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# Cyclophane-based tetra(resorcinarene) as a host for both histone and hydrophobic molecular guests

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**Abstract**—A cyclophane-based resorcinarene tetramer, which is constructed with a tetraaza[6.1.6.1]paracyclophane and four resorcinarenes bearing hepta(carboxylic acid) residues that connect the macrocycle through amide linkages, was prepared. The binding constant of the cyclophane-based resorcinarene with immobilized histone was determined to be $1.3 \times 10^7\ M^{-1}$ by surface plasmon resonance measurements, which was 31-fold larger than that of the resorcinarene monomer bearing octacarboxylic acids. Moreover, the cyclophane-based tetra(resorcinarene) acted as a host toward hydrophobic molecular guests such as 6-*p*-toluidinonaphthalene-2-sulfonate.
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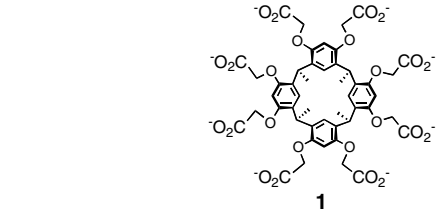
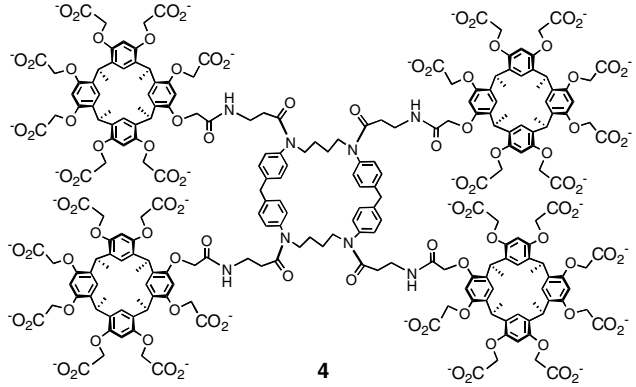
Histones are small basic proteins that make up a substantial portion of eukaryotic nucleoproteins. Recently, histones that adopt posttranslational modifications such as methylation, acetylation, and phosphorylation, have been suggested to play an important role in diverse biological processes such as gene regulation and chromosome condensation.[1,2] Although there are many approaches for isolating histones by immunoprecipitation methods using antibodies, there have been surprisingly few studies on the development of artificial compounds that interact with histones and recognize bioorganic and chemical modifications of histones. Naturally occurring histone has a high content of the amino acid lysine, and shows an isoelectric point of 10.8. Therefore, artificial compounds such as resorcinarene derivative having eight anionic polar side chains **1**[3] are expected to be candidates for receptors capable of binding histone through electrostatic interactions. Accordingly, in order to enhance the favorable interactions between these artificial compounds and histones, we designed a novel polytopic resorcinarene having 28 carboxylate residues **4** on the basis of a molecular design that allows the assembly of four anionic resorcinarenes on an azaparacyclophane skeleton. So-called cluster effects by tetramerization of resorcinarene moieties are expected to enhance the cooperative binding. In addition, the cyclophane cavity has intrinsic potential to act as a host for guest inclusion, and the resulting cyclophane-based tetra(resorcinarene) is expected to be



**1**



**4**

*Keywords*: Cyclophane; Resorcinarene; Histone; Guest-binding; Multivalency.
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utilized in molecular guest delivery systems toward histone. We describe herein the synthesis of cyclophane-based tetra(resorcinarene) **4**, which is constructed with a tetraaza[6.1.6.1]paracyclophane[4] and four resorcinarenes bearing heptacarboxylic acid residues that connect the macrocycle through amide linkages. In addition, the binding behavior of **4** with histone was examined by surface plasmon resonance (SPR) measurements, while its guest-binding abilities were evaluated in aqueous media by fluorescence spectroscopy.

Cyclophane-based resorcinarene **4** was prepared by following the reaction sequence given in Scheme 1. A resorcinarene derivative bearing a carboxylic acid residue **3** was obtained by partial hydrolysis from octa-ester[5] **2** in a 14% yield. Precursor **6** was synthesized by condensation of **3** with a tetraamine derivative of cyclophane **5**[6] in the presence of benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexa-fluorophosphate (BOP) in dry *N*,*N*-dimethylformamide. Cyclophane-based resorcinarene **4** was obtained by alkali hydrolysis of the ester groups of **6**. All the new compounds were fully characterized by means of spectroscopy ($^1$H and $^{13}$C NMR, and TOF-MS) and elemental analysis.[7]

Based on our investigation of the CPK molecular model, the molecular size of **4** in the extended conformation is 3.8–5.0 nm in the XY plane, while resorcinarene monomer **1** has a size of ca. 1.5 nm (Fig. 1). Cyclophane-based resorcinarene **4** provides a hydrophobic cavity suitable for encapsulating small, complementary organic molecules as a guest, as well as four heptaanionic resorcinarene derivatives with reasonably separated distances, which can be expected to confer the advantage of enhanced solubility in aqueous neutral media at biological pH. From a practical standpoint, cyclophane-based resorcinarene bearing 28 carboxylic acid residues **4** had good solubility of >0.4 g mL$^{-1}$ in aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) buffer (0.01 M, pH 7.4, with 0.15 M NaCl).

Cyclophane-based tetra(resorcinarene) **4** (10 μM) was agglutinated by histone (270 μg mL$^{-1}$), which was readily monitored by the visible turbidity of the solution due to a polyion complexation of these components to give an insoluble material. On the other hand, upon addition of histone to HEPES buffer containing **1**, the turbidity

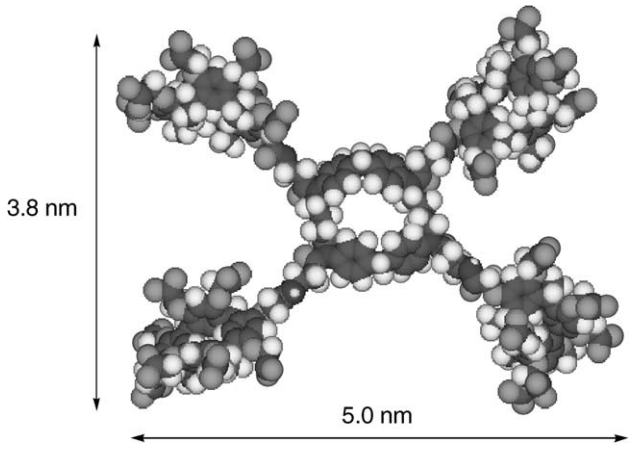
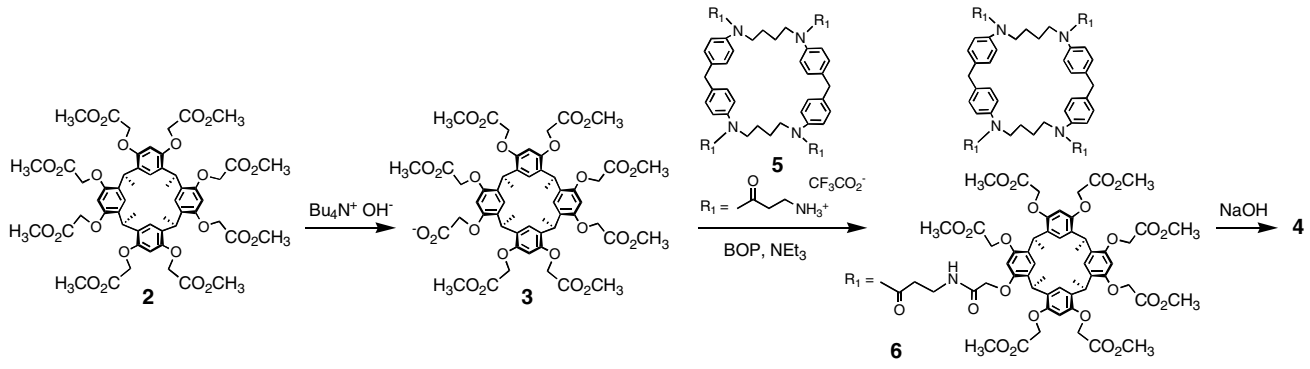



**Figure 1.** Probable computer-generated CPK model for cyclophane-based tetra(resorcinarene) **4**.

of the solution became almost negligible under identical conditions. Furthermore, we examined the binding interactions of **4** and resorcinarene monomer **1** to an immobilized histone on a sensor chip by SPR[8] measurements. First, immobilization of histone[9] to the carboxylated dextran sensor chip surface (CM5), which was set in BIAcore X (Pharmacia Biotech), was performed by utilizing the EDC–NHS coupling protocol.[10] The amount of immobilization of histone was given as a resonance signal of 6112 RU (resonance units). This value corresponds to an occupation density of 6.1 ng mm$^{-2}$ for histone on the chip, because a 1000 RU[11] corresponds to a surface concentration change of 1 ng mm$^{-2}$. Second, when a solution of **4** in HEPES buffer was injected over surfaces of immobilized histone, the association was observed as shown in Figure 2A (a, 0.78 μM) and 2B (d, 0.20 μM). Then, by changing the HEPES buffer to wash away the noncovalently bound **4**, the dissociation was initiated and observed as shown in Figure 2A(b) and B. The immobilized histone surface was regenerated with an injection of aqueous sodium hydroxide (50 mM) (see Fig. 2A(c)). The binding constant (*K*) of cyclophane-based resorcinarene **4** with immobilized histone was determined to be $1.3 \times 10^7$ M$^{-1}$ on the basis of kinetic analysis in a manner similar to that reported previously.[12] On the other hand, cyclophane-based resorcinarene **4** was hardly adsorbed on the surface of immobilized ovalbumin




**Scheme 1.** Preparation of cyclophane-based tetra(resorcinarene) **4.**

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