Diketopiperazine Receptors: A Novel Class of Highly Selective Receptors for Binding Small Peptides

Helma Wennemers,* Matteo Conza, Matthias Nold, and Philipp Krattiger[a]

Abstract: A novel class of receptors consisting of a rigid diketopiperazine backbone and peptidic side chains has been developed with the use of combinatorial chemistry. These diketopiperazine receptors interact with peptidic substrates with high specificity as shown in combinatorial on-bead assays. The central diketopiperazine moiety can be easily obtained from natural 4-hydroxyproline and serves as a rigidifying template for the peptidic modules which allow for structural as well as functional variations. Screenings of several dye-marked receptor prototypes against an encoded tripeptide library demonstrated not only the high binding specificities of the diketopiperazine receptors towards peptides but also revealed that small structural changes induce significant changes in their binding properties.

Keywords: combinatorial chemistry · diketopiperazine · molecular recognition · peptides · receptors

Introduction

The increasing need for therapeutics and sensors asks for the development of synthetic receptors recognizing small peptides selectively. Due to the many degrees of freedom of even simple di- or tripeptides the rational design of receptors for specific peptide sequences is an extremely challenging task. During the last decade, combinatorial chemistry has become a powerful tool for the search of suitable substrates for a given receptor and vice versa.[1] Using this stochastic approach, subtle differences in host–guest interactions have been disclosed which could have not been predicted by molecular modelling or found by conventional trial and error. Consequently, receptors capable of binding peptides have been established.[2–4] In particular, two-armed receptors consisting of a template with two modules have proven efficient in binding peptidic substrates.[3, 4] Yet, most of the examined receptors exhibit drawbacks such as either poor binding selectivities, rather laborious syntheses or limited possibilities for tuning the receptor structures. Thus, the need for receptors with easily variable structures that allow for the selection of a specific receptor for any desired peptide remains.

Here we introduce a novel class of two-armed receptors and report the highly selective binding of several structurally related receptor prototypes to different peptides within a tripeptide library.

A receptor class containing members capable of binding any desired peptide selectively should consist of a rigid, structure-directing backbone as well as functional groups that allow for the formation of non-covalent interactions such as hydrogen bonds, ionic and hydrophobic interactions. Furthermore, the receptor structure should offer opportunities for combinatorial structural and functional variations and should be accessible by a simple synthesis both in solution and on solid supports to ultimately permit the generation of a combinatorial receptor library using the split-and-mix protocol.[5]

The novel diketopiperazine receptors depicted in Figure 1 fulfil all of the above-mentioned requirements for a versatile class of receptors. Herein, the diketopiperazine derived from

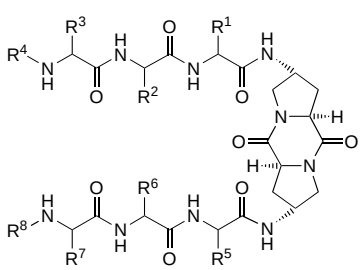


Figure 1. Two-armed diketopiperazine receptors.

4-hydroxyproline provides the rigid backbone and anchor for the peptidic side chains, that is "the arms". Natural L-, as well as D-amino acids, can be employed as building blocks for the

[a] Dr. H. Wennemers, Dipl.-Chem. M. Conza, Dipl.-Chem. M. Nold, Dipl.-Chem. P. Krattiger
Institute of Organic Chemistry, University of Basel
St. Johanns Ring 19, 4056 Basel (Switzerland)
Fax: (+41) 61-267-1105
E-mail: helma.wennemers@unibas.ch

Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/chemistry/ or from the author.

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001
0947-6539/01/0715-3342 $ 17.50+.50/0

receptor arms as they offer structural and functional variety. Standard peptide synthesis can be used to assemble these diketopiperazine receptors which could be easily attached to solid supports through side chain functional groups of the amino acids.

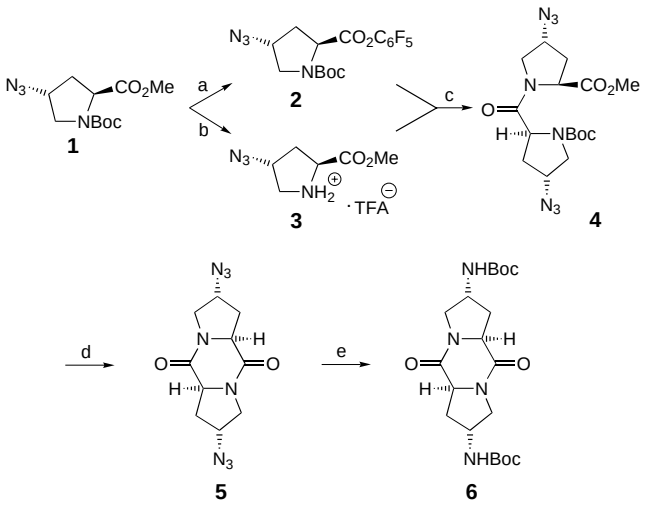
Results and Discussion

Since the diketopiperazine receptors are a novel class of receptors with unknown binding properties, we prepared several receptor prototypes, marked with dyes, and identical arms ($R^1 = R^5$, $R^2 = R^6$, $R^3 = R^7$, $R^3 = R^7 = Ac$) in order to examine their binding properties in on-bead screenings against a peptide library. As a dye we employed the red azo-dye Disperse Red 1 since it can be easily attached to the side chain functionality of an amino acid (such as tyrosine) and does not bind to peptides itself.[6]

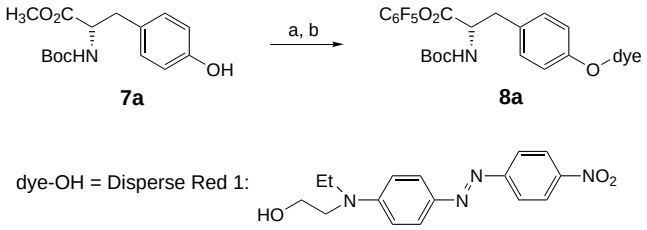
The synthesis of the diketopiperazine template starts from *N*-Boc-4-*trans*-azido-L-proline methyl ester (**1**) which is readily obtained from commercially available *N*-Boc-4-*cis*-hydroxyproline methyl ester by a S_N2 substitution with NaN_3 of the corresponding mesylate.[7] One portion of the *N*-Boc-4-*trans*-azido-L-proline methyl ester (**1**) is then hydrolyzed and transformed into the pentafluorophenyl (Pfp) ester **2** and the other portion is transformed into the trifluoroacetic acid (TFA) salt **3**. Mixing of **2** and **3** in the presence of Hünig's base yields the cyclization precursor **4**. *N*-Boc deprotection with TFA and addition of Hünig's base leads to the diketopiperazine **5**. The reduction of the azide functionalities with palladium on carbon in the presence of di-*tert*-butyl-dicarbonate (Boc_2O) yields the well storable *N*-Boc-protected diketopiperazine **6**.

The red azo-dye Disperse Red 1 is attached to the phenolic hydroxy group of *N*-Boc-L- or D-tyrosine methyl ester **7a** or **7b**, respectively, by a Mitsunobu reaction followed by conversion of the methyl esters into the Pfp esters to yield the dye-marked tyrosine derivatives **8a** and **8b** (Scheme 2).

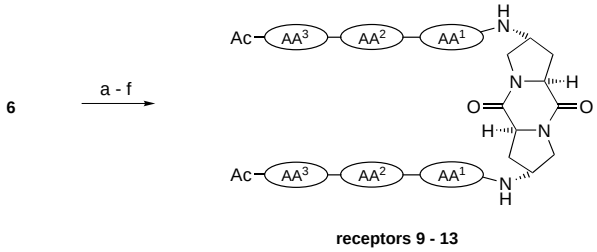
For the assembly of the receptors, the *tert*-butyl groups of **6** are removed and the resulting diamine is coupled with the dye-marked Pfp esters of *N*-Boc-D- or L-tyrosine **8a** or **8b**, respectively. After *N*-Boc deprotection with HCl the remaining amino acids of the arms are assembled by standard couplings of *N*-α-Fmoc-protected amino acids using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) as the coupling reagent and tris(2-aminoethyl)amine (TAEA) as the Fmoc deprotection reagent.[8] Acetylation with acetic anhydride in the presence of triethylamine after the final Fmoc deprotection leads to the diketopiperazine receptors **9**–**13** (Scheme 3).



Scheme 1. Synthesis of the diketopiperazine template **6**: a) i) NaOH, MeOH, THF, H_2O; ii) C_6F_5OH, EDC, CH_2Cl_2, 85%; b) TFA, CH_2Cl_2, quant.; c) iPr$_2$NEt, CH_2Cl_2, 89%; d) i) TFA, CH_2Cl_2; ii) iPr$_2$NEt, CH_2Cl_2, 79%; e) 10% Pd/C, H_2, MeOH, Boc_2O, 91%.



Scheme 2. Synthesis of *N*-Boc-L-Tyr(dye)-OC_6F_5 **8a**: a) Disperse Red 1, PPh_3, DEAD, toluene, 72%; b) i) NaOH, MeOH, THF, H_2O; ii) C_6F_5OH, EDC, CH_2Cl_2, 95%.



Scheme 3. Synthesis of the diketopiperazine receptors **9**–**13**: a) i) TFA, CH_2Cl_2; ii) iPr$_2$NEt, **7**, CH_2Cl_2; b) HCl, MeOH, dioxane; c) iPr$_2$NEt, *N*-α-Fmoc-amino acid, EDC, CH_2Cl_2; d) TAEA, CH_2Cl_2; e) repetition of c) and d) ; f) Ac_2O, NEt_3.

Using this synthesis route five receptor prototypes with closely related yet distinct structures were prepared. The two identical arms of each receptor consist of dye-marked L- or D-Tyr as initial amino acids followed by L-Phe and either L-Asn

Abstract in German: *Mit Hilfe von kombinatorischer Chemie wurde eine neue Klasse von Rezeptoren entwickelt, die aus einem rigiden Diketopiperazin und peptidischen Seitenketten bestehen. Diese Diketopiperazin Rezeptoren zeigen in kombinatorischen on-bead Screenings, dass sie mit peptidischen Substraten mit hoher Spezifität wechselwirken. Das zentrale Diketopiperazin ist aus 4-Hydroxyprolin synthetisch leicht zugänglich und dient als strukturgebendes Templat für die peptidischen Seitenketten, die die Einführung von struktureller als auch funktioneller Vielfalt ermöglichen. Die Screenings einiger farbstoffmarkierter Rezeptorprototypen gegen eine kodierte Tripeptidbibliothek zeigten nicht nur, dass die Diketopiperazin Rezeptoren Peptide mit hoher Selektivität erkennen, sondern auch, dass kleine strukturelle Unterschiede zu signifikanten Änderungen in den Rezeptorbindungseigenschaften führen.*

or L-Gln. Thus, the structural differences of the five prototypes (Figure 2) are as subtle as the opposite stereochemistry of tyrosine (e.g. **9** and **10**) or a single methylene group (e.g. **9** and **12** as well as **11** and **13**).

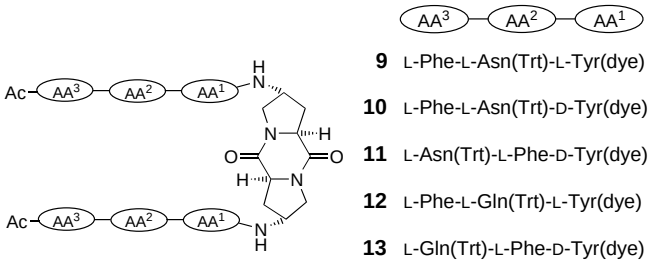


Figure 2. Diketopiperazine receptor prototypes **9**–**13**.

The dye-marked receptor prototypes were tested for their peptide binding affinities by screening them against an encoded resin-bound tripeptide library with the general structure Ac-AA3-AA2-AA1-NH$(CH_2)_6$-CONH-PS. The library had been synthesized on polystyrene resin by encoded split synthesis[9] employing 29 L- and D-amino acids at each position.[10] Thus, the library contained maximally $29^3 = 24\,389$ different acylated tripeptides. In order to ensure a representative screening result, an amount corresponding to at least five theoretical copies of the library were used per assay.[11]

Upon mixing the library with dilute solutions of the receptors ($\approx$20–40 μM) in chloroform and equilibration for at least 24 hours, several beads picked up the red color of the receptor in all five assays. The assays of receptor **9** and **13** indicated a particularly high level of binding specificity since only about 25 beads showed the red color of the receptor corresponding to a selectivity of one selected bead out of 5000. Isolation of the colored beads and analysis of the encoding electrophoric tag molecules by gas chromatography using electron capture detection[9] revealed remarkable specificities: Each of the five structurally similar receptor prototypes selects for different tripeptides within the library (Table 1).

While receptor **9** exclusively selects peptides containing a D-His following two hydrophobic D-amino acids, receptor **13** solely chooses peptides with an L-Asn following a hydrophobic L- and a hydrophobic D-amino acid. Receptor **10** differing from **9** in the configuration of the tyrosine residue, selects for sequences with an L-Asn in the middle or N-terminal position being flanked by a combination of a D- and L-hydrophobic amino acid. More interestingly, receptors **12** and **11** which differ from **9** and **13** only by a single methylene group (L-Asn is exchanged by L-Gln and vice versa) is not only selective for the tripeptides bound by **9** and **13** but also for two different peptide motifs. Thus, the novel diketopiperazine receptors are not only able to bind to peptides but small structural changes alter their ligand pattern to a significant extent.

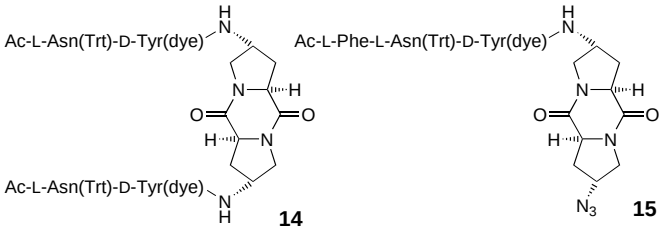
In order to obtain a measure for the strength of the observed intermolecular association we determined the binding affinities of receptors **9** and **12** towards the peptide selected in the on-bead assay and several non-selected peptides by solid-phase binding assays.[4] While receptor **9**, which has a strong preference for the peptide Ac-D-Val-D-Val-D-His, shows an affinity of $K_a = 1420 \pm 200\,M^{-1}$ ($\Delta G = -4.3 \pm 0.1$ kcal mol^{-1}), receptor **12**, which also recognizes other peptides, binds to Ac-D-Val-D-Val-D-His less tightly $K_a = 260 \pm 40\,M^{-1}$ ($\Delta G = -3.3 \pm 0.1$ kcal mol^{-1}). Small modifications of the ligand peptide such as inverting the stereochemistry of the central D-Val lead to a considerably decreased binding affinity of $K_a \leq 15\,M^{-1}$ ($\Delta G \leq 1.5$ kcal mol^{-1}). Thus, our diketopiperazine receptors are not only highly selective for certain tripeptides but also show a considerably higher binding affinity towards the selected peptides as compared to the non-selected peptides.

Table 1. Binding specificities of the receptor prototypes **9**–**13** for tripeptides within the library Ac-AA3-AA2-AA1-NH$(CH_2)_6$CONH-PS.

	AA3	AA2	AA1	freq. found [%][a]	freq. exp. [%][a]
9	D-Val/D-Ala	D-Hph[b]	D-His	100	0.04
10	D-Ala/D-Val	L-Asn/L-Gln	L-Ala/Gly	43	0.03
	L-Asn	D-Pro	L-Hph	57	0.02
11	D-Ala/D-Val	L-Hph	L-Ser/L-Thr	46	0.08
	D-Hph	L-Ala	L-Gln/L-Asn	23	0.04
	D-Hph	L-Hph	L-Ala	17	0.08
12	D-Ala/D-Val	D-Hph	D-His	34	0.04
	L-Ala/L-Leu	L-Gln	D-Hph	37	0.04
	D-Gln	D-Hph	D-Val/D-Leu	20	0.04
13	D-Ala/D-Val/D-Leu	L-Hph	L-Asn	100	0.06

[a] The column frequency found lists the percentage of beads selected in the receptor binding assay for the indicated peptide sequence. The column frequency expected lists the expected frequency for the particular tripeptide sequence if the beads were picked randomly. The comparison between the percentage of "frequency found" and "frequency expected" is a measure for the selectivity level of the receptor. [b] Hph = hydrophobic amino acid can be either Gly, Ala, Val, Leu or Phe.

To verify that the two-armed motif is the minimal structure necessary for binding peptides, truncated receptors with only two amino acids attached to the diketopiperazine **14**, a "single-arm" receptor **15** as well as an "isolated arm" **16** were synthesized and screened against the tripeptide library. Up to fragment concentrations of 500 μM none of the library beads turned red, thus, confirming that two arms consisting of three amino acid residues are crucial for the interactions between the ligand peptide and the receptor.



Ac-L-Phe-L-Asn(Trt)-D-Tyr(dye)-OCH_3 **16**

Figure 3. Receptor fragments **14**–**16**.

Conclusion

We have introduced a novel class of receptors which combine a particularly simple structure with remarkably high binding selectivities that are very responsive to subtle structural

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001
0947-6539/01/0715-3344 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 15

modifications. The novel diketopiperazine derived from natural 4-hydroxyproline has proven an ideal scaffold for guiding the receptor arms into a conformation providing for intermolecular binding. The simplicity of the structure—a simple octapeptide with the structure-directing central diketopiperazine—allows for an easy synthesis and for structural and functional variations. Additional diversity can be introduced into the receptor structure by using a diketopiperazine template with differently functionalized amino groups, therefore, allowing for the generation of two different arms. Thus, the novel diketopiperazine receptors present an ideal scaffold for the development of selective receptors for a given substrate that could be used for any application where selective molecular recognition is a prerequisite for example for the development of diagnostic sensors, therapeutics or catalysts.

Experimental Section

General methods: Materials and reagents were of the highest commercially available grade and used without further purification. Reactions were monitored by thin-layer chromatography using Merck silica gel 60 F_{254} covered aluminum plates. Compounds were visualized by UV, ceric ammonium molybdate (CAM) and ninhydrin. Flash chromatography was performed using Merck silica gel 60, particle size 40–63 μm. Gel filtrations were performed on Sephadex LH20 resin purchased from Sigma. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300, a Bruker DPX 400 or a Bruker DPX 500 spectrometer. Chemical shifts are reported in ppm using $CDCl_3$ as a reference. Infrared spectra were obtained on a Perkin–Elmer 1600 series; peaks are reported in cm^{-1}. Finnigan MAT LCQ and TSQ 700 instruments were used for electrospray ionization (ESI) mass spectrometry. HPLC analysis were carried out on a Nucleosil 100 5 (250 × 4.6 mm) from Macherey Nagel. The solid-phase binding assays were carried out with $CHCl_3$ freshly filtered through aluminum oxide.

***N*-Boc-*trans*-4-Azido-L-proline pentafluorophenyl ester (2)**: *N*-Boc-*trans*-4-Azido-L-proline methyl ester (**1**)[7] (5.56 g, 20.57 mmol) was dissolved in THF/MeOH 1:1 (50 mL). After the addition of NaOH (1.23 g, 30.86 mmol) dissolved in water (5 mL) the mixture was stirred for 1.5 h at room temperature and then carefully acidified with 1M HCl to pH 4. EtOAc (200 mL) and water (100 mL) were added and the mixture was extracted with additional 1M HCl (50 mL). The aqueous layer was extracted with EtOAc (100 mL), the combined organic layers were washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent at reduced pressure yielded a white powder which was suspended in CH_2Cl_2 (10 mL). Addition of pentafluorophenol (3.98 g, 21.60 mmol) and EDC (5.91 g, 30.85 mmol) yielded a solution which was stirred for 1 h at room temperature and then extracted with water (100 mL) and EtOAc (200 mL). The aqueous layer was extracted again with EtOAc (100 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 pentafluorophenyl ester **2** (7.38 g, 17.49 mmol, 85%) as a white solid. (^{1}H and ^{13}C NMR spectra show a double set of peaks (≈2:1) due to the s-*cis* and s-*trans* conformers around the tertiary carbamate.) ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 4.71 (m, 1H; Hα), 4.26 (m, 1H; Hγ), 3.77–3.55 (m, 2H; Hδ), 2.61–2.35 (m, 2H; Hβ), 1.49/1.46 (2s (2:1), 9H; *t*Bu); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25 °C): δ = 168.5, 168.2, 153.8, 153.2, 142.3, 139.7, 81.8, 81.4, 60.4, 59.3, 58.6, 57.3, 57.2, 51.4, 51.3, 36.7, 35.4, 28.2, 28.0; IR (KBr): $\tilde{\nu}$ = 3388, 2991, 2100, 1821, 1715, 1519, 1169; ESI-MS: *m*/*z*: calcd for $C_{16}H_{25}F_5N_4O_4Na$: 445 [*M*+Na]$^+$; found: 445; elemental analysis calcd (%) for $C_{16}H_{25}F_5N_4O_4$ (422.3): C 45.51, H 3.58, N 13.27; found: C 45.34, H 3.65, N 12.94.

TFA · *trans*-4-Azido-L-proline methyl ester (3): The *N*-Boc protected methyl ester **1** (2.00 g, 7.40 mmol) was dissolved in TFA/CH_2Cl_2 1:3 (20 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) and isolated by decantation followed by removal of all residual volatiles in vacuo to yield the TFA-salt **3** (2.10 g, 7.40 mmol, quant.) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.0–7.0 (broad, 2H; NH), 4.47 (m, 2H; Hα, Hγ), 3.82 (s, 3H; CH_3), 3.77 (dd, *J* = 12.6, 6.0 Hz, 1H; Hδ), 3.38 (dd, *J* = 12.6, 2.9 Hz, 1H; Hδ'), 2.45 (m, 2H, Hβ, Hβ'); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25 °C): δ = 168.3, 59.3, 58.0, 53.6, 50.3; IR (KBr): $\tilde{\nu}$ = 3006, 2112, 1748, 1674; elemental analysis calcd (%) for $C_9H_{11}F_3N_4O_4$ (284.2): C 33.81, H 3.90, N 19.71; found: C 33.76, H 3.90, N 19.53.

***N*-Boc-(*trans*-4-Azido-L-Pro)$_2$-OCH$_3$ (4)**: The Pfp-ester **2** (3.12 g, 7.40 mmol) was added to the solution of the TFA-salt **3** (2.10 g, 7.40 mmol) in CH_2Cl_2 (10 mL) and Hünig's base (2.68 mL, 14.80 mmol). After stirring at room temperature for 16 h the mixture was extracted with 0.5M HCl (100 mL) and EtOAc (150 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL) and the organic layers were washed with brine (50 mL) and dried over $MgSO_4$. Filtration and evaporation of the solvent at reduced pressure yielded the crude product which was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 100:0 to 100:5) to yield the dipeptide **4** (2.69 g, 6.59 mmol, 89%) as a slowly solidifying oil. (^{1}H 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 amide and carbamate.) ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.59–4.14 (m, 4H; Hα, Hγ), 3.67/3.66 (2s (2:1), 3H; CH_3), 3.79–3.39 (m, 4H; Hδ), 2.28–1.93 (m, 4H; Hβ), 1.37/1.34 (2s (2:1), 9H; *t*Bu); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25 °C): δ = 171.8, 171.6, 170.8, 170.7, 153.9, 153.1, 80.5, 80.4, 59.6, 59.3, 59.2, 58.7, 57.5, 57.5, 56.3, 56.0, 52.3, 58.3, 51.5, 51.4, 51.3, 51.2, 35.4, 34.7, 34.0, 33.9, 28.1, 28.0; IR (KBr): $\tilde{\nu}$ = 2977, 2105, 1748, 1698, 1665; ESI-MS: *m*/*z*: calcd for $C_{16}H_{24}N_8O_5Na$: 431 [*M*+Na]$^+$; found: 431; elemental analysis calcd (%) for $C_{16}H_{24}N_8O_5$ (408.4): C 47.05, H 5.92, N 27.44, O 19.59; found: C 47.18, H 5.90, N 27.07, O 19.33.

Diketopiperazine (5): Dipeptide **4** (2.15 g, 5.26 mmol) was dissolved in TFA/CH_2Cl_2 1:3 (20 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 (50 mL), Hünig's base (1.83 mL, 10.53 mmol) was added and the mixture was stirred at room temperature for 16 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 **5** (1.15 g, 4.16 mmol, 79%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.45 (dd, *J* = 10.2 Hz, 6.7 Hz, 2H; Hα), 4.34 (ψdt, *J* = 5.1 Hz, 1.4 Hz, 2H; Hγ), 3.70 (dd, *J* = 12.9 Hz, 5.1 Hz, 2H; Hδ), 3.61 (dd, *J* = 12.9 Hz, 1.3 Hz, 2H; Hδ'), 2.43 (ddd, *J* = 15.4 Hz, 6.6 Hz, 1.5 Hz, 2H; Hβ), 2.29 (ddd, *J* = 15.4 Hz, 10.4 Hz, 5.1 Hz, 2H; Hβ'); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25 °C): δ = 166.5, 58.8, 58.7, 50.7, 33.9; IR (KBr): $\tilde{\nu}$ = 2948, 2124, 1661, 1437, 1274; elemental analysis calcd (%) for $C_{10}H_{12}N_8O_2$ (276.3): C 43.48, H 4.38, O 11.58; found: C 43.26, H 4.50, O 11.64.

Diketopiperazine (6): Palladium on carbon (10%, 50 mg) was added to the solution of the diazide **5** (0.99 g, 3.59 mmol) and Boc_2O (2.35 g, 10.76 mmol) in MeOH (20 mL). The black suspension was evacuated, flushed with hydrogen and allowed to stir for 2 h at room temperature. After filtration over celite and removal of the solvent at reduced pressure, the residue was washed with Et_2O (50 mL) and purified by flash chromatography over silica gel (gradient of CH_2Cl_2/MeOH from 100:0 to 100:5) to afford the diketopiperazine **6** (1.38 g, 3.26 mmol, 91%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 5.95 (s, 2H; NH), 4.44 (ψt, *J* = 8.5 Hz, 2H; Hα), 4.22 (m, 2H; Hγ), 3.74 (dd, *J* = 12.8, 6.0 Hz, 2H; Hδ), 3.46 (ψd, *J* = 12.8 Hz, 2H; Hδ'), 2.30 (m, *J* = 8.5 Hz, 4H; Hβ, Hβ'), 1.46 (s, 18H; *t*Bu); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25 °C): δ = 166.5, 155.5, 58.6, 51.6, 33.8, 28.1; IR (KBr): $\tilde{\nu}$ = 3348, 2978, 1706, 1676, 1650, 1528, 1164; ESI-MS: *m*/*z*: calcd for $C_{20}H_{32}N_4O_5Na$: 447 [*M*+Na]$^+$; found: 447; elemental analysis calcd (%) for $C_{20}H_{32}N_4O_6 \cdot H_2O$ (442.5): C 54.28, H 7.74, N 12.66; found: C 54.42, H 7.70, N 12.57.

***N*-Boc-L-Tyr(O-Disperse Red)-OPfp (8a)**: (The D-tyrosine Pfp-ester **8b** was prepared analogously.) *N*-Boc-L-Tyrosine methyl ester **7a** (1.90 g, 6.44 mmol), Disperse Red (2.02 g, 6.44 mmol) and triphenylphosphine (1.69 g, 6.44 mmol) were dissolved in toluene (130 mL). Diethyl azodicarboxylate (1.17 mL, 6.44 mmol) was added dropwise over 15 min and the mixture was allowed to stir at room temperature for 16 h. After removal of the solvent at reduced pressure flash chromatography over silica gel (CH_2Cl_2 then CH_2Cl_2/acetone 10:1) afforded *N*-Boc-L-Tyr(O-Disperse Red) methyl ester (2.75 g, 4.65 mmol, 72%) as a red solid. ^{1}H NMR

(300 MHz, $CDCl_3$, 25 °C): $\delta = 8.27$ (d, $J = 9.1$ Hz, 2H; arom.), 7.88 (d, $J = 9.1$ Hz, 4H; arom.), 7.03 (d, $J = 8.5$ Hz, 2H; arom.), 6.82 (d, $J = 8.5$ Hz, 2H; arom.), 6.79 (d, $J = 9.2$ Hz, 2H; arom.), 5.01 (br d, $J = 7.7$ Hz, 1H; NH), 4.53 (m, 1H; Hα), 4.15 (t, $J = 5.6$ Hz, 2H; CH_2), 3.83 (t, $J = 5.6$ Hz, 2H; CH_2), 3.70 (s, 3H; CH_3), 3.59 (q, $J = 7.0$ Hz, 2H; CH_2), 3.02 (m, 2H; CH_2), 1.41 (s, 9H; *t*Bu), 1.27 (t, $J = 7.0$ Hz, 3H; CH_3); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25 °C): $\delta = 172.2$, 157.4, 156.6, 154.9, 151.2, 147.2, 143.6, 130.3, 128.4, 126.1, 124.5, 122.5, 114.3, 111.3, 79.7, 65.2, 54.4, 52.0, 49.7, 46.0, 37.3, 28.2, 12.2; ESI-MS: m/z: calcd for $C_{31}H_{37}N_5O_7Na$: 614 $[M+Na]^+$; found 614.

N-Boc-L-Tyr(O-Disperse Red)-OCH_3 (2.20 g, 3.72 mmol) was converted into the Pfp-ester **8a** (2.63 g, 3.53 mmol, 95%) following the procedure decribed for the formation of **2**. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta =$ 8.31 (d, $J = 9.2$ Hz, 2H; arom.), 7.92 (d, $J = 9.2$ Hz, 2H; arom.), 7.89 (d, $J =$ 9.1 Hz, 2H; arom.), 7.14 (d, $J = 8.7$ Hz, 2H; arom.), 6.87 (d, $J = 8.6$ Hz, 2H; arom.), 6.81 (d, $J = 9.1$ Hz, 2H; arom.), 4.95 (br d, $J = 8.8$ Hz, 1H; NH), 4.83 (m, 1H; Hα), 4.18 (t, $J = 5.8$ Hz, 2H; CH_2), 3.85 (t, $J = 5.8$ Hz, 2H; CH_2), 3.61 (q, $J = 7.0$ Hz, 2H; CH_2), 3.20 (m, 2H; CH_2), 1.43 (s, 9H; *t*Bu), 1.29 (t, $J = 7.0$ Hz, 3H; CH_3); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25 °C): $\delta = 168.3$, 157.9, 156.8, 155.0, 151.3, 147.4, 143.8, 130.5, 127.4, 126.3, 124.7, 122.6, 114.8, 111.4, 80.7, 65.4, 54.5, 49.9, 46.2, 37.0, 28.2, 12.3; ESI-MS: m/z: calcd for $C_{36}H_{35}F_5N_5O_7$: 744 $[M+H]^+$; found: 744.

Synthesis of the receptors (9 – 13)

Coupling of 7: The *tert*-butyl-protecting groups of bis-*N*-Boc-protected diketopiperazine **6** (50 mg, 0.12 mmol) were removed with TFA/CH_2Cl_2 1:3 (4 mL) as described for the formation of the TFA-salt **3**. The residue was dissolved in a solution of Hünig's base (90 µL, 0.47 mmol) in CH_2Cl_2 (2 mL), the pentafluorophenyl ester **8a** or **8b** (193 mg, 0.25 mmol) was added and the mixture was allowed to stir at room temperature for 16 h. After a filtration over silica gel (gradient of CH_2Cl_2/MeOH from 100:0 to 100:5) the diketopiperazine with the attached *N*-Boc protected tyrosine was isolated. The red solid was dissolved in MeOH (1 mL) and 4 M HCl in dioxane (2 mL) and allowed to react for 0.5 h. The corresponding HCl salt was isolated as described for **3**.

Coupling of the *N*-α-Fmoc protected amino acids: The desired *N*-α-Fmoc protected amino acid (0.48 mmol) was added along with EDC (92 mg, 0.48 mmol) to the solution of the diamino receptor precursor in CH_2Cl_2 (max. 1 mL) and the mixture was stirred at room temperature for 30 min. The mixture was extracted with EtOAc (15 mL) and 0.5 M HCl (5 mL), the aqueous layer was extracted with EtOAc (20 mL) and the organic layers were washed with brine and dried over Na_2SO_4. Filtration, removal of the solvent at reduced pressure and a filtration over silica gel (gradient of CH_2Cl_2/MeOH) afforded the *N*-Fmoc-protected receptor precursor which was used without further purification.

Fmoc deprotection: The *N*-Fmoc-protected receptor precursor was dissolved in CH_2Cl_2 (5 mL) followed by addition of tris(2-aminoethyl)amine (0.45 mL, 3.00 mmol) which caused the formation of a precipitate after 2 – 5 min. The suspension was stirred at room temperature for 0.5 h and then first extracted with brine (3×10 mL) followed by phosphate buffer pH 5.5 (3×10 mL). The aqueous layers were washed once with CH_2Cl_2 (10 mL) the organic layers were dried over $MgSO_4$ to yield the corresponding amine after removal of all volatiles at reduced pressure.

The coupling and Fmoc deprotection cycle was repeated until the desired tripeptidic arms were assembled. After the final Fmoc deprotection, the diamine was dissolved in CH_2Cl_2 (1 mL) followed by the addition of triethylamine (0.067 mL, 0.48 mmol) and acetic anhydride (45 µL, 0.48 mmol). After stirring the mixture for 30 min, the acetylated receptor was purified by flash chromatography (gradient of CH_2Cl_2/MeOH 100:0 to 100:10) and gel filtration (LH 20, CH_2Cl_2/MeOH 95:5). Receptors **9 – 13** were isolated in amounts of 25 – 40 mg (10 – 15% from **6**).

Receptor (9): ^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 8.17$ (d, $J = 9.1$ Hz, 4H; dye), 7.76 (d, $J = 9.1$ Hz, 4H; dye), 7.73 (d, $J = 9.3$ Hz, 4H; dye), 7.12 – 6.94 (m, 40H; trityl, Phe), 6.89 (d, $J = 8.7$ Hz, 4H; Tyr), 6.62 (d, $J = 9.3$ Hz, 4H; dye), 6.60 (d, $J = 8.7$ Hz, 4H; Tyr), 4.31 (dd, $J = 8.3$ Hz, 5.2 Hz, 2H; Tyr-Hα), 4.12 (dd, $J = 9.6$ Hz, 5.1 Hz, 2H; Phe-Hα), 4.17 (ψt, $J = 6.6$ Hz, 2H; Asn-Hα), 4.07 (m, 2H; Pro-Hγ), 3.91 (ψt, $J = 8.1$ Hz, 2H; Pro-Hα), 3.85 (t, $J = 5.7$ Hz, 4H; OCH_2CH_2N), 3.57 (t, $J = 5.7$ Hz, 4H; OCH_2CH_2N), 3.50 (dd, $J = 12.3$ Hz, 2.5 Hz, 2H; Pro-Hδ), 3.40 (q, $J =$ 7.1 Hz, 4H; CH_2CH_3), 3.02 (dd, $J = 12.3$ Hz, 4.0 Hz, 2H; Pro-Hδ'), 2.91 (dd, $J = 14.3$ Hz, 5.1 Hz, 2H; Tyr-Hβ), 2.75 (dd, $J = 14.3$ Hz, 8.3 Hz, 2H; Tyr-Hβ'), 2.70 (dd, $J = 14.0$ Hz, 5.0 Hz, 2H; Phe-Hβ), 2.63 (dd, $J = 15.2$ Hz, 6.6 Hz, 2H; Asn-Hβ), 2.47 (dd, $J = 14.0$ Hz, 9.6 Hz, 2H; Phe-Hβ'), 2.37 (dd, $J = 15.2$ Hz, 6.5 Hz, 2H; Asn-Hβ'), 2.10 (m, 2H; Pro-Hβ), 1.85 (m, 2H; Pro-Hβ'), 1.68 (s, 6H; $COCH_3$), 1.10 (t, $J = 7.0$ Hz, 6H; CH_2CH_3); ^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 172.4$, 171.7, 170.8, 170.6, 169.7, 166.4, 157.2, 156.6, 151.2, 147.0, 143.8, 143.4, 136.0, 130.0, 129.0, 128.7, 128.4, 128.3, 127.6, 126.7, 126.0, 124.4, 122.3, 114.1, 111.2, 70.2, 65.0, 58.3, 54.9, 54.4, 50.8, 49.8, 49.2, 47.2, 45.8, 37.1, 37.0, 35.6, 32.4, 22.0, 11.8; HRMS (ESI): m/z: calcd for $C_{128}H_{128}N_{20}O_{18}$: 1117.4931 $[M+2H]^{2+}$; found: 1117.4927.

Receptor (10): ^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 8.27$ (d, $J = 9.1$ Hz, 4H; dye), 7.87 (d, $J = 9.1$ Hz, 4H; dye), 7.85 (d, $J = 9.1$ Hz, 4H; dye), 7.24 – 7.04 (m, 44H; trityl, Phe, Tyr-2H), 6.75 (d, $J = 9.2$ Hz, 4H; dye), 6.71 (d, $J = 8.6$ Hz, 4H; Tyr), 4.49 (dd, $J = 10.3$ Hz, 4.0 Hz, 2H; Tyr-Hα), 4.32 (dd, $J = 8.1$ Hz, 5.4 Hz, 2H; Phe-Hα), 4.16 – 4.07 (m, 6H; Pro-Hα, Pro-Hγ, Asn-Hα), 4.06 (t, $J = 5.7$ Hz, 4H; OCH_2CH_2N), 3.77 (t, $J =$ 5.7 Hz, 4H; OCH_2CH_2N), 3.60 (dd, $J = 12.6$ Hz, 6.4 Hz, 2H; Pro-Hδ), 3.54 (q, $J = 7.0$ Hz, 4H; CH_2CH_3), 3.23 (dd, $J = 14.3$ Hz, 4.0 Hz, 2H; Tyr-Hβ), 3.01 (br d, $J = 12.6$ Hz, 2H; Pro-Hδ'), 2.93 (dd, $J = 14.0$ Hz, 5.4 Hz, 2H; Phe-Hβ), 2.81 (dd, $J = 14.1$ Hz, 8.2 Hz, 2H; Phe-Hβ'), 2.78 – 2.69 (m, 6H; Tyr-Hβ', Asn-Hβ, Asn-Hβ'), 2.18 – 2.11 (m, 4H; Pro-Hβ, Pro-Hβ'), 1.84 (s, 6H; $COCH_3$), 1.22 (t, $J = 7.0$ Hz, 6H; CH_2CH_3); ^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 172.8$, 172.1, 171.4, 170.8, 169.9, 166.2, 157.2, 156.8, 151.4, 147.3, 144.1, 143.6, 136.0, 130.2, 130.0, 129.2, 128.6, 128.5, 127.9, 127.1, 127.0, 126.3, 124.7, 114.3, 111.4, 70.1, 65.2, 58.6, 55.2, 54.7, 50.7, 50.7, 49.8, 47.6, 46.1, 37.2, 37.0, 35.6, 33.1, 22.3, 12.2; HRMS (ESI): m/z: calcd for $C_{128}H_{128}N_{20}O_{18}$: 1117.4931 $[M+2H]^{2+}$; found: 1117.4935.

Receptor (11): ^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 8.32$ (d, $J = 9.1$ Hz, 4H; dye), 7.91 (d, $J = 9.2$ Hz, 4H; dye), 7.89 (d, $J = 9.3$ Hz, 4H; dye), 7.28 – 7.14 (m, 36H; trityl, Phe-6H), 7.04 (d, $J = 6.9$ Hz, 4H; Phe), 6.81 (d, $J = 9.3$ Hz, 4H; dye), 6.73 (d, $J = 8.8$ Hz, 4H; Tyr), 6.70 (d, $J =$ 8.9 Hz, 4H; Tyr), 4.37 (m, 4H; Asn-Hα, Pro-Hα), 4.21 (m, 4H; Tyr-Hα, Pro-Hγ), 4.12 (t, $J = 5.8$ Hz, 4H; OCH_2CH_2N), 3.98 (ψt, $J = 7.1$ Hz, 2H; Phe-Hα), 3.83 (t, $J = 5.7$ Hz, 4H; OCH_2CH_2N), 3.60 (q, $J = 7.0$ Hz, 4H; CH_2CH_3), 3.45 (dd, $J = 12.4$ Hz, 4.5 Hz, 2H; Pro-Hδ), 3.18 (m, 2H; Pro-Hδ'), 2.91 (dd, $J = 13.7$ Hz, 7.1 Hz, 2H; Phe-Hβ), 2.84 (m, 4H; Tyr-Hβ, Asn-Hβ), 2.77 (dd, $J = 13.7$ Hz, 7.1 Hz, 2H; Phe-Hβ'), 2.71 (m, 2H; Tyr-Hβ'), 2.61 (dd, $J = 15.4$ Hz, 5.3 Hz, 2H; Asn-Hβ'), 2.35 (m, 2H; Pro-Hβ), 2.06 (m, 2H; Pro-Hβ'), 1.86 (s, 6H; $COCH_3$), 1.27 (t, $J = 7.0$ Hz, 6H; CH_2CH_3); ^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 171.8$, 171.5, 170.9, 170.9, 170.3, 166.4, 157.2, 156.7, 151.2, 147.3, 144.2, 143.6, 136.3, 130.3, 129.1, 128.7, 128.5, 127.7, 127.0, 126.9, 126.2, 124.6, 122.5, 114.3, 111.3, 70.5, 65.2, 58.8, 56.4, 54.0, 50.7, 50.6, 49.8, 48.1, 46.1, 37.1, 36.6, 35.1, 33.1, 22.7, 12.2; HRMS (ESI): m/z: calcd for $C_{128}H_{128}N_{20}O_{18}$: 1117.4931 $[M+2H]^{2+}$; found: 1117.4945.

Receptor (12): ^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 8.30$ (d, $J = 9.1$ Hz, 4H; dye), 7.87 (d, $J = 9.1$ Hz, 4H; dye), 7.83 (d, $J = 9.3$ Hz, 4H; dye), 7.32 – 7.09 (m, 44H; trityl, Phe, Tyr-4H), 6.76 (d, $J = 8.8$ Hz, 4H; Tyr), 6.72 (d, $J = 9.3$ Hz, 4H; dye), 4.63 (dd, $J = 11.8$ Hz, 3.4 Hz, 2H; Tyr-Hα), 4.58 (m, 2H; Pro-Hγ), 4.52 (dd, $J = 10.0$ Hz, 7.4 Hz, 2H; Pro-Hα), 4.12 (dd, $J = 10.6$ Hz, 3.9 Hz, 2H; Phe-Hα), 4.04 (t, $J = 5.7$ Hz, 4H; OCH_2CH_2N), 3.92 (m, 2H; Gln-Hα), 3.73 (t, $J = 5.7$ Hz, 4H; OCH_2CH_2N), 3.61 (dd, $J = 12.4$ Hz, 5.7 Hz, 2H; Pro-Hδ), 3.53 (q, $J = 7.1$ Hz, 4H; CH_2CH_3), 3.48 (br d, $J = 12.4$ Hz, 2H; Pro-Hδ'), 3.41 (dd, $J = 14.3$ Hz, 3.3 Hz, 2H; Tyr-Hβ), 3.07 (dd, $J = 14.3$ Hz, 3.8 Hz, 2H; Phe-Hβ), 2.79 (dd, $J = 14.3$ Hz, 11.8 Hz, 2H; Tyr-Hβ'), 2.52 (dd, $J = 14.3$ Hz, 10.7 Hz, 2H; Phe-Hβ'), 2.30 (m, 2H; Pro-Hβ), 2.15 (m, 2H; Pro-Hβ'), 1.95 (m, 2H; Gln-Hγ), 1.75 (m, 4H; Gln-Hγ', Gln-Hβ), 1.59 (m, 2H; Gln-Hβ'), 1.62 (s, 6H; $COCH_3$), 1.23 (t, $J = 7.1$ Hz, 6H; CH_2CH_3); ^{13}C NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 173.8$, 173.5, 172.6, 171.0, 170.4, 167.5, 157.1, 156.7, 151.2, 147.4, 144.2, 143.7, 136.1, 131.1, 130.4, 128.9, 128.7, 128.1, 127.5, 127.2, 126.3, 124.7, 122.6, 114.3, 111.4, 70.9, 65.5, 59.0, 56.4, 55.3, 54.6, 50.5, 49.8, 48.1, 46.1, 36.7, 36.1, 33.9, 32.9, 25.3, 22.9, 12.4; HRMS (ESI): m/z: calcd for $C_{130}H_{132}N_{20}O_{18}$ 1131.5087: $[M+2H]^{2+}$; found: 1131.5085.

Receptor (13): ^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25 °C): $\delta = 8.32$ (d, $J = 9.1$ Hz, 4H; dye), 7.91 (d, $J = 9.2$ Hz, 4H; dye), 7.89 (d, $J = 9.2$ Hz, 4H; dye), 7.26 – 7.16 (m, 36H; trityl, Phe-6H), 6.97 (d, $J = 7.0$ Hz, 4H; Phe), 6.82 (d, $J = 8.7$ Hz, 4H; Tyr), 6.80 (d, $J = 9.2$ Hz, 4H; dye), 6.71 (d, $J =$ 8.7 Hz, 4H; Tyr), 4.42 (dd, $J = 7.7$ Hz, 5.3 Hz, 2H, Tyr-Hα), 4.38 (dd, $J =$ 10.5 Hz, 7.6 Hz, 2H, Pro-Hα), 4.28 (ψt, $J = 5.7$ Hz, 2H; Pro-Hγ), 4.13 (ψt, $J = 7.1$ Hz, 2H; Gln-Hα), 4.09 (t, $J = 5.8$ Hz, 4H; OCH_2CH_2N), 3.87 (ψt, $J = 8.4$ Hz, 2H; Phe-Hα), 3.81 (t, $J = 5.8$ Hz, 4H; OCH_2CH_2N), 3.65 (dd,

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001
0947-6539/01/0715-3346 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 15

$J = 12.9, 5.7$ Hz, 2H; Pro-Hδ), 3.58 (m, $J = 7.1$ Hz, 6H; CH_2CH$_3$, Pro-Hδ'), 2.94 (dd, $J = 14.0, 7.7$ Hz, 2H, Tyr-Hβ), 2.82–2.77 (m, 4H; Phe-Hβ, Tyr-Hβ'), 2.72 (dd, $J = 13.6, 8.4$ Hz, 2H; Phe-Hβ'), 2.41–2.35 (m, 4H; Gln-Hγ, Pro-Hβ), 2.31 (m, 2H; Gln-Hγ'), 2.17 (m, 2H; Pro-Hβ'), 1.88 (s, 6H; COCH_3), 1.76 (m, 4H, Gln-Hβ, Gln-Hβ'), 1.26 (t, $J = 7.1$ Hz, 6H; CH$_2$CH_3); ^{13}C NMR (125.6 MHz, 5% CD$_3$OD in CDCl$_3$, 25 °C): $\delta = 173.1$, 172.5, 171.8, 171.3 (2 × C=O), 166.3, 157.4, 156.8, 151.3, 147.3, 144.3, 143.7, 136.6, 130.4, 129.3, 129.1, 128.7, 128.6, 127.8, 127.0, 126.3, 124.7, 122.6, 114.4, 111.4, 70.5, 65.3, 59.0, 56.6, 54.1, 52.4, 49.6, 49.1, 48.2, 46.1, 36.1, 35.2, 33.6, 32.1, 27.2, 22.5, 12.2; HRMS (ESI): m/z: calcd for $C_{130}H_{132}N_{20}O_{18}$: 1131.5087 $[M+2H]^{2+}$; found 1131.5068.

Receptorfragment (14): ^{1}H NMR (500 MHz, 5% CD$_3$OD in CDCl$_3$, 25 °C): $\delta = 8.31$ (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), 7.26–7.14 (m, 30H; trityl), 7.01 (d, $J = 8.7$ Hz, 4H; Tyr), 6.80 (d, $J = 9.3$ Hz, 4H; dye), 6.78 (d, $J = 8.7$ Hz, 4H; Tyr), 4.47 (ψt, $J = 6.4$ Hz, 2H; Tyr-Hα), 4.44 (ψt, $J = 5.6$ Hz, 2H; Asn-Hα), 4.15 (m, 2H; Pro-Hγ), 4.13 (t, $J = 5.8$ Hz, 4H; OCH_2CH$_2$N), 3.92 (ψt, $J = 8.3$ Hz, 2H; Pro-Hα), 3.82 (t, $J = 5.8$ Hz, 4H; OCH$_2$CH_2N), 3.74 (dd, $J = 12.4$ Hz, 6.8 Hz, 2H; Pro-Hδ), 3.60 (q, $J = 7.1$ Hz, 4H; CH_2CH$_3$), 3.07 (dd, $J = 14.1$ Hz, 6.7 Hz, 2H; Tyr-Hβ), 3.03 (dd, $J = 12.4$ Hz, 4.2 Hz, 2H; Pro-Hδ'), 2.95 (m, 4H; Tyr-Hβ', Asn-Hβ), 2.55 (dd, $J = 15.8$ Hz, 5.5 Hz, 2H; Asn-Hβ'), 2.48 (m, 2H; Pro-Hβ), 2.45 (m, 2H, Pro-Hβ'), 1.83 (s, 6H; COCH_3), 1.27 (t, $J = 7.1$ Hz, 6H; CH$_2$CH_3); ^{13}C NMR (125.6 MHz, 5% CD$_3$OD in CDCl$_3$, 25 °C): $\delta = 171.2$, 171.0, 170.6, 169.9, 166.0, 157.5, 156.8, 151.3, 147.3, 144.0, 143.7, 130.5, 128.9, 128.6, 128.0, 127.1, 126.3, 124.7, 122.6, 114.5, 111.4, 70.6, 65.4, 58.3, 53.9, 50.4, 50.4, 49.8, 47.3, 46.1, 37.9, 35.6, 32.7, 22.7, 12.2; HRMS (ESI): m/z: calcd for $C_{110}H_{110}N_{18}O_{16}$: 1939.8420 $[M+H]^+$; found: 1939.8405.

Receptorfragment (15): ^{1}H NMR (500 MHz, CDCl$_3$, 25 °C): $\delta = 8.32$ (d, $J = 9.1$ Hz, 2H; dye), 7.92 (d, $J = 9.1$ Hz, 2H; dye), 7.90 (d, $J = 9.3$ Hz, 2H; dye), 7.33–7.12 (m, 22H; trityl, Phe, Asn-NH, Phe-NH), 7.08 (d, $J = 8.6$ Hz, 2H; Tyr), 6.87 (d, $J = 8.6$ Hz, 1H, Pro-NH), 6.80 (m, 1H, Tyr-NH), 6.78 (d, $J = 9.3$ Hz, 2H; dye), 6.75 (d, $J = 8.6$ Hz, 2H; Tyr), 6.11 (m, 1H, Asn-NH), 4.63 (dd, $J = 14.6$ Hz, 6.3 Hz, 1H; Tyr-Hα), 4.47 (m, 1H; Phe-Hα), 4.40 (dd, $J = 11.6$ Hz, 5.6 Hz, 1H; Asn-Hα), 4.30 (ψt, $J = 4.9$ Hz, 1H; Pro-Hγ), 4.15 (m, 2H; Pro-Hα, Pro′-Hγ), 4.08 (t, $J = 5.8$ Hz, 2H; OCH_2CH$_2$N), 3.78 (t, $J = 5.7$ Hz, 2H; OCH$_2$CH_2N), 3.71 (ψt, $J = 8.4$ Hz, 1H; Pro′-Hα), 3.63 (dd, $J = 13.1, 5.1$ Hz, 1H; Pro-Hδ), 3.58 (q, $J = 7.0$ Hz, 2H; CH_2CH$_3$), 3.51 (br d, $J = 13.0$ Hz, 1H; Pro-Hδ'), 3.46 (m, 1H; Pro′-Hδ), 3.16 (dd, $J = 16.5$, 11.6 Hz, 1H; Asn-Hβ), 3.07 (m, 3H; Phe-Hβ, Tyr-Hβ, Tyr-Hβ'), 2.93 (dd, $J = 14.2$ Hz, 7.7 Hz, 1H; Phe-Hβ'), 2.88 (dd, $J = 13.9$ Hz, 3.0 Hz, 1H; Pro′-Hδ'), 2.64 (dd, $J = 16.5$, 5.5 Hz, 1H; Asn-Hβ'), 2.32 (br dd, $J = 13.8$, 6.7 Hz, 1H; Pro-Hβ), 2.22 (ddd, $J = 13.8$, 10.6, 4.9 Hz, 1H; Pro-Hβ'), 2.04 (m, 2H, Pro′-Hβ, Pro′-Hβ'), 1.69 (s, 6H; COCH_3), 1.26 (t, $J = 7.0$ Hz, 6H; CH$_2$CH_3); ^{13}C NMR (125.6 MHz, CDCl$_3$, 25 °C): $\delta = 171.5$, 171.2, 171.0, 170.7, 169.7, 166.0, 165.5, 157.4, 156.8, 151.3, 147.3, 144.1, 143.7, 135.8, 130.7, 129.3, 128.9, 128.6, 128.0, 127.4, 127.1, 126.3, 124.7, 122.6, 114.4, 111.4, 70.7, 65.2, 58.7, 58.5, 58.3, 54.9, 54.5, 50.6, 50.3, 50.0, 49.8, 47.8, 46.1, 37.3, 37.1, 35.9, 34.0, 33.4, 22.7, 12.3; IR (KBr): $\tilde{\nu} = 3311$, 2925, 2105, 1664, 1599, 1133; HRMS (ESI): m/z: calcd for $C_{69}H_{70}N_{14}O_{10}$: 1255.5472 $[M+H]^+$; found 1255.5474.

Ac-L-Phe-L-Asn(Trt)-D-Tyr(dye)-OCH$_3$ (16): ^{1}H NMR (500 MHz, CDCl$_3$, 25 °C): $\delta = 8.32$ (d, $J = 9.1$ Hz, 2H; dye), 7.91 (d, $J = 9.1$ Hz, 2H; dye), 7.90 (d, $J = 9.1$ Hz, 2H; dye), 7.53 (d, $J = 7.7$ Hz, 1H, Asn-NH), 7.27–7.10 (m, 21H; trityl, Phe, Tyr-NH), 7.03 (d, $J = 8.7$ Hz, 2H; Tyr), 7.01 (s, 1H, Asn-NHγ), 6.79 (d, $J = 9.3$ Hz, 2H; dye), 6.77 (d, $J = 8.7$ Hz, 2H; Tyr), 5.90 (d, $J = 6.8$ Hz, 1H, Phe-NH), 4.66 (ddd, $J = 7.6, 6.2, 3.8$ Hz, 1H; Asn-Hα), 4.62 (br ddd, $J = 7.9, 5.7, 2.0$ Hz, 1H; Tyr-Hα), 4.54 (ddd, $J = 6.8, 5.5$ Hz, 2.8 Hz, 1H; Phe-Hα), 4.11 (t, $J = 5.9$ Hz, 2H; OCH_2CH$_2$N), 3.81 (t, $J = 5.9$ Hz, 2H; OCH$_2$CH_2N), 3.62 (s, 3H, OCH$_3$), 3.59 (q, $J = 7.1$ Hz, 2H; CH_2CH$_3$), 3.07 (dd, $J = 10.1, 5.5$ Hz, 1H; Phe-Hβ), 3.05 (dd, $J = 10.1$ Hz, 5.6 Hz, 1H; Tyr-Hβ), 2.92 (dd, $J = 15.3, 3.8$ Hz, 1H; Asn-Hβ), 2.88 (m, 1H, Phe-Hβ'), 2.86 (dd, $J = 10.1$ Hz, 2.0 Hz, 1H; Tyr-Hβ'), 2.46 (dd, $J = 15.3$ Hz, 6.3 Hz, 1H; Asn-Hβ'), 1.80 (s, 6H; COCH_3), 1.27 (t, $J = 7.1$ Hz, 6H; CH$_2$CH_3); ^{13}C NMR (125.6 MHz, CDCl$_3$, 25 °C): $\delta = 171.3$, 171.3, 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, 128.7, 128.6, 127.9, 127.1, 127.0, 126.3, 124.7, 122.6, 114.4, 111.4, 70.1, 65.1, 54.8, 54.2, 52.2, 49.8, 49.7, 46.1, 37.5, 36.9, 22.9, 12.3; ESI-MS: m/z: calcd for $C_{60}H_{60}N_8O_9Na$: 1060 $[M+Na]^+$; found: 1060; elemental analysis calcd (%) for $C_{60}H_{60}N_8O_9$ (1037.2): C 69.48, H 5.83, N 10.80, O 13.88; found: C 69.15, H 6.07, N 10.63, O 14.11.

General procedure for the determination of binding constants on the solid support by UV: An accurately measured amount (ca. 5 mg) of resin-bound peptide was placed in a 1 mL UV cuvette. The UV cuvette was then charged with a solution of the receptor in CHCl$_3$, sealed with a teflon stopper and slightly agitated for 72 h. After this period of time the UV absorbance did not change anymore. The beads were allowed to float to the top of the CHCl$_3$ solution before determining the receptor concentration at equilibrium by UV. For the calculation of the binding constant it was assumed that all peptides on the resin are able to participate in the binding process.

Acknowledgements

This work was supported by the BACHEM company. We thank Dr. Mark Brönstrup from Aventis for recording the HRMS. H.W. is grateful to the Fonds der Chemischen Industrie for a Liebig Fellowship and an assistant professorship sponsored by the BACHEM company.

[1] For reviews see: a) W. C. Still, *Acc. Chem. Res.* **1996**, *29*, 155–163; b) C. Gennari, H. P. Nestler, U. Piarulli, B. Salom, *Liebigs Ann./Recueil* **1997**, 637–647; c) Y. R. de Miguel, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **1998**, *2*, 417–421; d) H. P. Nestler, R. Liu, *Comb. Chem. High Throughput Screening* **1998**, *1*, 113–126.

[2] a) A. Borchardt, W. C. Still, *J. Am. Chem. Soc.* **1994**, *116*, 373–374; b) H. Wennemers, S. S. Yoon, W. C. Still, *J. Org. Chem.* **1995**, *60*, 1108–1109; c) S. S. Yoon, W. C. Still, *Tetrahedron* **1995**, *51*, 567–578; d) M. Maletic, H. Wennemers, D. Q. McDonald, R. Breslow, W. C. Still, *Angew. Chem.* **1996**, *108*, 1594–1596; *Angew. Chem. Int. Ed.* **1996**, *35*, 1490–1492.

[3] a) R. C. Boyce, G. Li, H. P. Nestler, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1994**, *116*, 7955–7956; b) C. Gennari, H. P. Nestler, B. Salom, W. C. Still, *Angew. Chem.* **1995**, *107*, 1894–1896; *Angew. Chem. Int. Ed.* **1995**, *34*, 1765–1768; c) M. Davies, M. Bonnat, F. Guillier, J. D. Kilburn, M. Bradley, *J. Org. Chem.* **1998**, *63*, 8696–8703; d) H. Hioki, T. Yamada, C. Fujioka, M. Kodama, *Tetrahedron Lett.* **1999**, *40*, 6821–6825; e) T. Fessmann, J. D. Kilburn, *Angew. Chem.* **1999**, *111*, 2170–2174; *Angew. Chem. Int. Ed.* **1999**, *38*, 1993–1996; f) H. De Muynck, A. Madder, N. Farcy, P. J. De Clercq, M. N. Pérez-Payán, L. M. Öhberg, A. P. Davis, *Angew. Chem.* **2000**, *112*, 149–152; *Angew. Chem. Int. Ed.* **2000**, *39*, 145–148.

[4] a) Y. Cheng, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1996**, *118*, 1813–1814; b) D. W. P. M. Löwik, M. D. Weingarten, M. Broekema, A. J. Brouwet, W. C. Still, R. M. J. Liskamp, *Angew. Chem.* **1998**, *110*, 1947–1950; *Angew. Chem. Int. Ed.* **1998**, *37*, 1846–1850.

[5] a) Á. Furka, F. Sebestyén, M. Asgedom, G. Dibô, *Int. J. Pept. Protein Res.* **1991**, *37*, 487–493; b) K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, R. J. Knapp, *Nature* **1991**, *354*, 82–84.

[6] H. Wennemers, W. C. Still, *Tetrahedron Lett.* **1994**, *35*, 6413–6417.

[7] **1**: CAS [121147-97-5], a) B. P. Gangamani, V. A. Kumar, K. N. Ganesh, *Tetrahedron* **1996**, *52*, 15017–15030; b) M. L. Petersen, R. Vince, *J. Med. Chem.* **1991**, *34*, 2787–2797; c) K. Eva Ng, L. E. Orgel, *J. Med. Chem.* **1989**, *32*, 1754–1757.

[8] a) M. Beyermann, M. Bienert, H. Niedrich, L. A. Carpino, D. Sadat-Aalee, *J. Org. Chem.* **1990**, *55*, 721–728; b) L. A. Carpino, D. Sadat-Aalaee, M. Beyermann, *J. Org. Chem.* **1990**, *55*, 1673–1675.

[9] a) M. H. J. Ohlmeyer, R. N. Swanson, L. W. Dillard, J. C. Reader, G. Asouline, R. Kobayashi, M. H. Wigler, W. C. Still, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922–10926; b) H. P. Nestler, P. Bartlett, W. C. Still, *J. Org. Chem.* **1994**, *59*, 4723–4724.

[10] AA1–AA3 = Gly, D-Ala, L-Ala, D-Val, L-Val, D-Leu, L-Leu, D-Phe, L-Phe, D-Pro, L-Pro, D-Ser, L-Ser, D-Thr, L-Thr, D-Asp, L-Asp, D-Glu, L-Glu, D-Asn, L-Asn, D-Gln, L-Gln, D-His, L-His, D-Lys, L-Lys, D-Arg, L-Arg.

[11] a) K. Burgess, A. I. Liaw, N. Y. Wang, *J. Med. Chem.* **1994**, *37*, 2985–2987; b) P.-L. Zhao, R. Zambias, J. A. Bolognese, D. Boulton, K. T. Chapman, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 10212–10216.

Received: January 30, 2001 [F3053]