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A Sequence-Selective Peptide-Binding Receptor

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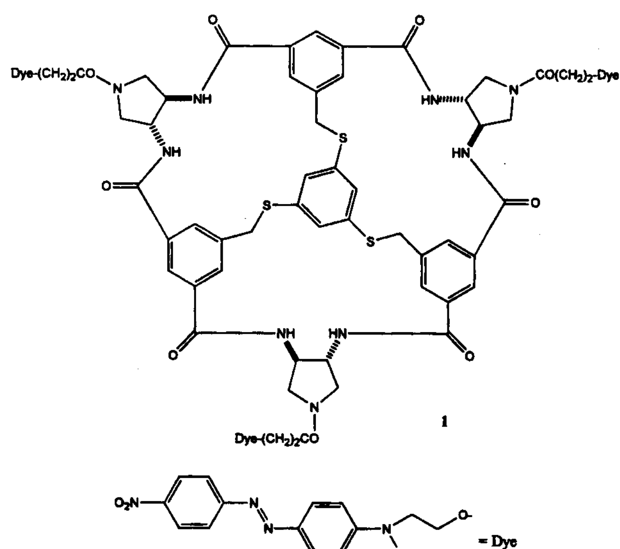
One of the remarkable features of many biological receptors, such as enzymes and antibodies, is their ability to recognize and selectively bind multi-residue peptide sequences. To discriminate between a vast array of different, and often closely related molecules in a biological medium, biological receptors use well-defined binding sites, with different sizes and arrays of functional groups, which are constructed by various combinations of the structurally related building blocks such as α-amino acids.

Recently, synthetic receptors have begun to reproduce such ability.[1] Among those are a class of cyclooligomeric receptors derived from trimesic acid (**A**) and chiral 1,2-diamines (**B**).[2] These molecules showed high enantio- and residue-selectivities in the binding of amino acids and small peptides. Furthermore, the peptide binding properties of these readily accessible receptors were markedly different, when the way in combinations of **A** and **B** are different. Obviously, there exist a number of way in which **A** and **B** can be combined. Here, to expand the scope of the peptide-binding selectivity of this class of receptors, a C_3 symmetric bowl-shaped receptor (**1**) is described.

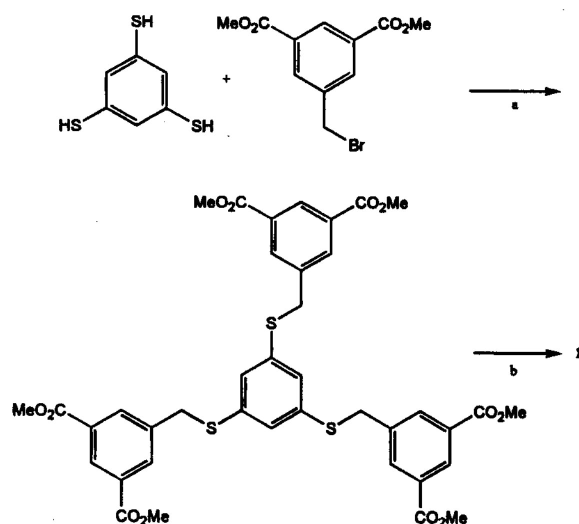
In receptor **1**, three molecules of **A** and **B** are linked through a 1,3,5-benzenetrimercaptane. As a result, **1** has a conformationally well-defined hydrophobic cavity composed of four *m*-substituted benzenes and periphery of hydrogen bond donors/acceptors. Therefore, receptor **1** might be expected to bind peptides selectively by hydrogen bondings and hydrophobic interactions.[3]

Synthesis of **1** begins with 1,3,5-benzenetrimercaptane as shown in Scheme 1. A triple alkylation with bromomethylbenzene 3,5-dicarboxylic acid dimethylester provided the hexamethylester **2**. Ester hydrolysis and subsequent EDC coupling with pentafluorophenol led to the cyclization precursor. The final step was an intermolecular macrolactamization which used hexakis (pentafluorophenyl) ester and (3R,4R)-N-(Disperse Red I)succinyl- pyrrolidine diamine di-TFA salts to close the macrocyclic ring. This cyclization provided the intensely red receptor **1** in 32% yield.

To survey the binding properties of receptor **1**, a solid phase color assay was employed with an encoded combinatorial library[4] of 50,625 acylated tetrapeptide substrates (R-AA3-AA2-AA1).[5] Substrate library was screened for



Structure of 1.



Scheme 1. (a) DIPEA, THF (77%). (b) i. NaOH, ii. C_6F_5OH/EDC (45%) and then Dye-linked pyrrolidine diamine diTFA salt/DIPEA in THF (32%).

Table 1. Residues found in substrates bound by receptor 1 and frequencies of occurrence of each residue (in braket)

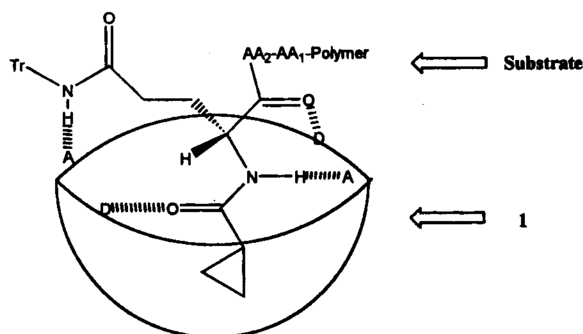
R	AA3	AA2	AA1
cyclopropyl [18]	(D)Gln [17]	(D)Ala [12]	(L)Pro [10]
Me_2N [5]	(D)Asn [4]	(D)Pro [3]	(L)Ala [3]
	(D)Lys [2]	(D)Val [3]	Gly [3]
		Gly [3]	(L)Ser [2]
		(D)Ser [2]	(L)Val [2]
			(L)Asn [2]
			(L)Gln [1]

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, extraordinary selectivity was observed for the terminal acylating groups. For example, the most frequently found substrates has cyclopropyl (18 of 23) and Me2N (5 of 23) at R position. Second, high selectivity was for the AA3 position. The redidue in AA3 was composed of D-amino acid with an amide group in the side chain. Third, selectivities were also found for AA2 and AA1 position. The substrates with D-Ala (12 of 23) and L-Pro (10 of 23) at AA2 and AA1 position were found to bind strongly. Such high selectivities for the N-terminal group and AA3 residue suggest that the binding mode of **1** is similar to that shown in Scheme 2 (D/A: hydrogen bond donor/acceptor).

It appears that the hydrogen bond donor/acceptors on the rim of **1** bind the peptide substrate by four hydrogen bonds and there are nonbonded hydrophobic interactions between the benzene-lined hydrophobic region of **1** and N-terminal group of peptide substrate. The residue at AA2 (D-Ala) and AA1 (L-Pro) positions in a tetrapeptide substrate might act as "a conformational lock" which enables the substrate to have the ideal conformation for the binding with **1**.[6]

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.



Scheme 2. The proposed structure of complex of 1 and peptide-substrate (A: hydrogen bond acceptor (C=O), D: hydrogen bond donor (N-H)).

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

Peptides	Binding Energy (kcal/mol)	Found in Assay?
cPr-(D)Gln-(D)Ala-(L)Pro-Polymer	−4.5	yes
Me_2N-(D)Gln-(D)Ala(L)Pro-Polymer	−4.1	yes
iPr-(D)Gln-(D)Ala-(L)Pro-Polymer	−1.0	no
cPr-(L)Gln-(D)Ala-(L)Pro-Polymer	−1.6	no
cPr-(D)Ala-(D)Ala-(L)Pro-Polymer	−2.4	no
cPr-(D)Gln-(D)Ala-(D)Pro-Polymer	−2.7	no
cPr-(D)Gln-(L)Ala-(L)Pro-Polymer	−2.5	no

These data showed that the changes in the N-terminal group from cyclopropyl and Me_2N to isopropyl reduce the binding energies by 3.5 and 3.1 kcal/mol, respectively. Stereochemical inversion of Gln at the AA3 site reduces binding energy by 2.9 kcal/mol. Also, stereochemical inversion of Ala and Pro at AA1 and AA2 site reduce binding energy by 1.5 and 1.4 kcal/mol, respectively. Removal of amide group in the side chain of substrate from Gln to Ala reduce binding energy by 2.1 kcal/mol. These data are well in accord with the picture emerged from the binding assay.

In summary, receptor **1**, a readily accessible hetero-oligomer from trimesic acid (**A**) and 1,2-diamine (**B**), has highly sequence-selective peptide binding properties. Also, this study demonstrates the power of directed screening of large chemical library as a method to study the binding properties.[8] Armed with this powerful method to evaluate the binding properties of receptor, work to develope receptors with the desired binding properties is in progress. Particularly, carbohydrate binding receptors are attracting because this area is still unexplored in spite of the increasing importance of carbohydrates in the field of chemistry, medicine and biology.[9]

Experimental

Hexamethyl ester. To a solution of 0.30 g of bromomethylbenzene 3,5-dicarboxylic acid dimethylester (1.04 mmol) and 55 mg of 1,3,5-benzene trimercaptane (0.314 mmol) in 10 mL of DMA was added 0.36 mL of DIPEA (1.11 mmol). After stirring for 12 hr at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 5% MeOH in methylene chloride to give an amorphous white solid (0.64 g, 77%): ^1H NMR ($CDCl_3$) δ 3.90 (s, 6H) 4.12 (s, 2H) 6.94 (s, 1H) 8.51 (s, 2H) 8.66 (s, 1H); MS (FAB) *m/z* 793 (M+1).

1. To a solution of 96 mg of hexamethylester (0.121 mmol) in 5 mL of THF, 3 mL of MeOH and 1 mL of water was added 0.85 mL of 1 N NaOH solution. After the stirring for 5 hr at room temperature, the reaction mixture was acidified with 1 N HCl solution and extracted into EtOAc. The crude hexacarboxyl acid was dissolved in 3 mL of THF and 10 mL of methylene chloride, and 0.15 g of pentafluorophenol (0.84 mmol) and 0.16 g of EDC (0.84 mmol) were added. After the stirring for 5 hr at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 20% acetone in methylene chloride to give hexakis (pentafluorophenyl)ester as an amorphous white solid (45

mg, 45%): ^{1}H NMR ($CDCl_3$) δ 4.12 (s, 2H) 6.94 (s, 1H) 8.31 (s, 2H) 8.56 (s, 1H).

A solution of 40 mg of hexakis(pentafluorophenyl)ester (0.0253 mmol) and 63 mg of N-(Disperse Red I)succinyl-(3R,4R)-pyrrolidine diamine diTFA salt[2(a)] (0.0835 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 5 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 (18 mg, 32%): ^{1}H NMR ($CDCl_3/CD_3OD$) δ 0.72 (t, 3H, J=7.0 Hz) 2.43 (m, 2H) 3.01 (q, 2H, J=7.0 Hz) 3.26 (s, 2H) 3.45 (m, 2H) 3.64 (m, 1H) 3.82 (m, 1H) 4.22 (m, 4H) 4.56 (m, 1H) 4.67 (m, 1H) 6.67 (d, 2H, J=9.1 Hz) 6.98 (m, 1H) 7.78 (m, 5H) 7.98 (m, 1H) 8.15 (d, 2H, J=9.1 Hz) 8.33 (m, 1H); ^{13}C NMR ($CDCl_3$) δ(ppm) 172.11, 170.54, 170.02, 168.23, 151.24, 141.61, 138.87, 137.97, 136.03, 135.66, 132.55, 128.07, 126.66, 126.22, 125.36, 124.65, 123.21, 122.21, 119.58, 111.23, 54.32, 50.34, 48.70, 45.34, 37.07, 28.77, 28.54, 28.18, 28.12; IR (neat) 3325, 2825, 1723, 1675, 1576 cm^{-1}; MS (FAB) m/z 2061 (M+1); HRMS (FAB) 2061.3164 (2061.3144 calcd for $C_{102}H_{109}O_{21}N_{21}S_3$)

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5. All possible combinations of R=methyl (Me), ethyl (Et), isopropyl (*i*Pr), *t*-butyl (*t*-Bu), neopentyl (neoPe), trifluoromethyl (TFA), methoxymethyl (MOM), acetoxymethyl (AcOM), cyclopropyl (cPr), cyclobutyl (cBu), cyclopentyl (cPe), phenyl (Ph), morpholino (Mor), dimethylamino (Me2N) and AA1-AA3=Gly, D-Ala, L-Ala, D-Ser(OtBu), L-Ser(OtBu), D-Val, L-Val, D-Pro, L-Pro, D-Asn(Tr), L-Asn(Tr), D-Gln(Tr), L-Gln(Tr), D-Lys(N-Boc), L-Lys(N-Boc).
6. Although **1** and a known receptor (**2**)[3] show the similar selectivities at R (Me and Me_2N) and AA3 site (Gln) of tetrapeptide substrate, there are selectivity differences at AA2 (**1** prefers Ala, **2** prefers Pro) and AA1 (**1** prefers Pro, **2** shows virtually no selectivity) site. These support the notion that this class of receptors recognize total structual features not just the direct interaction region of tetrapeptide substrate.
7. Since **1** was sparingly soluble in $CDCl_3$, it was not possible to study complexation properties of **1** in $CDCl_3$. See related approach; 2(a) and Smith, P.W.; Chang, G.; Still, W.C. *J. Org. Chem.* **1988**, *53*, 1587.
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An Enantioselective Peptide-Binding Receptor

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The enantioselective complexation of peptides by synthetic receptors has been widely studied because this would increase the understanding of basic principles on molecular recognition mechanism seen in biological systems such as enzyme and antibody, and lead to the selective catalysts, novel pharmaceuticals, novel analytical and separative tools.[1]

Learned from molecular recognition studies on receptor/substrate binding, criteria for the design of selective receptors were identified. The primary requirement to such receptors is a conformationally rigid system with proper arrays of functional groups complementary to those found in substrates.[2] In efforts to develop such receptors, chemists have constructed cyclic and multi-cyclic structures from conformationally rigid building blocks having the suitable functionalities for the binding with given substrates. Obviously, there exist a variety of readily available building blocks. Among those are cyclohexapeptides. Cyclohexapeptides are conformationally homogeneous due to intramolecular hydrogen bondings. Moreover, various functionalities can be