Synthesis and Sequence-selective Peptide-binding Properties of a C_2-symmetric Metallomacrocycle

Kum Hee Lee and Seung Soo Yoon*

*Department of Chemistry, SungKyunKwan University, Suwon 440-746, Korea. *E-mail: ssyoon@chem.skku.ac.kr*
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The development of synthetic receptors having selective binding properties with a variety of molecules has been an area of active research in recent years.[1] For effective recognition, a receptor must have the preorganized binding site having the shape and functionalities to complement with those of a given substrate. So far, the greatest difficulty in making such receptors is the multistep organic synthesis needed to create structurally distinct macrocyclic molecules.[2] Self-assembly by exploiting noncovalent interactions such as metal-ligand coordinate bond is now one of alternative strategies in construction of complex synthetic entities.[3] Besides the simplicity of synthetic procedure, one of several advantages using the strategy of intramolecular and intermolecular metal-templated self-assembly to synthesize artificial receptors is that changes in the coordination number and geometry of different metals and ligands can allow the modification of the shape of artificial recognition sites, and thus the fine-tuning of the binding properties of artificial receptors. Previously, we have shown that receptor-like self-assembly could be prepared by exploiting intramolecular coordinate bonds.[4] To expand the scope of these intramolecular self-assembly processes, we describe here a novel C_2-symmetric metallomacrocycle (**1**).

This receptor-like molecule (**1**) has a large nonpolar, conformationally rigidified cavity which is surrounded by polar functionalities. Thus it is reasoned that this can bind certain substrates selectively by hydrogen bondings and hydrophobic interactions. In this receptor-like molecule, metal ion acts to maintain macrocyclic structure, and thus makes potential substrate-binding site to be preorganized for the effective complexation with suitable substrates.[5] Furthermore, metal templated self-assembly **1** has the distinct colors originated from Fe(III) ion. Thus metal ion such as Fe(III) can act as the sensitive probes for binding studies using solid phase substrate library,[6] as well as the chromogenic center for the potential application to chemical sensor.

Synthesis of metallo-macrocycle **1** began with the preparation of the flexible ligand, as shown in Scheme 1. DIC-promoted amide coupling reaction between bis-carboxylic acid[7] and (L)phenylalanine methyl ester, and the subsequent hydrolysis of methyl esters and EDC-promoted ester formation reaction with pentafluorophenol provided bis-pentafluorophenyl ester **2**.

Amide forming reaction of bis-pentafluorophenyl ester **2** with mono-Boc 1,4-phenylene diamine provided **3**. Deprotection of Boc groups and the subsequent imine formation reaction with salicylic aldehyde provided ligand **4**. The

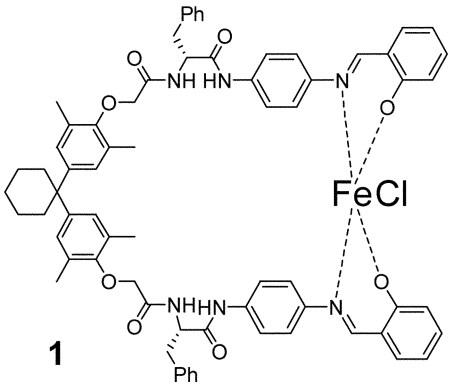
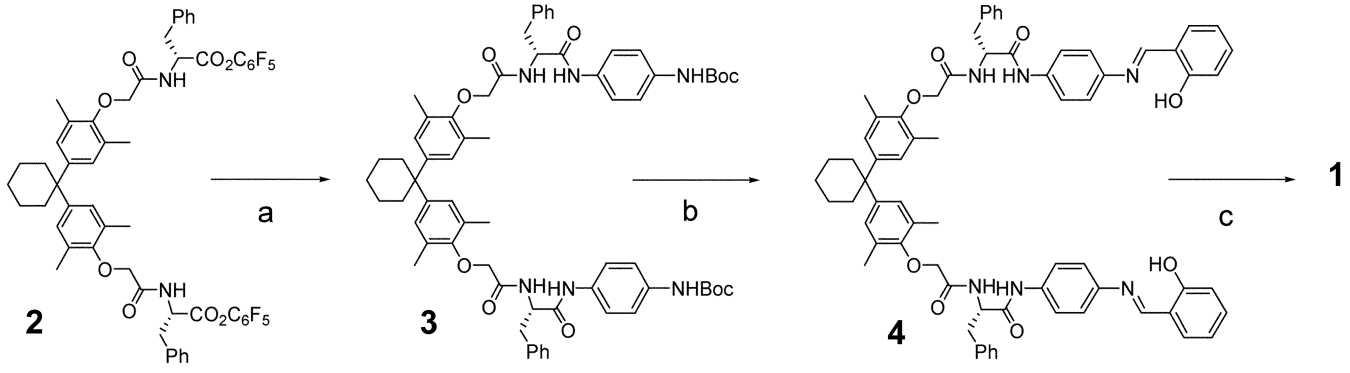


Figure 1. Structure of Metallomacrocycle (**1**).



Scheme 1. Synthesis of Metallomacrocycle (**1**); (a) mono-Boc-1,4-phenylene diamine. (b) TFA, then salicylic aldehyde in EtOH. (c) $FeCl_3$.

Fe(III) complex **1** was prepared as dark red solids with 52% yield by mixing 1 eq. of $FeCl_3$ and the corresponding ligand in ethanol, stirring for 5 hrs under refluxing condition, then adding diethyl ether. Although Fe(III) complexe **1** did not show clear ^{1}H NMR spectrum due to paramagnetic property of Fe(III) ion,[8] other spectroscopic methods such as IR and UV/VIS spectroscopy, and Mass spectroscopy support the proposed structure of **1**. In IR spectrum of **1**, upon complexation the absorption bands from the imine stretching mode shift from 1660 to 1655 cm^{-1}, respectively. Also, in UV/VIS spectra of **1**, upon complexation the absorption peaks of ligand show the red shift from 376 to 567 nm, respectively. These observations are well compatible with the proposed structure. In mass spectrum of **1**, the detection of peaks arising from 1177 $(M\text{-}Cl)^+$ confirms the proposed structure.

Recently, combinatorial chemistry has become a major tool in the elucidation of the binding properties of receptors.[7] Metallomacrocyclic compound (**1**) has the distinct color due to Fe(III) ions, and thus ideal for solid phase color binding assay using encoded combinatorial library of peptide substrates.

Metallomacrocyclic compound (**1**) was 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.[9] Decoding the tripeptides on the colored beads by using electron capture gas chromatography revealed selective peptides-binding properties of Metallomacrocyclic compound (**1**). The most tightly binding substrates 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 with the substrate with (L)Val (5 of 16), (D)Phe (5 of 16) and Gly (8 of 16) 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 peptide, Resin-(L)Val-(D)Phe-Gly-Ac was resynthesized and its associations with **1** measured in $CHCl_3$.[10] The binding energy was found to be –4.3 kcal/mol. The other substrates found by binding assay are expected to have the similar range of binding energies.

In conclusion, a receptor-like molecule with the well-defined binding cavity was successfully prepared by exploiting intramolecular coordinate bonds between transition metal and ligand. This study established that metal-templated self-assembling process is an efficient method to construct artificial receptor-like molecules. Furthermore, combinatorial binding studies revealed that these metallomacrocyclic receptors can have the highly selective peptide-binding properties.

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

Entry	Resin-AA1-AA2-AA3-Ac	Entry	Resin-AA1-AA2-AA3-Ac
1	(L)Ala-(D)Phe-(L)Leu	2	Gly-(D)Phe-Gly
3	(L)Ala-(D)Phe-Gly	4	(L)Val-(D)His-(L)Asp
5	(D)Val-(L)Phe-Gly	6	(L)Asp-(D)His-Gly
7	(D)Val-Gly-(L)Asp	8	(L)Val-Gly-(L)Ala
9	(L)Asp-(L)Glu-(L)Leu	10	(L)Val-Gly-(L)Asp
11	(L)Val-(L)Lys-(L)Asp	12	(L)Leu-(D)Phe-Gly
12	(L)Gln-(D)Phe-Gly	14	(L)Gln-(L)Lys-Gly
13	(L)Val-(D)Leu-(L)Leu	16	(L)Ala-Gly-Gly

Experimental Section

Synthesis of 2. To solution of 600 mg of bis-carboxylic acid (0.816 mmol) in 16 mL of methylene chloride : THF (1 : 1) were added 376 mg of pentafluorophenol (2.041 mmol) and 391 mg of EDC (2.041 mmol). After the stirring for 4 hrs at room temperature under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using (EtOAc : hexane) to give **2** (665 mg, 75%): ^{1}H NMR ($CDCl_3$) δ(ppm) 7.25 (m, 6H, ArH, NH), 6.84 (s, 2H, ArH), 4.25 (s, 2H, CH_2), 3.42 (m, 1H, CH_2), 3.32 (m, 1H, CH_2), 2.14 (br, 2H), 2.10 (s, 6H, CH_3), 1.49 (br, 3H); MS (FAB) *m/z* = 1067 $(MH)^+$.

Synthesis of 3. To solution of 225 mg of the bis-pentafluorophenyl ester **2** (0.211 mmol) in 8 mL of THF was added 96.6 mg of the mono-Boc-1,4-phyenylene diamine (0.464 mmol). After the stirring for 18 hrs at room temperature under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using (EtOAc : hexane) to give **3** (190 mg, 89.2%): ^{1}H NMR(DMSO-d_6) δ(ppm) 10.06 (s, 1H, NH), 9.25 (br, 1H, NH), 8.02 (d, J = 8.5 Hz, 1H, NH), 7.45-6.91 (m, 11H, ArH), 4.8-4.77 (q, J_{AB} = 3.5/J_{BC} = 5.0 Hz, 1H, CH), 4.20-4.06 (dd, $J_{AB,CD}$ = 14.5/J_{BC} = 38.5 Hz, 2H, CH_2), 3.12-3.01 (m, 2H, CH_2), 2.14 (br, 2H), 2.09 (s, 6H, CH_3), 1.45 (s, 9H, CH_3), 1.40 (br, 3H); MS (FAB) *m/z* = 1116 $(MH)^+$.

To a solution of 180 mg of **3** (0.156 mmol) in 8 mL of dichloromethane was slowly added 2 mL of TFA. After stirring for 4 hrs at room temperature, all volatiles were removed at reduced pressure. The crude di-TFA salts of **3** were used the next reaction without further purification.

Synthesis of 4. To solution of 180 mg of the di-TFA salts of **3** (0.156 mmol) in 35 mL of EtOH was added 0.1 mL of TEA (0.623 mmol). After the stirring for 10 min, 0.042 mL of salicyaldehyde (0.39 mmol) was added to the mixture solution. The mixture solution was stirred and heated to reflux for 18 hrs under a nitrogen atmosphere. All volatiles were removed at reduced pressure. The crude products were recrystallized from methylene chloride/hexane to give **4** (119 mg, 60%) that were collected by filtration: ^{1}H NMR (DMSO-d_6) δ(ppm) 13.17 (s, 1H, OH), 10.33 (s, 1H, NH), 8.95 (s, 1H, -CH=N), 8.13-6.92 (m, 16H, ArH, NH), 5.8 (m, 1H), 4.22-4.09 (m, 2H, CH_2), 3.18-3.0 (m, 2H, CH_2), 2.15-1.40 (m, 11H); ^{13}C-NMR (DMSO-d^6) δ(ppm) 9.215, 14.853 17.389, 17.427, 23.374, 23.609, 27.036 32.307, 37.920, 38.852, 45.764, 46.321, 55.683, 70.806, 117.909, 119.790, 119.913, 121.497, 122.400, 122.619, 127.923, 128.459

129.436, 129.531, 129.993, 130.377, 132.913, 133.754, 136.930, 137.223, 137.346, 145.162, 145.203, 145.566, 152.474, 161.754, 162.418, 169.400, 170.155, 170.373; IR (KBr) 1660, 1617, 1512, 1489 cm^{-1}; MS (FAB) *m/z* = 1124 $(MH)^+$.

Synthesis of 1. To a solution of 50 mg of **4** (0.0445 mmol) in 50 mL of EtOH was added 12 mg of iron(III) chloride (0.0445 mmol). After refluxing for 18 hrs under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The crude products were diluted with MC to give **1** as an amorphous brown solid (28 mg, 52%) that were collected by filtration: IR (KBr) 1655, 1708, 1537 cm^{-1}; UV/Vis ($CHCl_3$ soln) 236, 476, 567 nm; MS (FAB) *m/z* = 1177 $(M\text{-}Cl)^+$.

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9. 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; 8. A total of 15 tag molecules (five tags for AAn) were used to encode the library according to the method reported in *reference 6*.
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