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.\(^5\)

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. 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-\(\text{AA3-AA2-AA1-NH(CH}_2\text{)}_6\text{-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.
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 (6/15) and, (L)Ala and (L)Asn (4/15 and 3/15) at AA1, with the substrate with (L)Ala (5/15), (D)Leu and (L)Lys (6/15). The trends. For example, receptor resynthesized and their association with 15 and 7/15) and, (L)Ala and (L)Asn (4/15 and 3/15) at AA1, with the substrate with (L)Ala (5/15), (D)Leu and (L)Lys (6/15). The binding data in Table 1 showed clearly that the subtle changes in the coordination number and geometry of different metals can affect markedly the peptide-binding properties of metallomacro cyclic 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.

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'-methyleneedianiline (2.052 mmol), 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 mixture was dissolved in dichloromethane and organic layers was washed with 1 M HCl, saturated NaHCO₃, and brine and dried with MgSO₄. The residue was purified by flash chromatography on silica gel using 20% ethyl acetate in hexane to give 5 as an amorphous white solid (0.26 g, 49%): 1H NMR (CDCl₃) δ (ppm) 4.82 (br, 2H), 3.91 (s, 2H), 3.07 (m, 4H), 1.32 (s, 18H). The binding cavity was wash successfully prepared by exploiting flash chromatography on silica gel using 20% ethyl acetate in hexane to give 5 as an amorphous white solid (1.13 g, 65%): 1H NMR (CDCl₃) δ (ppm) 9.20 (br, 2H), 7.46 (m, 4H), 7.19 (m, 2H), 7.14 (m, 4H), 7.06 (m, 4H), 6.05 (br, 2H), 4.82 (br, 2H), 3.91 (s, 2H), 3.07 (m, 4H), 1.32 (s, 18H). To 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 0.5 g of the pentafluorophenyl ether 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 7 as an amorphous white solid (0.26 g, 49%): 1H NMR (CDCl₃) 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), 3.70 (s, 2H), 3.10 (m, 2H), 2.89 (m, 2H). The binding cavity was wash successfully prepared by exploiting flash chromatography on silica gel using 4% MeOH in dichloromethane to give bis-allyl protected intermediate 7 as an amorphous white solid (0.26 g, 49%): 1H NMR (CDCl₃) 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), 3.70 (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 was added palladium acetate 3.33 mg (10 mol%), triphenylphosphine 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 dilute with dichloromethane to give 8 as an amorphous white solid (74 mg, 59%) that were collected by filtration: 4H NMR (DMSO-d6) 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), 7.26 (m, 5H), 7.12 (m, 8H), 6.81 (m, 2H), 4.82 (m, 2H), 4.56 (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%): 4H NMR (DMSO-d6) 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), 7.46 (m, 10H), 7.24 (m, 10H), 7.19 (m, 8H), 7.10 (m, 10H), 4.82 (s, 2H), 4.74 (m, 2H), 4.48 (m, 4H), 3.83 (s, 2H), 3.06 (m, 2H), 2.87 (m, 2H); δC-NMR (DMSO-d6) 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, 128.692, 129.259, 129.771, 130.051, 130.340, 137.739, 138.780, 137.321, 139.579, 147.848, 151.886, 167.513, 168.973, 170.524; IR (KBr) 1659, 1632, 1538, 1533, 1467, 1438, 1303, 1275, 1284, 1294, 1293, 1365, 1382, 1451, 1474, 1492, 1514, 1519, 1532, 1553, 1619, 1619, 1639, 1645, 1647, 1438 cm−1; UV/Vis (CH2Cl2 soln) 333, 370, 424 nm; MS (FAB+) m/z = 1089 (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) and refluxed for 3 hr under a nitrogen atmosphere, all volatiles were removed at reduced pressure. The crude products were recrystallized from dichloromethane in 40 mL of MC : MeOH (1 : 1) was added 12.9 mg of vanadyl acetylacetoate (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 : MeOH (4 : 1) and evaporated to obtain 3 as an amorphous green solid (23 mg, 45%

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 4 as an amorphous green solid (26 mg, 49%): IR 1664, 1615, 1516, 1444 cm−1; UV/Vis (CH2Cl2 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 acetylacetoate (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%

References
7. AAn = Any possible combinations of 25 (o)-amino acid 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)His(OtBu), (D)His(OtBu), (L)Asn(Tr), (D)Asn(Tr), (L)Gln(Tr), (D)Gln(Tr), (L)Glu(Asn), (D)Glu(Asn).
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.