

A Peptide-binding Receptor Based on Resorc[4]arene and Peptide-Metal Complexes

Hyung Kyu Kang, Hyo Jeong Lim, and Seung Soo Yoon*

*Department of Chemistry, Sungkyunkwan University, Suwon 440-746, Korea. *E-mail: ssyoon@skku.edu*

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Recently, several synthetic receptors based on resorcarenes, readily accessible by condensation of resorcinol with various aldehydes, have played a significant role in the development of selective molecular receptors.^{1,2} In particular, resorc[4]arenes have been used directly as hosts³ and modified to become molecules for the construction of container molecules⁴ such as carcerands and hemicarcerands. A wide range of guests of different size, shape, and charge have been found to bind with these receptors, and many potential applications for these receptors can be envisioned, ranging from specific drug release to catalysis and memory storage devices.⁵ However, studies on peptide-binding properties of resorc[4]arene-derived receptors have been rare. Here, synthesis and peptide-binding properties of a novel resorc[4]arene derived receptor are described. Receptor **1** consists of two different parts: A benzene-lined hydrophobic cleft to which the flexible arms are attached, and the arms based on peptide-metal complexes derived from amino acids. This molecule has a potential substrate-binding cavity, having a benzene-lined hydrophobic surface with a periphery of hydrogen bond donor/acceptors. Thus, this molecule might be expected to be capable of interacting selectively with peptide substrates through hydrophobic interactions and hydrogen bonding. In particular, in this receptor-like molecule, metal ions act as sensitive probes for binding with substrates and providing additional interactions with peptide substrates.

Receptor **1** was prepared by following the standard amide bond formation reactions through pentafluorophenyl activated esters, as shown in Scheme 1. The amide coupling reaction between tetra-aminomethyl resorc[4]arenes⁶ and N-Boc-(L)alanine pentafluorophenyl ester, and the subsequent deprotection of Boc groups and amide formation with ferrocenecarboxylic

acid pentafluorophenyl ester, yielded **1** as a red solid with 47.0% yield. The structure of **1** was established by mass spectrum, ¹H NMR spectroscopy, IR and UV spectroscopy.

Recently, combinatorial chemistry has become a major tool in the elucidation of the binding properties of receptors.⁷ Receptor **1** has a distinct red color due to a transition metal ion (Fe(II)), and thus is ideal for a solid phase color binding assay using an encoded combinatorial library of peptide substrates. Receptor **1** was screened against a tripeptide library on hydrophobic polystyrene in CHCl₃. The library was prepared by encoded split synthesis and has the general structure Ac-AA3-AA2-AA1-NH(CH₂)₆-C(O)NH-Polystyrene.⁸ Decoding the tripeptides on the colored beads using electron capture gas chromatography revealed the selective peptide-binding properties of receptor **1**.⁹

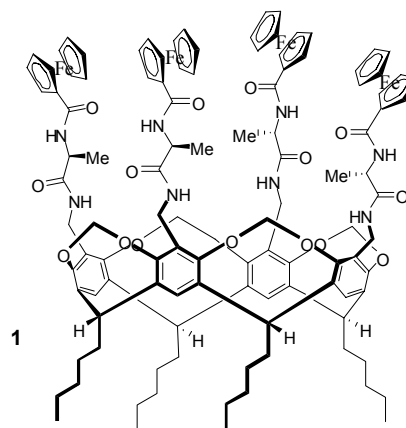
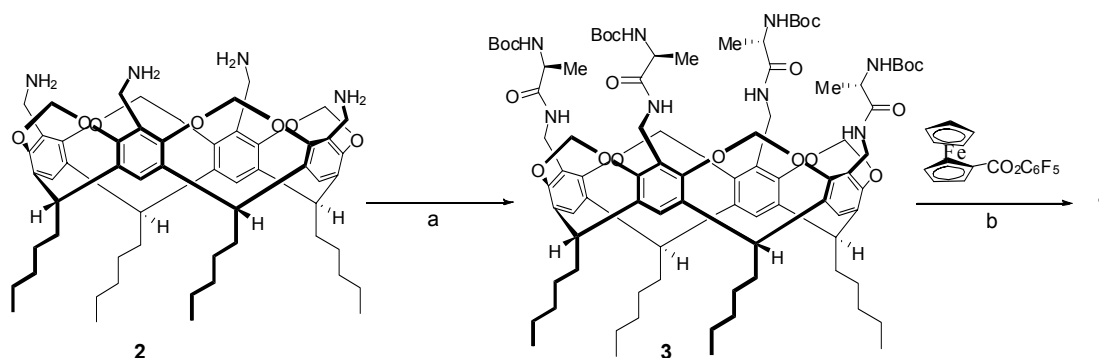


Figure 1. Structure of Receptor **1**



Scheme 1. Synthesis of Receptor **1**; (a) N-Boc-(L)Alanine pentafluorophenyl ester/NEt₃. (b) TFA, then ferrocene carboxylic acid pentafluorophenyl ester/NEt₃.

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

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

Table 2. Binding of **1** and Peptides in CHCl₃

Peptide	Binding Energy (kcal/mol)	Found in Assay?
Polymer-(L)Gln-(L)Pro-(D)Glu-Ac	-4.1	yes
Polymer-(L)Ala-(L)Pro-(D)Glu-Ac	-3.0	no
Polymer-(L)Gln-(L)Pro-(D)Ala-Ac	-3.1	no
Polymer-(L)Ala-(L)Pro-(D)Ala-Ac	-1.2	no
Polymer-(L)Gln-Gly-(D)Glu-Ac	-1.9	no

The substrates that were most tightly bound to receptor **1** are shown in Table 1.

The binding data in Table 1 reveal a number of notable trends in the substrates that bound most tightly to receptor **1**. First, high selectivity was observed for the AA1 position. For example, the substrates most frequently found had Gln (5 of 16) and Glu (4 of 16) at the AA1 position. The residues in AA1 were composed of D, L-glutamine and glutamic acid with a hydrogen bond donor/acceptor group in the side chain. Second, high selectivity was associated with the AA2 position. For example, receptor **1** was found to bind strongly with the substrate with (L,D)Pro (7/16) at AA2 position. Third, selectivities were also found with the AA3 position. The substrates with D-Glu (4 of 16) and D-Asp (3 of 16) at AA3 position were found to bind strongly.

To confirm the findings and to estimate the energetic extents of the selectivities observed, several peptides were resynthesized, and their association with **1** measured in CHCl₃.¹⁰ The results are summarized in Table 2.

These data showed that the most tightly bound peptide, Ac-(D)Glu-(L)Pro-(L)Gln-Polymer, was found to bind to **1** with -4.1 kcal/mol binding energy. The other substrates found by binding assay are expected to have a similar range of binding energies. Presumably, hydrogen bonds between hydrogen bond donor/acceptors on receptor **1** and peptide seem to be important in complexation. For examples, removal of amide group in the side chain of substrate from Gln and Glu to Ala at the AA3 and AA1 sites reduce the binding energies by 1.1 and 1.0 kcal/mol, respectively. Furthermore, removal of both amide groups in the side chain of substrate at AA3 and AA1 sites reduce the binding energy by ~2.9 kcal/mol. Also, hydrophobic interactions are crucial for complexation between receptor **1** and tripeptide substrates. The change in the side-chain from (L)Pro to Gly at AA2 site reduces the binding energy by 2.2 kcal/mol.

Although the exact nature of the peptide-receptor complex is not clear, the binding studies on peptide and receptor suggest a simple model of the structure of peptide-receptor complex. Hydrogen bonding between peptide arms in resorc[4]arene and side-chains of amino acid residues at AA1 and AA3 sites in the peptide substrate would contribute selective binding between receptor and peptide substrates. Also, hydrophobic interactions between the nonpolar cavity-surrounded aromatic groups in resorc[4]arene and the proline residue at AA2 site in peptide substrate seem to be important in complexation.

In conclusion, a cavitand derived from resorc[4]arene and flexible peptide-metal complexes has demonstrated highly selective peptide-binding properties. Further studies on the structures of receptor and peptide substrate complexes and the peptide-binding properties of other related synthetic receptors are in currently progress in this laboratory.

Experimental Section

Synthesis of 3. To a solution of 1.8 g of **2** (1.93 mmol) in 20 mL of DMF were added 3.3 g of N-Boc-(L)Alanine pentafluorophenyl ester (9.26 mmol) and 1.12 mL of NEt₃ (11.1 mmol) at 0 °C. After the stirring for 12 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 **3** as an amorphous white solid (1.50 g, 46.1%): ¹H NMR (CDCl₃) δ 0.91 (t, 12H, *J* = 7.0 Hz), 1.24 (s, 36H), 1.35 (d, 12H, *J* = 7.5 Hz), 1.54 (m, 24H), 2.16 (m, 8H), 4.11 (m, 12H), 4.37 (m, 4H), 4.76 (t, 4H, *J* = 8.0 Hz), 5.16 (s, 4H), 5.90 (d, 4H, *J* = 7.5 Hz), 6.58 (s, 4H), 7.05 (s, 4H); ¹³C NMR (DMSO-*d*₆) δ 15.2, 19.1, 22.1, 27.7, 28.9, 30.2, 32.1, 35.4, 43.8, 50.2, 95.3, 100.5, 124.8, 127.0, 128.9, 133.7, 151.5, 167.5, 171.3; Mass (FAB) *m/z* 1616 (MH)⁺.

Synthesis of 1. To a solution of 0.1 g of **3** (0.0618 mmol) and 0.1 mL of anisole in 10 mL of methylene chloride was added 3 mL of TFA. After stirring for 2 h at room temperature, all volatiles were removed at reduced pressure. The crude tetra-TFA salts were used the next reaction without further purification.

To a solution of the resulting tetra-TFA salts of **3** in 10 mL of DMA were added 0.1 mL of NEt₃ (0.99 mmol) and 0.27 g of ferrocenecarboxylic acid pentafluorophenyl ester (0.618 mmol) at 0 °C. After the stirring for 12 hr at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 3% MeOH in methylene chloride to give **1** as an amorphous red solid (60 mg, 47%): ¹H NMR (DMSO-*d*₆) δ 0.88 (t, 12H, *J* = 7.0 Hz), 1.25 (d, 12H, *J* = 7.5 Hz), 1.59 (m, 24H), 2.1 (q, 8H, *J* = 7.5 Hz), 4.12 (d, 4H, *J* = 7.5 Hz), 4.25 (m, 16H), 4.43 (m, 16H), 4.77 (m, 16H), 5.16 (s, 4H), 5.81 (d, 4H, *J* = 7.5 Hz), 6.58 (s, 4H), 7.06 (s, 4H), 7.59 (s, 4H); ¹³C NMR (DMSO-*d*₆) δ 14.2, 16.6, 22.3, 27.9, 31.2, 33.5, 35.4, 37.2, 51.3, 69.8, 70.1, 70.7, 72.3, 74.2, 77.5, 95.6, 121.7, 122.5, 123.3, 124.2, 153.8, 154.2, 173.6, 175.2; IR (KBr) 2929, 1633, 1520, 1464 cm⁻¹; UV (CHCl₃) 271, 507 nm; Mass (FAB) *m/z* 2088 (MNa)⁺.

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 8. 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.
 9. A total of 15 tag molecules (five tags for AAn) were used to encode the library according to the method reported in *reference 7*.
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