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Synthesis of Dabsyl-Appended Cyclophanes and Their Heterodimer Formation with Pyrene-Appended Cyclophanes

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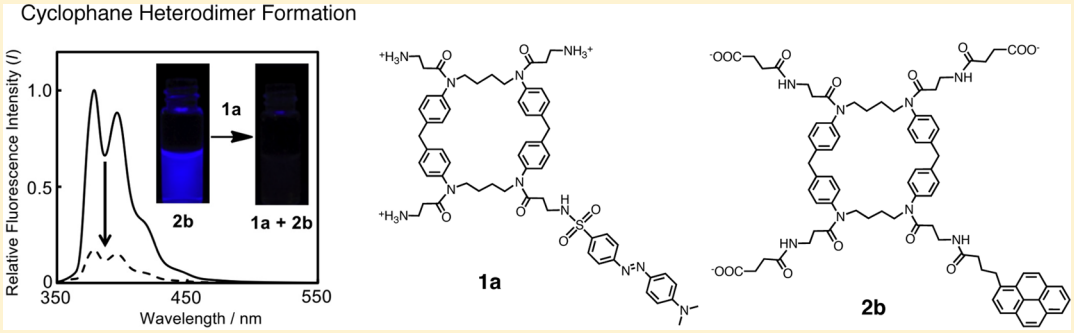
Supporting Information



ABSTRACT: As a quencher-type host, dabsyl-appended cyclophanes bearing positively and negatively charged side chains (**1a** and **1b**, respectively) were synthesized. Formation of cyclophane heterodimers of **1a** with anionic fluorescent cyclophane bearing a pyrene moiety **2b** was confirmed by fluorescence titration experiments. The 1:1 binding constant (K) of **1a** toward **2b** was calculated to be 1.6×10^5 M^{-1}. On the other hand, almost no complexation affinity of **1a** toward cationic analogue of fluorescent cyclophane **2a** was confirmed by the identical methods, indicating that electrostatic interactions became effective in the formation of cyclophane heterodimers. In addition, van't Hoff analysis applied to the temperature-dependent K values for the heterodimer formation gave negative enthalpy (ΔH) and entropy changes (ΔS). The large and negative ΔH values as well as small and also negative ΔS values showed that the complexation is an exothermic and enthalpy-controlled but not entropy-driven process. A similar trend of molecular recognition was also confirmed for formation of cyclophane heterodimers of **1b** with **2a** by the identical methods.

INTRODUCTION

Host–guest chemistry involves the formation of molecular complexes composed of host molecules noncovalently bound to guest molecules in a unique structural relationship.[1] Macrocyclic compounds[2] such as cyclophanes are widely studied because of their great technological importance in industrial application. In particular, functionalized cyclophanes show the following characteristic properties. Cyclophanes have an internal rigid cavity suitable for binding a guest molecule within.[3] In addition, a wide synthetic variation of the cyclophane molecules can be achieved so that sophisticated molecular discrimination becomes operative by the introduction of functional groups into appropriate sites of cyclophanes giving noncovalent interactions such as electrostatic forces or hydrogen bonding.[4] On these grounds, we have previously developed functionalized cyclophanes[5] by introducing side chains such as amino acid derivatives[6] and saccharide residues[7] into the cyclophane skeleton. It was found that the cyclophanes having positively or negatively charged side chains[6] act as a water-soluble host, while the cyclophanes having terminal saccharide residues[7] act as a ligand for lectins.

On the other hand, fluorescence techniques such as fluorescence resonance energy transfer (FRET) have been extensively used to investigate interactions of biomolecular complexes and assemblies.[8] Many types of fluorescent dyes such as fluorescein isothiocyanate,[9] rhodamine derivatines,[10] and molecular beacons[11] have been designed and developed for such purpose. Among them, 4-*N*,*N*-dimethylamino-azobenzene-4′-sulfonyl (dabsyl) derivatives[12] are a nonfluorescent chromophore frequently used as a dark quencher. Generally, fluorophore emission is influenced by interaction with the dabsyl derivatives, which may quench the emitted fluorescence from the excited fluorophore.[13] In the course of our ongoing research on water-soluble and functionalized cyclophanes as artificial hosts, we became interested in developing quencher-type cyclophanes capable of binding guest molecules selectively and monitoring by fluorescence spectroscopy. As a dark quencher host, we have now designed cationic and anionic cyclophanes bearing a dabsyl moiety (**1a** and **1b**, respectively) (Figure 1). In this context, we report preparation of **1a** and **1b** and their host–guest complexation as well as formation of cyclophane heterodimers[14] studies by fluorescence spectroscopy with emphasis on the molecular discrimination capabilities. In addition, their thermodynamic parameters were evaluated

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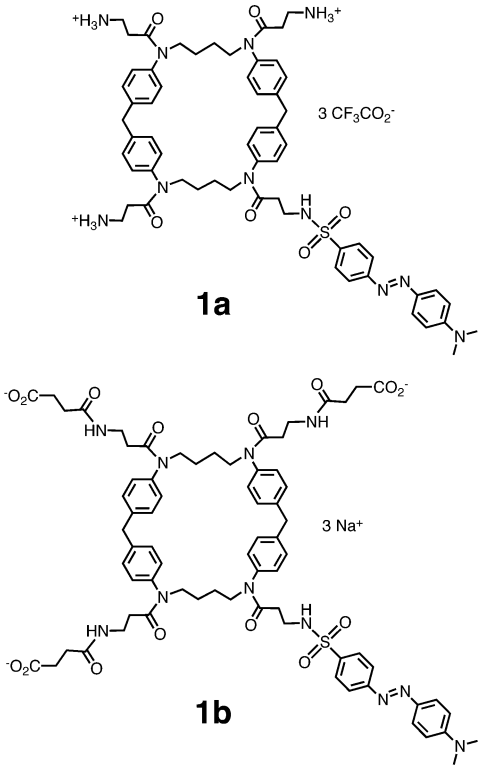


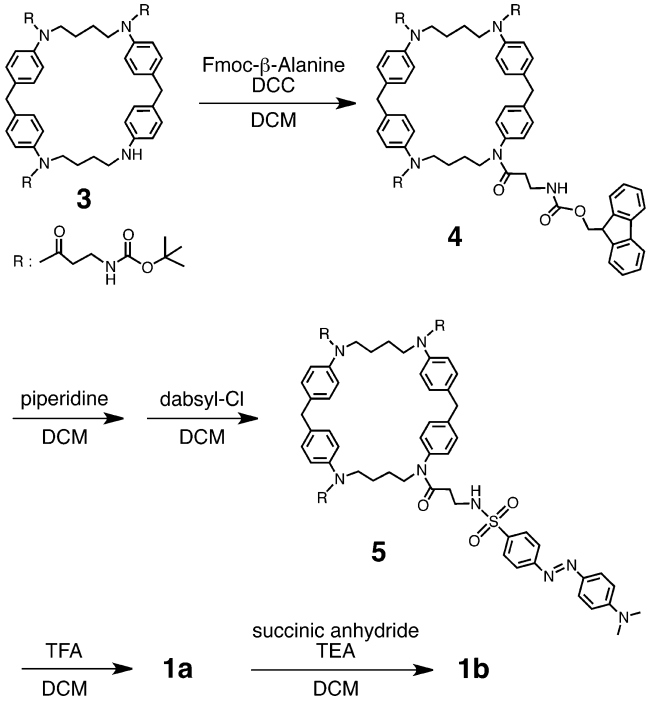
Figure 1. Cationic and anionic quencher-type cyclophanes bearing a dabsyl moiety **1a** and **1b**.

from temperature-dependent fluorescence titration experiments.

RESULTS AND DISCUSSION

Design and Syntheses of Dabsyl-Appended Cyclophanes. As mentioned above, we have previously prepared various functionalized tetraaza[6.1.6.1]paracyclophanes[15] having polar side chains such as amino acid derivatives and saccharide residues. In addition, dabsyl derivatives are a typical quencher[16] in FRET applications,[17] because it has a broad and intense visible absorption but no fluorescence. From a viewpoint of functionalization of cyclophanes, we have designed quencher-type cyclophanes having a dabsyl moiety. Actually, we have adopted a simple strategy to prepare dabsyl-appended cyclophanes by introducing a dabsyl moiety into tetraaza[6.1.6.1]paracyclophane through a *β*-alanine spacer. Dabsyl-appended cyclophanes bearing cationic and anionic polar side chains (**1a** and **1b**, respectively) were synthesized by following the reaction sequence shown in Scheme 1. In the preceding paper, we have synthesized a cyclophane derivative bearing three Boc-*β*-alanine residues **3**.[18] Cyclophane bearing *N*-protected amines **4** was synthesized by condensation of **3** with Fmoc-*β*-alanine in the presence of dicyclohexylcarbodiimide (DCC) in a 75% yield. A precursor (**5**) of **1a** was synthesized by a reaction of 4-*N*,*N*-dimethylamino-azobenzene-4′-sulfonylchloride (dabsyl-Cl) with a monoamine derivative of cyclophane, which was easily prepared from **4** by removal of the Fmoc protecting group with piperidine, in the presence of DCC in a 50% yield. Cationic cyclophane bearing a dabsyl moiety **1a** was derived from **5** by a treatment with trifluoroacetic acid (TFA). Then, **1a** was converted to a cyclophane having carboxylic acid residues **1b** by a reaction with succinic anhydride. New compounds were fully characterized by means of spectroscopy (IR, ^{1}H and ^{13}C NMR, and TOF-MS) and elemental analysis (see Supporting Information). Even though compounds **1a** and **1b** contain a hydrophobic cavity, both compounds were soluble in aqueous neutral media at biological pH owing to three polar side chains. From a practical standpoint, cyclophanes **1a** and **1b** had good H_2O-solubility of 0.33 and 0.18 g/mL, respectively. Judging from molecular mechanics studies of cyclophanes **1a** and **1b**, both compounds provide a rigid internal cavity and the peripheral polar side chains with reasonably separated distances from the cavity (see Supporting Information). These results indicate that **1a** and **1b** having hydrophobic cavities were expected to act as water-soluble hosts. As mentioned above, dabsyl derivatives have a broad and intense visible absorption. The UV−vis absorption spectrum of **1a** is between 350 and 550 nm in aqueous HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid) buffer (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K. In addition, the pH-dependency of the absorption spectra reveals that the pK_a of the diazo group of **1a** is ca. 2.2, which is almost comparable to that of dabsyl-L-alanine (pK_a 2.8)[19] (see Supporting Information).

Scheme 1. Preparation of Dabsyl-Appended Cyclophanes 1a and 1b



Fluorescent Study on Heterodimer Formation of Cyclophanes. Fluorescence titration experiments by using **1a** and **1b** as a dark quencher host applied to heterodimer formation of cyclophanes. In a preceding paper, we have also developed cationic and anionic fluorescent cyclophanes bearing a pyrene moiety **2a** and **2b**, respectively (Figure 2).[20] The former host provides three positively charged side chains, while the latter one provides three negatively charged side chains. Both cyclophanes **2a** and **2b** also showed characteristic fluorescence spectra originated from a pyrene moiety with wavelength bands at 379 and 396 nm in aqueous HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K (Figure 3). The fluorescence intensity originated from **2b** at 379 and 396 nm decreased upon addition of **1a**, reflecting formation of **1a**·**2b**

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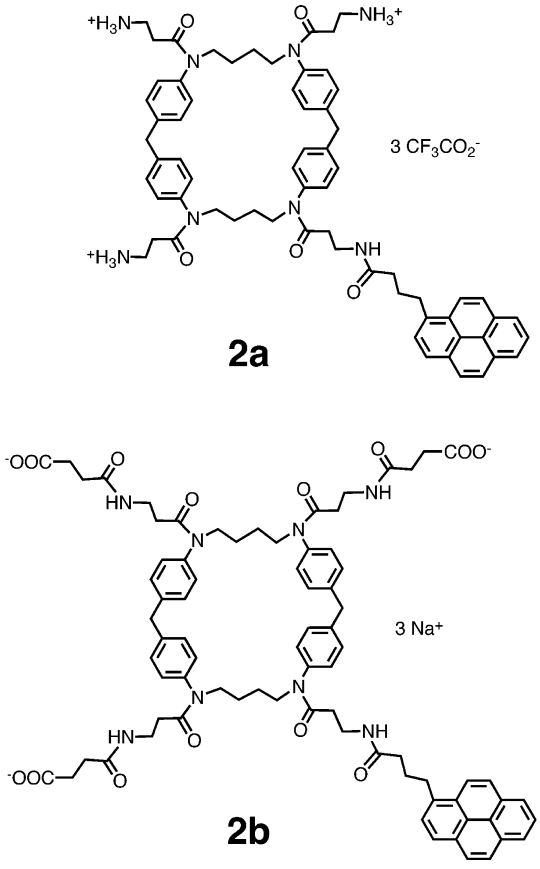

Figure 2. Cationic and anionic fluorescent cyclophanes bearing a pyrene moiety **2a** and **2b**.

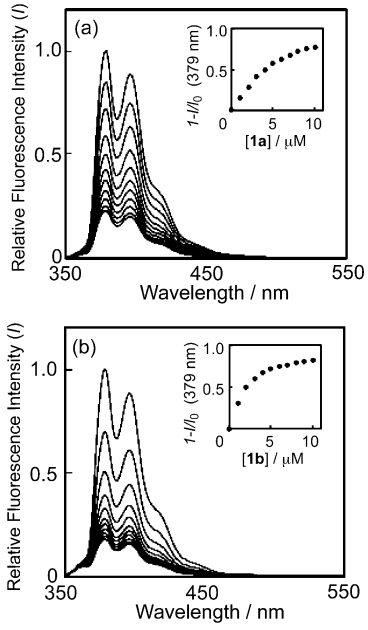

Figure 3. Fluorescence spectral changes for aqueous solutions of **2b** and **2a** upon addition of dabsyl-appended cyclophanes in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K: **1a** with **2b** (a), **1b** with **2a** (b). [**2a**] = [**2b**] = 0.5 μM. [**1a**] = [**1b**] = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 μM. (from top to bottom). Ex. 322 nm for both **2b** and **2a**. Insets: the corresponding titration curves.

complexes, as shown in Figure 3a. This complexation behavior can be also visually monitored by naked eye as the fluorescence originated from **2b** weakened upon addition of **1a** under UV irradiation (see the Supporting Information). The stoichiometry for the complex was confirmed to be 1:1 **1a**:**2b** by a Job plot (Figure 4). The 1:1 binding constant (K) of **1a** toward **2b**

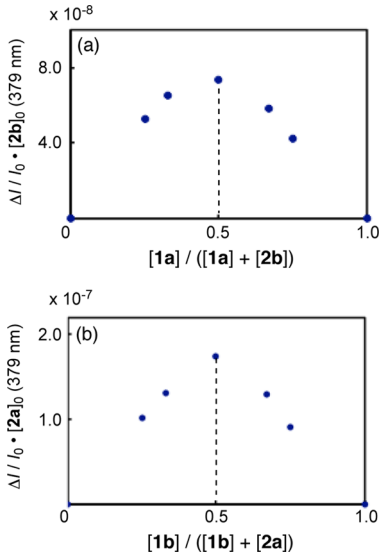

Figure 4. Job plots for complex of **1a** and **2b** (a), **1b** and **2a** (b): [**1a**] + [**2b**] = [**1b**] + [**2a**] = 1.0 μM.

was calculated to be 1.6×10^5 M^{-1} on the basis of the Benesi–Hildebrand relationship.[21] On the other hand, almost no complexation affinity of **1a** toward **2a** was confirmed by fluorescence spectroscopy. That is, upon the addition of **1a** to an aqueous solution containing trivalent and positively charged **2a**, the extent of change in the fluorescence intensity originating from the pyrene group of **2a** was almost negligible under the identical condition, indicating the electrostatic repulsion between **1a** and **2a** (Figure 5). A similar trend of molecular recognition was also confirmed for formation of cyclophane heterodimers of **1b** with **2a** and **2b** by the identical method (Figures 3b and 5b). The K value of **1b** toward **2a** was calculated to be 3.7×10^5 M^{-1}. Therefore, electrostatic interactions became effective in the formation of cyclophane heterodimers.

The guest-binding affinity of **1a** toward an anionic fluorescent guest, 4-(1-pyrene)butanoate (PBA) (Figure 6),[22] was also examined by fluorescence spectroscopy in aqueous HEPES at 298 K. The fluorescence intensity originated from PBA at 378 and 395 nm decreased upon addition of **1a**, reflecting formation of the host–guest complexes, as shown in Figure 7a. The stoichiometry for the complex was confirmed to be 1:1 host:guest by a Job plot (see the Supporting Information). The K of **1a** toward PBA was evaluated to be 3.6×10^4 M^{-1} on the basis of the Benesi–Hildebrand relationship. The K value of **1a** with trivalent charged **2b** was ca. 4-fold larger than that of **1a** with PBA. These results suggest that the increasing K value of **1a** with **2b** compared with that of **1a** with PBA was due to reflecting stability enhancement in complex formation through electrostatic interactions. The cyclophane–guest interaction of **1a** with PBA is schematically shown in Figure 8 together with the corresponding cyclophane–cyclophane interaction of **1a** with **2b**. Hydrophobic pyrene moieties of PBA and **2b** were located in the apolar cyclophane cavity of **1a** upon binding. In addition, the

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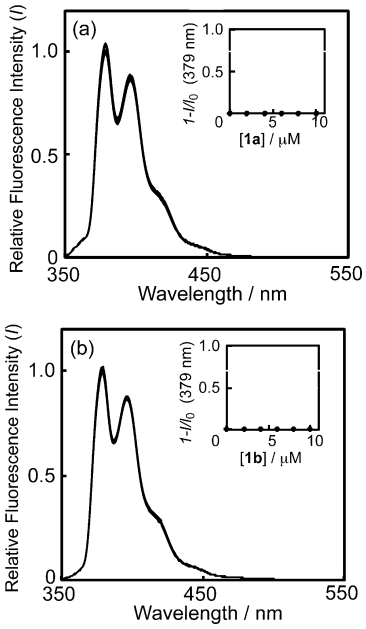

Figure 5. Fluorescence spectral changes for aqueous solutions of **2a** and **2b** upon addition of dabsyl-appended cyclophanes in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K: **1a** with **2a** (a), **1b** with **2b** (b). [**2a**] = [**2b**] = 0.5 μM. [**1a**] = [**1b**] = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 μM. Ex. 322 nm for both **2a** and **2b**. Insets: the corresponding titration curves.

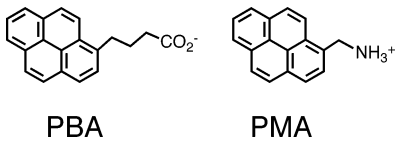

Figure 6. Fluorescent guests PBA and PMA.

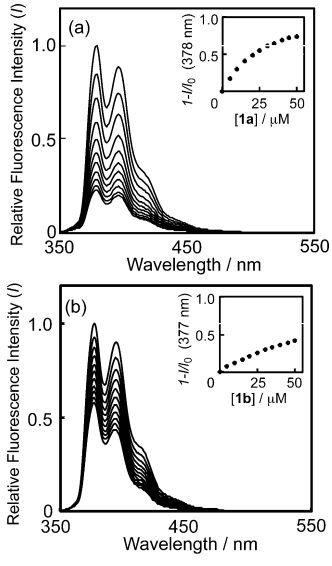

Figure 7. Fluorescence spectral changes for aqueous solutions of PBA and PMA upon addition of dabsyl-appended cyclophanes in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K: **1a** with PBA (a), **1b** with PMA (b). [PBA] = [PMA] = 0.5 μM. [**1a**] = [**1b**] = 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 μM (from bottom to top). Ex. 322 nm for both PBA and PMA. Insets: the corresponding titration curves.

electrostatic interactions of **1a** with trivalent charged **2b** were more favorable than that of **1a** with PBA, as shown in Figure 8. A similar molecular recognition behavior was confirmed for complexation of **1b** toward **2a** and 1-pyrenylmethylamine hydrochloride (PMA)[23] (Figure 6) by the identical method: K = 3.7 × 10^5 and 1.2 × 10^4 M^{-1} for **2a** and PMA, respectively (Figure 7).

Thermodynamic Parameters for Complexation of Cyclophanes. Thermodynamic parameters of **1a** for the formation of cyclophane heterodimers with **2b** were evaluated from temperature-dependent K values as determined by fluorescence spectroscopy in aqueous HEPES buffer at 288, 298, 308, and 318 K (see Supporting Information). Good linear correlations based on double-reciprocal plots of the extent of change in fluorescence intensity upon addition of the host against the total concentration of the hosts were obtained at various temperatures, and the K values are summarized in Table 1 together with the corresponding values of **1b** with **2a**. Thermodynamic parameters enthalpy (ΔH) and entropy changes (ΔS) on formation of host–guest complexes and then free energy change (ΔG) were calculated on the basis of van't Hoff analysis applied to the temperature-dependent K values (see Supporting Information) and are listed in Table 2 together with the corresponding values of **1b** with **2a**. Complexation of **1a** with **2b** gave negative ΔH and ΔS values. The large and negative ΔH values as well as small and also negative ΔS values showed that the complexation is an exothermic and enthalpy-controlled but not entropy-driven process. The negative ΔS values unfavorable to the complexation are overcome by the more negative values of ΔH leading to energetically favorable values. It seems that the large and negative ΔH values result from noncovalent interactions such as electrostatic interaction between **1a** and **2b**. Similar temperature-dependent K values and thermodynamic character were also confirmed for the formation of **1b** with **2a** (Tables 1 and 2).

Thermodynamic parameters of **1a** for complexation with PBA were evaluated by the identical methods (Tables 1 and 2). The analysis on the ΔH, ΔS, and ΔG summarized in Table 2 may help understanding the difference interaction pattern of **1a** with **2b** from those of **1a** with PBA. For the complexes of **1a** from **2b** to PBA at 298 K, $\Delta(\Delta H)$ = −8.5 kJ mol^{-1} and $\Delta(T\Delta S)$ = −4.8 kJ mol^{-1}. The large difference of $\Delta(\Delta H)$ is partially compensated by the $\Delta(T\Delta S)$, leading to the energetically favorable ΔG value. It seems that the large and negative ΔH values result from electrostatic interactions between **1a** with trivalent charged **2b**. Similar thermodynamic character was also confirmed for the formation of **1b** with trivalent charged **2a** (Tables 1 and 2).

■ CONCLUSION

Dabsyl-appended cyclophanes bearing three cationic polar side chains (**1a**) were successfully prepared by reaction of dabsyl chloride with a monoamine derivative of cyclophane, followed by removal of the protecting groups in a fairly good yield. Analogous anionic cyclophane **1b** was also derived from **1a** by a reaction with succinic anhydride. The heterodimer formation of the present cyclophanes with pyrene-appended cyclophanes was demonstrated by fluorescence quenching experiments. The effective molecular recognition through electrostatic interactions was performed by **1a** and **1b** in the formation of cyclophane heterodimers, as confirmed by temperature-dependent fluorescence spectroscopy.

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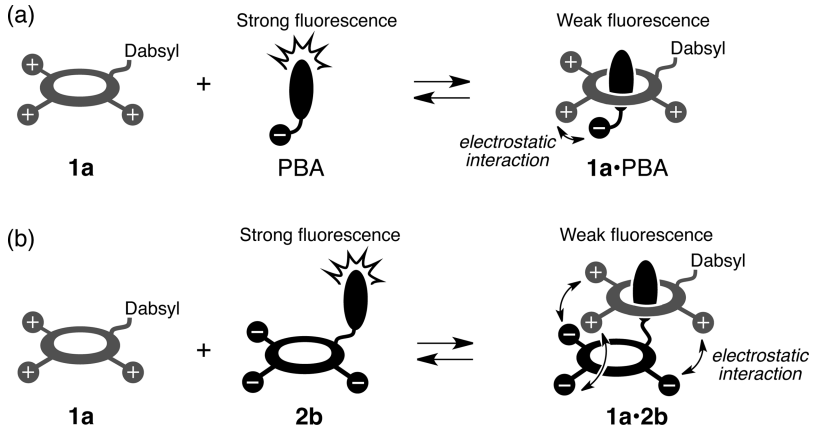

Figure 8. Schematic representation for cyclophane−guest interactions of **1a** with PBA (a) and cyclophane−cyclophane interactions of **1a** with **2b** (b).

Table 1. Binding Constants (K, M^{-1}) for Host−Guest Complexes in HEPES Buffer at Various Temperatures[a]

		T, K			
host	guest	288	298	308	318
1a	**2b**	2.8×10^5	1.6×10^5	9.7×10^4	5.8×10^4
1a	PBA	5.6×10^4	3.6×10^4	2.4×10^4	1.6×10^4
1b	**2a**	6.8×10^5	3.7×10^5	2.1×10^5	1.2×10^5
1b	PMA	2.0×10^4	1.2×10^4	7.8×10^3	5.0×10^3

[a]Excitation: 322 nm.

Table 2. Free Energy Change (ΔG, kJ mol^{-1}) and Thermodynamic Parameters for Formation of Host−Guest Complexes at 298 K; Enthalpy (ΔH, kJ mol^{-1}) and Entropy (ΔS, kJ mol^{-1} K^{-1}) Changes

host	guest	ΔG, kJ mol^{-1}	ΔH, kJ mol^{-1}	$T\Delta S$, kJ mol^{-1}
1a	**2b**	−29.7	−39.8	−10.1
1b	**2a**	−31.7	−43.9	−12.2
1a	PBA	−26.0	−31.3	−5.3
1b	PMA	−23.3	−34.9	−11.6

EXPERIMENTAL SECTION

Cyclophane Bearing *N*-Protected Amines (4). Dicyclohexylcarbodiimide (DCC, 113 mg, 0.55 mmol) was added to a solution of Fmoc-β-alanine (171 mg, 0.55 mmol) in dry dichloromethane (DCM, 5 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 cyclophane derivative bearing three Boc-β-alanine residues (**3**)[18] (506 mg, 0.5 mmol) in dry DCM (5 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, 20 mL). Insoluble materials were removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (SiO_2) with ethyl acetate as eluent. Evaporation of the product fraction under reduced pressure gave a white solid (493 mg, 75%): mp 113−114 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) δ 1.4 (m, 35H), 2.1 (m, 8H), 3.2 (m, 6H), 3.3 (m, 2H), 3.6 (m, 8H), 3.9 (s, 4H), 4.2 (m, 1H), 4.3 (m, 2H), 5.3 (m, 3H), 5.6 (m, 1H), 6.9 (m, 8H), 7.2 (m, 8H), 7.3 (m, 2H), 7.4 (m, 2H), 7.6 (d, J = 7.5 Hz, 2H), and 7.8 (d, J = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, 293 K) δ 25.0, 28.6, 35.0, 36.5, 41.2, 47.3, 48.8, 66.9, 79.2, 120.2, 125.4, 127, 128, 130, 140, 141, 144, 156, and 172. IR 1700, 1643 cm^{-1} (C═O). Found: C, 67.67; H, 7.21; N, 8.44. Calcd for $C_{76}H_{94}N_8O_{12}\cdot 2H_2O$: C, 67.73; H, 7.33; N, 8.31.

Precursor of 1a (5). Piperidine (1.0 mL) was added to a solution of cyclophane **4** (400 mg, 0.3 mmol) in dry DCM (2 mL), and the mixture was stirred for 5 h at room temperature. Then the solvent was evaporated off under reduced pressure to give a pale yellow solid (monoamine of cyclophane). The monoamine of cyclophane was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as an eluant. The precursor fraction was evaporated to dryness under reduced pressure to give a pale yellow solid (cyclophane monoamine, 264 mg). Triethylamine was added to a solution of 4-*N*,*N*-dimethylaminoazobenzene-4′-sulfonylchloride (Dabsyl-Cl, 58 mg, 0.18 mmol) in dry DCM (5 mL) at room temperature, and the mixture was allowed to stand at same temperature. The mixture was added to a solution of the monoamine of cyclophane (250 mg, 0.23 mmol) in dry DCM (2 mL), and the resulting mixture was stirred for 1 day at the same temperature. The residue was chromatographed on a column of silica gel (SiO_2) with chloroform/methanol = 9: 1 v/v as an eluant. Evaporation of the product fraction under reduced pressure gave a orange-red solid (204 mg, 50%): mp 114−115 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) δ 1.4 (m, 35H), 2.1 (m, 8H), 3.1 (m, 6H), 3.3 (m, 8H), 3.6 (m, 8H), 3.9 (d, J = 10 Hz, 4H), 5.3 (m, 4H), 6.8 (m, 2H), 6.9−7.0 (m, 8H), 7.1−7.2 (m, 8H), and 7.9 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 293 K) δ 25.1, 28.7, 34.5−36.5, 39.5−41.2, 48.8, 79.2, 112, 123, 126, 128−131, 140−141, 144, 153, 155.7, 171, and 172. IR 1634 cm^{-1} (C═O). Found: C, 62.98; H, 7.05; N, 10.79. Calcd for $C_{75}H_{97}N_{11}O_{12}S\cdot 3H_2O$: C, 62.96; H, 7.26; N, 10.77. ESI-TOF MS: m/z 1398 $[M + Na]^+$.

Cationic Cyclophane Bearing a Dabsyl Moiety (1a). Trifluoroacetic acid (1.0 mL) was added to a solution of **5** (85 mg, 0.062 mmol) in dry DCM (6 mL), and the mixture was stirred for 1 h at room temperature. Evaporation of the solvent under reduced pressure gave an orange-red solid. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as an eluant. Evaporation of the product fraction under reduced pressure gave an orange-red solid (88 mg, 98%): mp 145−146 °C. ^{1}H NMR (400 MHz, CD_3OD, 293 K) δ 1.4 (m, 8H), 2.4 (m, 8H), 3.1 (m, 6H), 3.3 (m, 8H), 3.6 (m, 8H), 4.0 (m, 4H), 6.8 (m, 8H), 7.1 (m, 2H), 7.2−7.4 (m, 12H), and 7.8 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD, 293K) δ 24.6, 26.6, 31.3, 35.8, 35.9, 39.1−40.7, 53.3, 113, 116, 128, 127−129, 130, 140, 142, 154, 158, 161, 170, and 171. IR 1634 cm^{-1} (C═O). Found: C, 51.46; H, 5.32; N, 9.51. Calcd for $C_{68}H_{76}F_{12}N_{11}O_{14}S\cdot 3H_2O$: C, 51.48; H, 5.27; N, 9.71. ESI-TOF MS: m/z 1077 $[M + Na]^+$, where M denotes triamine derivative of cyclophane as free base.

Anionic Cyclophane Bearing a Dabsyl Moiety (1b). Succinic anhydride (46 mg, 0.46 mmol) was added to a solution of cyclophane **1a** (72 mg, 0.05 mmol) and triethylamine (0.5 mL) in dry DCM (4 mL) at room temperature, and the mixture was stirred for 1 day. Ethylenediamine (0.1 mL, 1.5 mmol) was added to the mixture to quench the reaction. After being dried (Na_2SO_4), the solution was evaporated to dryness under reduced pressure to give an orange-red

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solid. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as an eluant. Evaporation of the product fraction under reduced pressure gave an orange-red solid. The resulting sodium salt was converted into the free acid by ion-exchange chromatography on a column of Amberlite IR-120B with methanol as eluent. Evaporation of the product fraction under reduced pressure gave an orange solid (59 mg, 68%): mp 99−100 °C. ^{1}H NMR (400 MHz, CD_3OD, 293 K) δ 1.4 (m, 17H) 2.1 (m, 8H), 2.4−2.6 (m, 12H), 3.1 (m, 12H), 3.3 (m, 16H), 4.0 (m, 4H), 36.8−7.3 (m, 18H), and 7.8−7.9 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD, 293K) δ 6.47, 24.4, 329.7−30.8, 34.0−35.6, 38.8−40.6, 52.4, 62.4, 112, 122, 126, 127−128, 130, 140−144, 154, 172−174, and 175−176. IR 1723, 1635 cm^{-1} (C═O). Found: C, 58.41; H, 6.58; N, 10.26. Calcd for $C_{72}H_{85}N_{11}O_{15}S{\cdot}6H_2O$: C, 58.25; H, 6.59; N, 10.38. ESI-TOF MS: m/z 1398 $[M + Na]^+$, where M denotes carboxylic acid of cyclophane.

Binding Constants of Cyclophane with Fluorescence Guests. To each solution of a fluorescent guest (0.5 μM) in HEPES buffer were added increasing amounts of the hosts, and the guest fluorescence intensity was monitored after each addition by excitation at 322 nm for PBA, PMA, **2a**, and **2b**. Aqueous stock solution of **1b** was prepared after addition of NaOH. The binding constants were calculated on the basis of the Benesi−Hildebrand method for titration data.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra for compounds **4**, **5**, **1a**, and **1b**. Computer-generated CPK models, UV−vis spectra, Job plots, additional fluorescence titration spectra, and van't Hoff plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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