Cyclooligomeric Receptors Based on Trimesic Acid and 1,2-Diamines. Minimal Structure for Sequence-Selective Peptide Binding

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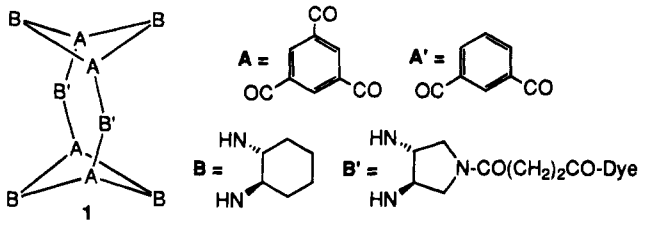
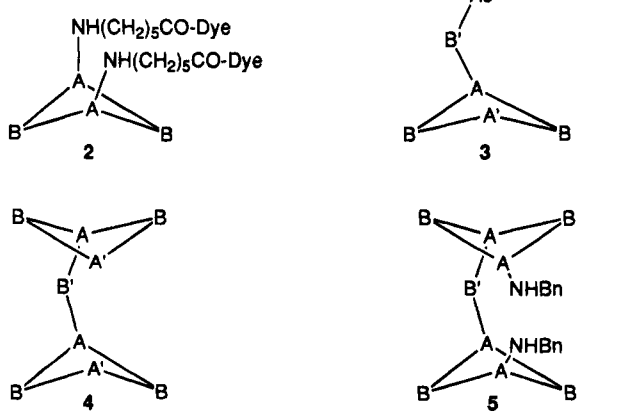
In recent reports from this laboratory, we have described a number of D_2-symmetric, macrotricyclic receptors (**1**) that are simple A_4B_6 cyclooligomers of trimesic acid ($A(OH)_3$) and 1,2-diamines (e.g., BH_2, $B'H_2$).[1] Such



receptors are easy to prepare and have been found to bind certain peptides in organic solvents with high enantioselectivity and sequence-selectivity. For example, **1** binds certain amino acid derivatives containing L-valine or L-phenylglycine residues with enantioselection exceeding 2 kcal/mol and displays a particular affinity for the side chain-protected tripeptide sequence D-Asn(*N*-Tr)-L-Val-L-Ser(O-tBu) in $CHCl_3$. Because **1** is among the most sequence-selective peptide receptors yet prepared by synthesis, we have carried out a study to determine the smallest, readily preparable substructure of **1** that retains selective peptide binding properties. Thus, we prepared and surveyed the peptide binding properties of the related macromonocycles **2** and **3** and macrobicycles **4** and **5**. As we will show, highly selective peptide binding properties are not limited to macrotricycles like **1**.



The dye-labeled receptor substructures **2**–**5** were prepared using methodology developed for the synthesis of **1**,[1c] and a detailed synthesis of **4** (dye = Disperse Red 1) is provided as supplementary material. To test each of these compounds for their general ability to bind peptidic substrates, we used our previously described solid phase color assay[2,3] employing a library of ~50 000 (maximally 15^4 = 50 625) acylated tripeptides prepared on polystyrene beads by encoded split synthesis.[4] Each member of the library was attached to a different bead and had the general structure

R(C=O)-AA3-AA2-AA1-NH$(CH_2)_5$CONH-polystyrene

Peptide binding surveys were performed by equilibrating 10–500 μM **2**–**5** in $CHCl_3$ with the substrate library for 48 h. Binding for a given substrate library bead was indicated when a bead concentrated the color of the receptor and its tethered dye. The structures of the acylated tripeptides on the most deeply colored beads were then determined by ECGC decoding.[4]

The results from these binding studies were very clear. The macromonocyclic receptor fragments **2** and **3** showed no evidence of binding to the peptide library even at receptor concentrations as high as 500 μM. This result indicates that no member of the library bound either of these single-armed receptor fragments with K_a's exceeding ~200 M^{-1} in $CHCl_3$.[5] In contrast, the double-armed, macrobicyclic receptors **4** and **5** did bind peptides in the substrate library. Indeed, both showed highly selective binding of certain peptides at receptor concentrations of 38 and 4 μM, respectively. The substrate selectivity with **4** was particularly high—there was a very large color contrast between receptor-bound and -unbound beads, and only one bead per ~2500 beads was stained by the dye-linked receptor. This level of selection indicates that only ~20 members of the entire 50 000-member peptide library are bound by **4** at ~40 μM concentration in $CHCl_3$. With **5**, there was a greater gradation of color intensities among the stained beads and one bead per ~500 was stained. In comparison, macrotricycle **1** has been reported to bind one bead per ~1000 at a receptor concentration of 20 μM.[1c] Thus, not only do these simplified receptors bind peptides effectively, but one of them (**4**) binds peptides with substantially more selectivity than does **1**.

Further simplifications of the structural motif found in **4** and **5** can be made. In particular, it is possible to eliminate the conformation-restraining ring of the central linker B′ and still retain selective binding properties. Thus, we replaced the pyrrolidine linker of **4** with a more flexible acyclic diamine derived from tartaric acid to create **6**.[1b] Like **4** and **5**, receptor **6** also showed highly selective binding. This selectivity corresponded to peptide binding at a level of one bead per ~900 (at [**6**] = 40 μM). These binding properties make **6** somewhat less selective than **4**, but they are still comparable to those of the structurally more complex **1**.

(1) (a) Yoon, S. S.; Still, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 823. (b) Yoon, S. S.; Still, W. C. *Tetrahedron Lett.* **1994**, *35*, 2117. (c) Yoon, S. S.; Still, W. C. *Tetrahedron* **1995**, *51*, 567.

(2) Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 373. Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7467.

(3) All possible combinations of R = methyl (Me), ethyl (Et), isopropyl (iPr), *tert*-butyl (tBu), neopentyl (neoPe), trifluoromethyl (CF_3), isobutyl (iBu), methoxymethyl (MOM), acetoxymethyl (AcOM), cyclopropyl (cPr), cyclobutyl (cBu), cyclopentyl (cPe), phenyl (Ph), morpholino (Morph), dimethylamino (Me_2N) and AA1–AA3 = Gly, D-Ala, L-Ala, D-Ser(O-tBu), L-Ser(O-tBu), D-Val, L-Val, D-Pro, L-Pro, D-Asn(*N*-trityl), L-Asn(*N*-trityl), D-Gln(*N*-trityl), L-Gln(*N*-trityl), D-Lys(*N*-Boc), L-Lys(*N*-Boc).

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

(5) Previous studies have shown that beads binding a dyed receptor can be readily detected when as little as 5–10% of the available substrates on the bead are bound.

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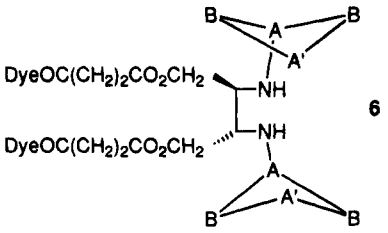

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We determined the structures of the peptide substrates preferentially bound by **4**–**6** by picking ~50 of the most deeply stained beads from the assay of each receptor and decoding by ECGC. The results are listed in Table 1 in terms of the frequencies of the most commonly occurring peptide sequences along with comparison data for the parent receptor **1**. Because the peptide library consists of all possible tripeptides having 15 different residues at each site, we can compute the expected occurrence frequencies of any di- or tripeptide for unselective binding, and those data are also listed in Table 1. As indicated, little selectivity for the terminal acyl group (R) is apparent, but the receptors do bind the peptidic domains of the substrate library with very high sequence selectivity. The peptide binding properties of **4** are particularly noteworthy in that 78% of the selected beads carried only two tripeptide sequences.

It is significant that the minimal structure having significant peptide binding properties is quite simple. In particular, each of the two-armed receptors **4**–**6** can be considered to be constructed from three distinct modules (a linker and two arms) that are held together by simple, acyclic amide bonds. As such, receptors of this type should be readily preparable as combinatorial libraries using split synthesis starting from a variety of linkers and arms.[6] It is also significant that each of these receptors had its own distinct peptide binding selectivity.

These results not only establish the minimal, two-armed structure that is necessary to bind peptides with **1**-like receptors but also suggest that libraries of such receptors may include members having substantial selectivities for given peptidic substrates. The work also demonstrates the first step of a protocol for finding receptors that bind desired substrates tightly and selectively. This protocol involves: (1) synthesis of examples of a new receptor family and screening against a library of the desired substrate type, (2) synthesis of a receptor library based on structures showing selective binding in step 1 and screening with the substrate for which selective binding is desired, and (3) resynthesis of individual receptors found in step 2 and screening against the substrate library of step 1 to establish selectivity. We are now preparing such a library of two-armed receptors related to **4**–**6** and will soon report its effectiveness as a source of sequence-selective receptors for specific peptides.

Table 1. Side Chain-Protected Peptides Selectively Bound by Receptors 4, 5, 6, and 1[a]

R	AA3	AA2	AA1	frequency found[b] (%)	frequency expected[c] (%)
		Macrobicyclic Receptor **4**			
X	D-Pro	L-Val	D-Gln	39	0.03
X	L-Lys	L-Val	D-Pro	39	0.03
		Macrobicyclic Receptor **5**			
X	Gly	L-Ala	L-Ser	16	0.03
X	X	L-Ala, L-Lys, L-Val	D-Gln	53	1.3
		Macrobicyclic Receptor **6**			
X	L-Asn	L-Pro	D-Gln	25	0.03
X	L-Pro	L-Asn	D-Gln	13	0.03
X	D-Pro, D-Val, D-Gln	L-Val	D-Gln	47	0.09
		Macrotricyclic Receptor **1**[c]			
X	D-Asn, D-Gln	L-Val	L-Ser,Gly	34	0.12
X	L-Val	D-Asn	X	10	0.44

[a] X indicates all possible residues (*i.e.*, little or no selectivity). [b] Percentage of beads selected by receptor binding assay with indicated peptide sequence. [c] Percentage of beads having indicated peptide sequence in library (expectation frequency for random bead picking).

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Supplementary Material Available: Experimental details for preparation of receptor **4** (3 pages).

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(6) For a more conformationally flexible, double-armed receptor library see: Boyce, R.; Li, G.; Nestler, H. P.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7955.