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Preparation and Multivalently Enhanced Guest-Binding Affinity of Water-Soluble Cyclophane Heptadecamers

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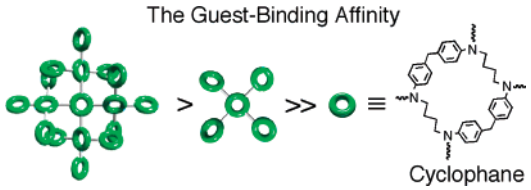

Water-soluble cyclophane heptadecamers (**17a** and **17b**), which were constructed with the core cyclophane heptadecamer and 36 polar side chains with a terminal galactose or glucose residue, respectively, were prepared. An analogous cyclophane pentamer (**5a**) was also prepared. The stoichiometry for the complex of the cyclophane oligomers with fluorescence guests such as TNS was confirmed to be 1:1 host:guest by a Job plot. The guest-binding affinity of cyclophane heptadecamers **17a** and **17b** was much enhanced relative to that of a corresponding monocyclic cyclophane (**1a**), i.e., the 1:1 binding constant (*K*) values for **17a** with TNS, 2,6-ANS, and 1,8-ANS were ca. 1700-, 1600-, and 1500-fold larger than those of **1a** for the identical guests, respectively, which reflects the multivalency effects in macrocycles. Meanwhile, the corresponding *K* values for the cyclophane pentamer **5a** with TNS, 2,6-ANS, and 1,8-ANS were ca. 250-, 250-, and 110-fold larger than those of **1a** for the identical guest, respectively.

Introduction

The hydrophobic interaction is the major driving force for molecular recognition in aqueous media, and other noncovalent intermolecular interactions such as electrostatic, charge-transfer, and hydrogen-bonding modes become effective[1] in hydrophobic and water-lacking microenvironments that are well-shielded from bulk aqueous phase. Cyclophanes with a sizable internal cavity[2] are typical hosts capable of providing such hydrophobic binding sites. In addition, the macrocyclic cavity generally provides a stable binding site that is scarcely affected by external factors such as pH, temperature, and ionic strength. Therefore, a wide synthetic variation of the macrocyclic cyclophanes[3,4] can be achieved, relative to the limited range of structural modifications of semiartificial cyclodextrins,[5] so that an appropriate recognition site with regard to size, shape, and microenvironmental properties can be provided for a target guest molecule. We have previously developed various water-soluble cyclophanes[6] composed of a single macrocyclic skeleton. For example, water-soluble saccharide cyclophane **1a**,[7] which was prepared by introducing four polar side chains with a terminal galactose residue into a tetraaza[6.1.6.1]paracyclophane[8] skeleton, exhibited binding capability toward fluorescent guest molecules such as 6-*p*-toluidinonaphthalene-2-sulfonate (TNS)

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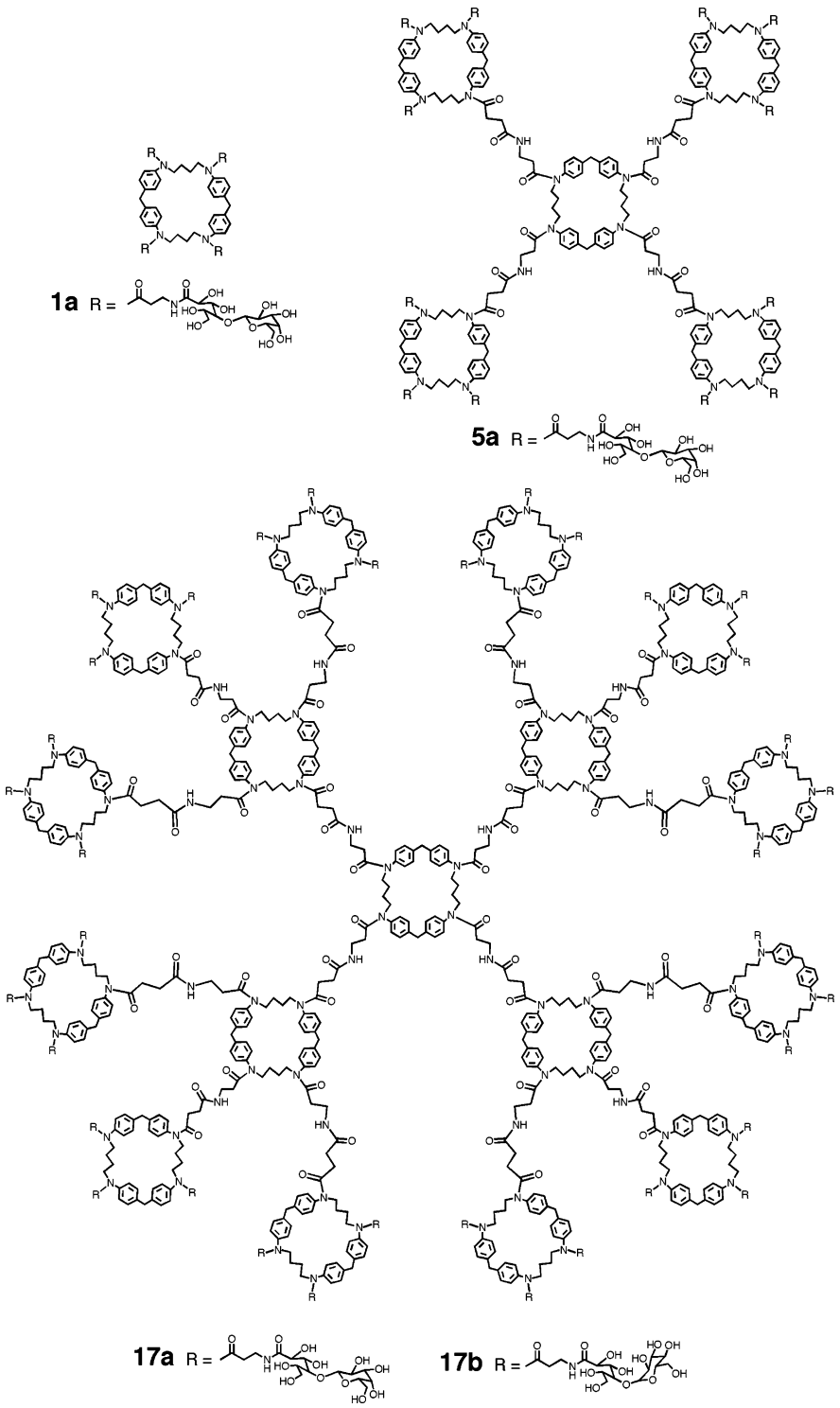

FIGURE 1. Water-soluble cyclophanes and cyclophane oligomers.

through hydrophobic interactions. Unfortunately, however, the guest-binding affinity of the water-soluble cyclophane composed of a single macrocyclic skeleton is only moderate.[8] That is, the (1:1) binding constants (*K*) between **1a** and TNS are on the order 10^3 M^{-1}.

In contrast, a so-called multivalent effect[9] of naturally occurring cell-surface receptors provides an important key to the development of a feasible strategy[10] to enhance the guest-binding abilities in aqueous media. That is, naturally occurring multivalent clusters of receptors are known to exhibit extremely strong binding capability toward substrates, even though these substrates individually bind only weakly to each other. On these grounds, we have previously developed polytopic cyclophanes such as linear-type cyclophane trimer,[11] which was constructed with three tetraaza[6.1.6.1]paracyclophane macrocycles and eight polar side chains with a terminal galactose residue connecting to the macrocyclic skeleton through amide linkages. The guest-binding affinity of the cyclophane trimer with TNS was enhanced 24-fold relative to that of the corresponding monocyclic cyclophane **1a** (Figure 1), as evaluated by fluores-

cence spectroscopy.[11] Since the cyclophane trimer has three binding sites, the guest-binding enhancement per binding site was estimated to be eightfold, which reflects multivalency effects in macrocycles. In the course of our ongoing research on polytopic cyclophanes, we became interested in developing divergent-type cyclophane oligomers in a multiple fashion. In order to enhance the multivalent effects of cyclophanes, we designed a divergent-type[12] cyclophane pentamer (**5a**) bearing 12 polar side chains with a terminal galactose residue (Figure 1). Furthermore, we have now designed an extended analogous heptadecamer (**17a**) bearing 36 polar side chains with a terminal galactose residue in order to enhance the multivalent effects of cyclophanes (Figure 1). We describe herein the synthesis of the divergent-type cyclophane oligomers and their binding affinity with fluorescence guests, as evaluated in aqueous media by fluorescence spectroscopy, with an emphasis on the multivalent effects in guest-binding.

Results and Discussion

Design and Synthesis of Cyclophane Oligomers. Cyclophane oligomers were developed on the basis of a molecular design that allows the assembly of five or 17 cyclophanes by an iterative divergent approach. First, we adopted a divergent cyclophane pentamer[12] as a core skeleton by assembling 4 tetraaza[6.1.6.1]paracyclophane derivatives onto a tetraaza-[6.1.6.1]paracyclophane through amide linkages. Water-soluble cyclophane pentamer (**5a**), which was constructed with the core cyclophane pentamer and 12 polar side chains with a terminal galactose residue that connect to the core skeleton through amide linkages, was designed (Figure 1). In addition, we designed an extended analogous heptadecamer (**17a**), which was constructed with a divergent cyclophane heptadecamer as a core skeleton and 36 polar side chains with a terminal galactose residue, by an iterative divergent approach in order to enhance the multivalent effects of cyclophanes (Figure 1). Similar cyclophane heptadecamer **17b** was also designed when a polar side chain with a terminal glucose residue was employed in place of that with a terminal galactose residue (Figure 1). It is desirable for these water-soluble cyclophanes oligomers as a host to provide hydrophobic binding sites that can be desolvated upon guest incorporation. It must be noted here that hydrophobicity and hydrophilicity are conflicting concepts. Therefore, hydrophobic binding sites must be reasonably separated from hydrophilic groups that are required for giving water solubility to cyclophanes, so that the hydrophobic efficiency is maintained for guest incorporation. Saccharide-derived substitutes[13] were introduced on the periphery of the cyclophane oligomers to confer water solubility on the resulting hosts.

Cyclophane oligomers **5a** and **17a** were prepared by following the reaction sequence given in Scheme 1. A cyclophane bearing three Boc-β-alanine residues (**2**)[14] was synthesized by condensation of a tetraaza[6.1.6.1]paracyclophane with Boc-β-alanine in the presence of dicyclohexylcarbodiimide (DCC) and then converted to a cyclophane having a carboxylic acid (**3**) by reaction with succinic anhydride. A precursor (**6**)[12] of **5a** was prepared by condensation of a tetraamine derivative of cyclophane **4**[7] with **3** in the presence of benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP). The use of dodecaamine derivative of cyclophane, which was prepared by removal of the Boc-protecting group of **6**, in place of tetraamine derivative of cyclophane **4** afforded the corresponding precursor (**7**) of **17a** using the same method applied to the preparation of **6**. Saccharide cyclophane pentamer **5a** and heptadecamer **17a** were obtained by the reaction of lactonolactone[15] with dodecaamine and hexatriacontaamine derivatives of cyclophane oligomers, which were prepared by removal of the boc-protecting groups of **6** and **7**, respectively. A cyclophane heptadecamer bearing polar side chains with a terminal glucose residue (**17b**) was prepared by the reaction of maltonolactone with the hexatriacontaamine derivative of cyclophane in a manner similar to that applied to the synthesis of **17a**.

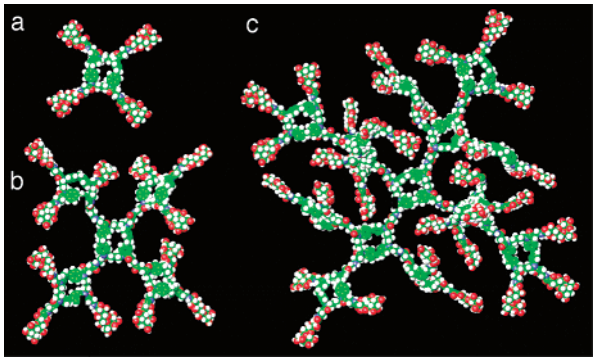

FIGURE 2. Computer-generated CPK models for **1a** (a) and cyclophane oligomers **5a** (b) and **17a** (c). Carbon, hydrogen, oxygen, and nitrogen atoms are shown in green, white, red, and blue, respectively.

Based on the molecular mechanics studies of cyclophane heptadecamer **17a**, followed by molecular dynamics simulations,[16] **17a** was found to provide 17 rigid macrocyclic cavities suitable for encapsulating small, complementary organic molecules as guests, as shown in Figure 2. The neighboring macrocyclic skeletons were separated from each other through a spacer including rigid amide linkages. In contrast, 36

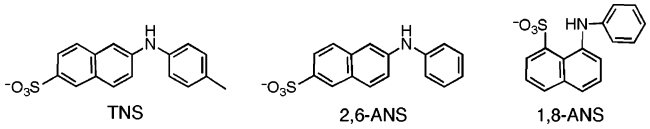

FIGURE 3. Hydrophobic fluorescent guests.

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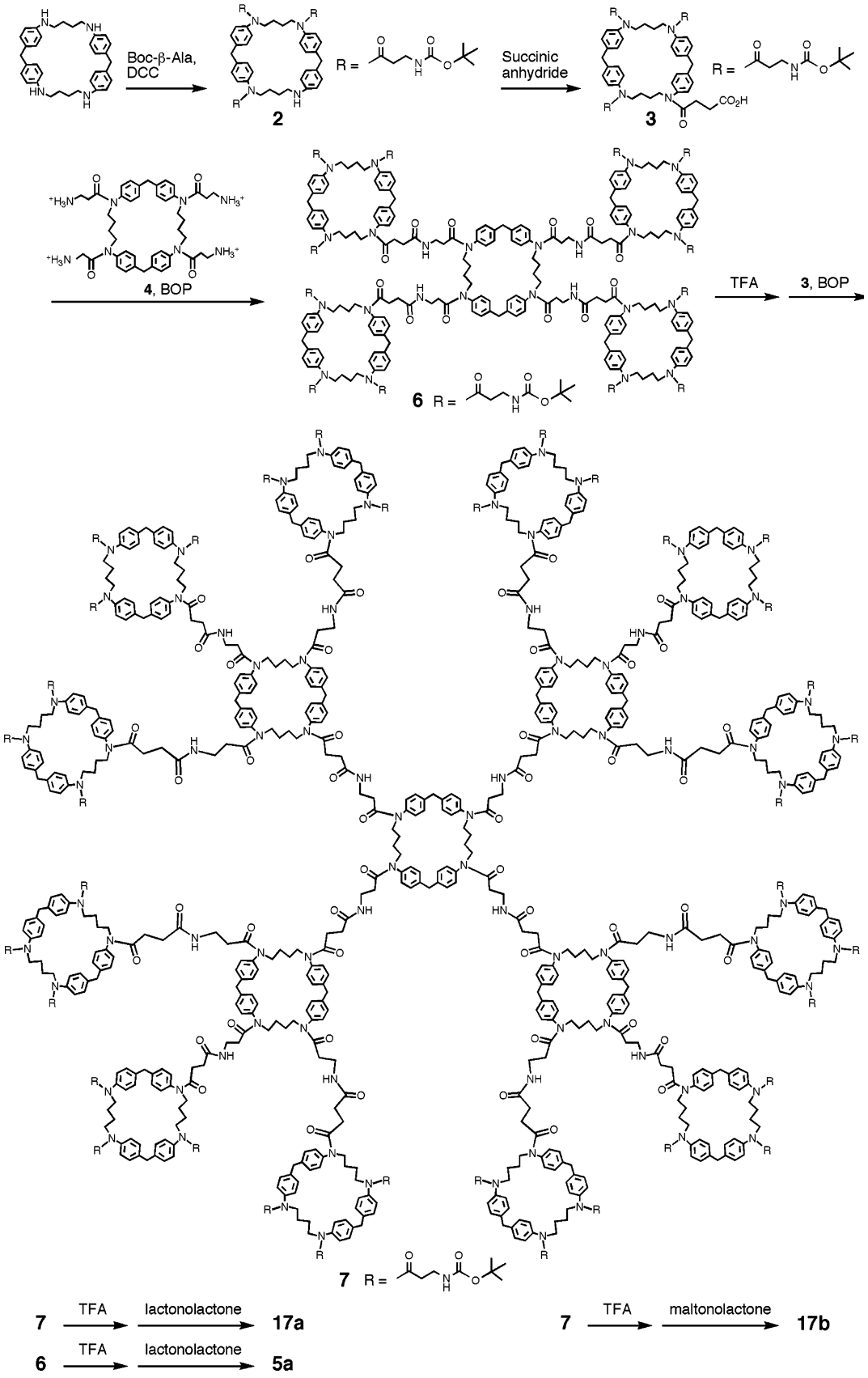
SCHEME 1. Preparation of Cyclophane Oligomers 5a, 17a, and 17b



peripheral polar side chains with reasonably separated distances were expected to confer the advantage of enhanced solubility in neutral aqueous media. From a practical standpoint, cyclophane heptadecamer **17a** had good H_2O-solubility of >1 g/mL. At least at concentrations below 2.0 μM of **17a**, a good linear Beer's plot of absorbance at 230 nm was observed. A similar

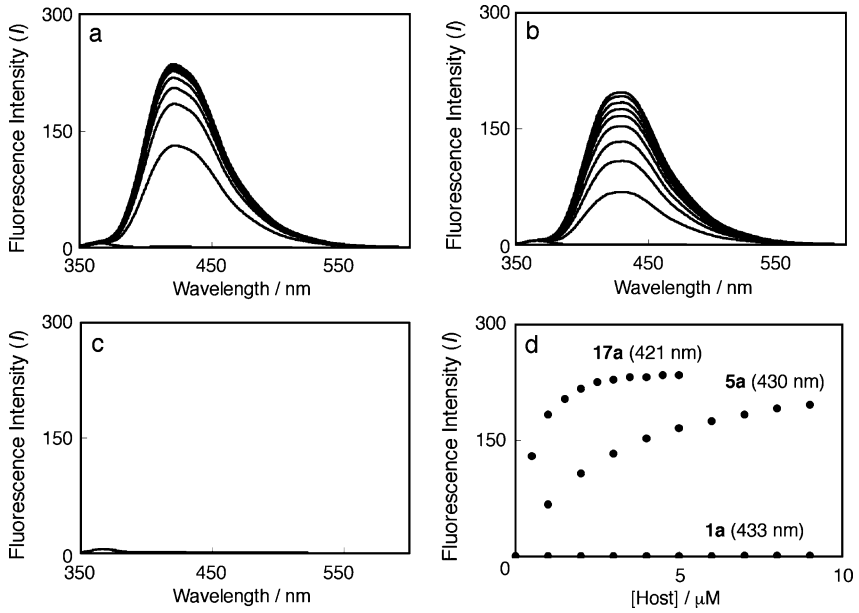


FIGURE 4. Fluorescence spectral changes for an aqueous solution of TNS (0.25 μM) upon addition of **17a** (a), **5b** (b), and **1a** (c) in H_2O at 303 K, **[17a]** = 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 μM, **[5a]** and **[1a]** = 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 μM (from bottom to top). The corresponding titration curves (d). Ex. 326 nm.

character in the H_2O-solubility of other cyclophane oigomers **17b** and **5a** was also confirmed by identical methods.

Fluorescent Study of the Guest-Binding of Cyclophane Oligomers. The guest-binding affinity of macrocyclic hosts was conveniently investigated by using well-known fluorescent guests[17] such as TNS (Figure 3) whose emissions are extremely sensitive to change in the microenvironmental polarity experienced by molecules in fluorescence spectroscopy. In general, the fluorescence intensity originating from guest molecules is subjected to increases along with a concomitant blue shift of the fluorescence maximum when the guest molecules are incorporated into the hydrophobic cavity provided by the hosts. As mentioned above, the guest-binding affinity of **1a** for TNS was moderate (K, 1.5×10^3 M^{-1}) (see the Supporting Information). When lower (0.25 μM) concentrations of TNS were employed, the extent of change in fluorescence intensity originating from the guest molecules was almost negligible upon addition of **1a** (up to 9.0 μM), as shown in Figure 4 (c). In the case of **17a**, however, the fluorescence intensity originating from TNS (0.25 μM) was much increased along with a concomitant blue shift of the fluorescence maximum upon addition of **17a** (up to 4.5 μM), as shown in Figure 4 (a). A similar fluorescent character was also confirmed for the complexation of **17a** with 2-anilinonaphthalene-6-sulfonate (2,6-ANS) and 1-anilinonaphthalene-8-sulfonate (1,8-ANS) (see the Supporting Information). In addition, upon addition of the cyclophane pentamer **5a,** the extents of change in the fluorescence intensity of fluorescent guests were somewhere between those of **17a** and **1a**, as shown in Figure 4b,d.

Relatively large fluorescence polarization values (P) were obtained for TNS, 2,6-ANS, and 1,8-ANS incorporated into **17a** (P, 0.20, 0.16, and 0.17, respectively). The obtained P values were somewhat larger than those for TNS bound to **1a** (P, 0.06, 0.06, and 0.05, respectively). On the other hand, the P values for TNS, 2,6-ANS, and 1,8-ANS incorporated into **5a** (P, 0.11, 0.12, and 0.09, respectively) were again between those of **17a** and **1a**. These results indicated that the tight host−guest interaction brings about more effective motional repression of the entrapped guest, in the case of **17a** with a large molecular weight of 25 839.

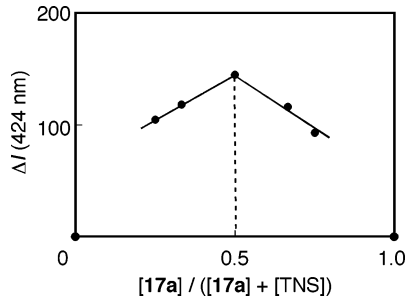


FIGURE 5. Job's plot for complex of **17a** with TNS; total concentration of **17a** and TNS, 3.0 μM.

Multivalent Effects on the Guest-Binding of Cyclophane Oligomers. First, the stoichiometry for the complexes formed with the host and the guest was investigated by Job's continuous variation methods.[18] The obtained Job's plot for TNS complexes of **17a** is shown in Figure 5 as a typical example. The result reveals that host **17a** forms a complex with TNS in a 1:1 molar ratio of host to guest. The same 1:1 stoichiometry was confirmed for other host−guest complexes of **17a** with 2,6-ANS and 1,8-ANS (see the Supporting Information). Although cyclophane heptadecamer **17a** contains multiple macrocyclic cavities, more than two anionic guest molecules were not incorporated simultaneously due to an electrostatic repulsion under these conditions.[19]A similar binding trend was also confirmed for the

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(19) When an aqueous HEPES buffer solution at high ionic strength of 0.5 (NaCl) was used in place of H_2O, two anionic guest molecules were incorporated simultaneously by host **17a**, as confirmed by Job's plot (see the Supporting Information). The result suggests that electrostatic repulsion between incorporated anionic guest molecules is not operative under the high ionic strength conditions employed.

TABLE 1. Binding Constants (K, M^{-1}) for Host−Guest Complexes of Cyclophanes with TNS, 2,6-ANS, and 1,8-ANS in H_2O at 303 K[a]

	K, M^{-1}		
host	TNS	2,6−ANS	1,8−ANS
17a	2.6×10^6	1.9×10^6	1.7×10^6
17b	4.5×10^6	4.0×10^6	3.6×10^6
5a	3.8×10^5	3.0×10^5	1.2×10^5
1a	1.5×10^3	1.2×10^3	1.1×10^3

[a] Excitation: TNS, 326 nm; 2,6-ANS, 318 nm; 1,8-ANS, 375 nm.

complexation of **5a** and **1a** with TNS, 2,6-ANS, and 1,8-ANS (see the Supporting Information).

The 1:1 binding constants (K) of **17a** toward these guests were evaluated on the basis of the Benesi-Hildebrand relationship[20] for the titration data under the condition of large excess amounts of the hosts, as described previously (see the Supporting Information), and are summarized in Table 1 together with the corresponding values for **17b**, **5a**, and **1a**. As regards the multivalent effect achieved by multiplying the cyclophane, the guest-binding affinity cyclophane heptadecamer **17a** and **17b** was much enhanced relative to that of a corresponding monocyclic cyclophane. That is to say, the K values for **17a** with TNS, 2,6-ANS, and 1,8-ANS were ca. 1700-, 1600-, and 1500-fold larger than those of **1a** for the identical guest, respectively. In other words, since the cyclophane heptadecamer has 17 binding sites, the guest-binding enhancement per binding site was estimated to be 100-, 93-, and 91-fold which reflects the multivalency effects in macrocycles.[21] A similar guest-binding enhancement was also confirmed for the complexation of **17b** with TNS, 2,6-ANS, and 1,8-ANS (Table 1). In contrast, the corresponding K values for the cyclophane pentamer **5a** with TNS, 2,6-ANS, and 1,8-ANS were ca. 250-, 250-, and 110-fold larger than those of **1a** for the identical guest, respectively. That is, the guest-binding enhancement per binding site was estimated to be 51-, 50-, and 22-fold, since the cyclophane pentamer has 5 binding sites, which were between those of **17a** and **1a**. Enhancements of the guest-binding ability[22] were successfully achieved by the divergent-type water-soluble cyclophane heptadecamer.

Conclusion

Core cyclophane pentamer and heptadecamer were designed on the basis of a molecular design that allows the assembly of five or 17 cyclophanes by an iterative divergent approach. Water-soluble cyclophane pentamer (**5a**) and heptadecamer (**17a**) were prepared by introducing 12 or 36 polar side chains with a terminal galactose residue into the core cyclophane pentamer and heptadecamer, respectively. The guest-binding ability was especially enhanced by the divergent-type water-soluble cyclophane heptadecamer, as demonstrated by fluorescence spectroscopy. These molecular design and unique guest-binding properties of the divergent-type cyclophane oligomers can provide important information for understanding the recognition mechanisms of naturally occurring cell-surface receptors. In addition, the present cyclophane oligomers have terminal galactose residues with reasonably separated distances that can be expected to act as multivalent ligands[23] for the specific carbohydrate-binding protein (lectin). Combined these characteristics suggest that the present cyclophane oligomers have the potential to act as a saccharide-directed molecular drug-delivery system. These subjects of interest will be explored further in the future.

Experimental Section

Binding Constants of Cyclophane Oligomers with Fluorescence Guests. To each solution of a fluorescent guest (0.025, 0.25, or 1.0 μM) in H_2O were added increasing amounts of the hosts at 303 K, and the guest fluorescence intensity was monitored after each addition by excitation at 326, 318, and 375 nm for TNS, 2,6-ANS, and 1,8-ANS, respectively. The binding constants were calculated on the basis of the Benesi-Hildebrand method for titration data.

***N,N′,N‴*-Tris[2-(*tert*-butoxycarbonylamino)propionyl]-1,6,-20,25-tetraaza[6.1.6.1]paracyclophane (2).** Dicyclohexylcarbodiimide (980 mg, 4.8 mmol) was added to a solution of Boc-β-alanine (230 mg, 4.8 mmol) in dry dichloromethane (DCM, 10 mL) at 0 °C, and the mixture was allowed to stand at the same temperature while being stirred for 20 min. The mixture was added to a solution of 1,6,20,25-tetraaza[6.1.6.1]paracyclophane (750 mg, 1.5 mmol) in dry DCM (25 mL), and the resulting mixture was stirred for 3 h at 0 °C. Precipitates that formed (*N,N′*-dicyclohexylurea) were removed by filtration, the solvent was eliminated under reduced pressure, and the residue was dissolved in ethyl acetate (EtOAc, 50 mL). The solution was then washed with 5% aqueous sodium hydrogen carbonate (60 mL) and saturated aqueous sodium chloride (30 mL) in this sequence. After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (SiO_2) with ethyl acetate−hexane as eluent. The product fraction was dried in vacuo to give a white solid. 0.37 g (25%): mp 109−110 °C. R_f (ethyl acetate) 0.60; ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.3 (s,31H), 1.5 (br,4H), 2.1 (m,6H), 3.0 (br,2H), 3.2 (m,6H), 3.6 (m,-6H), 3.7 (s,2H), 3.9 (s,2H), 5.3 (br,3H), 6.4, 6.9, and 7.2 (m,16H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 25.5, 25.8, 26.7, 28.8, 35.2, 36.7, 41.2, 44.0, 49.2, 79.4, 113.2, 128.6, 129.8, 130.7, 140.0, 140.8, 142.8, 146.9, 156.3, and 171.9. IR 1704, 1645 cm^{-1} (C═O). MALDI-TOF MS (positive mode, matrix: α-CHCA): *m/z*, 1041 $[M + Na]^+$. Found: C, 66.27; H, 7.67; N, 9.33. Calcd for $C_{58}H_{79}N_7O_9 \cdot 2H_2O$: C, 66.07; H, 7.93; N, 9.30.

***N,N′,N‴*-Tris[2-(*tert*-butoxycarbonylamino)propionyl]-*N‴*-(3-carboxypropionyl)-1,6,20,25-tetraaza[6.1.6.1]paracyclophane (3).** Succinic anhydride (27 mg, 0.27 mmol) was added to a solution of **2** (200 mg, 0.2 mmol) in dry DCM (2 mL) at room temperature, and the mixture was stirred for 12 h. Ethylenediamine (100 μL, 1.5 mmol) was added to the mixture to quench the reaction. DCM (30 mL) was added to the solution and then washed with 5% aqueous citric acid (10 mL) and saturated aqueous sodium chloride (30 mL) in this sequence. After being dried (Na_2SO_4), the solution was evaporated to dryness under reduced pressure to give a white solid. 190 mg (86%). mp 119−121 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.3 (s,35H), 2.0 (br,6H), 2.1(br,2H), 3.1 (br,6H), 3.5 (m,-8H), 3.9 (m,4H), 5.2 (br,3H), 3.9 (s,2H), 6.9 (m,8H), 7.1 (m,8H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 25.3, 28.8, 30.1, 35.2, 36.7, 41.4, 49.1, 49.4, 53.9, 79.4, 128.7, 130.6, 140.8, 140.9, 156.4, 171.9, and 175.5. IR 1706, 1646 cm^{-1} (C═O). MALDI-TOF MS (positive

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(21) It is possible that the cyclophane oligomer folds around the incorporated guest so that two or more macrocycles are around the guest molecule.

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mode, matrix: α-CHCA): *m/z*, 1141 $[M + Na]^+$. Found: C, 65.66; H, 7.43; N, 8.61. Calcd for $C_{62}H_{83}N_7O_{12}\cdot H_2O$: C, 65.53; H, 7.54; N, 8.63.

Cyclophane Pentamer Bearing Boc-Protected Amines (6). A solution of compound **3** (230 mg, 0.2 mmol) and triethylamine (0.2 mL) in dry *N*,*N*-dimethylformamide (DMF, 1 mL) was added dropwise to a solution of the tetraamine derivative of cyclophane **4** (40 mg, 0.03 mmol), benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP, 230 mg, 0.5 mmol), in dry DMF (1 mL) under nitrogen at room temperature, and the resulting mixture was stirred for 8 days at room temperature. EtOAc (50 mL) was added to the reaction mixture, and the mixture was then washed with 5% aqueous citric acid (20 mL), saturated aqueous sodium chloride (20 mL), and 5% aqueous sodium hydrogen carbonate (20 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 an eluant. The product fraction was evaporated to dryness under reduced pressure to give a pale yellow solid. 100 mg (60%): mp 151−152 °C. R_f (chloroform−methanol, 10:1 v/v) 0.6; ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.4 (s,140H), 1.4 (m,8H), 2.0 (br,-32H), 2.2 (m,8H), 2.3 (m,8H), 3.2 (br,24H), 3.3 (br,8H), 3.6 (br,-40H), 3.9 (s,20H), 5.3 (br,12H), 6.9, 7.0, 7.1, and 7.2 (m,80H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ 25.0, 28.4, 29.9, 31.3, 34.4, 35.1, 36.3, 41.1, 48.9, 77.4, 128.5, 130.2, 140.2, 140.5, 155.9, 171.4, and 171.9. IR 1710, 1646 cm^{-1} (C═O). MALDI-TOF MS (positive mode, matrix: sinapic acid): *m/z*, 5213 $[M + Na]^+$. Found: C, 68.01; H, 7.43; N, 9.73. Calcd for $C_{294}H_{384}N_{36}O_{48}$: C, 68.03; H, 7.46; N, 9.71.

Cyclophane Pentamer Having Branches with a Terminal Galactose Residue (5a). Trifluoroacetic acid (0.2 mL) was added to a solution of cyclophane heptadecamer **6** (50 mg, 9.6 μmol) in dry DCM (2 mL), and the mixture was stirred for 3 h at room temperature. After the solvent was evaporated off under reduced pressure, DCM (10 mL) was added to the residue, and this procedure was repeated 3 times to remove the remaining trifluoroacetic acid. Evaporation of the solvent under reduced pressure gave a dodecaamine derivative of cyclophane (50 mg) as the trifluoroacetic acid salt. A solution of the dodecaamine derivative (50 mg, 9.6 μmol) and triethylamine (0.05 mL, 0.4 mmol) in dry methanol (1 mL) was added to the methanol solution of lactonolactone (120 mg, 0.35 mmol), and the mixture was stirred at room temperature for 1 h. The precipitates which separated were collected by filtration, washed with methanol, and dried in vacuo. Insoluble materials were removed by filtration, and the filtrate was evaporated to dryness in vacuo to give a pale yellow solid (55 mg, 70%): mp 175−177 °C. ^{1}H NMR (600 MHz, D_2O, 298 K) δ 1.0−1.3, 2.0−2.2, 3.0−3.3, 3.5−4.0, 4.1, 4.3, 4.5, 4.6, 4.8, 6.8, and 7.5. ^{13}C NMR (150 MHz, D_2O, 298 K) δ 23.5, 31.5, 33.8, 35.7, 36.1, 41.0, 48.1, 61.5, 62.4, 69.0, 70.8, 71.5, 72.0, 72.7, 73.0, 75.8, 82.0, 103.9, 128.2, 130.5, 139.4, 141.7, 171.7, 172.7, 173.1, 173.9, and 174.1. IR 1634 cm^{-1} (C═O). Found: C, 54.80; H, 6.59; N, 6.35. Calcd for $C_{378}H_{528}N_{36}O_{156}\cdot 12H_2O$: C, 54.77; H, 6.71; N, 6.08.

Cyclophane Heptadecamer Bearing Boc-Protected Amines (7). Trifluoroacetic acid (0.2 mL) was added to a solution of cyclophane pentamer **6** (100 mg, 19 μmol) in dry DCM (3 mL), and the mixture was stirred for 3 h at room temperature. After the solvent was evaporated off under reduced pressure, DCM (20 mL) was added to the residue, and this procedure was repeated 3 times to remove the remaining trifluoroacetic acid. Evaporation of the solvent under reduced pressure gave a dodecaamine derivative of cyclophane (103 mg) as the trifluoroacetic acid salt. A solution of a cyclophane having a carboxylic acid (**3**) (431 mg, 0.39 mmol) in dry DMF (1.5 mL) was added dropwise to a solution of the dodecaamine derivative of cyclophane (103 mg, 19 μmol), BOP (170 mg, 0.39 mmol), and triethylamine (0.2 mL) in dry DMF (1.5 mL) under nitrogen at room temperature, and the resulting mixture was stirred for 14 days at room temperature. EtOAc (50 mL) was added to the reaction mixture, and the mixture was then washed with 5% aqueous citric acid (20 mL), saturated aqueous sodium chloride (20 mL), and 5% aqueous sodium hydrogen carbonate (20 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 an eluant. Evaporation of the product fraction under reduced pressure gave a pale yellow solid (150 mg, 45%): mp 152−154 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.3(m,460H), 2.1 (br,104H), 2.2 (m,32H), 2.3 (m,32H), 3.2 (br,104H), 3.6 (br,136H), 3.9 (s,68H), 5.3 (br,-36H), 6.9, 7.0, 7.1, and 7.2 (m,272H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 25.3, 28.5, 30.3, 31.7, 34.2, 34.5, 34.7, 38.3, 42.2, 50.6, 79.3, 129.0, 131.0, 140.2, 140.4, 156.0, 171.4, 171.8, 172.0, and 172.4. IR 1705, 1646 cm^{-1} (C═O). MALDI-TOF MS (positive mode, matrix: sinapic acid): *m/z*, 17193 $[M + H]^+$. Found: C, 67.55; H, 7.46; N, 9.60. Calcd for $C_{978}H_{1260}N_{120}O_{156}\cdot 8H_2O$: C, 67.75; H, 7.42; N, 9.69.

Cyclophane Heptadecamer Having Branches with a Terminal Galactose Residue (17a). Trifluoroacetic acid (0.2 mL) was added to a solution of cyclophane heptadecamer **7** (100 mg, 5.8 μmol) in dry DCM, 2 mL, and the mixture was stirred for 3 h at room temperature. After the solvent was evaporated off under reduced pressure, DCM (10 mL) was added to the residue, and this procedure was repeated 3 times to remove the remaining trifluoroacetic acid. Evaporation of the solvent under reduced pressure gave a dodecaamine derivative of cyclophane (105 mg) as the trifluoroacetic acid salt. A solution of the dodecaamine derivative (103 mg, 5.8 μmol) and triethylamine (0.1 mL, 0.8 mmol) in dry methanol (1 mL) was added to the methanol solution of lactonolactone (210 mg, 0.63 mmol), and the mixture was stirred at room temperature for 1 h. The precipitates which separated were collected by filtration, washed with methanol, and dried in vacuo. Insoluble materials were removed by filtration, and the filtrate was evaporated to dryness in vacuo to a pale yellow solid (95 mg, 63%): mp 178−180 °C. ^{1}H NMR (600 MHz, D_2O, 298 K) δ 1.0−1.3, 2.0−2.4, 3.0−3.3, 3.5−4.0, 4.1, 4.3, 4.5, 4.7, 4.8, 6.8, and 7.5. ^{13}C NMR (150 MHz, D_2O, 298 K) δ 23.6, 30.6, 30.7, 31.1, 33.8, 35.1, 35.8, 41.1, 47.9, 61.5, 62.4, 69.0, 70.8, 71.5, 71.6, 71.9, 73.0, 75.8, 81.5, 103.9, 127.0, 130.4, 139.2, 141.8, 172.8, 173.9, 174.2, and 178.7. IR 1634 cm^{-1} (C═O). Found: C, 55.87; H, 6.55; N, 6.42. Calcd for $C_{1230}H_{1692}N_{120}O_{480}\cdot 32H_2O$: C, 55.93; H, 6.70; N, 6.36.

Cyclophane Heptadecamer Having Branches with a Terminal Glucose Residue (17b). This compound was prepared by using maltonolactone in place of lactonolactone in a manner similar to that applied to the synthesis of **17a.** The precipitates which separated were collected by filtration, washed with methanol, and dried in vacuo. Insoluble materials were removed by filtration, and the filtrate was evaporated to dryness in vacuo to a pale yellow solid (102 mg, 44%): mp 179−181 °C. ^{1}H NMR (600 MHz, D_2O, 298 K) δ 1.0−1.3, 2.0−2.4, 3.0−4.0, 4.2, 4.6, 5.1, 5.3, 6.8, and 7.5. ^{13}C NMR (100 MHz, D_2O, 298 K) δ 25.2, 32.5, 32.7, 35.5, 35.8, 35.1, 39.0, 43.0, 50.5, 62.8, 64.0, 71.9, 74.2, 74.9, 75.1, 75.4, 78.8, 103.0, 130.4, 132.8, 141.2, 142.2, 174.2, 175.6, 176.2, and 177.0. IR 1634 cm^{-1} (C═O). Found: C, 55.00; H, 6.63; N, 6.16. Calcd for $C_{1230}H_{1692}N_{120}O_{480}\cdot 49H_2O$: C, 55.28; H, 6.75; N, 6.29.

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Supporting Information Available: NMR spectra for compounds **2**, **3**, **5a**, **6**, **7**, **17a**, and **17b**, Job plots, 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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