Syntheses and Peptide-binding Properties of Metal-templated Self-Assembly

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The development of synthetic molecules having the remarkable binding properties seen in biological receptors has been an area of active research in recent years.[1] Previous studies showed that such receptor-like compounds could be prepared by metal-templated self-assembly processes exploiting coordinate bonds between the suitable ligands and transition metals such as Pd(II), Zn(II) and Fe(III).[2] These molecules showed the selective binding properties to the certain organic and inorganic substrates. However, only a few of those had the selective binding properties to biologically relevant substrates such as peptides. Here, we describe syntheses and peptide binding properties of novel metal-templated self-assembly (**1** and **2**).

These receptor-like molecules have large nonpolar, conformationally rigidified clefts, which are surrounded by polar functionalities and aromatic surfaces. Thus it is reasoned that these can bind certain substrates selectively by hydrogen bondings and hydrophobic interactions. In these receptor-like molecules, metal ions act to maintain dimeric structures, and thus make potential substrate-binding sites to be preorganized for the effective complexation with suitable substrates.[3] Furthermore, metal templated self-assembly have the distinct colors originated from Fe ions. Thus metal ions can act as the sensitive probes for binding studies using solid phase substrate library,[4] as well as the chromogenic and catalytic centers for the potential applications to chemical sensors and catalysts.

Synthesis of metal-templated self-assembly (**1** and **2**) began with the preparation of monocyclic ligand as shown in Scheme 1.

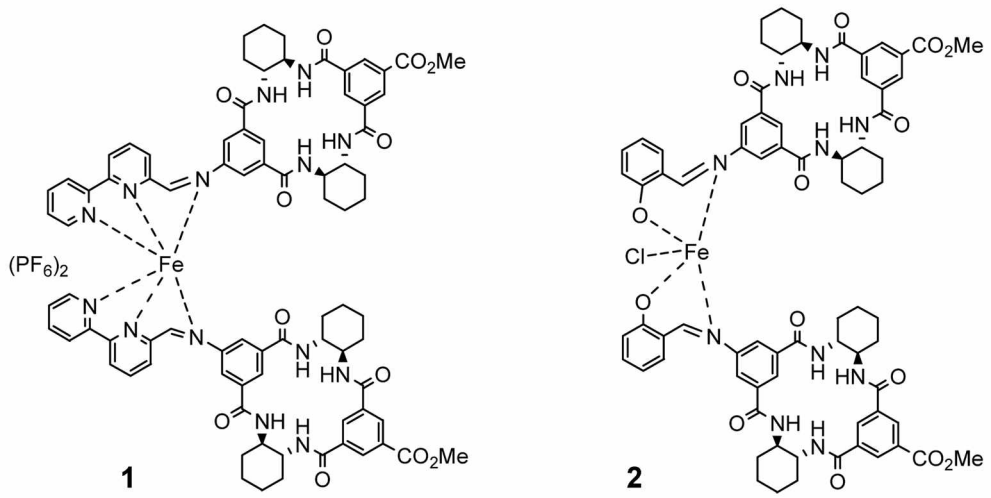
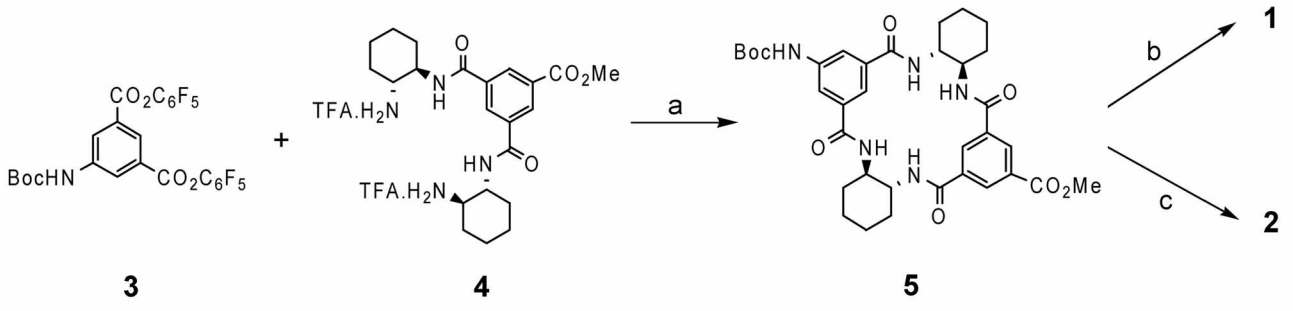


Figure 1. Metal templated self-assembly (**1** and **2**).



Scheme 1. Syntheses of metal-templated self-assembly (**1** and **2**); (a) slow addition to iPr$_2$NEt/THF, (b) TFA, then NEt_3, bipyridine-carboaldehyde and $FeCl_2$ in EtOH, (c) TFA, then NEt_3, salicylaldehyde and $FeCl_3$ in EtOH.

Intermolecular lactamization reaction between bis-pentafluorophenyl ester **3** and the corresponding diamine **4** under a high dilution condition provided the monocyclic intermediate **5**. Metal-templated self-assembly (**1** and **2**) was prepared by exploiting Fe(II)-bipyridylidene imine and Fe(III)-salicylidene imine coordinate bonds. The Fe(II) complex **1** was prepared as dark red solids in 35% yield by mixing $FeCl_2$, bipyridine carboaldehyde and the amine of monocyclic intermediate (**5**) in ethanol, stirring for 12 hrs under reflux condition, then adding the saturated NH_4PF_6 aq. solution. The Fe(III) complex **2** was prepared as dark red solids in 43% yield by mixing $FeCl_3$, salicylaldehyde and the amine of monocyclic intermediate (**5**) in ethanol, stirring for 12 hrs under reflux condition.

Recently, combinatorial chemistry has become a major tool in the elucidation of the binding properties of receptors.[5] Metal-templated self-assembling compounds (**1** and **2**) have the distinct color due to Fe(II) and Fe(III) ions, and thus ideal for solid phase color binding assay using encoded combinatorial library of peptide substrates.

Metal-templated self-assembling compounds (**1** and **2**) were 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.[6,7]

Decoding the tripeptides on the colored beads by using electron capture gas chromatography revealed selective peptides-binding properties of metal-templated self-assembling compounds (**1** and **2**). 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)Asn (8 of 15), (L)Ala (8 of 15) and Gly (6 of 15) at AA1, AA2 and AA3 position, respectively. Receptor **2** was found to bind strongly with the substrate with (L)Asn and (L)Gln (10 of 14), (L)Ala (7 of 14) and Gly (7 of 14) at AA1, AA2 and AA3 position, respectively.

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

Entry	Receptor 1	Receptor **2**
1	(L)Asn-(L)Ala-(L)Pro	(L)Asn-(L)Ala-Gly
2	(L)Asn-(L)Ala-(L)Pro	(L)Asn-(L)Ala-Gly
3	(L)Asn-(L)Ala-(L)Pro	(L)Asn-Gly-(L)Pro
4	(L)Asn-(L)Ala-(L)Ala	(L)Gln-Gly-(L)Ala
5	(L)Ser-(L)Ala-Gly	(L)Ser-(L)Ala-Gly
6	(L)Gln-(L)Ala-(L)Pro	(L)Gln-Gly-(L)Ala
7	(L)Gln-(L)Ala-(L)Ala	(L)Gln-(L)Ala-(L)Pro
8	(L)Lys-(L)Ala-(L)Pro	(L)Lys-(L)Ala-(L)Pro
9	(L)Asn-(L)Pro-Gly	(L)Asn-(L)Pro-Gly
10	(L)Asn-(L)Pro-(D)Ala	(L)Ser-Gly-(L)Ala
11	(L)Gln-(L)Pro-Gly	(L)Gln-(L)Ala-Gly
12	(L)Lys-(L)Pro-Gly	(L)Lys-(L)Ala-Gly
13	(L)Ser-(L)Pro-Gly	(L)Gln-(L)Asn-Gly
14	(L)Asn-(L)Asn-(L)Ala	(L)Asn-(L)Asn-(L)Ala
15	(L)Asn-(L)Asn-Gly	

Table 2. Binding of 1 and peptides in $CHCl_3$

Peptide	Binding Energy (kcal/mol)	Found in Assay ?
Resin-(L)Asn-(L)Ala-(L)Pro-Ac	−4.3	yes
Resin-(L)Ala-(L)Ala-(L)Pro-Ac	−2.8	no
Resin-(L)Asn-Gly-(L)Pro-Ac	−3.1	no

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

Peptide	Binding Energy (kcal/mol)	Found in Assay ?
Resin-(L)Asn-(L)Ala-Gly-Ac	−3.7	yes
Resin-(L)Ala-(L)Ala-Gly-Ac	−2.1	no
Resin-(L)Asn-Gly-Gly-Ac	−2.7	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**.

These data showed that the most tightly bound peptides, Resin-(L)Asn-(L)Ala-(L)Pro-Ac, Resin-(L)Asn-(L)Ala-Gly-Ac and Resin-(L)Asn-(L)Ala-(L)Gln-Ac were found to bind to **1** & **2** with −4.3 and −3.7 kcal/mol binding energy, respectively. Removal of amide group in the side chain of substrate from Asn to Ala at AA1 sites reduce binding energy by 1.5 and 1.6 kcal/mol, respectively. Also the changes in the side-chain from (L)Ala to Gly and (D)Ala at AA2 sites reduce the binding energies by 1.2 and 1.0 kcal/mol, respectively. 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 bonds between transition metals and ligands This study established that metal-templated self-assembling process is an efficient method to construct synthetic receptors. Furthermore, the highly selective peptide-binding properties of metal-templated self-assembling receptors were elucidated by using a combinatorial method. 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. A solution of 1.3 g of the bis-(pentafluorophenyl)ester **3** (2.11 mmol) and 1.21 g (1.92 mmol) of the di-TFA salts of amine intermediate **4** in 10 mL of DMA was added to a solution of 1.67 (9.60 mmol) ml of DIPEA in 200 mL of THF at room temperature for 20 hr by syringe pump. After the stirring for 5 hr at room temperature, all volatiles were removed at reduced pressure. The residue was

purified by flash chromatography on silica gel using 5% MeOH in methylene chloride to give **5** as an amorphous white solid (1.1 g, 86.3%): ^{1}H NMR (DMSO-d_6) δ (ppm) 9.57 (s, 1H), 8.58 (d, 2H, J = 4.32 Hz), 8.42 (m, 1H), 8.35 (m, 2H), 8.31 (d, 2H, J = 4.32 Hz), 7.83 (m, 2H), 7.78 (m, 1H), 3.90 (m, 7H), 1.92 (m, 4H), 1.75 (m, 4H), 1.53 (m, 4H), 1.45 (s, 9H), 1.31 (m, 4H); Mass (FAB) m/z = 662 $(MH)^+$.

To a solution of 0.10 g (0.15 mmol) of **5** and 0.1 mL of anisole in 20 mL of methylene chloride was added 10 mL of TFA. After stirring for 4 h 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 1. To a solution of 114 mg (0.15 mmol) of di-TFA salts of **5**, 0.21 mL (0.15 mmol) of Et_3N, 28 mg (0.15 mmol) of bipyridine carboaldehyde in 10 mL of ethanol was added 15 mg (0.075 mmol) of $FeCl_2{\cdot}4H_2O$. After stirring for 12 hr under refluxing condition, the reaction mixture was cooled to room temperature and then 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 (48 mg, 35.0%): ^{1}H NMR (DMSO-D_6) δ(ppm) 8.84-7.95 (br, 16H), 7.52 (m, 1H), 6.97 (s, 1H), 3.87 (m, 7H), 1.92 (m, 4H), 1.77 (m, 4H), 1.52 (m, 4H), 1.65 (m, 4H); IR (KBr) 3408, 3068, 2934, 2857, 1652, 1531, 1448, 1299, 1085 cm^{-1}; UV ($CHCl_3$) 258, 495, 592 nm; Mass (FAB) m/z = 1656 $(M\text{-}PF_6)^+$.

Synthesis of 2. To a solution of 125 mg (0.17 mmol) of di-TFA salts of **5**, 0.23 mL (0.17 mmol) of Et_3N, 18 mg (0.17 mmol) of salicylaldehyde in 10 mL of ethanol was added 13 mg (0.085 mmol) of $FeCl_3{\cdot}6H_2O$. After stirring for 12 hr under refluxing condition, the crude products were recrystallized from MeOH/ethyl ether to give **2** as an amorphous dark-red solid (48 mg, 43.0%): ^{1}H NMR (DMSO-D_6) δ (ppm) 8.58-8.14 (br, 13H), 6.97 (s, 1H), 3.88 (m, 7H), 1.92 (m, 4H), 1.76 (m, 4H), 1.53 (m, 4H), 1.65 (m, 4H); IR (KBr) 3377, 2937, 1724, 1649, 1536, 1445, 1265 cm^{-1}; UV ($CHCl_3$) 265, 488, 579 nm; Mass (FAB) m/z = 1385 $(M\text{-}Cl)^-$.

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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 4*.
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