Peptide-Binding Macrocycles Based on Trimesic Acid and 1,2-Diamines

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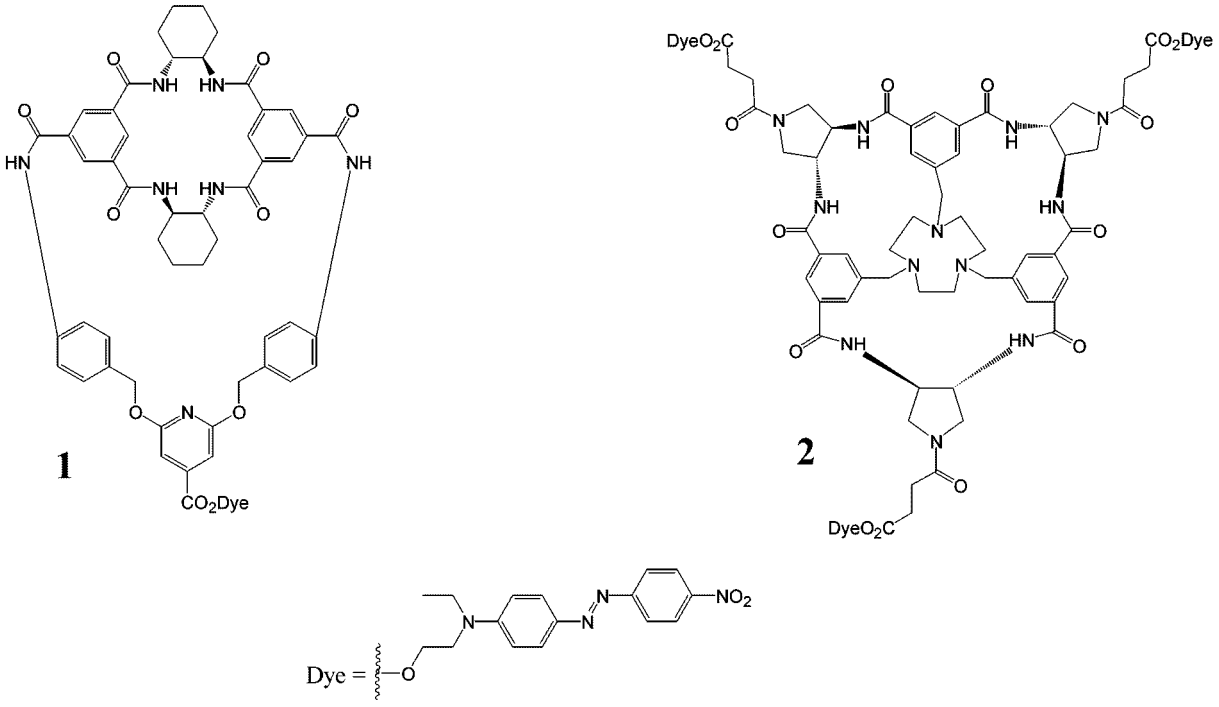
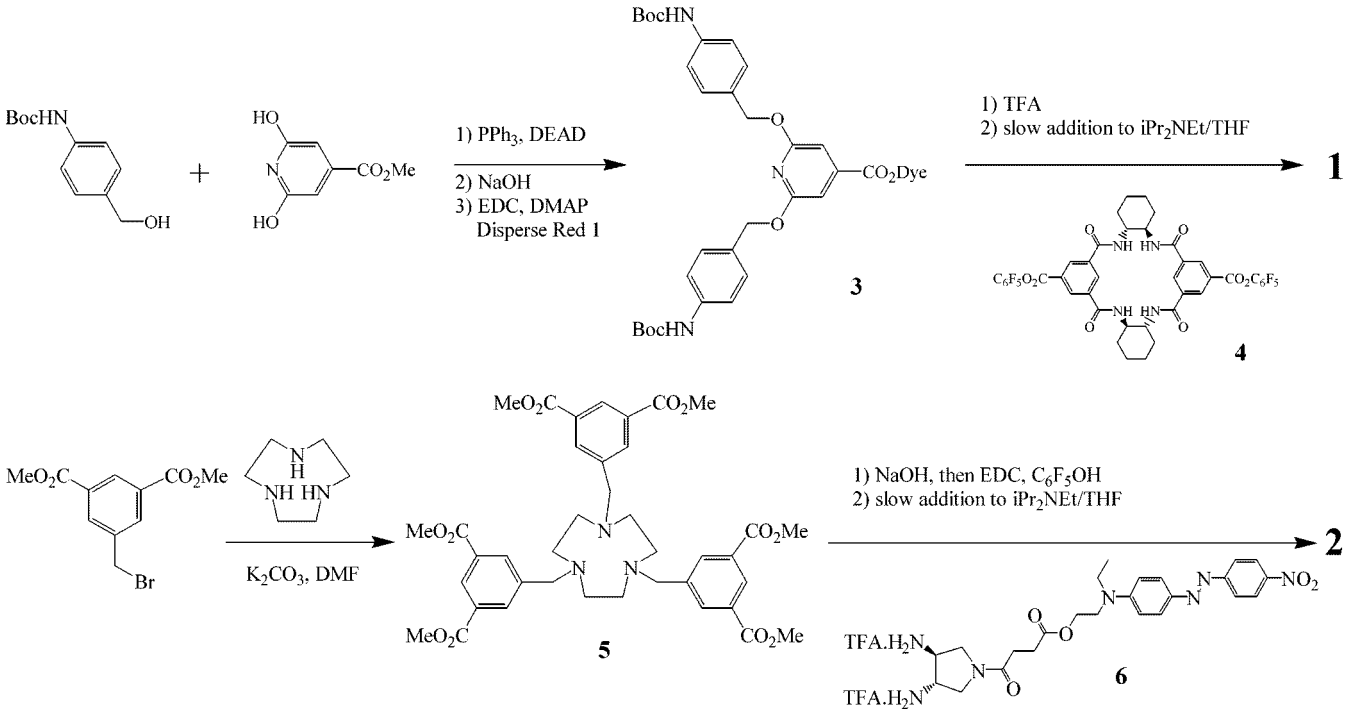
The development of selective peptide-binding receptors and thus the elucidation of the basic rules that govern non-covalent intermolecular interactions is of great importance for the improved understanding of molecular recognition mechanisms seen in biological systems, and the potential applications to synthetic, separative and analytical purposes.[1] Recently, molecular receptors capable of interacting selectively with peptides have been described.[2] For examples, a series of bridged macrocyclic compounds which were derived from trimesic acid and 1,2-diamines have been found to bind peptide highly selectively.[3] Also, macrocyclic and tweezer-like molecules derived from amino acids have been shown to have selective peptide-binding properties.[4] However, the search for new peptide-binding receptors is continuing to establish the basic principles for the design of receptors to a given substrate. Here, novel C_2 and C_3-symmetric macrocyclic receptors (**1** and **2**) from 1,2-diamine and trimesic acid are described. **1** and **2**, have the interesting structural features for the interaction with peptide substrates. Like the related receptors,[5,6] those have well-defined substrate-binding cavity having benzene-lined hydrophobic surface with periphery of hydrogen bond dornor/acceptors. Furthermore, relatively polar pyridine and azacrown moeities are placed at the bottom of the potential binding cavity of **1** and **2**. Thus **1** and **2** might be expected to be capable of interacting selectively with certain peptides through polar interactions between pyridine and azacrown of receptors, and acidic functionality of peptide substrate, as well as hydrophobic interactions and hydrogen bondings seen in the related receptors.

Synthesis were conducted by following the standard organic reaction procedures, as shown in Scheme 2. Synthesis of **1** began with Mistunobu reaction between N-Boc-aminobenzyl alcohol and citrazinic acid methyl ester. Hydrolysis of the resulting methyl ester, and subsequent EDC-promoted esterification reaction with dye molecule in the presence of DMAP provided the acyclic N-Boc protected intermediate **3**. After deprotection of Boc groups, macrocyclization between the dye-labled diTFA salts of **3** and bis-pentafluorophenyl ester (**4**) in high dilution condition provided **1** with 65% yield.

For synthesis of **2**, triple alkylation between 5-bromethyl-isophthalic acid dimethyl ester and 1,4,7-triazacyclononane in the presence of potassium carbonate provided hexametyl



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



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

ester intermediate **5**. Hydrolysis of **5**, and subsequent EDC-promoted esterification reaction with pentafluorophenol provided the cyclization precursor. Macrocyclization between the dye-labled diTFA salts of **6** and hexakis-pentafluorophenyl ester of **5** in high dilution condition provided **2** with 47% yield.

To test 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.[7,8] 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.

With receptor **1**, the binding data in Table 1 reveal a number of notable trends. First, high selectivity was observed for the residue in AA1, composed of amino acid with a hydrogen bond donor/acceptor group in the side chain such as Gln, Asn and Lys. Second, selectivities were also found for AA2 and AA3 position. The substrates with Pro (10 of 15) and Gly (7 of 15) at AA2 and AA3 position were found to bind strongly.

Also, the binding data in Table 1 reveal that receptor **2** show some peptide binding selectivities. Although there were little selectivity at AA3 position, high selectivities were observed for the residue in AA1 and AA2. For example, substrates with Pro (5 of 12) and Gly (7 of 12) at AA1, and amino acid with a hydrogen bond donor/acceptor group in the side chain such as (D)-Asp, (D)-Glu, (D)-Ser, (D)-Gln and (D)-Asn at AA2 position were found to bind strongly.

To confirm the findings and to estimate the energetic extents of the selectivities observed, the most tightly bound peptide with **1** and **2**, Resin-(L)Gln-(D)Pro-Gly-Ac and Resin-(D)Pro-(D)Asp-(L)Ala were resynthesized and their associations with **1** and **2** measured in $CHCl_3$.[9] The binding energies were found to be -4.0 and -4.2 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-(L)Ala-(L)Ala-(L)Ala ,which is not found in assay, were found to be both less than -0.5 kcal/mol.

In summary, receptors **1** and **2** which are readily accessible

Table 1. Sequences selected by binding assay with receptors (**1** and **2**)

	1	**2**
Entry	**Resin-AA1-AA2-AA3-Ac**	**Resin-AA1-AA2-AA3-Ac**
1	(L)Gln-(D)Pro-Gly	(D)Pro-(D)Asp-(L)Ala
2	(L)Gln-(D)Pro-Gly	(D)Pro-(D)Asp-(L)Leu
3	(D)Gln-(D)Pro-Gly	Gly-(D)Asp-(L)Phe
4	(D)Gln-(L)Pro-(L)Ala	Gly-(D)Asp-(L)Val
5	(L)Gln-(L)Pro-(L)Leu	(D)Pro-(D)Glu-Gly
6	(D)Gln-(D)Ala-Gly	Gly-(D)Glu-(L)Gln
7	(L)Gln-(D)Ala-(D)Ala	Gly-(D)Asn-(L)Ser
8	(D)Gln-(L)Ala-(D)Ala	(D)Pro-(D)Gln-(L)Ala
9	(L)Asn-(L)Ala-Gly	(D)Pro-(D)Ser-Gly
10	(D)Asn-(D)Ala-(L)Ala	Gly-(D)Ser-(L)Ala
11	(D)Asn-(L)Pro-(D)Ala	(D)Pro-(D)Ser-(L)Val
12	(L)Asn-(D)Pro-Gly	Gly-(D)Asn-(L)Leu
13	(L)Asn-(L)Pro-(D)Ala	
14	(L)Lys-(L)Pro-Gly	
15	(D)Lys-(D)Pro-(D)Ala	

C_2- and C_3-symmetric macrocycles from trimesic acid and 1,2-diaminocyclohexane 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 3. To a solution of 300 mg of citrazinic acid methyl ester (1.80 mmol), 855 mg of N-Boc-aminobenzyl alcohol (3.60 mmol), 1.04 g of triphenylphosphin (3.96 mmol) in 100 mL of CH_2Cl_2 was added 0.63 mL of DEAD (3.96 mmol) at 0 °C. After the stirring for 24 hr at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 1% MeOH in methylene chloride to give methyl ester of **3** as an amorphous white solid (790 mg, 72%): ^{1}H NMR ($CDCl_3$) δ (ppm) 1.35 (s, 18H), 3.95 (s, 3H), 4.33 (s, 4H), 6.95 (br, 2H), 7.16 (4H, d, *J* = 8.5 Hz), 7.65 (4H, d, *J* = 8.5 Hz), 7.90 (s, 2H).

To a solution of 300 mg of methylester of **3** (0.494 mmol) in 30 mL of THF, 10 mL of MeOH and 3 mL of water was added 1.0 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 to pH=4 and extracted into EtOAc. The crude carboxyl acid was dissolved in 20 mL of THF and 20 mL of methylene chloride, and 0.17 g of dimethylaminopyridine (DMAP, 0.74 mmol), 0.14 g of EDC (0.74 mmol) and 0.23 g of Disperse Red I (0.74 mmol) were added at 0 °C. After the stirring for 24 hr at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 5% methanol in methylene chloride to give **3** as an amorphous red solid (295 mg, 45%): ^{1}H NMR ($CDCl_3$) δ (ppm) 1.15 (3H, t, *J* = 7.5 Hz) 1.39 (s, 18H), 2.95 (2H, q, *J* = 7.5 Hz), 3.52 (t, 2H, *J* = 7.5 Hz), 3.95 (t, 2H, *J* = 7.0 Hz), 4.21 (s, 4H), 6.85 (br, 2H), 7.16 (4H, d, *J* = 8.5 Hz), 7.50 (2H, d, *J* = 7.5 Hz), 7.75 (4H, d, *J* = 8.5 Hz), 7.85 (4H, m), 7.95 (s, 2H), 8.21 (2H, d, *J* = 7.5 Hz); MS (FAB) *m/z* 859 (M+1).

Synthesis of 1. To a solution of 0.144 g of **3** (0.163 mmol) and 0.1 mL of anisole in 10 mL of methylene chloride was added 3 mL of TFA. After stirring for 2 h at r.t., all volatiles were removed at reduced pressure. The crude diTFA salts were used the next reaction without further purification. A solution of the resulting diTFA salts of **3** and 150 mg of bis(pentafluorophenyl)ester (**4**, 0.163 mmol) in 10 mL of DMA was added to a solution of 0.28 mL of DIPEA (1.63 mmol) in 200 mL of THF at room temperature for 20 hr by syringe pump. After the stirring for 24 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** (130 mg, 65.0%): ^{1}H NMR ($CDCl_3$/CD_3OD) δ (ppm) 1.05 (m, 4H), 1.21 (3H, t, *J* = 7.5 Hz) 1.32 (m, 4H), 1.67 (m, 4H), 2.06 (m, 4H), 2.84 (2H, q, *J* = 7.5 Hz), 3.72 (t, 2H, *J* = 7.5 Hz), 3.90 (t, 2H, *J* = 7.0 Hz), 4.32 (br, 4H), 4.50 (m, 2H), 4.73 (m, 2H), 7.21 (4H, d, *J* = 8.5 Hz), 7.35 (2H, d, *J* = 7.5 Hz), 7.54 (s, 2H), 1.05 (m, 4H), 1.21 (3H, t, *J* = 7.5 Hz)1.32 (m, 4H), 1.67 (m, 4H), 2.06 (m, 4H), 2.84 (2H, q, *J* = 7.5 Hz), 3.72 (t, 2H, *J* = 7.5 Hz), 3.90 (t, 2H, *J* = 7.0 Hz), 4.32 (br, 4H), 4.50 (m, 2H), 4.73 (m, 2H), 7.21 (4H, d, *J* = 8.5 Hz), 7.35 (2H, d, *J* = 7.5 Hz), 7.54 (s, 2H), 7.65 (4H, d, *J* = 8.5 Hz), 7.83 (s, 2H), 7.95 (4H, m), 8.04 (s, 2H), 8.15 (s, 2H), 8.31 (2H, d, *J* = 7.5 Hz); IR (neat) 3422, 2865, 1676, 1654, 1570 cm^{-1}; MS (FAB) *m/z* 1203 (M+1).

Synthesis of 5. To a solution of 100 mg of 1,4,7-triazanonane (0.775 mmol) and 380 mg of potassium carbonate (2.71 mmol) in 10 mL of DMF was added 0.78 g of 5-bromomethylisophthalic acid dimethyl ester (2.71 mmol). After the stirring for 24 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 methyl ester of **5** as an amorphous white solid (1.03 g, 51.0%): ^{1}H NMR ($CDCl_3$) δ (ppm) 2.56 (s, 4H), 2.78 (s, 2H), 3.98 (s, 6H), 7.41 (s, 2H), 7.75 (s, 1H).

Synthesis of 2. To a solution of 500 mg of methylester of **5** (0.669 mmol) in 20 mL of THF, 5 mL of MeOH and 3 mL of water was added 5 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 then all volatiles were removed at reduced pressure. The crude carboxyl acid was dissolved in 30 mL of THF and 5 mL of DMF, and then 0.89 g of EDC (4.68 mmol), and 0.95 g of pentafluorophenol (5.01 mmol) were added at 0 °C. After the stirring for 24 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 in the presence of 1% triethylamine to give pentafluoropheny ester of **5** as an amorphous white solid (0.65 g, 57.3%): ^{1}H NMR ($CDCl_3$) δ (ppm) 2.74 (s, 4H), 2.88 (s, 2H), 7.58 (s, 2H), 7.78 (s, 1H).

A solution of the resulting 300 mg of hexakis(pentafluorophenylester) (0.176 mmol) and 0.37 g of diTFA salt, **6** (0.528 mmol) in 10 mL of DMA was added to a solution of 0.3 mL of DIPEA (1.76 mmol) in 200 mL of THF at room temperature for 20 hr by syringe pump. After the stirring for 24 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, **2** (170 mg, 47.0%): ^{1}H NMR ($CDCl_3$/CD_3OD) δ (ppm) 1.09 (t, 3H, *J* = 7.2 Hz), 1.32 (3H, t, *J* = 7.5 Hz), 2.10 (t, 2H, *J* = 7.0 Hz), 2.21 (m, 4H), 2.30 (t, 2H, *J* = 7.0 Hz), 2.38 (d, 1H, *J* = 10.5 Hz), 2.45 (m, 4H), 2.56 (d, 2H, *J* = 10.5 Hz), 2.65 (m, 2H), 2.78 (m, 2H), 2.89 (q, 2H, *J* = 7.2 Hz), 3.33 (d, 2H, *J* = 7.5 Hz), 4.76 (m, 1H), 4.98 (m, 1H), 7.56 (s, 1H), 7.75 (s, 1H), 7.83 (s, 1H), 7.95 (4H, bs), 8.04 (d, 2H, *J* = 7.5 Hz); IR (neat) 3410, 2912, 1681, 1645, 1566 cm^{-1}; MS (FAB) *m/z* 2048 (M+1).

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7. AAn = Any possible combinations of 25 (α)-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, (D)Ser, (L)Asp, (D)Asp, (L)Glu, (D)Glu, (L)Asn, (D)Asn, (L)Gln, (D)Gln, (L)Lys, (D)Lys, (L)His, (D)His. The number of members in substrates library is $(25)^3$, 15625.
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