Simple Structural Requirements for Sequence-Selective Peptide Receptors? Tripeptide Binding by a Podand Ionophore

Matthew T. Burger and W. Clark Still*

Department of Chemistry, Columbia University, New York, New York 10027

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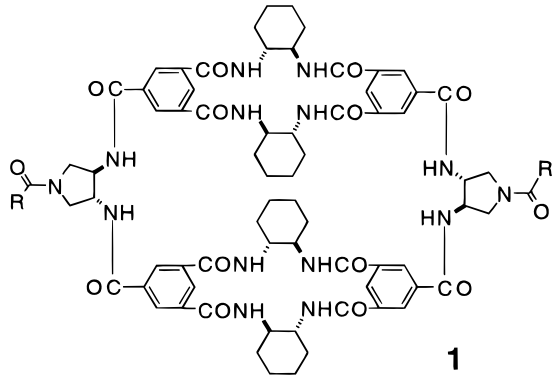
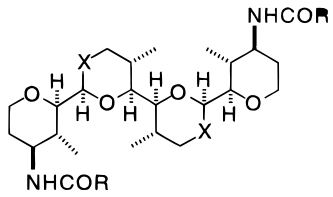
A simple, dye(R)-labeled and conformationally restricted ionophore **2** was prepared and screened for peptide binding using solid-supported combinatorial libraries of 24 389 protected and unprotected tripeptides. The ionophore was found generally to bind unhindered cationic peptides having arginines or N-terminal glycines. The podand was particularly selective in the case of unprotected tripeptides and was able to selectively bind a single tripeptide (L-Arg-L-Phe-D-Asp) from the unprotected ~24000-member tripeptide library. These results indicate that relatively simple organic molecules can make highly sequence-selective receptors for tripeptides. Structural features of such receptors are discussed.

One of the most exciting recent findings in synthetic receptor chemistry is that small organic molecules can bind oligopeptides sequence-selectively.[1,2] Though a few such receptors are flexible acyclics,[2] most have more typical host-like structures of which **1**[3] from this laboratory is a typical example. More generally, though, many of the most sequence-selective of these peptide-binding molecules are chiral and characterized by a conformationally restricted molecular surface (typically large and concave) that is studded with a well-defined pattern of polar functional groups for making specific electrostatic bonds (hydrogen bonds, ion pairing) to peptidic guests. Given the simplicity of this description, many chiral host-like molecules might be expected to have sequence-selective peptide-binding properties. In this paper, we study the oligopeptide-binding properties of **2** (X = SO_2), a podand ionophore[4] that was designed to bind chiral ammonium ions enantioselectively but that also seems to fit the above structural description of a sequence-selective peptide receptor (except perhaps for the large surface descriptor). As we will show, this small molecule not only binds cationic peptides sequence-selectively but is able to select a single tripeptide sequence from a 24000-member tripeptide library.

The podand ionophores we have studied here are built of linked tetrahydropyran (THP) rings.[4a] Through a particular array of chiral centers and methyl substituents, we are able to construct a conformational locking mechanism that sterically disfavors all conformations other than the desired one shown in Figure 1 in stereo for a simplified version of **2** (X = CH_2, NHCOR = H). This conformation fixes the relative positions of the four ring oxygens in an ideal geometry for binding an ammonium cation in the concave center of the podand. While this conformationally restricted molecule thus has four ether functional groups, all four ethers are involved in binding the same ammonium ion and additional functional groups are required for selectively binding polyfunctional guests such as peptides. The molecule we decided to study here was **2** (X = SO_2) which combined the conformationally locked podand ring system just described with sulfone and amide functional groups.[4b−d] These groups had other advantages: the SO_2 groups (X) simplified the podand synthesis by allowing a convergent route via the corresponding hemithioacetal and the amides provided convenient attachment points for the dye (R) that is used in our peptide-binding assay.



1



2

Podand **2** (X = SO_2) was synthesized in a manner analogous to that employed for a previous podand sulfone[4d] where the tetracyclic system was assembled by fusing the two identical outer rings to an acyclic intermediate via double thioacetalization of known dihydroxy dithiol **12**[4d] with aldehyde **11** with as shown in Scheme 1. Aldehyde **11** was prepared in seven steps from D-

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(1) Review: Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155 and references therein. See also: Albert, J. S.; Goodman, M. S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1995**, *117*, 1143. Waymark, C. P.; Kilburn, J. D.; Gillies, I. *Tetrahedron Lett.* **1995**, *17*, 3051. Maletic, M.; Wennemers, H.; McDonald, D. Q.; Breslow, R.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1490. Cheng, Y.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1813. Shao, Y.; Still, W. C. *J. Org. Chem.* **1996**, *61*, 6086.

(2) LaBrenz, S. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 3887. Gennari, C.; Nestler, H. P.; Salom, B.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1765. Lowik, D. W. P. M.; Mulders, S. J. E.; Cheng, Y.; Shao, Y.; Liskamp, R. M. J. *Tetrahedron Lett.* **1996**, *34*, 8253.

(3) Yoon, S. S.; Still, W. C. *Tetrahedron* **1995**, *51*, 567.

(4) (a) Wang, X.; Erickson, S. D.; Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* **1992**, *114*, 4128. (b) Armstrong, A.; Still, W. C. *J. Org. Chem.* **1992**, *57*, 4580. (c) Burger, M. T.; Armstrong, A.; Guarnieri, F.; McDonald, D. Q.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 3573. (d) Li, G.; Still, W. C. *J. Org. Chem.* **1991**, *56*, 6964.

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(6) Reetz, M. T.; Gansauer, A. *Tetrahedron* **1993**, *49*, 6025.

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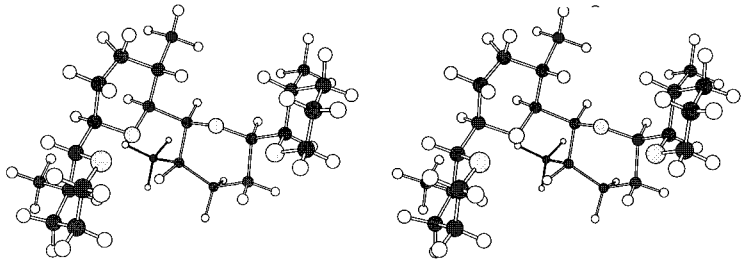
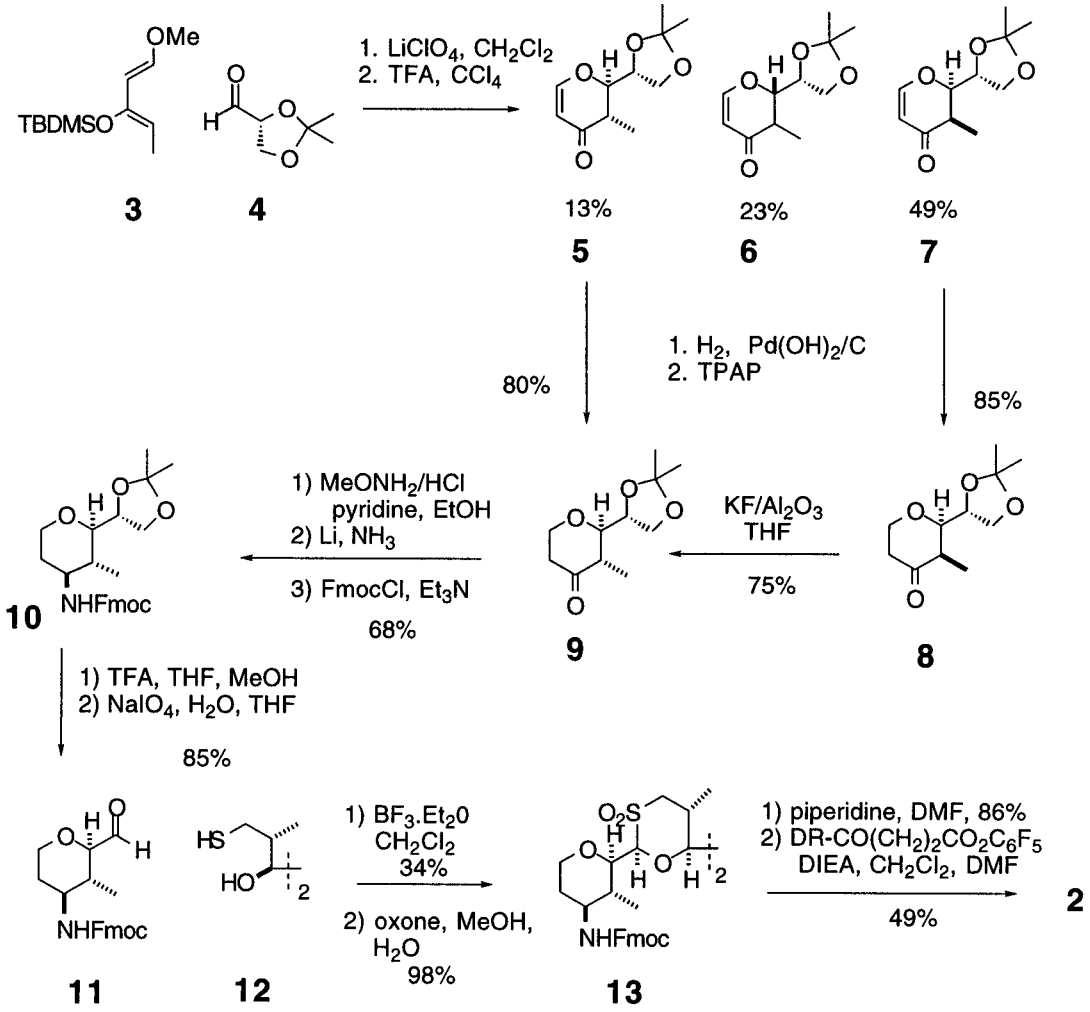


Figure 1.

Scheme 1. Synthesis of Dye-Labeled Podand 2 (X = SO_2)



glyceraldehyde acetonide (**4**).[5] A $LiClO_4$-catalyzed[6] Diels–Alder reaction of **4** with diene **3**[7] produced enones **5**, **6**, and **7** in 13%, 23%, and 49% yields, respectively. The roughly 3:1 ratio for the desired chelation-controlled adducts (**5**, **7**) to nonchelation-controlled adducts (**6**) is of note since **4** has been previously noted to be resistant to Diels–Alder reaction via a chelation-controlled transition state with $MgBr_2$ catalysis.[8] When we used $ZnCl_2$ (reported to favor nonchelation control) with **3** and **4**, the nonchelation-controlled products (**6**) were indeed favored to the extent of 6:1.[9] The stereochemistry of the major adduct (**7**) was confirmed by single-crystal X-ray crystallography. The two chelation-controlled enones could both be converted to the desired ketone **9** by direct reduction of **5**, and by reduction followed by KF/Al_2O_3-mediated epimerization[7] in the case of **7**. The *trans* arrangement of the six-membered ring substituents in **9** follows from observation of a diaxial coupling constant ($J_{4,5}$ = 10 Hz) for the associated THP methine hydrogens.

Conversion of ketone **9** to the methyl oxime and dissolving metal reduction yielded the corresponding equatorial amine. The *trans,trans* stereochemistry of the three ring substituents followed precedent[4b] and was supported by a diaxial H_3-H_4 coupling ($J_{3,4}$ = 12 Hz) and NOE signals between diaxial H_3-H_1 and H_3-H_5 in the NMR of the corresponding diacetamide. Fmoc protection led to **10** which was deketalized and cleaved with periodate to yield aldehyde **11**. The tetracyclic system was then assembled as were previous sulfonopodands by double hemithioketalization with **12**.[4d] The new stereo-

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(8) Danishefsky, S. J.; Pearson, W. H.; Harvey, D. F.; Maring, C. J.; Springer, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 1256.
(9) Danishefsky, S. J.; Kobayashi, S.; Kerwin, J. F. *J. Org. Chem.* **1982**, *47*, 1981.

centers followed precedent to yield a symmetrical molecule (by ^{1}H and ^{13}C NMR) showing NOE's from the hemithioacetal methines to the other axial α-protons on the central rings. Finally, oxidation with Oxone and replacement of the Fmoc protecting group with a succinate-linked red azo dye (Disperse Red 1) gave dye-labeled (red) **2** (R = $(CH_2)_2CO$-Disperse Red 1).

The peptide-binding properties of **2** were determined using a previously described solid phase parallel binding assay.[1] This assay involved equilibrating red **2** with a bead-supported library of 24 389 (29^3) different tripeptides in $CHCl_3$ containing 1% HOAc to insure protonation of all amines.[10] The library was prepared by encoded split synthesis,[11] and thus each bead should carry a single tripeptide sequence. Library beads that significantly bound **2** at its equilibrium concentration in the assay accumulated the red color of the dye label and could be manually picked under a low-power microscope and decoded by ECGC to reveal the structure of the bound peptide. Our tripeptide library was prepared on poly(ethylene glycol)/polystyrene copolymer beads (TentaGel, functional group loading ~0.3 mmol/g of dry beads)[12] and had the following form (AA3 is the N-terminal amino acid):

H-AA3-AA2-AA1-NHCH$_2$CH$_2$O-TentaGel

where AA*n* represents 29 different amino acids.[13] The library was screened in two forms: one with the amino acid side chains protected (the *protected library*) and one (after TFA deprotection) with the side chains free (the *deprotected library*).

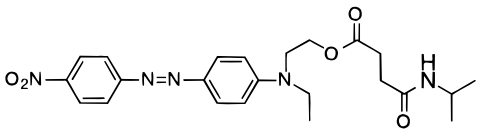
We first screened the protected library for side-chain-protected tripeptides that bound **2**. Thus we equilibrated 1.5 mL of **2** (initial concentration 230 μM) in 1% HOAc/$CHCl_3$ with 100 mg (~110 000 beads) of the protected library on a wrist-action shaker. After 24 h, <<1% (1 out of every ~2500) of the beads had picked up a bright red coloration while the vast majority of the beads remained colorless. This small percentage of red beads indicated highly selective peptide binding by **2**. The 39 reddest beads were picked and decoded to give the sequences of the bound peptides summarized in Table 1.

The strongest binding trend for **2** is its high selectivity for peptides with a glycine (Gly) N-terminal residue. Thus 90% of the red beads picked had AA3 = Gly (the other red beads had AA3 = Arg[14]). This Gly selectivity likely reflects the steric requirement for binding the ammonium ion in the electron rich but sterically hindered tetracyclic podand core. Thus the AA3 selectivity follows from the uniquely unhindered nature of the glycine residue. A similar preference for unhindered primary ammonium guests (e.g. $PhCH_2NH_3^+$) has been observed previously with simple podands.[4a] While AA3 = Gly is strongly selected, other amino acids at AA2 and AA1 also play a significant role as indicated by the fact that while 3.4% of the library has AA3 = Gly, **2** selected only 0.04% of the beads. In particular, **2** shows a significant preference for the N-terminal Gly to be followed by a D amino acid at AA2 and an L amino acid at AA1. This selectivity beyond AA3 cannot be readily explained by the structure of the podand core, and it is likely that AA2 and AA1 are folding back to make favorable contacts with more external podand functionality. Indeed, alternating D(AA2)-L(AA1) chiralities in peptides are known[15] to promote turn-like conformations that might favor podand-AA1 proximity and association. It is also possible that functional groups on the dye-label may play a role in binding AA2 or AA1 as peptide binding by dyes is well known (at least in water).[16] However, peptide−dye associations are not the sole determinants in the tripeptide binding of **2** as we could find no evidence of library peptide binding with the simple dye-linker **14** at concentrations as high as 2.5 mM.

Table 1. Binding of 2 to Side Chain-Protected Tripeptides

$(H_3N^+)AA_3$	AA_2	AA_1	found[a]	expected[b]
Gly	X[c]	X[c]	90%	3.4%
Gly	D-amino acid	X[c]	69%	1.7%
Gly	D-Ser(*O*-tBu)	X[c]	15%	0.1%
Gly	D-His(*N*-Trityl)	X[c]	21%	0.1%
Gly	D-amino acid	L-amino acid	44%	0.8%

[a] Percentage of red beads having the indicated amino acid sequence. [b] Percentage of beads having the indicated amino acid sequence expected from random bead picking. [c] X indicates any amino acid.



14

We also assayed **2** for binding of side-chain-deprotected tripeptides. Because preliminary experiments indicated exceptionally high peptide-binding selectivity, we equilibrated **2** (initially 140 μM) with ~13 copies of our deprotected library (~320 000 beads) under the same conditions described for the protected library. After 36 h of equilibration, we found only 20 red beads. Because our assay employed a number of beads corresponding to ~13 copies of the library, the selection for 20 beads implied that **2** was binding only 1−2 sequences out of the 24 389 possible sequences in the library. In fact,

(10) Actually, 24 389 is the maximum possible number of tripeptides in our library. In fact, the actual number of peptides we make and screen may be less than this due to library defects and sampling statistics. *Library Defects*. While solid phase peptide synthesis chemistry is very good, it is not perfect and some sequences may be present in significantly lower yields than others or even absent. *Sampling Statistics*. In any finite, random sample of a library (e.g. those we screen), probability dictates that some sequences will be overrepresented and others will be underrepresented (and in the extreme may even be absent). Thus in the screening assays we use here, any presence of binding is a significant result but any particular absence of binding may not be. We minimize such problems by using multiple couplings to drive each peptide coupling step to completion (as indicated by a negative Kaiser test) and by screening samples of the library containing ≥3 times as many beads as sequences (3 times should include >95% of all sequences in the library according to Poisson statistics).

(11) Ohlmeyer, M. J.; Swanson, R. J.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayahi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922.

(12) TentaGel S from Rapp Polymere. Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 113.

(13) AA*n* = Gly, D-Ala, L-Ala, D-Val, L-Val, D-Phe, L-Phe, D-Leu, L-Leu, D-Pro, L-Pro, D-Asp(*O*-tBu), L-Asp(*O*-tBu), D-Glu(*O*-tBu), L-Glu(*O*-tBu), D-Asn(*N*-Tr), L-Asn(*N*-Tr), D-Gln(*N*-Tr), L-Gln(*N*-Tr), D-His(*N*-Tr), L-His(*N*-Tr), D-Lys(*N*-Boc), L-Lys(*N*-Boc), D-Arg(*N*-Pmc), L-Arg(*N*-Pmc), D-Ser(*O*-tBu), L-Ser(*O*-tBu), D-Thr(*O*-tBu), L-Thr(*O*-tBu).

(14) It is possible that some of the acid-sensitive side chain-protecting groups were lost during the day-long assays in acidic (HOAc) chloroform. This may explain the minor selectivity trend for AA3 = Arg as it will be shown that the major selectivity with the deprotected library also involves AA3 = Arg. For Arg(*N*-Pmc) protection data, see: Ramage, R.; Green, J. *Tetrahedron Lett.* **1987**, *28*, 2287.

(15) Chalmers, D. K.; Marshall, G. R. *J. Am. Chem. Soc.* **1995**, *117*, 5927 and references therein.

(16) Wennemers, H.; Still, W. C. *Tetrahedron Lett.* **1994**, *35*, 6417.

Table 2. Binding of 2 to Side Chain-Deprotected Tripeptides

$(H_3N^+)AA_3$	AA_2	AA_1	found[a]	expected[b]
L-Arg	L-Phe	D-Asp	65%[d]	0.004%
L-Asn	D-amino acid	D,L-Arg	10%[e]	0.1%
Gly	D-amino acid	X[c]	20%[e]	1.7%

[a] Percentage of red beads having the indicated amino acid sequence. [b] Percentage of beads having the indicated amino acid sequence expected from random bead picking. [c] X indicates any amino acid. [d] This complete sequence was found 13 times in an assay with ~320 000 beads (~13 copies of the library). [e] No complete tripeptide sequence was duplicated.

decoding these 20 beads revealed that only one sequence was strongly preferred. That sequence was L-Arg-L-Phe-D-Asp, and it was found in 13 of the 20 red beads. The other seven beads carried various other sequences containing either AA3 = Gly (as found in the protected library) or AA3 or AA1 = Arg, but none of these sequences was ever duplicated (see Table 2).

While further structural studies would be needed to elucidate the origin of the striking preference for the L-Arg-L-Phe-D-Asp tripeptide sequence, the main shift from the protected library result of a preference for AA3 = Gly to the deprotected library result of AA3 = Arg likely follows from a preference of **2** for binding guanidinium ions over ammonium ions. Indeed, 80% of the red beads found in the deprotected library assay carried Arg-containing peptides (the remainder had AA3 = Gly).

While the fact that 13 of the 20 red beads carried the same tripeptide sequence shows that **2** is highly selective for a single tripeptide, the presence of seven other red beads having no sequence duplication deserves comment. In an ideal world where all beads carrying a given peptide are identical, each binding sequence would be replicated 13 times (on average) because we screened a sample of beads corresponding to 13 copies of the library. However, individual beads are actually not identical to one another and may have different loadings of tripeptide, differing compositions of the copolymer matrix, different extents of polymer cross-linking, bead swellability, etc. Each of these differences has an effect on binding strength in a solid phase assay like ours. Consequently, a set of beads bearing the same peptide sequence will not all bind a receptor to exactly the same extent. At certain receptor concentrations, these variations in binding affinities can make tightly binding bead-supported sequences be found more heavily replicated in an assay than are more weakly binding sequences. Thus if a solid phase assay is carried out at a given receptor concentration, a large proportion of supported sequences with (average) k_d's less than that concentration can bind the receptor while a small proportion of sequences with (average) k_d's greater than that concentration can also bind.

Finally, there are issues about the relationship of solid phase assays like ours to binding in free solution. In particular, it is possible that the support matrix (poly(ethylene glycol)/polystyrene) or the presence of multiple peptide molecules on the support is playing a roll in the binding we observe. While the former issue can be considered as a solvent effect for thermodynamically controlled binding, receptor binding of more than one peptide molecule on a solid support (the *average* concentration of peptide in a solvent-swollen bead is ~0.1 M) would be expected to differ from binding in a dilute solution. Indeed, experiments with large biological receptors and solid phase supported ligands often show considerable differences from the corresponding studies using ligands in free solution.[17] With small molecule receptors, however, our experience with peptide libraries in organic solvents is that solid phase selectivities correspond to solution phase selectivities though binding constants tend to be somewhat larger in dilute solution. Indeed, this has been the finding with every receptor we have examined in both media.[18] Nevertheless, it is possible that some of the results described above follow from the solid phase nature of our binding assays and if one is primarily interested in solution phase binding, then it is important to extend solid phase screening results to solution phase binding experiments. On the other hand, the ultimate goal of studies such as ours may be an application (e.g. in separations or chemical sensing) in which solid phase experiments are really the most relevant.

Regardless of the molecular details of the binding we have observed, our results show that small, relatively simple host molecules can bind peptides with surprisingly high sequence selectivity. While **2**'s selectivity for N-terminal glycine-containing peptides appears to be simply based on the steric requirements of the cationic binding cavity, associative interactions from outside the cavity must also be playing a very significant role in binding as residues far from strongly bound cationic sites are also strongly selected. It is likely that cognate peptides are ones having low-energy conformations with a characteristic shape and pattern of polar functionality that is recognized by a complementary surface of the receptor. Thus it is possible for a receptor to selectively recognize a complex substrate of comparable or even greater size if that substrate can fold to form a complementary molecular surface. Further work is needed to learn just how this occurs with **2** and L-Arg-L-Phe-D-Asp, but it is clear that relatively small molecules can make remarkably effective hosts for peptides.

Experimental Section

Diels−Alder Adducts 5−7. To 1.00 g of (*R*)-glyceraldehyde acetonide (**4**, 7.70 mmol, 1.0 equiv) and 1.31 g of $LiClO_4$ (12.3 mmol, 1.6 equiv) in 28 mL of CH_2Cl_2 under argon was added 3.50 g of diene **3**[7] in 20 mL of CH_2Cl_2. After 8 h of stirring, Et_2O (500 mL) and saturated aqueous $NaHCO_3$ (400 mL) were added. Upon separation, the aqueous layer was extracted with Et_2O (500 mL). The combined organic extracts were dried with $MgSO_4$. The crude product was dissolved in CCl_4 (100 mL), and 0.40 mL of trifluoroacetic acid (TFA) was added. After 4 min of stirring, CH_2Cl_2 (500 mL) was added and saturated aqueous $NaHCO_3$ (250 mL) was added in small portions to neutralize the acid. Separation followed by extraction with CH_2Cl_2 (500 mL), drying over $MgSO_4$, concentration, and flash chromatography on silica gel (35% ether in petroleum ether) yielded 437 mg of nonchelation-controlled enones (**6**, 23%), 213 mg of chelation-controlled α-methyl enone **5** (13%), and 805 mg of chelation-controlled β-methyl enone **7** (49%).

(17) For example: Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520.

(18) In published work, solid phase peptide binding results with small molecule receptors were compared with binding experiments in free solution: Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 373. Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7467. Carrasco, M. R.; Still, W. C. *Chem. Biol.* **1995**, *2*, 205. Maletic, M.; Wennemers, H.; McDonald, D. Q.; Breslow, R. C.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1490. In different systems, peptides on a solid phase binding to a small molecule receptor in solution were resynthesized and tested in solution for binding the receptor on a solid phase: Gasparrini, F.; Misiti, D.; Villani, C.; Borchardt, A.; Burger, M. T.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 4314. Chen, C.-T.; Wagner, H.; Still, W. C. *Science* **1997**, submitted.

5: R_f 0.22 (50:50 diethyl ether:petroleum ether, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 6.0 Hz, 1H), 5.39 (d, J = 6.0 Hz, 1H), 4.47−4.41 (m, 1H), 4.33 (dd, J = 8.0, 2.8 Hz, 1H), 4.13−4.09 (m, 1H), 3.67 (t, J = 7.6 Hz, 1H), 2.30 (m, 1H), 1.45 (s, 3H), 1.43 (s, 3H), 1.16 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.41, 162.29, 110.48, 105.76, 82.69, 74.86, 65.10, 41.84, 26.48, 25.61, 10.64; IR (neat) 1677.2, 1597.2 cm^{-1}; HRMS m/e calcd for $C_{11}H_{16}O_4$ 212.1049, found 212.1042.

7: R_f 0.29 (50:50 diethyl ether:petroleum ether, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 6.2 Hz, 1H), 5.41 (d, J = 6.2 Hz, 1H), 4.38 (td, J = 3.2, 6.8 Hz, 1H), 4.13−4.07 (m, 2H), 4.02 (dd, J = 12.0, 2.8 Hz, 1H), 2.81−2.76 (m, 1H), 1.46 (s, 3H), 1.39 (s, 3H), 1.21 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.48, 161.74, 109.75, 105.94, 82.46, 73.89, 64.63, 40.99, 25.96, 25.07, 10.04; IR (neat) 1678.0, 1602.8 cm^{-1}; HRMS m/e calcd for $C_{11}H_{16}O_4$ 212.1049, found 212.1047.

Tetrahydropyranone 8. 789 mg of **7** (3.72 mmol, 1.0 equiv) and 128 mg of $Pd(OH)_2$/C in 205 mL of EtOH were placed under a balloon of H_2. After 35 min of stirring, the mixture was poured onto a bed of Celite and eluted with Et_2O. All ceric ammonium nitrate (CAM) staining fractions were combined and concentrated. To the crude hydrogenation products were added 428 mg of *N*-methylmorpholine *N*-oxide (3.66 mmol, 0.98 equiv), 663 mg of 4 Å powdered molecular sieves, and 85 mL of CH_2Cl_2. After 5 min of stirring, 38 mg of tetrapropylammonium perruthenate (TPAP, 0.11 mmol, 0.03 equiv) was added. The reaction was stirred for 80 min, at which time it was applied directly to a 7 cm × 4 in. silica gel column, which after eluting with Et_2O, yielded 678 mg of **8**: R_f 0.50 (75:25 diethyl ether:petroleum ether, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.40−4.35 (m, 1H), 4.27 (dd, J = 7.6, 14.8 Hz, 1H), 4.04−4.00 (m, 1H), 3.78 (td, J = 3.6, 11.2 Hz, 1H), 3.66−3.60 (m, 1H), 2.80−2.72 (m, 1H), 2.40 (dd, J = 7.2, 4.0 Hz, 1H), 2.27 (bd, J = 14.8 Hz, 1H), 1.42 (s, 3H), 1.38 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.27, 109.39, 80.36, 75.38, 66.28, 64.58, 46.96, 38.40, 26.13, 25.05, 10.92; IR (neat) 1707.9 cm^{-1}; HRMS m/e calcd for $C_{11}H_{18}O_4$ 214.1205, found 214.12000.

Tetrahydropyranone 9. Via Reduction of 5. **5** (213 mg, 1.00 mmol, 1.0 equiv) and 34 mg of $Pd(OH)_2$/C in 55 mL of EtOH were placed under a balloon of H_2. After 30 min of stirring, the mixture was poured onto a bed of Celite and eluted with Et_2O. All CAM-staining fractions were combined and concentrated. To the crude hydrogenation products were added 112 mg of *N*-methylmorpholine *N*-oxide (0.96 mmol, 1.0 equiv), 330 mg of 4 Å powdered molecular sieves, and 5 mL of CH_2Cl_2. After 5 min of stirring, 11 mg of tetrapropylammonium perruthenate (0.03 mmol, 0.03 equiv) was added. The reaction was stirred for 40 min, at which time it was applied directly to a 5 × 5 cm silica gel column and eluted with 50% ether:petroleum ether to yield **9** (172 mg, 80%).

Via Epimerization of 8. **8** (294 mg, 1.40 mmol, 1.0 equiv) and 845 mg 40% KF/Al_2O_3 (8.9 mmol, 6.5 equiv) in 220 mL of THF were stirred at rt for 22 h. The reaction mixture was poured onto a bed of Celite and eluted with Et_2O. Chromatography (50% ether:petroleum ether) yielded 219 mg of **9** (75%): R_f 0.67 (75:25 diethyl ether:petroleum ether, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.40−4.38 (m, 1H), 4.26 (td, J = 2.4, 6.4 Hz, 1H), 4.09−4.00 (m, 2H), 3.64 (td, J = 2.8, 12.4 Hz, 1H), 3.21 (dd, J = 2.8, 10.4 Hz, 1H), 2.82−2.77 (m, 1H), 2.73−2.65 (m, 1H), 2.37 (bd, J = 13.2 Hz, 1H), 1.48 (s, 3H), 1.38 (s, 3H), 1.08 (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.56, 109.29, 82.06, 74.86, 66.93, 65.17, 47.25, 42.01, 25.96, 25.28, 8.90; IR (neat) 1714.5 cm^{-1}; HRMS m/e calcd for $C_{11}H_{19}O_4$ 215.1283, found 215.1278.

Fmoc Amine 10. Ketone **9** (400 mg, 1.87 mmol, 1.0 equiv), 438 mg of methoxylamine hydrochloride (5.24 mmol, 2.80 equiv) in 22 mL of pyridine, and 22 mL of EtOH was refluxed for 1 h. Upon cooling and concentration, Et_2O (500 mL) was added and the solution was washed with aqueous $CuSO_4$ (3 × 150 mL), dried over $MgSO_4$, and concentrated to yield 443 mg (98%) of oxime ethers, as a ∼7:1 mixture of isomers: R_f 0.82 (50:50 diethyl ether:petroleum ether, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.35−4.29 (m, 1H), 4.14−4.09 (m, 1H), 4.01 (t, J = 8.0 Hz, 1H), 3.95 (t, J = 7.6 Hz, 1H), 3.84 (s, 3H), 3.48 (td, J = 3.6 Hz, 11.6H), 3.13 (dd, J = 3.6, 8.8 Hz, 1H), 3.02 (dt, J = 3.6, 14.8 Hz, 1H), 2.58−2.54 (m, 1H), 2.24−2.16 (m, 1H), 1.45 (s, 3H), 1.37 (s, 3H), 1.15 (d, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.85, 109.25, 81.71, 74.68, 65.47, 65.43, 61.16, 37.76, 26.12, 25.70, 25.40, 11.90; IR (neat) 1637.8 cm^{-1}; HRMS m/e calcd for $C_{12}H_{21}NO_4$ 243.1470, found 243.1465.

To ∼50 mL of condensed NH_3 at −78 °C under argon was added 320 mg of Li wire (46.1 mmol, 24.3 equiv). To the resulting deep blue solution was added 461 mg of the above oxime ethers (1.90 mmol, 1.0 equiv) in 20 mL of anhydrous THF. The −78 °C bath was removed, and the solution was allowed to reflux for 1 h. After cooling back to −78 °C, MeOH (45 mL) was added cautiously to quench the reaction mixture. The solution was allowed to warm to rt. After concentrating, CH_2Cl_2 (500 mL) and H_2O (200 mL) were added. Extracting further with CH_2Cl_2 (2 × 200 mL), drying ($MgSO_4$), and concentration yielded the corresponding equatorial amine (372 mg, 91%).

To the crude amine (121 mg, 0.56 mmol, 1.0 equiv) in 4 mL of CH_2Cl_2 were added 133 μL of Et_3N (0.97 mmol, 1.7 equiv) and 248 mg of 9-fluorenylmethyl chloroformate (FmocCl, 0.97 mmol, 1.7 equiv) under argon. After 13 h of stirring, CH_2Cl_2 (60 mL) and H_2O (50 mL) were added. Further extraction with CH_2Cl_2 (2 × 50 mL), drying over $MgSO_4$, and flash chromatography yielded 188 mg of Fmoc-protected amine **10** (76%): R_f 0.21 (50:50 diethyl ether:petroleum ether, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.0, 27.58, d, 7.2 Hz, 2H), 7.40 (t, J = 7.2 Hz, 2H), 7.31 (t, J = 7.6 Hz, 2H), 4.58 (bd, J = 7.6 Hz, 1H), 4.51−4.46 (m, 1H), 4.40−4.36 (m, 1H), 4.24−4.20 (m, 1H), 4.06 (dd, J = 4.0, 12.0 Hz, 1H), 4.00−3.94 (m, 2H), 3.48−3.39 (m, 1H), 2.97 (dd, J = 2.0, 9.6 Hz, 1H), 1.89 (dd, J = 4.0, 12.8 Hz, 1H), 1.66−1.59 (m, 1H), 1.53−1.45 (m, 1H), 1.43 (s, 3H), 1.35 (s, 3H), 0.95 (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.20, 143.76, 141.18, 127.54, 126.90, 124.85, 119.86, 108.92, 80.61, 74.80, 66.54, 66.19, 65.15, 47.19, 38.65, 33.49, 29.15, 26.02, 25.35, 12.80; IR (neat) 3320.5, 1703.2 cm^{-1}; HRMS m/e calcd for $C_{26}H_{31}NO_5$ 437.2202, found 437.2202.

Tetrahydropyran Carboxaldehyde 11. To *N*-Fmoc amine **10** (186 mg, 0.43 mmol, 1.0 equiv) in 10 mL of 4:1 THF:H_2O was added 75 μL of TFA (0.44 mmol, 1.0 equiv). After 29 h of stirring, an additional 75 μL of TFA was added. After an additional 18 h, 2 mL of H_2O was added. After an additional 9 h of stirring, CH_2Cl_2 (150 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added. Phase separaton and further extraction with CH_2Cl_2 (3 × 50 mL), drying ($MgSO_4$), and silica gel chromatography (3−7% MeOH/CH_2Cl_2) yielded the deprotected diol (141 mg, 83%).

To the above diol (0.36 mmol, 1.0 equiv) in 10 mL of CH_2Cl_2 were added 0.45 mL of H_2O (24.9 mmol, 70.0 equiv) and 152 mg of $NaIO_4$ (0.72 mmol, 2.0 equiv). After 2 h of stirring, an additional 152 mg of $NaIO_4$ and 0.45 mL of H_2O were added. After an additional 24 h of stirring, 5 g of $MgSO_4$ was added. Concentration and silica gel chromatography (10−20% acetone/CH_2Cl_2) yielded carboxaldehyde **11** (110 mg, 85%): R_f 0.45 (7:93 methanol:methylene chloride, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.58 (s, 1H), 7.77 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 7.2 Hz, 2H), 7.4−7.39 (m, 2H), 7.34−7.30 (m, 2H), 4.61 (bs, 1H), 4.54−4.50 (m, 2H), 4.45−4.41 (m, 1H), 4.21 (t, J = 6.8 Hz, 1H), 4.11 (dd, J = 4.0, 11.6 Hz, 1H), 3.59 (3.49, m, 3H), 1.93 (bd, J = 12.8 Hz, 1H), 1.59−1.46 (m, 2H), 1.00 (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.30, 155.95, 143.76, 141.33, 127.70, 127.02, 124.85, 119.97, 85.24, 66.44, 66.33, 53.12, 47.20, 37.59, 33.05, 12.42; IR (neat) 3316.7, 1731.0, 1693.0 cm^{-1}; HRMS m/e calcd for $C_{22}H_{23}NO_4$ 365.1627, found 365.1618.

Fmoc Sulfonopodand 13. To 80.0 mg of aldehyde **11** (0.22 mmol, 2.5 equiv) and 18.4 mg of dihydroxy dithiol **12**[4d] (0.09 mmol, 1.0 equiv) in 1 mL of CH_2Cl_2 was added 42 μL of BF_3·Et_2O under argon. After 6 h of stirring, CH_2Cl_2 (55 mL) and saturated aqueous $NaHCO_3$ (55 mL) were added. Further extraction with CH_2Cl_2 (2 × 60 mL), drying over Na_2SO_4, silica gel chromatography (5−20% acetone/CH_2Cl_2), and size exclusion chromatography (LH-20, $CHCl_3$) yielded 26.7 mg of bishemithioacetal (34%): R_f 0.77 (20:80 acetone:methylene chloride, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.6 Hz, 4H), 7.61 (d, 7.4H, d), 7.42−7.39 (m, 4H), 7.34−7.31 (m,

4H), 4.94 (s, 2H), 4.60 (d, J = 8.8 Hz, 2H), 4.48−4.38 (m, 4H), 4.24 (t, J = 6.8 Hz, 2H), 4.11 (d, J = 9.6 Hz, 2H), 3.48−3.46 (m, 4H), 3.20 (t, J = 9.2 Hz, 4H), 2.80−2.69 (m, 4H), 2.19 (bs, 2H), 1.85 (bd, J = 10.0 Hz, 2H), 1.77−1.76 (m, 2H), 1.63−1.60 (m, 2H), 0.93 (d, J = 6.0 Hz, 6H), 0.88 (d, J = 6.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.08, 143.80, 141.31, 127.66, 127.01, 125.02, 119.97, 84.61, 84.20, 83.11, 67.07, 66.49, 53.52, 47.31, 37.83, 35.24, 33.22, 30.11, 17.53, 13.24; IR (KBr) 3329.3, 1718.5 cm^{-1}; HRMS m/e calcd for $C_{52}H_{61}N_2O_8S_2$ 905.3870, found 905.3852.

The above bis-hemithioacetal (27 mg, 0.03 mmol, 1.0 equiv) was oxidized in 2.6 mL of CH_3OH at 0 °C with 440 mg of Oxone (0.72 mmol, 24.0 equiv) as a slurry in 1.6 mL of H_2O. The solution was stirred for 24 h as it was allowed to warm to rt. Addition of CH_2Cl_2 (60 mL) and H_2O (35 mL), further extraction with CH_2Cl_2 (60 mL), and drying over Na_2SO_4 yielded 28 mg of Fmoc sulfonopodand **13** (98%): R_f 0.26 (20:80 acetone:methylene chloride, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 7.6 Hz, 4H), 7.61 (d, J = 7.2 Hz, 4H), 7.43−7.38 (m, 4H), 7.34 (7.30, m 4), 4.66 (d, J = 9.6 Hz, 2H), 4.49 (s, 2H), 4.44 (d, J = 6.4 Hz, 4H), 4.21 (t, J = 6.4 Hz, 2H), 4.14 (bd, J = 10.8 Hz, 2H), 3.82 (d, J =9.6 Hz, 2H), 3.53−3.48 (m, 6H), 3.24 (dd, J = 13.6, 3.6 Hz, 2H), 3.05 (bs, 2H), 2.86 (t, J = 12.4 Hz, 2H), 1.86 (bd, J = 10.0 Hz, 2H), 1.73−1.62 (m, 2H), 1.60−1.59 (m, 2H), 1.02 (d, J = 6.4 Hz, 6H), 0.92 (d, J = 6.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.06, 143.82, 141.35, 127.72, 127.05, 125.00, 119.99, 92.70, 83.37, 78.00, 67.09, 66.50, 57.25, 53.11, 47.31, 37.58, 32.86, 32.21, 16.79, 13.46; IR (KBr) 3397.6, 1718.2 cm^{-1}; HRMS m/e calcd for $C_{52}H_{61}N_2O_{12}S_2$ 969.3666, found 969.3644.

Disperse Red Sulfonopodand 2. The above Fmoc sulfonopodand **13** (28 mg) was deprotected by stirring in 20% piperidine in DMF for 3.5 h. After concentration, silica gel chromatography (15−20% 7 N NH_3/MeOH in CH_2Cl_2) yielded 13 mg (86%) of the corresponding amine that was immediately acylated with Disperse Red 1 succinate as follows.

To 5 mg (0.01 mmol, 1.0 equiv) of the above amine in 0.3 mL of CH_2Cl_2 was added 0.1 mL of DMF containing Disperse Red 1 succinate pentafluorphenyl ester[3] (22 mg, 0.04 mmol, 4.0 equiv) and diisopropylethylamine (7 μL, 0.4 mmol, 4.0 equiv). After 18 h of stirringand concentration, silica gel chromatography (0−5% MeOH/CH_2Cl_2) yielded red podand **2** (6.0 mg, 49%): R_f 0.21 (7:93 methanol:methylene chloride, silica gel); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35−8.31 (m, 4H), 7.94−7.87 (m, 8H), 6.79 (d, J = 9.2 Hz, 4H), 5.55 (d, J = 9.2 Hz, 4H), 4.46 (s, 2H), 4.33−4.27 (m, 4H), 4.04 (dd, J = 3.6, 11.6 Hz, 2H), 3.8−3.77 (m, 4H), 3.69 (t, J = 6.0 Hz, 4H), 3.56−3.44 (m, 8H), 3.22 (dd, J = 3.6, 13.6 Hz, 2H), 3.06−3.01 (m, 2H), 2.84 (t, J = 7.6 Hz, 2H), 2.79−2.61 (m, 4H), 2.47−2.44 (m, 4H), 1.79−1.72 (m, 4H), 1.64−1.52 (m, 2H), 1.25 (t, J = 7.2 Hz, 6H), 0.99 (d, J = 6.8 Hz, 6H), 0.88 (d, J = 6.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.89, 170.97, 156.67, 151.22, 147.69, 143.77, 126.22, 124.74, 122.69, 111.47, 92.81, 83.31, 78.07, 66.96, 61.54, 57.21, 51.00, 48.74, 45.76, 37.11, 32.39, 32.23, 30.81, 29.32, 16.78, 13.52, 12.29; IR (neat) 3367.0, 1734.4, 1654.3, 1599.6, 1516.0, 1339.2 cm^{-1}; HRMS m/e calcd for $C_{62}H_{81}N_{10}O_{18}S_2$ 1317.5170, found 1317.5210.

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