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A Peptide-Binding Receptor with the Extended Binding Site

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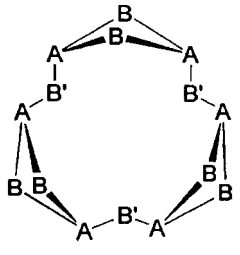
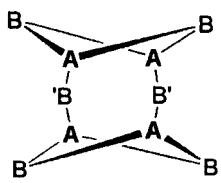
The last decade has witnessed an explosion in the field of molecular recognition. Since the pioneering work of Pederson, Cram and Lehn to define a new area of organic chemistry almost twenty years ago,[1] many molecular receptors capable of interacting selectively with other molecules have been described. For example, many crown ethers were developed for the selective recognition of metal ions and amine salts. Also, many molecular receptors including calixarenes, cyclodextrins, cyclophanes and cleft-shaped molecules for organic substrates such as nucleic acids, aromatics, peptidic molecules and carboxylic acids were described in recent literatures.[2] Among those are cyclooligomeric receptors derived from trimesic acid (**A**) and chiral 1,2-diamines (**B**).[3] From standpoint of the creation of synthetic receptors that have properties similar to those of such remarkable biological receptors as antibodies and enzymes, this class of receptors are attractive because of the following reasons. First, there are many ways in which **A** and **B** are combined. Thus many receptors having well-defined binding sites, with different sizes and arrays of functional groups, can be readily prepared by various combinations of **A** and **B** using macrolactamization reaction. Second, cyclooligomeric receptors derived from **A** and **B** have found to be capable of interacting with polypeptides sequence-selectively. Furthermore, peptide-binding selectivities of these receptors are sensitive to the way which **A** and **B** are combined. Therefore, careful design of cyclooligomers from **A** and **B** might lead to the development of synthetic receptors with the desired binding properties to a given substrate.

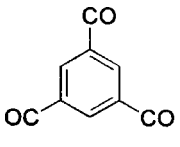
Here, as the continuing efforts to develop the selective peptide-binding synthetic receptors, a readily accessible cyclooligomeric receptor **1** is described. Receptor **1** has conformationally well-defined hydrophobic binding cavity composed of trisubstituted benzene rings and periphery of hydrogen bond donors/acceptors. Furthermore, CPK modeling studies on **1** ($A_6B_6B'_3$) and the known receptor **2** ($A_4B_4B'_2$)[4] receptor indicated that **1** have the extended



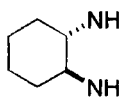
1



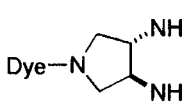
2



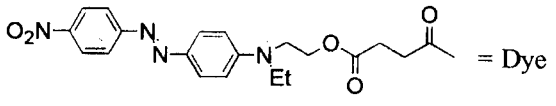
A

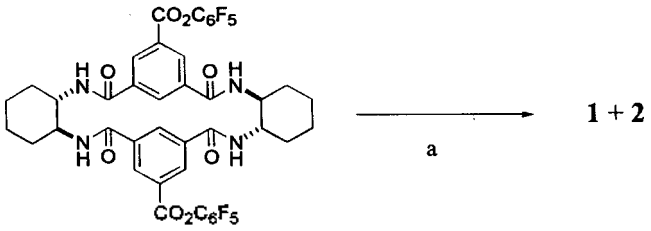


B



B'





Scheme 1. Synthesis of **1** (a) B', slow addition to iPr_2NEt/THF.

binding cavity with the similar size of cyclodextrines compare to the known receptor **2**. As will be shown **1** binds polypeptide substrate selectively.

As shown in Scheme 1, receptor **1** was synthesized by intermolecular macrolactamization reaction between diamine-diTFA salts and bis(pentafluorophenyl)-activated ester.

Synthesis of **1** began with the preparation[4] of monocyclic moiety ($A_2B_2(CO_2C_6F_5)_2$) of receptor. Macrocyclization using the 1 : 1 mixture of (3*R*,4*R*)-N-(Disperse Red I) succinyl- pyrrolidine diamine di-TFA salts and the bis-activated ester in high dilution condition afforded the cyclooligomeric receptor **1** ($A_6B_6B'_3$) with 6.3% yield as well as a known peptide receptor **2** ($A_4B_4B'_2$). Thus spontaneous six amide formation reaction between three molecules of 1,2-diamine and three molecules of bis (pentafluorophenyl) esters provided a new cyclooligomeric receptor **1** as well as the known receptor **2**.

To survey the binding properties of receptor **1**, a solid phase color assay[5] was employed with an encoded combinatorial library of 50,625 acylated tetrapeptide substrates (R-AA3-AA2-AA1).[6] Substrate library was screened for binding by mixing 0.1 g sample (about 10^6 beads) with 50 μM **1** in $CHCl_3$. After 24 hrs, 10% of the beads has become colored with 0.5% being very deep red. The most deeply stained beads were picked and decoded using gas chromotography to yield the sequences of the most tightly binding substrates. The residues found at each position of these substrates are summarized in Table 1 with the number of instances each residue found.

The binding data in Table 1 reveal a number of notable trends. First, high selectivity was observed for the terminal acylating groups. For example, the most frequently found substrates has Me_2N (14 of 27) and Morpholino (13 of 27) at R position. The residue in R was composed of groups with urea functionality. Second, high selectivity was for the AA3 position. The redidue in AA3 was composed of L-amino acid with a hydrogen bond donor/acceptor group in the side chain. Third, selectivities were also found for AA2 and AA1 position. The substrates with L-Pro (25 of 27) and

Table 1. Residues found in substrates bound by **1** and Frequencies of occurrence of each residue (in bracket)

R	AA3	AA2	AA1
Me_2N [14]	L-Asn [17]	L-Pro [25]	L-Asn [10], D-Asn
Mor [13]	L-Gln [8]	L-Ala [2]	L-Gln [5], D-Gln [2]
	L-Ser [2]		L-Ser [3], D-Ser [1]
			L-Lys [2]
			Gly [1]

Table 2. Binding of **1** and Peptides in $CHCl_3$

Peptide	Binding Energy (kcal/mol)	Found in Assay ?
Me_2N-(L)Asn-(L)Pro-(L)Asn-Polymer	-3.5	yes
iPr-(L)Asn-(L)Pro-(L)Asn-Polymer	-2.1	no
Me_2N-(D)Asn-(L)Pro-(L)Asn-Polymer	-1.4	no
Me_2N-(L)Ala-(L)Pro-(L)Asn-Polymer	-2.3	no
Me_2N-(L)Asn-(D)Pro-(L)Asn-Polymer	-1.5	no
Me_2N-(L)Asn-(L)Pro-(L)Ala-Polymer	-2.0	no

L-Asn (10 of 27) at AA2 and AA1 position were found to bind strongly. Thus the most tightly bound substrate with **1** was quite different from that with **2**. For example, while **2** bound tightly with MOM-(L)Ser(D-Pro)-Gly-(L)Ser, the most tightly bound substrate with **1** is Me_2N(Mor)-(L)Asn-(L)Pro-(L, D)Asn.[4]

Presumably, hydrogen bonds between hydrogen bond donor/acceptors on the rim of **1** and peptide substrate and nonbonded hydrophobic interactions between the benzene-lined hydrophobic region of **1** and peptide substrate seems to be important in complexation.

To confirm the findings and to estimate the energetic extents of the selectivities observed, several peptides were resynthesized and their association with **1** measured in $CHCl_3$.[7] The results are summarized in Table 2.

These data showed that the changes in the N-terminal group from Me_2N to isopropyl reduce the binding energies by 1.4 kcal/mol. Stereochemical inversion of Asn at the AA 3 site reduces binding energy by 2.1 kcal/mol. Also, stereochemical inversion of Pro at AA2 site reduce binding energy by 2.0 kcal/mol. Removal of amide group in the side chain of substrate from Asn to Ala at AA3 and AA1 sites reduce binding energy by 1.2 and 1.5 kcal/mol, respectively. These data in Table 2 are well in accord with the picture emerged from the binding assay.

In summary, cyclooligomeric receptors **1**, which have the large substrate binding sites, were successfully prepared using intermolecular macrolactamization reaction. Also, receptor **1** has highly sequence-selective peptide binding properties. This study establishes that synthetic receptors can bind selectively polypeptides, similar to biological receptors as antibodies and enzymes.

Experimental

$A_2B_2(CO_2Me)_2$, and $A_2B_2(CO_2C_6F_5)_2$ were prepared following the reported procedures.[4]

1. A solution of 40 mg of bis(pentafluorophenyl)ester (0.0440 mmol) and 15 mg of (1R,2R)-diaminocyclohexane diTFA salt (0.0440 mmol) in 10 mL of DMA was added to a solution of 0.19 mL of DIPEA (1.09 mmol) in 200 mL of THF at room temperature for 20 hr by syringe pump. After the stirring for 8 hr at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 10% MeOH in methylene chloride to give an amorphous red solid (1.8 mg, 6.3%): ^{1}H NMR ($CDCl_3$/CD_3OD) δ 1.82 (m, 4H), 1.85 (m, 8H), 2.11 (m, 4H), 3.82 (m, 4H), 4.10 (m, 6H), 4.24 (m,

2H), 4.31 (m, 2H), 7.20 (m, 4H), 7.44 (m, 2H), 7.60 (d, 2H, J=4.51 Hz), 8.33 (m, 4H), 8.43 (m, 2H); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 168.21, 167.97, 167.87, 167.56, 166.54, 165.54, 156.78, 143.23, 141.67, 134.88, 134.56, 134.45, 134.24, 133.56, 133.21, 130.46, 128.97, 127.56, 126.76, 125.09, 119.78, 67.86, 58.03, 57.54, 54.54, 54.09, 53.78, 53.03, 52.98, 47.88 (m), 47.23, 32.65, 31.44, 25.45, 24.76; IR (neat) 3324, 2877, 1685, 1665, 1575 cm^{-1}; MS (FAB) m/z 2591 (M+1).

Typical Procedure of Solid Phase Color Assay. 100 mg (about 10^6 beads) of substrate library was suspended in Eppendorf tube containing 0.5 mL $CHCl_3$ and then added 25 μL of a 0.5 mM $CHCl_3$ solution of dye-linked receptor. After 48 hr of agitation on a wrist-action shaker, about 1% of the beads were stained deep color with more having various lighter colorations.

Decoding Tagged Synthesis Supports. The mostly deep colored beads were selected manually under a 4X microscope, placed individually in separate melting point capilary tubes, and then washed with DMF (2×10 μL). The bead was suspended in 1 μL fresh, pure DMF and the tube was sealed using a flame. Next the bead was irradiated for 3 hr at room temperature at 366 nm with a Model UVL-56 ultraviolet light, and then warmed at 90 °C for 2 hr to complete release of the tag alcohols into solution. The tube was opened and bis(trimethylsilyl)acetamide (0.1 μL) was added for silylation to increase tag volatility. After centrifuging for 2 min, the tag solution (1 μL) over each bead was injected into an electron capture capilary gas chromatograph for tag analysis. The resulting chromatograms showed which tags were present and which were absent. Binary tag code used during library synthesis revealed the structure of each substrate which had been selected by the solid phase binding assay.

Measurement of Binding Energy of Receptor and Substrate Found in Solid Phase Assay. Substrate found in assay was resynthesized on Merrifield synthesis bead using the same methodology to the synthesis of substrate library without the tagging step. Known quantities of dye-linked receptor ($[R]_{total}$) were added to a constant quantity of bead-bound substrate with monitoring of $[R]_{free}$ by UV after 24 hr of equilibration. Because the concentration of bead-bound substrate is unknown, [R] data were collected at two points (A, B) and the two binding equations were solved simultaneously:

$$K=\{[Complex]_B/[R]_{free\text{-}B}\text{-}[Complex]_A/[R]_{free\text{-}A}\}/\{[Complex]_A\text{-}[Complex]_B\}$$

where $[Complex]=[R]_{total}\text{-}[R]_{free}$. Data was collected at three such points, and K's were calculated for each of the three pairs of points. The binding experiment was repeated. The resulting six K's were averaged, and error limits were computed from the different K's as 1 standard deviation.

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6. All possible combinations of R=methyl (Me), ethyl (Et), isopropyl (iPr), *t*-butyl (*t*-Bu), neopentyl (neoPe), trifluoromethyl (TFA), methoxymethyl (MOM), acetoxymethyl (AcOM), cyclopropyl (cPr), cyclobutyl (cBu), cyclopentyl (cPe), phenyl (Ph), morpholino (Mor), dimethylamino (Me_2N) and AA1-AA3=Gly, D-Ala, L-Ala, D-Ser, L-Ser, D-Val, L-Val, D-Pro, L-Pro, D-Asn, L-Asn, D-Gln, L-Gln, D-Lys, L-Lys.
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Influence of Wax on Migration of Antiozonants in NR Vulcanizates

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Ozone attack on rubber compounds causes characteristic cracking perpendicular to the direction of applied stresses. This degradation is caused by reactions of ozone with the double bonds in the rubber chains. These reactions lead to chain scission and the formation of various decomposition products. In order to control the effects of rubber ozonation,