Synthesis, guest-binding, and effective fluorescence quenching behaviors of a dabsyl-appended cyclophane tetramer

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Abstract

A water-soluble cyclophane tetramer having a dabsyl moiety (**4a**) was designed as a quencher-type host for fluorescence guests. Host **4a** was synthesized by stepwise condensation of a tetraaza[6.1.6.1]paracyclophane skeleton with a dabsyl moiety and three Boc-protected cyclophane derivatives in this sequence, followed by removal of the nine Boc-protecting groups. Host **4a** effectively quenched fluorescence emission of a fluorescent guest, 4-(1-pyrene) butanoate (PBA), in an aqueous HEPES buffer. A Stern-Volmer (SV) plot of the fluorescence quenching of PBA by **4a** was upward and nonlinear in proportion to the concentration of **4a**, indicating a major contribution of static (association) quenching of the fluorescence owing to the formation of the host-guest complexes. The evaluated association constant (K_A) of **4a** with PBA was 1.1 x 10^5 M^{-1} which was about 2-fold larger than that of dabsyl-appended monomeric cyclophane (**1a**) at 298K. On the other hand, good linear SV-plots indicating a simple dynamic quenching process were obtained for experiments both anionic dabsyl-appended cyclophane monomer (**1b**) and tetramer (**4b**) with PBA. Therefore, anionic hosts **1b** and **4b** showed very weak host-guest interactions with PBA due to an electrostatic repulsion.

Keywords: Cyclophane; Fluorescence quenching; Multivalent effect

1. Introduction

Host-guest interactions between artificial hosts and guests have been investigated by various analytical methodologies such as NMR, ITC, SPR, UV-Vis and fluorescence spectroscopy [1-5]. Among them, fluorescence spectroscopy is a simple, fast, and sensitive detection method [6]. Especially, it is convenient to study the host-guest interactions when the adopted guest is an environment-sensitive fluorescent probe, which exhibits intensive fluorescence in non-polar solvents, but with low fluorescence intensity in water [7]. In the case of other fluorescent guests, except for such environment-sensitive fluorescent probes, additional fluorescence techniques such as fluorescence resonance energy transfer (FRET) are required to detect the host-guest complexation [8]. In the FRET experiments, pairs of fluorophores and quenchers have been inevitably used [9]. If a donor fluorophore is brought closer to an acceptor quencher within a certain distance known as the FRET radius, the fluorescence intensity of the donor fluorophore decreases [10]. For such purpose, 4-*N*,*N*-dimethylamino-azobenzene-4'-sulfonyl (dabsyl) derivatives are frequently used as a non-fluorescent quencher. In the preceding paper, we have developed dabsyl-appended monomeric cyclophane (**1a**), as a cationic and quencher-type host (Figure 1) and examined guest-binding behavior toward an anionic fluorescent guest, 4-(1-pyrene)butanoate (PBA) by FRET experiments [11]. In fact, the fluorescence intensity of PBA was found to decrease upon the complexation with **1a**. However, shortcomings of the simple-monomeric cyclophane are moderately binding with the gest molecules. A feasible approach is multiplying in the macrocycles so as to increase the guest-binding abilities [12]. For instance, we found that the guest-binding ability of cyclophane tetramer was much larger than that of the corresponding monomeric cyclophane [13]. In order to develop quencher-type cyclophane oligomers having an enhanced guest-binding ability, we designed dabsyl-appended cyclophane tetramer having positively charged polar side chains (**4a**), which were constructed with four macrocycles and a dabsyl group (Figure 1). As a fluorescence quencher, **4a** has a dabsyl moiety to quench emission of an entrapped fluorescence donor guest. As a water-soluble host, **4a** has four hydrophobic cavities for incorporation of guest molecules and hydrophilic polar side chains to afford water-solubility

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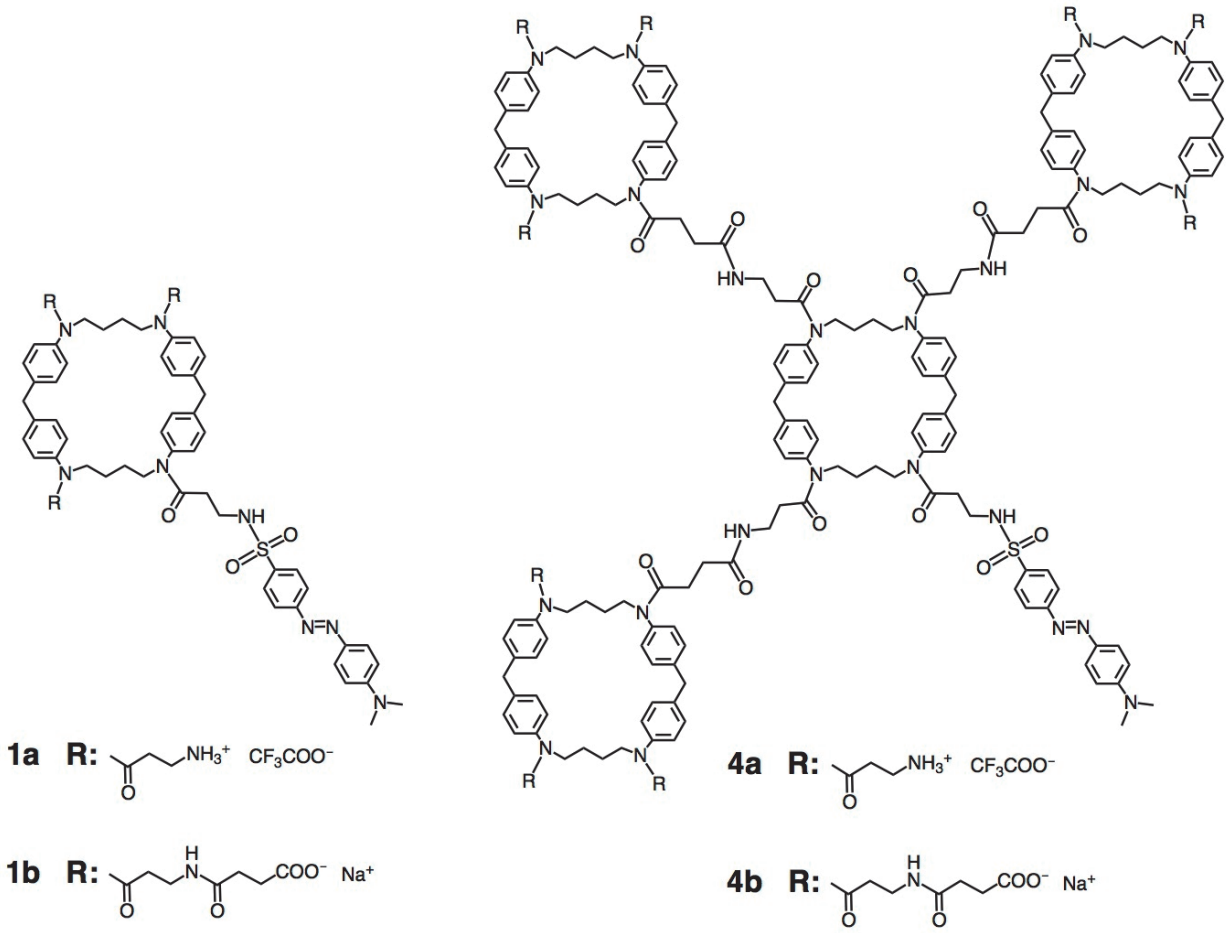


Figure 1. Dabsyl-appended cyclophane monomers (**1a** and **1b**) and tetramers (**4a** and **4b**).

on the resulting host. In addition, we also designed analogous anionic cyclophane tetramer (**4b**) as a control host in order to study electrostatic effects on the host-guest molecular recognition (Figure 1). In this context, we report synthesis and enhanced guest-binding ability of **4a** as well as its fluorescence quenching behavior upon the complexation with PBA.

2. Experimental section

2.1. A precursor of 4a, Boc-protected cyclophane tetramer 3

Dicyclohexylcarbodiimide (DCC, 139 mg, 0.68 mmol) was added to a solution of Boc-protected cyclophane having a carboxylic acid **2** (756 mg, 0.68 mmol) [16] in dry dichloromethane (DCM, 2 mL) at 0℃, 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 **1a** (192 mg, 0.14 mmol) in dry DCM (2 mL), and the resulting mixture was stirred for 3 days at room temperature. 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, 10 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 chloroform/methanol = 9:1 v/v as eluent. Evaporation of the product fraction under reduced pressure and the residue was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as an eluent. Evaporation of the product fraction under reduced pressure gave an orange solid (335 mg, 57 %) : ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.44 (m, 113H), 2.10 (m, 26H), 2.26 (m, 6H), 2.40 (m, 6H), 3.15 (s, 8H), 3.29 (m, 24H), 3.64 (m, 32H), 3.97 (m, 16H), 5.34 (s, 9H), 5.98 (s, 1H), 6.59 (s, 3H), 6.81 (d, 2H), 6.97 (m, 32H), 7.21 (m, 32H), and 7.92 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ 24.9, 28.4, 29.9, 31.3, 34.8, 36.4, 40.3, 41.0, 48.6, 78.9, 111, 122, 128, 130, 140, 144, 153, 156, and 171. IR 1645 cm^{-1} (C=O). Found: C, 65.04; H, 7.15; N, 9.82. Calcd for $C_{246}H_{316}N_{32}O_{39}S \cdot 9H_2O$: C, 65.09; H, 7.42; N, 9.87. MALDI-TOF MS: m/z 4400.2 $[M + Na]^+$,

where M shows $C_{246}H_{316}N_{32}O_{39}S$.

2.2. Cationic Cyclophane Tetramer 4a

Trifluoroacetic acid (1 mL) was added to a solution of **3** (120 mg, 0.027mmol) in dry DCM (3 mL), and the mixture was stirred for 2 hours at room temperature. Evaporation of the solvent under reduced pressure and the residue was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as an eluent. Evaporation of the product fraction under reduced pressure gave a red solid (125 mg, quantitative): ^{1}H NMR (400 MHz, CD_3OD, 298 K) δ 1.40 (m, 32H), 2.01 (m, 8H), 2.21 (m, 18H), 2.41 (m, 12H), 3.02 (m, 24H), 3.27 (m, 8H), 3.65 (m, 32H), 4.02 (s, 16H), 6.73 (m, 2H), 6.93 (m, 32H), 7.38 (m, 32H), and 7.71 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD, 298K) δ 24.1, 29.5, 31.1, 33.7, 35.3, 39.0, 40.4, 48.9, 111, 118, 122, 125, 128, 130, 139, 142, 144, 155, 170, 171, 172, 173. IR 1634 cm^{-1} (C=O). Found: C, 57.27; H, 5.77; N, 9.76. Calcd for $C_{219}H_{253}F_{27}N_{32}O_{39}S\cdot 5H_2O$: C, 57.39; H, 6.05 ; N, 9.71. MALDI-TOF MS: m/z 3476.9 $[M + H]^+$, 3498.8 $[M + Na]^+$, where M shows $C_{201}H_{244}N_{32}O_{21}S$ as a free amine.

2.3. Anionic Cyclophane Tetramer 4b

To a solution of cyclophane **4a** (174 mg, 0.040 mmol) and triethylamine (TEA, 1 mL) in dry. DCM (3 mL) was added succinic anhydride (70.8 mg, 0.71 mmol). The mixture was stirred for overnight at room temperature. The solution was distilled off under reduced pressure to give an orange solid. The residue was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as an eluent. The resulting salt was converted into the sodium salt by ion-exchange chromatography on a column of Amberlite IR-120 with methanol as eluent. Evaporation of the product fraction under reduced pressure gave a red solid. (142 mg, 84 %): ^{1}H NMR (400 MHz, CD_3OD, 298 K) δ 1.43 (m, 32H), 2.16 (m, 30H), 2.30 (m, 6H), 2.53 (t, 38H), 3.11 (s, 8H), 3.27 (m, 24H), 3.63 (m, 32H), 3.98 (m, 16H), 6.79 (m, 2H), 7.00 (m, 32H), 7.25 (m, 32H) and 7.83 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ 23.0, 25.5, 30.5-31.6, 35.1, 36.6, 41.7, 49.9, 129, 131, 141, 142, 172-174, and 176. IR 1633 cm^{-1} (C=O). Found : C, 59.86 ; H, 6.45; N, 9.43. Calcd for $C_{237}H_{271}N_{32}Na_9O_{48}S\cdot 10H_2O$: C, 59.60; H, 6.45; N, 9.26. MALDI-TOF MS: m/z 4375.6 $[M - H]^-$, where M shows $C_{237}H_{280}N_{32}O_{48}S$.

2.4. Fluorescence titration experiments and their analysis

To a solution of PBA (0.5 μM) in HEPES buffer were added increasing amounts of the hosts (Hs) such as **4a**, **1a**, **4b**, and **1b**. The fluorescence spectra were recorded after each addition of the hosts. Stern-Volmer quenching constant (K_D) of **4b** and **1b** with PBA was calculated by Stern-Volmer equation (1), where I and I_0 stand the intensities of fluorescence at 378 nm in the absence and presence of the host (H).

$$\frac{I_0}{I} = 1 + K_D\,[H] \qquad (1)$$

In the case of a combined dynamic and static quenching such as combination of **4a** and **1a** with PBA, the following equation was used to determine the K_D and association constant (K_A) [20].

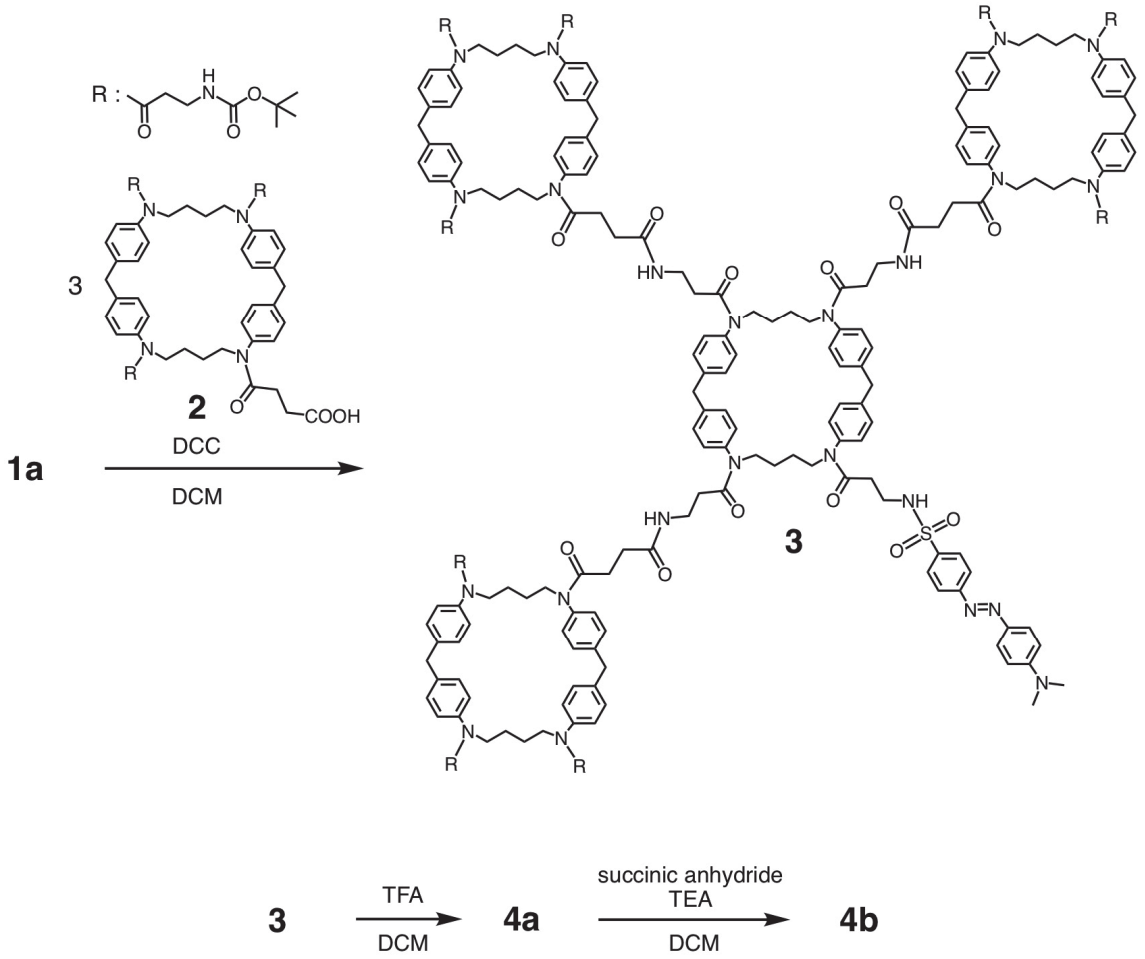
$$\frac{I_0}{I} = (1 + K_D\,[H])\,(1 + K_A\,[H]) = 1 + (K_D + K_A)\,[H] + K_D\,K_A\,[H]^2 \qquad (2)$$

3. Results and discussion

3.1. Design and synthesis of dabsyl-appended cyclophane tetramers

Various functionalized macrocyclic hosts have been prepared by introducing side chains into a cyclophane skeleton [14]. For instance, water-soluble cyclophanes bearing positively or negatively charged side chains recognize and bind hydrophobic guests through electrostatic and hydrophobic interactions in aqueous media [12]. In addition, various functional groups such as biotin, dansyl, pyrene, rhodamine, and coumarin were appended to the macrocyclic skeletons and their host-functions were investigated [15]. Considering the importance of multivalency on the host-guest interactions, we have previously developed cyclophane oligomers such as dimer, trimer, tetramer, pentamer, and hexadecamer by connecting several cyclophane skeletons through amide linkages [16]. On these grounds, we synthesized dabsyl-appended cyclophane tetramers having positively or negatively charged polar side chains, **4a** and **4b**, respectively, which were constructed with four macrocycles and a dabsyl group by following the reaction sequence given in the Scheme 1.

In the preceding paper, we have synthesized dabsyl-appended cyclophane monomer **1a**, by introducing a dabsyl moiety into tetraaza[6.1.6.1]paracyclophane derivative bearing three Boc-β-alanine residues, followed by removal of the Boc-protecting groups with TFA [11]. By using **1a** as the starting material, a precursor (**3**) of **4a** was synthesized by condensation of **1a** with three equivalents of Boc-



Scheme 1. Preparation of cyclophane tetramers, **4a** and **4b**.

protected cyclophane having a carboxylic acid **2** [16] in the presence of dicyclohexylcarbodiimide (DCC). Cationic dabsyl-appended cyclophane tetramer **4a** was obtained by removal of the protecting groups with TFA. Anionic cyclophane tetramer **4b** was obtained from **4a** by a reaction with succinic anhydride. All new compounds were identified by ^{1}H and ^{13}C NMR and MALDI-TOF MS spectroscopy as well as by elemental analysis. Cationic cyclophane tetramer **4a** had good water-solubility of 0.25 g/mL. At least at concentrations below 50 μM of **4a**, a good linear Beer's plot of absorbance at 475 nm was obtained, which indicates that **4a** was not self-aggregated. A similar property in the water-solubility of **4b** was also observed by identical methods.

3.2. Fluorescence quenching behavior of dabsyl-appended cyclophanes

Generally, a quenching process between quenchers and fluorophores is attributed to a dynamic quenching or a static (association) quenching [17]. The dynamic quenching is due to collisions between quenchers and fluorophores, while the static quenching is ascribed to a formation of non-fluorescent complexes of the quenchers and the fluorophores [18]. Hence, it is interesting to study the quenching processes of dabsyl-appended cyclophane tetramers having guest-binding ability. As mentioned above, dabsyl derivatives are a frequently used quencher in FRET applications, because it has an absorption wavelength of a visible region in 350-550 nm but no fluorescence. Cyclophane tetramer **4a** has a dabsyl moiety to quench emission of an excited fluorescence donor. Actually, we confirmed a certain spectral overlap between the emission spectrum of PBA and the absorbance spectrum of **4a**, as shown in Figure 2. We expected quenching of PBA by **4a** via FRET mechanism.

In order to evaluate the quenching processes and guest-binding behavior of **4a**, fluorescence titration experiments were executed by using PBA as an anionic

fluorescent guest at 298 K in an aqueous HEPES (0.01 M, pH 7.4, with 0.15 M NaCl) buffer (Figure 3). Upon addition of **4a** to the HEPES buffer containing PBA, a fluorescence intensity of PBA at around 378 nm decreased with a saturation behavior, as shown in Figure 3a. Interestingly, Stern-Volmer plot of fluorescence quenching of PBA by **4a** was upward and nonlinear in proportion to the host concentration, as shown in Figure 4. According to the

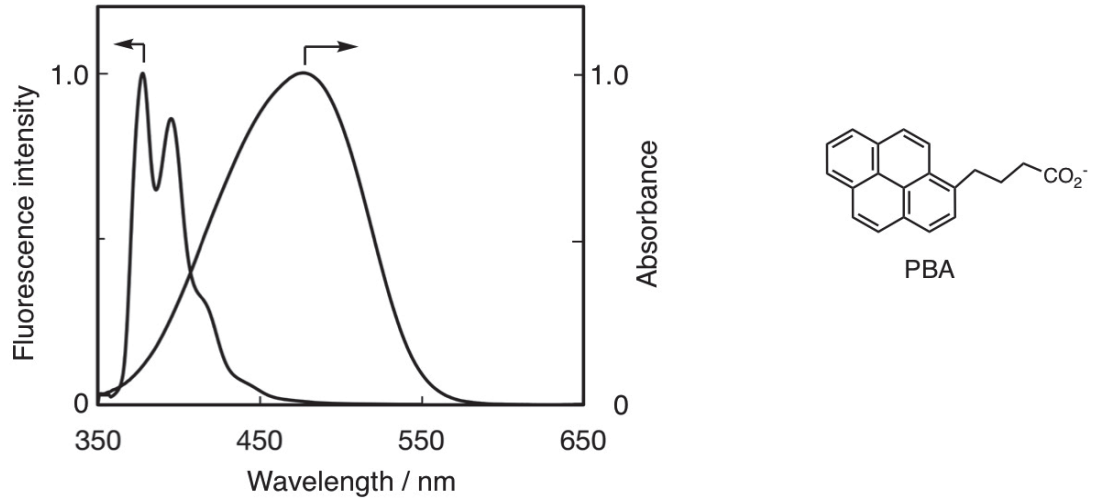


Figure 2. Normalized fluorescence spectrum of PBA and absorption spectrum of **4a** in HEPES buffer at 298K.

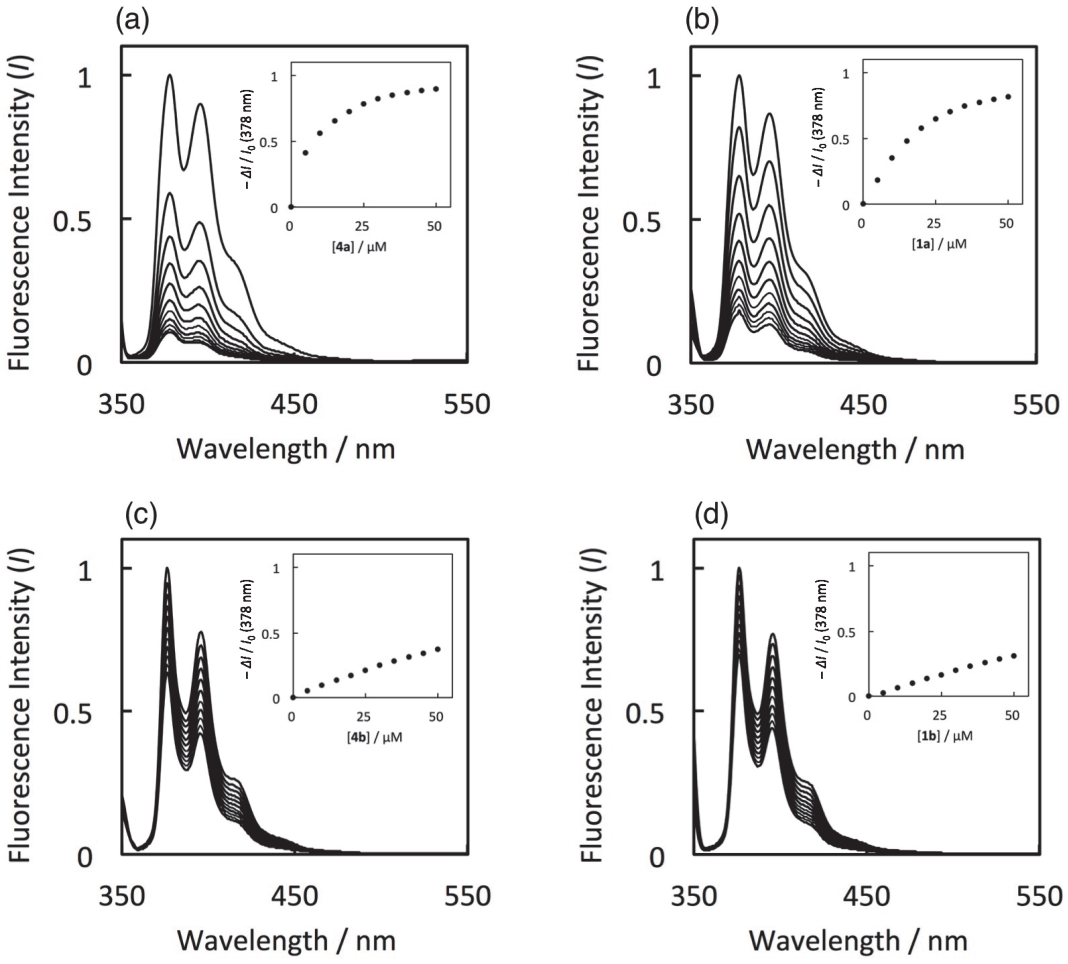


Figure 3. Fluorescence spectra for aqueous solutions of PBA (0.5 μM) upon addition of the hosts: **4a** (a), **1a** (b), **4b** (c), and **1b** (d) in HEPES buffer at 298K: [host] (from top to bottom) = 0. 5.0, 10, 15, 20, 25, 30, 35, 40, 45, and 50 μM. Ex, 346 nm. Inset: correlations between fluorescence intensity and host concentrations.

literature [19-20], such nonlinear S-V plot arises from both dynamic and static quenching. Hence, quenching process of PBA by **4a** was attributed to the combination of both the dynamic and static quenching. By means of computer-aided least-squares curve fitting method applied to the fluorescence titration data, Stern-Volmer quenching constant (K_D) and association constant (K_A) for the interaction of PBA with **4a** were quantitatively evaluated and listed in Table 1. Concerning the interactions of PBA with **4a**, evaluated K_A was about 10 times larger than K_D at 298 K (Table 1). These results showed that static quenching of fluorescence is a major contribution for the interaction of PBA with **4a**, because the constants K_D and K_A represent the dynamic and static quenching processes, respectively. In addition, the fluorescence titration experiments of PBA with **4a** at a lower temperature (288 K) resulted in both an increase in K_A value and a decrease in K_D value (Table 1). Such a lower temperature condition was found to be favorable for formation of the host-guest complexes and unfavorable for collisions between the host and the guest. Therefore, static quenching of fluorescence is a major contribution for the interaction of PBA with **4a**, owing to a formation of host-guest complexes.

Table 1. Stern-Volmer quenching constants (K_D, M^{-1}) and association constants (K_A, M^{-1}) for the interaction of PBA with hosts in HEPES buffer.

Host	Tem. / K	K_D	K_A
4a	298	1.1×10^4	1.1×10^5
1a	298	1.3×10^4	4.8×10^4
4b	298	1.1×10^4	
1b	298	8.5×10^3	
4a	288	1.0×10^4	1.3×10^5
1a	288	1.1×10^4	6.8×10^4

In the case of an analogous cationic dabsyl-appended cyclophane monomer (**1a**) with PBA, A similar spectral change in fluorescence spectroscopy was obtained, even though its SV-plot was slightly upward as shown Figure 4. In comparison with **4a** and **1a**, the obtained K_A value of **4a** with PBA was about 2-times larger than that of **1a**, while K_D value of **4a** was almost comparable to that of **1a**. These results indicated that the guest-binding ability of **4a** is larger than that of **1a**, reflecting multivalent effect in macrocycles. In the case of an anionic dabsyl-appended cyclophane monomer (**1b**) and a tetramer (**4b**) with PBA (Figure 3c, d), good linear SV-plots were obtained for the fluorescence titration experiments of both **1b** and **4b** with PBA, indicating a simple dynamic quenching process (Table 1). Therefore, both anionic dabsyl-appended cyclophane monomer **1b** and tetramer **4b** showed very weak host-guest interactions with PBA due to an electrostatic repulsion.

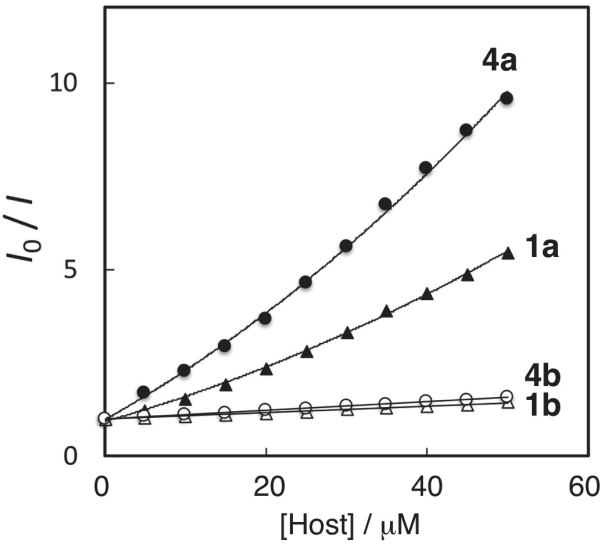


Figure 4. Stern-Volmer plots of fluorescence quenching of PBA by **4a**, **1a**, **4b**, and **1b**.

4. Conclusions

We have synthesized a cationic and quencher-type cyclophane tetramer **4a** by introducing three Boc-protected cyclophane derivatives into a tetraaza[6.1.6.1] para-cyclophane having a dabsyl moiety as a core skeleton through DCC condensation, followed by removal of the external Boc-protecting groups. Cationic cyclophane tetramer **4a** effectively quenched fluorescence emission of an anionic fluorescent guest, PBA. A Stern-Volmer plot of the fluorescence quenching of PBA by **4a** was upward and nonlinear in proportion to the concentration of **4a**, indicating a major contribution of static (association) quenching of the fluorescence owing to the formation of the host-guest complexes. In the case of an analogous cationic dabsyl-appended cyclophane monomer **1a** with PBA, slightly upward SV-plot was observed. The evaluated K_A of **4a** with PBA was about 2-fold larger than that of **1a**, while the K_D value based on dynamic quenching of **4a** was almost comparable to that of **1a**. On the other hand, both anionic dabsyl-appended cyclophane monomer **1b** and tetramer **4b** showed very weak host-guest interactions with PBA due to an electrostatic repulsion. Therefore, good linear SV-plots

were obtained for experiments of both **1b** and **4b** with PBA, indicating a simple dynamic quenching process.

Acknowledgment

The present work is partially supported by Grant-in-Aid (No. 16K05761 and 19K05448) from the Ministry of Education, Culture, Science, Sports and Technology of Japan.

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