Synthesis of Guanidinium-Derived Receptor Libraries and Screening for Selective Peptide Receptors in Water

Kim B. Jensen, Tobias M. Braxmeier, Mariangela Demarcus, Jeremy G. Frey, and Jeremy D. Kilburn*[a]

Abstract: A library of "tweezer" receptors, incorporating a guanidinium "head group" and two peptide derived side arms has been prepared on the solid-phase using an orthogonally protected guanidinium scaffold **12**. The library was screened with various tripeptide derivatives in an aqueous solvent system. A tweezer receptor **25** for the side chain protected tripeptide **19** was identified from the screening experiments. Receptor **25** was resynthesised and solution binding studies were carried out, which revealed that **25** binds to tripeptide **19** with $K_a = 8.2 \times 10^4 \pm 2.5 \times 10^4$ (15% DMSO/H_2O, pH 8.75) and with appreciable selectivity over the tripeptide enantiomer **22** and the side chain deprotected tripeptide **20**.

Keywords: combinatorial chemistry · guanidinium · peptides · receptors · screening

Introduction

Synthetic receptors for specific peptide sequences provide model systems for biological protein–peptide complexes as well as having many potential applications, including for separation of peptide mixtures, biosensors, and new therapeutics. Much recent work in the area of peptide receptors[1] has focused on "tweezer" receptors (Figure 1)[2] which, despite their inherent flexibility, have proved to be highly selective for certain peptide sequences in both nonpolar[3] and aqueous solvent systems.[4]

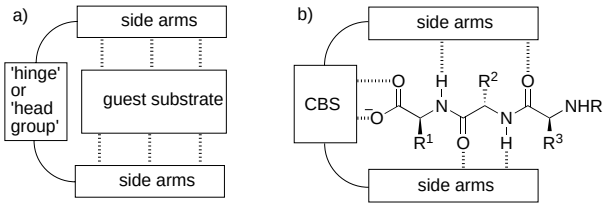

Figure 1. a) Schematic of a tweezer receptor with "hinge" or "head group" and side arms, which provide binding interactions with a suitable guest substrate. b) Schematic of a tweezer receptor with a carboxylic acid binding site (CBS) as the "head group".

We have focused on tweezer receptors, which incorporate a "head group" with a specific recognition site for the C-terminus of a peptide (carboxylate binding site, CBS), and peptidic side arms. For example we have synthesised individual tweezer receptors incorporating a guanidinium head group, which bind to peptides with a free carboxylate terminus in aqueous media[4a]—with the strong interaction between carboxylate and guanidinium proving to be essential for binding.[5] Thus, receptor **3** was prepared on the solid-phase using a guanidine scaffold **1** attached to the solid-phase through a tosyl group (Figure 2). The resulting dye-labelled receptor **3** was screened with a solid-phase library of potential peptidic guests to identify a suitable substrate (L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH).[4a]

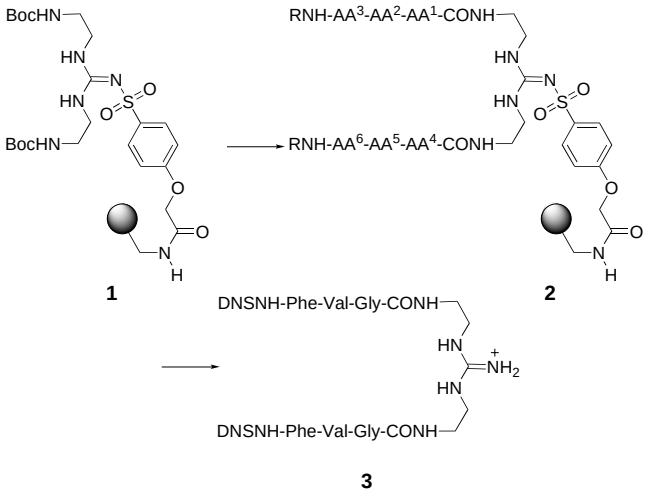

Figure 2. Schematic view of the synthesis of tweezer receptors.

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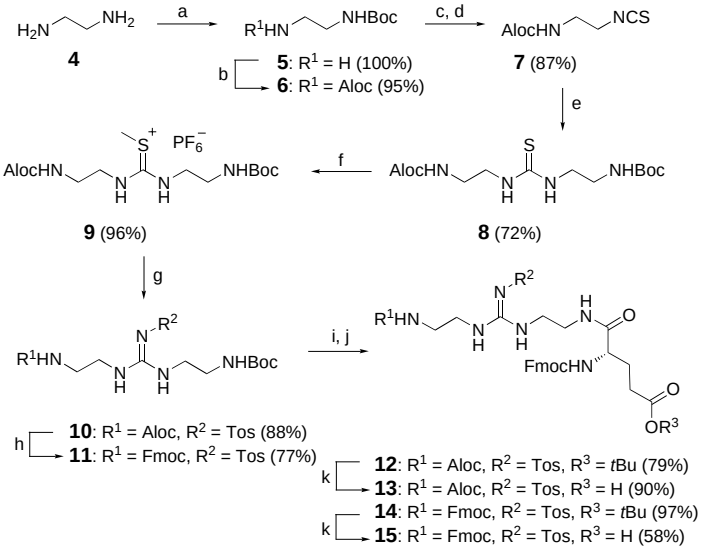
We have also successfully prepared libraries of tweezer receptors with a diamidopyridine head group as a carboxylic acid binding site, using split-and-mix synthesis,[6] and screened the library, on the solid-phase, to identify receptors for a chosen peptide guest with a carboxylic acid terminus, in nonpolar solvents.[3a,b, 7] The diamidopyridine – carboxylic acid interaction is too weak to promote peptide recognition in more competitive solvents, but clearly it would be highly desirable to extend the concept of screening such receptor libraries to libraries suitable for peptide recognition in aqueous media. Indeed we have previously prepared a library of tweezers **2** from scaffold **1**, but the use of a tosylated guanidine linker meant that the tweezers, on the solid-phase, lacked the essential guanidinium unit and screening this library for peptide receptors in water was not successful.[4a] In this paper we describe an alternative approach, which has allowed us to prepare libraries of tweezer receptors with an unprotected guanidinium head group, which, in turn, have been successfully screened to identify a receptor for a chosen peptidic guest in aqueous media.

Results and Discussion

In order to prepare the desired libraries, we synthesised an orthogonally protected guanidine derivative, suitably functionalised with a carboxylic acid moiety to allow attachment to resin beads. The guanidine derivative **13** was prepared in eleven steps with an overall yield of 37% starting from diamine **4** (Scheme 1). Diamine **4** was first converted into the



Scheme 1. a) $(Boc)_2O$, CH_2Cl_2; b) AlocCl, dioxane, pyridine; c) 40% CF_3CO_2H, CH_2Cl_2; d) $CSCl_2$, $NaHCO_3$(aq), $CHCl_3$; e) **5**, $CHCl_3$, reflux; f) CH_3I, acetone, NH_4PF_6, CH_2Cl_2, CH_3OH; g) $TosNH_2$, DBU, toluene, $CHCl_3$, reflux; h) FmocCl, $[Pd(PPh_3)_4]$, Bu_3SnH, CH_2Cl_2; i) 20% CF_3CO_2H, CH_2Cl_2; j) Fmoc-L-Glu(O*t*Bu)-OH, PyBOP, HOBt, DIPEA, DMF; k) 70% CF_3CO_2H, CH_2Cl_2.

mono-Boc protected amine **5** followed by Aloc protection of the second amino function to give bis-protected diamine **6** in 95% yield over two steps. Boc deprotection of **6** gave mono-Aloc protected diamine,[8] which was treated with thiophosgene to give isothiocyanate **7**. Subsequent reaction of **7** with amine **5** gave orthogonally protected thiourea **8** in 62% overall yield from **6**. Alkylation of the thiourea **8** with methyl iodide and counterion exchange gave the thiouronium hexafluorophosphorate **9**, which on treament with tosyl amide, in the presence of DBU, led to the orthogonally protected guanidine **10**. Boc deprotection of **10** and coupling with *N*-α-Fmoc-L-glutamic acid γ-*tert*-butyl ester using PyBOP/HOBt gave **12** which was hydrolysed, using TFA, to give acid **13** in 62% overall yield from **8**. The Aloc protected guanidine **10** could also be converted into the corresponding Fmoc analogue **11** and hence to the bis-Fmoc protected acid **15** using identical procedures.

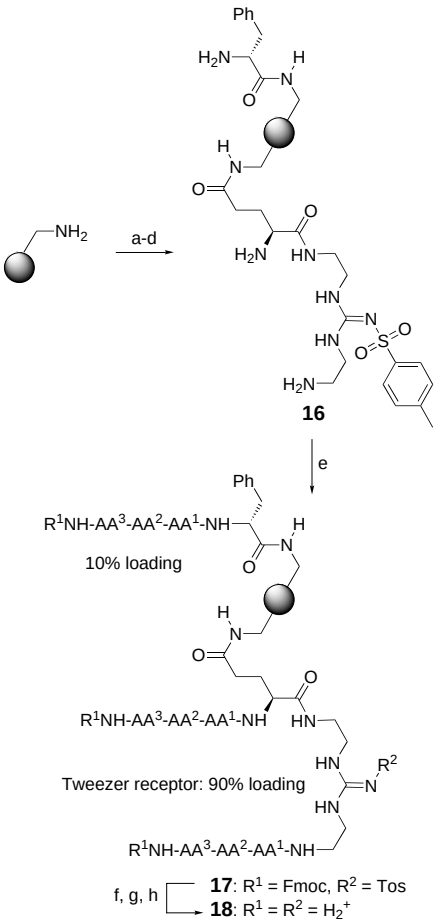
The orthogonal protecting groups used in the synthesis of **13** allows for the synthesis of libraries of "unsymmetrical" tweezer receptors, that is tweezer structures where the two peptide arms can be randomised (by split-and-mix synthesis) independently. Initially, in order to demonstrate the potential of such libraries in aqueous screening experiments, we chose to prepare a relatively small and structurally less diverse library of "symmetrical" tweezers, with identical peptide sequences appended to both sides of the starting guanidine scaffold.

For this library synthesis a coding strand was first introduced,[9] by coupling Fmoc-protected phenylalanine on to 10% of the amine sites on TentaGel resin, followed by coupling protected guanidine **13** on to the remaining amine sites. Subsequent Aloc and Fmoc deprotection yielded resin **16** ready for library generation. A 2197-member library of Fmoc-protected tweezer receptors **17** was prepared by a threefold coupling of thirteen Fmoc-protected amino acids to the free amine groups using the split-and-mix strategy (Scheme 2). Fmoc deprotection, Boc and *tert*-butyl deprotection of relevant amino acid side chains and cleavage of the tosyl function using HF, generated the resin bound library of tweezer receptors **18** with a guanidinium head group.

Screening experiments were carried out with the tweezer library **18** using a range of dye-labelled guests **19**–**23** (Figure 3). Compound **19** was chosen as it had been identified as a guest for receptor **3** in previous studies (see above), but it was also of interest to consider the side chain deprotected (and more hydrophilic) analogue **20**, as well as the dipeptide D-Ala-D-Ala-OH **21**, given its well-known biological relevance.[10]

Screening experiments were carried out using an aqueous buffer solution (pH 9.2, borate). At this pH the guanidinium moiety is still protonated and the peptidic guests exist as deprotonated carboxylates. The screening experiments are simple to perform: a library sample is equilibrated in the buffer system; the peptide guest, dissolved in water and 20% DMSO, is added; after a second equilibration time, the selectivity in binding the peptide can be judged by observation of stained beads, visualised under a microscope. Additional aliquots of peptide guest can be added to increase the peptide concentration to provide optimal selectivity, as judged by the number of highly stained beads against a background of non or lightly stained beads.

Using this strategy, tweezer receptor library **18** was screened with dye-labelled peptide **19**. The selectivity (at



Scheme 2. a) 10 mol % Fmoc-L-Phe-OH, HOBt, PyBOP, DIPEA, DMF; b) **13**, HOBt, PyBOP, DIPEA, DMF; c) $[Pd(PPh_3)_4]$, Bu_3SnH, HOAc, CH_2Cl_2; d) 20 % piperidine in DMF; e) three-fold split-and-mix Fmoc-peptide synthesis using Gly, L-Ala, L-Val, L-Phe, L-Leu, L-Lys(Boc), L-Pro, L-Glu(O*t*Bu), L-Ser(*t*Bu), L-Met, L-Trp, L-Asn, L-Gln; f) 20 % piperidine in DMF; g) 45 % CF_3CO_2H, CH_2Cl_2; h) liquid HF.

peptide concentration 12.5 μM) was good, showing <1 % highly red coloured beads. Five of the most intensively stained beads were selected and sequenced by Edman degradation (Table 1).[11]

At first sight the structures identified from the screening experiments appear to lack much consensus. However, the third amino acid (AA^3) was methionine in three cases. The second position (AA^2) was leucine or closely related valine in three cases, and there is a clear preference for a branched hydrophobic side chain at this position. The first position (AA^1) was less well conserved, but proline was found in two cases.

Screening was also carried out with peptides **20** and **21**, but a much lower selectivity was observed (>10 % of beads were

Table 1. Sequencing data for five highly stained beads selected from the screening experiment of peptide **19** with library **25** in water.

Bead	AA1	AA2	AA3
1	Pro	Leu	Met
2	Phe	Leu	Met
3	Lys	Phe	Met
4	Pro	Ala	Gly
5	Gln	Val	Phe

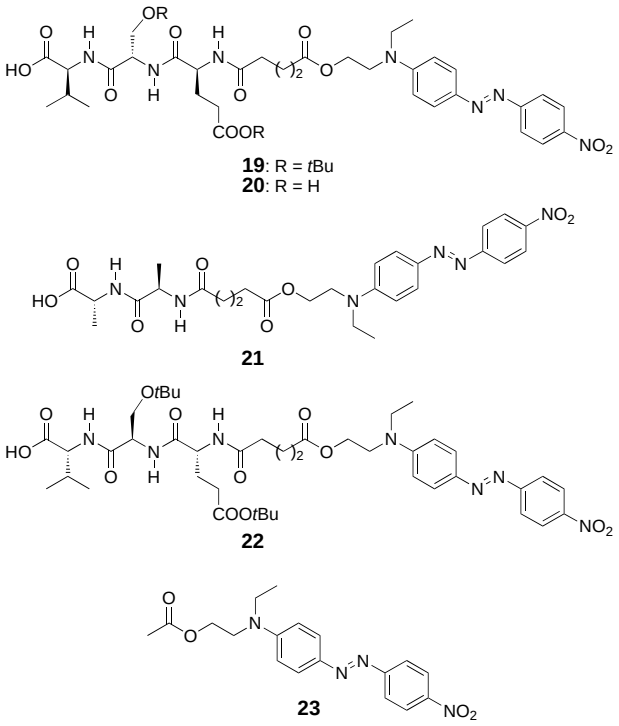


Figure 3. Red dye labelled guests.

significantly stained). The simple acylated red dye **23** was also incubated with library **18**, but no selectivity or strong staining was observed, confirming that the selectivity observed with the dye-labelled peptide was not a consequence of selective recognition of the dye moiety alone.

From the screening results with peptide **19**, tweezer receptor **25**, with peptide arms incorporating the amino acids L-Pro, L-Leu and L-Met (at positions AA^1-AA^3, respectively) was identified as a consensus structure, and was resynthesised on the solid-phase (Scheme 3).

Coupling of CBS **15** on to Rink amide resin and Fmoc deprotection was followed by sequential coupling of Fmoc-proline, Fmoc-leucine and Fmoc-methionine, and final Fmoc deprotection. Cleavage from the resin gave the tosylated guanidine tweezer **24**, which was detosylated with liquid HF. Purification on reversed-phase HPLC yielded receptor **25** as the TFA salt.

Binding studies using tweezer **25** and various peptide guests were carried out using a UV titration experiment. The intensity of the UV absorption maximum (at 500 nm) of the red dye moiety of the peptide guests was monitored as aliquots of the tweezer **25** were added. Titration of peptide **19** with tweezer receptor **25** resulted in a decrease in the absorption at 500 nm, with a clean isobestic point at 400 nm. The data from this experiment showed a good fit for the presumed 1:1 binding and allowed an estimate of the binding constant,[12] $K_a = 8.2 \times 10^4 \pm 2.5 \times 10^4\,M^{-1}$. Titration of the enantiomeric peptide **22** with **25** also resulted in a decrease in the absorption at 500 nm, with isobestic points at 410 and 590 nm, giving a binding constant, $K_a = 8.0 \times 10^3 \pm 1.5 \times 10^3\,M^{-1}$. The overall change in absorbance in these experiments was relatively small, presumably reflecting the fact that the dye moiety is not intimately involved in the complexation

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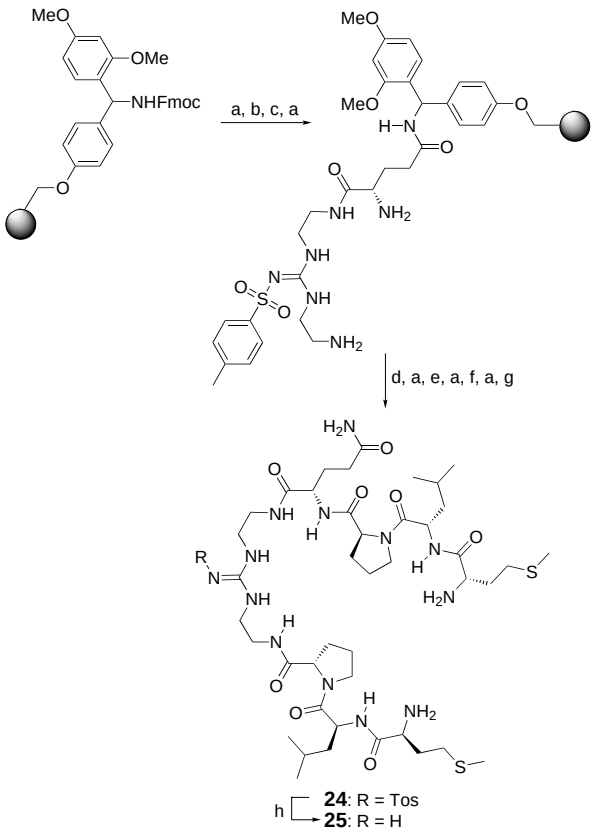

Scheme 3. a) 20% Piperidine in DMF; b) **15**, HOBt, PyBOP, DIPEA, DMF; c) Ac_2O, DMAP, CH_2Cl_2; d) Fmoc-L-Pro-OH, DIC, HOBt, DIPEA, DMF; e) Fmoc-L-Leu-OH, DIC, HOBt, DIPEA, DMF; f) Fmoc-L-Met-OH, DIC, HOBt, DIPEA, DMF; g) 10–45% CF_3CO_2H, TIS, CH_2Cl_2; h) liquid HF.

of peptide with tweezer. Titration experiments using tweezer **25** with the deprotected peptide **20** resulted in no detectable change to the UV absorption associated with the red dye-labelled peptide, confirming that tweezer **25** is not a good receptor for the side chain deprotected peptide. Titration experiments were also carried out using the tosylated tweezer **24** and peptide **19**, but again no detectable change in the UV absorption was observed, confirming that interaction between the free guanidinium of the receptor and the carboxylate terminus of the guest is essential for strong binding.

The binding studies serve to confirm that the tweezer structure identified from the screening experiments, is indeed able to bind the side chain protected peptide **19** in water, but also indicate that the tweezer receptor so identified is also truly selective, as it binds with good enantioselectivity ($\approx$10:1) and very high selectivity ($>$100:1) over the side chain deprotected peptide **20**.

Conclusion

Thus, we have devised a strategy for the synthesis of tweezer receptor libraries with a guanidinium head group, and successfully screened a small exemplar library to identify a selective receptor for a protected tripeptide. Selective receptors for less hydrophobic peptides **20** and **21** were not identified from this library, but we can expect to find such receptors using larger and more diverse (unsymmetrical) tweezer libraries, which are accessible from the orthogonally protected precursor **13**. Such studies are currently underway in our laboratory.

Experimental Section

General methods: Commercially available compounds were used without further purification. When necessary solvents were dried according to literature procedures.[13] Dimethylformamide for peptide synthesis was purchased from Rathburn Chemicals, HPLC grade solvents from Riedel-de-Haën. TentaGel S NH_2 resin was purchased from Rapp Polymere, Tübingen (Germany). Rink amide resin, *N*-Fmoc- and *N*-Boc-amino acids and coupling reagents were purchased from NovaBiochem. All other chemicals were purchased from Aldrich or Fluka. Peptide and library synthesis on solid-phase were performed in glass vessels with sinter frits or polypropylene filtration tubes with polyethylene frits on a Visiprep SPE Vacuum Manifold (Supelco). Reaction vessels were agitated either on a shaker (Stuart Scientific Flash Shaker SF1) or on a blood tube rotator (Stuart Scientific Blood Tube Rotator SB1). Thin-layer chromatography (TLC) was performed on aluminium-backed plates Merck silica gel 60 F_{254}. Column chromatography was performed on Sorbsil C60, 40–60 mesh silica. All melting points were determined in open capillary tubes using a Gallenkamp electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded on a Bio-Rad FT-IR spectrometer. Optical rotations were measured on a AA-100 Polarimeter. Proton NMR spectra were obtained on a Bruker AC 300 and on a Bruker DPX 400. Carbon NMR spectra were recorded at 75 MHz on a Bruker AC 300 and at 100 MHz on a Bruker DPX 400. Chemical shifts are reported in ppm on the δ scale relatively to TMS as internal standard or to the signal of the solvent used. Coupling constants are given in Hz. Signal multiplicities were determined using the distortionless enhancement by phase transfer (DEPT) spectral editing technique. Mass spectra were obtained on a VG analytical 70–250-SE normal geometry double focussing mass spectrometer. High resolution accurate mass measurements were carried out at 10000 resolution using mixtures of polyethylene glycols and/or polyethylene glycolmethyl ethers as mass calibrates for FAB. All electrospray (ES) spectra were recorded on a Micromass Platform quadrupole mass analyser with an electrospray ion source using acetonitrile as solvent. UV titration experiments were recorded on an Agilent 8453 UV-Visible spectrophotometer. UV absorbance of ninhydrin and Fmoc assays were measured on a Hewlett-Packard 8452A Diode Array Spectrometer using two way quartz cells. Absorbance values were recorded at 570 nm (ninhydrin) and 302 nm (Fmoc). The purification of red dye labelled tripeptides and the tweezer receptor **25** was achieved by semipreparative reversed-phase HPLC (Phenomenex Prodigy ODS(3) C-18, 250 $\times$ 10 mm) using a linear gradient from water +0.1% TFA to acetonitrile +0.042% TFA over 40 min, acetonitrile +0.042% TFA for 10 min, a linear gradient from acetonitrile +0.042% TFA to water +0.1% TFA over 5 min, and water +0.1% TFA for 5 min, with a flow rate of 2.5 mL min^{-1}, monitoring at 220 nm.

Abbreviations: Aloc, allyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; DIC, *N*,*N*′-diisopropylcarbodiimide; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DIPEA, diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMS, dimethyl sulphide; DMSO, dimethyl sulphoxide; EDT, 1,2-ethanedithiol; Fmoc, 9-fluorenyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolino-phosphonium hexafluorophosphate; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3,-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIS, triisopropyl silane; TMS, trimethylsilyl; Tos, 4-toluenesulfonyl.

1-(*tert*-Butyloxycarbonyl)ethyldiamine (5): Compund **5** was synthesised in according to literature procedures.[14] A solution of di-*tert*-butyl dicarbonate (6.1 g, 28 mmol) in dichloromethane (400 mL) was added dropwise to a solution of ethylenediamine (**4**, 11.2 mL, 166.7 mmol) in dichloromethane (50 mL) over 6 h with vigorous stirring. Stirring was continued for a further

24 h at room temperature. After concentration to an oily residue, the reaction mixture was dissolved in aqueous sodium carbonate (2 M, 300 mL) and extracted with dichloromethane (2 × 300 mL). The organic layer was dried (anhydrous $MgSO_4$) and the solvent evaporated under reduced pressure to yield **5** (4.47 g, 100 %) as a colourless viscous liquid. IR (film): $\tilde{\nu}$ = 3362, 3323, 2973, 2928, 2864, 1687, 1520, 1454, 1391, 1364, 1247, 1166, 867 cm^{-1}; MS (ES^+): *m*/*z* (%): 161 (28) $[M+H]^+$, 201 (8) $[M+CH_3CN]^+$, 321 (15) $[2M+H]^+$. All structural assignments were in agreement with the ^1H and ^{13}C NMR data available from the literature.[14]

1-(Allyloxycarbonyl)-4-(*tert*-butyloxycarbonyl)ethyldiamine (6): Allylchloroformate (5.1 mL, 48.4 mmol) was added slowly in small portions (about 0.5 mL) to a solution of **5** (3.1 g, 19.4 mmol) in a mixture of dioxane (15 mL) and pyridine (15 mL) at −5 °C and stirred at room temperature for 18 h. Evaporation of the solvents yielded a yellow solid. The crude product was dissolved in dichloromethane (60 mL) and washed with a concentrated aqueous solution of potassium carbonate (80 mL) and water (100 mL) followed by another wash with an aqueous solution of potassium hydrogen sulfate (60 mL, pH 3) and water (100 mL). The organic phase was dried (anhydrous $MgSO_4$) and evaporation of the solvent yielded pure **6** (4.48 g, 95 %) as a white solid. M.p. 111 – 112 °C; IR (CH_2Cl_2): $\tilde{\nu}$ = 3328, 2978, 2933, 1683, 1539, 1450, 1365, 1322, 1265, 1239, 1147, 990, 922, 870 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.44 (s, 9H, $C(CH_3)_3$), 3.28 (m, 4H, CH_2CH_2), 4.56 (d, *J* = 5.0 Hz, 2H, CH_2=CHCH_2O), 4.82 (brs, 1H, N*H*CO), 5.11 (brs, 1H, N*H*CO), 5.21 (dd, *J* = 10.5, 1.3 Hz, 1H, C*H*H=CHCH_2O), 5.30 (dd, *J* = 17.1, 1.3 Hz, 1H, C*H*H=CHCH_2O), 5.92 (m, 1H, CHH=C*H*CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4 (CH_3), 40.7 (CH_2), 41.5 (CH_2), 65.7 (CH_2), 79.7 (C), 117.7 (CH_2), 132.8 (CH), 156.4 (C), 156.7 (C); MS (ES^+): *m*/*z* (%): 511 (100) $[2M+Na]^+$, 527 (40) $[2M+K]^+$.

2-(Allyloxycarbonylamino)ethylisothiocyanate (7): Orthogonal protected diamine **6** (1.62 g, 6.66 mmol) was dissolved in a 40 % solution of TFA in dichloromethane (60 mL) and stirred for 2 h at room temperature. After addition of toluene (300 mL) all solvents were evaporated under reduced pressure. The TFA salt (1.71 g, 100 %) was obtained as a pale yellow foam. IR (CH_2Cl_2): $\tilde{\nu}$ = 3008, 1780, 1670, 1527, 1459, 1332, 1262, 1135, 1053, 983, 934, 838 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ = 3.05 (t, *J* = 5.8 Hz, 2H, $NHCH_2CH_2NH_3$), 3.39 (t, *J* = 5.8 Hz, 2H, $NHCH_2CH_2NH_3$), 4.56 (d, *J* = 6.8 Hz, 2H, CH_2=CHCH_2O), 4.88 (s, 4H, NH_3, N*H*CO), 5.19 (d, *J* = 10.5 Hz, 1H, C*H*H=CHCH_2O), 5.31 (dd, *J* = 17.3, 1.8 Hz, 1H, CH*H*=CHCH_2O), 5.94 (m, 1H, CH_2=C*H*CH_2O); ^{13}C NMR (100 MHz, CD_3OD): δ = 39.5 (CH_2), 41.1 (CH_2), 66.8 (CH_2), 117.7 (q, *J* = 291 Hz, CF_3), 117.8 (CH_2), 134.2 (CH), 159.3 (C), 162.1 (q, *J* = 36 Hz, C).

Thiophosgene (0.9 mL, 11.6 mmol) was added to a solution of the TFA salt (2.30 g, 8.91 mmol) in chloroform (530 mL) and aqueous sodium hydrogen carbonate (4.1 g, 49.0 mmol, in 220 mL water) at 0 °C. The reaction mixture was stirred at room temperature for 18 h. After separation of the organic layer the aqueous layer was extracted with chloroform (200 mL). The combined organic layers were dried (anhydrous $MgSO_4$) and the solvent removed under reduced pressure to afford **7** (1.45 g, 87 %) as a pale orange oil with no notable impurities. IR (film): $\tilde{\nu}$ = 3331, 2940, 2194, 2112, 2088, 1694, 1520, 1441, 1346, 1251, 1150, 991, 922, 774 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 3.45 (q, *J* = 5.6 Hz, 2H, $NHCH_2CH_2NCS$), 3.68 (t, *J* = 5.6 Hz, 2H, $NHCH_2CH_2NCS$), 4.59 (d, *J* = 5.0 Hz, 2H, CH_2=CHCH_2O), 5.24 (dd, *J* = 10.5, 1.3 Hz, 1H, C*H*H=CHCH_2O), 5.29 (brs, 1H, N*H*CO), 5.32 (dd, *J* = 17.1, 1.3 Hz, 1H, CH*H*=CHCH_2O), 5.93 (m, 1H, CH_2=C*H*CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 41.2 (CH_2), 45.5 (CH_2), 66.1 (CH_2), 118.2 (CH_2), 131.2 (C), 132.7 (CH), 156.3 (C).[15]

***N*-[2-(Allyloxycarbonyl)ethyl]-*N*′-[2-(*tert*-butyloxycarbonyl)-ethyl]thiourea (8)**: 1-(*tert*-Butyloxycarbonyl)ethyldiamine (**5**, 1.22 g, 7.63 mmol) was added to a solution of 2-(allyloxycarbonylamino)ethylisothiocyanate (**7**, 1.29 g, 6.94 mmol) in chloroform (60 mL) and the reaction mixture was refluxed for 18 h. The solvent was removed under reduced pressure to give a brown, oily residue. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH 95:5) or (CH_2Cl_2/EtOAc 50:50) afforded **8** (1.72 g, 72 %) as a pale brown oil. R_f = 0.22 (CH_2Cl_2/MeOH 95:5); IR (film): $\tilde{\nu}$ = 3318, 2974, 2932, 2362, 2337, 1690, 1525, 1392, 1366, 1253, 1166, 992, 911 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.45 (s, 9H, $C(CH_3)_3$), 3.31 (q, *J* = 5.7 Hz, 2H, $NHCH_2CH_2NHCS$), 3.42 (q, *J* = 5.7 Hz, 2H, $NHCH_2CH_2NHCS$), 3.54 (brs, 2H, $NHCH_2CH_2NHCS$), 3.62 (brs, 2H, $NHCH_2CH_2NHCS$), 4.57 (d, *J* = 5 Hz, 2H, CH_2=CHCH_2O), 5.09 (brs, 1H, N*H*CO), 5.22 (dd, *J* = 10.6, 1.3 Hz, 1H, C*H*H=CHCH_2O), 5.30 (dd, *J* = 17.1, 1.3 Hz, 1H, CH*H*=CHCH_2O), 5.45 (brs, 1H, N*H*CO), 5.90 (m, 1H, CH_2=C*H*CH_2O), 6.83 (brs, 2H, N*H*CS); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4 (CH_3), 39.7 (CH_2), 40.3 (CH_2), 44.9 (CH_2), 45.2 (CH_2), 65.9 (CH_2), 80.4 (C), 117.9 (CH_2), 132.7 (CH), 157.3 (C), 182.3 (C); MS (ES^+): *m*/*z* (%): 347 (5) $[M+H]^+$, 693 (5) $[2M+H]^+$, 715 (27) $[2M+Na]^+$.

***N*-[2-(Allyloxycarbonyl)ethyl]-*N*′-[2-(*tert*-butyloxycarbonyl)-ethyl]-*S*-methylthiouronium hexafluorophosphate (9)**: Methyl iodide (0.62 mL, 9.93 mmol) was added to a solution of **8** (1.72 g, 4.96 mmol) in acetone (50 mL) and the reaction mixture was stirred for 18 h at room temperature. The solvent and other volatile compounds were removed under reduced pressure to give a pale yellow foam. After dissolution in a mixture of dichloromethane (30 mL) and methanol (30 mL), ammonium hexafluorophosphate (1.62 g, 9.92 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvent was evaporated and the oily residue redissolved in dichloromethane (50 mL). After washing with water (70 mL) and drying over magnesium sulfate the solvent was removed under reduced pressure to give **9** (2.39 g, 96 %) as a colourless foam. R_f = 0.08 (CH_2Cl_2/MeOH 95:5); IR (CH_2Cl_2): $\tilde{\nu}$ = 1688, 1616, 1525, 1445, 1254, 1163, 994, 838 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 9H, $C(CH_3)_3$), 2.63 (s, 3H, $CSCH_3$), 3.45 (brs, 4H, $NHCH_2CH_2$), 3.54 (brs, 4H, $NHCH_2CH_2NH$), 4.57 (d, *J* = 4.5 Hz, 2H, CH_2=CHCH_2O), 5.21 (d, *J* = 10.4 Hz, 1H, C*H*H=CHCH_2O), 5.30 (d, *J* = 17.4 Hz, 1H, CH*H*=CHCH_2O), 5.56 (brs, 1H, N*H*CO), 5.89 (m, 2H, CH_2=C*H*CH_2O, N*H*CO), 8.12 (brs, 2H, N*H*CSCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.3 (CH_3), 39.5 (br, CH_2), 45.9 (br, CH_2), 47.0 (CH_3), 66.2 (CH_2), 67.5 (br, CH_2), 118.0 (CH_2), 120.0 (CH), 125.2 (CH), 127.2 (CH), 127.8 (CH), 132.4 (CH), 141.3 (C), 143.7 (C), 167.2 (C).

***N*1-{1-[2′-(Allyloxycarbonyl)ethylamino]-1-[2″-(*tert*-butyloxycarbonyl)-ethylamino]methylidene}-4-methyl-1-benzenesulfonamide (10)**: DBU (150 μL, 0.88 mmol) was added to a solution of tosyl amide (377 mg, 2.2 mmol) and **9** (233 mg, 0.44 mmol) in a mixture of toluene (20 mL) and chloroform (5 mL), and refluxed for 24 h. The solvents were removed under reduced pressure and the residue purified by column chromatography on silica gel (petroleum ether/EtOAc 10:90) to obtain **10** (196 mg, 92 %) as a pale yellow oil. R_f = 0.34 (petroleum ether/EtOAc 10:90); IR (film): $\tilde{\nu}$ = 3328, 2983, 2928, 1690, 1648, 1571, 1552, 1244, 1158, 1079, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.44 (s, 9H, $OC(CH_3)_3$), 2.39 (s, 3H, ArCH_3), 3.31 (brs, 8H, $(NHCH_2CH_2NH)_2CNR$), 4.53 (d, *J* = 5.0 Hz, 2H, CH_2=CHCH_2O), 5.19 (d, *J* = 10.0 Hz, 1H, C*H*H=CHCH_2O), 5.23 (brs, 2H, N*H*CO), 5.27 (dd, *J* = 17.1, 1.5 Hz, 1H, CH*H*=CHCH_2O), 5.88 (m, 1H, CH_2=C*H*CH_2O), 7.25 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.72 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.93 (brs, 2H, N*H*CNR); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.8 (CH_3), 28.7 (3CH_3), 40.0 (br, CH_2), 40.6 (br, CH_2), 41.4 (br, CH_2), 66.2 (CH_2), 80.5 (br, C), 118.1 (CH_2), 126.3 (CH), 129.8 (CH), 133.1 (CH), 140.9 (C), 142.7 (C), 156.1 (C), 157.6 (br, C); MS (ES^+): *m*/*z* (%): 507 (100) $[M+Na]^+$, 990 (40) $[2M+Na]^+$. Performed later on a larger scale, DBU (1.8 mL, 11.0 mmol) was added to a solution of tosyl amide (2.6 g, 13.7 mmol) and **9** (2.78 g, 5.49 mmol) in a mixture of toluene (50 mL) and chloroform (10 mL). After refluxing for 48 h the solvent was evaporated under reduced pressure. Column chromatography afforded **10** as a pale yellow oil in a reduced yield (71 %).

***N*1-{1-[2′-(*tert*-butyloxycarbonyl)ethylamino]-1-[2″-[(9*H*-fluorenyl)methoxycarbonyl)]ethylamino]methylidene}-4-methyl-1-benzenesulfonamide (11)**: 9-Fluorenylmethyl chloroformate (86 mg, 0.33 mmol) was added to a solution of **10** (152 mg, 0.32 mmol) in dichloromethane followed by the addition of a $[Pd(PPh_3)_4]$ solution (18 mg, 16 μmol in 2 mL dichloromethane) and tributyltin hydride (110 μL, 0.41 mmol).[16] After the additions the solution was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel (CH_2Cl_2/MeOH 98:2) to afford **11** (150 mg, 77 %) as a pale yellow foam. R_f = 0.4 (CH_2Cl_2/MeOH 90:10); IR (film): $\tilde{\nu}$ = 3329, 2967, 1699, 1580, 1520, 1443, 1362, 1255, 1132, 1076 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.40 (s, 9H, $C(CH_3)_3$), 2.36 (s, 3H, ArCH_3), 3.20 (brs, 2H, $NHCH_2CH_2$), 3.33 (brs, 6H, $NHCH_2CH_2$), 4.19 (t, *J* = 6.5 Hz, 1H, $NHCOOCH_2CHR_2$), 4.38 (d, *J* = 6.5 Hz, 2H, $NHCOOCH_2CHR_2$), 5.05 (brs, 2H, *NH*CO), 7.21 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.29 (t, *J* = 7.5 Hz, 2H, Ar*H*), 7.39 (t, *J* = 7.5 Hz, 2H, Ar*H*), 7.57 (d, *J* = 7.5 Hz, 2H, Ar*H*), 7.75 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.78 (d, *J* = 8.4 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.4 (CH_3), 28.3 (CH_3), 39.9 (CH_2), 40.3 (CH_2), 41.7 (CH_2), 42.1 (CH_2), 47.2 (CH), 67.2 (CH_2), 80.6 (C), 120.0 (CH), 125.1 (CH), 126.1 (CH), 127.2 (CH), 127.8 (CH), 129.3 (CH),

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140.6 (C), 141.3 (C), 142.2 (C), 143.8 (C), 155.3 (C), 157.3 (br, C); MS (ES^+): *m/z* (%): 621 (32) $[M+H]^+$, 643 (100) $[M+Na]^+$, 644 (25) $[M+H+Na]^+$.

***tert*-Butyl (4*S*)-5-({2-[({2-{[(allyloxy)carbonyl]amino}ethyl)-amino]{[(4-methylphenyl)sulfonyl]imino}methyl)amino]ethyl}-amino)-4-{[(9*H*-fluorenylmethyloxy)carbonyl]amino}-5-oxo-pentanoate (12)**: Compound **10** (436 mg, 0.9 mmol) was stirred in 20% TFA in dichloromethane (25 mL) at room temperature for 2 h. After addition of toluene (50 mL) the solvents were removed under reduced pressure to yield the corresponding TFA salt as a light brown oil.

A solution of *N*-α-Fmoc-L-glutamic acid γ-*tert*-butyl ester (422 mg, 0.99 mmol), PyBOP (517 mg, 0.99 mmol), and HOBt (152 mg, 0.99 mmol) in dichloromethane (15 mL) was stirred at room temperature for 10 min and then added to a solution of the TFA salt in dichloromethane (5 mL). After addition of DIPEA (0.39 mL, 2.26 mmol) the resulting reaction mixture was stirred for 18 h. More dichloromethane (50 mL) was added and the mixture washed with water (100 mL) to remove unwanted salts. The organic layer was dried (anhydrous $MgSO_4$) and the solvent removed under reduced pressure to give a brown oil. Purification by gradient column chromatography on silica gel (petroleum ether/EtOAc 20:80 to pure EtOAc) afforded **12** (562 mg, 79%) as a white solid. M.p. 175 °C; $[\alpha]_D^{RT} = -6.1°$ ($c = 9.5$ mg mL^{-1}, $l = 0.5$ dm, CH_3CN); $R_f = 0.34$ (CH_2Cl_2/MeOH 95:5); IR (CH_2Cl_2): $\tilde{\nu}$ = 3329, 2979, 2361, 2341, 1712, 1522, 1449, 1264, 1242, 1149, 1130, 1081 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.43$ (s, 9H, C(CH_3)$_3$), 1.93 (m, 1H, CHRCH_aH$_b$CH$_2$CO), 2.10 (m, 1H, CHRCH$_a$$H_bCH_2$CO), 2.35 (brs, 5H, ArC$H_3$, CHRCH$_2CH_2$COO), 3.28 (brs, 8H, (NHCH_2CH_2NH)$_2$CNR), 4.19 (m, 2H, NHCOOCH$_2$CHR$_2$, CHRCH$_2$CH$_2$COO), 4.33 (brd, $J = 8.6$ Hz, 2H, NHCOOCH_2CHR$_2$), 4.50 (brd, $J = 6.0$ Hz, 2H, CH$_2$=CHCH_2O), 5.15 (d, $J = 10.5$ Hz, 1H, CHH=CHCH$_2$O), 5.24 (d, $J = 17.6$ Hz, 1H, CHH=CHCH$_2$O), 5.85 (m, 1H, CH$_2$=CHCH$_2$O), 6.22 (brs, 3H, NHCO), 7.20 (d, $J = 8.0$ Hz, 2H, ArH), 7.28 (t, $J = 7.5$ Hz, 2H, ArH), 7.37 (t, $J = 7.4$ Hz, 2H, ArH), 7.53 (brs, 2H, NHCNR), 7.61 (d, $J = 7.0$ Hz, 2H, ArH), 7.74 (m, 4H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.1 (CH_3), 27.6 (CH_2), 27.8 (CH_3), 31.4 (CH_2), 39.4 (br, CH_2), 40.0 (br, CH_2), 41.1 (br, CH_2), 46.8 (CH), 54.5 (br, CH), 65.3 (CH_2), 66.8 (CH_2), 80.3 (C), 117.2 (CH_2), 119.6 (CH), 125.0 (CH), 125.6 (CH), 126.8 (CH), 127.4 (CH), 128.9 (CH), 132.7 (CH), 140.9 (C), 141.6 (C), 143.5 (C), 143.7 (C), 155.6 (C), 156.0 (C), 156.7 (C), 172.1 (br, C), 173.0 (br, C); MS (ES^+): *m/z* (%): 792 (41) $[M+H]^+$, 814 (8) $[M+Na]^+$; HRMS (FAB^+): *m/z*: calcd for $C_{40}H_{51}N_6O_9S$ $[M+H]^+$: 791.3438; found: 791.3412.

(4*S*)-5-({2-[({2-{(Allyloxy)carbonyl]amino}ethyl)amino]{[(4-methylphenyl)-sulfonyl]imino}methyl)amino]ethyl}amino)-4-{[(9*H*-fluorenylmethyloxy)carbonyl]amino}-5-oxopentanoic acid (13): Compound **12** (562 mg, 0.71 mmol) was stirred vigorously in 70% TFA in dichloromethane (20 mL) for 24 h at room temperature. Toluene (100 mL) was added and the solvents removed under reduced pressure. The resulting brown oil was purified by gradient column chromatography on silica gel (CH_2Cl_2/MeOH 95:5 to 90:10) to yield **13** (471 mg, 90%) as a white foam. $[\alpha]_D^{RT} = -6.1°$ ($c = 5.3$ mg mL^{-1}, $l = 0.5$ dm, CH_3CN); $R_f = 0.34$ (CH_2Cl_2/MeOH 90:10); IR (film): $\tilde{\nu}$ = 3334, 2938, 2358, 2336, 1709, 1571, 1529, 1449, 1413, 1345, 1245, 1190, 1130, 1082 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.91$ (m, 1H, CHRCH_aH$_b$CH$_2$CO$_2$), 2.07 (m, 1H, NHCOCHRCH$_a$$H_bCH_2CO_2$), 2.38 (s, 5H, ArC$H_3$, CHRCH$_2CH_2CO_2$), 3.23–3.38 (brm, 8H, (NHCH_2CH_2-NH)$_2$CNR), 4.16 (m, 2H, CHRCH$_2$CH$_2$CO$_2$, NHCOOCH$_2$CHR$_2$), 4.37 (t, $J = 7.5$ Hz, 2H, NHCOOCH_2CHR$_2$), 4.52 (d, $J = 5.0$ Hz, 2H, CH$_2$=CHCH_2O), 5.18 (d, $J = 10.5$ Hz, 1H, CHH=CHCH$_2$O), 5.27 (dd, $J = 17.1$, 1.5 Hz, 1H, CHH=CHCH$_2$O), 5.87 (m, 1H, CH$_2$=CHCH$_2$O), 6.32 (brs, 3H, NHCO), 7.24 (d, $J = 8.0$ Hz, 2H, ArH), 7.30 (t, $J = 7.5$ Hz, 2H, ArH), 7.40 (m, 2H, ArH), 7.61 (m, 2H, ArH), 7.73 (d, $J = 8.0$ Hz, 2H, ArH), 7.76 (d, $J = 7.5$ Hz, 2H, ArH), 7.92 (brs, 2H, NHCNR); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.4 (CH_3), 28.0 (CH_2), 30.9 (CH_2), 39.4 (CH_2), 41.0 (CH_2), 41.3 (CH_2), 42.6 (CH_2), 47.3 (CH), 54.4 (CH), 65.9 (CH_2), 67.3 (CH_2), 117.7 (CH_2), 120.1 (CH), 125.2 (CH), 126.0 (CH), 127.3 (CH), 127.9 (CH), 129.5 (CH), 132.8 (CH), 141.4 (C), 142.5 (C), 143.9 (C), 144.0 (C), 155.0 (C), 155.8 (C), 157.0 (C), 160.2 (C), 173.7 (C); MS (ES^+): *m/z*: 757 (10) $[M+Na]^+$; HRMS (ES^+): *m/z*: calcd for $C_{36}H_{42}N_6NaO_9S$ $[M+Na]^+$: 757.2626; found: 757.2618.

***tert*-Butyl (4*S*)-4-{[(9*H*-fluorenylmethoxy)carbonyl]amino}-5-({2-[({2-{[(9*H*-fluorenylmethyloxy)carbonyl]amino}ethyl)-amino]{[(4-methylphenyl)-sulfonyl]imino}methyl)-amino]ethyl}amino)-5-oxopentanoate (14)**: Compound **11** (290 mg, 0.47 mmol) was stirred in a 20% TFA in dichloromethane (12 mL) at room temperature for 2 h. After addition of toluene (75 mL), the solvents were removed under reduced pressure to yield the corresponding TFA salt as a light brown oil.

A solution of *N*-α-Fmoc-L-glutamic acid γ-*tert*-butyl ester (239 mg, 0.56 mmol), PyBOP (292 mg, 0.56 mmol), and HOBt (86 mg, 0.56 mmol) in dichloromethane (8 mL) was stirred at room temperature for 10 min and then added to a solution of the TFA salt in dichloromethane (5 mL) followed by the addition of DIPEA (0.22 mL, 1.26 mmol). After the reaction have been stirred for 18 h the solvent was removed under reduced pressure to give a brown oil. Purification by gradient column chromatography on silica gel (petroleum ether/EtOAc 20:80 to pure EtOAc) gave **14** (420 mg, 97%) as a white solid. M.p. 138 °C; $[\alpha]_D^{RT} = -5.9°$ ($c = 8.8$ mg mL^{-1}, $l = 0.5$ dm, CH_3CN); $R_f = 0.41$ (CH_2Cl_2/MeOH 90:10); IR (CH_2Cl_2): $\tilde{\nu}$ = 3332, 2924, 1719, 1572, 1450, 1151, 1132, 1083 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.41$ (s, 9H, C(CH_3)$_3$), 1.94 (m, 1H, CHRCH_aH$_b$CH$_2$CO), 2.09 (m, 1H, CHRCH$_a$$H_bCH_2$CO), 2.32 (brs, 5H, ArC$H_3$, CHRCH$_2CH_2$COO), 3.27 (brs, 8H, (NHCH_2CH_2NH)$_2$CNR), 4.13 (m, 3H, NHCOOCH$_2$CHR$_2$, CHRCH$_2$CH$_2$COO), 4.34 (brd, $J = 7.4$ Hz, 4H, NHCOOCH_2CHR$_2$), 5.60 (brs, 3H, NHCO), 7.16 (d, $J = 8.0$ Hz, 2H, ArH), 7.26 (t, $J = 7.5$ Hz, 4H, ArH), 7.35 (t, $J = 7.4$ Hz, 2H, ArH), 7.36 (t, $J = 7.4$ Hz, 2H, ArH), 7.54 (d, $J = 8.0$ Hz, 2H, ArH), 7.56 (d, $J = 8.0$ Hz, 2H, ArH), 7.73 (d, $J = 8.0$ Hz, 4H, ArH), 7.75 (d, $J = 8.0$ Hz, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.5 (CH_3), 28.1 (CH_3), 29.8 (CH_2), 31.7 (CH_2), 39.6 (br, CH_2), 40.4 (br, CH_2), 41.4 (br, CH_2), 47.2 (CH), 54.9 (br, CH), 67.2 (CH_2), 81.2 (C), 120.1 (CH), 125.2 (CH), 126.0 (CH), 127.2 (CH), 127.8 (CH), 129.3 (CH), 140.9 (C), 141.4 (C), 142.2 (C), 143.9 (C), 155.8 (C), 156.5 (br, C), 157.4 (br, C), 173.0 (br, C), 173.1 (br, C); MS (ES^+): *m/z* (%): 950 (25) $[M+Na]^+$, 951 (12) $[M+Na+H]^+$; HRMS (FAB^+): *m/z*: calcd for $C_{51}H_{57}N_6O_9S$ $[M+H]^+$: 929.3908; found: 929.3923.

(4*S*)-4-{[(9-Fluorenylmethyloxy)carbonyl]amino}-5({2-[({2-{[(9*H*-fluorenylmethoxy)carbonyl]amino}ethyl)amino]{[(4-methylphenyl)-sulfonyl]-imino}methyl)amino]ethyl}amino)-5-oxopentanoic acid (15): Compound **14** (400 mg, 0.43 mmol) was stirred vigorously in 70% TFA in dichloromethane (11 mL) for 24 h at room temperature. Toluene (50 mL) was added and the solvents removed under reduced pressure. The resulting brown oil was purified by gradient column chromatography on silica gel (CH_2Cl_2/MeOH 95:5 to 90:10) to yield **15** (216 mg, 58%) as a white solid. M.p. 125–130 °C; $[\alpha]_D^{RT} = -6.5°$ ($c = 3.4$ mg mL^{-1}, $l = 0.5$ dm, CH_3CN); $R_f = 0.22$ (CH_2Cl_2/MeOH 90:10); IR (film): $\tilde{\nu}$ = 3346, 2938, 2362, 2343, 1714, 1573, 1531, 1449, 1417, 1344, 1260, 1191, 1130, 1081 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.95$ (m, 1H, CHRCH_aH$_b$CH$_2$CO$_2$), 2.09 (m, 1H, CHRCH$_a$$H_bCH_2CO_2$), 2.27 (s, 3H, ArC$H_3$), 2.40 (brm, C$H_2CO_2$H), 3.00–3.50 (brm, 8H, (NHCH_2CH_2NH)$_2$CNR), 4.10 (m, 3H, NHCOOCH$_2$CHR$_2$, CHRCH$_2$CH$_2$COO), 4.31 (m, 4H, NHCOOCH_2CHR$_2$), 5.73 (brs, 1H, NHCO), 6.29 (brs, 2H, NHCO), 7.11 (d, $J = 8.0$ Hz, 2H, ArH), 7.21 (t, $J = 6.5$ Hz, 4H, ArH), 7.31 (t, $J = 7.0$ Hz, 4H, ArH), 7.50 (t, $J = 7.50$ Hz, 4H, ArH), 7.68 (d, $J = 7.5$ Hz, 4H, ArH), 7.73 (d, $J = 8.0$ Hz, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.3 (CH_3), 27.6 (CH_2), 29.9 (CH_2), 40.2 (br, CH_2), 40.9 (br, CH_2), 47.0 (CH), 54.2 (CH), 67.1 (CH_2), 67.3 (CH_2), 119.9 (CH), 125.1 (CH), 125.9 (CH), 127.1 (CH), 127.7 (CH), 129.4 (CH), 140.6 (C), 141.2 (C), 142.2 (C), 143.72 (C), 155.8 (C), 156.5 (C), 157.4 (C), 173.1 (C), 176.0 (C); MS (ES^+): *m/z*: 874 (30) $[M+H]^+$; HRMS (FAB^+): *m/z*: calcd for $C_{47}H_{49}N_6O_9S$ $[M+H]^+$: 873.3282; found: 873.3286.

General procedure for aloc deprotection on the solid-phase: A solution of $[Pd(PPh_3)_4]$ (0.04 equiv) and acetic acid (3.5 equiv) in dichloromethane (3 mL per g resin) was added to the pre-swollen resin (1 equiv of Aloc protected amino sites) followed by addition of tributyltin hydride (3 equiv) and the reaction mixture was agitated for 60 min.[17] The resin was drained, washed with dichloromethane (3×), a solution of triethylamine in dichloromethane (10% *w*/*w*) (3×), a solution of sodium diethyldithiocarbamate in DMF (0.5% *w*/*w*) (3×), methanol (3×), and dichloromethane (3×) (10 mL solvent per g resin). The progress of the deprotection was monitored by the ninhydrin test.

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 drained and washed with dichloromethane (3×), DMF (3×) and dichloromethane (3×) (10 mL solvent per g resin). The procedure was repeated once and the progress of the deprotection monitored by the ninhydrin test.

General procedure for Boc deprotection on the solid-phase: The Boc-protected resin was suspended in a mixture of 45% dichloromethane, 45%

TFA, 4% EDT, 3% DMS, and 3% anisole (20 mL per g resin) and agitated for 60 to 120 min. The resin was drained and washed with dichloromethane (3 ×), DMF (3 ×), a 20% solution of DIPEA in dichloromethane (3 ×), methanol (3 ×), DMF (3 ×) and dichloromethane (3 ×) (10 mL solvent per g resin). The DIPEA solution wash was omitted for samples requiring prolonged storage. The progress of the deprotection was monitored by the 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 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 ×) and finally 20% DIPEA in dichloromethane (3 ×), methanol (3 ×) and dichloromethane (3 ×) (20 mL solvent per g resin). The DIPEA treatment was omitted for samples requiring prolonged storage.

Synthesis of the tweezer receptor library (18): A solution of Fmoc-L-Phe-OH (14 mg, 35.7 μmol), PyBOP (19 mg, 35.7 μmol), and HOBt (5 mg, 35.7 μmol) in DMF (7 mL) was stirred for 10 min and then added to pre-swollen TentaGel S NH_2 resin (1.23 g, 0.36 mmol free NH_2) in DMF (10 mL) followed by addition of neat DIPEA (18 mg, 0.14 mmol, 50 μL). After agitation on a tube rotator for 24 h at room temperature the resin was drained, washed with dichloromethane (3 × 15 mL), DMF (3 × 15 mL), dichloromethane (3 × 15 mL) and dried. A quantitative ninhydrin test indicated a loading of approximately 10%.

A solution of CBS **13** (354 mg, 0.48 mmol), PyBOP (250 mg, 0.48 mmol) and HOBt (74 mg, 0.48 mmol) in DMF (4 mL) was stirred for 10 min and added to the pre-swollen resin in DMF (10 mL) followed by addition of neat DIPEA (145 mg, 1.12 mmol, 190 μL). After agitation on a tube rotator for 24 h at room temperature the resin was drained, washed with dichloromethane (3 × 15 mL), DMF (3 × 15 mL), dichloromethane (3 × 15 mL) and dried. A qualitative ninhydrin test showed no free amino functions.

After performing an Aloc deprotection step (as described in the general procedure) a qualitative ninhydrin test showed free amino functions, as expected. Following a subsequent Fmoc deprotection (see general procedure) the resin was divided into 13 equal portions. Each portion was pre-swollen in DMF (2 mL). A solution of a Fmoc-protected amino acid (0.11 mmol, 2 equiv), TBTU (32 mg, 0.1 mmol), and HOBt (2 mg, 0.01 mmol) in DMF (2 mL) was pre-activated for a few minutes and then added to each portion followed by DIPEA (36 mg, 0.28 mmol, 48 μL). Each portion was agitated on a tube rotator for at least 2–24 h at room temperature. The portions were washed with dichloromethane (3 × 5 mL), DMF (3 × 5 mL), and dichloromethane (3 × 5 mL) and dried. The success of the coupling step was monitored by a qualitative ninhydrin test. Each coupling cycle was repeated until a ninhydrin test showed no free amino functions. The quantities of Fmoc-amino acid used per coupling cycle were as follows: L-Ala (35 mg), L-Asn (39 mg), L-Glu(O*t*Bu) (47 mg), L-Gln (41 mg), L-Gly (33 mg), L-Leu (39 mg), L-Lys(Boc) (52 mg), L-Met (41 mg), L-Phe (43 mg), L-Pro (37 mg), L-Ser(*t*Bu) (43 mg), L-Trp (47 mg), L-Val (38 mg). Alternatively, PyBOP (52 mg, 0.1 mmol) or HATU (38 mg, 0.1 mmol) were employed as alternative coupling reagents.

After each successful coupling step, the resin was re-combined and Fmoc-deprotected as described in the general procedure and monitored by a qualitative ninhydrin test. This split-and-mix procedure was then repeated twice in order to build up the tripeptide side arms. Finally, the resin was mixed together and subsequently Fmoc-, Boc-, and tosyl-deprotected as described in the general procedures to yield tweezer receptor library **18**.

Red dye-spacer-L-Glu(OtBu)-L-Ser(tBu)-L-Val-OH (19):[18] A solution of 4-(4-hydroxymethyl-2-methoxyphenyl) butanoic acid (HMPB-linker) (1.08 mmol, 347 mg), HOBt (1.08 mmol, 146 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 μL) in DMF (25 mL) was added to a TentaGel S NH_2 resin (2 g, 0.54 mmol NH_2 sites) and agitated overnight. After washing the resin with dichloromethane (3 × 15 mL), DMF (3 × 15 mL) and dichloromethane (3 × 15 mL), a symmetrical anhydride solution (generated in situ from Fmoc-L-Val-OH (2.7 mmol, 916 mg) and DIC (2.7 mmol, 340 mg) in dichloromethane (25 mL)) was added to the resin followed by the addition of DMAP (6.0 mg, 0.05 mmol) and agitated overnight. After washing with dichloromethane (3 × 5 mL), DMF (3 × 5 mL) and dichloromethane (3 × 5 mL) the completion of the coupling step was monitored by a qualitative ninhydrin test. The Fmoc group was removed using the general procedure and a qualitative ninhydrin test confirmed the presence of free amino functions.

In the second amino acid coupling, a solution of Fmoc-L-Ser(*t*Bu)-OH (1.08 mmol, 414 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 μL) in DMF (25 mL) was added to the resin and agitated for 4 h. The resin was washed as before and a qualitative ninhydrin test indicated complete coupling. Fmoc deprotection, subsequent coupling of Fmoc-L-Glu(O*t*Bu)-OH (460 mg, 1.08 mmol) followed by further Fmoc deprotection provided the resin-bound tripeptide. A solution of red dye-linked glutaric acid[19] (1.08 mmol, 462 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 μL) in DMF (25 mL) was added to the resin bound tripeptide and agitated overnight. After washing with dichloromethane (3 × 5 mL), DMF (3 × 5 mL) and dichloromethane (3 × 5 mL) the resin was dried and divided into two equal portions. One half was used in the synthesis of red dye-spacer-L-Glu-L-Ser-L-Val-OH (**20**), whereas the second half was agitated again overnight with 1% TFA in DMF. The solvent was removed by filtration and the solvent evaporated under reduced pressure to give the crude product **19**. Purification by column chromatography afforded the pure compound **19** (23 mg, 20%). M.p. 98–100 °C; $R_f = 0.37$ (CH_2Cl_2/MeOH 90:10); IR (CH_2Cl_2): $\tilde{\nu} = 3344$, 2966, 2918, 1728, 1701, 1672, 1645, 1627, 1600, 1512, 1422, 1388, 1336, 1250, 1228, 1194, 1137, 1104, 1016, 945, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.87$ (d, $J = 6.7$ Hz, 3H, CH_3CHCH_3), 0.90 (d, $J = 6.7$ Hz, 3H, CH_3CHCH_3), 1.13 (s, 9H, $C(CH_3)_3$), 1.18 (t, $J = 7.2$ 3H, NCH_2CH_3), 1.37 (s, 9H, $C(CH_3)_3$), 1.87 (qv, $J = 7.3$ Hz, 3H, $CH_2CH_2CH_2CO$, βCHGlu), 2.01 (m, 1H, βCHGlu), 2.15–2.23 (m, 3H, CH_3CHCH_3, $CH_2CH_2CH_2CONH$), 2.29–2.33 (m, 4H, $OCOCH_2CH_2CH_2CO$, γCHGlu), 3.36 (t, $J = 8.0$ Hz, 1H, αCHSer), 3.46 (q, $J = 7.0$ Hz, 2H, NCH_2CH_3), 3.61 (t, $J = 6.0$ Hz, 2H, NCH_2CH_2), 3.72 (m, 1H, αCHGlu), 4.23 (t, $J = 6.0$ Hz, 1H, OCH_2CH_2), 4.41 (m, 3H, αCHVal, CH_2OtBu), 6.73 (d, $J = 9.0$ Hz, 2H, Ar*H*), 6.88 (br s, 1H, N*H*CO), 7.25 (m, 2H, N*H*CO), 7.84 (m, 4H, Ar*H*), 8.25 (d, $J = 9.0$ Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.6$ (CH_3), 18.0 (CH_3), 20.9 (CH_2), 20.9 (CH_2), 27.7 (CH_2), 28.3 (CH_2), 28.4 (CH_2), 29.6 (CH_3), 29.8 (CH_3), 32.2 (CH_2), 34.3 (CH_2), 46.1 (CH_2), 49.1 (CH_2), 53.3 (CH), 53.7 (CH), 58.0 (CH), 61.6 (CH), 61.3 (CH_2), 74.7 (C), 81.6 (C), 111.8 (2CH), 123.0 (2CH), 125.0 (2CH), 126.6 (2CH), 144.2 (C), 147.8 (C), 151.6 (C), 157.1 (C), 163.2 (C), 170.7 (C), 172.0 (C), 173.3 (C), 175.1 (C), 176.0 (C); MS (ES^+): *m*/*z* (%): 856 (100%) $[M+H]^+$; HRMS (ES^+): *m*/*z*: calcd for $C_{42}H_{62}N_7O_{12}$ $[M+H]^+$: 856.4451; found: 856.4437.

Red dye-spacer-L-Glu-L-Ser-L-Val-OH (20): The resin bound red dye-spacer-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH (300 mg) was agitated overnight in 50% TFA in DMF. The solvent was removed by filtration and the solvent evaporated under reduced pressure to afford crude **20**. Characterisation was achieved following purification by semipreparative reversed-phase HPLC (for conditions see general methods). Under these conditions, the TFA salt of **20** eluted after 18.78 min. The TFA salt of **20** was obtained as a red foam (15 mg). $R_f = 0.90$ (CH_2Cl_2/MeOH 90:10); IR (CH_2Cl_2): $\tilde{\nu} = 3283$, 2920, 1666, 1600, 1550, 1515, 1434, 1389, 1337, 1256, 1185, 1130, 1006, 851, 800, 753, 722 cm^{-1}; ^1H NMR (400 MHz, DMSO): $\delta = 0.72$ (d, $J = 7.0$ Hz, 6H, CH_3CHCH_3), 1.02 (t, $J = 8.0$ Hz, 3H, NCH_2CH_3), 1.60 (m, 3H, $CH_2CH_2CH_2$, $CHRCHHCH_2CO$), 1.75 (m, 1H, $CHRCHHCH_2CO$), 1.90 (m, 1H, CH_3CHCH_3), 2.02 (t, $J = 7.5$ Hz, 2H, $CH_2CH_2CH_2CON$), 2.11 (t, $J = 7.8$ Hz, 2H, $CHRCH_2CH_2CO$), 2.17 (t, $J = 7.5$, 2H, $CH_2OCOCH_2CH_2CH_2$), 3.39–3.45 (m, 4H, NCH_2CH_3, CH_2OH), 3.58 (br t, 2H, NCH_2CH_2), 4.01 (t, $J = 8.0$ Hz, 1H, αCHVal), 4.10 (br t, 2H, $CH_2CH_2OCOCH_2$), 4.16 (m, 1H, αCHGlu), 4.21 (m, 1H, αCHSer), 4.71 (br s, 1H, N*H*Glu), 6.78 (d, $J = 9.0$ Hz, 2H, Ar*H*), 7.66 (d, $J = 8.5$ Hz, 1H, N*H*Val), 7.71 (d, $J = 9.0$ Hz, 2H, Ar*H*), 7.80 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.78 (d, $J = 7.8$ Hz, 1H, N*H*Ser), 8.22 (d, $J = 8.8$ Hz, 2H, Ar*H*); ^{13}C NMR (400 MHz, DMSO): $\delta = 12.8$ (CH_3), 16.7 (CH_3), 20.9 (CH_2), 27.5 (CH_2), 29.0 (CH_2), 31.7 (CH_2), 32.9 (CH_2), 34.6 (CH_2), 44.0 (CH_2), 47.1 (CH_2), 50.7 (CH), 53.7 (CH), 55.9 (CH), 60.0 (CH_2), 60.3 (CH_2), 110.5 (CH), 121.4 (CH), 123.8 (CH), 124.9 (CH), 141.7 (C), 145.8 (C), 150.4 (C), 156.6 (C), 168.8 (C), 170.2 (C), 170.6 (C), 171.5 (C), 171.6 (C), 172.8 (C); MS (ES^+): *m*/*z* (%): 744 (10) $[M+H]^+$, 766 (10) $[M+Na]^+$; HRMS (ES^+): *m*/*z*: calcd for $C_{34}H_{46}N_7O_{12}$ $[M+H]^+$: 744.3199; found: 744.3198.

Red dye-spacer-D-Glu(OtBu)-D-Ser(tBu)-D-Val-OH (22):[18] To a suspension of Rink acid resin (500 mg, 0.215 mmol) in dry THF was added triphenylphosphine (310 mg, 1.18 mmol) and hexachloroethane (280 mg,

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1.18 mmol) and the resulting mixture was stirred for 6 h at room temperature. After washing the resin with THF (3 × 10 mL) the resin was pre-swollen in dichloromethane. Fmoc-D-Val-OH (233 mg, 0.68 mmol) together with DIPEA was added to the resin and agitated for 20 h. Subsequent washing with dichloromethane (3 × 10 mL), methanol (3 × 10 mL) and dichloromethane (3 × 10 mL) yield a resin which gave a negative ninhydrin test. Fmoc deprotection was achieved as described in the general procedure and subsequent washing with dichloromethane (3 × 10 mL), DMF (3 × 10 mL) and dichloromethane (3 × 10 mL) yielded a resin which gave a positive ninhydrin test. A solution of Fmoc-D-Ser(*t*Bu)-OH (247 mg, 0.64 mmol), DIC (101 μL, 0.64 mmol) and HOBt (98 mg, 0.64 mmol) in DMF was stirred for 10 min and added to the resin followed by DIPEA (168 μL, 0.96 mmol), and the resulting mixture was agitated at room temperature for 20 h. The resin was washed as before and a qualitative ninhydrin test showed complete coupling. Fmoc deprotection and subsequent coupling of Fmoc-D-Glu(O*t*Bu)-OH (275 mg, 0.64 mmol) followed by Fmoc deprotection as described above provided the resin-bound tripeptide **22**. A solution of red dye-linked glutaric acid[19] (110 mg, 0.26 mmol), HOBt (47 mg, 0.26 mmol), HBTU (98 mg, 0.26 mmol) in DMF (10 mL) was stirred for 10 min and added to the resin followed by DIPEA (150 μL, 0.86 mmol) and agitated for 20 h. After this time the resin was washed with dichloromethane (3 × 10 mL), DMF (3 × 10 mL) and dichloromethane (3 × 10 mL). Cleavage of the product from the Rink acid resin was performed by agitating with 10% acetic acid in dichloromethane for 2 h. The solvent collected from the cleavage step was evaporated and the crude was purified by column chromatography (CH_2Cl_2/MeOH 90:10) to afford compound **22** (133 mg, 73%). The ^1H, and ^{13}C NMR spectra were all in agreement with the structural data from the red dye-spacer-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH. HRMS (ES^+): *m*/*z*: calcd for $C_{42}H_{62}N_7O_{12}$ $[M+H]^+$: 856.4451; found: 856.4441.

Screening of the tweezer receptor library 18: Preparation of the buffer solution: aqueous borax (0.025 M, 50 mL) was added to aqueous sodium hydroxide (0.1 M, 0.9 mL) and the pH was adjusted to 9.2.

A sample of library **18** (27.5 mg) was equilibrated in borax buffer solution (300 μL) for 24 h. A solution of guest **19** (20 μM, 500 μL) in a 20% DMSO aqueous solution was added to the library sample to give a 12.5 μM concentration in guest. Equilibration was continued for 24 h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (magnification × 40) and eight highly red stained beads were selected and submitted for Edman sequencing.[11]

The same screening experiment was performed with guests **20** and **21**. However, a lower selectivity was observed.

2-{*N*-Ethyl-4-[2-(4-nitrophenyl)-1-diazenyl]anilino}ethyl acetate (23): Acetic anhydride (60 μL, 0.64 mmol) and DMAP (8 mg, 64 μmol) was added to a solution of Disperse Red 1 (100 mg, 0.32 mmol) in dichloromethane (10 mL) and the reaction mixture was stirred at room temperature for 48 h. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (CH_2Cl_2/MeOH 99:1) to provide **23** (112 mg, 99%) as red crystals. M.p. 122 °C; $R_f = 0.91$ (CH_2Cl_2/MeOH 90:10); IR (film): $\tilde{\nu} = 3105$, 2962, 1748, 1597, 1510, 1388, 1334, 1241, 1224, 1134, 1101 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.26$ (t, $J = 7.0$ Hz, 3H, $NRCH_2CH_3$), 2.07 (s, 3H, $COCH_3$), 3.53 (q, $J = 7.0$ Hz, 2H, $NRCH_2CH_3$), 3.69 (t, $J = 6.6$, 2H, $NRCH_2CH_2O$), 4.30 (t, $J = 6.6$ Hz, 2H, $NRCH_2CH_2O$), 6.80 (d, $J = 9.0$ Hz, 2H, Ar*H*$NRCH_2CH_3$), 7.90 (d, $J = 8.0$ Hz, 2H, Ar*H*$NRCH_2CH_3$), 7.92 (d, $J = 8.0$ Hz, 2H, Ar*H*N_2R), 8.34 (d, $J = 9.0$ Hz, 2H, Ar*H*N_2R); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.70$ (CH_3), 21.26 (CH_3), 46.12 (CH_2), 49.25 (CH_2), 61.71 (CH_2), 111.87 (CH), 123.06 (CH), 125.09 (CH), 126.64 (CH), 144.30 (CH), 147.90 (CH), 151.65 (CH), 157.18 (CH), 171.27 (C); MS (ES^+): *m*/*z*(%): 357 (12) $[M+H]^+$.

Control experiments: A sample of the library **18** was equilibrated in borax buffer solution for 24 h and a solution of dye derivative **23** was added to the equilibrated solution. The concentrations and conditions were the same as described above for the screening experiments. After 48 h (to allow for equilibration time) no selective staining was observed.

Preparation of tweezer receptor (25): A solution of CBS **15** (160 mg, 0.18 mmol), PyBOP (95 mg, 0.18 mmol), and HOBt (28 mg, 0.183 mmol) in DMF (3 mL) was stirred for 5 min and then added to pre-swollen and Fmoc-deprotected Rink amide resin (370 mg, 0.17 mmol free NH_2) in DMF (2 mL) followed by addition of neat DIPEA (70 μL, 0.41 mmol). After agitation on a tube rotator for 24 h at room temperature the resin was drained, washed with dichloromethane (3 × 5 mL), DMF (3 × 5 mL), dichloromethane (3 × 5 mL) and dried. A quantitative ninhydrin test indicated the presence of free amino groups on the resin (0.03 mmol in total). The coupling was repeated in the same manner on a 0.033 mmol scale CBS **15** (29 mg), PyBOP (17 mg), HOBt (5 mg), and DIPEA (13 μL).

In order to cap remaining free amino functions the resin was agitated on a tube rotator for further 18 h after addition of acetic anhydride (14 μL, 0.15 mmol) and DMAP (0.4 mg, 3 μmol) in dichloromethane (3 × 5 mL). Subsequent washing with dichloromethane (3 × 5 mL), DMF (3 × 5 mL) and dichloromethane (3 × 5 mL) afforded a resin which gave a negative ninhydrin test. Fmoc deprotection was achieved as described above in the general procedures and subsequent washing with dichloromethane (3 × 5 mL), DMF (3 × 5 mL), and dichloromethane (3 × 5 mL) yielded a resin which gave a positive ninhydrin test.

Quantities of reagents required were calculated on basis of the loading of the commercially available Rink amide resin (370 mg, 0.17 mmol, two amino sites per guanidinine unit: 0.34 mmol free NH_2). All deprotection steps were monitored by qualitative ninhydrin tests. A solution of Fmoc-L-proline (226 mg, 0.67 mmol), DIC (105 μL, 0.67 mmol), and HOBt (102 mg, 0.67 mmol) in DMF (2 mL) was stirred for 5 min and then added to the pre-swollen resin in DMF (2 mL) followed by addition of DIPEA (257 μL, 1.50 mmol). After agitation on a tube rotator for 18 h at room temperature the resin was drained, washed with dichloromethane (3 × 5 mL), DMF (3 × 5 mL), dichloromethane (3 × 5 mL) and dried. A ninhydrin test indicated complete coupling. A Fmoc deprotection was followed by coupling of Fmoc-L-leucine (236 mg, 0.67 mmol). A further Fmoc deprotection and coupling of Fmoc-L-methionine (248 mg, 0.67 mmol) provided the resin-bound tosylated tweezer receptor **24** after final Fmoc deprotection. Cleavage of the tweezer receptor from the Rink amide resin was performed in a glass funnel with a fine sinter by letting a cleaving mixture percolate slowly through the resin. A cleavage mixture of 10% TFA, 1% TIS and 89% dichloromethane (50 mL) was first used followed by a 50% TFA, 1% TIS, 49% dichloromethane cleavage solution (25 mL). Solvents were removed under reduced pressure and a white TFA salt of **24** precipitated after addition of diethyl ether to the oily crude. The TFA salt was triturated with diethyl ether and centrifuged. The precipitate was washed and centrifuged three times more, the diethyl ether was decanted from the precipitate each time. Thus, the TFA salt of **24** was obtained in high purity and a quantitative yield (207 mg).

The tosyl-protected TFA salt **24** (109 mg) was treated with liquid HF (see general procedure) to give the deprotected tweezer **25** (66 mg). Characterisation was achieved after purification by semipreparative reversed-phase HPLC (see general methods). Under these conditions the TFA salt of the tweezer receptor **25** eluted after 15.49 min. The TFA salt of **25** was obtained as a white solid (45.7 mg). M.p. 140 °C; $[\alpha]_D^{RT} = -42.8°$ ($c = 4.3$ mg mL^{-1}, $l = 0.5$ dm, MeOH); ^1H NMR (400 MHz, CD_3COD): $\delta = 0.89$ (m, 12H, δLeu), 1.46–1.75 (m, 6H, γC*H*Leu, βC*H*Leu), 1.78–2.08 (m, 10H, βC*H*Glu, γC*H*Pro, βC*H*Met), 2.00 (s, 3H, SCH_3), 2.02 (s, 3H, SCH_3), 2.14 (m, 2H, γC*H*Glu), 2.25 (m, 4H, βC*H*Pro), 2.49 (m, 2H, ?γC*H*Met), 3.15–3.45 (m, 2H, γC*H*Met), (m, 8H, RNHCH_2CH_2NHR), 3.57 (m, 2H, δC*H*Pro), 3.79 (m, 2H, δC*H*Pro), 3.91 (t, $J = 6.5$ Hz 2H, αC*H*Met), 4.14 (dd, $J = 9.5$, 4.0 Hz, 1H, αC*H*Glu), 4.24–4.32 (m, 2H, αC*H*Pro), 4.60 (m, 2H, αC*H*Leu); ^{13}C NMR (100 MHz, CD_3CN): $\delta = 15.53$ (CH_2), 22.06 (CH_3), 24.15 (CH_3), 26.28 (CH), 26.58 (CH_2), 29.06 (CH_2), 30.00 (CH_2), 30.92 (CH_2), 31.26 (CH_2), 32.77 (CH_3), 39.76 (CH_2), 40.99 (CH_2), 41.19 (CH_2), 42.50 (br, CH_2), 51.75 (CH_2), 53.81 (CH), 54.98 (CH), 62.16 (CH), 62.44 (CH), 156.39 (C), 162.00 (br, C), 170.24 (C), 173.26 (C), 173.58 (C), 175.02 (C), 178.32 (C); MS (ES^+): *m*/*z* (%): 479 (100) $[M+H]^{2+}$, 957 (25) $[M+H]^+$.

Solution binding studies of tweezer receptor 25: Preparation of buffer solution: Sodium borate (38.12 mg, 0.1 mmol) was dissolved in water (HPLC grade, 85 mL) and 15% DMSO (spectroscopy grade, 15 mL) to give a sodium borate concentration of 1 mM and pH 8.75.

Preparation of tripeptide solutions: The different red dye labelled tripeptide solutions were all made up by dissolving the red dye labelled tripeptide in a pre-made buffer. Solutions were sonicated for 2–3 h at 0–25 °C prior to their use in UV binding studies. The red dye labelled tripeptide concentrations employed were as follows: **19** (0.65 mg, 0.76 μmol, 30.4 μM, 25 mL), **20** (0.8 mg, 1.08 μmol, 43.0 μM, 25 mL) and **22** (0.80 mg, 0.93 μmol, 37.4 μM, 25 mL).

Preparation of tweezer solutions: Tweezer receptor **25** was dissolved in the red dye labelled tripeptide solution (1 or 2 mL) at room temperature at a concentration to give a solution about 20 times that of the red dye labelled tripeptide. The tweezer receptor **25** dissolved instantly and after 1 h the UV experiment was started. To avoid possible precipitation of the guest (on account of its poor solubility) or hydrolysis of its ester bond, solutions were prepared shortly prior to commencement of the titration. Throughout the titration UV absorbance was recorded at 500 nm (A_{max} of the red dye labelled tripeptide). After a blank of the buffer solution. The red dye labelled tripeptide curvette solution (1 mL) were titrated with tweezer receptor **25**. Typically was added 2×5 μL, followed by 2×10 μL, 3×20 μL, 1×30 μL and 2×40 μL additions. Hence a total of 4 molar equivalents of tweezer receptor **25** was added to the guest solution (Table 2).

Table 2. UV absorption for guest **19** and **22** when titrated with tweezer receptor **25**.

Titration 1 **19/25**		Titration 2 **19/25**		Titration 3 **22/25**		Titration 4 **22/25**	
Vol. [μL]	Abs.	Vol. [μL]	Abs.	Vol. [μL]	Abs.	Vol. [μL]	Abs.
0	0.53531	0	0.58280	0	0.77955	0	0.74494
5	0.53421	5	0.58193	10	0.76564	5	0.74143
10	0.53274	10	0.57918	20	0.75665	10	0.74339
15	0.53085	15	0.57932	40	0.74741	20	0.74032
20	0.52945	20	0.57861	60	0.74205	30	0.73861
25	0.52815	25	0.57617	80	0.74058	50	0.73490
30	0.52775	30	0.57527	100	0.73543	70	0.73289
40	0.52629	40	0.57329	120	0.72518	100	0.72838
50	0.52535	50	0.57128	160	0.71798	120	0.72651
60	0.52430	60	0.57036	200	0.70881	160	0.72288
70	0.52395	70	0.56927			200	0.71982
80	0.52348	80	0.56841				
90	0.52300	100	0.56648				
110	0.52192						
130	0.52153						
150	0.52012						
200	0.51763						

The UV spectrum (200 – 600 nm) was also recorded after each addition and the overlaid UV spectra for each enantiomer of the red dye labelled tripeptide **19** and **22**. An isobestic point was observed at 400 nm for **19** and at 410 and 590 nm for **22** which indicates a 1:1 binding stoichiometry between tweezer receptor **25** and the red dye labelled tripeptide **19** and its enantiomer **22**. Data from Table 2 showed a good fit for the presumed 1:1 binding and allowed an estimation of the binding constants:[12]

19; tweezer receptor **25**; $K_a = 8.2 \times 10^4 \pm 2.5 \times 10^4\,\text{M}^{-1}$

20; tweezer receptor **25**; $K_a =$ no binding

22; tweezer receptor **25**; $K_a = 8.0 \times 10^3 \pm 1.5 + 10^3\,\text{M}^{-1}$

19; tweezer receptor **24**; $K_a =$ no binding

Dilution control experiments for both the red dye labelled tripeptide **19** and **20** were performed. In both cases no evidence for dimerisation was observed.

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[19] Red dye-linked glutaric acid was synthesised from the corresponding Disperse Red 1 and glutaric anhydride in presence of DMAP and triethylamine.

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