A Tweezer-like Peptide-binding Receptor

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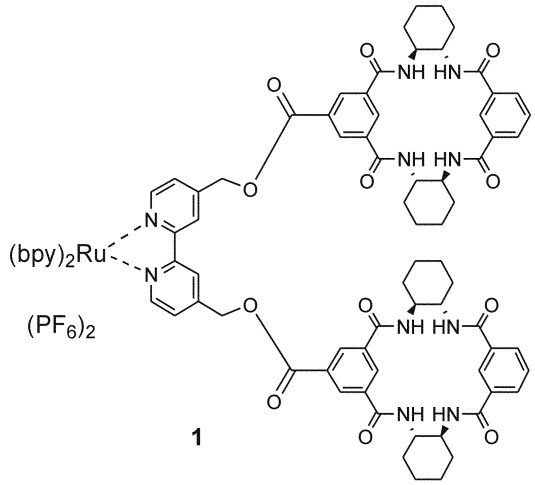
In recent years, significant progress has been made in the construction of synthetic molecules that selectively bind peptide substrates and other small molecules.[1] Some such molecules have the complex, cage-like structures, but a different and simple structural motif such as molecular tweezer with two substrate binding arms has emerged as having selective peptide-binding properties.[2] Among those are two-armed receptors based on cyclic oligomers of 1,2-diamine and isophthalic acid. Although such receptors showed remarkable binding properties, to have further possible applications its binding properties had to be improved. Here, a novel two-armed tweezer-like peptide-binding receptor is described.

Receptor (**1**) consist of two different parts: A linker to which the tweezer arms are attached; and two substrate binding arms based on cyclic tetramer of two isophthalic acids and two 1,2-diaminocyclohexane. Particularly, linker in receptor (**1**) has transition metal ion (Ru^{2+}). In this receptor-like molecule, metal ion acts to make potential substrate-binding sites to be preorganized for the effective complexation with suitable substrates. Furthermore, metal ion such as Ru can act as the sensitive probes for binding with substrates and provide the additional interactions between linkers and peptide substrates.

Synthesis of **1** began with the preparation of monocyclic intermediates of 1,2-diaminocyclohexanes and isophthalic acid derivatives by the known procedures for the related molecules.[3] Ester bond formation reaction between bis-(pentafluorophenyl)ester (**2**) and 4,4'-hydroxymethyl-2,2'-dipyridine (**3**),[4] and the subsequent reaction with $Ru(bpy)_2Cl_2$ in refluxing EtOH provided **1** with 48.2% yield.

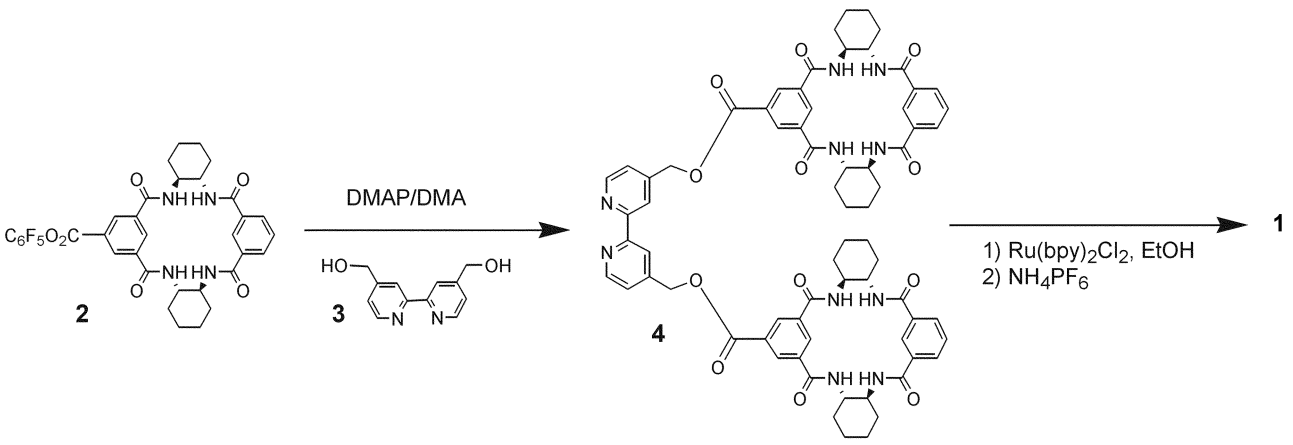


Scheme 1. Two-armed Tweezerlike Receptor (**1**).

Recently, combinatorial chemistry has become a major tool in the elucidation of the binding properties of receptors.[5] By using such methods it has become possible to find the binding properties of receptors efficiently, and furthermore to detect subtle differences in receptor-substrate binding that could not have been studied by conventional experiments.

Receptor **1** has the distinct red color due to transition metal ion (Ru), and thus ideal for solid phase color binding assay using encoded combinatorial library of peptide substrates.

Receptor **1** was screened against a tripeptide library on



Scheme 2. Synthesis of **1**

Table 1. Sequences selected by binding assay with receptor (**1**)

1	(L)Gln-(L)Val-(L)Pro	8	(L)Lys-(L)Val-(L)Pro
2	(L)Gln-(L)Val-(L)Pro	9	(L)Asn-(L)Pro-(L)Ser
3	(L)Gln-(L)Pro-(L)Ser	10	(L)Asn-(L)Pro-(L)Pro
4	(L)Asn-(L)Pro-(L)Ser	11	(L)Gln-(L)Pro-(L)Pro
5	(L)Gln-(L)Val-(L)Asn	12	(L)Lys-(L)Pro-(L)Ser
6	(L)Gln-(L)Val-(L)Pro	13	(L)His-(L)Val-(L)Gln
7	(L)Asn-(L)Pro-(L)Gln	14	(D)Asn-(L)Val-(L)Asn

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] Decoding the tripeptides on the colored beads by using electron capture gas chromatography revealed selective peptides- binding properties of molecular tweezer (**1**). The most tightly binding substrates with **1** are shown in Table 1.

The binding data in Table 1 reveal a number of notable trends. First, high selectivity was observed for the residue in AA1 composed of amino acids such as (L)Gln and (L)Asn which have hydrogen bonding dornor/acceptors in the side chain. Second, selectivities were also found for AA2 and AA3 position. The substrates with (L)Pro and (L)Val, and (L)Pro at AA2 and AA3 position were found to bind strongly.

To confirm the findings and to estimate the energetic extents of the selectivities observed, the most tightly bound peptide, Resin-(L)Gln-(L)Val-(L)Pro was resynthesized and its associations with **1** measured in $CHCl_3$.[7] The binding energy was found to be -4.2 kcal/mol. The other substrates found by binding assay are expected to have the similar range of binding energies. The binding energy between **1** and (L)Gln-(L)Val-Gly, which is not found in assay, were found to be -2.9 kcal/mol. Thus molecular tweezer (**1**) shows highly selective and efficient binding properties for certain tripeptides.

In conclusion, metallomolecular tweezer with two substrate binding arms was readily prepared from 1,2-diaminocyclohexane and isophthalic acid. Combinatorial binding assay revealed that this receptor has remarkable sequence-selective tripeptide binding properties.

Experimental Section

Synthesis of 1. To a solution of 0.1 g of **3** (0.463 mmol) and 0.17 g of DMAP (1.37 mmol) in 10 mL of DMA was added 0.71 g of **2** (1.01 mmol) at room temperature. 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 10% MeOH in methylene chloride to give **4** as an amorphous white solid (0.29 g, 51.0%): ^{1}H NMR ($CDCl_3/CD_3OD$) δ (ppm) 1.21 (bs, 4H), 1.32 (bs, 2H), 1.59 (bs, 2H), 3.94 (bs, 2H), 4.77 (s, 2H), 7.31 (d, 1H, J = 5.0 Hz), 8.02 (d, 2H, J = 6.2 Hz), 8.24 (t, 1H, J = 6.2 Hz), 8.42 (s, 1H), 8.52 (s, 2H), 8.62 (s, 1H), 8.71 (d, 1H, J = 5.0 Hz), 8.82 (s, 1H);); MS (FAB) *m/z* 1244 (MH^+).

To a solution of 200 mg of **4** (0.161 mmol) in 10 mL of ethanol was added 78 mg of $RuCl_2(bpy)_2$ (0.161 mmol). After stirring for 12 hr under reflux condition, 100 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 (130 mg, 48.2%): ^{1}H NMR (DMSO-D_6) δ (ppm) 1.15 (bs, 4H), 1.43 (bs, 2H), 1.69 (bs, 2H), 4.14 (bs, 2H), 4.98 (m, 2H), 7.52 (m, 2H), 7.82 (m, 2H), 8.21-8.43 (4H), 8.72-8.80 (m, 6H), 8.88-8.95 (m, 4H); UV ($CHCl_3$) 231, 487, 586 nm; MS (FAB) *m/z* 1658 (M^+).

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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. 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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