s, 6H; $COCH_3$);

^{13}C-NMR: (75 MHz, $CDCl_3$ +1%d4-MeOH): δ = 171.2, 170.5, 166.3, 151.4, 130.1, 128.8, 113.8, 58.0, 55.2, 53.9, 50.0, 48.8, 36.2, 31.7, 23.3.

ESI-MS: *m/z*: calcd for $C_{34}H_{42}N_6O_3Na$ $[M+Na]^+$ 685; found 685;

6. 7. 2. Two armed molecule 37

$C_{34}H_{42}N_6O_8$: 662.73

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.15 (UV)

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 7.77 (d, *J* = 4.3 Hz, 2H; NH-DKP), 7.04 (d, *J* = 8.6 Hz, 4H; Tyr-Hδ), 6.73 (d, *J* = 8.7 Hz, 4H; Tyr-Hε), 6.04 (d, *J* = 7.5 Hz, 2H; NH-Tyr), 4.72 (q, *J* = 7.5 Hz, 2H; Tyr-Hα), 4.14 (dd, *J* = 1.5, 10.1 Hz, 2H; Pro-Hα), 4.09 (ddd, *J* = 4.5, 9.0, 9.0 Hz, 2H; Pro-Hγ), 3.79 (dd, *J* = 1.0, 12.2 Hz, 2H; Pro-Hδ'), 3.74 (s, 3H; OCH_3), 3.21 (dd, *J* = 4.5, 12.2 Hz, 2H; Pro-Hδ), 3.14 (dd, *J* = 1.0, 14.3 Hz, 2H; Pro-Hβ'),3.03 (dd, *J* = 67.6, 14.0 Hz, 2H; Tyr-Hβ), 2.86 (dd, *J* = 7.5, 14.0 Hz, 2H; Tyr-Hβ'), 2.23 (ddd, *J* = 5.1, 10.3, 15.3 Hz, 2H; Pro-Hβ), 1.95 (s, 6H; COCH_3);

^{13}C-NMR: (75 MHz, $CDCl_3$ +5%d4-MeOH): δ = 171.3, 170.9, 170.6, 158.4, 149.6, 130.2, 128.8, 114.1, 58.9, 55.3, 51.4, 47.9, 36.8, 23.1;

ESI-MS: *m/z*: calcd for $C_{34}H_{42}N_6O_3Na$ $[M+Na]^+$ 685; found 685;

6. 7. 3. Two armed molecule 38

$C_{48}H_{48}N_8O_{12}$: 949.10

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.10 (UV)

^{1}H NMR (500 MHz, $CDCl_3$ + 5%d4-MeOH, 25°C): δ = 7.53 - 7.50 (m, 2H; NH), 7.41 (d, *J* = 6.8 Hz, 2H; NH), 7.01 (d, *J* = 7.2 Hz, 2H; NH), 6.93 (d, *J* = 8.4 Hz, 4H; Tyr-Hδ), 6.75 (d, *J* = 8.6 Hz, 4H; Tyr-Hε), 4.38 - 4.29 (m, 6H, Tyr-Hα, Ser-Hα, Pro-Hγ), 4.26 (dd, *J* = 7.3, 7.3 Hz, 2H; Pro-Hα), 3.74 - 3.72 (m, 2H; Pro-Hδ), 3.73 (s, 6H; OCH_3), 3.62 (dd, *J* = 4.3, 8.9 Hz, 2H; Ser-Hβ), 3.39 - 3.33 (m, *J* = 2H; Pro-Hδ), 3.27 (dd, *J* = 8.8, 8.8 Hz, 2H; Ser-Hβ') 2.95 (dd, *J* = 6.6, 13.6 Hz, 2H; Tyr-Hβ), 2.83 (dd, *J* = 7.4, 13.9 Hz, 2H; Tyr-Hβ'), 2.48 - 2.42 (m, 4H; Pro-Hβ), 1.97 (s, 6H; COCH_3), 1.19 (s, 18H, OtBu);

^{13}C-NMR: (75 MHz, $CDCl_3$ +5%d4-MeOH): δ = 171.5, 171.2, 170.4, 165.6, 158.6, 129.9, 128.4, 114.0, 75.0, 61.3, 58.3, 55.5, 55.2, 52.5, 51.2, 47.6, 35.7, 32.1, 27.4, 22.8.

ESI-MS: *m/z*: calcd for $C_{48}H_{48}N_8O_{12}$**:** Na $[M+Na]^+$ 972; found 972;

6. 7. 4. Two armed molecule 39

$C_{48}H_{48}N_8O_{12}$: 949.10

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.10 (UV)

^{1}H NMR (500 MHz, $CDCl_3$+5%d4-MeOH, 25°C): δ =7.84 (d, *J* = 8.6 Hz, 2H; Ser-NH), 7.51 (d, *J* = 5.5 Hz, 2H; Tyr-NH), 7.27 - 7.25 (m, 2H; DKP-NH), 6.83 (d, *J* = 8.6 Hz, 4H; Tyr-Hδ), 6.68 (d, *J* = 8.7 Hz, 4H; Tyr-Hε), 4.45 - 4.36 (m, 4H, Tyr-Hα, Ser-Hα) 4.17 (dd, *J* = 3.1, 10.1 Hz, 2H; Pro-Hα), 4.15 (m, 2H, Pro-Hγ), 3.99 (dd, *J* = 0.3, 12.0 Hz, 2H; Pro-Hδ'), 3.69 (s, 6H; OCH_3), 3.64 (dd, *J* = 4.2, 8.9 Hz, 2H; Ser-Hβ), 3.41 (dd, *J* = 9.0, 9.0 Hz, 2H; Ser-Hβ'), 3.28 (dd, *J* = 4.7, 11.8 Hz, 2H; Pro-Hδ), 2.94 (dd, *J* = 7.4, 13.8 Hz, 2H; Tyr-Hβ), 2.79 - 2.71 (m, 4H; Tyr-Hβ', Pro-Hβ'), 2.32 (ddd, *J* = 5.3, 10.1, 14.2 Hz 2H; Pro-Hβ), 1.97 (s, 6H; $COCH_3$), 1.27 (s, 18H, OtBu);

^{13}C-NMR: (75 MHz, $CDCl_3$ +5%d4-MeOH): δ = 171.9, 171.8, 169.9, 166.1, 160.0, 129.9, 130.0, 128.4, 114.2, 74.9, 61.3, 57.7, 55.5, 55.1, 50.1, 49.2, 48.0, 35.8, 33.0, 27.4, 22.9;

ESI-MS: *m/z*: calcd for $C_{48}H_{48}N_8O_{12}$**:** Na $[M+Na]^+$ 972; found 972.

6. 7. 5. Two armed molecule 40

$C_{40}H_{68}N_8O_{10}$: 821.02

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.17 (Ninhydrin)

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 7.88 (d, *J* = 6.8 Hz, 2H; Pro-NH), 7.85 (d, *J* = 7.8 Hz, 2H; Leu-NH), 7.39 (d, *J* = 7.0 Hz, Ser-NH), 4.48 (ddd, *J* = 10.0 Hz, 7.1 Hz, 4.3 Hz, 2H; Ser-Hα), 4.35 (dd, *J* = 10.5 Hz, 4.3 Hz, 2H; Pro-Hα), 4.30 (m, 2H; Pro-Hγ), 4.20 (q, *J* = 7.7 Hz, 2H; Leu-Hα), 4.03 (d (broad), *J* = 12.1 Hz, 2H; Pro-Hδ´), 3.66 (dd, *J* = 8.7 Hz, 4.3 Hz, 2H; Ser-Hβ), 3.30 (dd, *J* = 9.8 Hz, 9.0 Hz, 2H; Ser-Hβ´), 3.22 (dd, *J* = 12.1 Hz, 3.6 Hz, 2H; Pro-Hδ), 2.71 (ddd, *J* = 14.3 Hz, 4.0 Hz, ≈ 1.5 Hz, 2H; Pro-Hβ´), 2.60 (ddd, *J* = 14.4 Hz, 10.8 Hz, 6.8 Hz, 2H; Pro-Hβ), 2.09 (s, 6H; COCH_3), 1.65 (dt, *J* = 13.3 Hz, 7.0 Hz, 2H; Leu-Hβ), 1.52 (m, *J* = 6.6 Hz, 2H; Leu-Hγ), 1.41 (dt, *J* = 13.3 Hz, 7.1 Hz, 2H; Leu-Hβ´), 1.26 (s, 18H; tBu), 0.82 (2t, *J* = 6.6 Hz, 12H; Leu-Hδ, Leu-Hδ´);

^{13}C NMR (125.6 MHz, $CDCl_3$, 25°C): δ = 172.0, 171.9, 170.3, 165.5, 75.1, 61.4, 58.1, 52.4, 52.0, 51.9, 47.6, 39.1, 32.0, 27.5, 24.6, 23.0, 22.3, 22.2;

ESI-MS: *m/z*: calcd for $C_{40}H_{63}N_3O_{10}Na$ $[M+Na]^+$ 843; found 843;

6. 7. 6. Two armed molecule 41

$C_{40}H_{68}N_8O_{10}$: 821.02

TLC: CH_2Cl_2:MeOH (10:1); Rf: 0.15 (Ninhydrin)

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 8.01 (d, *J* = 8.3 Hz, 2H; Leu-NH), 7.93 (d, *J* = 5.2 Hz, 2H; NH), 7.75 (d, *J* = 7.3 Hz, Ser-NH), 4.53 (ddd, *J* = 9.9 Hz, 7.5 Hz, 4.3 Hz, 2H; Ser-Hα), 4.38 (dd, *J* = 11.8 Hz, 1.6 Hz, 2H; Pro-Hδ´), 4.29 (yq, *J* = 7.8 Hz, 2H; Leu-Hα), 4.23 (q (broad), *J* = 4.8 Hz, 2H; Pro-Hγ), 4.22 (d (broad), *J* = 11.0 Hz, 2H; Pro-Hα), 3.65 (dd, *J* = 8.8 Hz, 4.3 Hz, 2H; Ser-Hβ), 3.42 (ψt, *J* = 9.3 Hz, 2H; Ser-Hβ´), 3.32 (dd, *J* = 11.8 Hz, 4.4 Hz, 2H; Pro-Hδ), 2.95 (dd, *J* = 14.4 Hz, ≈ 1.5 Hz, 2H; Pro-Hβ´), 2.38 (ddd, *J* = 14.4 Hz, 11.0 Hz, 5.1 Hz, 2H; Pro-Hβ), 2.07 (s, 6H; COCH_3), 1.66 (ddd, *J* = 13.8 Hz, 7.1 Hz, 6.6 Hz, 2H; Leu-Hβ), 1.52 (m, *J* = 6.6 Hz, 2H; Leu-Hγ), 1.40

(m, J = 13.5 Hz, 7.5 Hz, 2H; Leu-Hβ´), 1.27 (s, 18H; tBu), 0.82 (2t, J = 6.6 Hz, 12H; Leu-Hδ, Leu-Hδ´);

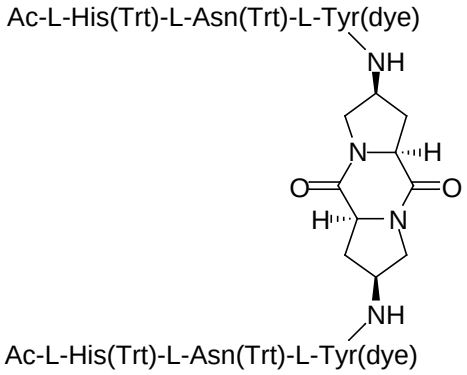
^{13}C NMR (125.6 MHz, $CDCl_3$, 25°C): δ = 171.3, 171.3, 169.9, 166.4, 75.1, 61.2, 57.7, 52.0, 51.9, 50.3, 48.2, 39.1, 33.4, 27.5, 24.7, 23.1, 22.4, 22.2;

ESI-MS: *m/z*: calcd for $C_{40}H_{63}N_3O_{10}Na$ $[M+Na]^+$ 843; found 843;

6. 8. Synthesis of two armed molecule 42

The side chain protected two armed molecule **42'** was obtained following the general procedure described in chapter 6. 4. 1. starting with bis-Boc protected *cis*-diketopiperazine **9**.

6. 8. 1. Two-armed molecule 42' :



$C_{160}H_{152}N_{24}O_{18}$: 2699.07

TLC: CH_2Cl_2:MeOH (20:1); Rf: 0.13

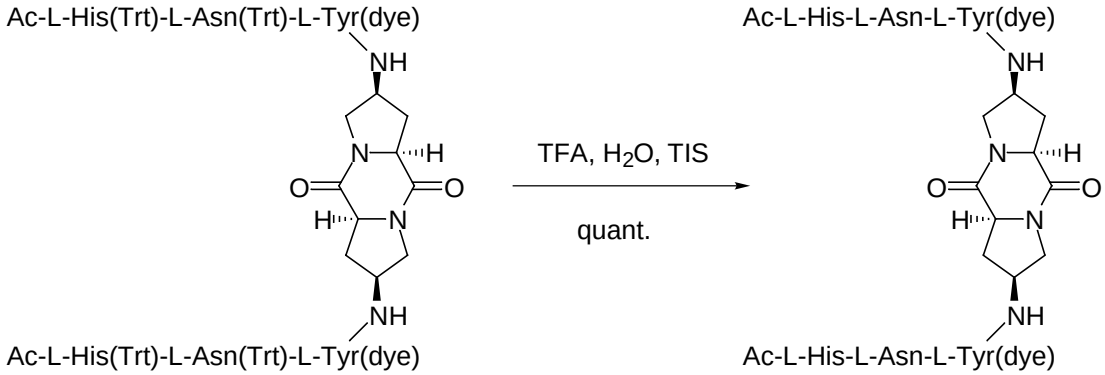
^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 8.31 (d, *J* = 9.0 Hz, 4H; dye), 7.90 (d, *J* = 9.0 Hz, 4H; dye), 7.89 (d, *J* = 9.2 Hz, 4H; dye), 7.54 - 6.93 (m, 70H; Trityl, NH, His-Hε), 6.89 (d, *J* = 8.6 Hz, 4H; Tyr), 6.78 (d, *J* = 9.3 Hz, 4H; dye), 6.58 (d, *J* = 8.7 Hz, 4H; Tyr), 6.57 (s, 2H; His-Hδ), 4.64 - 4.57 (m, 2H; Tyr-Hα), 4.53 (q, 2H, *J* = 5.5 Hz; Asn-Hα), 4.32 - 4.27 (m, 4H; Pro-Hγ, His-Hα), 4.09 (t, *J* = 7.8 Hz, 2H; Pro-Hα), 4.02 (t, *J* = 5.9 Hz, 4H; OCH_2CH_2N), 3.77 (t, *J* = 5.8 Hz, 4H; OCH_2CH_2N), 3.66 - 3.61 (m, 2H; Pro-Hδ'), 3.57 (q, *J* = 7.2 Hz, 4H; CH_2CH_3), 3.35 - 3.28 (m, 2H; Pro-Hδ), 3.11 (dd, *J* = 5.0, 14.5 Hz, 2H; Tyr-Hβ), 2.86 (dd, *J* = 5.8, 14.6 Hz, 2H; His-Hβ), 2.76 (dd, *J* = 9.14, 14.0 Hz, 2H; Tyr-Hβ), 2.71 (dd, *J* = 4.4, 14.8 Hz, 2H; His-Hβ), 2.66 - 2.58 (m, 2H; Asn-Hβ), 2.54

- 2.46 (m, 2H; Asn-Hβ), (td, J = 1.9, 5.0 Hz, 4H; Pro-Hβ), 1.89 (s, 6H; $COCH_3$), 1.26 (t, J = 6.9 Hz, 6H; CH_2CH_3);

^{13}C NMR (125.8 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): δ = 171.6, 171.4, 171.1, 170.4, 170.1, 166.1, 156.9, 156.8, 151.3, 147.4, 144.2, 143.7, 138.6, 135.5, 130.0, 129.7, 128.65, 128.2, 128.0, 126.3, 124.7, 122.6, 120.6, 114.2, 111.4, 70.8, 65.1, 58.9, 54.5, 53.5, 51.5, 50.2, 49.8, 47.2, 46.1, 37.3, 35.3, 32.5, 30.3, 23.1, 12.3;

ESI-MS: *m/z*: calcd for $C_{160}H_{152}N_{24}O_{18}$ $[M+Na]^+$ 2720.2; found 2720.6;

6. 8. 2. Two-armed molecule 42 :



42' **42**

15 mg (5.6 μmol, 1 eq) of the side chain protected two armed molecule **42'** were dissolved in 1 ml TFA containing 2.5% of water and 2.5% of TIS. After 20 minutes of stirring at r.t., the volatiles were removed at reduced pressure and the residue was triturated three times with diethylether and excess ether was evaporated in vacuo. This yielded 9.5 mg (100%) of the deprotected receptor **42**.

$C_{84}H_{96}N_{24}O_{18}$: 1729.81

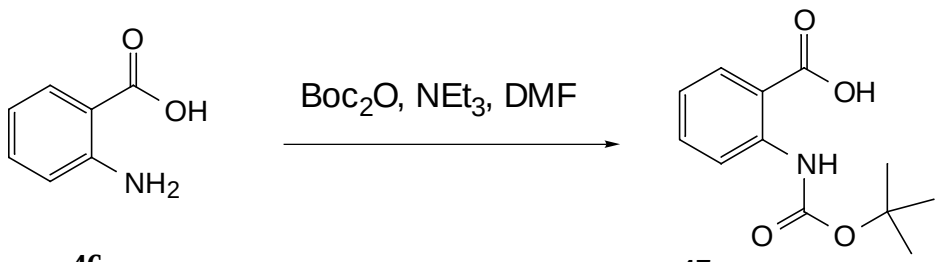
^{1}H NMR (500 MHz, DMSO, 25°C): δ = 8.36 (d, *J* = 9.1 Hz, 4H; dye), 8.24 (d, *J* = 7.0 Hz, 2H; NH-Asn), 8.14 (d, *J* = 8.2 Hz, 2H; NH-His), 8.10 (d, *J* = 8.6 Hz, 2H; NH-Tyr), 7.93 (d, *J* = 9.1 Hz, 4H; dye), 7.84 (d, *J* = 9.2 Hz, 4H; dye), 7.47 (s, 2H, NHδ-Asn), 7.14 (s, 2H; His-Hε), 7.10 (d, *J* = 8.5 Hz, 4H; Tyr-Hδ),6.98 (s, 2H; His-Hδ), 7.92 (d, *J* = 9.4 Hz, 4H; dye), 6.81 (d, *J* = 8.7 Hz, 4H; Tyr-Hε), 4.57 (dd, *J* = 5.6, 7.8 Hz, 2H; His-Hα), 4.48 (dd, *J* = 7.4, 9.1 Hz, 2H; Asn-Hα), 4.40 - 4.26 (m, 6H; Pro-Hα, Pro-Hγ, Tyr-Hα), 4.12 (t, *J* = 5.4 Hz, 4H; OC*H*$_2$CH$_2$N), 3.82 (t, *J* = 5.4 Hz, 4H; OCH$_2$C*H*$_2$N), 3.58 (q, *J* = 7.3 Hz, 4H; C*H*$_2$CH$_3$), 3.57 - 3.53 (m, 2H; Pro-Hδ'), 2.98 - 2.88 (m, 6H; Pro-Hδ, His-Hβ, Tyr-Hβ), 2.82 (dd, *J* = 8.4, 15.3 Hz, 2H; His-Hb'), 2.76 (dd, *J* = 8.2, 13.4 Hz, 2H; Tyr-Hβ'), 2.58 - 2.50 (m, 4H; Asn-Hβ), 2.42 (dd, *J* = 6.6, 15.4 Hz, 2H; Asn-Hβ'), 2.34 - 2.27 (m, 2H; Pro-Hβ'), 1.93 - 1.84 (m, 2H; Pro-Hβ), 1.81 (s, 6H; COC*H*$_3$), 1.17 (t, *J* = 7.3 Hz, 6H; CH$_2$C*H*$_3$);

^{13}C-NMR: (125.8 MHz, DMSO): δ = 172.2, 171. 3, 171.2, 170.9, 170.1, 165.9, 157.3, 156.6, 152.1, 147.3, 143.2, 138.9, 135.8, 130.7, 130.2, 126.6, 125.5, 122.9, 120.6, 114.5, 112.2, 65.7, 58.8, 55.0, 52.2, 50.1, 49.6, 48.1, 46.9, 46.6, 37.2, 37.1, 33.1, 28.9, 23.0, 12.5;

ESI-MS: *m/z*: calcd for $C_{84}H_{96}N_{24}O_{18}$ $[M+Na]^+$ 1752; found 1752;

6. 9. Synthesis of the building blocks for the fluorophore quencher library

6. 9. 1. Synthesis of N-Boc-anthranilic acid 47



46 **47**

Anthranilic acid **46** (10 g, 72.9 mmol, 1 eq) was dissolved in 30 ml DMF and 19.91 g (91.2 mmol, 1.25 eq) of Boc_2O and 19.33 ml (109.5 mmol, 1.5 eq) NEt_3 was added. After stirring 24 hours, the solvent was removed at reduced pressure. The residue was dissolved in 20 ml of CH_2Cl_2 and extracted 10 times with 40 ml of saturated potassium carbonate. All the aquous phases were acidified with 60 ml 2M HCl each and extracted three times with 60 ml Et_2O. After removal of the solvent, the product was precipitated with pentane to yield 9.5 g (55%) of **47**.

$C_{12}H_{15}NO_4$: 237.25

TLC: EtOAc R_f:0.58 (Ninhydrin)

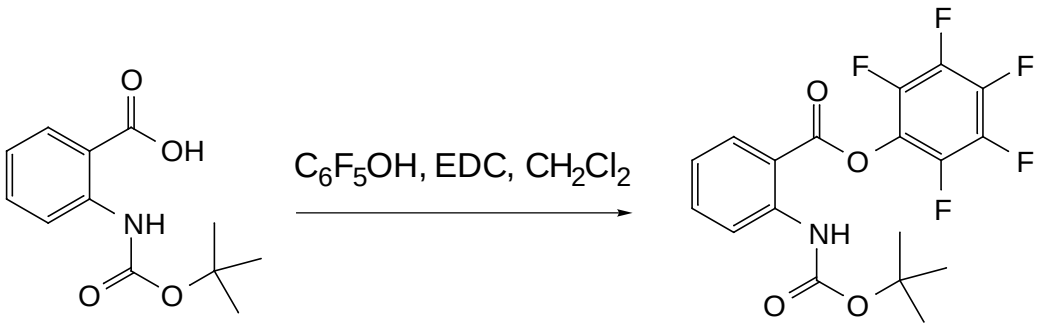
^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ (ppm) = 10.03, (s, 1H; NH), 8.47 (d, J = 7.5 Hz, 1H; H ar *o*-carbonyl), 8.10 (d, J = 8.0 Hz, 1H; H ar *o*-amino), 7.57 (t, J = 7.3 Hz, 1H; H ar *p*-carbonyl), 7.04 (t, J = 8.2 Hz, 1H; H ar *p*-amino), 1.55 (s, 9H; *t*-Bu);

^{13}C NMR (100.5 MHz, $CDCl_3$, 25°C): δ (ppm) = 172.2, 152.8, 142.9, 135.6, 131.9, 121.3, 118.9, 80.8, 28.3;

ESI-MS: *m/z*: calcd for $C_{12}H_{15}NO_4Na$ $[M+Na]^+$ 260; found 260.

elemental analysis calcd (%) for $C_{12}H_{15}NO_4$ (237.26): C 60.75, H 6.37, N 5.90; found C 60.70, H 6.35, N 5.88.

6. 9. 2. Synthesis of N-Boc-anthranilic acid pentafluorophenylester 47'



47 **47'**

3.01 g (12.7 mmol, 1 eq) of **47** was dissolved in 30 ml of absolute DCM followed by 2.45 g (13.3 mmol, 1.05 eq) pentafluorophenol and 3.65 g (19.05 mmol, 1.5 eq) EDC. The solution was stirred for 1 hour and then extracted with 80 ml water and 80 ml Et2O. After purification over silica gel (Pentane:Ethyl acetate 20:1) 3.2 g (63%) of **47'** were obtained.

$C_{18}H_{14}F_5NO_4$: 403.30

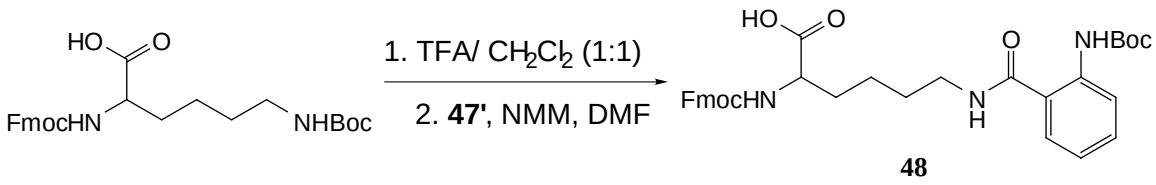
TLC: CH_2Cl_2/MeOH (50:1); Rf: 0.80 (Ninhydrin)

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ (ppm) = 9.74, (s, 1H; NH), 8.55 (d, J = 9.2 Hz, 1H; H ar *o*-carbonyl), 8.24 (d, J = 7.9 Hz, 1H; H ar *o*-amino), 7.65 (t, J = 9.0 Hz, 1H; H ar *p*-carbonyl), 7.11 (t, J = 8.2 Hz, 1H; H ar *p*-amino), 1.51 (s, 9H; *t*-Bu);

^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ (ppm) = 164.1, 152.4, 143.7, 136.5, 131.68, 121.5, 119.1, 111.0, 81.1, 18.2;

ESI-MS: *m/z*: calcd for $C_{18}H_{14}F_5NO_4Na$ $[M+Na]^+$ 426; found 426;

6. 9. 3. Synthesis of fluorophore building block 48



48

FMOC-Lys(BOC)-OH (3.70 g, 7.90 mmol, 1 eq) was dissolved in a 1:1 mixture of TFA and CH2Cl2 and stirred at room temperature for 1 hour. After removal of the volatile components, the residue was triturated three times with Et_2O. The Boc-deprotected lysine was then dissolved in 15 ml of DMF and 3.2 g (7.90 mmol, 1 eq) of pentafluorophenylester **47'** as well as 8.7 ml (79 mmol, 10 eq) N-methylmorpholine were added. After stirring the mixture for 1 hour, it was extracted with 20 ml of EtOAc and 20 ml of water. The organic layers were washed with sat. NaCl solution, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification over silica gel (gradient of CH_2Cl_2 / MeOH from 0 to 5% of methanol) yielded 4.03 g (87%) of **48**.

$C_{33}H_{37}N_3O_7$: 587.66

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.37 (Ninhydrin)

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 10.04 (s, 1H, NHBoc), 9.80 (s br, 1H, OH), 8.25 (d, *J* = 7.9 Hz, 1H; H ar *o*-carbonyl), 7.72 (d, *J* = 7.5 Hz, 2H, Fmoc-H(ar)), 7.54 (d, *J* = 7.2 Hz, 2H, Fmoc-H(ar)), 7.42 - 7.28 (m, 4H, H ar *o*-amino, H ar *p*-carbonyl, Fmoc-2H(ar)), 7.26 (t, *J* = 7.6 Hz, 2H, Fmoc-H(ar)), 6.85 (dd, *J* = 7.7 Hz, 7.7 Hz 1H; H ar *p*-amino), 6.64 (t, *J* = 4.9 Hz, 1H, NH-Lysε), 5.61 (d, *J* = 8.2 Hz, 1H, NH-Lysα), 4.42 - 4.28 (m, 3H; Lys-Hα, FMOC-CH_2), 4.16 (t, *J* = 7.1 Hz, 1H, FMOC-CH), 3.42 - 3.28 (m,

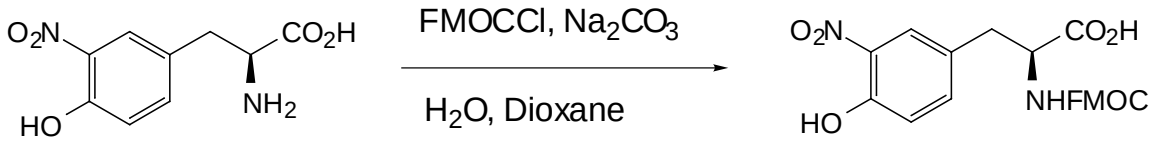
2H; Lys-ε), 1.94 - 1.82 (m, 1H; Lys-β), 1.78 - 1.70 (m, 1H; Lys-β'), 1.68 - 1.52 (m, 2H; Lys-δ), 1.50 (s, 9H, Boc), 1.45 - 1.37 (m, 2H; Lys-γ);

^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 175.8, 169.2, 156.3, 153.2, 143.6, 143.5, 141.2, 139.6, 132.2, 127.7, 127.0, 126.8, 124.9, 121.5, 120.2, 119.9, 80.4, 67.1, 53.4, 46.9, 39.5, 31.9, 31.5, 28.5, 28.2;

ESI-MS: *m/z*: calcd for $C_{33}H_{37}N_3O_7$ $[M+Na]^+$ 610; found 610.

Elemental analysis: Calcd (%) for $C_{33}H_{37}N_3O_7$ (587.7):
C 67.45, H 6.35, N 7.15; found C 67.37, H 6.38, N 7.07.

6. 9. 4. Synthesis of quencher building block 50



49 **50**

1.35 g of Nitrotyrosine **49** (7.73 mmol, 1 eq) was dissolved in 10 ml dioxane and 20 ml 10% aquous Na_2CO_3 solution. A solution of 2.0 g (7.73 mmol, 1 eq) of FMOC-Cl in 20 ml dioxane was added and the mixture was allowed to stir at room temperature for two hours. After extracting with 20 ml of EtOAc and 20 ml of 0.1 M HCl, the organic layers were washed with sat. NaCl solution, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification over silica gel (gradient of CH_2Cl_2 / MeOH from 0 to 5% of methanol) yielded 1.70 g (49%) of **50**.

$C_{24}H_{20}N_2O_7$: 448.42

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.31 (Ninhydrin)

^{1}H NMR (300 MHz, DMSO, 25°C): δ = 7.86 (d, *J* = 8.4 Hz, 2H, Fmoc-H(ar)), 7.85 (s, 1H, H ar *o*-NO_2), 7.76 (d, *J* = 8.2 Hz, 2H, H ar *p*-NO_2), 7.62 (d, *J* = 7.0 Hz, 2H, Fmoc-H(ar)), 7.47 - 7.33 (m, 3H, NH, Fmoc-2H(ar)), 7.27 (td, *J* = 7.3, 7.3 Hz, 2H, Fmoc-H(ar)), 7.06 (d, *J* = 8.2 Hz, 1H; H ar *m*-NO_2), 4.29 - 4.09 (m, 4H, Hα, FMOC-CH_2, FMOC-CH), 3.09 (dd, *J* = 4.1, 13.7 Hz, 1H, Hβ), 3.85 (dd, *J* = 10.9, 13.5 Hz, 1H, Hβ);

^{13}C NMR (75 MHz, DMSO, 25°C): δ = 174.0, 157.0, 151.9, 144.7, 141.7, 137.2, 137.0, 130.2, 128.6, 128.0, 126.5, 126.2, 121.1, 120.0, 66.6, 56.2, 47.5, 36.0;

ESI-MS: *m/z*: calcd for $C_{33}H_{37}N_3O_7$ $[M+Na]^+$ 610; found 610.

Elemental analysis: Calcd (%) for $C_{33}H_{37}N_3O_7$ (587.7):
(+1/2 H_2O) C 63.02, H 4.63, N 6.12; found C 62.64, H 4.38, N 5.97.

6. 10. Solid Phase Peptide Synthesis (SPPS)

6. 10. 1. General FMOC-Strategy for Peptide Synthesis on Solid Support Exemplified by the Synthesis of Ac-L-Glu-L-Glu-εAhx-Resin

6. 10. 1. 1. FMOC-Amino Acid Coupling onto Amino-Functionalized Resin

300 mg (0.078 mmol) of amino methyl polystyrene resin (TentaGel, purchased by Rapp Polymere, loading 0.26 mmol/g) was placed in a 25 ml Merrifield vessel and washed three times with DCM (10 ml) for 1 minute. The resin is suspended in the smallest amount of dry DCM that still allows efficient shaking (4 ml) and 83 mg (234 µmol, 3 eq) of FMOC-εAhx was added. After the addition of a solution of 32 mg (234 µmol, 3 eq) of HOBt in 200 µl of DMF the mixture was agitated for 2 minutes. Then 36 µl (234 µmol, 3 eq) of DIC is added and the mixture was shaken for 2 hours. The resin was then washed five times with DMF and five times with DCM for 1 minute each. Then the absence of free amino groups was ensured by performing the Kaiser test with a small amount of beads. A negative Kaiser test indicates that no more free amino groups are present and thus the coupling has been exhaustive. However, if the Kaiser test was positive, the coupling had to bee repeated until no more free amino groups were detected.

6. 10. 1. 2. FMOC-Deprotection

After washing the FMOC-functionalized resin three times with DMF, it was shaken twice with 8 ml of a 4:1 mixture of DMF and Piperidine first for five and then a second time for ten minutes. Then it was washed five times with DMF and five times with DCM. The successful deprotection of the FMOC-group was confirmed by the Kaiser test.

Steps 6. 10. 1. 1. and 6. 10. 1. 2. were repeated until the desired peptide sequence was accomplished

6. 10. 1. 3. Acetylation of free amines

The resin was suspended in 5 ml of dry DCM and 54 µl (390 µmol, 5 eq) of Et_3N are added followed by 37 µl (390 µmol, 5 eq) of Ac_2O. After shaking the mixture for 1 hour, it was washed three times with DMF and five times with DCM. The completion of the reaction was confirmed by a negative Kaiser test.

6. 10. 1. 4. Cleavage of the side-chain protecting groups

The side-chain protecting groups are removed by shaking the resin twice with 10 ml of a 1:1:0.1 mixture of TFA, DCM and TIS first for 2 minutes and then for 1 hour. The resin is then washed five times with DCM, five times with a 4:1 mixture of DCM / Et_3N followed by another five times with DCM. Then the resin is dried *in vacuo* and stored at 4°C.

6. 10. 2. General FMOC-Strategy for Solid Phase Peptide Synthesis on Wang Resin Exemplified by the Synthesis of Ac-L-Glu-L-Glu-NHPr

6. 10. 2. 1. Coupling onto Hydroxy-Functionalized Resin

If an amino acid had to be coupled to hydroxy-functionalized resin as Wang resin, it was proceeded as described in 6.10.1.1. but 0.05 eq of DMAP was added to the reaction mixture. To ensure optimal reaction yields, the coupling to the resin was repeated twice and the loading after the coupling was determined by cleavage of the FMOC group of an aliquot and quantify by UV-measurement.

The rest of the peptide synthesis was performed according to 6. 10. 1. 1. - 6. 10. 1. 3.

6. 10. 2. 2. Cleavage from the Resin as Propyl Amide

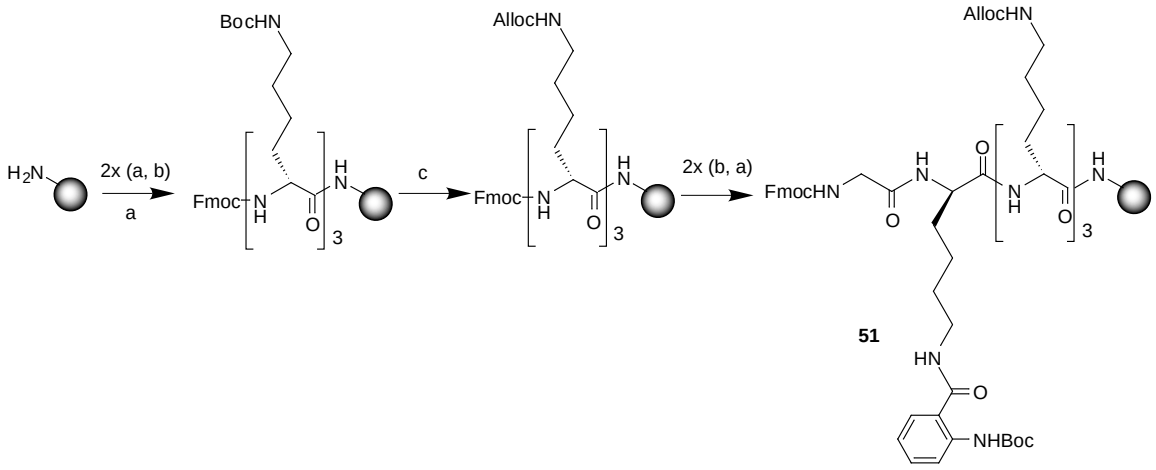
After washing three times with DCM the resin was dried in vacuo and placed in a 50 ml round bottom flask with magnetic stirrer and 10 ml of DCM and 5 ml of propylamine was added. After stirring for 72 hours the mixture was filtered and all the volatiles were removed at reduced pressure. The peptide was purified by extraction with DCM, HCl (0.5%) and brine. (If the peptides were water soluble gel filtration (LH 20, DCM/MeOH 90:10) was used for purification instead of extraction). Drying over Na2SO4 and evaporation of the solvent yielded 240 mg (0.51 mmol) of the propylamide of the side chain protected diamine.

6. 10. 2. 3. Removal of the Side Chain Protecting Groups

123 mg (0.26 mmol, 1 eq) of the side chain protected peptide was placed into a 25 ml round bottom flask and dissolved in 5ml of TFA containing 2.5% water and 2.5 % TIS. After 1 hour of stirring at r.t. the TFA was removed at reduced pressure and the peptide was purified by precipitating five times with diethyl ether and adjacent drying at reduced pressure. If necessary the peptide was further purified by preparative HPLC.

6. 11. Synthesis of the fluorophore quencher library 43 using oxidative cleavable tags

6. 11. 1. Synthesis of library precursor 51



25 g (6.5 mmol, 1 eq) of amino functionalized PEG-PS resin (TentaGel loading 0.26 mmol/g) was placed in a 500 ml Merrifield shaking vessel and washed three times with CH_2Cl_2 (100 ml). Then three NεBoc-NαFMOC protected lysin moieties were coupled according to 6.10.1.1. and 6.10.1.2. The Lys(Boc)-groups were removed by shaking twice (2 minutes and 1 hour) with a mixture of CH_2Cl_2 and TFA (3:1) with subsequent washing of the beads six times with CH_2Cl_2, twice with CH_2Cl_2 : NEt_3 (9:1) and again six times with CH_2Cl_2. The free lysine ε-amino groups were then Alloc protected by suspending the resin in 50 ml DCM and adding allylchloroformiate (10.15 ml, 95.5 mmol, 15 eq) and NEt_3 (19.96 ml, 143 mmol, 22 eq). The coupling was repeated twice until the Kaiser test was negative. After FMOC-deprotection (see 6.10.1.2.), the fluorophore building block **6** was coupled (4.99 g, 8.5 mmol, 1.33 eq) according to 6.10.1.1. followed by another FMOC-deprotection and coupling of FMOC-Glycin-OH (5.68 g, 19.11 mmol, 3 eq) was coupled to yield library precursor **5**.

6. 11. 2. Split-and-mix synthesis of the combinatorially varied peptide sequence

6. 11. 2. 1. Deprotection of the lysine Nε-Alloc protecting groups

The resin with library precursor 5 (25 g, 6.37 mmol, 1 eq) was placed in a 250 ml Merrifield vessel and washed three times with 100 ml DCM. Then it was washed again under nitrogen using 100 ml dry DCM. Then the resin was suspended in 50 ml of dry DCM in a nitrogen athmosphere and 1.50 g (5.7 mmol, 0.9 eq) of PPh3 and 4.01 g (28.7 mmol, 4.5 eq) of dimedone was added followed by 220.7 mg (0.063 mmol, 0.01 eq) of Pd(PPh3)4. After shaking for 20 minutes it was washed five times with DMF and three times with DCM. Successful deprotection of the alloc groups was confirmed by Kaiser test.

6. 11. 2. 2. Splitting and Encoding

The alloc deprotected resin was devided into 31 equal portions of 806 mg (0.21 mmol) and each portion was placed into a 25 ml Merrifield shaking vessel. After washing three times with 10 ml of DCM, the resin was suspended in 10 ml of dry DCM. 4 mol % of oxidative cleavable tags (See 6.11.2.5) were dissolved in 200µl of DMF and added to the resin followed by 56 mg (420µmol, 50 eq) of HOBt per tag dissolved in 200µl of DMF. To ensure an equal distribution of the tags, the mixture was then shaken vigorously for five minutes and 132µl (840µmol, 100 eq) of DIC per tag were added and each mixture was agitated immidiately after the addition. After shaking the reaction mixtures over night, they all were washed ten times with DMF.

6. 11. 2. 3. Check of Successful Encoding and Alloc Protection

From each reaction vessel three beads were separated and each single bead was placed into a 25 ml micropipette that was then sealed at one end. (H.P. Nestler, Paul A. Bartlett,

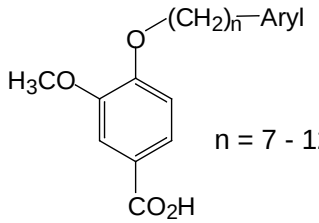
W.C. Still, J.O.C. 1994, 59, 4723 - 4724) The beads were washed three times with water, then 1 µl of 0.5 M ceric ammonium nitrate in 1 : 1 water : acetonitrile and 3 µl of hexane were added and the micropipette was sealed at the other end. After treating in a sonic bath for 2 hours the released tags were extracted into the hexane phase by centrifugating the capillary several times. The tag alcohols were silylated and the corresponding trimethylsilylethers were analysed by ECGC. If the amount of tags was insufficient, the tag coupling process was repeated, otherwise the resin was washed three times with DCM and the remaining free Nε-aminogroups were alloc protected by suspending the resin in 5 ml of DCM and adding 130ml (0.99mmol, 4.5 eq) of NEt_3 followed by 100µl (0.99mmol, 4.5 eq) of allyl chloroformiate. After shaking for 1 hour and washing five times with DCM, it was made sure by Kaiser test, that no more free amino groups were present.

6. 11. 2. 4. FMOC-Deprotection and Amino Acid Coupling

The α–FMOC group was removed according to 6.10.1.2. Then the amino acid coupling was performed following the general protocol described in chapter 6.10.1.1. using 3 eq of each amino acid (see 6.11.2.5. for details), 84 mg (0.617 mmol, 3 eq) of HOBt and 100µl (0.617 mmol, 3 eq) of DIC. Each coupling was repeated twice to ensure complete coupling. After that, the resin was combined in a 250 ml Merrifield vessel.

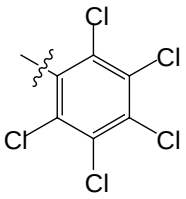
The steps 6. 11. 2. 1. to 6. 11. 2. 4. were repeated twice with with different sets of tags leading to a library with $31^3 = 29791$ members.

6. 11. 2. 5. Tags used for the Fluorophore Quencher Library

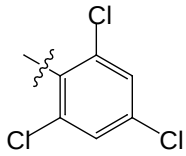


n = 7 - 12

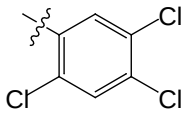
Aryl =



Cl_5



2,4,6-Cl_3



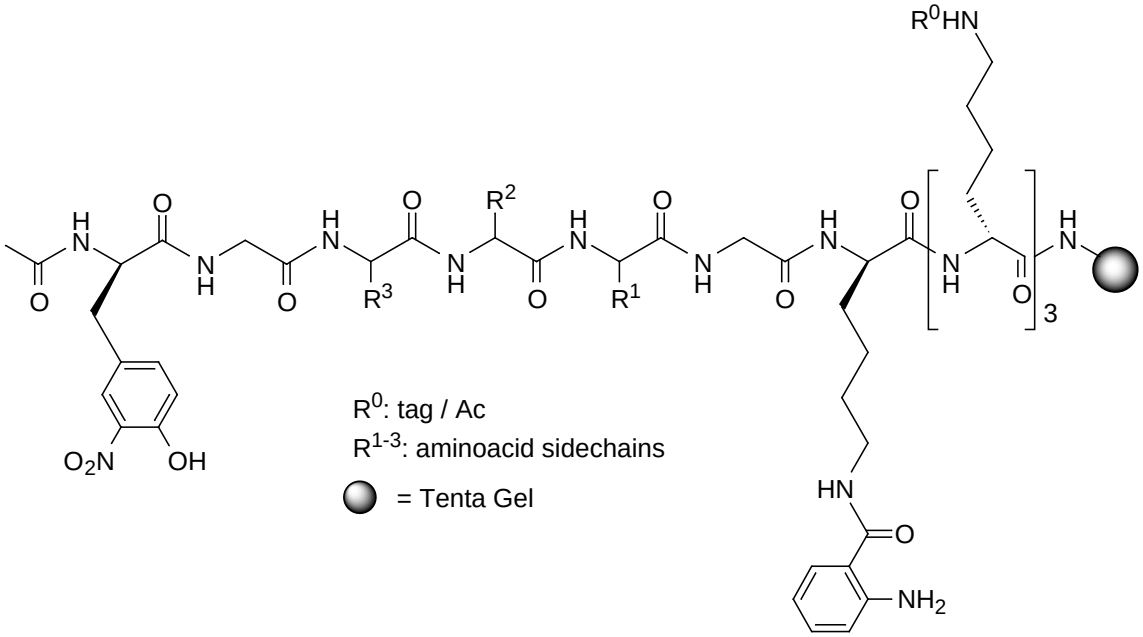
2,4,5-Cl_3

1st step:	$(CH_2)_{12}$-2-4-5-Cl_3	(4.33 mg, 8.40 µmol) = tag No 1
	$(CH_2)_9$-Cl_5	(4.56 mg, 8.40 µmol) = tag No 2
	$(CH_2)_{10}$-Cl_5	(4.68 mg, 8.40 µmol) = tag No 3
	$(CH_2)_{11}$-Cl_5	(4.79 mg, 8.40 µmol) = tag No 4
	$(CH_2)_{12}$-Cl_5	(4.91 mg, 8.40 µmol) = tag No 5
2nd step:	$(CH_2)_9$-2-4-5-Cl_3	(3.98 mg, 8.40 µmol) = tag No 1
	$(CH_2)_{10}$-2-4-6-Cl_3	(4.10 mg, 8.40 µmol) = tag No 2
	$(CH_2)_{10}$-2-4-5-Cl_3	(4.10 mg, 8.40 µmol) = tag No 3
	$(CH_2)_{11}$-2-4-6-Cl_3	(4.22 mg, 8.40 µmol) = tag No 4
	$(CH_2)_{11}$-2-4-5-Cl_3	(4.22 mg, 8.40 µmol) = tag No 5
3rd step:	$(CH_2)_7$-2-4-6-Cl_3	(3.74 mg, 8.40 µmol) = tag No 1
	$(CH_2)_7$-2-4-5-Cl_3	(3.74 mg, 8.40 µmol) = tag No 2
	$(CH_2)_8$-2-4-6-Cl_3	(3.86 mg, 8.40 µmol) = tag No 3
	$(CH_2)_8$-2-4-5-Cl_3	(3.86 mg, 8.40 µmol) = tag No 4
	$(CH_2)_9$-2-4-6-Cl_3	(3.98 mg, 8.40 µmol) = tag No 5

Encoding sheme and amounts of amino acids used

Tag No 1	Tag No 2	Tag No 3	Tag No 4	Tag No 5	amino acid	0.617 mmol
1	0	0	0	0	Gly	183 mg
0	1	0	0	0	L-Ala	192 mg
0	0	1	0	0	D-Ala	192 mg
0	0	0	1	0	L-Val	209 mg
0	0	0	0	1	D-Val	209 mg
1	1	0	0	0	L-Leu	218 mg
1	0	1	0	0	D-Leu	218 mg
1	0	0	1	0	L-Phe	239 mg
1	0	0	0	1	D-Phe	239 mg
0	1	1	0	0	L-Ser	236 mg
0	1	0	1	0	D-Ser	236 mg
0	1	0	0	1	L-Thr	245 mg
0	0	1	1	0	D-Thr	245 mg
0	0	1	0	1	L-Asn	368 mg
0	0	0	1	1	D-Asn	368 mg
1	1	1	0	0	L-Gln	377 mg
1	1	0	1	0	D-Gln	377 mg
1	1	0	0	1	L-Asp	254 mg
1	0	1	1	0	D-Asp	254 mg
1	0	1	0	1	L-Glu	262 mg
1	0	0	1	1	D-Glu	262 mg
0	1	1	1	0	L-Lys	289 mg
0	1	1	0	1	D-Lys	289 mg
0	1	0	1	1	L-Pro	208 mg
0	0	1	1	1	D-Pro	208 mg
1	1	1	1	0	L-His	382 mg
1	1	1	0	1	D-His	382 mg
1	1	0	1	1	L-Arg	409 mg
1	0	1	1	1	D-Arg	409 mg
0	1	1	1	1	L-Cys	361 mg
1	1	1	1	1	D-Cys	361 mg

6. 11. 2. 6. Fluorophore-quencher library 43



After removal of the FMOC-group of the third combinatorially varied amino acid FMOC-Glycin followed by the quencher building block **50** was coupled according to 6. 10. 1. 1. and 6. 10. 1. 2. The alloc groups were deprotected as described in 6. 11. 2. 1. and the free amino groups were acetylated according to 6. 10. 1. 3. Finally the terminal FMOC groups were removed and the according amino groups were acetylated as well. After deprotection of the side chain protecting groups the resin was washed three times with DCM, three times with methanol, six times with 0.1 M NaOH, again three times with methanol and three times with DCM. Then the resin was dried at reduced pressure and library y was stored at 4°C.

6. 12. Analytical data of the model peptides 54' , 54, 57 and 58

6. 12. 1. Ac-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-NHPr 54'

$C_{23}H_{41}N_3O_7$: 471.59

TLC: CH_2Cl_2/MeOH (10:1); Rf: 0.52 (Cer)

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.52 (d, *J* = 7.5 Hz, 1H; NH Glu), 6.91 (d, *J* = 5.9 Hz, 1H; NH Glu), 6.73 (t, *J* = 5.7 Hz, 1H; NH Pr), 4.40 (ψtd, *J* = 5.7, 7.2 Hz, 1H; Glu-Hα), 4.31 (ddd, *J* = 5.2, 6.2, 8.2 Hz, 1H; Glu-Hα), 3.20 (ψtd, *J* = 5.9, 7.5 Hz, 2H; NHC**H**$_2$$CH_2CH_3$), 2.49 - 2.25 (m, 4H; Glu-Hγ), 2.13 - 1.92 (m, 4H; Glu-Hβ), 2.04 (s, 3H; Ac), 1.53 (ψq, *J* = 7.0 Hz, 2H; $NHCH_2$C**H**$_2$$CH_3$), 0.91 (t, *J* = 7.2 Hz, 3H, $NHCH_2CH_2$C**H**$_3$);

^{13}C-NMR: (100.6 MHz, $CDCl_3$, 25°C): δ = 173.3, 173.2, 171.3, 170.8, 170.6, 81.0, 80.9, 53.7, 52.8, 41.2, 31.9, 31.8, 28.0, 28.0, 27.3, 27.2, 23.1, 22.5, 11.3;

ESI-MS: *m/z*: calcd for $C_{23}H_{41}N_3O_7$ $[M+Na]^+$ 494; found 494.

Elemental analysis: Calcd (%) for $C_{23}H_{41}N_3O_7$ (471.60):
C 58.58, H 8.76, N 8.91; found C 58.57, H 8.54, N 8.75.

6. 12. 2. Ac-L-Glu-L-Glu-NHPr 54

$C_{15}H_{25}N_3O_7$: 359.38

^{1}H NMR (400 MHz, DMSO, 25°C): δ = 8.04 (d, *J* = 7.2 Hz, 1H; NH Glu), 7.93 (d, *J* = 8.2 Hz, 1H; NH Glu), 7.77 (t, *J* = 5.7 Hz, 1H; NH Pr), 4.21 - 4.13 (m, 2H; Glu-Hα), 2.98 (m, 2H; NHC**H**$_2$$CH_2CH_3$), 2.24 - 2.15 (m, 4H; Glu-Hγ), 1.91 - 1.63 (m, 4H; Glu-Hβ),

1.83 (s, 3H; Ac), 1.53 (td, *J* = 7.3, 14.6 Hz, 2H; $NHCH_2C\mathbf{H}_2CH_3$), 0.80 (t, *J* = 7.6 Hz, 3H, $NHCH_2CH_2C\mathbf{H}_3$);

^{13}C-NMR: (100.6 MHz, $CDCl_3$, 25°C): δ = 174.4, 174.3, 171.7, 171.1, 170.1, 52.6, 52.4, 40.7, 30.6, 30.5, 27.7, 27.6, 22.9, 22.7, 11.7;

ESI-MS: *m/z*: calcd for $C_{15}H_{25}N_3O_7$ $[M\text{-}H]^-$ 358; found 358;

6. 12. 3. Ac-F-Gly-Gly-Gly-L-Glu-L-Glu-Ahx-NHPr 57

$C_{40}H_{62}N_{10}O_{13}$: 890.98

^{1}H NMR (500 MHz, d4-MeOH, 25°C): δ = 7.55 (d, *J* = 7.9 Hz, 1H; H ar *o*-carbonyl), 7.32 (ψt, *J* = 7.6 Hz, 1H; H ar *p*-carbonyl), 6.95 (d, *J* = 7.9 Hz, 1H; H ar *o*-amino), 6.89 (d, *J* = 6.6 Hz 1H; H ar *p*-amino), 4.34 - 4.30 (m, 2H; Glu Ha's), 4.26 (dd, *J* = 5.8, 8.5 Hz, 1H; Lys-α), 3.95-3.83 (m, 6H; Gly-α), 3.36 (t, *J* = 6.8 Hz, 2H; Lys-ε), 3.21 - 3.12 (m, 2H; Ahx-ε), 3.11 ((ψt, *J* = 7.2 Hz, 2H; $NHC\mathbf{H}_2CH_2CH_3$), 2.45-2.35 (m, 4H; Glu-γ), 2.17 (t, *J* = 7.5 Hz, 2H; Ahx–α), 2.17-2.07 (m, 2H; Glu-β), 2.03 - 1.92 (m, 2H; Glu-β), 1.98 (s, 3H; Ac), 1.89 - 1.81 (m, 1H; Lys–β), 1.77 - 1.68 (m, 1H; Lys-β), 1.64 (ψq, *J* = 7.0 Hz, 2H; Lys-δ), 1.60 (ψq, *J* = 7.6 Hz, 2H; Ahx-β), 1.54 - 1.42 (m, 6H; $NHCH_2C\mathbf{H}_2CH_3$, Ahx-δ, Lys-γ), 1.36 - 1.29 (m, 2H; Ahx-γ), 0.91 (t, *J* = 7.5 Hz, 3H, $NHCH_2CH_2C\mathbf{H}_3$);

^{13}C NMR (100.5 MHz, d4-MeOH, 25°C): δ = 175.2, 175.1, 174.7, 174.6, 174.1, 172.6, 172.3, 172.0, 171.2, 171.1, 170.9, 146.4, 131.9, 127.8, 126.5, 119.8, 119.7, 54.0, 53.4, 53.0, 42.5, 42.4, 40.7, 38.9, 38.8, 35.6, 30.8, 29.9, 29.8, 28.6, 28.5, 26.6, 26.0, 25.3, 22.8, 22.2, 21.0, 10.3;

ESI-MS: *m/z*: calcd for $C_{40}H_{62}N_{10}O_{13}$ $[M+]^+$ 891; found 891.

RP-HPLC: retention time: 19.6 minutes (gradient: water (+ 0.1 % TFA): acetonitrile (+ 0.1 % TFA) 100:0 -> 60:40 in 30 minutes).

6. 12. 4. Ac-F-Gly-Gly-Gly-Gly-Gly-Ahx-NHPr 58

$C_{34}H_{54}N_{10}O_9$: 746.41

^{1}H NMR (500 MHz, D_2O, 25°C): δ = 7.25 (dd, *J* = 1.3, 7.6 Hz, 1H; H ar *o*-carbonyl), 7.18 (td, *J* = 1.5, 8.2 Hz, 1H; H ar *p*-carbonyl), 6.74 (d, *J* = 8.1 Hz, 1H; H ar *o*-amino), 6.69 (td, *J* = 1.2, 8.6 Hz, 1H; H ar *p*-amino), 4.12 (dd, *J* = 5.8, 8.5 Hz, 1H; Lys-α), 3.86 - 3.70 (m, 10H; Gly-α), 3.22 (t, *J* = 6.7 Hz, 2H; Ahx-ε), 3.03 (t, *J* = 7.3 Hz, 2H; Lys-ε), 2.97 ((t, *J* = 6.9 Hz, 2H; NHC**H**$_2$CH$_2$CH$_3$), 2.06 (t, *J* = 6.3 Hz, 2H; Ahx–α), 1.86 (s, 3H; Ac), 1.75 - 1.66 (m, 1H; Lys–β), 1.66 - 1.56 (m, 1H; Lys-β), 1.54 - 1.46 (m, 2H, Ahx-δ), 1.45 - 1.39 (m, 2H, Ahx-β), 1.37 - 1.29 (m, 6H; NHCH$_2$C**H**$_2$CH$_3$, Lys-γ, Lys-δ), 1.16 - 1.09 (m, 2H; Ahx-γ), 0.72 (t, *J* = 7.4 Hz, 3H, NHCH$_2$CH$_2$C**H**$_3$);

^{13}C NMR (100.5 MHz, D_2O, 25°C): δ = 175.8, 175.4, 175.2, 172. 2, 172.1, 172.0, 171.9, 132.4, 128.0, 118.7, 118.3, 54.0, 46.6, 42.4, 41.0, 39.1, 39.0, 35.6, 30.3, 27.9, 25.3, 25.0, 22.2, 21.7, 21.5, 10.5.

ESI-MS: m/z: calcd for $C_{34}H_{55}N_{10}O_9$ $[M+1]^+$ 747; found 747*;*

RP-HPLC: retention time: 18.4 minutes (gradient: water (+ 0.1 % TFA): acetonitrile (+ 0.1 % TFA) 100:0 -> 60:40 in 30 minutes*)*

6. 13. Studies on the sequence dependence of peptide cleavage caused by the Fenton reaction

6. 13. 1. Combinatorial assay with fluorophore-quencher library 43

In a 2 ml siringe with filter frit, ca. 10 mg of library **43** was weighted. It was washed twice with water, followed by 1 ml of a 0.3 M $FeCl_3$ solution (10 seconds). It was washed five times with water. The iron complexed beads were placed into a 1.5 ml Eppendorff tube and dried in a Speedvac.Then, 2 mg beads were placed in an Eppendorff tube and 650 µl water was added, followed by 500 µl of 0.1 M ascorbic acid solution and 100 µl of 1 M hydrogen peroxide (final pH of the reaction mixture: 3.0). After 20 minutes, the reaction was quenched by washing five times with water. The beads were then studied under a fluorescence microscope.

6. 13. 2. Solid phase complexation assay (excess)

The resin (0.23 mmol/g) with the peptide of interest (**52** or **53**) was first dried over high vacuum. Then ca. 15 mg (3.5 µmol) was weighed into a 5 ml syringe containing a filter frit. The resin was washed five times with water and then equilibrated for 10 seconds with 2 ml of a 0.3 M solution of $FeCl_3$ in water. The resin was then washed six times with 2 ml water each. Then it was washed 10 times with 2 ml of 0.1 M HCl each. The acidic washing phases were collected in a 25 ml graduated flask, 1250 µl of 1M KSCN was added and it was filled up to the mark with 0.1 M HCl and UV was measured at 480 nm to determine the iron content.

6. 13. 3. Solid phase complexation assay (equilibration)

After drying over high vacuum, ca. 8 mg (1.8 µmol) of the resin bound (0.23 mmol/g) peptide of interest (**52** or **53**) was weighed into a 5 ml syringe containing a filter frit.

After the addition of 3 ml (1 eq) of a 0.6 mM solution of iron(III)chloride in 0.5 M MES (pH 5.0) or 0.5 M acetate (pH 5.0) respectively it was equilibrated for 24 hours. Then the reaction solution was filtered into a 25 ml graduated flask. Into the flask ca. 20 ml of 0.1 M HCl was added followed by 1250 µl of 1M KSCN and it was filled up to the mark with 0.1 M HCl. The UV of this solution was measured at 480 nm to determine the iron concentration in the solution.
The iron content of the resin was determined by washing the resin 10 times with 2 ml of 0.1 M HCl each. The washing phases were collected in a 25 ml graduated flask, 1250 µl of 1M KSCN was added and it was filled up to the mark with 0.1 M HCl and UV was measured at 480 nm.

6. 13. 4. Quantitative study of the cleavage by fluorescence spectroscopy

Into a sirynge (2 ml) with filter frit ca. 1 mg of the resin carrying the peptide of interest (**55** or **56**) was exactly weighted. The resin was equilibrated with 1 ml 0.3 M $FeCl_3$ and washed five times with water. The iron complexed resin was then equilibrated for 20 minutes with 0.05 M ascorbic acid and 0.5 M hydrogen peroxide in a final volume of 1000 µl. After 20 minutes, the beads were removed by filtering and it was washed with 250 µl of water. Ca. 5 mg MnO_2 was added to the reaction mixture. After 5 minutes, the $MnO_2$was removed by zentrifugation. 1000 ml of the solution was taken and placed into a fluorescence cuvette followed by 2000 ml of 0.5 M acetate buffer (pH 4) and measuring of the fluorescence with 320 nm exitation energy.

6. 13. 5. Quantitative study of the cleavage in solution

From a stock solution, 0.05 µmol of the peptide of interest (**57** or **58**) was placed into a 1 ml eppendorf tube. After removal of the solvent on a speed vac, 5 µl (0.15 µmol, 3 eq) of a 30mM solution of iron in 0.5 M MES (pH 5.0) was added. After five minutes 100 µl of 0.5 M MES (pH 5.0) and 35 µl of water was added and it was waited for another five

minutes. Then 100 µl (0.5 µmol, 10 eq) of a 5mM sodium ascorbate solution in water was added followed immediately by 10 µl (10 µmol, 200 eq) of 1M hydrogen peroxide. After 20 minutes of reaction time, ca. 5 mg of MnO_2 was added and it was waited 5 minutes before the reaction mixture was filtered and injected into HPLC.

Appendix

Notes and References

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

*Sequences found in the screening against side chain deprotected acetylated tripeptide library **18** on polystyrene in chloroform*

Receptor **10** (2 assays)

AS3	**AS2**	**AS1**	found
D-Val	D-Val	D-His	4x
D-Val	D-Ala	D-His	3x
D-Ala	D-Val	D-His	2x
D-Ala	L-Asn	L-Ala	2x
D-Ala	L-Asn	L-Val	2x
D-Phe	D-Ala	D-His	2x
D-Phe	D-Phe	D-His	2x
D-Phe	D-Val	D-His	2x
D-Ala	D-Ala	D-His	
D-Ala	L-Asn	Gly	
D-Ala	L-Leu	D-His	
D-Asn	D-Leu	D-Val	
D-Asn	D-Phe	D-Val	
D-Asn	D-Val	Gly	
D-Gln	D-Leu	D-His	
D-Leu	D-Phe	D-His	
D-Leu	D-Val	D-His	
D-Phe	D-Leu	D-His	
D-Thr	D-Val	D-His	
D-Val	D-Leu	D-His	
D-Val	L-Ala	D-His	
L-Ala	L-Asn	Gly	
L-His	D-Leu	D-Val	

Receptor **13** (2 assays)

AS3	**AS2**	**AS1**	found
D-Val	D-Phe	D-His	4x
D-Val	D-Leu	D-His	3x
D-Val	D-Val	D-His	2x
D-Gln	D-Phe	D-Val	2x
D-Phe	D-Phe	D-His	
L-Ala	L-Gln	D-Ala	

D-Phe	D-Ala	D-His
D-Ala	D-Phe	D-His
D-Gln	D-Val	D-Leu

Receptor **20** (2 assays)

AS3	**AS2**	**AS1**	found
L-Asn	L-Phe	L-Asn	3x
L-Asn	L-Val	L-Asn	
L-Asn	L-Leu	L-Asn	
L-Asn	L-Ala	L-Asn	
D-Leu	D-Asn	D-Phe	
D-Val	D-Asn	D-Asn	
L-Val	L-Ala	D-Ser	
Gly	L-Ala	L-Asn	
D-Asn	L-Val	L-Asn	

*Sequences found in the screening against the fluorophore-quencher library **43** in water*

Receptor **47**

AA3	**AA2**	**AA1**
L-Asp	L-Asp	D-Lys
D-Glu	D-Glu	Gly
D-Asn	L-Asp	L-Asn
D-Asp	D-Phe	L-Asn
L-Glu	L-Glu	Gly
L-Phe	L-Asp	L-Asp
L-Glu	D-Arg	D-Phe
L-Thr	D-Asp	L-Asn
L-Asp	L-Gln	L-Asp
D-Asp	L-Asp	L-Glu
L-Asp	L-Gln	L-Glu
L-Asp	L-Leu	D-Asn
L-Ser	L-Glu	L-Asn
D-Glu	D-Arg	D-Leu
D-Asp	L-Asp	L-Glu
L-Glu	D-Glu	L-Ala
L-Asn	D-Asp	Gly

*Sequences found by applying the Fenton reaction to fluorophore-quencher library **43***

AA3	**AA2**	**AA1**
D-Lys	L-Asp	L-Asp
L-Lys	D-Asp	L-Asp
L-Gln	L-Asp	D-Asp
L-Pro	L-Asp	L-Glu
Gly	L-Asp	L-Glu
L-Thr	L-Asp	D-Glu
L-Cys	D-Asp	D-Glu
D-Cys	D-Glu	L-Asp
D-Leu	L-Glu	L-Asp
L-Gln	L-Glu	D-Asp
L-Pro	D-Glu	L-Asp
D-Ser	L-Glu	D-Glu
L-Phe	L-Glu	D-Glu
L-Asn	D-Glu	L-Glu
D-Asn	D-Glu	L-Glu
D-Gln	D-Glu	L-Glu
Gly	D-Glu	L-Glu
L-Phe	D-Glu	D-Glu
L-Asp	L-Asp	L-Lys
L-Asp	D-Asp	L-Gln
L-Asp	D-Asp	L-Pro
L-Asp	L-Glu	D-Ala
L-Asp	D-Glu	L-Ala
L-Asp	D-Glu	D-Cys
D-Asp	L-Asp	Gly
D-Asp	L-Glu	L-Leu
D-Asp	L-Glu	L-Cys
L-Glu	L-Asp	D-Glu
L-Glu	L-Asp	L-Thr
L-Glu	D-Asp	D-Leu
L-Glu	D-Asp	D-Pro
D-Glu	L-Glu	D-Cys
D-Glu	D-Glu	L-His
L-Asp	L-Cys	L-Glu
D-Asp	D-Ser	D-Asp
L-Glu	L-Thr	L-Asp
L-Glu	D-Gln	D-Asp
L-Glu	D-Gln	L-Glu
D-Glu	D-Ala	L-Glu
D-Asp	L-Ala	L-Asn
L-Glu	L-Gln	D-Asp
D-Glu	L-Ser	L-Ala

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Publikationen:

- H. Wennemers, M. Conza, M. Nold, P. Krattiger "Diketopiperazine Receptors: A Novel Class of Highly Selective Receptors for Binding Small Peptides", *Chem. Eur. J.* **2001**, *7*, 3342.

- R.R.French, P.Holzer, M.Leuenberger, M.C.Nold, W.D.Woggon "A Supramolecular Enzyme Model Catalyzing the Central Cleavage of Carotenoids", *J.of Inorg. Biochem*. **2002**, 88, 295-304.

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- M. Nold, H. Wennemers “Peptide Damage under Fenton conditions is sequence-dependent”, *Chem. Comm.* **2004**, 16, 1800 – 1801. ("RSC hot article ")

- M. Nold, K. Koch, H. Wennemers “Acid-Rich Peptides are Prone to Damage under Fenton Conditions - Split-and-Mix Libraries for the Detection of Selective Peptide Cleavage”, *Synthesis* **2005**, 1455-1458.

Während meiner Ausbildung an der Universität Basel besuchte ich Vorlesungen und Praktika bei folgenden Dozenten:

E. Constable, T. Boller, G. Gescheidt, B. Giese, P. Hauser, A. Hermann, C. Housecroft, H.P. Huber, M. Jungen, T.A. Kaden, C. Körner, J.P. Maier, W. Meier, M. Oehme, A. Pfaltz, H. Rudin, B. Scarpellini, P. Schiess, U. Séquin, H. Sigel, L. Tauscher, C. Ullrich H. Wennemers, A.M. Wiemken, T. Wirth, J. Wirz, W.D. Woggon, M. Zehnder, A.D. Zuberbühler.

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich meine Dissertation selbstständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Ich erkläre ausserdem, dass ich diese Dissertation an keiner anderen Fakultät eingereicht habe.

Basel, den 2. Dezember 2003

Matthias Nold