Syntheses and Peptide-binding Properties of C_2-symmetric Metallomacrocycles

Chang-Yeon Lee, 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 11, 2005

Key Words : Molecular recognition, Metallomacrocycle, Peptide-binding

Over the last decade, much effort in supramolecular chemistry has been devoted to developing efficient synthetic receptors for peptides.[1] Peptides as a substrate are interesting for their biological significance. There are many biochemical or medicinal processes, for examples enzymatic activity, antibody action and bacterial infections, in which a selective molecular interaction of peptide plays a decisive role. Thus study of synthetic receptors capable of selective binding to a specific peptide are not only useful for better understanding of biological processes, but also facilitate the design of sensors, catalysts and new medicinal therapeutics.

Recently, metallomacrocycles which are self-assembled from a flexible ligand by exploiting metal-ligand coordinate bond are recognized as the emerging class of new synthetic receptors for peptide substrates.[2] In metallo-macrocylic receptors, metal acts to maintain macrocyclic structure and thus makes the receptor preorganized for the effective complexation with the corresponding substrates. Besides, certain metals can act chromogenic and catalytic center and thus have the potential applicability for chemical sensors and catalysts. Here, we describe syntheses and peptide binding properties of novel metallomacrocyclic receptors (**1** and **2**).

Syntheses of receptor **1** and **2** began with the preparation of the flexible ligands (**4** and **6**), as shown on Scheme 1. Compounds **3** was prepared by following the standard amide coupling procedures through pentafluorophenyl activated esters from **5**.[3] Amide coupling reaction between bis-pentafluorophenyl ester of **3** and 5-aminomethyl-2,2':6',2''-terpyridine[4] provided the bis-terpyridine ligand of **4**. Metallomacrocycle **1** was prepared as dark red solids with 70.0% yield by mixing $FeCl_2$ and ligand **4** in ethanol, stirring for 12 hrs under reflux condition, then adding the saturated NH_4PF_6 aq. solution. Amide coupling reaction between **5**[3] and (L)-phenylalanine 5-aminomethyl-2,2':6',2''-terpyridine amide provided the bis-terpyridine ligand of **6**. Metallomacrocycle **2** was prepared as dark red solids with 80.0% yield by following the similar synthetic procedures using ligand **6**.

Metallomacrocycles (**1** and **2**) have the well-defined, potential substrate binding cavities having the convergent hydrogen bonding donor/acceptors and the hydrophobic surfaces.

To examine the peptide-binding properties of receptors, **1** and **2** were screened against a tripeptide library on hydrophobic polystyrene in $CHCl_3$.[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.[4] Decoding the tripeptides on the colored beads by using electron capture gas chromatography revealed selective peptides-binding properties of macrocyclic compounds (**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 with the substrate with Gly (8/15) at AA1 position, while there are no significant selectivities for the residues at AA2 and AA3 positions. However, receptor **2** shows different selectivity for the residue at each position of tripeptide

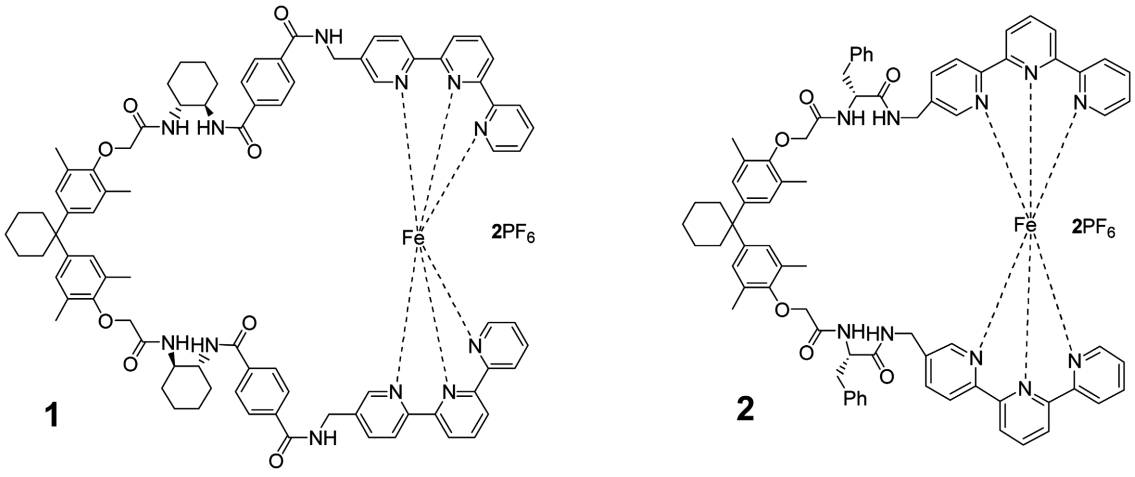
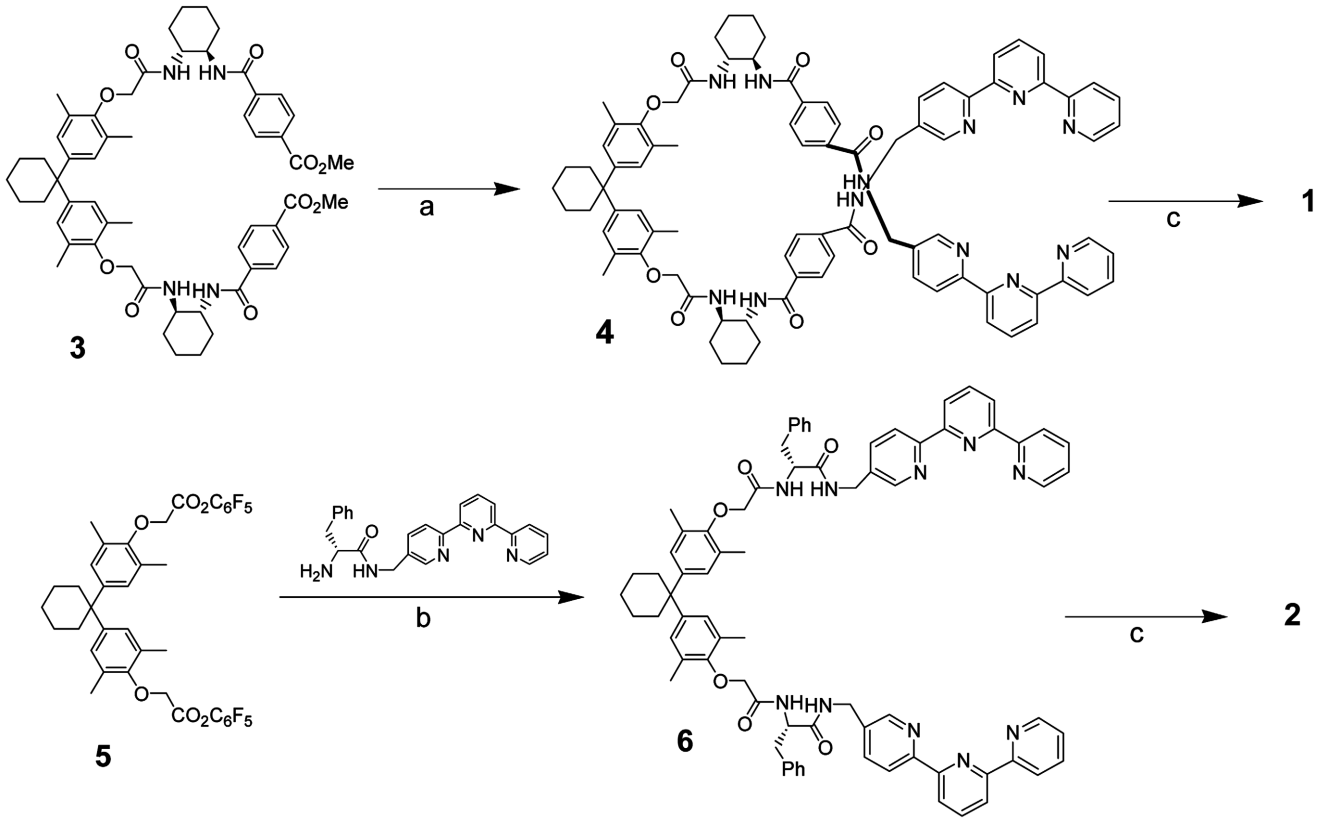


Figure 1. Structures of Metallomacrocycles (**1** and **2**).



Scheme 1. Syntheses of Metallomacrocycles (**1** and **2**); (a) i. NaOH, then C_6F_5OH/EDC, ii. Aminomethylterpyridine/iPr_2NEt/DMA. (b). iPr_2NEt/DMA. (c) $FeCl_2$ then NH_4PF_6.

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

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

substrates. For example, receptor **2** was found to bind strongly with the substrate with Gly (13 of 18), (L)Leu (7 of 18) and (L)Leu (10 of 18) at AA1, AA2 and AA3 position, respectively.

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** measured in $CHCl_3$.[5] The results are summarized in Table 2 and 3.

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

Peptide	Binding Energy (kcal/mol)	Found in Assay ?
Ac-(D)Val-(L)Asp-Gly-Polymer	–4.7	yes
Ac-(L)Val-(L)Asp-Gly-Polymer	–4.6	yes
Ac-(L)Val-Gly-Gly-Polymer	> –0.5	no
Ac-Gly-Gly-Gly-Polymer	> –0.5	no

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

Peptide	Binding Energy (kcal/mol)	Found in Assay ?
Ac-(L)Leu-(L)Leu-Gly-Polymer	–4.5	yes
Ac-(L)Ala-(L)Ala-Gly-Polymer	–4.0	yes
Ac-(L)Ala-Gly-Gly-Polymer	> –0.5	no
Ac-Gly-Gly-Gly-Polymer	> –0.5	no

These data showed that the most tightly bound peptides, Ac-(D)Val-(L)Asp-Gly-Polymer and Ac-(L)Leu-(L)Leu-Gly-Polymer were found to bind to **1** and **2** with –4.7 and –4.5 kcal/mol binding energy, respectively. The binding energy with Polymer-Gly-Gly-Gly-Ac, which was not bound with receptors **1** and **2** in assay, was found to be both less than –0.5 kcal/mol. These data suggest that hydrogen

bondings and hydrobobic interactions are crucial for complexation between receptors (**1** and **2**) and tripeptide substrates.

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

Spectroscopic data of **4**: ^{1}H NMR (DMSO-D_6) δ 1.27-1.44 (br, 7H), 1.69 (s, 2H), 1.89-1.97 (br, 8H), 2.05 (s, 2H), 3.80 (m, 1H), 3.96 (m, 1H), 3.95 (d, 1H, J = 14.5 Hz), 4.06 (d, 1H, J = 14.5 Hz), 4.59 (d, 2H, J = 5.5 Hz), 6.81 (s, 2H), 7.47 (m, 1H), 7.78 (d, 1H, J = 8.0 Hz), 7.86 (d, 2H, J = 8.5 Hz), 7.96 (m, 4H), 8.06 (t, 1H, J = 8.3 Hz), 8.39 (m, 3H), 8.58 (m, 2H), 8.70 (m, 2H), 9.26 (t, 1H, J = 5.8 Hz); ^{13}C NMR (DMSO-d_6) δ 17.27, 23.64, 25.72, 32.83, 37.89, 41.61, 45.40, 53.34, 53.62, 71.31, 121.59, 121.75, 121.82, 125.52, 128.13, 128.27, 128.48, 130.65, 136.77, 137.33, 137.66, 138.28, 138.51, 139.63, 149.78, 150.48, 153.13, 155.09, 155.89, 155.95, 156.23, 166.77, 166.95, 168.95; IR (KBr) 3395, 3268, 2934, 1656, 1639, 1561, 1543, 1493, 1454, 1430 cm^{-1}; UV ($CHCl_3$/MeOH) 224, 280 nm; Mass (FAB) m/z = 1418 $(MH)^+$.

Synthesis of 1. To a solution of 50 mg of **4** (0.0353 mmol) in 10 mL of methanol was added 7 mg of $FeCl_2 \cdot 4H_2O$ (0.0353 mmol). After stirring for 5 hr at room temperature, 50 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 (44 mg, 70.0%): ^{1}H NMR (DMSO-D_6) δ 1.30 (m, 5H), 1.53 (m, 2H), 1.73 (m, 2H), 2.10 (m, 10H), 3.86 (m, 1H), 4.15 (m, 3H), 4.18 (m, 2H), 6.85 (m, 3H), 7.09 (m, 2H), 7.74 (m, 2H), 7.91 (m, 5H), 8.41 (m, 2H), 8.71 (m, 2H), 8.87 (m, 1H), 9.00 (m, 2H); IR (KBr) 3425, 1642, 1542, 1494, 1453 cm^{-1}; UV ($CHCl_3$/MeOH) 224, 276, 325, 553 nm; Mass (FAB) m/z = 1618 $(M\text{-}PF_6)^+$.

Spectroscopic data of **6**: ^{1}H NMR (DMSO-D_6) δ 1.37 (s, 6H), 2.07 (s, 12H), 2.11 (s, 4H), 2.98 (m, 2H), 3.09 (m, 2H), 4.06 (d, 2H, J = 14.5 Hz), 4.19 (d, 2H, J = 14.5 Hz), 4.42 (d, 4H, J = 5.5 Hz), 4.69 (m, 2H), 6.88 (s, 4H), 7.21 (m, 10H), 7.47 (m, 2H), 7.80 (d, 2H, J = 8.0 Hz), 8.01 (m, 4H), 8.08 (t, 2H, J = 7.5 Hz), 8.42 (d, 2H, J = 8.0 Hz), 8.56 (d, 2H, J = 8.0 Hz), 8.61 (m, 4H), 8.71 (d, 2H, J = 5.0 Hz), 8.76 (t, 2H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 17.36, 23.59, 27.01, 37.91, 39.07, 41.61, 45.73, 54.89, 70.83, 121.64, 121.67, 121.69, 121.86, 124.48, 127.82, 128.41, 129.45, 129.96, 130.31, 134.21, 136.96, 137.05, 137.57, 138.58, 145.45, 149.21, 149.83, 152.52, 155.65, 156.01, 156.17, 156.84, 169.91, 171.30; IR (KBr) 3403, 3274, 2924, 1651, 1649, 1551, 1540, 1497, 1455, 1425 cm^{-1}; UV ($CHCl_3$/MeOH) 235, 283 nm; Mass (FAB) m/z = 1224 $(MH)^+$.

Synthesis of 2. To a solution of 83 mg of **4** (0.0678 mmol) in 20 mL of methanol was added 15 mg of $FeCl_2 \cdot 4H_2O$ (0.0690 mmol). After stirring for 3 hr at room temperature, 50 mg of NH_4PF_6 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 (85 mg, 70.0%): ^{1}H NMR (DMSO-D_6) δ 1.42 (s, 6H), 2.02 (s, 12H), 2.15 (s, 4H), 2.75 (d, 1H, J = 8.5 Hz), 2.81 (d, 1H, J = 13.0 Hz), 2.87 (d, 1H, J = 13.5 Hz), 2.99 (d, 1H, J = 9.0 Hz), 4.14 (br, 8H), 4.44 (s, 2H), 6.89 (br, 6H), 7.13 (br, 14H), 7.43 (d, 1H, J = 7.5 Hz), 7.58 (d, 1H, J = 8.0 Hz), 7.85 (d, 1H, J = 6.0 Hz), 7.96 (d, 2H, J = 7.0 Hz), 8.07 (d, 1H, J = 8.0 Hz), 8.38 (m, 2H), 8.69 (m, 6H), 9.17 (m, 4H); IR (KBr) 3412, 2390, 1676, 1527, 1453 cm^{-1}; UV ($CHCl_3$/MeOH) 243, 276, 302, 324, 552 nm; Mass (FAB) m/z = 1423 $(M\text{-}PF_6)^+$.

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

References

1. *Highlights in Bioorganic Chemistry*; Schmuck, C.; Wennermers, H., Eds.; Wiley-VCH: 2004; p 140; Wennemers, H. *Chimia* **2003**, *57*, 237; Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155; Matteo, C.; Wennemers, H. *J. Org Chem.* **2002**, *67*, 2696; Philipp, K.; Wennemers, H. *Synlett* **2005**, 706.
2. For reviews, See: Conn, M. M.; Rebek, Jr. J. *Chem. Rev.* **1997**, *97*, 1647; Linton, B.; Hamilton, A. D. *Chem. Rev.* **1997**, *97*, 1669; Linton, B. R. *Methods in Molecular Biology* **2002**, *201*, 111 and references therein; Kim, K.-J.; Yoon, S. S. *Bull. Korean Chem. Soc.* **1999**, 1387; Lee, K. H.; Yoon, S. S. *Bull. Korean Chem. Soc.* **2003**, *24*, 1692; Du, M.; Bu, X.-H.; Guo, Y.-M.; Ribas, J. *Chemistry-A European J.* **2004**, *10*, 1345; Chichak, K. S.; Cantrill, S. J.; Pease, A. R.; Chiu, S.-H.; Cave, G. W.; Atwood, J. R.; Stoddart, J. R. *Science* **2004**, *304*, 1308.
3. 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.
4. Scott Goodman, M.; Jubian, V.; Hamilton, A. D. *Tetrahedron Lett.* **1995**, *36*, 2551.
5. (a) 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; (b) Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 373; (c) Jung, J. E.; Yoon, S. S. *Bull. Korean Chem. Soc.* **2003**, *24*, 1525.
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; A total of 15 tag molecules (five tags for AAn) were used to encode the library according to the method reported in *reference 5*.
7. Yoon, S. S.; Still, W. C. *Tetrahedron* **1995**, *51*, 567.