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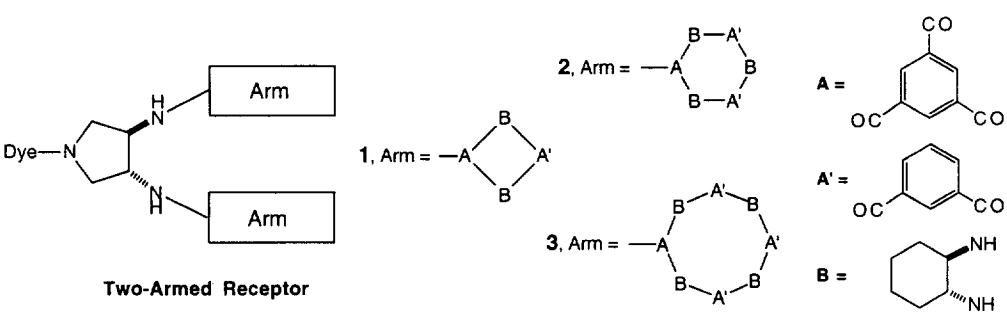
Macrocyclic Oligomers of Isophthalic Acid and *trans*-1,2-Diaminocyclohexane - Building Blocks for Synthetic Peptide Receptors.

Zhengying Pan and W. Clark Still
Department of Chemistry, Columbia University, New York, NY 10027

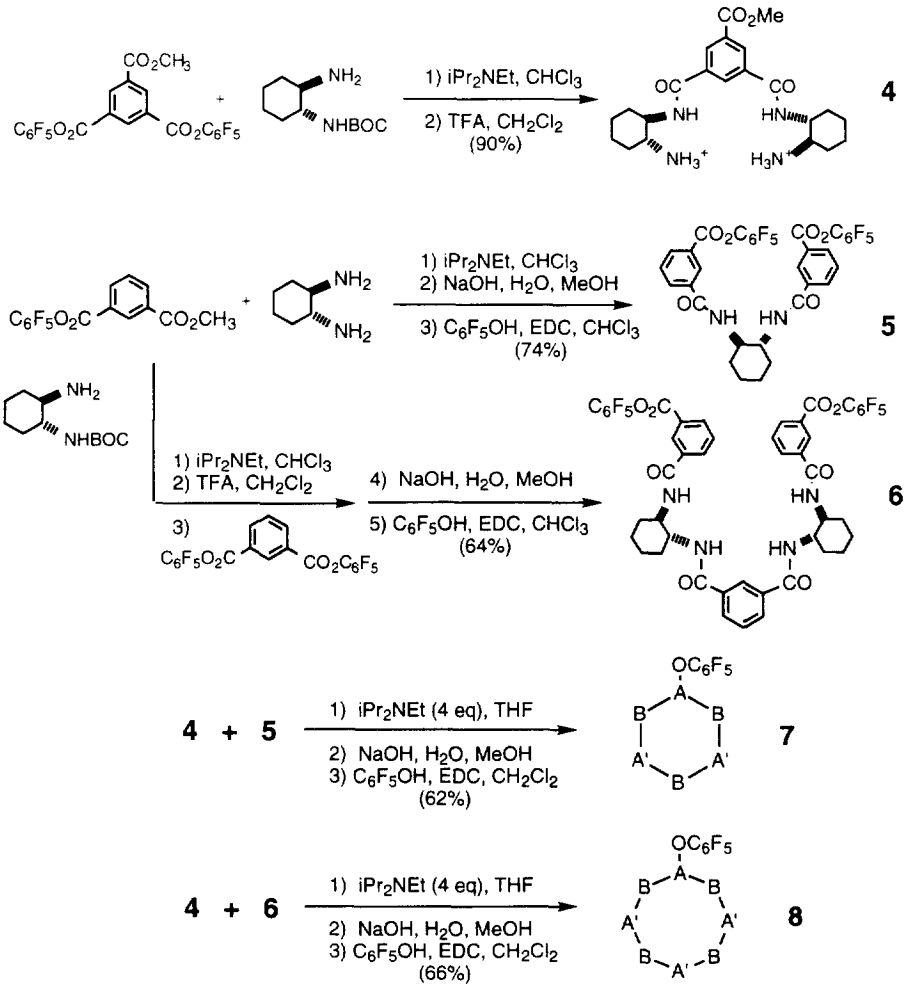
Abstract: *New macrocyclic oligomers of isophthalic acid and trans-1,2-diaminocyclohexane are readily prepared and useful in the synthesis of new, sequence-selective receptors for peptides. Such receptors have a simple, two-armed structural motif and the peptide sequences they bind vary with the ring size of the macrocyclic arms.*

The construction of synthetic receptors that selectively bind small biological molecules has made substantial progress in recent years. One particularly simple design for receptors that bind peptides has a tweezer-like motif that we describe as a *two-armed receptor*.[1] As shown below, it consists of a *linker* (here a *trans*-1,2-diaminopyrrolidine) that is conformationally restricted and directs two functionalized, substrate-binding *arms* toward one another to form a binding cleft. Of the various arms we have investigated, macrocyclic $(AB)_2$ dimers of isophthalic acid (**A'**$(OH)_2$) and a 1,2-diamine (*e.g. trans*-1,2-diaminocyclohexane, **B**H_2) make particularly effective building blocks for receptors that bind peptides sequence-selectively. One such receptor, **1**, is remarkably selective for two tripeptide sequences, (D)Pro-(L)Val-(D)Gln(*N*-Tr) and (L)Lys(*N*-Boc)-(L)Val-(D)Pro, in chloroform.[1b] Because the two-armed design with macrocyclic $(AB)_2$ arms (**1**) is so simple and effective, we investigated the synthesis and properties of related receptors (**2**, **3**) having ring-expanded $(AB)_3$ and $(AB)_4$ macrocyclic arm units. Here we show that the synthesis of such macrocyclic $(AB)_n$ arms is efficient and that the resulting receptors have unique peptide-binding properties.[2]



Syntheses of the new macrocyclic arms **7** and **8** for receptors **2** and **3** respectively are shown below. Since the new macrocycles have common fragments, their syntheses share some of the same building blocks. Thus compound **4** is an intermediate in preparation of both

7 and **8**. The macrocycles are formed in good yield (78% and 89% respectively for the methyl esters of **7** and **8**) in a high dilution (0.5 mM) coupling reaction that both joins fragments and closes the large rings. Receptors **2** and **3** are prepared by simple coupling of these macrocycles to a dye-labeled 1*R*,2R-diaminopyrrolidine as described previously.[1b]



The binding studies were performed by equilibrating the dye-labeled receptors with an encoded combinatorial library of 3,375 (15^3) N-acetylated tripeptides on Merrifield polystyrene. Different members of the library were on different synthesis beads and had the following general structure: Ac-AA_3-AA_2-AA_1-$NH(CH_2)_5CONH$-polystyrene.[4] Equilibrations were carried out with agitation for 96-192 hours in $CHCl_3$ and binding was indicated when library beads developed the red color of the dye. These binding assays were conducted at the lowest

receptor concentration (~10 μM) where significant dye-staining of library beads was observed. Under these conditions, receptor **2** bound 1-2% of the peptide-bearing beads in the library while receptor **3** was more selective and bound ~0.1% peptides in the library. In both cases, the red beads were picked under a low power microscope and the structures of their attached peptides were determined as described previously.[5] The peptide sequences bound by the receptors **2** and **3** are summarized below along with previously reported data for receptor **1**.

AA_3	AA_2	AA_1	Frequency Found	Frequency Expected
Receptor **1** (40 μM, 48 hrs eq.)[1b]				
(D)Pro	(L)Val	(D)Gln	39%	0.03%
(L)Lys	(L)Val	(D)Pro	39%	0.03%
Receptor **2** (9 μM, 96 hrs eq.)				
(D)Ala	(L)X	(L)Ser	37%	0.21%
Receptor **3** (8 μM, 192 hrs eq.)				
(D)Gln	(L)Val	(D)Gln	40%	0.03%
(D)Gln	(L)Lys	(D)Gln	30%	0.03%

While receptor **2** is a less selective binder of peptides than is **1** or **3**, **2** is unusual in that it avoids the strong selectivity for (L)Val-containing peptides that is found with many derivatives of **1** (and also **3**). Instead, **2** preferentially binds peptides having (D)Ala-(L)X-(L)Ser sequences where X represents any amino acid. Other sequences bound by **2** included X-(L)X-(D)Gln (23% of the red beads) where (L)X is frequently (L)Pro. The mediocre sequence selectivity of **2** was indicated not only by the relatively large number of deep red beads seen in the solid phase binding assay but also by the even greater number of less intensely colored beads carrying more weakly binding peptides. In comparison, receptor **3** showed a large color contrast between the red receptor-bound beads and the other beads. Thus, except for the very few deep red/orange beads whose peptides are given in the table above, all the other beads were visually colorless. This high visual contrast indicates a significant gap in the binding constants between the peptides found to bind **3** and the other peptides. As shown in the Table, 70% of the red beads stained by receptor **3** carried only two tripeptide sequences: (D)Gln-(L)Val-(D)Gln and (D)Gln-(L)Lys-(D)Gln. In this regard, **3** and **1** had similar though not identical sequence selectivities. Most of the remaining red beads carried closely related sequences with AA_3 occasionally being (D)Asn or Gly. AA_2-AA_1 was (L)X-(D)Gln in 100% of the red beads we picked using **3**. The concentrations used in our binding assays indicate that **2** and **3** complex their preferred peptide sequences with binding constants in the range of 10^4-10^5 M^{-1}.

These findings show that $(AB)_{n=2-4}$ cyclooligomers of isophthalic acid (A) and *trans*-1,2-diaminocyclohexane (B) are both readily prepared and useful as building blocks for the construction of simple receptor molecules for peptides in organic solvents. The success of

these structures in selectively binding peptides likely follows from the conformational restraints in these molecules that are provided by the 6-membered cyclic subunits, conjugation and the macrocyclic ring closure. It is expected that other binding selectivities can be engineered into these molecules by additional functionalization of the A and/or B fragments as we have already shown with certain $(AB)_2$-based receptors.[1b] Here we have found that simple receptors based on $(AB)_4$ cyclooligomers are as highly selective binders of peptides as are the previously studied $(AB)_2$-based receptors and that their preferred binding sequences are different. Thus, $(AB)_4$-based receptors should make excellent candidates for inclusion in the combinatorial receptor libraries that are the ultimate goal of this work.

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Notes and References:

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3. Receptor **2**: ^{1}H-NMR (400 MHz, $CDCl_3$:CD_3OD = 9:1, ppm): 1.27 (t, J=7.0, 3H), 1.41 and 1.46 (m, 24H), 1.84 (m, 12H), 2.04 and 2.12 (m, 12H), 2.65 and 2.70 (m, 4H), several peaks under CD_3OD, 3.55 (m, 2H), 3.73 (m, 2H), 3.96 and 4.01 (m, 12H), 4.34 and 4.41 (m, 4H), 4.77 (m, 2H), 6.83 (d, J=9.2, 2H), 7.28 (m, 4H), 7.72 (m, 8H), 7.91 (m, 4H), 8.03 (m, 4H), 8.10. (m, 2H), 8.31 (m, 2H), 8.40 (m, 4H); HRMS ($M+H^+$) : calculated for 2014.9320, found 2014.9330.

Receptor **3**: ^{1}H-NMR (400 MHz, $CDCl_3$:CD_3OD = 9:1, ppm): 1.26 (t, J=7.0, 3H), 1.47 and 1.54 (m, 32H), 1.88 (m, 16H), 2.20 (m, 16H), 2.58 and 2.66 (m, 4H), 3.05 (m, 1H), 3.54 (m, 2H), 3.72 (m, 2H), 3.90 and 4.05 (m, 19H), 4.31 (m, 2H), 4.40 and 4.50 (m, 2H), 6.81 (d, J=9.2, 2H), 7.18 (m, 6H), 7.70 (m, 12H), 7.91 (m, 4H), 8.17 (m, 2H), 8.30, 8.38 and 8.43 (m, 12H); HRMS ($M+Na^+$): calculated for 2525.1560, found 2525.1620.

4. AA_N = Gly, (D and L) (Ala, Val, Pro, Ser(*O*-tBu), Lys(*N*-Boc), Asn(*N*-Tr), Gln (*N*-Tr)).

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