Identification of Sequence Selective Receptors for Peptides with a Carboxylic Acid Terminus

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Abstract: Split-and-mix libraries of resin-bound "tweezer" receptors have been prepared and screened to identify receptors for dye-labelled tripeptides. The receptors incorporate a diamidopyridine unit to serve as a specific recognition site for the CO_2H group, leading to strong and selective receptors for peptide guests with a CO_2H terminus. The role of the dye-label, attached to the peptide guest to allow visualisation of selective recognition events in the screening experiments, has also been examined and was found to have a significant influence on the binding selectivities.

Keywords: combinatorial chemistry · peptides · receptors · tweezers

Introduction

The ability to prepare synthetic receptors for specific ligands is a highly desirable goal. In particular, selective receptors for specific peptide sequences would have potential applications for separation of peptide mixtures, as biosensors and as new therapeutics, as well as providing model systems for biological protein – peptide complexes. Much recent work in the area of peptide receptors has focused on "tweezer" receptors[1, 2] which, despite their inherent flexibility, have proved to be highly selective for certain peptide sequences in both non-polar[3] and aqueous solvent systems.[4] Many of these tweezer receptors have been screened against combinatorial libraries of resin-bound peptides allowing a rapid evaluation of the binding properties of the receptor.[3, 4a] The reverse process, that is screening of a library of receptors with a chosen peptide substrate, has been less well developed, although such a strategy was pioneered by Still, some years ago.[5]

The basic design of tweezer receptors incorporates a "head group" or "hinge" bearing two side arms which incorporate appropriate functionality for binding with the backbone of suitable substrates (Figure 1). In contrast to many of the tweezer receptors reported to date we are interested in incorporating a "head group" with a specific recognition site for the terminal functional group of the peptide guests.[4a, 6] Incorporation of such a binding site or "anchor point", in addition to binding interactions from the tweezer side arms, should greatly increase the binding affinity of such tweezer receptors with suitable substrates, and should ultimately lead to receptors for the C-terminal sequence of larger peptides. We recently described[6] our preliminary studies with a combinatorial library of "tweezer" receptors, incorporating a 2,6-diamidopyridine unit[7] as the tweezer "head group", thus providing a binding site for the carboxylic acid terminus of peptide guests. Both arms of the tweezer were themselves simple peptides, which can potentially provide selective interactions with the backbone of the guests. This library was successfully screened to identify a sequence selective receptor for a dye-labelled peptide guest with a carboxylic acid terminus. We have now extended our studies to evaluate the binding properties of such libraries with several peptide guests, both side chain protected and unprotected and with several different dye-label attachments. These studies demonstrate the power of the combinatorial approach in identifying sequence selective receptors for tripeptides in non-polar solvents, with this class of tweezer compounds, but the role of the attached dye in the recognition process cannot be ignored. Herein we describe these studies in full as well as providing full details of the synthesis of the tweezer libraries.

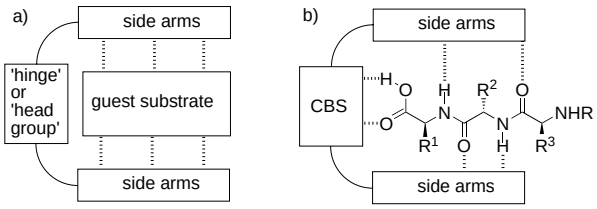

Figure 1. a) Schematic of a tweezer receptor with "hinge" or "head group" and side arms which provide binding interactions with a suitable guest substrate. b) Schematic of a tweezer receptor with a carboxylic acid binding site (CBS) as the "head group".

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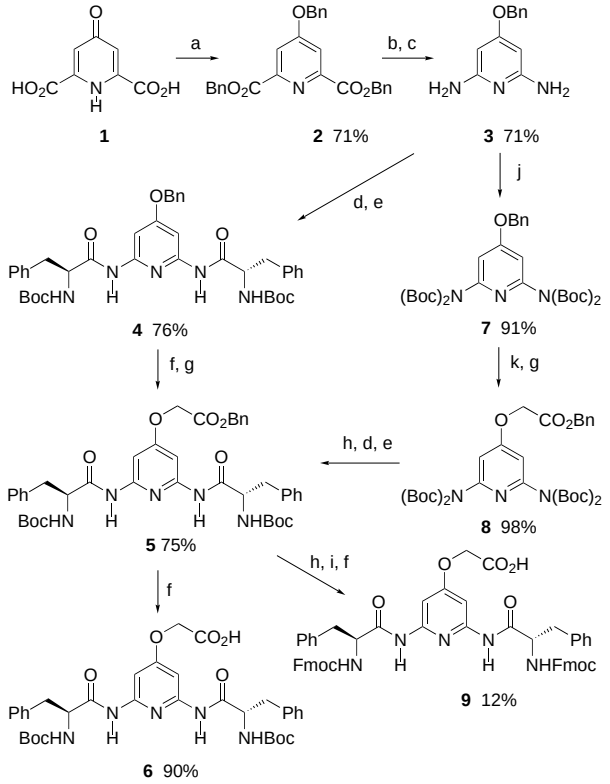
Results and Discussion

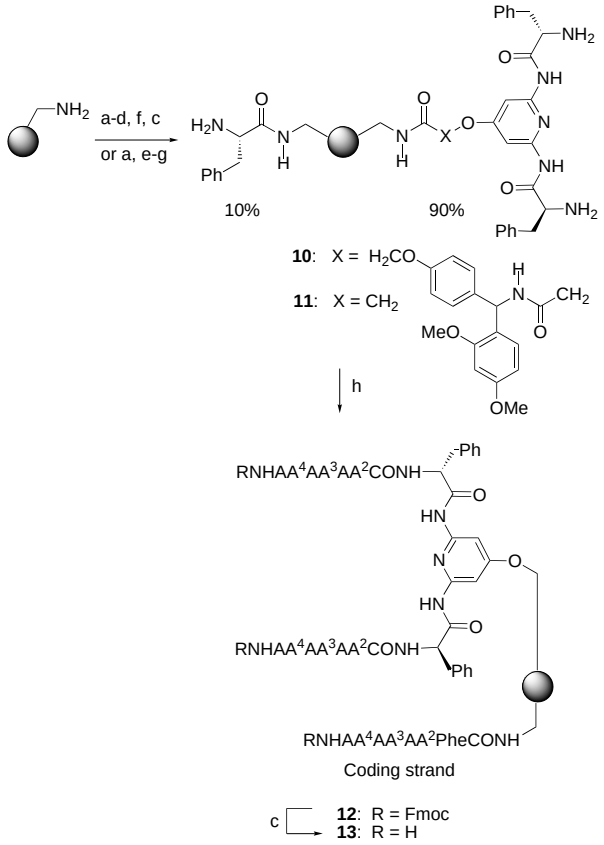
Synthesis: In order to prepare libraries of tweezer structures we synthesised a 2,6-diamidopyridine derivative suitably functionalised with a carboxylic acid moiety to allow attachment to resin beads. In our initial work, such a diamidopyridine derivative, **6**, was prepared by first converting chelidamic acid **1** to the trisbenzyl derivative **2**, followed by aminolysis of the resulting benzyl esters and subsequent Hofmann rearrangement to give diaminopyridine **3**, in 50% overall yield (Scheme 1).[8] Treatment of **3** with *N*,*O*-bis(trimethylsilyl)acetamide,[9] followed by addition of Boc-phenylalanine acid fluoride[10] gave **4** in 76% yield. Hydrogenolysis of the benzyl ether, and alkylation with benzyl bromoacetate gave **5**, and finally further hydrogenolysis of the benzyl ester, gave **6** in 68% overall yield from **4**. An alternative approach to **6** was achieved by treatment of the diaminopyridine **3** with Boc-anhydride, to give **7**, followed by hydrogenolysis of the benzyl ether and alkylation with benzyl bromoacetate as before to give **8**, which is a convenient intermediate for the preparation of a range of diamidopyridine derivatives. For example, **8** was readily Boc deprotected using TFA and acylated with Boc-phenylalanine acid fluoride. Hydrogenolysis of the benzyl ester then gave the desired diamidopyridine **6** for use in library synthesis.



Scheme 1. a) K_2CO_3, BnBr, acetone, reflux; b) NH_3, MeOH; c) KOH, Br_2, 90 °C; d) *N*,*O*-bis(trimethylsilyl)acetamide; e) Boc-Phe-F; f) 10% Pd/C, NH_4CO_2H, MeOH; g) $BrCH_2CO_2CH_2Ph$, K_2CO_3, DMF; h) 20% CF_3CO_2H, CH_2Cl_2; i) Fmoc-ONSu, Na_2CO_3, H_2O, dioxan; j) $(Boc)_2O$, DMAP, CH_3CN; k) H_2, 10% Pd/C, EtOH.

In initial studies the Boc-protected diamidopyridine derivative **6** was successfully coupled directly to TentaGel resin (without a linker) and after removal of the Boc-protecting groups, standard solid-phase "split-and-mix" synthesis,[11] using eight Fmoc-amino acids (Gly, L-Ala, L-Val, L-Phe, D-Phe, L-Leu, L-Glu(O*t*Bu), L-Ser(*t*Bu)) gave a 512-membered library of tweezer receptors with tetrapetide side arms. Attempted sequencing of individual beads using Edman degradation was, however, only partially successful (typically only the first two amino acids could be unambiguously identified) and we concluded that under the conditions of the Edman degradation the amide bonds to the diamidopyridine core were being cleaved, leading to loss of the peptide side arm.[12]

In order to circumvent this problem we adopted a strategy using a peptide coding strand, and with the tweezer attached via the Rink-amide linker,[13] which would be cleaved and washed away, under the conditions for the Edman sequencing of the coding strand. The use of the acid labile Rink linker is not compatible with Boc protection of the diamidopyridine derivative **6** for attachment and library generation. Instead we prepared the Fmoc-protected diamidopyridine derivative **9**, which was accomplished by Boc deprotection of **5** followed by reprotection with Fmoc (Scheme 1). A peptide coding strand was conveniently incorporated by first coupling Aloc phenylalanine onto 10% of the amine sites on TentaGel-NH_2 resin beads followed by coupling Fmoc-Rink amide linker[13] to the remaining 90% of amine sites (Scheme 2). Removal of the



Scheme 2. a) Aloc-Phe-OH, HOBt, DIC, DMAP, CH_2Cl_2 (10% of amine sites); b) Fmoc-Rink amide linker, HOBt, DIC, DMAP, CH_2Cl_2; c) 20% piperidine, DMF; d) **9**, HOBt, DIC, DMAP, CH_2Cl_2; e) **6**, HOBt, DIC, DMAP, CH_2Cl_2; f) $Pd(PPh_3)_4$, dimedone, CH_2Cl_2, THF; g) 20% CF_3CO_2H, CH_2Cl_2; h) 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.

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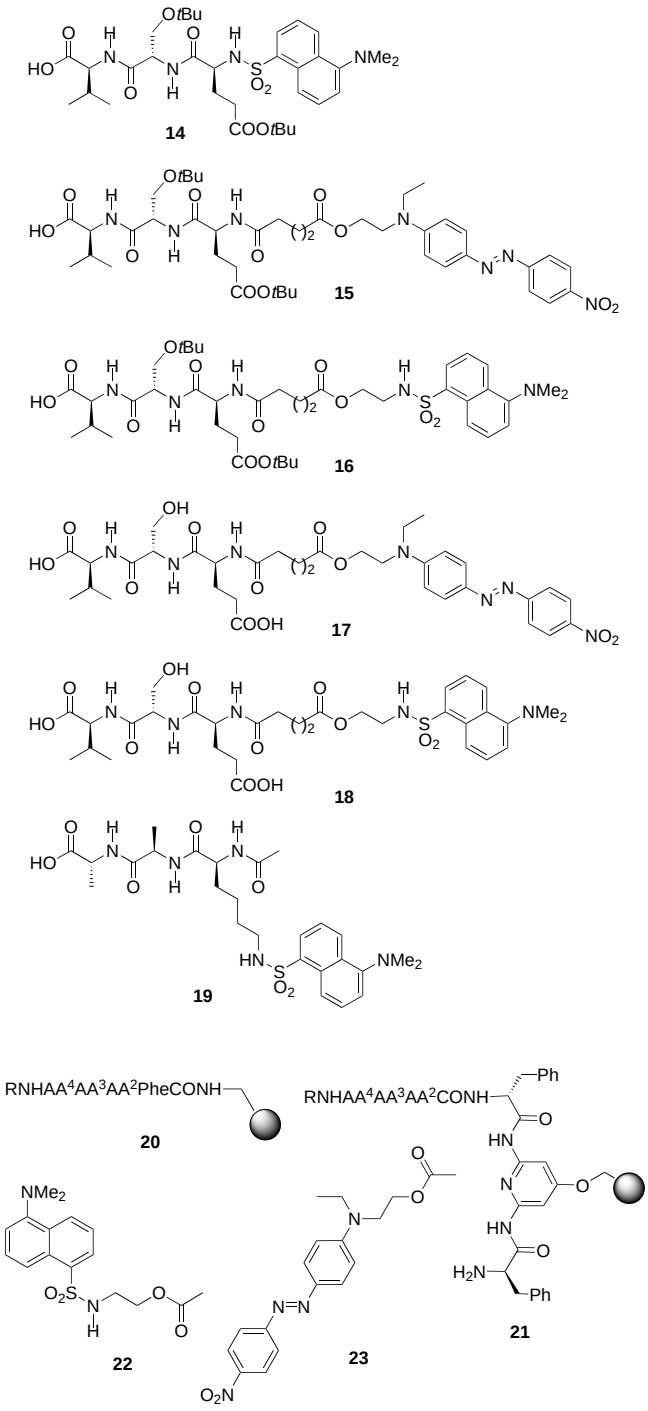
Fmoc group from the Rink linker was followed by coupling of diamidopyridine **9** and further deprotection of both the Fmoc- and Aloc-protecting groups to yield resin **10** ready for library generation. Further studies revealed that attachment of the tweezer receptors to the solid support via a linker was not necessary as neither its presence, nor its apparent degradation under Edman sequencing conditions, compromised the reading of the coding strand. Thus, after coupling of Aloc-phenylalanine onto 10% of the amine sites, Boc-protected **6** was attached directly to the remaining 90% of amine sites, and after deprotection yielded, again, a resin **11** ready for library generation.

A 2197-member library of Fmoc-protected tweezers **12** was prepared by a three-fold coupling of thirteen Fmoc-protected amino acids (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) to the free amine groups of both **10** or **11**, using the "split-and-mix" strategy.[13] A second library of tweezers, **13**, with free terminal amino groups, was prepared by treatment of a portion of library **12** with piperidine.

Screening experiments and binding studies: Screening experiments were carried out with the tweezer libraries using a range of dye-labelled peptide guests **14**–**19**.[14] In a typical screening experiment a sample of 4–5 mg (4000–5000 beads) of the library was equilibrated in a chosen solvent system (160 μL) for 24 h, followed by addition of dye-labelled tripeptide, as a solution in the same solvent system (160 μL), and incubation for a further 24 h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (magnification ×40). For screening experiments with a dansylated (DNS) guest, a filter cube that contained a suppression filter at 425 nm and excitation filter at 340–380 nm was employed. The screening experiments are simple, and allow rapid evaluation of the binding potential and selectivity of the libraries with the guest, in a range of solvent systems.

Several control tests were also carried out. In order to show that any observed selectivity was not a consequence of interaction of the tripeptide guest simply with the peptide side arm of the tweezer receptor, or with the coding strand on the library beads, a simple 2197-membered peptide library **20** directly attached to TentaGel resin (analogous to the coding strand) was prepared. Incubation of this library with the dye-labelled peptide guests showed no selectivity. Similarly incubation of the guests with a library **21** of diamidopyridines with a single tweezer side arm showed no selectivity. In order to probe the role of the dye in the recognition process the simple acetylated dansyl and red-dye substrates **22** and **23** were prepared, but incubation of these compounds with the tweezer libraries again showed no selective binding properties. Taken together these results confirm that any observed selectivity in the screening of the tweezer libraries with the dye-labelled peptide guests are a consequence of selective binding between the tweezer and the dye-labelled peptide guest as desired.

Finally, screening the libraries with the methyl esters of peptide guests **14** and **15**, showed no selective binding, confirming that the observed selectivity with the analogous peptide guests, with a free carboxylic acid at the C-terminus, (see below) was critically dependent on the presence of the carboxylic acid functionality, presumably interacting with the diamidopyridine moiety as desired.



Our initial studies focussed on using libraries **12** and **13** and the protected tripeptide DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH (**14**) as the guest.[14] In practice we observed no selective binding with either of the guest-library combinations in a range of buffered aqueous solvents. With chloroform as solvent, however, good selectivity was observed for DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH with both the protected library **12** and deprotected library **13** (approximately 1% of beads were highly fluorescent in both cases against a background of low fluorescence beads).

From each of the successful screening experiments highly fluorescent beads were selected and sequenced by Edman degradation.[15] (Edman sequencing was carried out in all cases for a fourth cycle, which always showed the presence of phenylalanine for all beads, which was expected as it was introduced by the synthesis, and provided a useful check that the coding strand was operating correctly). The results of the sequencing experiments had the highest level of consensus for screening of DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH against the protected library **12** (Table 1) with the sequence Phe-Val-AA^3-Trp found for six of the ten beads analysed, and more specifically Phe-Val-Leu-Trp was found for three of the beads. With the deprotected library **13** (Table 2) a strong consensus for valine or leucine at the second and third positions was also found, but the terminal amino acid residue was less well defined with phenylalanine detected for four of the beads. Significantly tryptophan was not detected at the terminal position for any of the beads from deprotected library **13**.

The effect of solvent polarity on the binding selectivity of these libraries could be readily established by simple addition of more polar solvents to the screening experiments set up in $CHCl_3$ solution. Thus, addition of DMSO led to a rapid loss of selective fluorescence, with both protected and deprotected libraries **12** and **13**, when the solvent composition reached 10% DMSO/90% $CHCl_3$. Likewise addition of 10% MeOH effectively destroyed the selectivity. Addition of CH_3CN, however, did not have any noticeable effect on the selectivity observed for the screening of DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH with the Fmoc-protected library **12**, although addition of 50% CH_3CN did destroy the selectivity for the screening with the deprotected library **13**. Indeed, screening DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH against the Fmoc-protected library **12** in neat CH_3CN showed good selectivity (again, approximately 1% of beads were highly fluorescent). Selection of five highly fluorescent beads from this screening experiment and sequencing by Edman degradation gave the identical consensus sequence, Phe-Val-Leu-Trp, as found for the same screen in $CHCl_3$, for two of the five beads, and Phe-Val-Met-Trp for a third bead (Table 3). The strong consensus for tryptophan at the terminal position when screening with the Fmoc-protected library **12** in both $CHCl_3$ and CH_3CN, the absence of tryptophan at the terminal position when screening with the deprotected library **13** in $CHCl_3$ and absence of any selectivity for the latter screen in CH_3CN, suggests that the combination of Fmoc-protecting group and the tryptophan residue play an important role in binding of this peptide guest.

From these preliminary, and highly encouraging results we sought to establish that the observed binding with resin-bound tweezers was also operating in free solution. Thus tweezer **24** was prepared by solid-phase synthesis (Scheme 3), as a single

Table 1. Sequencing data for ten fluorescent beads selected from screening experiment of DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH (**14**) with protected library **12** in $CHCl_3$.

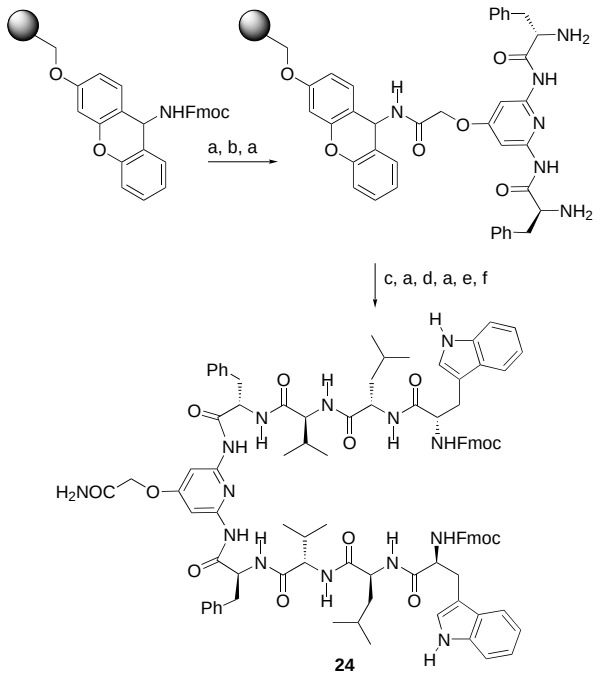
Bead	AA^1	AA^2	AA^3	AA^4
1	Phe	Val	Leu	Trp
2	Phe	Val	Leu	Trp
3	Phe	Val	Leu	Trp
4	Phe	Val	Ala	Trp
5	Phe	Val	Val	Trp
6	Phe	Val	Met	Trp
7	Phe	Val	Gly	Phe
8	Phe	Val	Ala	Val
9	Phe	Val	Gly	Val
10	Phe	Gln	Val	Gln

Table 2. Sequencing data for ten fluorescent beads selected from screening experiment of DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH (**14**) with deprotected library **13** in $CHCl_3$.

Bead	AA^1	AA^2	AA^3	AA^4
1	Phe	Val	Leu	Met
2	Phe	Val	Leu	Met
3	Phe	Val	Leu	Leu
4	Phe	Val	Leu	Leu
5	Phe	Val	Gly	Ala
6	Phe	Val	Met	Met
7	Phe	Leu	Val	Phe
8	Phe	Leu	Val	Phe
9	Phe	Phe	Val	Phe
10	Phe	Ala	Ala	Phe

Table 3. Sequencing data for five fluorescent beads selected from screening experiment of DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH (**14**) with protected library **12** in CH_3CN.

Bead	AA^1	AA^2	AA^3	AA^4
1	Phe	Val	Leu	Trp
2	Phe	Val	Leu	Trp
3	Phe	Val	Met	Trp
4	Phe	Phe	Val	Ala
5	Phe	Phe	Met/	Met/
		Val	Trp	



Scheme 3. a) 20% piperidine, DMF; b) **9**, HOBt, DIC, DMAP, CH_2Cl_2; c) Fmoc-L-Val-OH, HOBt, DIC, DMAP, CH_2Cl_2; d) Fmoc-L-Leu-OH, HOBt, DIC, DMAP, CH_2Cl_2; e) Fmoc-L-Trp-OH, HOBt, DIC, DMAP, CH_2Cl_2; f) 1% CF_3CO_2H, CH_2Cl_2.

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compound, with tetrapeptide side arms Phe-Val-Leu-Trp, corresponding to the most commonly found sequence in the screening experiments. The synthesis of **24** was most easily achieved using the Sieber amide resin,[16] which ultimately allowed cleavage of the tweezer from the resin with 1% CF_3CO_2H in CH_2Cl_2 (Scheme 3). Unfortunately tweezer **24** was essentially insoluble in neat $CDCl_3$ or neat CD_3CN and therefore did not allow NMR studies on the formation of a complex with DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH. A solution of **24** in 2% DMSO/$CHCl_3$ could, however, be prepared and allowed us to study the complexation of **24** with DNS-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH, by fluorescence spectroscopy. The intensity of the fluorescence emission maximum (at 494 nm) for the dansyl group of the peptide guest decreased as aliquots of the tweezer receptor **24** were added and the drop in intensity exhibited typical saturation. The data from this experiment showed a good fit for the presumed 1:1 binding and allowed an estimation of the binding constant as $2.6 \times 10^5\,\text{M}^{-1}$ ($-\Delta G_a = 30.4\,\text{kJ mol}^{-1}$).[17]

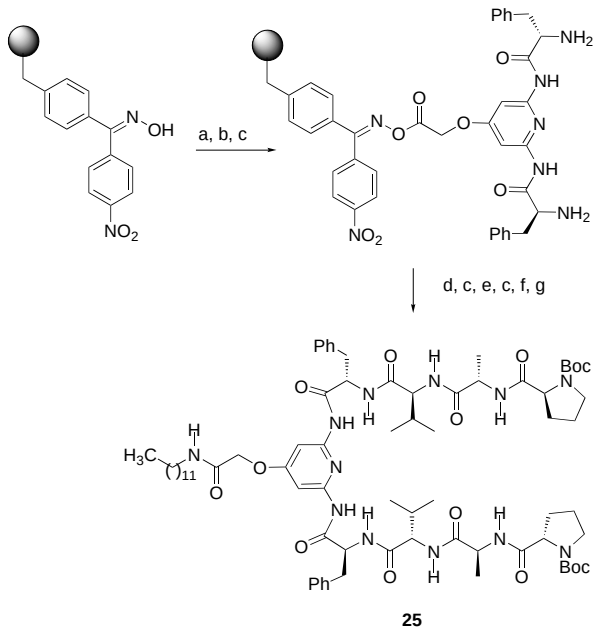
Although the experiments with DNS-L-Glu(O*t*Bu)-L-Ser-(*t*Bu)-L-Val-OH gave pleasing results with good selectivity, the proximity of the dansyl dye on the peptide guest clearly raises questions about the role of the dye in the overall recognition process. Furthermore the observed binding was with a peptide guest with side chain protecting groups and therefore it was of interest to establish whether these tweezers could selectively bind deprotected peptides. Thus we carried out screening experiments with the additional guest molecules, red dye-spacer-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH (**15**), DNS-spacer-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH (**16**), red dye-spacer-L-Glu-L-Ser-L-Val-OH (**17**), DNS-spacer-L-Glu-L-Ser-L-Val-OH (**18**) and Ac-L-Lys(DNS)-D-Ala-D-Ala-OH (**19**).[18]

Screening of library **12** with red dye-labelled protected peptide **15** gave particularly good selectivity. Four beads were sequenced and three gave identical results for the tweezer side arm structure: Phe-Val-Ala-Pro; and the fourth bead yielded the closely related sequence Phe-Val-Met-Pro (Table 4).

As before, with dansylated peptide **14**, in order to establish that the observed binding of a red dye-labelled peptide, with resin-bound tweezers, was also operating in free solution, we synthesised tweezer **25** with side arm sequence Phe-Val-Ala-Pro, using the oxime resin;[19] this type of resin allowed cleavage of the tweezer using dodecyl amine (Scheme 4). The presence of the dodecyl chain meant that **25** was soluble in $CHCl_3$, as hoped, although the ^{1}H NMR spectrum of **25** in $CDCl_3$ (2 mM solution) was poorly resolved, presumably due to aggregation. Binding studies were carried out by titrating aliquots of the red dye-labelled peptide in to a solution of

Table 4. Sequencing data for four red beads selected from screening experiment of red dye-labelled peptide **15** with protected library **12** in $CHCl_3$.

Bead	AA^1	AA^2	AA^3	AA^4
1	Phe	Val	Ala	Pro
2	Phe	Val	Ala	Pro
3	Phe	Val	Ala	Pro
4	Phe	Val	Met	Pro



Scheme 4. a) **6**, PyBOP, DIPEA, CH_2Cl_2; b) Ac_2O, DIPEA; c) 20% CF_3CO_2H, CH_2Cl_2; d) Boc-L-Val-OH, HOBt, DIC, DMAP, CH_2Cl_2; e) Boc-L-Ala-OH, HOBt, DIC, DMAP, CH_2Cl_2; f) Boc-L-Pro-OH, HOBt, DIC, DMAP, CH_2Cl_2; g) $CH_3(CH_2)_{11}NH_2$ (0.5 M in $CHCl_3$).

tweezer **25** in $CHCl_3$, (final concentration of tweezer **25** was ≈ 0.01 mM, with no detectable aggregation at this concentration) and monitoring the change in UV at 240 nm. The data from this experiment gave an excellent fit for the presumed 1:1 binding and allowed an estimation of the binding constant as $9.1 \times 10^3\,\text{M}^{-1}$ ($-\Delta G_a = 22.6\,\text{kJ mol}^{-1}$).[17]

The binding constant for tweezer **25** with peptide **15** is somewhat lower than the binding constant for tweezer **24** with peptide **14**, although the difference in binding energies ($\approx 8\,\text{kJ mol}^{-1}$) can be accounted for with just two hydrogen bond interactions. Alternatively, binding of the dansylated peptide may be strengthened by a specific interaction of the dansyl group with the Fmoc-Trp terminus of the tweezer side arm—a reasonable assumption in view of the strong consensus for this residue in the screening experiment (Table 1).

Screening of the same library **12**, but using the analogous dansyl-labelled protected peptide **16** gave somewhat poorer selectivity and, rather surprisingly, different tweezer structures were identified. Thus from seven beads, three beads gave the sequence Phe-Val-Glu(O*t*Bu)-Leu and two others with the sequence Phe-AA^2-Val-Ala (Table 5).

Table 5. Sequencing data for seven fluorescent beads selected from screening experiment of dansylated peptide **16** with protected library **12** in $CHCl_3$.

Bead	AA^1	AA^2	AA^3	AA^4
1	Phe	Val	Glu	Leu
2	Phe	Val	Glu	Leu
3	Phe	Val	Glu	Leu
4	Phe	Leu	Glu	Ala
5	Phe	Leu	Val	Ala
6	Phe	Trp	Val	Ala
7	Phe	Met	Leu	Lys

Screening library **12** with the red dye-labelled, side chain deprotected peptide **17** also gave good selectivity, but significantly different sequences for the tweezer side arms were identified in comparison with the screening results with the structurally related red dye-labelled protected peptide **15**. Ala was found at the fourth position in each of five beads sequenced, and Val or closely related Leu at the third position in each case (Table 6). For this peptide, however, Val was not found at the second position with any of the beads as it had been for some or all selected beads in each of the previous screening experiments. Of course, with the deprotected peptide **17**, the free acid of the glutamic acid side chain may now be bound to the diamidopyridine in preference to the C-terminus carboxylic acid group, leading to the different sequences observed in this experiment. Nonetheless, it is clear that selective binding of the side chain deprotected peptide **17** is possible with this set of tweezers.

Table 6. Sequencing data for five red beads selected from screening experiment of red dye-labelled peptide **17** with protected library **12** in $CHCl_3$.

Bead	AA^1	AA^2	AA^3	AA^4
1	Phe	Ala	Leu	Ala
2	Phe	Ala	Leu	Ala
3	Phe	Gln	Leu	Ala
4	Phe	Gln	Val	Ala
5	Phe	Met	Val	Ala

Screening of the same library but using the analogous dansyl-labelled deprotected peptide **18**, on the other hand, gave poor selectivity as did the screening of either **17** or **18** with library **13**. Again, with the deprotected peptides **17** and **18**, the free acid of the glutamic acid side chain may now compete for the diamidopyridine binding site so a different mode of binding for these guests may be operating in comparison with the protected peptides **15** and **16**.

With Ac-L-Lys(DNS)-D-Ala-D-Ala-OH **19** as the guest tripeptide no selectivity was observed when screened with the Fmoc-protected library **12**, but high selectivity was again observed when screened against the deprotected library **13** (approximately 1–2% of beads were highly fluorescent, although there were also a number of beads with intermediate levels of fluorescence suggesting that the library was less selective for this peptide guest than for some of the other guest peptides assayed previously). Indeed Edman sequencing of nine selected beads from this latter screen showed only a moderate overall consensus, but there was considerable consensus for the third position with seven of the nine sequences having Asn or Gln (Table 7). Significantly the observed sequences were essentially completely different to those found for the screening with dye-labelled-L-Glu(O*t*Bu)-L-Ser(*t*Bu)-L-Val-OH, which confirms that the tweezer receptors are selective for different peptide substrates.

Table 7. Sequencing data for nine fluorescent beads selected from screening experiment of Ac-L-Lys(DNS)-D-Ala-D-Ala-OH (**19**) with deprotected library **13** in $CHCl_3$.

Bead	AA^1	AA^2	AA^3	AA^4
1	Phe	Trp	Asn	Gly
2	Phe	Trp	Asn	Gly
3	Phe	Trp	Val	Phe
4	Phe	Leu	Val	Phe
5	Phe	Leu	Gln	Leu
6	Phe	Val	Gln	Leu
7	Phe	Phe	Gln	Val
8	Phe	Gln	Gln	Gln
9	Phe	Met	Gln	Lys

Interpretation of these results is not straightforward. The different selectivities observed for the binding of different guests clearly supports the notion that binding involves selective recognition of the tripeptide structure. Furthermore the control experiments clearly confirm that the observed selectivity in the screening experiments is not a consequence of selective binding to the dye moiety on its own. However, comparison of binding results with the two dansylated peptides **14** and **16** show that binding selectivity is very sensitive to the location of the dye-label and changing the structure of the dye moiety in two otherwise identical peptide guests **15** and **16** clearly shows that even when the dye is removed from the peptide by an appropriate spacer, the dye label plays a significant role in the recognition process. In fact these observations are not so surprising and the notion that the tweezer will bind the peptide organised in a linear strand with the dye as a remote spectator is probably unrealistic. Instead, particularly in non-polar solvents, both the peptide guest and the tweezer structure are likely to form a variety of folded structures stabilised by intramolecular hydrogen bonds and binding between the two is likely to be much more complicated than that represented in Figure 1b. In addition, in free solution and removed from a resin, the tweezers can be anticipated to form aggregated structures, borne out by the low solubility of tweezer **24** in non-polar solvents (see above) and the poorly resolved ^1H NMR spectrum of **25**.

Dye-labelled substrates have been used by several groups to screen libraries of receptors for selective binding,[2–5] and selectivity assays have been developed using competitive binding with a red dye-labelled substrate and a blue dye-labelled substrate.[5b, 20] In these latter experiments it was reported that observed binding selectivities were not a consequence of differential binding of the different dye moieties, but our results described here show that this cannot be taken for granted. A practicable solution to this problem is to attach a fluorescent dye reporter group to the receptor rather than the guest molecule as has been demonstrated by several groups recently.[21, 22] Nevertheless, the combinatorial synthesis of receptor libraries does provide a powerful method for identifying selective receptors for a chosen dye-labelled guest molecule, and the incorporation of diamidopyridine unit into our tweezer structures does lead to the identification of potent receptors for dye-labelled peptides—both side chain protected and deprotected. Our continuing efforts in this area are now directed at the preparation of larger libraries of tweezers with non-identical side arms, which should allow the discovery of even more selective receptors for biologically relevant peptide sequences in both organic and aqueous solvent systems.

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Experimental Section

Materials and general methods: Whenever possible all solvents and reagents were purified according to literature procedures. Solvents for peptide syntheses were purchased from Rathburn Chemicals, HPLC grade solvents from Riedel-de-Haën. TentaGel-S-NH_2 resin was used as solid support in screening experiments and in peptide syntheses unless otherwise indicated and purchased from Rapp Polymere, Germany. Oxime resin, Fmoc- and Boc-amino acids as well as coupling reagents were purchased from NovaBiochem. All other chemicals were purchased from Aldrich or Fluka. Peptide and library syntheses on solid phase were performed in glass vessels with scinter frits or polypropylene filtration tubes with polyethylene frits on a Visiprep SPE Vacuum Manifold from Supelco. The reaction containers 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}. Sorbsil C60, 40–60 mesh silica was used for column chromatography. 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 Perkin–Elmer 1600 FT-IR spectrophotometer or on a Bio-Rad FT-IR spectrometer. Proton NMR spectra were obtained at 300 MHz on a Bruker AC 300 and at 400 MHz 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 solvent signal used. Coupling constants are given in Hz. The multiplicities of the signals 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. All electrospray (ES) spectra were recorded on a Micromass Platform quadrupole mass analyser with an electrospray ion source using acetonitrile as solvent. Fluorescence titration experiments were carried out on a Perkin–Elmer LS-5 fluorescence spectrometer using four way quartz cells. UV titration experiments were recorded on a Shimadzu UV-1601 UV/Visible spectrophotometer. UV absorbance of ninhydrin tests were measured on a Hewlett–Packard 8452A diode array spectrometer using two way quartz cells. Values were read at 570 nm.

Abbrevations: Boc, *tert*-butyloxycarbonyl; DIC, *N*,*N*′-diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DNS, dansyl; Fmoc, 9-fluorenyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolino-phosphonium hexafluorophosphate; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafuoroborate.

Dibenzyl 4-(benzyloxy)-2,6-pyridinedicarboxylate (2): Chelidamic acid (4.47 g, 22 mmol), dry potassium carbonate (13.8 g, 0.1 mol) and benzyl bromide (8.9 mL, 75 mmol) were refluxed in dry acetone for 3 d. After addition of hydrochloric acid (2 N, 100 mL) the aqueous layer was extracted with ethyl acetate (3 × 150 mL) and the combined organic layers were dried over magnesium sulfate. The solvent was removed under reduced pressure to give a yellow oil. After trituration of the resulting oil with petrol ether **2** was obtained as light yellow solid (7.08 g, 71%). M.p. 65 °C; IR (KBr): $\tilde{\nu}$ = 1725, 1716, 1597, 1351, 1072 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (s, 2H, Ar*H*), 7.42–7.32 (m, 15H, Ar*H*), 5.40 (s, 4H, CO_2CH_2), 5.11 (s, 2H, $ArOCH_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.7 (0), 164.5 (0), 150.2 (0), 135.6 (0), 134.8 (0), 129.1 (1), 128.9 (1), 128.7 (1), 128.6 (1), 128.5 (1), 127.9 (1), 115.0 (1), 70.9 (2), 67.9 (2); MS (ES$^+$): *m*/*z* (%): 454 (45) $[M+H]^+$, 929 (100) $[2M+Na]^+$; elemental analysis calcd (%) for $C_{28}H_{23}NO_5$ (453.5): C 74.16, H 5.11, N 3.09; found: C 74.21, H 5.07, N 3.03.

4-Benzyloxy-2,6-pyridinediamine (3): Dibenzyl ester **2** (5.0 g, 11 mmol) was stirred in a saturated solution of ammonia in methanol (60 mL) for 3 h at room temperature. The solvent was removed under reduced pressure to give the corresponding diamide as white powder (5.56 g, 93%). M.p. >220 °C; IR (KBr): $\tilde{\nu}$ = 3431, 1696, 1662, 1576, 1560 cm^{-1}; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.34 (s, 2H, Ar*H*), 7.75 (s, 4H, NH_2), 7.43 (m, 5H, C_6H_5), 4.85 (s, 2H, CH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 166.9 (0), 165.2 (0), 151.3 (0), 135.9 (0), 128.7 (1), 128.3 (1), 127.8 (1), 110.6 (1), 69.9 (2); MS (ES$^+$): *m*/*z* (%): 272 (33) $[M+H]^+$, 295 (87) $[M+Na]^+$, 565 (100) $[2M+Na]^+$.

The diamide (5 g, 18.44 mmol) was added to an aqueous solution of potassium hydroxide (5 M, 50 mL) and bromine (2.4 mL, 46 mmol). The resulting reaction mixture was heated to 90 °C for 5 h. After extraction with dichloromethane (7 × 75 mL) the combined organic layers were dried over magnesium sulfate and the solvent was removed under reduced pressure to yield **3** as light brown solid (3.04 g, 76%). M.p. 164–166 °C; IR (KBr): $\tilde{\nu}$ = 3433, 1634, 1446, 1184 cm^{-1}; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.40–7.20 (m, 5H, OCH_2ArH), 5.35 (s, 2H, C_5H_2N), 5.30 (s, 4H, NH_2), 4.90 (s, 2H, OCH_2Ar); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 167.0 (0), 160.0 (0), 137.2 (0), 128.4 (1), 127.8 (1), 127.5 (1), 82.4 (1), 68.3 (2); MS (ES$^+$): *m*/*z* (%): 216 (100) $[M+H]^+$.

4-Benzyloxy-2,6-bis(*N*-*tert*-butoxycarbonyl-L-phenylalaninylamino)pyridine (4): Diamine **3** (162 mg, 0.5 mmol) was dissolved in dry acetonitrile (5 mL). After addition of *N*,*O*-bis(trimethylsilyl)acetamide (123 µL, 0.5 mmol) the reaction mixture was stirred at room temperature for 2 h. Then, *N*-Boc-phenylalanyl fluoride[10] (326 mg, 1 mmol) and a catalytic amount of TBAF (10 µL) were added and the reaction mixture was stirred for additional 12 h. The solvent was removed under reduced pressure and the residue redissolved in dichloromethane (40 mL). The organic layer was washed with an aqueous solution of sodium hydrogencarbonate (5%, 2 × 40 mL), hydrochloric acid (4 N, 2 × 40 mL), and water (40 mL) and dried over magnesium sulfate. The solvent was removed under reduced pressure to yield **4** as orange solid (269 mg, 76%). M.p. 88–90 °C; $[\alpha]_D$ = 6.2 (ethanol, *c* = 1); IR (KBr): $\tilde{\nu}$ = 3429, 1685, 1440, 1165, 699 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (br s, 2H, N*H*), 7.30 (br m, 7H, $OCH_2C_6H_5$, ArH_{py}), 7.13 (m, 10H, $CHCH_2C_6H_5$), 5.43 (s, 2H, N*H*), 4.98 (s, 2H, OCH_2Ph), 3.17 (br s, 2H, $CHCH_aH_bPh$), 2.95 (br s, 2H, $CHCH_aH_bPh$), 1.32–1.25 (br s, 18H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.6 (0), 168.0 (0), 156.1 (0), 150.2 (0), 136.6 (0), 136.0 (0), 129.5 (1), 128.8 (1), 128.7 (1), 128.3 (1), 127.9 (1), 127.1 (1), 96.9 (1), 80.7 (0), 70.2 (2), 56.8 (1), 38.4 (2), 28.5 (3); MS (ES$^+$): *m*/*z* (%): 710 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{40}H_{47}N_5O_7$ (709.8): C 67.68, H 6.67, N 9.87; found: C 67.17, H 6.70, N 9.80.

Benzyl 2-{[2,6-bis(*N*-*tert*-butoxycarbonyl-L-phenylalanylamino)-4-pyridyl]oxy} acetate (5): Benzyl ether **4** (640 mg, 0.9 mmol), 10% palladium on charcoal (150 mg) and ammonium formate (300 mg) were suspended in dry and degassed methanol (15 mL) and refluxed for 30 min. The reaction mixture was filtered over celite and the solvent removed under reduced pressure to give the corresponding diamidopyridone as a white solid (523 mg, 94%) which was recrystallised from ethanol. M.p. 115–118 °C; $[\alpha]_D$ = 68.0 (DMSO, *c* = 1); IR (KBr): $\tilde{\nu}$ = 3400, 1684, 1455, 1440, 1165 cm^{-1}; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.65 (s, 1H, NH_{py}), 9.95 (s, 2H, NH_{amide}), 7.40–7.15 (br m, 12H, Ar*H*), 4.45 (br m, 2H, $CHCH_2Ph$), 3.05–2.77 (m, 4H, $CHCH_2Ph$), 1.29 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.6 (0), 167.0 (0), 155.6 (0), 151.1 (0), 138.0 (0), 129.4 (1), 128.1 (1), 126.4 (1), 97.1 (1), 78.3 (0), 56.6 (1), 37.1 (2), 28.2 (3); MS (ES$^+$): *m*/*z* (%): 620 (100) $[M+H]^+$, 1240 (20) $[2M+H]^+$; elemental analysis calcd (%) for $C_{40}H_{47}N_5O_7$ (709.8): C 63.99, H 6.67, N 11.30; found: C 63.89, H 6.62, N 11.14.

Potassium carbonate (41 mg, 290 mmol) and benzyl bromoacetate (46.5 µL, 290 mmol) were added to a solution of the pyridone (200 mg, 323 mmol) in DMF (5 mL) and stirred 15 h at room temperature. The solvent was removed under reduced pressure and the residue purified by column chromatography (20–30% ethyl acetate in petroleum ether) to yield benzyl ester **5** as white solid (186 mg, 75%) which was recrystallised from ethanol/water. M.p. 65–67 °C; $[\alpha]_D$ = 4.2 (ethanol, *c* = 1); IR (KBr): $\tilde{\nu}$ = 3441, 1691, 1586, 1513, 1437, 1161 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.03–8.60 (br s, 2H, ArN*H*CO), 7.60–7.00 (m, 17H, Ar*H*), 5.52 (br s, 2H, $NHCO_2$), 5.24 (s, 2H, OCH_2CO), 4.67 (s, 2H, OCH_2Ph), 4.55 (br s, 2H, $NHCHCO$), 3.10 (m, 4H, $CHCH_2Ph$), 1.33 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.5 (0), 167.8 (0), 167.1 (0), 156.0 (0), 150.2 (0), 136.5 (0), 135.2 (0), 129.9 (1), 129.6 (1), 129.5 (1), 128.1 (1), 128.6 (1), 127.1 (1), 96.7 (1), 80.7 (0), 67.3 (2), 65.1 (2), 56.7 (1), 38.3 (2), 28.4 (3); MS (ES$^+$): *m*/*z* (%): 768 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{42}H_{49}N_5O_9$ (767.9): C 65.70, H 6.43, N 9.12; found: C 66.05, H 6.34, N 8.80.

2-[2,6-Bis(*N*-*tert*-butoxycarbonyl-L-phenylalanyl)-4-pyridyloxy] acetic acid (6): Benzyl ester **5** (527 mg, 0.686 mmol) was refluxed in degassed methanol (20 mL) in the presence of ammonium formate (1 g) and 10% palladium on charcoal (70 mg) for 1 h. The reaction mixture was filtered over celite and the solvent removed under reduced pressure to give acid **6** as white solid (422 mg, 90%). M.p. 100–102 °C; IR (KBr): $\tilde{\nu}$ = 3440, 2360, 1684, 1436, 1163 cm^{-1}; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.90 (s, 1H, COO*H*), 7.47 (s, 2H, C_5H_2N), 7.45–7.10 (br m, 14H, OCH_2ArH, N*H*), 6.46

(brs, 2H, CONHCH), 4.38 (s, 2H, OCH_2CO), 3.10–2.50 (brm, 4H, $CHCH_2Ph$), 1.11 (brs, 9H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta =$ 171.7 (0), 170.2 (0), 167.5 (0), 155.6 (0), 151.1 (0), 138.0 (0), 129.4 (1), 128.1 (1), 126.4 (1), 96.2 (1), 78.3 (0), 65.0 (2), 56.5 (1), 37.1 (2), 28.2 (3); MS (ES$^+$): *m*/*z* (%): 678 (100) $[M+H]^+$; HRMS calcd for $C_{35}H_{44}N_5O_9$ $[M+H]^+$: 678.3139, found: 678.3149; elemental analysis calcd (%) for $C_{35}H_{43}N_5O_9$ (677.8): C 62.03, H 6.40, N 10.33; found: C 61.47, H 6.20, N 9.99.

2-{2,6-Bis[*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-4-pyridyloxy} acetic acid (9): TFA (5 mL) was added to a solution of **5** (1 g, 1.3 mmol) in dichloromethane (25 mL) and stirred for 30 min at room temperature. Toluene (25 mL) was added to the reaction mixture and the solvent and excess TFA were removed under reduced pressure to give a yellow oil. Trituration with diethyl ether gave the corresponding TFA salt (532 mg, 51%) as a white solid. IR (KBr): $\tilde{\nu} = 3429, 1675, 1437, 1200\,cm^{-1}$; ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.47$ (s, 6H, NH_3^+), 7.61–7.10 (brm, 19H, ArH, NHCO), 5.73 (s, 2H, OCH_2CO_2), 4.95 (s, 2H, OCH_2Ph), 4.60–4.30 (brm, 2H, $COCHNH_3^+$), 3.30–2.95 (brm, 4H, $CHCH_2Ph$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta =$ 168.2 (0), 168.0 (0), 166.8 (0), 158.5 (q, $J = 38$, 0), 151.0 (0), 137.9 (0), 135.5 (0), 129.6 (1), 129.4 (1), 128.7 (1), 128.6 (1), 128.0 (1), 127.4 (0), 115.1 (q, $J = 293$, 0), 97.0 (1), 66.4 (2), 64.9 (2), 54.1 (1), 37.2 (2); MS (ES$^+$): *m*/*z* (%): 568 (100) $[M+H]^+$.

N-Fmoc-succinimide (250 mg, 0.75 mmol) in 1,4-dioxane (5 mL) was added to a solution of the TFA salt (500 mg, 0.63 mmol) in an aqueous solution of sodium carbonate (9%, 10 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirring was continued for 15 h. After extraction with ethyl acetate (4 × 50 mL) the combined organic layers were dried over magnesium sulfate and the solvent was removed under reduced pressure to give a yellow oil. After purification by column chromatography on silica gel (dichloromethane) the Fmoc-protected system (248 mg, 39%) was obtained as a white solid. M.p. 94–96 °C; IR (KBr): $\tilde{\nu} = 3424, 1695, 1585, 1437, 1215\,cm^{-1}$; ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta = 7.97$ (d, $J = 7$, 2H, ArH), 7.64 (t, $J = 7$, 2H, ArH), 7.50–7.18 (brm, 29H, ArH, NH), 5.23 (s, 2H, OCH_2CO_2), 4.98 (s, 2H, OCH_2Ph), 4.61 (m, 2H, $CHCH_2Ph$), 4.25–4.10 (brm, 6H, OCH_2CH), 2.95–2.80 (brm, 4H, $CHCH_2Ph$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta =$ 171.8 (0), 168.1 (0), 166.6 (0), 156.1 (0), 151.3 (0), 143.8 (0), 140.7 (0), 137.9 (0), 135.6 (0), 129.4 (1), 128.6 (1), 128.2 (1), 128.0 (1), 127.7 (1), 127.1 (1), 126.5 (1), 125.4 (1), 125.3 (1), 120.2 (1), 96.0 (1), 66.3 (2), 65.8 (2), 64.7 (2), 56.8 (1), 46.6 (1), 37.1 (2); MS (ES$^+$): *m*/*z* (%): 1012 (40) $[M+H]^+$.

10% Palladium on charcoal (5 mg) was added to a solution of Fmoc-protected benzyl ester (51 mg, 0.05 mmol) in dichloromethane (5 mL) and methanol (5 mL). The solution was degassed and then stirred in a hydrogen atmosphere for 1 h at room temperature. The catalyst was removed by filtration and the solvent removed under reduced pressure to give **9** as a white solid (28 mg, 60%). M.p. 143–145 °C; $[\alpha]_D = 8.2$ (DMSO, $c = 0.3$); IR (KBr): $\tilde{\nu} = 3020, 1686, 1450, 1216, 770\,cm^{-1}$; ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta = 7.80–7.10$ (brm, 28H, ArH), 4.68 (s, 2H, OCH_2CO_2), 4.50 (m, 2H, $CHCH_2Ph$), 4.35–4.08 (brm, 6H, OCH_2CH), 3.25–2.75 (brm, 4H, CH_2Ph); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta =$ 173.0 (0), 172.7 (0), 169.0 (0), 159.0 (0), 152.2 (0), 145.1 (0), 142.5 (0), 138.3 (0), 130.6 (1), 129.5 (1), 128.8 (1), 128.1 (1), 127.8 (1), 126.1 (1), 120.9 (1), 97.7 (1), 68.1 (2), 67.0 (2), 58.0 (1), 39.0 (1), 28.6 (2); MS (ES$^+$): *m*/*z* (%): 922 (50) $[M+H]^+$, 944 (20) $[M+Na]^+$; HRMS calcd for $C_{55}H_{48}N_5O_9$ $[M+H]^+$: 922.3452, found: 922.345; elemental analysis calcd (%) for $C_{55}H_{47}N_5O_9 \cdot H_2O$ (940.0): C 70.28, H 5.25, N 7.45; found: C 69.68, H 4.88, N 7.37.

4-Benzyloxy-2,6-bis[bis(*N*-*tert*-butoxycarbonyl)amino]pyridine(7): 4-Dimethylaminopyridine (298 mg, 2.45 mmol) and a solution of di-*tert*-butyl dicarbonate (6.7 g, 30.6 mmol) in acetonitrile (10 mL) was added to a solution of 4-benzyloxy-2,6-diaminopyridine (1.315 g, 6.12 mmol) in a mixture of acetonitrile (25 mL) and dichloromethane (25 mL). The resulting reaction mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (dichloromethane+1% methanol to dichloromethane+5% methanol) to give **7** as colourless solid (3.42 g, 91%). M.p. 148 °C; IR (CH_2Cl_2): $\tilde{\nu} = 2982, 1783, 1770, 1735, 1714, 1601, 1572, 1368, 1306, 1251, 1153, 1101, 1002, 848, 736\,cm^{-1}$; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.41$ (m, 5H, Ph), 6.81 (s, 2H, PyrH), 5.13 (s, 2H, CH_2), 1.43 (s, 36H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 167.76 (0), 152.51 (0), 151.32 (0), 135.76 (0), 129.21 (1), 128.91 (1), 127.94 (1), 106.97 (1), 83.51(0), 70.78 (2), 28.26 (3); MS (ES$^+$): *m*/*z* (%): 616.3 (100) $[M+H]^+$, 638.2 (99) $[M+Na]^+$, 654.2 (24) $[M+K]^+$; HRMS calcd for $C_{32}H_{45}N_3O_9$ $[M+H]^+$: 615.31558, found: 615.31776.

Benzyl 2-{[2,6-bis(bis(*N*-*tert*-butoxycarbonyl)amino)]-4-pyridyloxy} acetate (8): 10% Palladium on charcoal (450 mg, 0.43 mmol Pd) was added to a solution of **7** (2623 mg, 4.27 mmol) in a mixture of ethanol (20 mL) and dichloromethane (10 mL). The reaction mixture was stirred vigorously for 18 h at room temperature under a hydrogen atmosphere. The catalyst was separated by filtration through a plug of celite. Evaporation of the solvent and drying of the residue at high vacuum yielded the corresponding pyridone as a colourless solid (2.24 g, 99%). M.p. >230 °C; IR (CH_2Cl_2): $\tilde{\nu} = 2979, 1773, 1749, 1728, 1715, 1613, 1367, 1308, 1276, 1247, 1153, 1116, 854, 778, 738\,cm^{-1}$; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.42$ (brs, 1H, NH), 6.68 (brs, 2H, CH), 1.45 (s, 36H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 166.50 (0), 151.80 (0), 151.28 (0), 108.68 (br, 1), 83.45 (br, 0), 27.82 (3); MS (ES$^+$): *m*/*z* (%): 526 (54) $[M+H]^+$, 548 (100) $[M+Na]^+$, 564 (18) $[M+K]^+$; HRMS calcd for $C_{25}H_{39}N_3O_9$ $[M+H]^+$: 525.26863, found: 525.26675.

Benzyl bromoacetate (1.12 g, 4.9 mmol) was added to a suspension of the pyridone (2.24 mg, 4.26 mmol), potassium carbonate (2.47 g, 17.9 mmol) and tetrabutylammonium bromide (1.37 g, 4.26 mmol) in DMSO (30 mL). The resulting reaction mixture was stirred for 18 h at room temperature and then partitioned between diethyl ether (200 mL) and water (200 mL). After separation of the organic layer, the aqueous layer was extracted with diethyl ether (200 mL). The combined organic layers were washed with water (2 × 200 mL) and dried over magnesium sulfate. Evaporation of the solvent and drying of the residue at high vacuum yielded **9** (2.39 g, 99%) as a colourless solid. M.p. 114–117 °C; IR (CH_2Cl_2): $\tilde{\nu} = 2982, 1782, 1753, 1740, 1707, 1600, 1395, 1366, 1284, 1251, 1152, 950, 850\,cm^{-1}$; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.38$ (m, 5H, Ph), 6.77 (s, 2H, PyrH), 5.25 (s, 2H, OCH_2CO_2), 4.71 (s, 2H, OCH_2Ph), 1.44 (s, 36H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 167.24 (0), 166.34 (0), 152.11 (0), 150.75 (0), 134.78 (0), 128.74 (1), 128.56 (1), 128.50 (1), 105.76 (1), 83.20 (0), 67.39 (2), 65.11 (2), 27.83 (3); MS (ES$^+$): *m*/*z* (%): 675 (100) $[M+H]^+$, 697 (85) $[M+Na]^+$, 1370 (20) $[2M+Na]^+$; HRMS calcd for $C_{34}H_{47}N_3O_{11}$ $[M+H]^+$: 673.32106, found: 673.32052.

Synthesis of tweezer receptor library 12: A solution of *N*-Aloc-phenylalanine (8.25 mg, 33 μmol) and HOBt (22 mg, 0.17 mmol) in dichloromethane (25 mL) was added to TentaGel S NH_2 resin (1.00 g, 0.33 mmol NH_2) followed by DIC (26 μL, 0.17 mmol) and DMAP (10 mg, 82 μmol). The suspension was shaken by a nitrogen stream for 3 h. A solution of Rink amide linker (178 mg, 0.33 mmol) and HOBt (223 mg, 1.65 mmol) in dichloromethane (25 mL) was added to the resin followed by DIC (260 μL, 1.65 mmol) and DMAP (10 mg, 82 μmol) and the suspension was shaken by a nitrogen stream for 3 h. Subsequent Fmoc deprotection was achieved with a solution of 20% piperidine in DMF. A solution of **9** (275 mg, 0.3 mmol) and HOBt (203 mg, 1.5 mmol) in dichloromethane (25 mL) was added to the resin followed by DIC (235 μL, 1.5 mmol) and DMAP (10 mg, 82 μmol) and the suspension was shaken by a nitrogen stream for 3 h. This coupling cycle was repeated to give a negative ninhydrin test. The resin was suspended in a mixture of THF/methanol (1:1, 40 mL) and shaken by an argon stream. A degassed solution of $Pd(PPh_3)_4$ (190 mg, 0.17 mmol) and dimedone (462 mg, 3.3 mmol) was added to the resin suspension and mechanical shaking was continued for 15 h shielding the reaction vessel from strong light. The reagents were washed off with a solution of diethyldithiocarbamic acid sodium salt (0.5 g) and DIPEA (0.5 mL) in DMF (100 mL). The Fmoc groups were removed with 20% piperidine in DMF. The resulting resin was divided in 13 equal portions. To each resin portion one of the following Fmoc-amino acids was added: Gly, Ala, Val, Phe, Leu, Lys(Boc), Pro, Glu(O*t*Bu), Ser(*t*Bu), Met, Trp, Asn, Gln (average mass: 371 g mol^{-1}, two amino sites per diamidopyridine unit: 0.66 mmol NH_2 per g resin = 50 mg amino acid per resin portion), along with HOBt (70 mg, 0.66 mmol), DIC (50 μL, 0.66 mmol) and DMAP (1 mg) in dichloromethane (3 mL). The reaction mixtures were shaken for 15 h. Ninhydrin tests were carried out to check all transformations were complete. The resin was mixed and the terminal Fmoc groups removed with 20% piperidine in DMF. The resin was split again into 13 equal portions and the procedure repeated twice in order to build up the tweezer receptor library **12**. After a final Fmoc deprotection with 20% piperidine in DMF a sublibrary **13** with free amino terminus was obtained.

Preparation of tweezer receptor 24: A solution of **9** (690 mg, 0.75 mmol) and HOBt (101 mg, 0.75 mmol) in dichloromethane (5 mL) was added to

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pre-swollen Sieber amide resin (250 mg, 0.15 mmol NH_2) followed by DIC (117 μL, 0.75 mmol) and DMAP (10 mg). The suspension was shaken for 3 h at room temperature. Removal of the Fmoc groups with 20% piperidine in DMF and subsequent coupling of the Fmoc-protected amino acids valine, leucine and tryptophan, using the same methods and conditions as described for the library synthesis above, yielded the resin-bound receptor **24**. Cleavage from resin was achieved by treatment of the resin with a solution of 2% TFA in dichloromethane (50 mL) for 15 h. Removal of solvent and purification by column chromatography on silica gel (dichloromethane+1% methanol to dichloromethane+5% methanol) gave tweezer receptor **24** (44 mg, 17%) as a white solid. M.p. 210–212 °C; IR (KBr): $\tilde{\nu}$ = 3447, 2365, 1684, 1617, 1576, 1509 cm^{-1}; ^1H NMR (300 MHz, [D_6]DMSO): δ = 10.80 (s, 2H, NH_2), 10.19 (s, 2H, NH(Trp)), 8.30–6.90 (m, 48H, ArH, NH), 4.85 (m, 2H, COC*H*NH), 4.46 (s, 2H, OCH_2CO), 4.32 (m, 4H, OCH_2CH), 4.17 (t, J = 8, 2H, OCH_2CH), 4.15–4.05 (m, 6H, COC*H*NH), 3.10–2.80 (m, 8H, CH_2Ar), 1.92 (m, 2H, $CHCH(CH_3)_2$), 1.60–1.40 (m, 6H, $CH_2CH(CH_3)_2$), 0.90–0.75 (m, 24H, CH_3); ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 171.9 (0), 171.6 (0), 171.0 (0), 170.8 (0), 168.7 (0), 166.7 (0), 155.8 (0), 151.1 (0), 143.7 (0), 140.6 (0), 137.2 (0), 136.1 (0), 129.1 (0), 128.0 (1), 127.6 (1), 127.3 (1), 127.1 (1), 126.3 (1), 125.3 (1), 123.8 (1), 120.8 (1), 120.0 (1), 118.6 (1), 118.2 (1), 111.3 (1), 110.3 (0), 96.0 (1), 66.3 (2), 65.7 (2), 57.3 (1), 55.4 (1), 54.3 (1), 51.1 (1), 46.6 (1), 40.5 (2), 37.2 (2), 30.9 (1), 27.7 (2), 24.1 (1), 23.0 (3), 21.7 (3), 19.1 (3), 17.9 (3); MS (ES^+): m/z (%): 1718 (10) $[M+H]^+$, 1741 (10) $[M+Na]^+$.

Preparation of tweezer receptor 25: A solution of **6** (80 mg, 0.12 mmol) and PyBOP (61 mg, 0.12 mmol) in dichloromethane (4 mL) was added to pre-swollen oxime resin (200 mg, 0.12 mmol NH_2) followed by DIPEA (41 μL, 0.24 mmol). The suspension was agitated on a tube rotator for 48 h, drained and then washed with dichloromethane (3 × 10 mL), DMF (3 × 10 mL), and dichloromethane (3 × 10 mL). In order to cap remaining free amino functions the resin was agitated on a tube rotator for further 18 h after addition of a solution of acetic anhydride (223 μL, 2.36 mmol) in dichloromethane (5 mL). Subsequent washing with dichloromethane (3 × 10 mL), DMF (3 × 10 mL), and dichloromethane (3 × 10 mL) and drying yielded a resin which gave a negative ninhydrin test. Removal of the Boc groups was achieved by adding a solution of 25% TFA in dichloromethane and agitating for 2 h. Subsequent washing with dichloromethane (3 × 10 mL), DMF (3 × 10 mL), methanol (3 × 10 mL), and dichloromethane (3 × 10 mL) and drying yielded a resin which gave a positive ninhydrin test. A solution of BocVal (51 mg, 0.24 mmol), TBTU (76 mg, 0.24 mmol), HOBt (36 mg, 0.24 mmol), and DIPEA (93 μL, 0.53 mmol) in DMF (5 mL) was added to the pre-swollen resin and the resulting suspension was agitated on a tube rotator for 18 h. After washing with dichloromethane (3 × 10 mL), DMF (3 × 10 mL), and dichloromethane (3 × 10 mL) the coupling cycle was repeated to yield a resin which gave a negative ninhydrin test. Subsequent Boc removal as described above followed by coupling cycles using Boc-Ala-OH and Boc-Pro in alteration with Boc deprotection, each coupling and deprotection step monitored by ninhydrin tests, yielded the resin-bound tweezer receptor **25** with Boc-protected N-terminus. Cleavage from solid support was achieved by agitating the pre-swollen resin with a solution of dodecylamine (0.5 M) in chloroform. The resin beads were filtered off and washed with dichloromethane (3 × 10 mL) and methanol (3 × 10 mL). Concentration of the filtrate under reduced pressure yielded an yellowish oil (166 mg). Further purification by column chromatography on silica (dichloromethane+10% methanol) produced a colourless film (25 mg, 15%). Characterisation was achieved after purification by semi-preparative reverse-phase HPLC (Phenomenex Prodigy ODS(3) C-18, 250 × 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. Under these conditions receptor **25** eluted after 45.93 min. ^1H NMR (300 MHz, $CDCl_3$) (poorly resolved due to Boc-proline rotamers): δ = 7.70–6.95 (m, 21H, ArH, NH), 4.85–3.85 (m, 10H, COC*H*NH, OCH_2CO), 3.45–2.90 (m, 10H, CH_2Ph, $CONHCH_2$, CH_2NBoc), 2.20–1.10 (m, 54H), 0.80–0.60 (m, 15H, CH_2CH_3, $CH(CH_3)_2$); MS (ES^+): m/z (%): 1379 (8) $[M+H]^+$, 1401 (8) $[M+Na]^+$.

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