Novel Macrocyclic Receptors for Peptides

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Received July 3, 2002

Key Words : Molecular recognition, Peptide-binding, Macrocyclic receptor

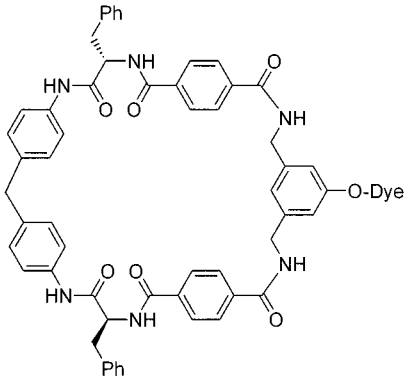
The creation of synthetic molecules having selective peptide-binding properties seen in biological systems such as enzymes and antibodies is a challenging problems both in chemistry and biology.[1] Through studies on the selective peptide-binding receptors, understanding of molecular recognition mechanisms seen in biological systems would be improved, and the potential applications to synthetic, separative and analytical purposes would be anticipated. Although it is known that several synthetic receptors bind with the certain peptides selectively,[2] the search for new peptide substrates is continuing to establish the underlining principles in the design of selective receptors for a given peptide substrate.

Recently, 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.[3] Also, many others including calixarenes, cyclodextrins, cyclophanes, cryptophanes, carcerands and, molecular tweezers and clefts for organic substrates such as nucleic acids, aromatics, peptidic molecules and carboxylic acids were described.[4] Typically, synthetic receptors have the macrocyclic structures with the binding cavities having the convergent functionalities. Yet, macrocyclic receptors derived from amino acids and aromatic spacers have not been explored intensively. There are many available amino acids and aromatic spacer molecules. Thus many receptors having well-defined binding sites, with different sizes and arrays of functional groups, can be readily prepared by various combinations of building blocks using macrolactamization reaction. Careful design of macrocycles from amino acids and aromatic spacers might lead to the development of synthetic receptors with the desired binding properties to a given peptide substrate.

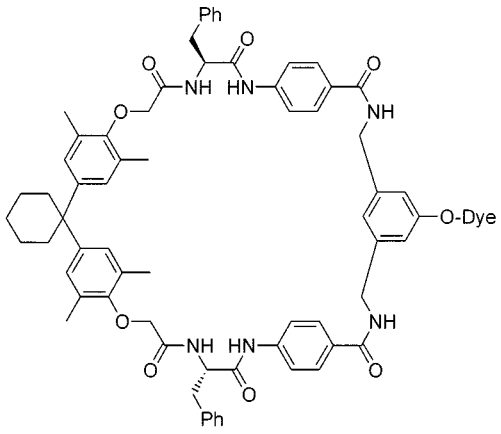
Here, to test the synthetic feasibility and peptide-binding properties of macrocyclic receptors derived from amino acids and aromatic spacer molecules, a novel class of synthetic receptors (**1**, **2**) are described.

These molecules have well-defined substrate-binding cavity having benzene-lined hydrophobic surface with periphery of hydrogen bond dornor/acceptors. Thus these molecules might be expected to be capable of interacting selectively with peptide substrates through hydrophobic interactions and hydrogen bondings.

Synthesis of macrocyclic peptides (**1** and **2**) were conducted by following the standard organic reactions, as shown in Scheme 2. Synthesis of **1** began with amide bond formation



1



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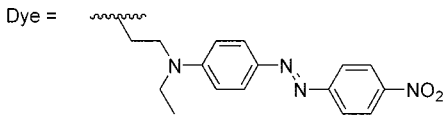
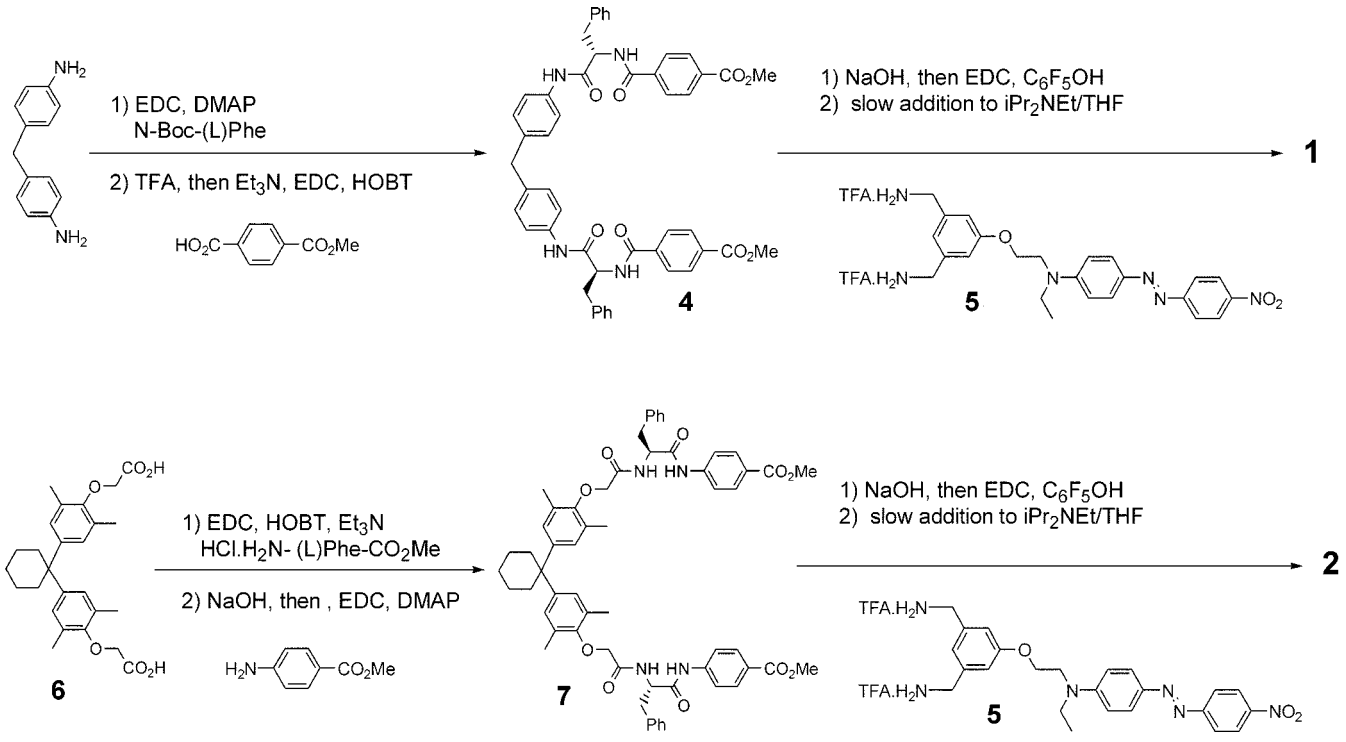


Disperse Red 1

Scheme 1. Macrocyclic Receptors (**1**, **2**).



Scheme 2. Synthesis of **1** and **2**.

reaction between bis(4-aminophenyl)metane and N-Boc-(L)phenylalanine. Deprotection of Boc groups by TFA and EDC-promoted amide bond formation reaction with terephthalic acid monomethyl ester provided the dimethyl ester intermediate **4**. Macrocyclization between bis(pentafluorophenyl)ester of **4** and diTFA salts of dye-linked spacer, **5**, under high dilution condition provided **1** with 67.3% yield. Synthesis of **2** began with the preparation of the known dicarboxyl acid **6**. EDC-promoted amide bond formation between **6** and (L)phenylalanine methyl ester, and then subsequent coupling with 4-aminobenzoic acid methyl ester provided the acyclic intermediate **7**. Macrocyclization between bis(pentafluorophenyl)ester of **7** and diTFA salts of dye-linked spacer, **5**, under high dilution condition provided **2** with 42.3% yield.

Table 1. Sequences (Resin-AA1-AA2-AA3-Ac) selected by binding assay with receptors (**1**, **2**)

	1	**2**
1	Gly-(L)Ala-(L)Ala	(L)Ala-(L)Val-Gly
2	Gly-(L)Ala-Gly	(L)Ala-(L)Leu-Gly
3	Gly-(L)Ala-(L)Ala	(L)Ala-(L)Val-(L)Ala
4	Gly-(L)Ala-(L)Ala	(L)Ala-(L)Leu-(L)Ala
5	Gly-(L)Pro-Gly	Gly-(L)Leu-(L)Ala
6	(L)Asn-(L)Ala-Gly	Gly-(L)Val-Gly
7	(L)Asn-(L)Pro-Gly	Gly-(L)Ala-Gly
8	(L)Asn-(L)Pro-(L)Ala	(L)Ser-(L)Val-Gly
9	(L)Gln-(L)Pro-Gly	(L)Ser-(L)Leu-Gly
10	(L)Gln-(L)Pro-(L)Ala	Gly-(L)Ser-(L)Ala
11	(L)Lys-(L)Pro-Gly	Gly-(L)Ser-Gly
12		(L)Pro-(L)Val-Gly

To establish the peptide-binding properties of receptors, **1** and **2** were screened against a tripeptide library on hydrophobic polystyrene in $CHCl_3$. The library was prepared by encoded split synthesis and has the general structure Ac-AA3-AA2-AA1-NH$(CH_2)_6$-C(O)NH-Polystyrene.[6,7] Decoding the tripeptides on the colored beads by using electron capture gas chromatography revealed selective peptides-binding properties of macrocyclic compounds. The most tightly binding substrates with macrocyclic compounds are shown in Table 1, and the residues found at each position of these substrates are summarized in Table 2.

The binding data in Table 1 and 2 reveal a number of notable trends. For example, C_2-symmetric receptor **1** were found to bind strongly with the substrate with Gly (5 of 11), (L)Ala (5 of 11) and (L)Pro (6 of 11), and Gly (6 of 11) and (L)Ala (5 of 11) at AA1, AA2 and AA3 position. Also, receptor **2** shows the strong binding selectivity with the substrate having Gly and (L)Ala, (L)Val and (L)Leu, and Gly at AA1, AA2 and AA3 position, respectively.

To confirm the findings and to estimate the energetic extents of the selectivities observed, the most tightly bound

Table 2. Frequencies of Residues of Substrates (Resin-AA1-AA2-AA3-Ac) Bound by Receptors (**1**, **2**)

	AA1	**AA2**	**AA3**
Receptor 1	Gly (5) (L)Asn (3), (L)Gln (2) (L)Lys (1)	(L)Ala (5) (L)Pro (6)	Gly (6) (L)Ala (5)
Receptor 2	Gly (5) (L)Ala (4), (L)Ser (2) (L)Pro (1)	(L)Val (5) (L)Leu (4) (L)Ser (2), (L)Ala (1)	Gly (8) (L)Ala (4)

peptide with **1** and **2**, Resin-Gly-(L)Pro-Gly-Ac and Resin-Gly-(L)Val-Gly were resynthesized and their associations with **1** and **2** measured in $CHCl_3$.[8] The binding energies were found to be -3.8 and -3.9 kcal/mol, respectively. The other substrates found by binding assay are expected to have the similar range of binding energies. The binding energies with Resin-Gly-Gly-Gly-Ac, which is not found in assay, were found to be both less than -0.5 kcal/mol.

In summary, receptors **1** and **2** have highly sequence-selective peptide binding properties. Further studies on peptide binding properties of the other related synthetic receptors are in progress in this laboratory.

Experimental Section

Synthesis of 1. A solution of 0.20 g the crude bis(pentafluorophenyl)ester of **4** (0.179 mmol) and 0.12 g of bis-TFA salts, **5**, in 10 mL of DMA was added to a solution of 0.25 mL of DIPEA (1.79 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 **1** as an amorphous red solid (145 mg, 67.3 %): ^{1}H NMR ($CDCl_3$/CD_3OD) δ (ppm) 1.28 (t, 3H, J = 7.0 Hz), 2.98 (dd, 2H, J = 10.5, 7.2 Hz), 3.12 (dd, 2H, J = 10.5, 5.5 Hz), 3.64 (q, 2H, J = 7.0 Hz), 3.75 (s, 2H), 3.87 (t, 2H, J = 6.0 Hz), 4.19 (t, 2H, J = 6.0 Hz), 4.71 (d, 2H, J = 10.5 Hz), 4.78 (m, 2H), 4.87 (d, 2H, J = 10.5 Hz), 6.70 (s, 2H), 6.82 (s, 1H), 6.90 (d, 2H, J = 9.5 Hz), 7.12 (d, 4H, J = 9.0 Hz), 7.24 (m, 6H), 7.35 (m, 4H), 7.52 (d, 4H, J = 9.0 Hz), 7.90 (d, 2H, J = 9.5 Hz), 7.95 (d, 2H, J = 9.5 Hz), 8.05 (d, 4H, J = 10.0 Hz), 8.12 (d, 4H, J = 10.0 Hz), 8.33 (d, 2H, J = 9.5 Hz); IR (KBr) 3415, 3340, 2990, 1690, 1645, 1607 cm^{-1}; MS (FAB) *m/z* 1202 (MH^+).

Synthesis of 2. A solution of 0.22 g of bis(pentafluorophenyl)ester of **7** (0.118 mmol) and 0.12 g of bis-TFA salts, **5**, in 10 mL of DMA was added to a solution of 0.15 mL of DIPEA (1.2 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 **2** as an amorphous red solid (83 mg, 42.3%): ^{1}H NMR ($CDCl_3$/CD_3OD) δ (ppm) 1.31 (t, 3H, J = 7.0 Hz), 1.65 (m, 10H), 2.45 (s, 12H), 2.96 (dd, 2H, J = 10.0, 7.5 Hz), 3.10 (dd, 2H, J = 10.0, 6.0 Hz), 3.54 (q, 2H, J = 7.0 Hz), 3.90 (t, 2H, J = 6.2 Hz), 4.12 (t, 2H, J = 6.2 Hz), 4.21 (dd, 4H, J = 10.2, 6.2 Hz), 4.32 (dd, 4H, J = 10.2, 6.2 Hz), 4.91 (t, 2H, J = 7.5 Hz), 6.95 (s, 4H), 7.09 (d, 4H, J = 9.2 Hz), 7.15 (d, 4H, J = 10.0 Hz), 7.23 (s, 2H), 7.31 (m, 10H), 7.50 (s, 1H), 7.95 (d, 2H, J = 9.5 Hz), 8.00 (d, 2H, J = 9.5 Hz), 8.21 (d, 6H, J = 10.0 Hz), 8.35 (d, 2H, J = 9.5 Hz); IR (KBr) 3410, 3375, 2976, 1705, 1665, 1622 cm^{-1}; MS (FAB) *m/z* 1386 (MH^+).

Acknowledgment. Financial support from Korea Science & Engineering Foundation (Grant #1999-2-123-001-3) is gratefully acknowledged.

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6. AAn = Any possible combinations of 25 (a)-amino acids such as Gly, (L)Ala, (D)Ala, (L)Val, (D)Val, (L)Leu, (D)Leu, (L)Phe, (D)Phe, (L)Pro, (D)Pro, (L)Ser(OtBu), (D)Ser(OtBu), (L)Asp(OtBu), (D)Asp(OtBu), (L)Glu(OtBu), (D)Glu(OtBu), (L)Asn(Tr), (D) Asn(Tr), (L)Gln(Tr), (D)Gln(Tr), (L)Lys(Boc), (D)Lys(Boc), (L)His(Tr), (D)His(Tr). The number of members in substrates library is $(25)^3$, 15625.
7. A total of 15 tag molecules (five tags for AAn) were used to encode the library according to the method reported in *reference 5*.
8. Yoon, S. S.; Still, W. C. *Tetrahedron* **1995**, *51*, 567.