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Multivalent Macrocyclic Hosts: Histone Surface Recognition, Guest Binding, and Delivery by Cyclophane-Based Resorcinarene Oligomers

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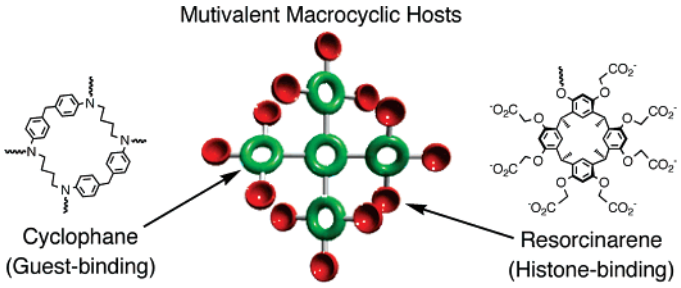

As a new class of host for both specific proteins and hydrophobic molecular guests, cyclophane-based resorcinarene oligomers were designed on the basis of a molecular design that allows the assembly of four or 12 anionic resorcinarenes on a cyclophane skeleton. We prepared a cyclophane-based resorcinarene tetramer (**4**), constructed with a tetraaza[6.1.6.1]-paracyclophane skeleton and four resorcinarenes bearing heptacarboxylic acid residues that connect to the macrocycle through amide linkages. In addition, we prepared an extended analogical dodecamer (**12**), which was constructed with a pentakis(cyclophane) skeleton and 12 resorcinarenes. The cyclophane-based resorcinarene oligomers exhibited potent recognition capabilities toward histone, a small basic protein of eukaryotic chromatins. The binding constants (*K*) of cyclophane-based resorcinarene tetramer **4** and dodecamer **12** with histone were determined to be 1.3×10^7 and 8.4×10^7 M^{-1}, respectively, by means of surface plasmon resonance measurements. The *K* values of **4** and **12** with histone were 31- and 200-fold larger than that of an untethered reference resorcinarene, reflecting the multivalency effects in resorcinarenes. In addition to that, cyclophane-based resorcinarene tetramer **4** and dodecamer **12** captured hydrophobic guests such as 6-*p*-toluidinonaphthalene-2-sulfonate, with respective binding constants of 2.4×10^3 and 2.5×10^4 M^{-1} in an aqueous HEPES buffer as evaluated by fluorescence spectroscopy. Furthermore, the resorcinarene oligomers were also found to act as guest carriers from the bulk aqueous phase to histone surfaces, as confirmed by fluorescence spectroscopy.

Introduction

Cyclophanes play a broad and prominent role in host−guest chemistry, supramolecular chemistry, and molecular recognition.[1] Numerous attempts have been made by organic chemists to develop functionalized cyclophanes, capable of acting as artificial receptors,[2] carriers,[3] and enzyme models.[4] A tetraaza-[6.1.6.1]paracyclophane bearing diphenylmethane moieties,[5] prepared by Koga et al., is a particularly attractive molecular skeleton because durene, a guest molecule, was accommodated

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in its cavity as confirmed by X-ray structural analysis.[6] We have previously clarified that saccharide cyclophanes based on this skeleton were utilized for saccharide-directed guest delivery[7] toward a carbohydrate-binding protein,[8] lectin. The saccharide cyclophanes, having four branches with a terminal glucose residue, exhibited unique functionality[7] in that (i) fluorescent guest molecules such as perylene were effectively incorporated by the saccharide cyclophanes through hydrophobic interactions and (ii) fluorescent dye molecules were carried from the aqueous phase to the glucoside-binding site of Concanavalin A, a glucoside- and mannoside-binding lectin,[8] by the saccharide cyclophanes. On these grounds, we became interested in whether our strategy in guest-binding and delivery systems could be utilized for other proteins besides lectins.

Histones are small basic proteins that make up a substantial portion of eukaryotic chromatins. Recently, histones that adopt post-translational 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.[9] Therefore, histone is very attractive as a target protein,[10] and the ability of artificial compounds to detect it is valuable. Naturally occurring histone has a high content of the amino acid lysine and shows an isoelectric point of 10.8. Hence, artificial compounds such as a resorcinarene derivative having eight anionic polar side chains **1** are expected to be candidates for receptors capable of binding histone through electrostatic interactions (Figure 1). Accordingly, we have developed polytopic resorcinarene having 28 carboxylate residues **4** on the basis of a molecular design that allows the assembly of four anionic resorcinarenes as binding sites for histone on the tetraaza[6.1.6.1]paracyclophane skeleton as a binding site for hydrophobic guests (Figure 1). In addition, to enhance the favorable interactions of these artificial compounds both for histones and for molecular guests, we designed a novel polytopic resorcinarene (**12**) having 84 carboxylate residues on the basis of a molecular design that allows the assembly of 12 anionic resorcinarenes on a pentakis(cyclophane) skeleton (Figure 1).[13] So-called multivalent effects[14] by the cyclophane-based resorcinarene oligomers are expected to enhance the cooperative binding.[15] We describe herein the synthesis of the cyclophane-based resorcinarene oligomers, their binding and recognition behavior with histone as examined by surface plasmon resonance (SPR) measurements, and their guest-binding and delivery abilities as evaluated in aqueous media by fluorescence spectroscopy, with an emphasis on the multivalent effects in the binding.

Results and Discussion

Design and Synthesis of Cyclophane-Based Resorcinarene Oligomers. Cyclophane-based resorcinarene oligomers were developed on the basis of a molecular design that allows the assembly of four or 12 anionic resorcinarenes on cyclophane skeletons. First, we designed a cyclophane-based resorcinarene tetramer (**4**), which was constructed with a tetraaza[6.1.6.1]-paracyclophane skeleton and four resorcinarenes having heptacarboxylic acid residues that connect to the macrocycle through amide linkages. In addition, we designed an extended analogical dodecamer (**12**), which was constructed with a pentakis(cyclophane) skeleton[13] and 12 resorcinarenes, to enhance the multivalent effects of both cyclophanes and resorcinarenes. Cyclophane-based resorcinarene oligomers **4** and **12** were prepared by following the reaction sequence given in Scheme 1. A resorcinarene derivative (**3**) bearing a carboxylic acid residue was synthesized by partial hydrolysis from octa-ester[16] **2**. Precursor **6** was obtained by condensation of **3** with a tetraamine derivative of cyclophane **5** in the presence of benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP) in dry *N*,*N*-dimethylformamide. The cyclophane-based resorcinarene **4** was obtained by alkali hydrolysis of the ester groups of **6**. The use of a dodecaamine derivative of pentakis(cyclophane) **7**[13] in place of **5** afforded the corresponding precursor **8** bearing 12 resorcinarenes, and **8** was converted to **12** using the same method applied to the preparation of **4**. All the new compounds were fully characterized by means of spectroscopy (^{1}H and ^{13}C NMR and TOF−MS) and elemental analysis.

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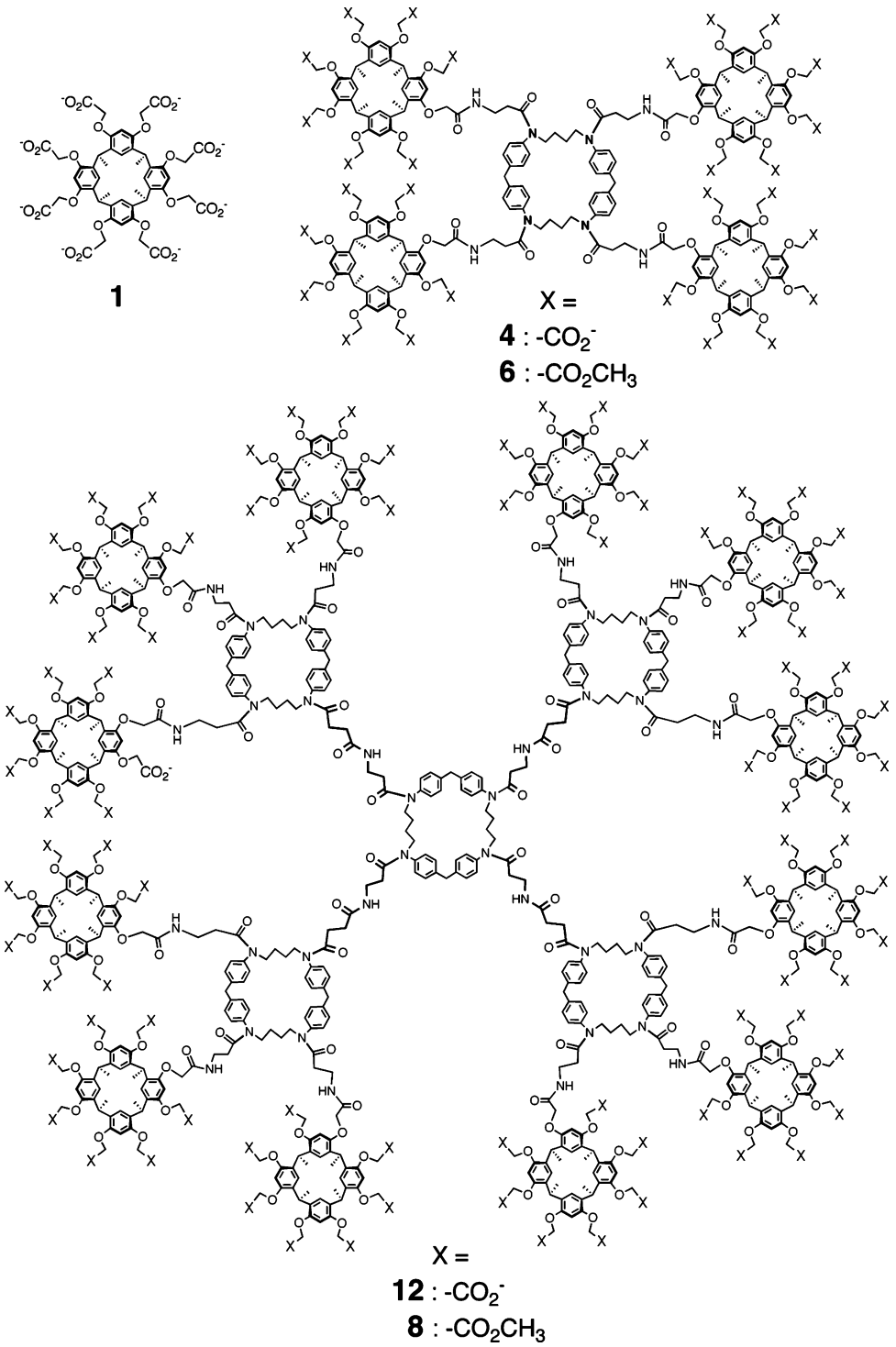

FIGURE 1. Anionic resorcinarene and cyclophane-based resorcinarene oligomers.

On the basis of our investigation of the CPK molecular model,[18] the molecular size of **12** in extended conformation is 6.5−7.7 nm in the XY plane, while **4** and resorcinarene monomer **1** have sizes of ca. 3.8−5.0 and 1.5 nm, respectively, as shown in Figure 2. Cyclophane-based resorcinarene oligomers **12** and **4** provide hydrophobic cavities[13] suitable for encapsulating small, complementary organic molecules as guests. On the other hand, 12 and four peripheral heptaanionic resorcinarene derivatives with reasonably separated distances were expected to confer the advantage of enhanced solubility in aqueous, neutral media at biological pH as well as anionic domains suitable for histone binding. From a practical standpoint, the cyclophane-based resorcinarene oligomers **4** and **12** both had good H_2O solubilities of >0.4 g mL^{-1}.

SPR Study for Interaction of Cyclophane-Based Resorcinarene Oligomers with Immobilized Histone. Both of the cyclophane-based resorcinarene oligomers were agglutinated by

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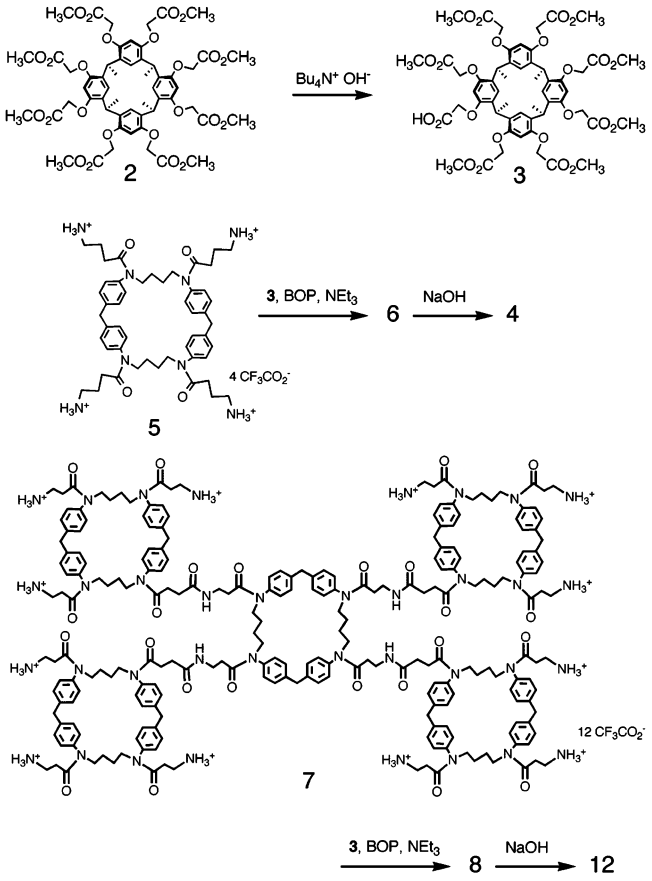
SCHEME 1. Preparation of Cyclophane-Based Resorcinarene Oligomers



histone, which was readily monitored by the visible turbidity of the solution due to the polyion complexation[19] of these components, to yield an insoluble material. On the other hand, upon the addition of histone to HEPES buffer containing **1** under identical conditions, the turbidity of the solution became almost negligible. To obtain further insights into the interactions between the cyclophane-based resorcinarene oligomers and histone, we investigated the binding behavior of the resorcinarene oligomers to an immobilized histone on a sensor chip by SPR measurements.[20] First, immobilization of histone to the carboxylated dextran sensor chip surface (CM5) was performed by utilizing the EDC-NHS coupling protocol.[21] The degree of immobilization of histone was given as a resonance signal of 6112 RU[22] (resonance units). Second, when a solution of **12** in HEPES buffer was injected over surfaces of immobilized histone, the association shown in Figures 3a and 4a was observed. Then, by changing the HEPES buffer to wash away the noncovalently bound **12**, the dissociation was initiated and observed as shown in Figure 3b. The immobilized histone surface was regenerated with an injection of aqueous sodium hydroxide (50 mM) (Figure 3c).

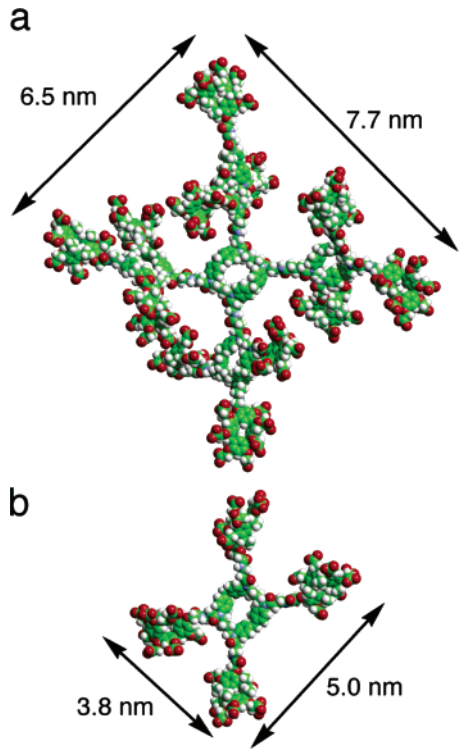


FIGURE 2. Computer-generated CPK models for the cyclophane-based resorcinarene oligomers **12** (a) and **4** (b). Carbon, hydrogen, oxygen, and nitrogen atoms are shown in green, white, red, and blue, respectively.

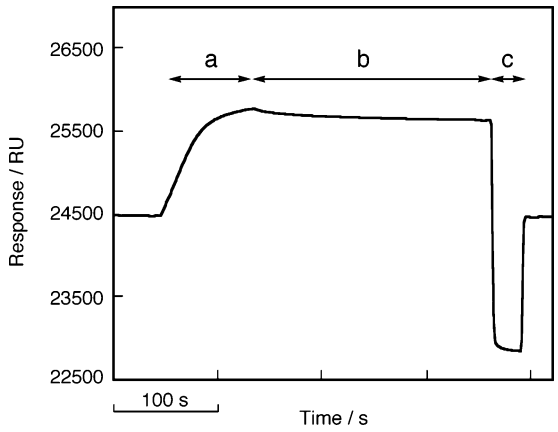


FIGURE 3. Response curve obtained during (a) and after (b) injection of **12** (0.20 μM) on immobilized histone surfaces. Aqueous sodium hydroxide (c, 50 mM) was used to regenerate the surface.

As regards the cluster effect achieved by multiplying the resorcinarenes, the binding constant (K) of **12** with immobilized histone was determined to be 8.4×10^7 M^{-1} on the basis of kinetic analysis in a manner similar to that reported previously.[23] A similar binding behavior of **4** with immobilized histone was also confirmed by the same method, as shown in Figure 4b, even though the K value was evaluated to be 1.3×10^7 M^{-1} with ca. 1/6.5 of the affinity of **12**. On the other hand, resorcinarene octacarboxylic acid **1** showed poor RU responses under identical conditions (0.20 μM) (Figure 4c). An association

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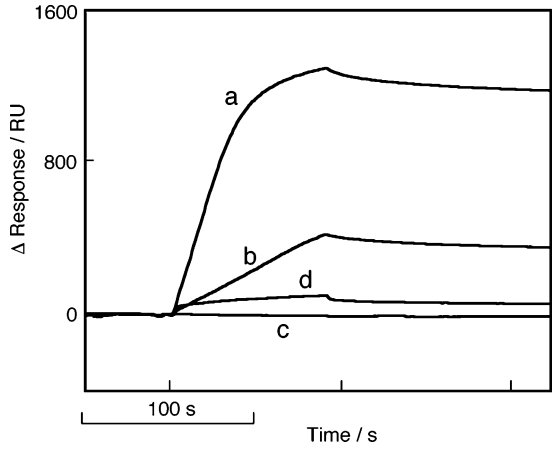

FIGURE 4. Overlay SPR sensorgrams of **12** (a, 0.20 μM), **4** (b, 0.20 μM), and **1** (c, 0.20 μM and d, 13 μM) on an immobilized histone surface. Flow rate: 20 μL min^{-1} in HEPES buffer.

curve was observed (Figure 4d); however, when a solution of **1** was injected at higher concentration (13 μM), the binding affinity of **1** to histone was much weaker than those of **12** and **4**: K, 4.2×10^5 M^{-1}. Therefore, the respective binding affinities of **12** and **4** to immobilized histone were enhanced 200- and 31-fold relative to that of **1**. The enhancement observed for **12** and **4** was one of the multivalent effects.

To examine the specificity of the binding, we also evaluated the binding abilities of **12** and **4** with immobilized ovalbumin and lysozyme, having isoelectric points of 4.6 and 11.0, respectively. Sensor chip surfaces of immobilized ovalbumin and lysozyme were prepared in a manner similar to that of histone immobilization, using 5900 and 5800 RU, respectively. Cyclophane-based resorcinarene oligomers **12** and **4** were hardly adsorbed on the surfaces of immobilized ovalbumin or lysozyme. Therefore, **12** and **4** exhibited potent recognition capability[24] toward histone. Clearly, structural characteristics of **12** and **4** were responsible for the histone binding because poly(acrylic acid) sodium salt having an average MW of 2100 showed poor binding affinity toward histone under the same conditions.

Guest-Binding Behavior and Guest Delivery to Histone. We have previously clarified that the guest-binding affinity for hydrophobic guest molecules of a pentakis(cyclophane) having 12 polar side chains with terminal glucose residues[13] was much enhanced relative to that of a corresponding monocyclic cyclophane,[25] reflecting the multivalency effects of macrocycles. That is to say, the K values for the former host with hydrophobic guests were greater by at least 3 orders of magnitude than those for the latter host. Thus, we investigated the guest-binding ability of **12** as a host to well-known fluorescent guests[26] such as 6-*p*-toluidinonaphthalene-2-sulfonate (TNS), 2-anilinonaphthalene-6-sulfonate (2,6-ANS), 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-*N*,*N*,*N*-trimethylethanaminium (DASP), and 1-pyrenemethylamine hydrochloride (PMA), all of whose emissions were extremely sensitive to change in the microenvironmental polarity experienced by molecules in fluorescence spectroscopy (Figure

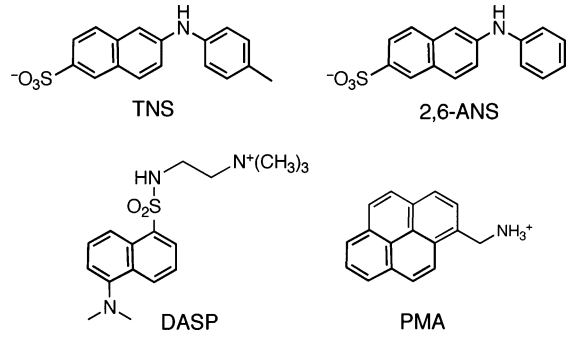

FIGURE 5. Hydrophobic fluorescent guests.

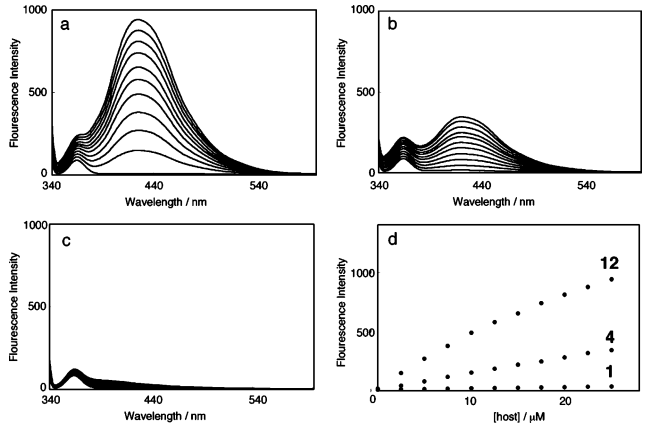

FIGURE 6. Fluorescence spectral changes for an aqueous solution of TNS (0.25 μM) upon addition of **12** (a), **4** (b), and **1** (c) in a HEPES buffer (0.01 M, pH 7.4, with 0.15 M NaCl) at 293 K: [**4**] = 0, 2.5, 5.0, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, and 25 μM (from bottom to top). The corresponding titration curves (d). Excitation 321 nm.

5). Upon addition of **12** to aqueous solutions containing TNS, the fluorescence intensity originating from the guest molecules was subjected to increase along with a concomitant blue shift of the fluorescence maximum, showing that the guest molecules were incorporated into the hydrophobic cavity provided by the hosts, as shown in Figure 6a. A similar fluorescent character was also confirmed for the complexation of **4** with TNS, as shown in Figure 6b. The present hosts were found to undergo complexation with the guests in a 1:1 molar ratio of host/guest as confirmed by Job's continuous variation method. A similar binding trend was also confirmed for the complexation of **12** and **4** with 2,6-ANS, DASP, and PMA, even though the fluorescence intensity of protonated PMA decreased due to an ionic complex formation between the protonated guest molecule and the anionic hosts. The 1:1 binding constants (K) of **12** toward these guests were evaluated on the basis of the Benesi−Hildebrand relationship[27] in the manner described previously[28] and are summarized in Table 1. The K values between **12** and guests were on the order of 10^4 to 10^5 M^{-1} and were only greater by 1 order of magnitude than those of **4** toward these guests. The enhancements in multivalency effects in macrocycles were relatively smaller than that of the pentakis(cyclophane) with glucose residues.[13] It seems that the insufficient enhancement of multivalency effects by **12** resulted from, to some extent,

(24) Resorcinarene oligomers **12** and **4** also have binding capabilities toward synthetic polylysine, which was immobilized on a sensor chip: K, 7.5×10^7 and 7.3×10^6 M^{-1}, respectively.

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TABLE 1. Binding Constants (K (M^{-1})) for Host−Guest Complexes of Cyclophane-Based Resorcinarene Oligomers with Fluorescence Guests

	K (M^{-1})			
host	TNS	2,6-ANS	DASP	PMA
4[12]	2.4×10^3	2.5×10^3	4.7×10^3	5.8×10^4
12	2.5×10^4	1.3×10^4	3.6×10^4	4.3×10^5

the steric hindrance by its resorcinarene moieties of the neighboring branched cyclophane cavities. Naturally, the fluorescence spectral changes upon addition of resorcinarene octacarboxylic acid **1** to an aqueous HEPES buffer containing TNS are almost negligible, as shown in Figure 6c, reflecting the lack of a cyclophane moiety.

Regarding the microenviroments experienced by the entrapped guest molecules, the microenvironmental polarity was evaluated on the basis of the correlation between λ_{max} and the solvent polarity parameter (E_T^N)[29] as described previously.[30] The E_T^N values for TNS placed in **12** and **4** were almost the same and were estimated to be 0.62 (λ_{max}, 425 nm), which was equivalent to the value for 1-propanol (Figure 6a,b). Therefore, the enhancements in binding affinity of **12** toward the guests relative to those of **4** originated from the multivalency effects in macrocycles but not from the hydrophobic effects of the cavities. Furthermore, relatively large fluorescence polarization values (P) were obtained for identical guests incorporated into **12** and **4** (P, 0.18 and 0.15, respectively). The obtained P values were somewhat larger than that for TNS bound to a tetraaza-[6.1.6.1]paracyclophane[30] bearing four L-valine residues (P, 0.09). This indicates that the tight host−guest interaction brings about effective motional repression of the entrapped guest.

These results, combined with the previously mentioned histone-binding capabilities, indicate that the resorcinarene oligomers have potential as guest carriers to histone surfaces. Consequently, DASP and histone were used in a proof of principle experiment. Upon the addition of histone to solutions containing host−guest complexes of **4** and **12** with DASP, insoluble materials were readily formed, due to the polyion complexation mentioned previously. After removal of the insoluble materials by filtration, there was a 19−21% decrease in fluorescence intensity originating from the entrapped guest molecules. These results indicate that a certain percentage of the complexes was precipitated as ternary complexes with histone when the hosts delivered guest molecules from the bulk aqueous phase.

Conclusion

A novel cyclophane-based resorcinarene tetramer and dodecamer were developed and showed unique properties as multivalent hosts of histone as well as of hydrophobic molecular guests, as demonstrated by SPR measurements and fluorescence spectroscopy, respectively. Naturally, histones adopt post-translational modifications, such as methylation, which play important roles in diverse biological processes, as mentioned previously. Particularly in the case of methylation at histone surface, the resulting trimethylated ϵ-ammonium groups of lysine residues are analogues to a good guest[31] for resorcinarene-type hosts. Therefore, the present cyclophane-based resorcinarene oligomers are favorable candidates as receptors recognizing trimethylated histones. In addition, the development of supramolecules formed with cyclophane-based resorcinarene oligomers and a fluorescence probe as a guest may be quite promising to detect trimethylated histones. When an amine-coupling fluorescent molecule such as 6-(5-dimethyl-aminonaphthalene-1-sulfonylamino)-hexanoic acid succinimidyl ester (dansyl-SE) was adopted as a guest, the fluorescent dye molecule was carried by the hosts from the aqueous phase to the histone surface and reacted at the proximal binding site. These subjects of interest will be explored further in the future.

Experimental Section

General Methods. Elemental analyses were performed at the Microanalysis Center of Kyushu University. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer, and ^{1}H and ^{13}C NMR spectra were taken on Bruker DRX 600 and JEOL JNM-EX400 spectrometers. A Bruker Autoflex was used for MALDI−TOF mass spectrometry. Fluorescence spectra were recorded on a JASCO FP-750 spectrophotometer. Surface plasmon resonance (SPR) response curves were recorded on a BIAcore X system docked with a sensor chip (CM5) under the flow of degassed 2-[4-(2-hydroxy-ethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) buffer (0.01 M, pH 7.4, with 0.15 M NaCl).

Materials. The following compounds were obtained from commercial sources as guaranteed reagents and used without further purification: potassium 6-*p*-toluidinonaphthalene-2-sulfonate [K(TNS)] (from a commercial supplier), 2-anilinonaphthalene-6-sulfonic acid (2,6-ANS) (from a commercial supplier), 2-(5-(dimethylamino)-naphthalene-1-sulfonamido)-*N*,*N*,*N*-tri-methylethanaminium (DASP) (from a commercial supplier), and 1-pyrenemethylamine hydrochloride (PMA) (from a commercial supplier). 2,11,20−-1,6,20,-25-Tetraaza-[6.1.6.1]paracyclophane (**4**) was prepared after a method reported previously.[5] The following proteins were obtained from commercial sources and used without further purification: calf thymus histone, egg white ovalbumin (both from a commercial supplier), and egg white lysozyme (from a commercial supplier).

SPR Measurements. Preparation of sensor surface: the carboxyl groups on the sensor surfaces of CM5 were activated with an injection of a solution containing 0.4 M *N*-ethyl-*N*′-(3-diethylaminopropyl)carbodiimide (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS), at a flow rate of 5 μL min^{-1}. Specific surfaces were obtained by injecting histone or other proteins. These proteins were diluted in 10 mM acetate buffer at pH 4 and used at a concentration of 100 μg mL^{-1}. The immobilization procedure was completed by a 7 min injection of 1 M ethanolamine hydrochloride to block the remaining ester groups. Interaction of hosts with immobilized proteins: HEPES buffer solutions containing hosts were injected for 1.5 min at a flow rate of 20 μL min^{-1}.

Binding Constants of Cyclophane-Based Resorcinarene Oligomers with Fluorescence Guests. To each solution of a fluorescent guest (0.25×10^{-6} M) in HEPES buffer (0.01 M, pH 7.4, with 0.15 M NaCl) was added increasing amounts of the hosts at 298 K, and the guest fluorescence intensity was monitored after each addition by excitation at 321, 318, 329, and 326 nm for TNS, 2,6-ANS, DASP, and PMA, respectively. The binding constants were calculated on the basis of the Benesi−Hildebrand method for titration data.

Resorcinarene Derivative Bearing a Carboxylic Acid Residue (3). A mixture of **2** (3.0 g, 2.7 mmol), aqueous tetra-*n*-butylammonium hydroxide (1.7 mL, 2.7 mmol), and tetrahydrofuran (500

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mL) was refluxed for 0.5 h and cooled to room temperature. The reaction mixture was evaporated to dryness under reduced pressure, and 100 mL of methanol was added to the residue. The mixture was then stirred at room temperature for 1 h, insoluble materials were removed by filtration, and the solvent was evaporated off reduced pressure. The residue was chromatographed on a column of silica gel (SiO_2) with chloroform/methanol (9:1 v/v) as eluent. The product fraction was dried in vacuo to give a white solid. 0.4 g (14%): R_f (Wako Silica Gel 70FM, ethyl acetate) 0.35; ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ 1.49 (d, 12H), 3.69 − 3.80 (m, 21H), 4.59 (br, 14H), 4.71−4.73 (q, 8H), 6.15−6.30 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 20.16, 20.22, 31.08, 31.13, 31.19, 31.23, 52.09, 52.32, 52.34, 52.44, 52.63, 66.86, 67.27, 67.35, 67.40, 67.48, 67.55, 67.64, 67.78, 100.13, 100.86, 101.92, 125.91, 126.03, 126.24, 126.37, 126.49, 129.90, 130.60, 153.56, 154.36, 154.48, 154.78, 154.91, 170.09, 170.18, 170.26. MS (MALDI−TOF) *m*/*z*, 1108 $[M + H]^+$, 1130 $[M + Na]^+$, 1145 $[M + K]^+$. Found: C, 59.23; H, 5.64. Calcd for $C_{55}H_{62}O_{24}$•0.5H_2O: C, 59.19; H, 5.69%.

Precursor of Cyclophane-Based Resorcinarene Tetramer (6). A solution of **5** (50 mg, 34 μmol) in dry *N*,*N*-dimethylformamide (DMF, 7 mL) was added dropwise to a solution of **3** (320 mg, 0.29 mmol), benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP, 260 mg, 0.59 mmol), and triethylamine (0.08 mL, 0.6 mmol) in dry DMF (10 mL) under nitrogen at room temperature, and the resulting mixture was stirred for 16 h at room temperature. EtOAc (150 mL) was added to the reaction mixture, and the mixture was then washed with 5% aqueous citric acid (70 mL), saturated aqueous sodium chloride (70 mL), and 5% aqueous sodium hydrogen carbonate (70 mL) in this sequence. After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol/chloroform (1:1 v/v) as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (130 mg, 69%): ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ 1.2 (m, 8H), 1.4−1.6 (m, 48H), 3.2−3.4 (m, 8H), 3.6−3.8 (m, 8H), 4.0−4.3 (m, 8H), 4.3−4.6 (m, 56H), 4.6−4.8 (m, 16 H), 6.0−6.4 (m, 16H), 6.96−6.98 (d, J = 8 Hz, 8H), 7.16−7.18 (d, J = 8 Hz, 8H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 18.65, 18.76, 18.88, 24.10, 29.76, 29.84, 29.87, 29.95, 33.35, 34.29, 40.02, 47.95, 50.04, 50.94, 50.97, 51.01, 51.10, 51.39, 65.90, 65.99, 66.04, 66.86, 98.50, 124.7, 124.9, 127.3, 129.3, 139.3, 139.5, 152.3, 152.9, 153.5, 167.3, 168.5, 168.6, 168.7, 168.8, 169.4. MALDI−TOF MS (positive mode, matrix: sinapic acid) *m*/*z*, 5168 $[M + Na]^+$. Found: C, 60.93; H, 5.79; N, 2.26. Calcd for $C_{266}H_{300}N_8O_{96}$•4H_2O: C, 61.24; H, 5.95; N, 2.15.

Cyclophane-Based Resorcinarene Tetramer (4). A mixture of **6** (50 mg, 9.7 μmol), aqueous sodium hydroxide (0.56 mL, 0.56 mmol), tetrahydrofuran (4 mL), and water (4 mL) was stirred for 14 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The residue was purified by gel filtration chromatography on a column of Sephadex G-50 with water as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (50 mg, 96%): ^{1}H NMR (400 MHz, D_2O, 298 K) δ 1.4 (m), 1.5 (m), 2.0 (m), 3.5 (m), 3.7 (m), 5.7 (m), 6.0 (m), 6.4 (m), 6.9 (m). ^{13}C NMR (150 MHz, D_2O, 298K) δ 19.66, 19.97, 20.10, 20.4, 24.5, 30.5, 35.8, 39.1, 49.1, 68.9, 100.5, 125.8, 128.1, 130.9, 139.7, 141.3, 154.3, 155.2, 171.7, 177.5. IR 1590 cm^{-1} (C═O). MALDI−TOF MS (negative mode, matrix: sinapic acid) *m*/*z*, 4752 $[M - H]^-$. Found: C, 58.39; H, 5.18; N, 2.41. Calcd for $C_{238}H_{244}N_8O_{96}$•7H_2O: C, 58.59; H, 5.33; N, 2.30%.

Precursor of Cyclophane-Based Resorcinarene Dodecamer (8). Solution of **7** (52 mg, 10 μmol) in dry *N*,*N*-dimethylformamide (DMF, 1 mL) was added dropwise to a solution of **3** (256 mg, 0.23 mmol), BOP (200 mg, 0.46 mmol), and triethylamine (0.07 mL, 0.6 mmol) in dry DMF (1 mL) under nitrogen at room temperature, and the resulting mixture was stirred for 40 h at room temperature. EtOAc (200 mL) was added to the reaction mixture, and the mixture was then washed with 5% aqueous citric acid (50 mL), saturated aqueous sodium chloride (50 mL), and 5% aqueous sodium hydrogen carbonate (50 mL) in this sequence. After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol/chloroform (1:1 v/v) as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (113 mg, 69%): ^{1}H NMR (600 MHz, $CDCl_3$, 298 K) δ 1.5 (m, 40H), 1.5−1.6 (m, 144H), 2.0−2.5 (m, 32H), 3.5 (m, 8H), 3.6 (m, 24H), 3.8 (m, 40H), 3.8−3.9 (m, 252 H), 4.0 (m, 20H), 4.8 (m, 24H), 4.8 (m, 48H), 6.2−6.6 (m, 48H), 7.1 (m, 40H), 7.3 (m, 40H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 1.9, 2.4, 3.9, 33.3, 34.1, 34.0, 40.0, 47.9, 50.9, 65.9, 97.8, 124.7, 127.2, 129.2, 139.3, 152.2, 153.0, 167.2, 168.6, 169.4, 170.4, 170.8. IR 1738, 1645 cm^{-1} (C═O) 1645. MALDI−TOF MS (positive mode, matrix: sinapic acid) *m*/*z*, 1760 $[M + H]^+$. Found: C, 61.13; H, 6.01; N, 2.97. Calcd for $C_{894}H_{1008}N_{36}O_{300}$•24$H_2O$: C, 61.39; H, 6.09; N, 2.88.

Cyclophane-Based Resorcinarene Dodecamer (12). A mixture of **8** (53 mg, 3 μmol), aqueous sodium hydroxide (0.52 mL, 0.52 mmol), tetrahydrofuran (2 mL), and water (2 mL) was stirred for 48 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The residue was purified by gel filtration chromatography on a column of Sephadex G-50 with water as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (44 mg, 88%): ^{1}H NMR (400 MHz, D_2O, 298 K) δ 1.1 (m), 2.0 (m), 3.8 (m), 4.36 (m), 4.43 (m), 5.7 (m), 5.9 (m) 6.3 (m), 7.0 (m). ^{13}C NMR (150 MHz, D_2O, 298 K) δ 20.0, 24.5, 31.0, 34.2, 35.7, 40.2, 48.5, 68.9, 100.4, 125.9, 128.0, 130.6, 139.4, 141.5, 154.0, 155.1, 171.0, 177.3. IR 1587 cm^{-1} (C═O). MALDI−TOF MS (positive mode, matrix: sinapic acid) *m*/*z*, 16103. Found: C, 60.07; H, 5.48; N, 3.14. Calcd for $C_{810}H_{840}N_{36}O_{300}$•14$H_2O$: C, 60.31; H, 5.42; N, 3.13%.

Supporting Information Available: NMR spectra for compounds **3**, **4**, **6**, **8**, and **12**. SPR sensorgrams for the immobilization of histone and the control experiments. Additional titration curves and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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