DOI: 10.1002/chem.200500876

Synthesis of Unsymmetrical Tweezer Receptor Libraries and Identification of Receptors for Lys-D-Ala-D-Ala in Aqueous Solution

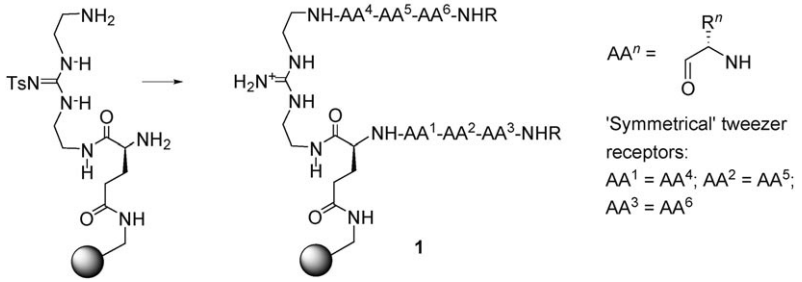
Jon Shepherd, Tom Gale, Kim B. Jensen, and Jeremy D. Kilburn*[a]

Abstract: Libraries of "unsymmetrical" tweezer receptors, featuring a guanidinium head group as a carboxylate binding site and two independently synthesized peptidic arms, have been prepared and screened to identify receptors for the *N*-Ac-Lys-D-Ala-D-Ala tripeptide sequence. The binding properties of one such receptor structure, with dye-labeled *N*-Ac-Lys-D-Ala-D-Ala, were investigated. These studies demonstrated that when attached to the solid-phase, the receptor binds dye-labeled *N*-Ac-Lys-D-Ala-D-Ala, in buffered aqueous media, with mM binding affinity.

Keywords: library · peptides · receptors · solid-phase synthesis

Introduction

The selective binding of peptides with synthetic receptors remains a major challenge, particularly, if binding is to be successful in competitive aqueous media,[1] which is essential if such systems are to be of use in the physiological milieu. In recent years, a variety of receptors, featuring one or more peptidic arms attached to a rigid template, have proved to be effective and sequence selective for peptides, despite the inherent flexibility of many of these receptor systems.[2-4] In addition, the use of a combinatorial, split-and-mix approach to randomize the amino acid content in the peptidic arms has provided a powerful method for the construction of libraries of potential peptide receptors,[5] and this approach has been used successfully for the identification of sequence selective receptors for a variety of peptides, in both nonpolar organic[3] and aqueous solvents.[2,4] The incorporation of a specific recognition site for the carboxylate functionality into the general receptor structure has facilitated the identification of receptors for peptides with a free carboxylate terminus. In this context, we have used bis(aminoalkyl)guanindiniums as a scaffold to prepare libraries of two-armed "tweezer" receptor structures **1** (Scheme 1) and hence identified receptors for a side-chain-protected tripeptide in aqueous media.[2e]



Scheme 1. Two-armed tweezer receptor structures **1**.

Recently, Schmuck has used linear peptides, capped with a guanidinopyrrole as a carboxylate binding site, to identify receptors that bind the amyloid peptide (Val-Val-Ile-Ala) in aqueous solution.[2c,d,6] Although not incorporating a specific carboxylate binding site, libraries of three-armed receptors, based on a cyclotriveratrylene scaffold, have also been screened by Liskamp who utilized the dye-labeled dipeptides D-Ala-D-Ala and D-Ala-D-Lac, with a free carboxylate

[a] J. Shepherd, Dr. T. Gale, Dr. K. B. Jensen, Prof. J. D. Kilburn
School of Chemistry
University of Southampton
Southampton, SO17 1BJ (UK)
Fax: (+44) 2380-596-805
E-mail: jdk1@soton.ac.uk

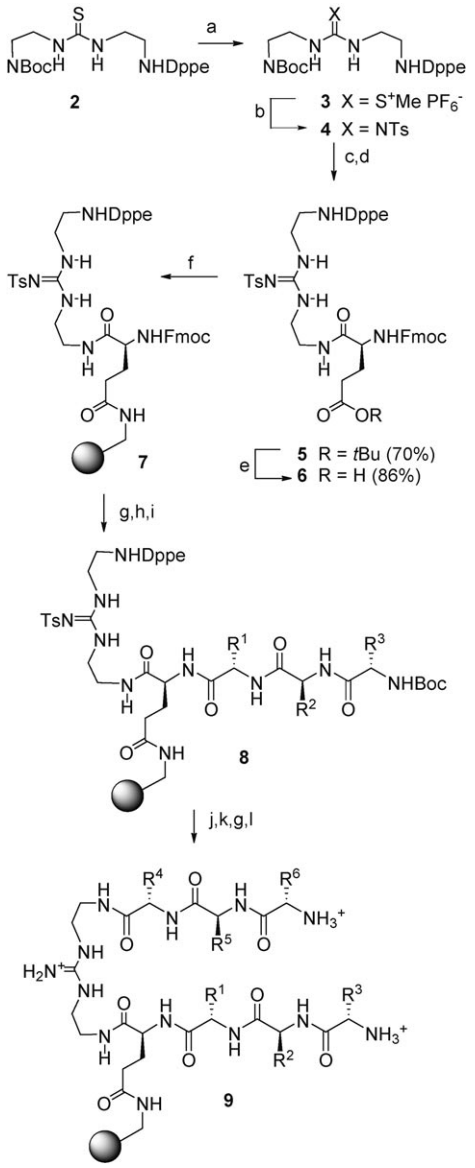
terminus, as substrates in phosphate buffer.[2a,b] These developments demonstrate the potential of such receptor systems to provide potent and sequence selective peptide receptors for use as novel therapeutics or biosensors.

In our own work with two-armed "tweezer" receptors, based around a bis(aminoalkyl)guanindinium scaffold, the libraries prepared to date have limited diversity; this is because both arms of the receptor are synthesized simultaneously on the solid support, which leads to "symmetrical" tweezer receptors containing two arms with an identical amino acid sequence.[2e] To increase the diversity of structures that can be prepared by using this combinatorial approach, we have now developed routes to libraries of analogous "unsymmetrical" tweezer receptors in which the two arms are synthesized independently. These libraries have been screened to identify receptor structures that are able to bind to the bacterial cell wall precursor peptide *N*-Ac-Lys-D-Ala-D-Ala.[7] One such receptor structure was resynthesized and used to determine the association constant with *N*-Ac-Lys-D-Ala-D-Ala. In free solution, although a UV binding study provides evidence for an association between the receptor and the tripeptide, the data does not allow for the determination of a simple 1:1 (receptor:substrate) binding constant. When attached to the solid support, however, the receptor binds to the tripeptide in an aqueous buffered solution with a mM association constant. Herein we describe these studies in detail.

Results and Discussion

Our approach to tweezer receptor libraries has relied on Edman degradation to identify the amino acid components in selected "hit" beads from screening experiments. While this proved to be relatively straightforward for the "symmetrical" receptor structures reported previously,[2e] the construction of "unsymmetrical" structures requires a more complicated procedure and careful use of orthogonal protecting groups. We have used two strategies for the construction of such libraries, with both cases utilizing the orthogonally protected guanidinium derivative **6**, prepared in five steps from the previously described[8] thiourea **2**, as the starting point.

In the first strategy, the guanidinium **6** was attached to tentagel resin and the Fmoc-protecting group was removed (Scheme 2). Two rounds of split-and-mix synthesis using Fmoc-protected amino acids (Fmoc = 9-fluorenyloxycarbonyl), with acid-sensitive side chain protection where appropriate, were then employed, followed by a third round of split-and-mix synthesis using Boc-protected amino acids to give **8** (Boc = *tert*-butyloxycarbonyl), thus, completing the synthesis of the first peptidic arm of the receptor structures. Subsequent removal of the Dppe-protecting group (Dppe = 1-(4,4-dimethyl-2,6-dicyclohexylidene)phenylethyl)[9] was followed by three more rounds of split-and-mix synthesis using Fmoc-protected amino acids. Finally, treatment with piperidine removed the Fmoc-protecting group on the last amino acid



Scheme 2. a) i) CH_3I, acetone; ii) NH_4PF_6, CH_2Cl_2, CH_3OH; b) $TsNH_2$, DBU, toluene, $CHCl_3$, reflux; c) 30% CF_3CO_2H, CH_2Cl_2; d) Fmoc-L-Glu-(O*t*Bu)-OH, EDC, HOBt, DIPEA, DMF; e) 60% CF_3CO_2H, CH_2Cl_2; f) Tentagel resin, PyBOP, HOBt, DIPEA, DMF; g) 20% piperidine in DMF; h) two-fold split-and-mix Fmoc peptide synthesis using Gly, L-Leu, L-Phe, L-Glu(O*t*Bu), and L-Met; i) split-and-mix Boc peptide synthesis using Gly, L-Leu, L-Phe, L-Glu(O*t*Bu), and L-Met; j) 35% aqueous NH_2NH_2; k) three-fold split-and-mix Fmoc peptide synthesis using L-Ala, L-Val, L-Gln, L-Hist, and L-Lys(Boc); l) liquid HF.

and treatment with HF, simultaneously, removed all acid-sensitive protecting groups, including the tosyl group on the guanidinium unit, producing library **9**.

Identifying the structure of a receptor on a single bead from this library involved Edman sequencing[10] of both tweezer arms simultaneously, so that each round of Edman sequencing identified two amino acids. Therefore, to avoid any ambiguity as to which arm the respective amino acids are derived from, the choice of amino acids used in the split-and-mix steps is restricted, that is, the set of amino

acids used at the first position of the first arm (AA^1) must be different from those incorporated at the first position of the second arm (AA^4). The same restriction applies to AA^2 versus AA^5 and AA^3 versus AA^6.

Following this approach, a library containing 15625 members was prepared by using the amino acids Gly, L-Leu, L-Phe, L-Glu(O*t*Bu), and L-Pro for positions AA^1–AA^3 and the amino acids L-Ala, L-Val, L-Gln, L-Ser(O*t*Bu), and L-Lys(Boc) for positions AA^4–AA^6. Edman sequencing on randomly selected beads from the library confirmed that the structure of the receptor on each bead could be unambiguously determined for each case.

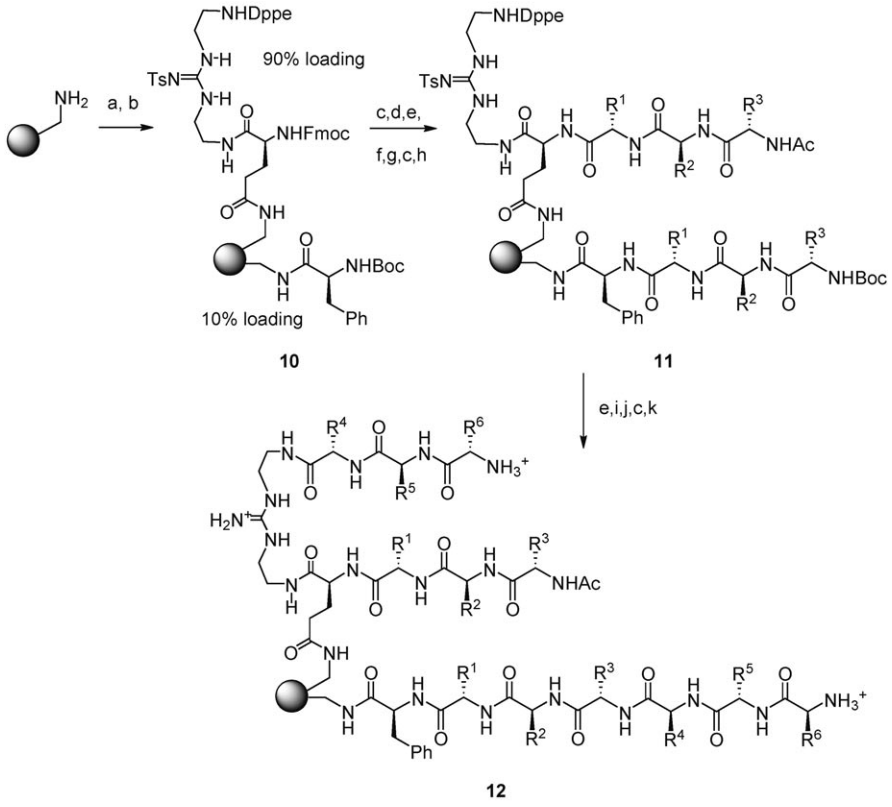
An alternative strategy also started from the guanidinium **6**, but required the introduction of an independent coding strand for the structural identification of individual library members. Thus, tentagel resin was first coupled with *N*-Boc phenylalanine (10 mol%), followed by the coupling of the remaining free amine sites (90 mol%) with the guanidinium **6** (Scheme 3). After the removal of the Fmoc-protecting group, the resin was split into the desired number of portions and coupled with different Fmoc-protected amino acids. Each separate portion was then treated with TFA (TFA = trifluoroacetic acid) to remove the Boc-protecting group on the coding strand, and the resulting free amine was coupled with the same amino acid as that used previously, but with a Boc-protecting group rather than an Fmoc-protecting group. Thus, on each individual bead the coding strand contains the same amino acid sequence as on the peptide arm. On the third cycle of couplings, the Fmoc-protected amino acids were treated with piperidine and capped with acetic anhydride, prior to the Boc deprotection and coupling of the Boc-protected amino acid onto the coding strand. Synthesis of the second peptidic arm of the receptors required the removal of the dppe group and Boc deprotection of the coding strand. Subsequent split-and-mix synthesis with Fmoc-protected amino acids produced the second peptidic arm with simultaneous extension of the coding strand. The use of this procedure avoids restricting the diversity of library **9**, but does, on the other hand, mean that amino acids with acid-sensitive side-chain protection cannot used in the construction of the first peptidic arm.

Identifying the structure of a receptor on a single bead now involved Edman sequencing of the heptapeptide coding strand. For every bead, the seventh residue from the sequencing procedure should be phenylalanine, as this was introduced on all of the beads, providing a useful check that the library synthesis and sequencing chemistry has worked efficiently.

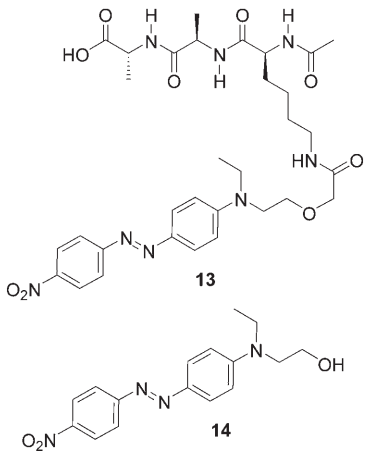
Following this approach, a library containing 15625 members was constructed by using the amino acids Gly, L-Ala, L-Val, L-Phe, and L-Gln for positions AA^1–AA^3 and amino acids Gly, L-Ala, L-Ser, L-Met, and L-Hist for positions AA^4–AA^6. Edman sequencing on randomly selected beads from the library confirmed that the structure of the receptor on each bead could be unambiguously determined for each case.

To demonstrate the potential of these libraries for the identification of new receptors, they were screened in aqueous buffer (pH 8.5, borate) with the dye-labeled tripeptide *N*-Ac-Lys-D-Ala-D-Ala (**13**). At this pH the guanidinium moiety is still protonated and the carboxylate of the peptidic guest is deprotonated.

The screening experiments are simple to perform. First, a library sample is equilibrated in the buffer system, and then a solution of the peptide guest, dissolved in water and 10% DMSO, is added. After a second equilibration, the selectivity of the peptide binding can be judged by the observation of stained beads, visualized under



Scheme 3. a) 10 mol% Boc-L-Phe-OH, PyBOP, HOBt, DIPEA, DMF; b) **6**, PyBOP, HOBt, DIPEA, DMF; c) 20% piperidine in DMF; d) split-and-mix Fmoc peptide synthesis using Gly, L-Ala, L-Val, L-Phe, and L-Gln; e) 30% CF_3CO_2H, CH_2Cl_2; f) split-and-mix Boc peptide synthesis using Gly, L-Ala, L-Val, L-Phe, and L-Gln; g) repeat c–f twice; h) Ac_2O, DMAP; i) 35% aqueous NH_2NH_2; j) three-fold split-and-mix Fmoc peptide synthesis using Gly, L-Ala, L-Ser, L-Met, and L-Hist; k) liquid HF.



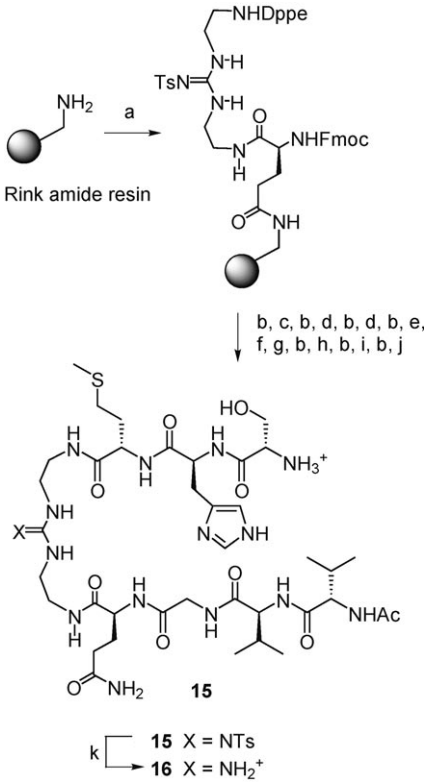
a microscope. Additional aliquots of the peptide guest can be added to increase the peptide concentration, and thus, provide optimal selectivity, as adjudged by the number of highly stained beads against a background of lightly or unstained beads.

By using this strategy, both tweezer receptor libraries **9** and **12** were screened with dye-labeled **13**. For library **9**, the selectivity was disappointing and there were a large number of beads stained, with little discrimination between the degree of staining. By using library **12**, however, the selectivity after equilibration (48 h, at a peptide concentration of 30 μM) was high, showing $<2\%$ of the highly red-colored beads. As a control experiment, the library was also incubated with the disperse red dye **14** (30 μM), but selective binding of the dye was not observed. Ten of the most intensively stained beads from the screening experiment of **13** with library **12** were selected and sequenced by Edman degradation[10] (Table 1).

Table 1. Sequencing results for ten "hit" beads identified from the screening experiments of library **9** and dye-labeled peptide **12**, in aqueous buffer (pH 8.5, borate).

Bead	AA^0	AA^1	AA^2	AA^3	AA^4	AA^5	AA^6
1	Phe	Gly	Val	Val	Met	His	Ser
2	Phe	Gly	Phe	Ala	His	His	Ser
3	Phe	Ala	Val	Gln	Gly	His	Ser
4	Phe	Gln	Ala	Val	Gly	Met	Ser
5	Phe	Val	Ala	Val	Ser	Met	Ser
6	Phe	Gln	Phe	Ala	Met	Gly	Ser
7	Phe	Ala	Ala	Ala	Met	Ser	Ser
8	Phe	Phe	Val	Val	Gly	Met	His
9	Phe	Gly	Ala	Ala	Met	Met	His
10	Phe	Ala	Val	Phe	Met	–	–

The sequencing results were successful for each bead, with the exception of one, for which AA^5 and AA^6 could not be unambiguously determined. As expected, for each bead, the seventh residue identified by Edman sequencing was phenylalanine. Inspection of the sequencing data reveals that AA^6 has a very high proportion (70%) of serine residues, AA^5 has a strong preference for histidine (30%) or methionine (40%), and AA^4 has a strong preference for methionine (50%). AA^3 and AA^2, however, have a strong preference for hydrophobic residues valine (40%) or alanine (40%). AA^1 is the least well-defined with glycine (30%) and alanine (30%). Therefore, receptor structure **16** was chosen for resynthesis and binding studies, since it incorporated the observed preferences, and was also the exact structure identified for bead 1 (Scheme 4).



Scheme 4. a) **6**, PyBOP, HOBt, DIPEA, DMF; b) 20% piperidine in DMF; c) Fmoc-Gly, DIC, HOBt, DMF; d) Fmoc-L-Val, DIC, HOBt, DMF; e) Ac_2O, DMAP, DMF; f) 35% aqueous NH_2NH_2; g) Fmoc-L-Met, DIC, HOBt, DMF; h) Fmoc-L-His, DIC, HOBt, DMF; i) Fmoc-L-Ser(O*t*Bu), DIC, HOBt, DMF; j) 95% CF_3CO_2H, CH_2Cl_2; k) liquid HF.

Receptor **16** was prepared on acid-labile Rink amide resin.[11] Orthogonally protected guanidine **6**[8] was then attached, and the peptide arms constructed by using standard Fmoc-protected amino acid coupling chemistry. Cleavage from the resin (TFA) and treatment with HF, to remove the tosyl group from the guanidine, produced the desired receptor, which was purified by HPLC.

A UV titration experiment was used to study the binding of tweezer **16** with dye-labeled **13**. As aliquots of the tweezer **16** were added, the intensity of the UV absorption maximum (at 500 nm) of the red dye moiety of the peptide guest was monitored. This resulted in a decrease in the absorption at 500 nm, with a clean isobestic point at 400 nm. However, the data from this experiment did not fit with the presumed 1:1 binding, and the titration curve (Figure 1) suggests that

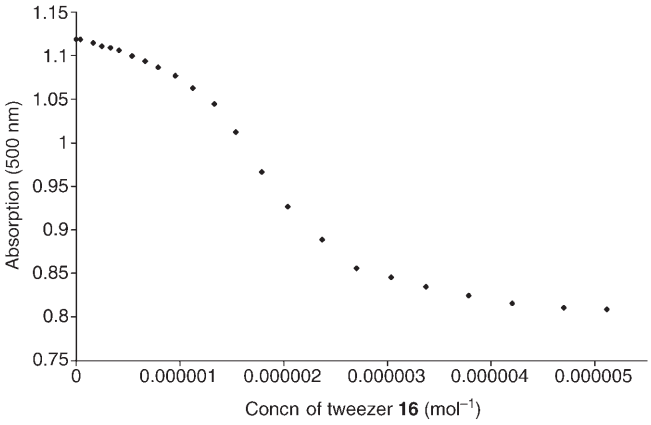
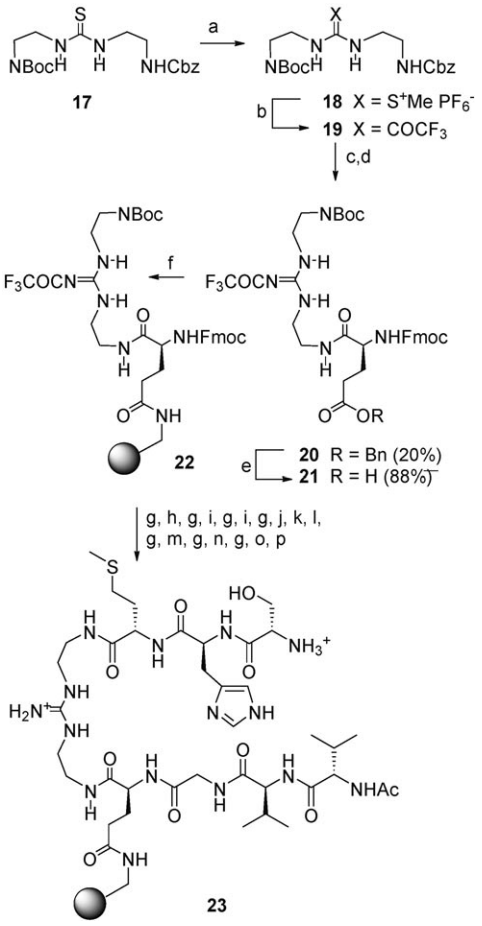


Figure 1. UV titration for tweezer **16** with peptide **13**.

both 1:1 and 1:2 (host:guest) binding stoichiometries are present. Notably, however, when titration experiments were also carried out with the tosylated tweezer **15** and peptide **13**, no detectable change in the UV absorption was observed, confirming that an interaction between the free guanidinium of the receptor and the carboxylate terminus of the guest is essential for strong binding. Complexation was also investigated by ^{1}H NMR spectroscopy, and the addition of one equivalent of dye-labeled **13** to a solution of the tweezer receptor **16** did lead to changes in the spectrum of the latter; however, the limited solubility and poor resolution precluded further analysis.

Therefore, the receptor structure **16** was resynthesized for binding studies on the solid-phase. The synthesis essentially followed the route described above, but used the trifluoroacetyl-protected guanidinium as the scaffold for initial attachment to the tentagel resin, since the trifluoroacetyl protection of guanidines, recently introduced by us,[8] is much easier to remove than tosyl protection. Thus, guanidine **19** was attached to tentagel resin (0.2 mmol g^{-1}), and the peptide arms constructed by using the standard Fmoc-protected amino acid coupling chemistry as before (Scheme 5). Final treatment with K_2CO_3 (0.15 M) cleaved the trifluoroacetyl group to give the resin-bound tweezer receptor **23**.

The binding affinity of the resin-bound receptor **23** for dye-labeled *N*-Ac-Lys-D-Ala-D-Ala (**13**) was measured by determining the ability of the resin-bound receptor to absorb the peptide guest from solution, following a previously described methodology.[3c,12] Thus, a known mass of resin **23** was incubated with a solution of guest **13** in an aqueous buffer and the change in the concentration of the guest in free solution was monitored by UV spectroscopy.[12] To account for nonspecific absorption of **13** to the polystyrene resin matrix, an equivalent mass of underivatized tentagel resin was also incubated with **13**. This allowed the amount of **13** specifically bound by the tweezer receptor to be determined, and hence the association constant, based on a 1:1 receptor–substrate stoichiometry, was estimated as $K_{ass} \sim 1350\,M^{-1}$. An identical binding experiment was carried out again by using the resin-bound receptor **23**, but with the diastereomeric dye-labeled *N*-Ac-Lys-L-Ala-L-Ala, producing a value for K_{ass} of $\sim 250\,M^{-1}$.



Scheme 5. a) i) CH_3I, acetone; ii) NH_4PF_6, CH_2Cl_2, CH_3OH; b) CF_3CONH_2, DBU, toluene, $CHCl_3$, reflux; c) H_2, Pd/C, DMF; d) Fmoc-L-Glu(OBn)-OH, EDC, HOBt, DIPEA, DMAP, DMF; e) H_2, Pd/C, DMF; f) Tentagel resin, DIC, HOBt, DIPEA, DMF; g) 20% piperidine in DMF; h) Fmoc-Gly, DIC, HOBt, DIPEA, DMF; i) Fmoc-L-Val, DIC, HOBt, DIPEA, DMF; j) Ac_2O, Et_3N, DMF; k) 20% CF_3CO_2H, CH_2Cl_2; l) Fmoc-L-Met, DIC, HOBt, DIPEA, DMF; m) Fmoc-L-His(Trt), DIC, HOBt, DIPEA, DMF; n) Fmoc-L-Ser(O*t*Bu), DIC, HOBt, DIPEA, DMF; o) 95% TFA, CH_2Cl_2, p) K_2CO_3 (0.15 M) in MeOH/DMF/H_2O (2:2:1).

Conclusion

We have described novel solid-phase synthesis methodology for the construction of "unsymmetrical" gaunidinyl tweezer receptor structures. A relatively small library of these receptors was prepared and screened to identify a receptor for dye-labeled *N*-Ac-Lys-D-Ala-D-Ala. Binding studies on the solid-phase confirm that the resin-bound receptor has significant (mM) affinity for the dye-labeled peptide, and reduced affinity for the diastereoisomeric peptide, dye-labeled *N*-Ac-Lys-L-Ala-L-Ala. Binding in free solution, however, proved hard to establish, and although both UV and NMR spectroscopic studies indicated that complexation had occurred,

binding affinities could not be determined. The failure of binding on the solid-phase to translate to equivalent binding in free solution suggests that the environment created by the resin may have a significant role in influencing binding affinity—possibly by excluding or organizing solvent molecules within the resin matrix—or may suppress aggregation of the tweezer receptor molecules, which occurs readily in free solution. These difficulties might be addressed by attaching a polyethylene glycol chain to the receptor in free solution to mimic more precisely the environment of the resin-bound receptors[2d] or by linking a number of such tweezer structures to a dendrimer. This latter strategy might, ultimately, have an added benefit in creating multivalent Lys-D-Ala-D-Ala receptors. Such studies are underway in our laboratory. Furthermore, the library described herein was relatively small given the diversity available just by using the natural L-amino acids, let alone the structures that may be produced by introducing other building blocks. These limitations notwithstanding the results described herein further emphasize the potential of these tweezer structures to act as strong and selective peptide receptors, even in competitive aqueous solvents.

Experimental Section

General: Solvents for synthesis were purchased from Fisher or Rathburn Chemicals. Tentagel S NH_2 or Rink Amide resin was used as the solid support in peptide synthesis, and was purchased from Rapp Polymere (Germany) or Novabiochem. Fmoc- and Boc-protected amino acids were purchased from NovaBiochem or BAChem. All other chemicals were purchased from Aldrich, Fluka, Lancaster, or NovaBiochem. Peptide synthesis on the solid-phase was performed in polypropylene filtration tubes with polyethylene frits on a Visiprep SPE vacuum manifold from Supelco. The reaction containers were agitated on a blood tube rotator (Stuart Scientific Blood Tune Rotator SB1). Coupling was monitored by using the Kaiser ninhydrin test. TLC was performed on aluminum-backed plates (Merck silica gel 60 F254). Sorbisil C60, 40–60-mesh silica was used for column chromatography. Melting points were determined in open capillary tubes by using a Gallenkamp electrothermal melting point apparatus. IR spectra were recorded on a Bio-Rad FT-IR spectrometer as neat compounds. ^{1}H NMR spectra were obtained at 300 MHz on a Bruker AC 300 and at 400 MHz on a Bruker DPX 400. ^{13}C NMR spectra were recorded at 75 MHz on a Bruker AC300 and at 100 MHz on a Bruker DPX 400. Coupling constants are given in Hz. The multiplicities of the signals were determined by using the distortionless enhancement phase transfer (DEPT) spectral editing technique. ES-MS were obtained on a Micromass Platform II with a quadrupole mass analyzer or a Waters ZMD with a quadrupole mass analyzer. UV titration experiments were recorded on a Shimadzu UV-1601 UV/visible spectrophotometer.

Abbreviations: Ac, acetyl; Boc, *tert*-butyloxycarbonyl; DIC, *N*,*N*-diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; dppe, 1-(4,4-dimethyl-2,6-dicyclohexylidene)phenylethyl; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Fmoc, 9-fluorenyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolino-phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; Trt, trityl; Ts, tosyl.

***tert*-Butyl ester of 4-{2-{{{2-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-2-phenylethylamino]ethylamino}(toluene-4-sulfonylimino)methyl}amino}-ethylcarbamoyl}-4-(9H-fluoren-9-ylmethoxycarbonylamino)butyric acid (5)**: Carbamate **4**[8] (500 mg, 0.75 mmol) was stirred in a 20% solution of trifluoroacetic acid in CH_2Cl_2 (25 mL) at room temperature for 3 h. After the addition of toluene, the solvents were removed under reduced pressure to give a yellow oil that was redissolved in CH_2Cl_2 (5 mL) and added to a solution of *N*-α-Fmoc-L-glutamic acid-*ω*-*tert*-butyl ester (320 mg, 0.75 mmol), EDC (144 mg, 0.75 mmol), and HOBt (203 mg, 0.75 mmol) in CH_2Cl_2 (15 mL) at room temperature. After the addition of DIPEA (260 μL, 1.5 mmol), the resulting reaction mixture was stirred for 36 h. CH_2Cl_2 (50 mL) was then added and the mixture washed with water (60 mL). The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure to give a yellow solid. Purification by column chromatography on silica gel (EtOAc/PE, 8:1→EtOAc) produced **5** as a white hygroscopic foam (510 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 13.48$ (br s, 1H; NH), 7.67 (d, $J = 7.5$ Hz, 2H; ArH), 7.63 (d, $J = 8.0$ Hz, 2H; ArH), 7.49 (d, $J = 7.5$ Hz, 2H; ArH), 7.35–7.05 (m, 9H; ArH), 6.99 (d, $J = 7.5$ Hz, 2H; ArH), 6.35 (br s, 1H; NH), 4.39 (m, 2H; $PhCH_2$), 4.28 (m, 2H; CH_2), 4.13–4.00 (m, 2H), 3.45 (m, 2H), 3.30–3.10 (m, 6H), 2.40–1.80 (m, 11H), 1.36 (s, 9H; $(CH_3)_3C$), 0.91 ppm (s, 6H; $(CH_3)_2C$); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): $\delta = 198.1$ (C), 174.2 (C), 174.0 (C), 173.0 (C), 156.6 (C), 155.6 (C), 143.8 (C), 142.1 (C), 141.4 (C), 141.1 (C), 135.8 (C), 129.3 (CH), 128.9 (CH), 128.1 (CH), 127.9 (CH), 127.2 (CH), 126.8 (CH), 126.0 (CH), 125.2 (CH), 120.2 (CH), 108.4 (C), 81.3 (C), 67.2 (CH_2), 60.5 (CH_2), 54.6 (CH_2), 53.0 (CH), 47.3 (CH), 42.4 (CH_2), 40.9 (CH_2), 39.7 (CH_2), 35.0 (CH_2), 31.7 (CH_2), 30.1 (C), 28.4 (CH_3), 28.2 (CH_3), 27.5 (CH_2), 21.6 ppm (CH_3); IR (neat): $\tilde{\nu} = 3322$ (br), 2949 (w), 2926 (w), 1720 (m), 1561 (s), 1496 (w), 1450 (m), 1419 (w), 1365 (w), 1342 (m), 1245 (s), 1182 (w), 1131 (s), 1081 cm^{-1} (s); LRMS (ES): *m/z*: 947 $[M+H]^+$, 969 $[M+Na]^+$.

4-{2-{{{2-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-2-phenylethylamino]ethylamino}(toluene-4-sulfonylimino)methyl}amino}ethylcarbamoyl}-4-(9H-fluoren-9-ylmethoxycarbonylamino)butyric acid (6): TFA (60% in CH_2Cl_2, 11 mL) was added to a solution of ester **5** (470 mg, 0.48 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred vigorously for 18 h at room temperature. Toluene (150 mL) was then added and the solvents were removed under reduced pressure to give a yellow oil. Column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded acid **6** as a foam (380 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.77$ (d, $J = 8.0$ Hz, 2H; ArH), 6.50 (br s, 1H; NH), 7.73 (d, $J = 8.0$ Hz, 2H; ArH), 7.60–7.05 (m, 11H; ArH), 6.98 (d, $J = 7.0$ Hz, 2H; ArH), 4.74 (s, 2H; $PhCH_2$), 3.55–3.30 (m, 8H), 2.45–2.20 (m, 9H), 2.15 (m, 1H), 2.03 (m, 1H), 1.02 ppm (s, 6H; $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 198.3$ (C), 176.1 (C), 174.7 (C), 173.5 (C), 156.6 (C), 155.6 (C), 143.8 (C), 142.4 (C), 141.4 (C), 140.8 (C), 135.6 (C), 129.4 (CH), 129.0 (CH), 128.1 (CH), 127.9 (CH), 127.2 (CH), 126.8 (CH), 126.0 (CH), 125.2 (CH), 120.2 (CH), 108.3 (C), 67.4 (CH_2), 54.2 (CH), 53.6 (CH_2), 52.8 (CH_2), 47.2 (CH), 42.7 (CH_2), 38.7 (CH_2), 40.8 (CH_2), 36.2 (CH_2), 35.0 (CH_2), 30.1 (C), 28.3 (CH_3) 27.8 (CH_2), 21.6 ppm (CH_3); IR (neat): $\tilde{\nu} = 3319$ (br), 2951 (w), 1705 (m), 1561 (s), 1496 (w), 1449 (m), 1341 (m), 1241 (m), 1181 (m), 1128 (s), 1079 cm^{-1} (s); LRMS (ES): *m/z*: 914 $[M+Na]^+$.

General procedure for Fmoc deprotection on the solid-phase: The Fmoc-protected resin was suspended in a solution of 20% piperidine in DMF (20 mL per g resin) and agitated for 30 to 45 min. The resin was then drained and washed with CH_2Cl_2 (3×10 mL), DMF (3×10 mL), and additional CH_2Cl_2 (3×10 mL). The procedure was repeated once and the progress of the deprotection monitored by a ninhydrin test.

General procedure for Boc deprotection on the solid-phase: The Boc-protected resin was suspended in a mixture of 45% CH_2Cl_2, 45% TFA, 4% EDT, 3% DMS, and 3% anisole (20 mL per g resin) and agitated for 60 to 120 min. The resin was then drained and washed with CH_2Cl_2 (3×10 mL), DMF (3×10 mL), a 20% solution of DIPEA in CH_2Cl_2 (3×10 mL), MeOH (3×10 mL), DMF (3×10 mL), and additional CH_2Cl_2 (3×10 mL). The DIPEA solution wash was omitted for samples requiring prolonged storage. The progress of the deprotection was monitored by a ninhydrin test.

General procedure for tosyl deprotection on the solid-phase using the HF procedure: The tosyl-protected resin was dried overnight under high vacuum and placed in a Teflon apparatus. After the addition of *p*-thiocresol and cresol, the reaction vessel was cooled with liquid nitrogen, and liquid HF was condensed into the vessel (about 30 mL per g resin). The cleavage mixture was stirred for 120 min at 0°C, after which time the HF

was evaporated under a stream of nitrogen. The resin was then washed with diethyl ether (4×20 mL), 20% DIPEA in CH_2Cl_2 (3×20 mL), MeOH (3×20 mL), and CH_2Cl_2 (3×20 mL).

Receptor library 9: TentaGel S NH_2 resin (900 mg, 0.26 mmol g^{-1}, 0.23 mmol) was preswollen in CH_2Cl_2 and then drained. A solution of acid **6** (314 mg, 0.35 mmol), PyBOP (182 mg, 0.35 mmol), HOBt (47 mg, 0.35 mmol), and DIPEA (60 μl, 0.35 mmol) in DMF was added to the resin and the mixture agitated on a tube rotator for 18 h, before being drained and washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), and additional CH_2Cl_2 (3×5 mL). A ninhydrin test gave a negative result. Following Fmoc deprotection (see general procedure) the resin was divided into five equal portions, and each portion was preswollen in DMF (2 mL). A solution of Fmoc-protected amino acid (Gly, Leu, Phe, Glu(O*t*Bu), or Pro; 0.14 mmol), PyBOP (73 mg, 0.14 mmol), and HOBt (17 mg, 0.14 mmol) in DMF (3 mL) was preactivated for a few minutes, and then added to each portion of the resin, followed by the addition of DIPEA (18 mg, 0.14 mmol). Each portion was agitated on a tube rotator for at least 24 h at room temperature. After this time, the portions were washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), additional CH_2Cl_2 (3×5 mL), and then dried. The success of the coupling step was monitored by a qualitative ninhydrin test. Each coupling cycle was repeated until the ninhydrin test showed that no free amino functions were present. After the successful coupling step, the resin was recombined and Fmoc-deprotected, as described in the general procedure, with monitoring by a qualitative ninhydrin test. This split-and-mix procedure was then repeated with the same five amino acids (first, Fmoc-protected and then Boc-protected) to build up the first tripeptide side arm. For the next step in the synthesis, the recombined resin was swollen in DMF (10 mL), treated with hydrazine hydrate (15 mL of a 35% aq solution), and agitated on a tube rotator for 4 h, to remove the dppe-protecting group.[9] The resin was then divided into five portions and each portion was preswollen in DMF (2 mL). A solution of Fmoc-protected amino acid (Ala, Val, Gln, Ser(O*t*Bu), or Lys(Boc); 0.14 mmol), PyBOP (73 mg, 0.14 mmol), and HOBt (17 mg, 0.14 mmol) in DMF (3 mL) was preactivated for a few minutes and then added to each portion of resin, followed by the addition of DIPEA (18 mg, 0.14 mmol). Each portion was agitated on a tube rotator for at least 24 h at room temperature. The portions were washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), additional CH_2Cl_2 (3×5 mL), and then dried. The success of the coupling step was monitored by a qualitative ninhydrin test. Each coupling cycle was repeated until the ninhydrin test showed that no free amino functions were present. After the successful coupling step, the resin was recombined and Fmoc-deprotected, as described in the general procedure, with monitoring by the qualitative ninhydrin test. This split-and-mix procedure was then repeated twice more with the same five Fmoc-protected amino acids to build up the second tripeptide side arm. After the recombination of all of the portions of the resin, the protecting groups were removed by liquid HF treatment (see general procedure) to give library **9**.

Receptor library incorporating coding strand 12: TentaGel S NH_2 resin (900 mg, 0.26 mmol g^{-1}, 0.23 mmol), was preswollen in CH_2Cl_2 and then drained. A solution of Boc-Phe (6 mg, 23 μmol), PyBOP (12 mg, 23 μmol), HOBt (31 mg, 23 μmol), and DIPEA (40 μl, 23 μmol) in DMF (3 mL) was added to the resin and the mixture agitated on a tube rotator for 18 h, before being drained and washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), and additional CH_2Cl_2 (3×5 mL). A solution of acid **6** (314 mg, 0.35 mmol), PyBOP (182 mg, 0.35 mmol), HOBt (47 mg, 0.35 mmol), and DIPEA (60 μL, 0.35 mmol) in DMF was then added to the resin and the mixture agitated on a tube rotator for a further 18 h, before being drained and washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), and additional CH_2Cl_2 (3×5 mL). A ninhydrin test gave a negative result. Following Fmoc deprotection (see general procedure), the resin was divided into five equal portions, and each portion was preswollen in DMF (2 mL). A solution of Fmoc-protected amino acid (Gly, Ala, Phe, Val, or Leu; 0.14 mmol), PyBOP (73 mg, 0.14 mmol), and HOBt (17 mg, 0.14 mmol) in DMF (3 mL) was preactivated for a few minutes and then added to each portion of the resin, followed by the addition of DIPEA (18 mg, 0.14 mmol). Each portion was agitated on a tube rotator for 24 h at room temperature. After this time, the portions were washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), additional CH_2Cl_2 (3×5 mL), and then dried. The success of the coupling step was monitored by a qualitative ninhydrin test. Each coupling cycle was repeated until the ninhydrin test showed that no free amino functions were present. Each portion was then Boc-deprotected to release the free amine of the coding strand (see general procedure) and then coupled with a Boc-protected amino acid (Gly, Ala, Phe, Val, and then Leu, corresponding with the Fmoc-protected amino acids used in the previous coupling; 14 μmol) by using PyBOP (7 mg, 14 μmol), HOBt (2 mg, 14 μmol), and DIPEA (2 mg, 14 μmol). Once complete, the portions were washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), additional CH_2Cl_2 (3×5 mL), and then dried. After the successful coupling step, the resin was recombined and Fmoc-deprotected, as described in the general procedure, with monitoring by the qualitative ninhydrin test. This split-and-mix procedure was then repeated twice, with the same five amino acids, to build up the first tripeptide side arm and the coding strand. For the next step in the synthesis, the recombined resin was swollen in DMF, Fmoc-deprotected (see general procedure), treated with excess acetic anhydride and DMAP, and then agitated on a tube rotator for 4 h to cap the first tripeptide arm. The resin was then treated with hydrazine hydrate (15 mL of a 35% aq solution) and agitated on a tube rotator for 4 h, to remove the dppe-protecting group.[9] Once complete, the resin was divided into five portions, preswollen in DMF (2 mL), and subjected to three rounds of split-and-mix synthesis to build up the second tripeptide arm (Fmoc-protected amino acids, Gly, Ala, Ser(O*t*Bu), Hist(Trt), and then Met were used) and the coding strand (with the corresponding Boc-protected amino acids), following the exact same procedures described above for the synthesis of the first tripeptide arm and coding strand. Next, a solution of Fmoc-protected amino acid (Ala, Val, Gln, Ser(O*t*Bu), and then Lys(Boc); 0.14 mmol), PyBOP (73 mg, 0.14 mmol), and HOBt (17 mg, 0.14 mmol) in DMF (3 mL) was preactivated for a few minutes and then added to each portion of resin, followed by the addition of DIPEA (18 mg, 0.14 mmol). Each portion was then agitated on a tube rotator for 24 h at room temperature. After this time, the portions were washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), additional CH_2Cl_2 (3×5 mL), and then dried. The success of the coupling step was monitored by a qualitative ninhydrin test. As before, each coupling cycle was repeated until the ninhydrin test showed that no free amino functions were present. After the successful coupling step, the resin was recombined and Fmoc-deprotected, as described in the general procedure, with monitoring by a qualitative ninhydrin test. This split-and-mix procedure was then repeated twice more, with the same five Fmoc-protected amino acids, to build up the second tripeptide side arm. After the recombination of all of the portions of the resin, the protecting groups were removed by liquid HF treatment (see general procedure) to give library **12**.

Screening experiments:

Screening of the tweezer receptor library 18: A sample of library **12** (15 mg) was equilibrated in borax buffer solution (300 μL) for 24 h. A solution of the tripeptide guest **13** (20 μM, 500 μL) in a 15% solution of DMSO/borax buffer was then added to the library sample to give a guest concentration of 12.5 μM. Equilibration was continued for 24 h. Analysis of the beads was carried out in flat-bottomed glass pots, under a Leica inverted DML microscope (magnification×40), and 10 highly red stained beads were selected and submitted for Edman sequencing.[10]

Benzyl ester of 4-{2-[*N*′-(2-*tert*-butoxycarbonylaminoethyl)-*N*′-(2,2,2-trifluoroacetyl)guanidino]ethylcarbamoyl}-4-(9H-fluoren-9-ylmethoxycarbonylamino)butyric acid (20): Carbamate **19**[8] (224 mg, 0.47 mmol) was dissolved in DMF (10 mL). Palladium on charcoal (10% by wt, 10 mol%, 50 mg) was then added, and the mixture was stirred under a hydrogen atmosphere for 16 h. After this time, the mixture was filtered through Celite and the resulting filtrates were evaporated to give a pale yellow oil. *N*-α-Fmoc-L-glutamic acid-ω-benzyl ester (238 mg, 0.52 mmol), HOBt (127 mg, 0.94 mmol), DIPEA (410 μl, 2.36 mmol), DMAP (6 mg, 47 μmol), and EDC (99 mg, 0.52 mmol) were added to a solution of this oil in DMF (10 mL), and the resulting mixture was stirred for 18 h. After this time, evaporation of all of the solvent and purification of the residue by column chromatography (EtOAc/PE 1:1) produced **20** as a white hygroscopic foam (73 mg, 20%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.65 (brs, 1H; NH), 7.76 (d, 2H, J=7.5 Hz; ArH), 7.57 (d, 2H, J=

7.0 Hz; ArH), 7.42–7.16 (m, 9H; ArH), 7.16 (brs, 1H; NH), 7.01 (brs, 1H; NH), 5.76 (brs, 1H; NH), 5.12 (s, 2H; CH_2Ph), 4.45–4.30 (m, 2H), 4.19 (m, 2H; CH_2), 3.60–3.15 (m, 8H; CH_2CH_2), 2.49 (m, 2H; CH_2COO), 2.13 (m, 1H), 2.01 (m, 1H), 1.40 ppm (s, 9H; $(CH_3)_3C$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 173.3$ (C), 173.2 (C), 162.7 (C), 157.6 (C), 157.0 (C), 156.6 (C), 143.8 (C), 141.4 (C), 135.7 (C), 128.7 (CH), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.2 (CH), 125.3 (CH), 120.1 (CH), 116.7 (C), 80.6 (C), 67.3 (CH_2), 66.8 (CH_2), 54.8 (CH), 47.1 (CH_2), 41.9 (CH_2), 41.0 (CH_2), 39.6 (CH_2), 38.9 (CH_2), 30.4 (CH_2), 28.4 ppm (CH_3); IR (neat): $\tilde{\nu} = 1629$ (m), 1522 (m), 1449 (m), 1242 (m), 1166 (m), 1140 (m), 848 (w), 739 (m), 514 cm^{-1} (s); LRMS (ES): *m/z*: 783 $[M+H]^+$, 805 $[M+Na]^+$.

4-{2-[*N′*-(2-*tert*-Butoxycarbonylaminoethyl)-*N″*-(2,2,2-trifluoroacetyl)guanidino]ethylcarbamoyl}-4-(9H-fluoren-9-ylmethoxycarbonylamino)butyric acid (21): Palladium on charcoal (10% by wt, 10 mol%, 8 mg) was added to a solution of ester **20** (55 mg, 70 μmol) in EtOH (5 mL). The mixture was stirred under a hydrogen atmosphere for 2 h and then filtered through Celite. Finally, the solvent was removed by evaporation under reduced pressure to give **21** as an off-white hygroscopic foam (43 mg, 88%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.47$ (brs, 1H; NH), 7.74 (d, 2H, $J = 7.5$ Hz; ArH), 7.55 (d, 2H, $J = 6.5$ Hz; ArH), 7.44 (brs, 1H; NH), 7.42–7.22 (m, 4H; ArH), 6.18 (brs, 1H; NH), 5.88 (brs, 1H; NH), 4.45–4.38 (m, 3H), 4.17 (m, 1H; CH), 3.70–2.95 (m, 8H; CH_2CH_2), 2.44 (m, 2H; CH_2COO), 2.18–1.82 (m, 2H), 1.40 ppm (s, 9H; $(CH_3)_3C$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 180.1$ (C), 176.2 (C), 173.7 (C), 171.7 (C), 159.9 (C), 157.8 (C), 156.7 (C), 143.7 (C), 141.4 (C), 127.9 (CH), 127.3 (CH), 125.3 (CH), 120.1 (CH), 80.7 (C), 67.5 (CH_2), 55.7 (CH), 54.6 (CH), 47.1 (CH_2), 41.9 (CH_2), 41.1 (CH_2), 39.6 (CH_2), 38.7 (CH_2), 30.0 (CH_2), 28.4 ppm (CH_3); IR (neat): $\tilde{\nu} = 2977$ (br, w), 1628 (s), 1524 (m), 1449 (m), 1243 (m), 1139 (s), 909 (m), 844 (m), 734 cm^{-1} (s); LRMS (ES): *m/z*: 693 $[M+H]^+$, 715 $[M+Na]^+$.

Resin-bound receptor 23: TentaGel S NH_2 resin (0.2 mmol g^{-1}, 162.2 mg, 32.4 μmol) was swollen in CH_2Cl_2 and drained. A solution of acid **21** (34 mg, 48.7 μmol), DIC (15 μl, 97.3 μmol), HOBt (13 mg, 97.3 μmol), and DIPEA (17 μl, 97.3 μmol) in DMF (2 mL) was then added to the resin, and the mixture agitated on a tube rotator for 18 h. Any remaining amine residues were capped by treating the resin with an excess of acetic anhydride. The resin was then filtered and washed with CH_2Cl_2 (3×5 mL), DMF (3×5 mL), and additional CH_2Cl_2 (3×5 mL). A ninhydrin test was negative. There then followed three sequential cycles of coupling/Fmoc deprotection to add Fmoc-Gly, Fmoc-Val, and Fmoc-Val to the resin, and to synthesize the first arm of the receptor. This was achieved by using Fmoc-protected amino acid (97 μmol), DIC (12 mg, 97 μmol), HOBt (13 mg, 97 μmol), and DIPEA (17 μl, 97 μmol) in DMF (2 mL). Ninhydrin tests were used to monitor progress of each coupling reaction and the coupling reactions were repeated until complete. After deprotection of the second Val residue, the chain was capped by the treatment of the resin with acetic anhydride (9 μl, 97.3 μmol) and DIPEA (17 μl, 97.3 μmol) in DMF (2 mL). Boc deprotection (see general procedure) was followed by three sequential coupling/deprotection cycles to add Fmoc-Met, Fmoc-His(Trt), and Fmoc-Ser(O*t*Bu) to the resin also by using the Fmoc-protected amino acid (97 μmol), DIC (12 mg, 97 μmol), HOBt (13 mg, 97 μmol), and DIPEA (17 μl, 97 μmol) in DMF (2 mL); this time to synthesize the second arm of the receptor. Ninhydrin tests were used to monitor the progress of each coupling reaction. Trt deprotection was achieved by the treatment of the resin with 95% TFA/CH_2Cl_2 (2×1 h), followed by washing with CH_2Cl_2 (3×5 mL), 50% DIPEA/DMF (3×5 mL), DMF (3×5 mL), and additional CH_2Cl_2 (3×5 mL). Trifluoroacetyl deprotection was carried out by the treatment of the resin with K_2CO_3 (0.15 M) in MeOH/DMF/water (2:2:1, 2×3 h). Finally, the resin was washed as above, rinsed with diethyl ether, and dried in vacuo.

Acknowledgements

We wish to thank EPSRC for supporting this work (GR L⁻98916/01) and the BBSRC for a studentship (JS).

[1] For a recent review on peptide receptors see: a) M. W. Peczuh, A. D. Hamilton, *Chem. Rev.* **2000**, *100*, 2479–2493.
[2] a) C. Chamorro, R. M. J. Liskamp, *Tetrahedron* **2004**, *60*, 11145–11157; b) C. Chamorro, J.-W. Hofman, R. M. J. Liskamp, *Tetrahedron* **2004**, *60*, 8691–8699; c) C. Schmuck, M. Heil, *Org. Biomol. Chem.* **2003**, *1*, 633–636; d) C. Schmuck, M. Heil, *ChemBioChem* **2003**, *4*, 1232–1238; e) K. B. Jensen, T. M. Braxmeier, M. Demarcus, J. D. Kilburn, *Chem. Eur. J.* **2002**, *8*, 1300–1309.
[3] a) H. Wennemers, M. C. Nold, M. Conza, K. J. Kulicke, M. Neuburger, *Chem. Eur. J.* **2003**, *9*, 442–448; b) H. Wennemers, M. Conza, *J. Org. Chem.* **2002**, *67*, 2696–2698; c) R. Arienzo, J. D. Kilburn, *Tetrahedron* **2002**, *58*, 711–719; d) T. Braxmeier, M. Demarcus, T. Fessmann, S. McAteer, J. D. Kilburn, *Chem. Eur. J.* **2001**, *7*, 1889–1898; e) J. D. Kilburn, T. Fessmann, *Angew. Chem.* **1999**, *111*, 2170–2174; *Angew. Chem. Int. Ed.* **1999**, *38*, 1993–1996.
[4] Other recent examples of sequence selective peptide receptors: a) C. Schmuck, L. Geiger, *J. Am. Chem. Soc.* **2004**, *126*, 8898–8899; b) S. Rensing, T. Schrader, *Org. Lett.* **2002**, *4*, 2161–2164; c) R. Xu, G. Greiveldinger, L. E. Marenus, A. Cooper, J. A. Ellman, *J. Am. Chem. Soc.* **1999**, *121*, 4898–4899; d) M. Sirish, H.-J. Schneider, *J. Chem. Soc. Chem. Commun.* **1999**, 907–908; e) Md. A. Hossain, H.-J. Schneider, *J. Am. Chem. Soc.* **1998**, *120*, 11208–11209; f) R. Breslow, Z. Yang, R. Ching, G. Trojandt, F. Odobel, *J. Am. Chem. Soc.* **1998**, *120*, 3536–3537.
[5] a) N. Srinivasan, J. D. Kilburn, *Curr. Opin. Chem. Biol.* **2004**, *8*, 305–310; b) B. Linton, A. D. Hamilton, *Curr. Opin. Chem. Biol.* **1999**, *3*, 307–312.
[6] C. Schmuck, P. Frey, M. Heil, *ChemBioChem* **2005**, *6*, 628–631.
[7] a) D. H. Williams, *Nat. Prod. Rep.* **1996**, *13,* 469–477; b) M. L. Cohen, *Science* **1992**, *257*, 1050–1055; c) H. C. Neu, *Science* **1992**, *257*, 1064–1073.
[8] S. Bartoli, K. B. Jensen, J. D. Kilburn, *J. Org. Chem.* **2003**, *68*, 9416–9422.
[9] S. R. Chhabra, B. Hothi, D. J. Evans, P. D. Wite, B. W. Bycroft, W. C. Chan, *Tetrahedron Lett.* **1998**, *39*, 1603–1606.
[10] P. Edman, G. Begg, *Eur. J. Biochem.* **1967**, *1*, 80–91; sequencing was performed by using an applied biosystems 477 A pulsed liquid-phase sequencer, coupled online to an applied biosystems 120 A phenylthiohydantoin (PTH) derivative analyzer.
[11] H. Rink, *Tetrahedron Lett.* **1987**, *28*, 3787–3790.
[12] a) M. Conza, H. Wennemers, *Chem. Commun.* **2003**, 866–867; b) M. C. F. Monnee, A. J. Brouwer, L. M. Verbeek, A. M. A. van-Wageningen, R. M. J. Liskamp, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1521–1525; c) Y. Cheng, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1996**, *118*, 1813–1814.

Received: July 25, 2005
Published online: October 14, 2005

