Synthesis and Peptide-binding Properties of C_3-symmetric Metallomacrocycles

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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,[1] many molecular receptors capable of interacting selectively with various substrates have been described.[2] To construct such receptors, chemists use the multi-step synthetic methodology to make macrocyclic structures that have the shape and functionalities complement to those of a given substrate. However, the macrocyclization reactions required much synthetic effort and the general applicability of these reactions still is limited because these are good for certain types of substrates only. Recently, an alternative method in which a metallomacrocyclic receptor self-assembles from a flexible ligand by exploiting metal-ligand coordinate bond is recognized as an efficient method to construct molecular receptors.[3]

Here, we describe C_3-symmetric metallomacrocyclic receptors with binding sites having convergent hydrogen bonding donor/acceptor functionalities, as well as hydrophobic surface.

Synthesis of receptor **1** began with the preparation of the flexible ligand (**5**), as shown in Scheme 1. DMAP-promoted amide coupling reaction between tris-pentafluorophenyl ester **3** and (L)-phenylalanine bipyridyl amide **4** provided the flexible ligand **5**. Metallomacrocycle **1** was prepared as dark red solids in 39.1% yield by mixing $FeCl_2 \cdot 4H_2O$ (1/3 eq) and ligand **5** in ethylene glycol, stirring for 12 hrs under a reflux condition, then adding the saturated NH_4PF_6 aq. solution. Similar amide coupling reaction using **6** and subsequent metalation reaction provided metallomacrocycle **2** as dark red solids in 43.2% yield.

The structures of **1** and **2** were established by mass spectrum, ^{1}H NMR spectroscopy and UV spectroscopy. In ^{1}H NMR spectra of **1** and **2**, upon complexation with Fe(II) the resonance peaks from all protons of ligand turn to be very broad due to Fe(II) ion.[4] In UV/VIS spectra of **1** and **2**, there are bands at 532 nm typical of metal-to-ligand charge transfer band of [Fe(bpy)$_3$] type complexes. This observation is well compatible with the proposed structrures. In mass spectra of **1** and **2**, the detection of peaks arising from 1666 $(M\text{-}PF_6)^+$ and 1811 $(M\text{-}PF_6)^+$ confirms the proposed structures.

Recently, combinatorial chemistry has become a major tool in the elucidation of the binding properties of receptors.[5] Receptors **1** and **2** have the distinct red colors due to transition metal ion (Fe(II)), and thus ideal for solid phase color binding assay using encoded combinatorial library of peptide substrates.

Receptors **1** and **2** were screened against a tripeptide library on hydrophobic polystyrene in $CHCl_3$.[6] 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] Decoding the tripeptides on the colored beads by using electron capture gas chromatography revealed selective peptide-binding property of receptors (**1** and **2**). The most tightly binding substrates with macrocyclic compounds (**1** and **2**) are shown in Table 1.

The binding data in Table 1 reveal a number of notable trends. For example, receptor **1** was found to bind strongly

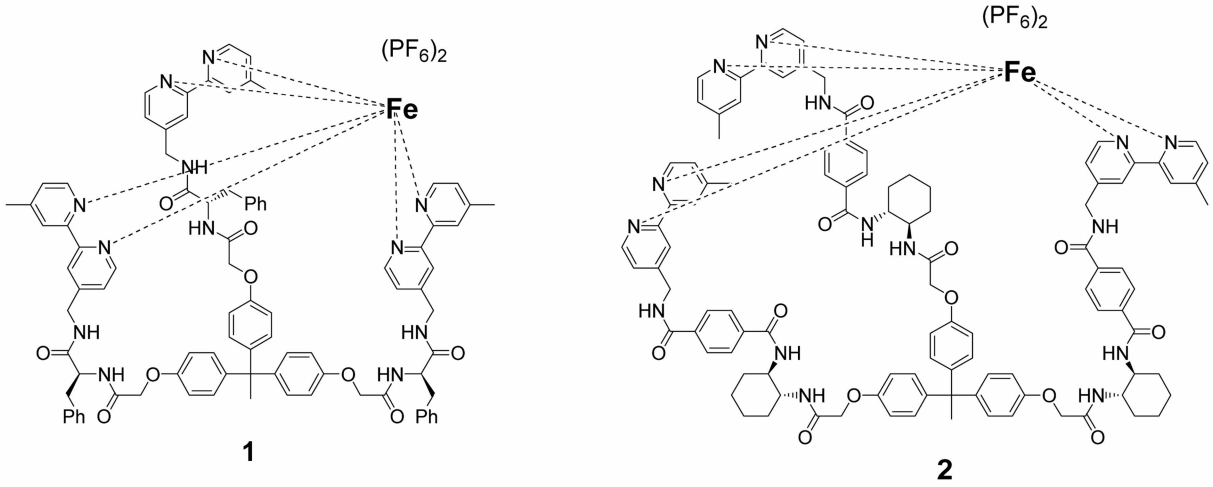
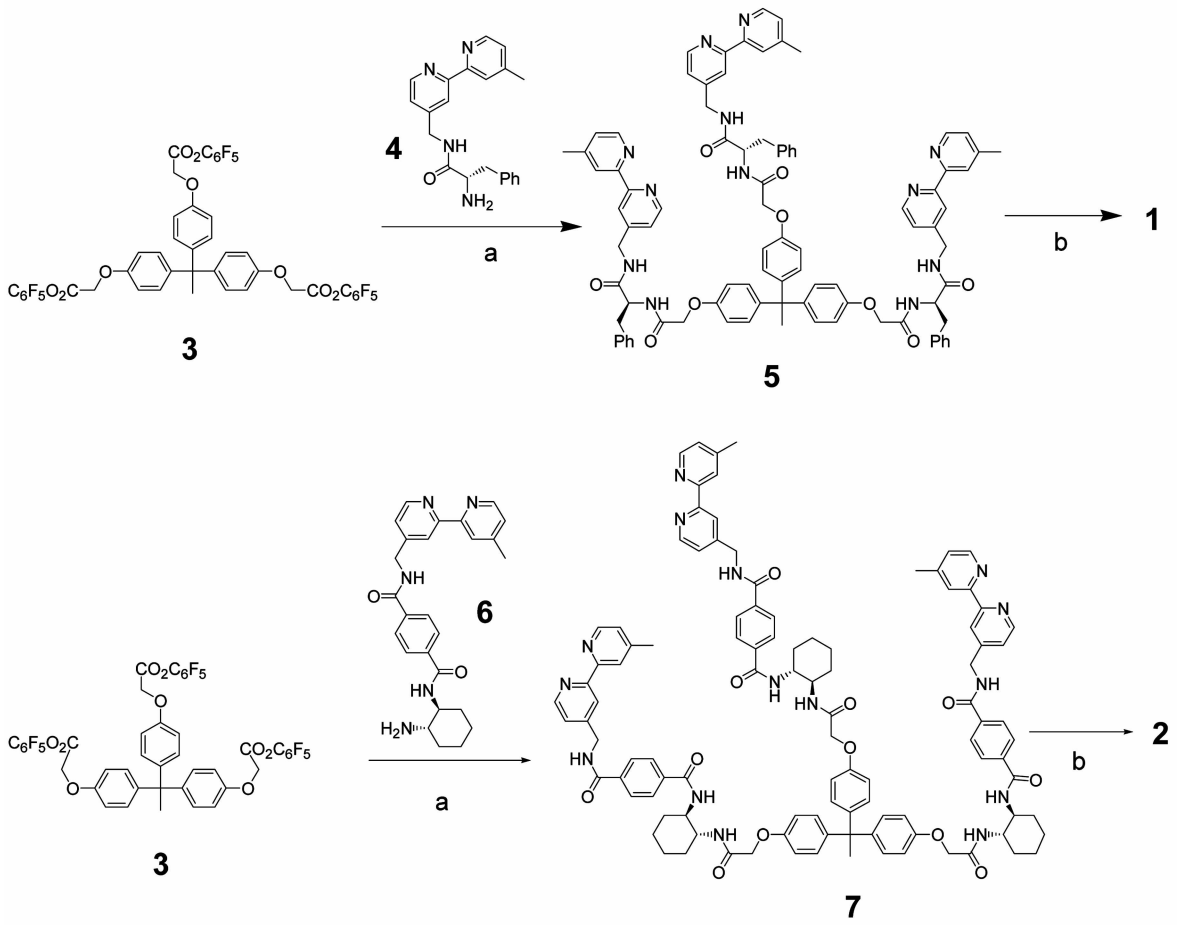


Figure 1. Structures of Metallomacrocycles (**1** and **2**).



Scheme 1. Synthesis of Metallomacrocycles **1** and **2**: (a) NEt_3/DMAP, (b) $FeCl_2$, then NH_4PF_6.

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

Entry	1	Entry	2
1	Gly-(D)Pro-Gly	1	(L)Gln-(D)Pro-(L)Ala
2	Gly-(D)Pro-Gly	2	(L)Gln-(D)Pro-(L)Ala
3	(D)Gln-(D)Pro-Gly	3	(L)Gln-(D)Pro-(L)Ala
4	Gly-(D)Pro-Gly	4	(L)Gln-(D)Pro-(L)Ala
5	(D)Gln-(D)Pro-Gly	5	(L)Gln-(D)Pro-(D)Ala
6	Gly-(D)Pro-Gly	6	(L)Gln-(D)Ala-(L)Ala
7	Gly-(D)Pro-Gly	7	(L)Gln-(L)Ala-(D)Leu
8	(D)Leu-(L)Gln-(D)Gln	8	(L)Asn-(D)Pro-(L)Ala
9	(D)Leu-(D)Pro-Gly	9	(L)Asn-(D)Pro-(L)Ala
10	(D)Gln-(D)Pro-(L)Phe	10	(L)Asn-(L)Ala-(L)Ala
11	(L)Leu-(L)Gln-Gly	11	(L)Asn-(D)Ala-(L)Ala
12	Gly-(L)Gln-Gly	12	(L)Asn-(L)Ala-Gly
13	(D)Leu-(D)Lys-Gly	13	(L)Lys-(D)Pro-(D)Val
14	(D)Leu-(D)His-Gly	14	(L)Lys-(D)Ala-(L)Val
15	(L)Phe-(L)Gln-(L)Phe	15	(L)Lys-(L)Ala-(D)Ala
16	(D)Leu-(D)Pro-(L)Phe		
17	Gly-(L)Pro-Gly		

with the substrate with Gly (7/17), (D)Pro (9/17) and, Gly (13/17) at AA1, AA2 and AA3 positions, respectively. Also, receptor **2** was found to bind strongly with the substrate with (L)Gln (7/15), (D)Pro (8/15) and, (L)Ala (10/15) at AA1, AA2 and AA3 positions, respectively.

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

Peptide	Binding Energy (kcal/mol)	Found in Assay
Polymer-Gly-(D)Pro-Gly-Ac	−5.04	Yes
Polymer-(D)Gln-(D)Pro-Gly-Ac	−4.52	Yes

Table 3. Binding of **2** and Peptides in $CHCl_3$

Peptide	Binding Energy (kcal/mol)	Found in assay
Polymer-(L)Gln-(D)Pro-(L)Ala-Ac	−4.10	Yes
Polymer-(L)Ala-(D)Pro-(L)Ala-Ac	−3.00	No
Polymer-(L)Gln-(D)Pro-(L)Gln-Ac	−2.10	No

To confirm the findings and to estimate the energetic extents of the selectivities observed, several peptides were resynthesized and their association with **1** and **2** was measured in $CHCl_3$.[8] The results are summarized in Table 2 and 3.

The binding energies of peptides with **1** were found to be −4.52-5.04 kcal/mol. The other substrates found by binding assay are expected to have the similar range of binding energies.

The binding data in Table 3 showed that the most tightly

bound peptides, Resin-(L)Gln-(D)Pro-(L)Ala-Ac was found to bind to **2** with −4.1 kcal/mol binding energy. Removal of amide group in the side chain of substrate from Gln to Ala at AA1 sites reduce binding energy by 1.1 kcal/mol. Furthermore, the changes in the side-chain from (L)Ala to (L)Gln and (D)Ala at AA3 sites reduce the binding energies by 2.0 kcal/mol. These data suggest that hydrogen bondings and hydrobobic interactions are crucial for complexation between receptor **2** and tripeptide substrates

In conclusion, a receptor-like molecule with the well-defined binding cavity was successfully prepared by exploiting coordinate bond between transition metal and ligand. Furthermore, combinatorial binding studies revealed that these metal-templated self-assembling receptors have the highly selective peptide-binding properties. Further studies on the structures of complexes between receptor and peptide substrates, and the peptide-binding properties of the other related synthetic receptors are in progress in this laboratory.

Experimental Section

Synthesis of 5. To a solution of 0.47 g of **4** (1.10 mmol) in 15 mL of THF were added 0.29 g of **3** (0.290 mmol), 0.46 ml of NEt_3 (3.30 mmol) and 12.2 mg of DMAP (0.110 mmol) at room temperature. 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 3% MeOH in methylene chloride to give **5** as an amorphous white solid (0.23 g, 54.0%): ^{1}H NMR ($CDCl_3$) δ (ppm) 2.073 (s, 1H), 2.411 (s, 3H), 3.099 (m, 1H), 3.185 (m, 1H), 4.427 (M, 4H), 4.811 (m, 1H), 6.631 (d, 2H, J = 12.0 Hz), 6.953 (d, 2H, J = 9.50 Hz), 7.101 (m, 1H), 7.188 (m, 5H), 7.266 (m, 1H), 8.146 (d, 1H, J = 1.0 Hz), 8.184 (t, 1H, J = 1.0 Hz), 8.512 (m, 1H), 8.546 (m, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 21.943, 31.408, 38.690, 43.208, 46.294, 54.894, 67.869, 114.723, 120.450, 122.876, 125.649, 127.922, 129.544, 130.014, 136.801, 143.543, 149.402, 150.145, 155.892, 156.977, 169.211, 171.200; IR (KBr) 3395, 3268, 2934, 1656, 1639, 1561, 1543, 1493, 1454, 1430 cm^{-1}; UV ($CHCl_3$) 230, 300 nm; Mass (FAB) m/z = 1467 $(MH)^+$.

Synthesis of 1. To a solution of 50 mg of **5** (0.0340 mmol) in 60 mL of ethylene glycol was added 7 mg of $FeCl_2{\cdot}4H_2O$ (0.0353 mmol). After stirring for 12 hr at 160 °C, 100 mg of NH_4PF_6 was added to precipitate the crude products. The crude products were recrystallized from MeOH/ethyl ether to give **1** as an amorphous dark-red solid (23 mg, 39.1%): ^{1}H NMR ($CDCl_3$) δ (ppm) 1.925-2.073 (br, 1H), 2.523 (m, 1H), 2.960-3.161 (br, 2H), 4.031-4.829 (br, 5H), 6.517-6.837 (br, 4H), 7.039-7.530 (br, 11H), 8.683-8.832 (br, 2H); IR (KBr) 3402, 3256, 2945, 1645, 1625, 1560, 1547 cm^{-1}; UV ($CHCl_3$) 230, 300, 357, 532 nm; Mass (FAB) m/z = 1666 $(M\text{-}PF_6)^-$.

Synthesis of 7. To a solution of 0.41 g of **6** (0.75 mmol) in 15 mL of THF were added 0.19 g of **3** (0.225 mmol) and 0.40 mL of iPr$_2$NEt (2.25 mmol) at room temperature. 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 3% MeOH in methylene chloride to give **7** as an amorphous white solid (0.25 g, 58.0%): ^{1}H NMR (DMSO-d_6) δ(ppm) 1.23 (m, 2H), 1.42 (m, 2H), 1.68 (m, 2H), 1.86 (m, 2H), 2.38 (s, 3H), 2.43 (m, 1H), 3.75 (m, 1H), 3.87 (m, 1H), 4.32 (dd, 2H, J = 15.0, 8.0 Hz), 4.58 (d, 2H, J = 2.96 Hz), 6.68 (m, 4H), 7.22 (m, 1H), 7.34 (m, 1H), 7.84 (dd, 2H, J = 4.40, 4.11 Hz), 7.93 (m, 3H), 8.21 (m, 1H), 8.33 (m, 2H), 8.45 (dd, 1H, J = 2.35, 2.93 Hz), 8.57 (dd, 1H, J = 2.35, 2.93 Hz), 8.69 (t, 1H, J = 6.16 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 21.849, 25.681, 32.688, 32.762, 43.296, 46.888, 51.148, 53.598, 53.767, 56.060, 68.091, 114.993, 120.017, 122.421, 123.733, 125.071, 128.319, 128.484, 130.159, 137.141, 138.378, 142.870, 149.036, 150.104, 150.306, 150.756, 156.146, 156.550, 156.756, 166.803, 166.915, 168.804; Mass (FAB) m/z = 1758 $(MH)^-$.

Synthesis of 2. To a solution of 50 mg of **7** (0.0320 mmol) in 30 mL of ethylene glycol was added 6.4 mg of $FeCl_2{\cdot}4H_2O$ (0.0320 mmol). After stirring for 12 hr at 160 °C, a solution of 100 mg of NH_4PF_6 in 5 mL of H_2O was added to precipitate the crude products. The crude products were recrystallized from MeOH/ethyl ether to give **2** as an amorphous dark red solid (24 mg, 43.2%): ^{1}H NMR (DMSO-d_6) δ (ppm) 1.29 (m, 2H), 1.38 (m, 2H), 1.58 (m, 2H), 1.81 (m, 2H), 2.42 (m, 4H), 3.81 (m, 2H), 4.43 (br, 4H), 6.97-7.21 (br, 6H), 7.80-7.99 (br, 5H), 8.21-8.41 (br, 2H), 8.63-8.92 (br, 4H); UV ($CHCl_3$) 232, 302, 537 nm; Mass (FAB) m/z = 1811 $(M\text{-}PF_6)^+$.

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6. 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(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*.
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