Screening an Inverted Peptide Library in Water with a Guanidinium-Based Tweezer Receptor

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A 1000-member, biased library of tripeptides, attached to TentaGel resin via the amino terminus, has been screened with dansyl-labeled tweezer receptor **4** in water. The tweezer receptor was found to bind to ~3% of the library members and, following sequencing of 20 beads using a novel coding strategy, showed 95% selectivity for Val at the carboxy terminus of the tripeptides and 40% selectivity for Glu(O^{t}Bu) at the amino terminus. Although complicated by solubility issues, binding of one of the tripeptides selected from the screening experiments, Z-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH, to tweezer **4** was measured by microcalorimetry to have an association constant, $K_{assoc} = 4 \times 10^5 \pm 5 \times 10^4$ M^{-1} (in sodium borate buffer containing 16.7% DMSO, pH 9.2) and presumably results from a combination of a carboxylate−guanidinium interaction, β-sheetlike hydrogen bonding with the sidearms of the tweezer, and hydrophobic interactions.

Noncovalent intermolecular interactions play a fundamental role in many physiological processes. Understanding the basis of these interactions and developing synthetic molecules able to mimic and block the action of natural compounds with a high degree of specificity are of paramount importance in the development of new therapeutic agents for treating a range of diseases. An approach that is increasingly being used in these studies is combinatorial chemistry, in particular the "split-and-mix" technique, which provides a powerful tool for generating chemical diversity.[1] The strength of this methodology is that in essence it employs a "statistical design process" to identify potential lead compounds rapidly. This is enhanced by the recent development of a range of techniques for the screening of resin-bound libraries[2] that has revolutionized the way in which ligands for a range of natural and synthetic[3] receptors can be pinpointed.

Most synthetic receptors for polar substrates described to date only bind effectively in relatively nonpolar solvents, relying principally on hydrogen-bonding interactions to provide the driving force for binding.[4] Selective binding of polar substrates, such as peptides, in water has proved much more demanding.[3b,5] This is unfortunate if one considers the wider medicinal applications of the success of such an undertaking. For example, receptors for the carboxy terminus of small peptides might lead to mimics for the family of antibiotics based on vancomycin, which selectively bind to the bacterial cell wall precursor tripeptide -L-Lys-D-Ala-D-Ala-OH.[6]

We recently described[7] the solid-phase synthesis of tweezer receptor **3**, which was designed to bind to the carboxy terminus of peptides in aqueous media, using a guanidinium binding site for the carboxylate as the primary binding interaction, with the tweezer arms providing the potential to form both hydrophobic and β-sheetlike hydrogen-bonding interactions with the backbone of the peptide substrate.[8] To establish the binding properties of **1**, we wished to screen it against a library of resin-bound, carboxyl-terminating tripeptides. In this paper, we describe the screening of the fluorescently labeled tweezer receptor **4** in a buffered aqueous medium against a library of peptides attached to TentaGel resin via the amino terminus and related studies that demonstrate that structures such as **4** can indeed bind selectively to peptides with a carboxy terminus in an aqueous medium, through a combination of a carboxylate−guanidinium interaction and hydrophobic and hydrogen-bonding interactions.

A 1000-member inverted tripeptide library was prepared on TentaGel-S-NH$_2$ resin (130 μm beads, 0.29 mmol g^{-1} NH$_2$) using the methodology we have recently

(1) (a) Balkenhohl, F.; von dem Bussche-Hünnefield, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288. (b) Fruchtel, J. S.; Jung, G. *Ibid.* **1996**, *35*, 17. (c) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Prot. Res.* **1991**, *36*, 487. (d) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82. (e) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Ibid.* **1991**, *354*, 84.

(2) Gissel, B.; Jensen, M. R.; Gregorius, K.; Elsner, H. I.; Svendsen, I.; Mouritsen, S. *J. Peptide Sci.* **1995**, *1*, 217.

(3) Screening of peptide receptors against libraries of potential substrates: (a) Löwik, D. W. P. M.; Weingarten, M. D.; Broekema, M.; Brouwer, A. J.; Still, W. C.; Liskamp, R. M. J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1846. (b) Torneiro, M.; Still, W. C. *Tetrahedron* **1997**, *53*, 8739. (c) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155. (d) Löwik, D. W. P. M.; Mulders, S. J. E.; Cheng, Y.; Shao, Y.; Liskamp, R. M. J. *Tetrahedron Lett.* **1996**, *37*, 8253. (e) Gennari, C.; Nestler, H. P.; Salom, B.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1765.

(4) For recent examples of peptide receptors that bind in nonpolar solvents, see ref 3 and references therein. For receptors specifically for peptides with a carboxylic acid terminus, see: (a) Waymark, C. P.; Kilburn, J. D.; Gillies, I. *Tetrahedron Lett.* **1995**, *36*, 3051. (b) Dowden, J.; Edwards, P.; Kilburn, J. D. *ibid.* **1997**, *38*, 1095.

(5) (a) Hinzen, B.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **1996**, *79*. (b) Torneiro, M.; Still, W. C. *J. Am. Chem. Soc.* **1995**, *117*, 5887. (c) LaBrenz, S. R.; Kelly, J. W. *Ibid.* **1995**, *117*, 1655. (d) Jung, G.; Hofstetter, H.; Feiertag, S.; Stoll, D.; Hofstetter, O.; Wiesmuller, K.-H.; Schurig, V. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2148.

(6) (a) Cohen, M. L. *Science* **1992**, *257*, 1050. (b) Neu, H. C. *Ibid.* **1992**, *257*, 1064.

(7) Bonnat, M.; Bradley, M.; Kilburn, J. D. *Tetrahedron Lett.* **1996**, *37*, 5409.

(8) For related tweezer receptor that forms a complex with a complementary peptidic guest in water, using a combination of β-sheet, hydrophobic, and electrostatic interactions, see ref 5c; for recent related work on screening a library of (non-carboxy-terminating) peptides with a tweezer receptor in water, see ref 3b; for other work with tweezer receptors as peptide receptors see ref 3d,e; for original work on tweezer receptors, see: Zimmerman, S. C.; Wu, W.; Zeng, Z. *J. Am. Chem. Soc.* **1991**, *113*, 196.

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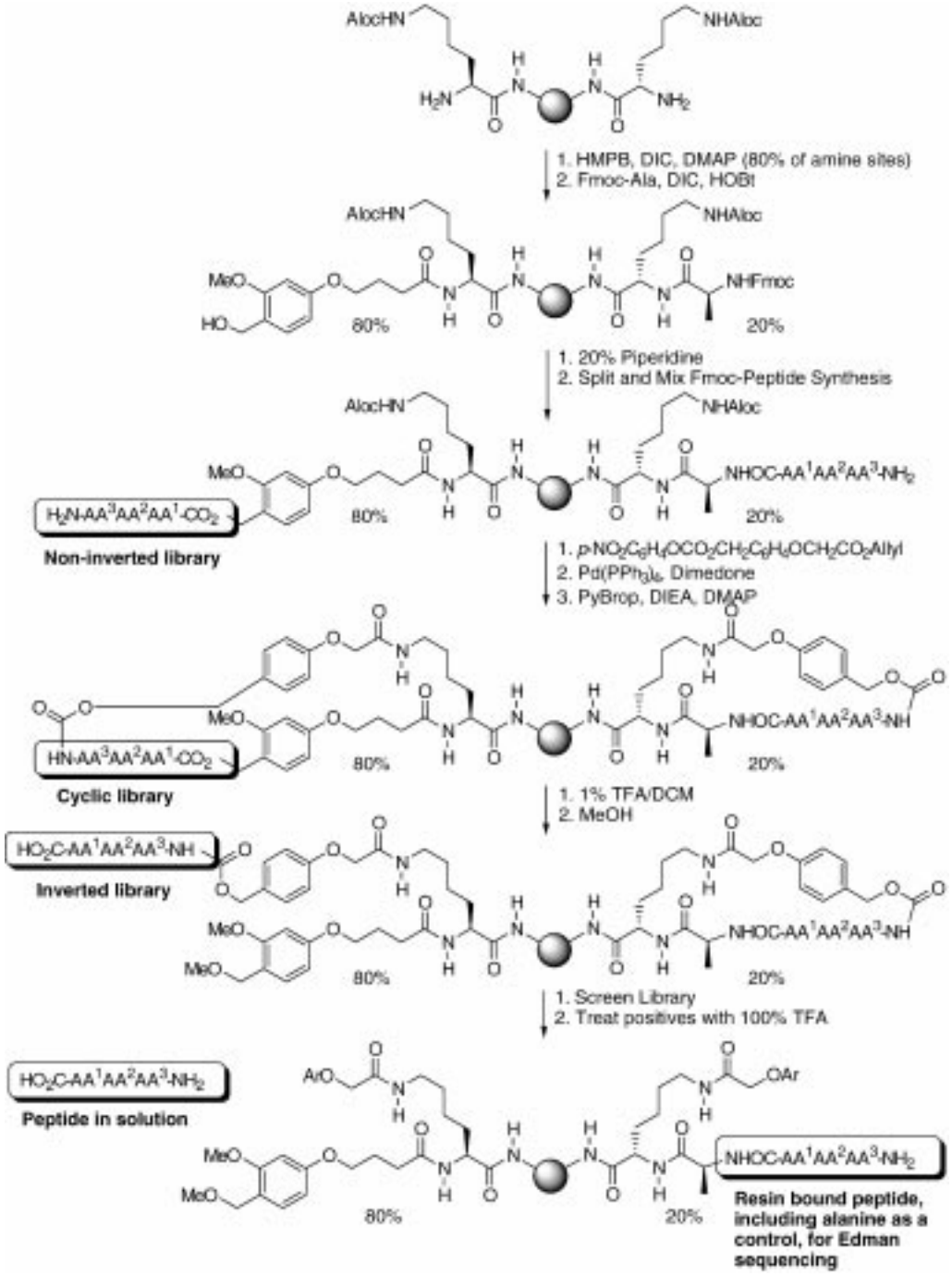
Scheme 1[a]



[a] Coupling 80% of the HMPB linker and 20% alanine to the resin prior to peptide synthesis leads to a sequenceable "coding strand" remaining attached to the resin via alanine after peptide cleavage. Cleavage of the HMPB linker (1% TFA/DCM, then MeOH) opens the cyclic library, affording the inverted library attached to the beads for screening. Beads selected from the screen are further treated (100% TFA) to free the coding strand which is analyzed by edman degradation. nonstandard peptide nomenclature has been used for the sake of clarity.

described,[9] but with a modified strategy for incorporating the coding strand (Scheme 1).

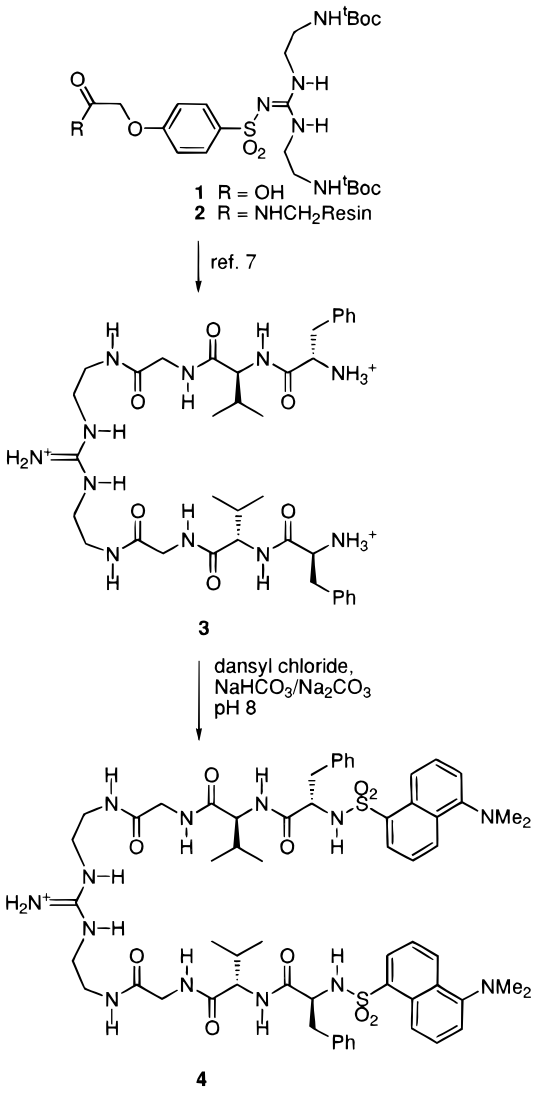
Thus, titration coupling of HMPB[10] to a level of 80% (four couplings of 20% each monitored by the ninhydrin assay[11]) onto H-Lys(Aloc)-NH-TentaGel resin was followed by coupling the remaining 20% of sites with Fmoc-Ala-OH (Scheme 1). This afforded Ala at the final position of the coding strand as an internal standard, but not in the inverted peptide sequence. The first step in the library synthesis, the coupling of 10 Fmoc amino acids to 10 portions of resin, was monitored individually to ensure efficient incorporation of all residues at the initial esterification stage by Fmoc deprotection and ninhydrin assay.[11] The inverted library was then pre-

(9) Davies, M.; Bradley, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1097.

(10) (a) Flörsheimer, A.; Riniker, B. *Pept. 1990 Proc. Eur. Peptide Symp. 21st 1990* **1991**, 131. (b) Riniker, B.; Flörsheimer, A.; Fretz, H.; Sieber, P.; Kamber, B. *Tetrahedron* **1993**, *49*, 9307.

(11) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**,*117*, 147.

Scheme 2. Synthesis of the fluorescently labeled tweezer receptor 4



pared using split-and-mix synthesis and peptide inversion methodology.[9] To maximize the chance of finding a suitable substrate for the tweezer receptor, the library was biased toward the formation of hydrophobic interactions with the receptor arms by the incorporation of 10 hydrophobic amino acid residues into the library Ala, Gly, Glu(O^{t}Bu), Leu, Met, Phe, D-Phg, Pro, Ser(O^{t}Bu), and Val.

Tweezer receptor **3** was prepared from the resin-bound sulfonated guanidine derivative **2**, as previously reported,[7] and **3** was fluorescently labeled with dansyl chloride at pH 8 to give **4** (Scheme 2) and purified by reversed-phase HPLC.

Incubation of the dansylated receptor **4** with the inverted peptide library was attempted under a range of conditions, varying concentration, solvent, pH, and buffer (see the Experimental Section), but the best conditions for selective binding were found when dansylated receptor **4** (13.5 μM) was equilibrated with a sample of approximately 7000 beads of the inverted library in aqueous sodium borate buffer containing 16.7% DMSO (to dissolve the receptor) at pH 9.2 for 24 h at room temperature.

Beads were analyzed in flat-bottomed cell culture plates under a Leica inverted DML microscope (magnification × 40) with a filter cube that contained a suppression filter at 425 nm and excitation filter at 340−380 nm. Approximately 3% of the beads were observed to be brilliantly fluorescent, which was considered acceptable considering the biased nature of the library. Four types of beads were observed: (i) those with essentially no staining, (ii) those with a small amount of background staining, (iii) those with two-tone fluorescence with a high-intensity nucleus and less intense periphery, and (iv) those that were brilliantly fluorescent (Figure 1).

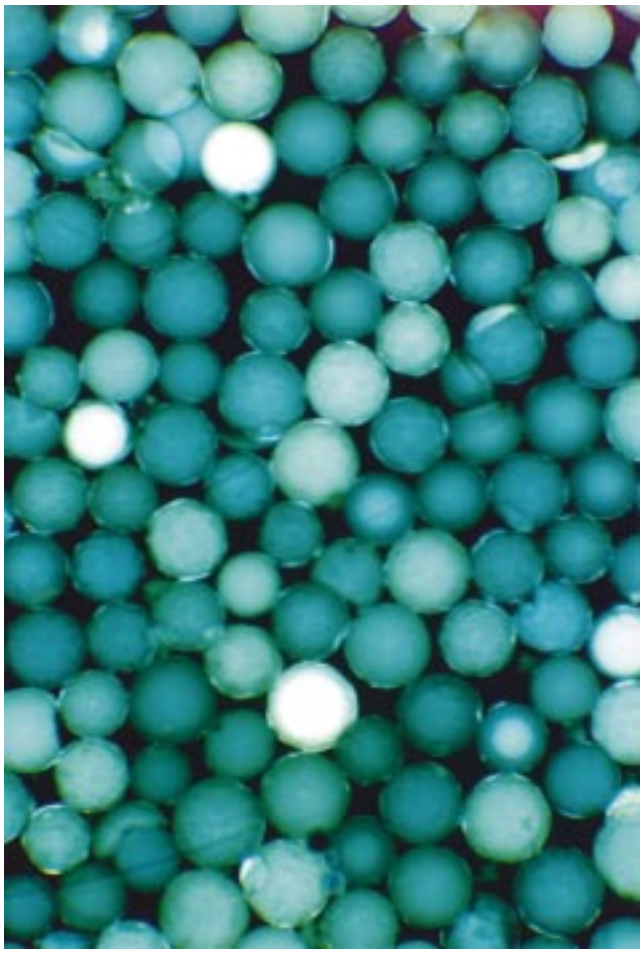


Figure 1. Portion of the resin-bound inverted peptide library incubated with dansylated tweezer receptor **4**, showing ~3% of highly fluorescent beads.

Highly fluorescent beads were removed manually, and peptide identification was achieved by Edman sequencing the material still attached to the bead via the Ala "linker" following a TFA pretreatment to remove the side-chain protecting groups (Glu(O^{t}Bu), Ser(O^{t}Bu)). Twenty beads were removed from the 7000 screened and sequenced by Edman degradation, giving the data shown in Table 1.

Several control experiments were carried out to verify that the observed binding selectivity was indeed for the carboxylate-terminating peptide. Thus, receptor **4** was incubated with underivatized TentaGel-S-NH$_2$ beads with the noninverted library and with the cyclic library (Scheme 1). In all these control experiments no selectivity was observed with beads showing only background staining. In addition, the inverted peptide library was incubated with dansylated tripeptide DNS-Phe-Val-Gly-OH, corresponding to the receptor arms, and again no

Table 1. H_2N---peptide---CO_2H

Glu(O^{t}Bu)	Glu(O^{t}Bu)	Leu
Glu(O^{t}Bu)	Glu(O^{t}Bu)	Val
Glu(O^{t}Bu)	Glu(O^{t}Bu)	Val
Glu(O^{t}Bu)	Ser(O^{t}Bu)	Val
Glu(O^{t}Bu)	Ser(O^{t}Bu)	Val
Glu(O^{t}Bu)	Val	Val
Glu(O^{t}Bu)	Gly	Val
Glu(O^{t}Bu)	Pro	Val
Pro	Leu	Val
Pro	Leu	Val
Pro	Val	Val
Pro	Phe	Val
Pro	Glu(O^{t}Bu)	Val
Leu	Ser(O^{t}Bu)	Val
Leu	Ser(O^{t}Bu)	Val
Leu	Gly	Val
Leu	Phe	Val
Phe	Pro	Val
Val	Pro	Val
Gly	Glu(O^{t}Bu)	Val

selectivity was observed. This control experiment was important since it rules out the possibility that the binding selectivity could be attributed to the dansyl moiety.[12] In a competition experiment, addition of the peptide Z-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH (20 equiv, 240 μM) (selected as a good substrate from the screening results, Table 1) to a sample of the inverted peptide library, which had been preincubated with the receptor **4**, showed no fluorescently stained beads, above background, after an overnight incubation.

From the decoding data (Table 1), it is clear that the tweezer receptor is highly selective for the first residue with 95% of sequences determined having Val in the carboxyl position (corresponding to that nearest to the guanidinium-binding site), the only other residue being the structurally similar Leu. Specificity is less well defined at the second position, which had two dominant residues, Glu(O^{t}Bu) (25%) and Ser(O^{t}Bu) (20%). The third position showed increased specificity over the second, being occupied predominantly by Glu(O^{t}Bu) (40%); however, Pro (25%) also featured strongly. Ala was always observed at the fourth position, confirming the integrity of the coding strand. The observed selectivity, particularly for Val, is remarkable given the lack of preorganization in the tweezer structure and that the library contains structurally quite similar amino acids.

Having successfully demonstrated that tweezer **4** had the desired property of binding carboxylate-terminating peptides in an essentially aqueous environment, it was of interest to determine if the process could be reversed, i.e., whether a solid-phase library of tweezers could be screened to identify a selective receptor for a specific peptide sequence. We therefore prepared a 125-membered library of tweezers, using the methodology previously described for the synthesis of **3** and using split-and-mix synthesis with incorporation of the five amino acids (Ala, Gly, Phe, Ser(O^{t}Bu), and Val) (Scheme 3).

The dansylated tripeptide DNS-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH was equilibrated with the 125-member library under identical conditions for the screening of tweezer **3** with the inverted peptide library (sodium borate buffer containing 16.7% DMSO, pH 9.2), with the expectation that the peptide would bind to, among others, the resin bound tweezer **3**, which was one of the structures present in the 125 member library. In practice, no binding was

(12) Wennemers, H.; Still, W. C. *Tetrahedron Lett.* **1994**, *35*, 6413.

Scheme 3. Synthesis of a resin bound library of tweezer receptors

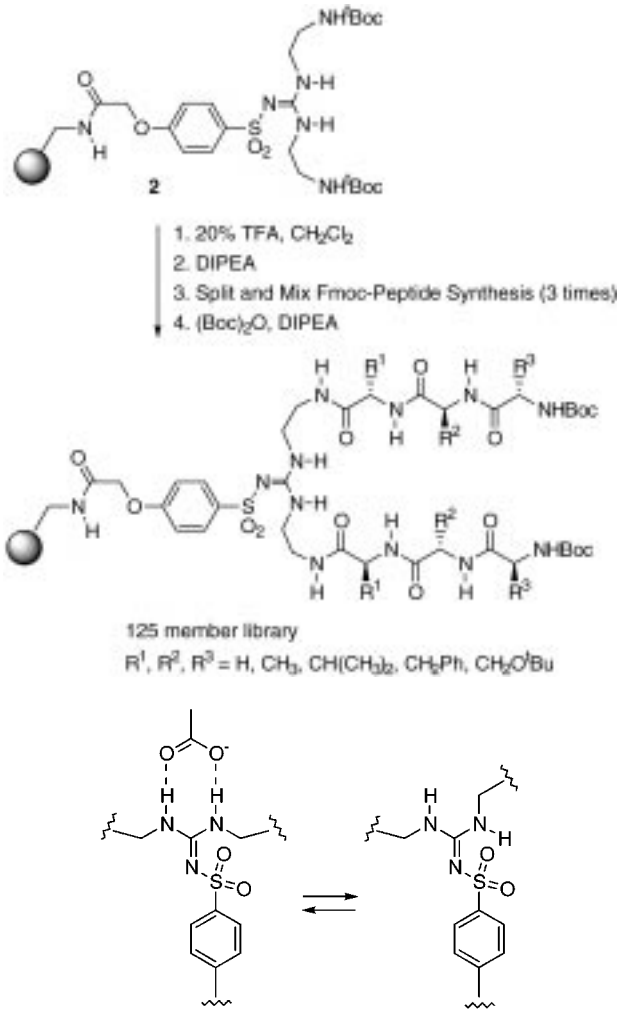


Figure 2. Conformations of sulfonylated guanidine.

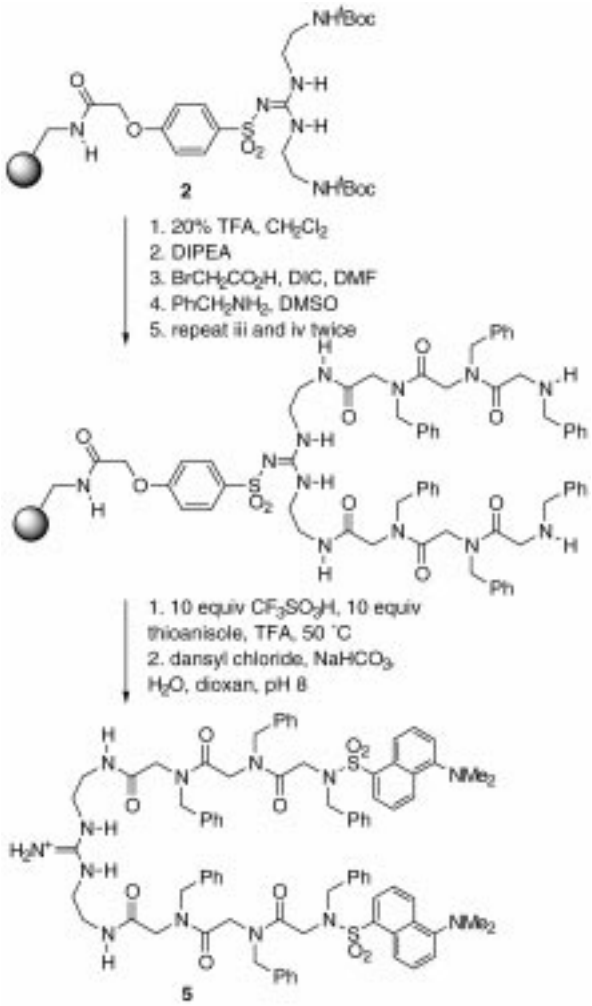
observed, even after prolonged incubation, with essentially no staining for any beads. In retrospect, this result can be readily understood as the resin bound tweezers exist with a weakly basic sulfonylated guanidine rather than an unprotected guanidinium, and additionally, the preferred conformation of the sulfonylated guanidine may well be unsuitable for carboxylate binding (Figure 2). The negative result from the screen therefore serves to emphasize the importance of the guanidinium−carboxylate binding interaction in the observed binding of **4** with the inverted peptide library.

In a further experiment, we extended the methodology for the preparation of tweezer **3** to the solid-phase synthesis of a dansylated peptoid tweezer **5**, using standard conditions for the construction of the *N*-alkylglycine sidearms,[13] cleavage from the resin as previously described for **3**, and dansylation (Scheme 4).

Peptoid tweezer **5** was incubated with the 1000-member inverted tripeptide library (sodium borate buffer containing 16.7% DMSO, pH 9.2), but again no binding

(13) (a) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moss, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646. (b) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R. Y.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.

Scheme 4. Synthesis of peptoid tweezer 5



was observed in this experiment, with essentially no staining for any beads. While the previous experiment (screening of the tweezer library with the dansylated tripeptide) demonstrated the importance of the guanidinium−carboxylate binding interaction in the observed binding of **4** with the inverted peptide library, the failure of **5** to bind to the inverted peptide library indicates that such an interaction is insufficient in itself for binding under these conditions. Clearly, the structure of the tweezer sidearms, and presumably most significantly the hydrogen-bonding capabilities of the peptide sidearms in **4**, play an important role in the binding strength and selectivity.

While the above results conclusively prove that tweezer **4** binds with considerable selectivity to resin-bound tripeptides, it was obviously also of interest to establish that such binding is also operating in free solution, to quantify the strength of the binding, and to try to determine the structure of such tweezer−peptide complexes. However, under conditions used for the screening experiments (sodium borate buffer containing 16.7% DMSO, pH 9.2), tweezer **4** was insufficiently soluble to allow studies by ^{1}H NMR. Under conditions that did allow sufficient quantity of tweezer **4** to be dissolved (50% CD_3CN/H_2O/phosphate buffer, pH 3, 6.5, or 9 or 70%$(CD_3)_2SO/H_2O$, borate buffer pH 9.2) ^{1}H NMR spectra of a 1:1 mixture of tweezer **4** and tripeptide Z-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH showed no changes compared to the ^{1}H NMR spectra of the tweezer or the peptide on their own. The apparent lack of binding under these conditions was confirmed by incubating tweezer **4** with the 1000-member inverted peptide library under the same solution conditions (50% CH_3CN/H_2O/phosphate buffer, pH 3, 6.5, or 9 or 70% $(CH_3)_2SO/H_2O$, borate buffer pH 9.2) where no staining of beads was observed. The result is also not surprising in view of the known ability of acetonitrile to disrupt peptide−peptide interactions[14] and was further confirmed in another experiment in which the inverted peptide, resin-linker-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH, was prepared on TentaGel resin using the conditions described above for the inverted peptide library synthesis. Under the screening conditions described above (sodium borate buffer containing 16.7% DMSO, pH 9.2), with receptor **4**, these beads became strongly fluorescent, as expected. A 50 mm × 4.5 mm column was packed with resin-linker-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH material and was thoroughly washed and packed by pumping water through the column at 2 mL/min overnight. Following injection of tweezer receptor **4**, no elution of the tweezer was observed after 24 h of elution with water. The tweezer was finally released by running a gradient elution with acetonitrile, with the tweezer coming off as a broad peak at approximately 60% acetonitrile. Running isocratically at 50% water/acetonitrile still led to retention of the tweezer and no elution even after several hours. Running isocratically at 60% acetonitrile led to immediate elution of the tweezer. Elution of **4** with intermediate solvent mixtures (50−60% acetonitrile) gave very broad peaks. Thus, the binding of tweezer **4**, at least with Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH, is very sensitive to the precise solvent composition and appears to require predominantly aqueous solutions to be effective—which indicates that hydrophobic interactions are a major component of the observed binding.

To date, the limited solubility of the tweezer receptor in predominantly aqueous solutions has therefore precluded an NMR investigation of the binding properties of tweezer **4**. A study of the binding of tweezer receptor **4** with peptide Z-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH using circular dichroism was also precluded by the need for DMSO to solubilize the receptor. However, we were able to obtain an estimate of the association constant for the binding of **4** with Z-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH (in sodium borate buffer containing 16.7% DMSO, pH 9.2), using isothermal microcalorimetry. The data obtained from these experiments (see the Experimental Section) could be fitted to a simple 1:1 binding model and gave an apparent association constant $K_{assoc} = 4 \times 10^5 \pm 5 \times 10^4$ mol^{-1}. In the absence of definitive NMR data, one can only speculate on the mode of binding of the selected tripeptides with tweezer receptor **4**. However, the absence of binding with the sulfonylated tweezer library and with the peptoid tweezer **5** certainly supports the notion that binding involves a carboxylate−guanidinium interaction and hydrogen bonding with the sidearms of the tweezers as well as a strong hydrophobic component (which is evidenced by the limited solubility of the tweezer in predominantly aqueous solutions and the sensitivity of the binding to replacement of water with organic solvents). It cannot be ruled out, however, in the absence of definitive NMR evidence, that the observed

(14) (a) Arunkumar, A. I.; Kumar, T. K. S.; Sivaraman, T.; Yu, C. *Int. J. Biol. Macromol.* **1997**, *21*, 299. (b) Gevaert, K.; Demol, H.; Puype, M.; Broekaert, D.; DeBoeck, S.; Houthaeve, T.; Vandekerckhove, J. *Electrophoresis* **1997**, *18*, 2950.

selective binding of **4** with the inverted peptide library in the screening experiments is strongly influenced by the environment presented by the TentaGel resin.

In conclusion, we have prepared an inverted tripeptide library using split-and-mix methodology and shown that, with its novel coding strategy, it can be screened and decoded to identify substrates for receptors such as tweezer **4**, which was observed to be a selective receptor for carboxylic acid terminating peptides in an aqueous medium. Further work is under way to determine the exact mode of binding in solution and to extend this work to make libraries of tweezers with unprotected guanidinium units in an effort to identify medicinally relevant receptors.

Experimental Section

Synthesis of Inverted Peptide Library. TentaGel (2.0 g, 0.29 mmol/g) was treated with Fmoc-Lys(Aloc)-OH (0.53 g, 2 equiv), DIC (182 μL, 2 equiv), and HOBt (0.16 g, 2 equiv) in DCM (10 mL) and the resin gently shaken overnight. The resin was washed with DCM (5 × 40 mL), DMF (5 × 40 mL), MeOH (5 × 40 mL), and DCM (5 × 40 mL) and dried in vacuo. The Fmoc group was removed using two treatments of 20% piperidine in DMF (20 mL) for 1 × 5 min and 1 × 20 min and the resin washed with DMF (5 × 40 mL) and DCM (5 × 40 mL). 4-(Hydroxymethyl)-3-(methoxyphenoxy)butanoic acid (HMPB) (30 mg, 0.2 equiv), DIC (19 μL, 0.2 equiv), and HOBt (15 mg, 0.2 equiv) were dissolved in DCM (20 mL) and added to the resin and the reaction shaken for 4 h. The resin was then washed in the normal manner. This reaction was repeated with 0.2 equiv × 3 of HMPB linker to give approximately 80% reaction of the available amine sites as determined by ninhydrin analysis. Fmoc-Ala-OH (93 mg, 0.5 equiv), DIC (48 μL, 0.5 equiv), and HOBt (40 mg, 0.5 equiv) were dissolved in DCM (10 mL) and after 5 min added to the resin, and the reaction was shaken for four h. The resin was washed with DCM (5 × 40 mL), DMF (5 × 40 mL), MeOH (5 × 40 mL), and DCM (5 × 40 mL) and gave a negative ninhydrin test. The Fmoc group was removed from the resin (400 mg), which was then swollen in a mixture of DCM/DMF (2:1) and pipetted as a slurry, using a cut-off pipet tip, into 10 Supelco syringe barrels each fitted with a frit and arranged on a Supelco vacuum manifold, following which the resin was dried under vacuum. Library synthesis was then perfomed using the split-and-mix process and the following 10 Fmoc amino acids Ala, Phe, Gly, Val, Leu, D-Pg, Met, Pro, Ser(O^{t}Bu), and Glu(O^{t}Bu). For the initial coupling, 4 equiv of each amino acid, 4 equiv of DIC, and 1 equiv of DMAP in DCM (1 mL) were used, and the coupling was repeated (2 × 12 h), with each resin sample being analyzed to ensure suitable loading of the HMPB linker (>80%). The Fmoc group was removed and the resin mixed and realiquoted as previously described. Subsequent couplings used 4 equiv of each amino acid, 4 equiv of DIC, and 4 equiv of HOBt in DCM (1 mL) for 12 h but no DMAP. The resin was pooled after the addition of the third amino acid, globally Fmoc deprotected, and washed and the amine coupled with 4-(nitrophenyl) [4′-(hydroxymethyl)phenyl-1′-oxyallyl acetate]carbonate (0.07 g, 2 equiv) with pyridine (23 μL, 3 equiv) in DMF (4 mL) at 50 °C for 12 h, following which the resin gave a negative ninhydrin test. The allyl groups were removed by treatment with dimedone (0.11 g, 10 equiv) and $Pd(PPh_3)_4$ (0.1 g, 1 equiv) in thoroughly degassed DCM/THF (1:1) (4 mL) and the reaction shaken in the dark for 4 h. The resin was then washed with DMF (5 × 20 mL), DCM (5 × 20 mL), 0.5% DIPEA, 0.5% sodium ethyl thiocarbonate in DMF (5 × 20 mL), DMF (5 × 20 mL), and DCM (5 × 20 mL) and the resin dried in vacuo. (Note the order of addition is very important. The resin was added to the solvent and degassed for 30 min under a stream of nitrogen, and the dimedone was then added followed by Pd(0)). The peptide was cyclized by the addition of PyBrop (0.17 g, 4 equiv), DMAP (0.01 g, 1.1 equiv), and DIPEA (70 μL, 4 equiv) in DCM (4 mL) for 24 h, following which the resin was washed in the usual manner. The resin was swollen in DCM (4 mL), and to this was added 1% TFA in DCM (50 mL) and shaken for 12 h (the beads become pink). The resin was then washed with DCM−5% MeOH (5 × 20 mL), DCM (5 × 20 mL), DMF (5 × 20 mL), and DCM (5 × 20 mL) and the resin dried in vacuo.

Preparation of Resin-Bound Bis(aminoethyl)guanidine 2. Aminomethylated polystyrene resin (0.82 g, 0.75 mmol/g) previously swollen in DMF was treated with a solution of acid **1** (0.37 g, 1.1 equiv), DIC (108 μL, 1.1 equiv), and HOBt (0.105 g, 1.1 equiv) in DCM (5 mL) and the resin shaken overnight. The resin was washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and DCM (5 × 20 mL), and any remaining free amino sites were capped using 10% acetic anhydride in DCM (10 mL) for 30 min. The resin was then washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and Et_2O (2 × 20 mL) and dried in vacuo, affording the bis(aminoethyl)guanidine-derived functionalized resin **2**.

Preparation of Tweezer Receptor 3. The Boc groups were removed from **2** (1 g, 0.4 mmol/g) using three treatments of 20% TFA in DCM for 3 × 15 min and the resin washed with DCM (3 × 20 mL) and DMF (3 × 20 mL) and then neutralized with a solution of 10% diisopropylethylamine in DCM for 3 × 10 min. The resin was then washed with DCM (5 × 20 mL), DMF (5 × 20 mL) MeOH (5 × 20 mL), and Et_2O (2 × 20 mL) and dried in vacuo.

Fmoc-Gly-OH (713 mg, 3 equiv), DIC (375 μL, 3 equiv), and HOBt (367 mg, 3 equiv) were dissolved in a mixture of DMF/DCM 1:1 (15 mL) and after 5 min added to the bis(aminoethyl)-guanidine-based functionalized resin **2** previously swollen in DMF, and the reaction was shaken for 15 h. The resin was washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and DCM (5 × 20 mL) and gave a negative ninhydrin test. The Fmoc groups were removed from the resin using three treatments of 20% piperidine in DMF (20 mL) for 1 × 5 min and 2 × 20 min, and the resin was washed with DCM (5 × 20 mL) and DMF (5 × 20 mL). Subsequent coupling used Fmoc-Val-OH for the second residue and Fmoc-Phe-OH for the third residue and were performed in a similar manner. The bis-tripeptide resin-bound tweezer receptor was obtained after Fmoc deprotection of the third residue, final washing with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and Et_2O (2 × 20 mL), and drying in vacuo.

The resin was placed in a 25 mL flask charged with 10 mL of TFA and placed under a nitrogen atmosphere. Trifluoromethanesulfonic acid (355 μL, 10 equiv) and thioanisole (470 μL, 10 equiv) were added, and the mixture was vigorously stirred and heated at 50 °C for 36 h. The resin was filtered and washed with TFA (3 × 2 mL) and DCM (3 × 2 mL), and the filtrate was concentrated in vacuo. The crude product was dissolved in a minimum amount of TFA and dripped into cold diethyl ether (20 mL). The precipitate was isolated by centrifugation and afforded 950 mg of crude oil. Characterization was achieved after purification by semipreparative reversed-phase HPLC (Zorbax ODS, 25 cm × 9.4 mm) of a portion (50 mg) of the crude oil using a linear gradient from H_2O + 0.1% TFA to acetonitrile + 0.1% TFA over 50 min, with a flow rate of 2.5 mL/min, monitoring at 220 nm. Under these conditions, tweezer **3** eluted after 29 min.

3: ^{1}H NMR (360 MHz, MeOH-d_4) δ 7.75 (10H, m, Ph), 4.28 (2H, dd, J = 5.5, 8.3 Hz, CHCH$_2$Ph), 4.23 (2H, d, J = 7.4 Hz, CHCH(CH$_3$)$_2$), 3.96 (2H, d, J = 16.7 Hz, CH_AH$_B$CO), 3.88 (2H, d, J = 16.7 Hz, CH$_A$$H_B$CO), 3.40 (8H, m, C$H_2CH_2$), 3.32 (2H, dd, J = 5.4, 14.3 Hz, CH_AH$_B$Ph), 3.14 (2H, dd, J = 8.3, 14.3 Hz, CH$_A$$H_B$Ph), 2.14 (2H, m, C$H$(CH$_3$)$_2$), 1.07 (6H, d, J = 6.7 Hz, CH(CH_3)$_2$), 1.05 (6H, d, J = 6.7 Hz, CH(CH_3)$_2$); ^{13}C NMR (75 MHz, MeOH-d_4) δ 173.5, 172.1, 167.0, 161.7, 135.4, 130.5 (1), 130.0 (1), 128.8 (1), 60.9 (1), 55.3 (1), 43.3 (1), 42.1 (2), 39.2 (2), 38.5 (2), 31.3 (1), 19.5 (3), 18.8 (3); m/z (ES$^+$) 377.1 $(MH_2)^{2+}$ 100, 752.5 $(MH)^+$; (ES$^-$) 113.0 (TFA).

Preparation of Dansylated Tweezer Receptor 4. Dansyl chloride (561 mg, 0.208 mmol) in acetone (1 mL) was added dropwise over 10 min at 20 °C to tweezer **3** (91 mg, 0.080 mmol) in acetone/H_2O (1:1, 4 mL). The mixture was stirred

for 4 h at room temperature and was maintained at pH 8 by the addition of a solution of $NaHCO_3/Na_2CO_3$ (1:1, 1 N). The solvent was removed in vacuo and afforded a yellow oil that was poured into cold water (30 mL). The precipitate formed was isolated by centrifugation and was further purified by semipreparative reversed-phase HPLC (Zorbax ODS, 25 cm × 9 mm) eluting with H_2O + 0.1% TFA (solvent A) for 10 min followed by a linear gradient from H_2O + 0.1% TFA to acetonitrile + 0.1% TFA over 30 min with a flow rate of 2.5 mL/min, monitoring at 220 nm. Under these conditions, the dye-labeled tweezer **4** (38 mg) eluted after 32 min: ^{1}H NMR (360 MHz, MeOH-d_4) δ 8.43 (2 H, d, J = 8.6 Hz, arom dansyl), 8.23 (2H, d, J = 8.6 Hz, arom dansyl), 8.06 (2H, dd, J = 7.4, 1.2 Hz, arom dansyl), 7.52 (4H, dd, J = 7.5, 8.6 Hz, arom dansyl), 7.37 (2H, dd, J = 7.7, 0.9 Hz, arom dansyl), 6.78 (10 H, m, Ph), 4.09 (2H, dd, J = 4.0, 10.9 Hz, *CH*CH_2Ph), 4.08 (2H, m, C*H*$CH(CH_3)_2$), 4.00 (2H, d, J = 16.8 Hz, CH_AH_BCO), 3.82 (2H, d, J = 16.8 Hz, CH_AH_BCO), 3.39 (8H, m, CH_2CH_2), 3.05 (12H, s, N(CH_3)$_2$), 2.99 (2H, dd, J = 4.1, 14.1 Hz, CH_AH_B-Ph), 2.63 (2H, dd, J = 10.3, 14.1 Hz, CH_AH_BPh), 2.12 (2H, m, C*H*$(CH_3)_2$), 1.01 (6H, d, J = 6.8 Hz, CH(CH_3)$_2$), 1.00 (6H, d, J = 6.8 Hz, CH(CH_3)$_2$); ^{13}C (90.5 MHz, MeOH-d_4) δ 174.5, 174.0, 172.3, 158.9, 143.1, 137.3, 137.0, 130.6, 130.4, 129.6, 128.8, 128.6, 127.2, 125.8, 124.9, 118.3, 60.9, 59.6, 46.7, 43.6, 42.1, 39.2, 39.1, 31.5, 19.6, 18.8; m/z (ES$^+$) 610.2 $(MH_2)^{2+}$ 100, 1218.6 $(MH)^+$ 30; ES$^-$ 113.0 (TFA) 100. Purity was also established using HPLC: (Phenomenex Prodigy ODS, 25 cm × 4.6 mm) eluting with 75% H_2O + 25% acetonitrile + 0.1% TFA for 5 min followed by a linear gradient 75% H_2O + 25% acetonitrile + 0.1% TFA to acetonitrile + 0.1% TFA (solvent B) over 15 min with a flow rate of 1.0 mL/min, monitoring at 220 nm. Under these conditions, a single compound, tweezer **4**, was eluted after 7.7 min.

Preparation of Dansylated Peptoid[13] Tweezer 5. The Boc groups were removed from resin **2** (500 mg, 0.4 mmol/g) using three treatments of 20% TFA in DCM for 3 × 15 min, and the resin was washed with DCM (3 × 20 mL) and DMF (3 × 20 mL) and then neutralized with a solution of 10% diisopropylethylamine in DCM for 3 × 10 min. The resin was then washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and Et_2O (2 × 20 mL) and dried in vacuo.

Bromoacetic acid (0.667 g, 12 equiv) in DMF (7 mL) was added, followed by addition of DIC (750 μL, 12 equiv) in DMF (7 mL). The reaction mixture was shaken for 1 h. The resin was then washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and DCM (5 × 20 mL), and the acylation and washing procedure was repeated. A solution of benzylamine (1.66 mL, 40 equiv) in dimethyl sulfoxide (10 mL) was added, followed by shaking for 2 h at room temperature. The resin was then washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and Et_2O (2 × 20 mL) and dried in vacuo. The growth of the tripeptoid chains was checked by the ninhydrin test for the first acylation reaction, which gave a negative result, and by mass spectrometry (ES$^+$) analysis of a small fraction cleaved from the solid support after each reaction.

The resin (460 mg) was placed in a 25 mL flask charged with TFA (10 mL) and placed under a nitrogen atmosphere. Trifluoromethanesulfonic acid (230 μL, 10 equiv) and thioanisole (177 μL, 10 equiv) were added, and the mixture was vigorously stirred and heated at 50 °C for 6 h. The resin was filtered and washed with TFA (3 × 2 mL) and DCM (3 × 2 mL), and the filtrate was concentrated in vacuo. The crude product was dissolved in a minimum amount of TFA and dripped into cold diethyl ether (20 mL). The precipitate was isolated by centrifugation and afforded 142 mg of crude product. Purification was achieved by semipreparative reversed-phase HPLC (Phenomenex Prodigy, ODS (3), 250 mm × 10 mm) eluting with 90% H_2O + 10% acetonitrile + 0.1% TFA for 10 min, followed by a linear gradient from 90% H_2O + 10% acetonitrile + 0.1% TFA to 100% acetonitrile + 0.1% TFA over 35 min, with a flow rate of 2.5 mL/min, monitoring at 220 nm. Under these conditions, the peptoid tweezer was eluted after 32 min: m/z (ES$^+$) 515.2 $(MH_2)^{2+}$ 100, 1028.3 $(MH)^+$.

Dansyl chloride (12 mg, 2.2 equiv) in dioxane was added dropwise to the peptoid receptor (19 mg) solubilized in a mixture of dioxane/water 1:1 (4 mL) previously adjusted to pH 8 with aqueous $NaHCO_3$, and the mixture was stirred overnight at room temperature. The resulting solution was evaporated to dryness under reduced pressure. Water (30 mL) and DCM (30 mL) were added. The aqueous layer was extracted with DCM. The organic layers were combined, dried with magnesium sulfate, and concentrated to afford 18 mg of crude dansylated peptoid tweezer.

Purification was achieved by semipreparative reversed-phase HPLC (Phenomenex Prodigy, ODS (3), 250 cm × 10 mm) eluting with 80% H_2O + 20% acetonitrile + 0.1% TFA for 10 min followed by a linear gradient from 80% H_2O + 20% acetonitrile + 0.1% TFA to 100% acetonitrile + 0.1% TFA over 35 min, with a flow rate of 2.5 mL/min, monitoring at 220 nm. Under these conditions, the dansylated peptoid tweezer **5** was eluted after 40 min. Purity was confirmed when only one peak was obtained using an analytical column (Phenomenex Prodigy ODS, 250 cm × 4.6 mm) eluting with 80% H_2O + 20% acetonitrile + 0.1% TFA for 5 min followed by a linear gradient from 80% H_2O + 20% acetonitrile + 0.1% TFA to 100% acetonitrile + 0.1% TFA over 35 min, with a flow rate of 1.0 mL/min, monitoring at 220 nm. Under these conditions, **5** was eluted after 32 min: m/z (ES$^+$) 1494.6 $(MH)^+$ 100.

Preparation of Tweezer Library. Tentagel (0.5 g, 0.25 mmol/g) previously swollen in DMF was treated with acid **1** (0.175 g, 1.25 equiv), DIC (49 μL, 1.25 equiv), and HOBt (48 mg, 1.25 equiv) in a mixture of DMF/DCM 1:1 (10 mL) and the resin shaken for 5 h. The resin was washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and DCM (5 × 20 mL) and gave a negative ninhydrin test. The Boc groups were removed from the resin using three treatments of 20% TFA in DCM for 3 × 15 min, and the resin was neutralized and washed in the normal manner. The resin was then swollen in DCM (7.5 mL) and pipetted as a slurry into five Supelco syringe barrels each fitted with a frit and arranged on a Supelco vacuum manifold, following which the resin was dried under vacuum. Library synthesis was then performed using the split-and-mix process and the following five Fmoc amino acids (Ala, Val, Phe, Gly, and Ser(O^{t}Bu). For the coupling reactions, 3 equiv of each amino acid, 3 equiv of DIC, and 3 equiv of HOBt in a mixture of DMF/DCM 1:1 (2 mL) were used, and the mixture was shaken for 3 h. Each sample of resin was washed with DCM (5 × 2 mL), DMF (5 × 2 mL), MeOH (5 × 2 mL), and DCM (5 × 2 mL). The Fmoc group was removed and the resin realiquoted as before to perform the second and then the third coupling. The resin-bound tweezer library was obtained after Fmoc deprotection of the third residue, final washing with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and Et_2O (2 × 20 mL), and drying in vacuo. The resin (136 mg) preswollen in DCM was treated with diisopropylethylamine (18 mL, 3 equiv) and di-*tert*-butyl dicarbonate (22 mg, 3 equiv) in dioxane (10 mL) and the mixture shaked overnight. A negative ninhydrin test validated the protection reaction. The resin was finally washed with DCM (5 × 20 mL), DMF (5 × 20 mL), MeOH (5 × 20 mL), and Et_2O (2 × 20 mL) and finally dried in vacuo.

Screening the Inverted Peptide Library with Dansylated Tweezer 4. In preliminary experiments (see the Supporting Information), solutions of tweezer receptor **4** at a range of concentrations and with a range of buffers (sodium phosphate, pH 8.0; glycine, pH 8.5; sodium borate, pH 9.2; sodium carbonate, pH 9.0) and a range of concentrations (100, 10, 1 mM) were assayed. From these preliminary experiments, it was adjudged that the best binding selectivity was observed using 1 mM sodium borate buffer, and this was used in the definitive screening experiment described below.

Buffer solution (1 mM sodium borate, pH 9.2) containing 16.7% DMSO was prepared. Tweezer receptor **4** was dissolved in DMSO at a concentration of 10 mg/mL, and 5 μL of this solution was added to 1495 μL of the buffer/16.7% DMSO solution to give 1500 μL of receptor working solution (27 μM receptor). Resin beads (20 mg, 350 beads per mg) were placed into the wells of a flat-bottomed culture plate and equilibrated

with 650 μL of the buffer/16.7% DMSO solution with agitation overnight. The buffer/16.7% DMSO solution was decanted, and a receptor working solution (650 μL) and further buffer/16.7% DMSO solution (650 μL) were added (total volume = 1.3 mL, final receptor concentration = 13.5 μM) and left to equilibrate overnight with agitation. Beads were analyzed in flat bottomed cell culture plates under a Leica inverted DML microscope (magnification × 40) with a filter cube that contained a suppression filter at 425 nm and excitation filter at 340−380 nm. Prolonged exposure resulted in bleaching of the dye. (There are ~300 pmol of sites per bead. Therefore, 20 mg of beads (~7000 beads) provides ~2.1 μmol of inverted peptide material. The final solution of receptor used (1.3 mL, 13.5 μM) contains 17.5 nmol of receptor, which is considerably less than the total on the ~7000 beads.)

Four types of beads were observed: (1) identical to TentaGel blank; (2) uniform and slightly increased level compared to the TentaGel blank; (3) two tone fluorescence with high-intensity nucleus and less intense periphery; and (4) uniform high-intensity fluorescence corresponding to 3% of the total beads.

Highly fluorescent beads were removed manually and sequenced by Edman degradation.

Control Experiments. (a) Acetonitrile or DMSO was added to wells containing beads previously incubated with the receptor and showing selectivity. The stepwise additon of these solvents up to a 50% led to complete and rapid (a few minutes) loss of fluorescence.

(b) Addition of the peptide Z-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH (20 equiv, 240 μM) to a sample of the inverted peptide library, which had been preincubated with the tweezer receptor **4** and which gave fluorescent beads, showed no fluorescently stained beads, above background, after an overnight incubation.

(c) A solution of dansyl-Phe-Val-Gly-OH (13.5 μM in 1 mM sodium borate, pH 9.2/16.7% DMSO) was incubated with resin beads of (i) unmodified TentaGel resin or (ii) inverted peptide library, as described above. No specific binding was observed for either the inverted peptide library or unmodified TentaGel resin.

(d) Incubation of receptor solutions under conditions identical to those above (13.5 μM in 1 mM sodium borate, pH 9.2/16.7% DMSO) with resin beads of (i) unmodified TentaGel resin (ii) the cyclized library (20 mg) and (iii) the uninverted peptide library (20 mg) showed no selectivity under the conditions described above.

Microcalorimetry. Microcalorimetry was carried out on a Micro Cal Inc Micro Calorimetry system consisting of their ITC unit, MCS Observer, and Origin ITC Data Analysis Software. The ITC cell conditions were 26 injections at 30 °C with an initial delay of 60 s. The initial injection to prime the system was 2.00 μL over 5.02 s and then 25 injections of 10.02 μL over 25.15 s with a 250 s delay between injections. The experiments were run using a 20 μM solution of the receptor in the cell and 200 μM of the ligand in the syringe, both in 16% DMSO, 0.001 sodium borate pH 9.2. The cell was equilibrated overnight before running the expriment. These experiments were complicated by the insolubility of the ligand and the receptor in buffers and necessitated the use of DMSO in all experiments. Corrected data for one experiment are given in Table 2.

Table 2[a]

area (μcal)	injection (μL)	[ligand] (mM) (before injection)	[receptor] (mM) (before injection)	[ligand]/[receptor] (after injection)	cal/mol of injected ligand
	2.00		0.0200	0.0150	
-64.66	10.02	0.00030	0.01997	0.0900	-32262
-68.63	10.02	0.00178	0.01982	0.1656	-34241
-66.84	10.02	0.00326	0.01967	0.2419	-33350
-65.88	10.02	0.00472	0.01953	0.3186	-32872
-64.64	10.02	0.00618	0.01938	0.3959	-32251
-62.93	10.02	0.00762	0.01924	0.4738	-31397
-58.48	10.02	0.00905	0.01909	0.5523	-29181
-53.90	10.02	0.01047	0.01895	0.6313	-26895
-49.26	10.02	0.01188	0.01881	0.7108	-24578
-45.90	10.02	0.01327	0.01867	0.7909	-22904
-43.89	10.02	0.01466	0.01853	0.8716	-21901
-42.42	10.02	0.01603	0.01839	0.9528	-21165
-40.25	10.02	0.01740	0.01826	1.0346	-20081
-38.25	10.02	0.01875	0.01812	1.1169	-19084
-37.59	10.02	0.02009	0.01799	1.1998	-18755
-35.99	10.02	0.02142	0.01785	1.2833	-17 956
-34.97	10.02	0.02274	0.01772	1.3673	-17449
-33.67	10.02	0.02405	0.01759	1.4519	-16802
-32.98	10.02	0.02534	0.01745	1.5370	-16456
-31.88	10.02	0.02663	0.01732	1.6227	-15906
-30.96	10.02	0.02790	0.01719	1.7090	-15447
-30.66	10.02	0.02916	0.01707	1.7958	-15299
-29.00	10.02	0.03042	0.01694	1.8832	-14471
-29.51	10.02	0.03166	0.01681	1.9711	-14723
-28.42	10.02	0.03289	0.01668	2.0596	-14181

[a] Using a one-site model and correcting this data for a blank injection of the DMSO/buffer solution into the cell gave a binding constant of 4×10^5 M $\pm$ 5×10^4.

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Supporting Information Available: Details of preliminary screening experiments with the inverted peptide library and dansylated tweezer receptor **4** (2 pages).

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