Sequence-selective Peptide-binding Metallomacrocycles

Kum Hee Lee and Seung Soo Yoon*

*Department of Chemistry, SungKyunKwan University, Suwon 440-746, Korea. *E-mail: ssyoon@chem.skku.ac.kr*

Received August 17, 2005

Key Words : Molecular recognition, Metallomacrocycle, Peptide-binding

Since the pioneering work[1] of Pederson, Cram and Lehn, many molecular receptors capable of interacting selectively with various substrates have been described.[2] Particularly, the development of peptide-binding receptors[3] is of great interest because of its relevance to peptide-protein recognition processes in biological systems.

Recently, self-assembly by exploiting noncovalent interactions such as metal-ligand coordinate bond is emerging as a novel strategy in construction of peptide-binding synthetic molecules.[4]

Here, as the continuing efforts to develop selective peptide-binding receptors, novel C_2-symmetric metallomacrocycles are described.

Syntheses of receptors **1**-**4** began with the preparation of the flexible ligand (**9**), as shown in Scheme 1. DIC-promoted amide coupling reaction between N-Boc-(L)-phenylalanine and 4,4'-methylenedianiline provided the starting material **5**. DMAP-catalyzed amide coupling reaction between Boc-deprotected bis-amine of **5** and bis-pentafluorophenyl ester **6**,[5] and the subsequent deprotection of allyl groups and imine formation with benzyl amine provided the ligand **9**. Metallomacrocycles (**1**-**4**) were prepared by mixing ligand **9** and the corresponding metal chloride, acetate or acetoacetonate in ethanol, stirring for 12 hrs under reflux conditions with 55, 52, 49 and 45%, respectively.

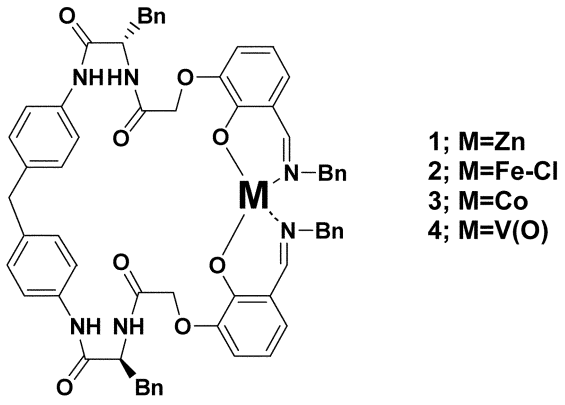
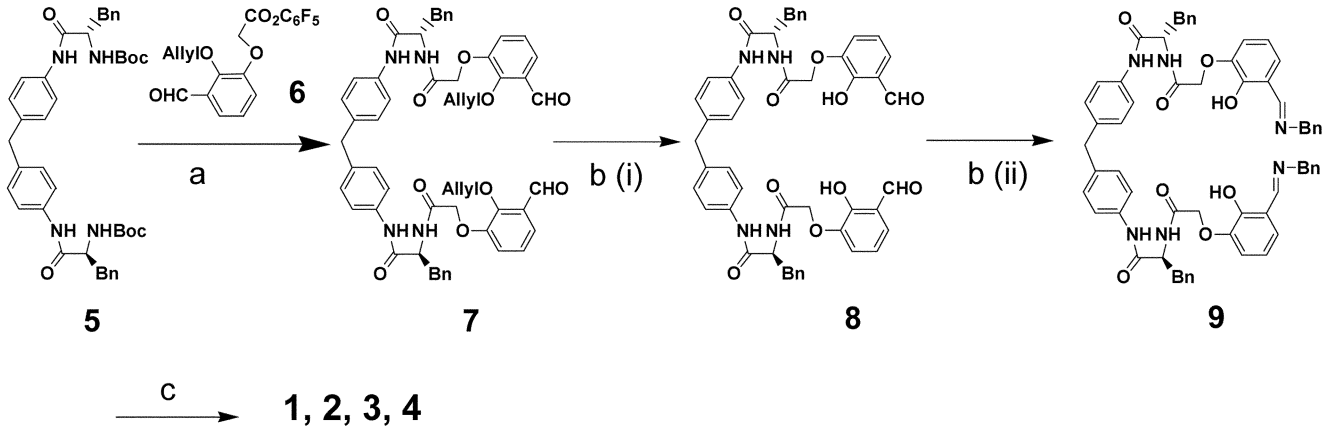


Figure 1. Structures of Metallomacrocycles (**1**-**4**).

The products, metal complexes (**1**-**4**) are air-stable and moisture-insensitive, and the structures of **1**-**4** were established by mass spectrum, ^{1}H NMR spectroscopy, IR and UV spectroscopy.

Recently, combinatorial chemistry has become a major tool in the elucidation of the binding properties of receptors.[6] Receptor **2** has the distinct red color due to transition metal ion (Fe(III)), and thus ideal for solid phase color binding assay using encoded combinatorial library of peptide substrates.

Receptor **2** was screened against a tripeptide library on hydrophobic polystyrene in $CHCl_3$.[7] 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.[8] Decoding the tripeptides on the colored beads by using electron capture gas chromatography revealed selective peptides-binding properties of receptor (**2**). The most tightly binding substrates with macrocyclic compounds (**2**) are shown in Table 1.



Scheme 1. Syntheses of Metallomacrocycles (**1**-**4**); (a) TFA, then NEt_3/DMAP with **6**. (b) i. $Pd(OAc)_2$, $P(Ph)_3$, $HCOONHEt_3$, ii. Benzyl amine in EtOH. (c) reflux in EtOH with $Zn(OAc)_2$ for **1**, $FeCl_3$ for **2**, $Co(OAc)_2$ for **3** and V(O)$(acac)_2$ for **4**.

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

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

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

Peptide	Binding Energy (kcal/mol)
Polymer-(L)Ala-(D)Leu-(L)Ala-Ac	−4.45
Polymer-(L)Ala-(D)Leu-(L)Asn-Ac	−4.58
Polymer-(D)Val-(D)Leu-(L)Ala-Ac	−4.74
Polymer-(D)Val-(D)Leu-(L)Asn-Ac	−4.86
Polymer-Gly-Gly-Gly-Ac	< −0.5

The binding data in Table 1 reveal a number of notable trends. For example, receptor **2** was found to bind strongly with the substrate with (L)Ala (5/15), (D)Leu and (L)Lys (6/15and 7/15) and, (L)Ala and (L)Asn (4/15 and 3/15) at AA1, AA2 and AA3 positions, respectively.

To confirm the findings and to estimate the energetic extents of the selectivities observed, several peptides were resynthesized and their association with **2** measured in $CHCl_3$.[9] The results are summarized in Table 2. The binding energies were found to be −4.45 ~ −4.86 kcal/mol. The other substrates found by binding assay are expected to have the similar range of binding energies. The binding energy with Polymer-Gly-Gly-Gly-Ac, which was not bound with receptor **2** in assay, was found to be both less than −0.5 kcal/mol.

To test the notion that the modification of the shape of artificial recognition sites using the different metal ions in metallomacrocycles can allow to change the peptide-binding properties of artificial receptors, a polypeptide was synthesized and its association with **1-4** were measured in $CHCl_3$. The results are summarized in Table 3.

The binding data in Table 3 showed clearly that the subtle changes in the coordination number and geometry of different metals can affect markedly the peptide-binding properties of metallomacrocyclic receptors. For example, these data showed that the changes in metal ion from Fe(III) to Zn(II) reduce the binding energies by ~4.7 kcal/mol. Also, changes in metal ion from Fe(III) to Co(II), V(IV) reduce the binding energies by 1.5 and 1.8 kcal/mol, respectively.

Table 3. Binding of **1-4** and Peptide in $CHCl_3$

Peptide	Receptor	Binding Energy (kcal/mol)
Polymer-(D)Val-(D)Leu-(L)Ala-Ac	**1**	~0
	2	−4.74
	3	−3.15
	4	−2.91

In conclusion, receptor-like molecules with the well-defined binding cavity were successfully prepared by exploiting coordinate bond between transition metal and ligands. 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 receptors 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 solution of 1.54 g of N-Boc-(L)-phenylalanine (5.800 mmol) in 6 mL of dichloromethane were added 0.5 g of 4,4'-methylenedianiline (2.052 mmol), 0.783 g of HOBT (5.800 mmol) and 0.91 mL of DIC (5.800 mmol) at 0 °C. After the stirring for 5 hr at room temperature, all volatiles were removed at reduced pressure. The mixture was dissolved in dichloromethane and organic layers was washed with 1 M HCl, saturated $NaHCO_3$, and brine and dried with $MgSO_4$. The residue was purified by flash chromatography on silica gel using 20% ethyl acetate in hexane to give **5** as an amorphous white solid (1.13 g, 65%): ^{1}H NMR ($CDCl_3$) δ (ppm) 9.20 (br, 2H), 7.46 (m, 4H), 7.19 (m, 4H), 7.12 (m, 6H), 7.01 (m, 4H), 6.05 (br, 2H), 4.82 (br, 2H), 3.91 (s, 2H), 3.07 (m, 4H), 1.32 (s, 18H).

To a solution of 0.4 g of **5** (0.577 mmol) in 8 mL of dichloromethane was slowly added 2 mL of TFA. After stirring for 4 hr at room temperature, all volatiles were removed at reduced pressure. The crude di-TFA salts of **5** were used the next reaction without further purification.

Synthesis of 7. To solution of 0.4 g of the di-TFA salts of amine intermediate (0.577 mmol) and 0.58 g of the pentafluorophenylester **6** (1.443 mmol) in 7 mL of DMA was added 0.14 g of DMAP (1.155 mmol) and 0.6 mL of DIEA (3.462 mmol) at 0 °C. After the stirring for 18 hr at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 4% MeOH in dichloromethane to give bis-allyl protected intermediate of **7** as an amorphous white solid (0.26 g, 49%): ^{1}H NMR (DMSO-d_6) δ (ppm) 10.28 (s, 2H), 10.13 (s, 2H), 8.37 (d, J = 8.0 Hz, 2H), 7.47 (m, 4H), 7.24 (m, 10H), 7.18 (m, 2H), 7.14 (m, 4H), 7.06 (m, 4H), 6.05 (m, 2H), 5.31 (d, J = 17.0 Hz, 2H), 5.19 (d, J = 10.5 Hz, 2H), 4.77 (m, 2H), 4.64 (m, 8H), 3.83 (s, 2H), 3.10 (m, 2H), 2.89 (m, 2H).

Synthesis of 8. To solution of 0.138 g of bis-allyl protected compound **7** (0.149 mmol) and 0.6 mL of DIEA (3.462 mmol) in 20 mL of MeOH were added palladium acetate 3.33 mg (10 mol%), triphenyl phosphine 15.58 mg (40 mol%), TEA 0.124 mL (0.891 mmol), and formic acid

0.034 mL (0.891 mmol) and refluxed for 3 hr under a nitrogen atmosphere. The solution was acidified with 1 M HCl and extracted with dichloromethane. The crude products were diluted with dichloromethane to give **8** as an amorphous white solid (74 mg, 59%) that were collected by filtration: ^{1}H NMR (DMSO-d_6) δ(ppm) 10.40 (s, 2H), 10.38 (s, 2H), 10.16 (s, 2H), 8.75 (d, J = 8.5 Hz, 2H), 7.49 (d, J = 5.0 Hz, 4H), 7.29 (m, 5H), 7.22 (m, 5H), 7.12 (m, 8H), 6.81 (m, 2H), 4.82 (m, 2H), 4.56 (d, J = 15.0 Hz, 2H), 4.48 (d, J = 15.0 Hz, 2H), 3.83 (s, 2H), 3.13 (m, 2H), 2.93 (m, 2H).

Synthesis of 9. To a solution of 0.2 g of **8** (0.236 mmol) in 40 mL of EtOH : DMF (1 : 1) was added 0.059 mL of benzylamine (0.542 mmol). After refluxing for 14 hr under a nitrogen atmosphere, the crude products precipitated by adding ethyl ether. The crude products were recrystallized from EtOH/ethyl ether to give **9** as an amorphous yellow solid (184 mg, 76%): ^{1}H- NMR (DMSO-d_6) δ(ppm) 13.80 (br, 2H), 10.07 (s, 2H), 8.71 (s, 2H), 8.27 (d, J = 8.5 Hz, 2H), 7.46 (d, J = 6.5 Hz, 4H), 7.36 (m, 10H), 7.24 (m, 2H), 7.19 (m, 8H), 7.10 (m, 10H), 4.82 (s, 4H), 4.74 (m, 2H), 4.48 (m 4H), 3.83 (s, 2H) 3.06 (m, 2H), 2.87 (m, 2H); ^{13}C-NMR (DMSO-d_6) δ (ppm) 39.043, 41.163, 55.368, 62.263, 69.551, 118.697, 119.184, 119.959, 120.759, 125.795, 127.581, 128.422, 128.892, 129.259, 129.771, 130.051, 130.340, 137.739, 137.780, 138.321, 139.579, 147.848, 153.886, 167.513, 168.973, 170.524; IR (KBr) 1659, 1632, 1521, 1467, 1438 cm^{-1}; MS (FAB) m/z = 1027 $(MH)^+$.

Synthesis of 1. To a solution of 50 mg of **9** (0.0487 mmol) in 40 mL of dichloromethane : MeOH (1 : 1) was added 10.68 mg of zinc(II) acetate dihydrate (0.0487 mmol). After refluxing for 18 hr under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The crude products were recrystallized from dichloromethane/hexane to give **1** as an amorphous white yellow solid (29 mg, 55%): ^{1}H NMR (DMSO-d_6) δ(ppm) 9.95 (m, 2H), 8.37 (s, 2H), 8.26 (d, J = 8.5 Hz, 2H), 7.37 (m, 6H), 7.18 (m, 10H), 7.06 (m, 10H), 6.87 (m, 6H), 6.33 (m, 2H), 4.65 (d, J = 5.5 Hz, 2H), 4.41 (m, 4H), 4.26 (m, 4H), 3.79 (m, 2H), 2.99 (m, 2H), 2.85 (m, 2H); ^{13}C-NMR (DMSO-d_6) δ(ppm) 31.377, 38.479, 54.995, 63.902, 70.505, 113.604, 119.611, 120.291, 126.938, 128.289, 128.677, 129.013, 129.158, 129.516, 129.586, 129.772, 129.887, 130.540, 137.174, 137.310, 137.809, 137.889, 151.227, 162.265, 169.314, 170.025; IR 1658, 1619, 1543, 1514 cm^{-1}; UV/Vis (CH_2Cl_2 soln) 327, 370 nm; MS (FAB) m/z = 1089 $(MH)^+$.

Synthesis of 2. To a solution of 50 mg of **9** (0.0487 mmol) in 48 mL of MC : MeOH (1 : 1) was added 13.15 mg of iron(III) chloride (0.0487 mmol). After refluxing for 18 hr under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The crude products were recrystallized from dichloromethane to give **2** as an amorphous brown solid (28 mg, 52%) that were collected by filtration: IR 1658, 1608, 1541, 1516, 1215 cm^{-1}; UV/Vis (CH_2Cl_2 soln) 333, 520 nm; MS (FAB) m/z = 1081 M^+.

Synthesis of 3. To a solution of 50 mg of **9** (0.0487 mmol) in 40 mL of MC : MeOH (1 : 1) was added 10.54 mg of cobalt(II) acetate tetrahydrate (0.0487 mmol). After refluxing for 18 hr under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The crude products were recrystallized from dichloromethane/ethyl ether to give **3** as an amorphous green solid (26 mg, 49%): IR 1664, 1615, 1516, 1444 cm^{-1}; UV/Vis (CH_2Cl_2 soln) 303, 380 nm; MS (FAB) m/z = 1084 $(MH)^+$.

Synthesis of 4. To a solution of 50 mg of **9** (0.0487 mmol) in 40 mL of MC : MeOH (1 : 1) was added 12.9 mg of vanadyl acetylacetate (0.0487 mmol). After refluxing for 18 hr under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The crude products were dissolved in EtOH and obtained after evaporating from solvent to give **4** as an amorphous green solid (23 mg, 45%): ^{1}H NMR (DMSO-d_6) δ(ppm) 10.15 (d, J = 15.5 Hz, 2H), 8.60 (s, 2H), 8.30 (d, J = 8.5 Hz, 2H), 7.49 (m, 8H), 7.32 (m, 8H), 7.21 (m, 8H), 7.16 (m, 4H), 7.02 (d, J = 3.5 Hz, 2H), 6.97 (d, J = 3.5 Hz, 2H), 6.64 (t, J = 7.0 Hz, 2H), (m, 34H), 4.93 (s, 4H), 4.76 (m, 2H), 4.59 (s, 4H) 3.83 (s, 2H), 3.09 (m, 2H), 2.89 (m, 2H); IR 1658, 1607, 1517, 1454, 997 (V=O) cm^{-1}; UV/Vis (CH_2Cl_2 soln) 333, 370, 424 nm; MS (FAB^+) m/z = 1092 $(MH)^+$.

Acknowledgement. This work was supported by Korea Research Foundation (Grant No. 2000-015-DP0262).

References

1. Cram, D. J. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1093; Lehn, J.-M. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89; Perderson, C. J. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1021.
2. *Comprehensive Supramolecular Chemistry*; Lehn, J.-M., Chair ed.; Pergamon Press: Oxford, 1996; Vol. 1-12; Cho, H.-K.; Kim, H.-J.; Lee, K. H.; Hong, J.-I. *Bull. Korean Chem. Soc.* **2004**, *25*, 1714.
3. Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155 and references therein.
4. For reviews, See: Linton, B.; Hamilton, A. D. *Chem. Rev.* **1997**, *97*, 1669.
5. van Starveren, C. J.; van Eerden, J.; van Veggel, F. C. J. M.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1988**, *110*, 4994.
6. Ohlmeyer, M. H. L.; Swanson, R. T.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922; Jung, J. E.; Yoon, S. S. *Bull. Korean Chem. Soc.* **2003**, *24*, 1525; Lee, K. H.; Yoon, S. S. *Bull. Korean Chem. Soc.* **2003**, *24*, 1692.
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(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*.
9. Yoon, S. S.; Still, W. C. *Tetrahedron* **1995**, *51*, 567.