Cooperative Multiple Recognition by Novel Calix[4]arene-Tethered *β*-Cyclodextrin and Calix[4]arene-Bridged Bis(*β*-cyclodextrin)

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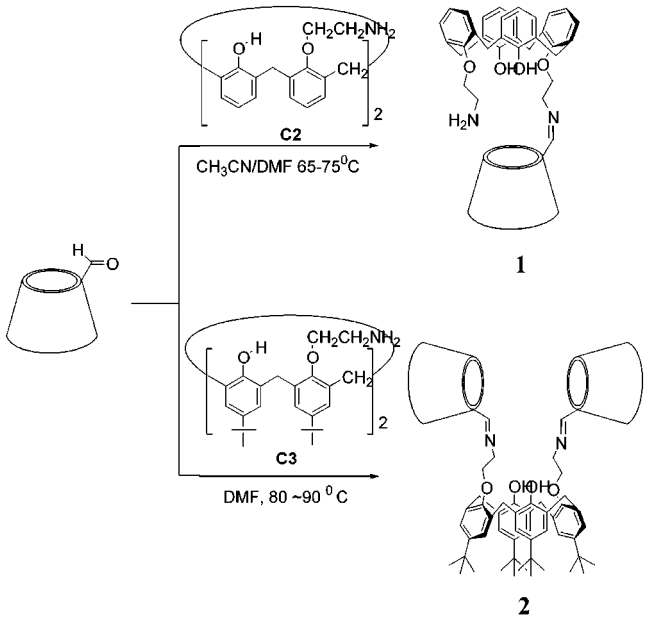
Introduction

Possessing dual hydrophobic cavities in close proximity, cyclodextrin dimers connected with a tether of different length and structure can bind diverse molecules to form supramolecular sandwich complexes with much enhanced binding abilities and molecular selectivities than those exhibited by the parent cyclodextrin. This is made possible through the cooperative binding of one guest molecule by two adjacent hydrophobic cyclodextrin cavities, which may serve as a model system mimicking the substrate-specific interaction of enzymes. Accordingly, a number of bis(*β*-cyclodextrin)s linked by simple tethers have recently been designed and synthesized to increase the original molecular binding ability and selectivity of native *β*-cyclodextrin and also to gain insights into the factors and mechanism controlling the inclusion complexation phenomena.[1−7] More recently, Reinhoudt and co-workers have demonstrated that some water-soluble *β*-cyclodextrin−calix[4]arene dyads dramatically enhance the original binding ability of native *β*-cyclodextrin with a series of organic guests.[8]

More sophisticated triad hosts, such as calixarene-bridged bis(cyclodextrin), have rarely been reported so far. However, a comparative study, using dyad and triad hosts, will certainly lead not only to enhancement of both affinity and selectivity for specific guests but also to deeper and more precise understanding of cooperative multiple interactions in natural and artificial supramolecular recognition systems. Now, we report the results of our study on the syntheses of novel calix[4]arene-tethered mono- (**1**) and bis(*β*-cyclodextrin) (**2**) (Scheme 1) and their molecular recognition behavior with fluorescence dyes, i.e., acridine red (AR) and sodium 2-(*p*-toluidino)-6-naphthalenesulfonate (TNS), as well as some structurally related guests, i.e., methyl orange (MO), ethyl orange (EO), tropaeolin OO (TOO), brilliant green (BG), crystal violet (CV), and rhodamine B (RhB) in aqueous buffer solution (Chart 1). It is of our particular interest to mimic and understand the cooperative "multimode, multipoint" bindings often observed in biological systems, by comparatively investigating the cooperative binding of the organic guests by the "two-mode, two-site" (**1**) and "two-mode, three-site" receptor models (**2**), which carry entirely different recognition sites, i.e., calixarene and *β*-cyclodextrin, since a general conclusion has been drawn that the complexation behaviors of the calixarenes are largely driven by electrostatic interactions rather than the hydrophobic interaction in the cyclodextrin's complexation.

Scheme 1



Experimental Section

Apparatus. Mass spectra were obtained by using a JEOL JMS-DX-303 instrument. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC−P200 instrument using tetramethylsilane as an internal reference. Infrared and ultraviolet spectra were recorded on Shimadzu IR-435 and UV-2401/PC instruments, respectively. Elemental analysis was performed on a Perkin-Elmer 2400C instrument. Fluorescence spectra were measured in a quartz cell (10 × 10 × 45 mm) thermostated at 25 °C on a JASCO FP-750 spectrometer with excitation and emission slit widths of 5 nm. Circular dichroism spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) thermostated at 25 °C on a JASCO J-720S spectropolarimeter. Fluorescence lifetimes were determined by the time-correlated single-photon-

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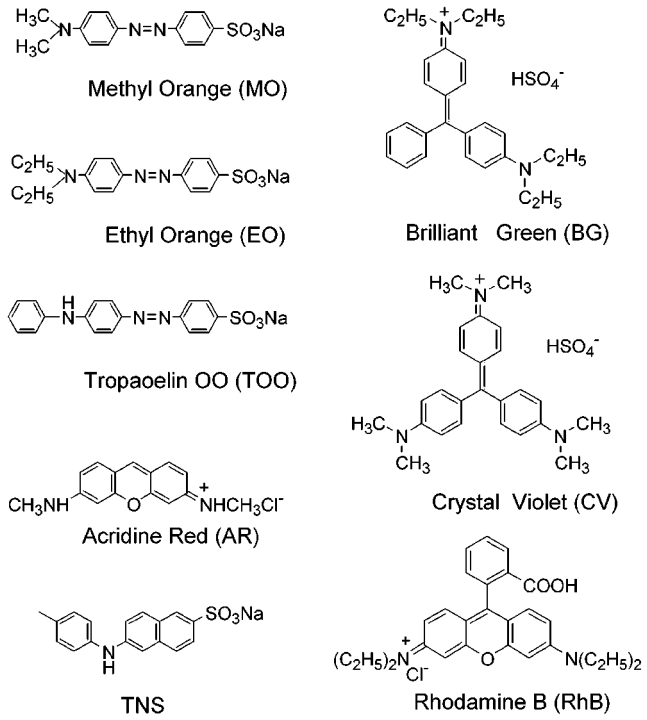
Chart 1



counting method using a Horiba NAES-550 instrument with a time resolution of <0.5 ns. A self-oscillating discharge lamp filled with hydrogen gas was employed as the pulsed light source, and the excitation light was made monochromatic by a 10 cm monochromator. The emission from the sample was passed through an appropriate filter (Toshiba UV-33) placed before the detector unit in order to eliminate scattered excitation light. Maximum counts of up to 10000 were collected for each measurement. The accumulated signals were then processed and the lifetime was determined by deconvolution by the nonlinear least-squares fit method.

Materials. Guest dyes, i.e., acridine red, sodium 2-(*p*-toluidino)-6-naphthalenesulfonate, methyl orange, ethyl orange, tropaeolin OO, brilliant green, crystal violet, and rhodamine B, were commercially available and used without further purification. Reagent grade β-cyclodextrin was recrystallized twice from water and dried in vacuo at 100 °C for 24 h. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and distilled under reduced pressure prior to use. Disodium hydrogen and sodium dihydrogen phosphate were dissolved in deionized, distilled water to make a 0.10 M phosphate buffer solution of pH 7.20.

Synthesis of 25,27-Bis(cyanomethoxy)-26,28-dihydroxycalix[4]arene (C1). C1 was prepared by the reaction of 25,26,27,28-tetrahydroxycalix[4]arene (6.5 g) with chloroacetonitrile (4.2 mL) in the presence of potassium carbonate (8.53 g) and sodium iodide (10.0 g) in anhydrous acetone (250 mL). The mixture was stirred and heated under refluxing for 7 h, and the resulting suspension was cooled to room temperature to give a precipitate, which was filtered and washed with a small amount of dichloromethane. The precipitate was dried under reduced pressure and then added to anhydrous ethanol (40 mL) to obtain a white precipitate. The white precipitate was collected by filtration and recrystallized from anhydrous ethanol to give pure colorless crystal of **C1** (3.54 g, 47%): mp 240−241 °C; ^{1}H NMR ($CDCl_3$, TMS) δ 2.14 (s, ArOH), 3.4−4.3 (m, 8H, $ArCH_2Ar$), 4.82 (m, 4H, OCH_2), 5.98 (s, ArOH), 6.7−7.2 (m, 12H, ArH). Anal. Calcd for $C_{32}H_{26}N_2O_4 \cdot C_2H_5OH$: C, 74.43, H, 5.88, N, 5.11. Found: C, 74.10, H, 5.78, N, 5.07.

Synthesis of 25,27-Bis(aminoethoxy)-26,28-dihydroxycalix[4]arene (C2). C2 was prepared by the reaction of **C1** (2.1 g) with lithium aluminum hydride (0.6 g) in anhydrous tetrahydrofuran (40 mL) at 0 °C for 4 h. Sodium hydroxide (5%) solution (4.2 mL) was added to the mixture, and the filtrate was evaporated under reduced pressure. Then the residue was recrystallized from anhydrous methanol to give pure white solid of **C2** (1.49 g, 71%): mp 216−218 °C; FAB-MS *m*/*z* 510.6 (M^+); ^{1}H NMR ($CDCl_3$, TMS) δ 3.42 (4H, t, NCH_2), 3.92 (4H, d, $ArCH_2Ar$), 4.07 (4H, t, OCH_2), 4.26 (4H, d, $ArCH_2Ar$), 6.68−7.03 (12H, m, ArH). Anal. Calcd for $C_{32}H_{34}N_2O_4(CH_3OH)$: C, 73.04, H, 7.06, N, 5.16. Found: C, 72.90, H, 6.78, N, 5.09.

Synthesis of Calix[4]arene-β-cyclodextrin Dyad 1. 6-Formyl-β-cyclodextrin[9] (1.2 g) and **C2** (0.5 g) was allowed to react under nitrogen atmosphere for 48 h with stirring in 1:1 CH_3CN-DMF solution (50 mL) at 65−75 °C. The precipitate obtained was washed repeatedly with chloroform and deionized, distilled water to give pure product **1** (0.18 g, 10% yield) as an off-white solid: FAB-MS *m*/*z* 1626 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, TMS) δ 3.1−3.9 (m, 46H), 4.0−4.5 (m, 12H), 4.8−5.1 (m, 7H), 6.5−7.2 (m, 12H); FT-IR (KBr) ν 3393.3, 2931.1, 1654.9, 1462.2, 1249.6, 1198.8, 1152.8, 1081.5, 1029.8, 929.2, 762.4, 578.3 cm^{-1}. Anal. Calcd for $C_{74}H_{100}O_{38}N_2 \cdot 4H_2O$: C, 52.35; H, 6.41; N, 1.65. Found: C, 52.02; H, 6.13; N, 1.85.

Synthesis of Calix[4]arene-Bridged Bis(β-cyclodextrin) 2. 6-Formyl-β-cyclodextrin[9] (2.4 g) and 5,11,17,23-tetra-*tert*-butyl-25,27-bis(aminoethoxy)-26,28-dihydroxycalix[4]arene[10] (**C3**) (0.37 g) were allowed to react under nitrogen atmosphere for 4 days with stirring in dry DMF (50 mL) at 80−90 °C. Then DMF was removed under reduced pressure and the residue was washed with chloroform. The solid obtained was dissolved in DMF (50 mL) again and 6-formyl-β-cyclodextrin (0.6 g) was added. The resultant mixture was stirred for 3 days at 80−90 °C under nitrogen atmosphere. Then the solvent was evaporated under reduced pressure to dryness. The residue was dissolved in a minimum amount of hot water, and then acetone was added to the solution. The precipitate obtained was collected by filtration and purified by column chromatography over Sephadex G-25 with deionized, distilled water as eluent to give 0.18 g (10% yield) of **2** as a yellowish solid; FAB-MS *m*/*z* 2964 $(M)^+$; ^{1}H NMR (DMSO-d_6, TMS) δ 1.13 (s, 18H), 1.63 (s, 18H), 3.26−3.67 (m, 92H), 4.04−4.33 (m, 8H), 4.81−5.00 (m, 14H), 7.13 (s, 4H), 7.16 (s, 4H); FT-IR (KBr) ν 3369.6, 2931.6, 1637.7, 1410.4, 1155.3, 1028.1, 938.2, 858.3, 758.4, 707.1, 577.9 cm^{-1}. Anal. Calcd for $C_{132}H_{198}O_{72}N_2 \cdot 17H_2O$: C, 48.47; H, 7.15; N, 0.86. Found: C, 48.40; H, 7.32; N, 1.00.

Measurement. Since host **2** is water-soluble but host **1** is insoluble in water, the comparative study on the inclusion complexation behavior of hosts **1** and **2** with selected guests, i.e., AR and TNS, was investigated in 1:3 (v/v) DMSO−H_2O mixture. On the other hand, the quantitative study on the molecular recognition behavior of cyclodextrin−calixarene triad **2** with structurally related guests was examined in aqueous phosphate buffer solution at pH 7.20.

Results and Discussion

Synthesis. The cyclodextrin−calixarene dyad **1** and triad **2** were synthesized by the reactions of 6-formyl-β-cyclodextrin with the corresponding bis(aminoethoxy)-modified calix[4]arenes. Unexpectedly, only 5,11,17,23-tetra-*tert*-butyl-25,27-bis(aminoethoxy)-26,28-dihydroxycalix[4]arene (**C3**) reacted with two formylcyclodextrins to give the calixarene-bridged bis(cyclodextrin) **2**, whereas 25,27-bis(aminoethoxy)-26,28-dihydroxycalix[4]arene (**C2**) afforded only mono-cyclodextrin derivative **1**. This significant difference in reactivity would be attributed to the conformational flexibility of **C2**, which allows the calixarene moiety to adopt a "tilt-in" conformation of the aminoethoxyphenyl moiety, reducing its reactivity toward formylcyclodextrin, and this conformational flexibility of **C2** would allow the free amino group of dyad **1** to be included in the cyclodextrin cavity of this molecule, which consequently prevents further reaction of **1** with another formyl-functionalized cyclodextrin.

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Another possible reason would be the difference in solvent, $CH_3CN-DMSO$ versus DMF, as the former solvents are likely to be included in hydrophobic cavities, although its consequence upon reactivity of the amino group is not necessarily clear.

Fluorescence Lifetime. The fluorescence lifetime of calixarene-bridged bis(cyclodextrin) **2** was investigated by the time-correlated single-photon-counting method. Since the rate of complexation/decomplexation is much slower than that of the fluorescence decay rate, the decay profile of fluorescence intensity ($F(t)$) can be expressed as the sum of unimolecular decays for all fluorescing species present in the solution:

$$F(t) = \sum_{i=1}^{n} A_i \exp(-t/\tau_i) \ (n = 1, 2, \text{etc.})$$

where A_i and τ_i represent the initial abundance and lifetime of the ith species.

It is interesting to note that the calixarene moiety of host **2** in diluted aqueous buffer solution (10 μmol dm^{-3}) gives two different fluorescence lifetimes with an excitation wavelength of 300 nm, indicating the presence of two independent fluorescing species in the host solution. The shorter lifetime (τ_S) is 0.5 ns with a relative quantum yield (Φ_S) of 60.5%, while the longer lifetime (τ_L) is 4.1 ns with a relative quantum yield (Φ_L) of 39.5%. Judging from the two independent lifetimes (τ_S and τ_L), the short- and long-lived fluorescing species are most likely assigned to the hydroxyphenyl and aminoethoxyphenyl groups of host **2**, which independently fluoresce giving different lifetimes.

Fluorescence Spectral Titration. We have recently reported the contrasting behavior of fluorescent dye acridine red (AR) upon inclusion complexation with β-cyclodextrin and calixarenesulfonate, where the former host induces a marked enhancement in fluorescence intensity, while the latter causes a significantly reduced fluorescence intensity.[11] In the present case, possessing both cyclodextrin and calixarene moieties in a single host molecule, the dyad host **1** and triad host **2** induced the contrasting fluorescent behavior of AR upon inclusion complexation. Under our experimental conditions using a dilute AR solution, the fluorescence of AR was gradually quenched by the stepwise addition of host **1**, as shown in Figure 1. In sharp contrast, the gradual addition of host **2** to a dilute solution of AR significantly enhanced the fluorescence intensity under the comparable conditions, as illustrated in Figure 2. In the control experiment, the fluorescence intensity change of guest (AR or TNS) upon addition of the parent calixarene derivatives (**C2** and **C3**) is negligible under comparable conditions. This clearly demonstrates that the fluorescence intensity change of AR upon addition of host **1** or **2** is attributable not to the complexation by the calixarene moiety (**C2** or **C3**) but mostly to the inclusion in the cyclodextrin moiety/ies. Furthermore, such contrasting fluorescence behavior of AR upon addition of **1** and **2** may indicate that distinctly different binding modes are operative in the complexation of AR with dyad **1** and triad **2**. In the previous study,[11] we revealed that the decreased fluorescence intensity of AR arises primarily from the host−guest complex formation between the positively charged

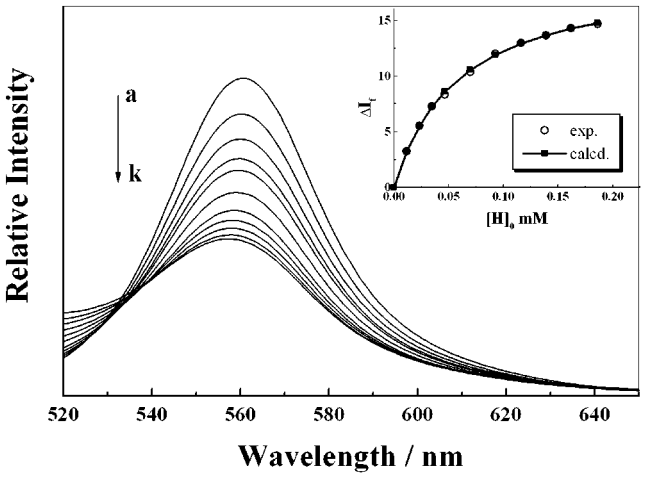

Figure 1. Fluorescence spectral changes of AR (2.1 μmol dm^{-3}) and curve-fitting analysis (inset) of the differential intensity (ΔI_f) to calculate the complex stability constant (K_s) upon addition of β-cyclodextrin−calix[4]arene **1** (0−183 × μmol dm^{-3} from a to k) in 1:3 DMSO−water solution (v/v). Excitation wavelength (λ_{ex}) = 490 nm.

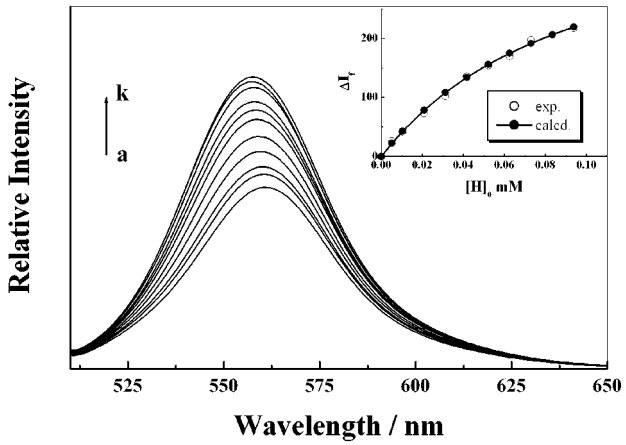

Figure 2. Fluorescence spectral changes of AR (1.6 μmol dm^{-3}) and curve-fitting analysis (inset) of the differential intensity (ΔI_f) to calculate the complex stability constant (K_s) upon addition of calix[4]arene-bridged bis(β-cyclodextrin) **2** (0−94 μmol dm^{-3} from a to k) in 1:3 DMSO−water solution (v/v). λ_{ex} = 490 nm.

AR and calix[4]arene. On the other hand, the sulfonate group on the calixarene may also play a role in the fluorescence quenching of AR. If a similar mode of interaction and mechanism of fluorescence quenching are operative in the present case, the calixarene cavity of dyad **1** is actively incorporated in the inclusion complexation with AR. On the other hand, the enhanced fluorescence upon addition of triad **2** is rationalized if the calixarene moiety in **2** does not actively participate in the binding of AR but merely acts as a rigid tether to facilitate the cooperative binding by two cyclodextrin cavities.

Assuming the 1:1 stoichiometry, the inclusion complexation of a guest (G) with a host (H) is expressed by eq 1.

$$H + G \overset{K_S}{\rightleftharpoons} G{\cdot}H \qquad (1)$$

Herewith, the relative fluorescence intensity change of guest molecule (ΔI_f) upon addition of host, where ΔI_f

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Table 1. Complex Stability Constant (K_s) and Gibbs Free Energy Change ($-\Delta G°$) for 1:1 Inclusion Complexation of AR and TNS with Calix[4]arene-Tethered β-Cyclodextrin 1 and Calix[4]arene-Bridged Bis(β-cyclodextrin) 2 in 1:3 DMSO−H_2O Solution (v/v) at 25 °C

host	guest	α	K_s/mol dm^{-3}	log K_s	$-\Delta G°$, kJ mol^{-1}	$\Delta\Delta G°$, kJ mol^{-1} [a]
β-cyclodextrin	AR	7249600	319 ± 10	2.50	14.3	0
	TNS	8290440	91 ± 5	1.96	11.2	0
1	AR	9274010	17600 ± 600	4.24	24.2	9.9
	TNS	7164960	8120 ± 400	3.91	22.3	11.1
2	AR	6609560	10480 ± 500	4.02	23.0	8.7
	TNS	2877130	2550 ± 100	3.41	19.4	8.2

[a] Differential complex stability, relative to the relevant value obtained with β-cyclodextrin for each guest series.

$= I_f$ (with host) $- I_f$ (without host), is assumed to be proportional to the concentration of inclusion complex formed by host molecule with model substrate, i.e., $\Delta I_f = \alpha[H\cdot G]$. The proportionality coefficient α is taken as a sensitivity factor for the fluorescence change upon inclusion complexation. Then, the effective stability constant (K_s) can be expressed by eq 2:

$$K_s = \frac{[H\cdot G]}{[H][G]} = \frac{\Delta I_f/\alpha}{([H]_0 - \Delta I_f/\alpha)([G]_0 - \Delta I_f/\alpha)} \quad (2)$$

where $[G]_0$ and $[H]_0$ refer to the initial concentrations of organic dye and host, respectively. Subsequently, eq 2 can be solved for ΔI_f to give eq 3.

$$\Delta I_f = \{\alpha([H]_0 + [G]_0 + 1/K_s) \pm \sqrt{\alpha^2([H]_0 + [G]_0 + 1/K_s)^2 - 4\alpha^2[H]_0[G]_0}\}/2 \quad (3)$$

Employing a nonlinear least-squares method according to the curve-fitting eq 3,[12] we obtained the complexation stability constant for each host−guest combination from the analysis of the sequential changes of fluorescence intensity (ΔI_f) at various host concentrations. For each host−guest system examined, an excellent curve fit between experimental and calculated data was found, verifying the 1:1 complex stoichiometry assumed above. In repeated measurements, the K_s values were reproducible within an error of ±5%. The K_s values thus obtained are listed in Table 1, along with the free energy change of complex formation ($-\Delta G°$).

The quantitative molecular binding ability and selectivity of triad host **2** toward representative structurally related dyes were investigated in aqueous buffer solution at 25 °C by means of circular dichroism, UV−vis, and fluorescence spectral titration methods. Previous reports have shown that the inclusion of chromophoric achiral guest in chiral host such as cyclodextrin produces induced circular dichroism (ICD) signals at the wavelengths absorbed by the guest chromophore.[13−15] Hence, the circular dichroism spectrometric titration technique can be employed in the investigation of inclusion complexation of some azo dyes with bis(β-cyclodextrin) **2**.[16,17] As can be seen from Figure 3, the stepwise addition of host **2** to a dilute aqueous buffer solution of ethyl orange causes weak but steady development of ICD intensity over the wavelength range 350−500 nm. On the other hand, UV−vis spectral titration is applicable to the

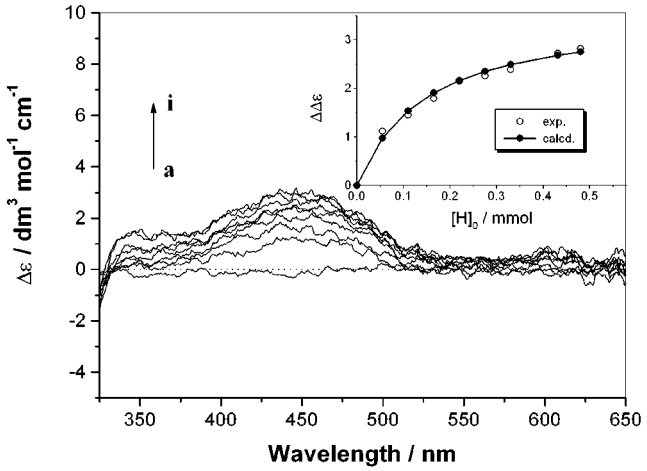

Figure 3. ICD spectral changes of ethyl orange (13 μmol dm^{-3}) and the nonlinear least-squares analysis (inset) of the differential intensity ($\Delta\Delta\epsilon$) to calculate the complex stability constant (K_s) upon addition of **2** (0−480 μmol dm^{-3} from a to i) in aqueous buffer solution.

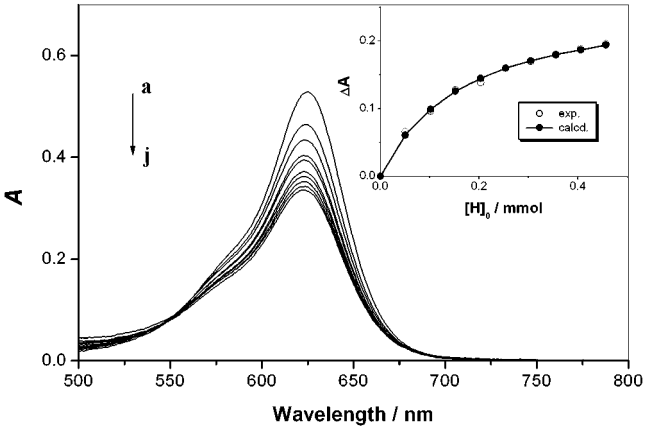

Figure 4. UV−vis spectral changes of brilliant green (11 μmol dm^{-3}) and the nonlinear least-squares analysis (inset) of the differential intensity (ΔA) to calculate the complex stability constant (K_s) upon addition of **2** (0−457 μmol dm^{-3} from a to j) in aqueous buffer solution.

examination of inclusion complexation behavior of host **2** with some tri/diphenylmethane dyes, such as brilliant green (BG) and CV, which displays no appreciable ICD or fluorescence in aqueous solution. The stability constant (K_s) can be determined by analogous nonlinear least-squares method using the curve-fitting eq 2, where relative fluorescence intensity change (ΔI_f) is replaced by the ICD intensity change ($\Delta\Delta\epsilon$) or absorbance intensity change (ΔA). Typical spectrophotometric titration plots of BG with host **2** is shown in Figure 4, where excellent fit between the observed and calculated values is found.

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Table 2. Complex Stability Constant (K_s) and Gibbs Free Energy Change ($-\Delta G°$) for 1:1 Inclusion Complexation of Various Guest Dyes with β-Cyclodextrin and Calix[4]arene-Bridged Bis(β-Cyclodextrin) (2) in Aqueous Buffer Solution (pH 7.20) at 25.0 °C

host	guest	α	K_s	log K_s	$-\Delta G°$, kJ mol^{-1}	method[a]	ref
β-cyclodextrin	MO		3560	3.55	20.3	CD	*c*
	EO	242570	4580 ± 200	3.66	20.5	CD	*b*
	CV	13570	1870 ± 50	3.27	18.7	UV	*b*
	BG	13520	2187 ± 100	3.34	19.1	UV	*b*
	TOO	1770	5530 ± 250	3.74	21.4	CD	*b*
	AR		2630	3.42	19.5	FL	*d*
	TNS		3670	3.56	20.4	FL	*c*
	RhB		4240	3.63	20.7	FL	*d*
2	MO	35490	18130 ± 750	4.26	24.3	CD	*b*
	EO	278680	7330 ± 300	3.87	22.1	CD	*b*
	CV	8420	22300 ± 1000	4.35	24.8	UV	*b*
	BG	24350	6270 ± 250	3.80	21.7	UV	*b*
	TOO	95910	10410 ± 500	4.02	22.9	CD	*b*
	AR	19441250	12450 ± 500	4.10	23.4	FL	*b*
	TNS	2789000	9570 ± 450	3.98	23.1	FL	*b*
	RhB	4655900	17920 ± 750	4.25	24.3	FL	*b*

[a] Method employed: CD, circular dichroism; UV, spectrophotometry; FL, fluorometry. [b]This work. [c]Reference 14. [d]Reference 20.

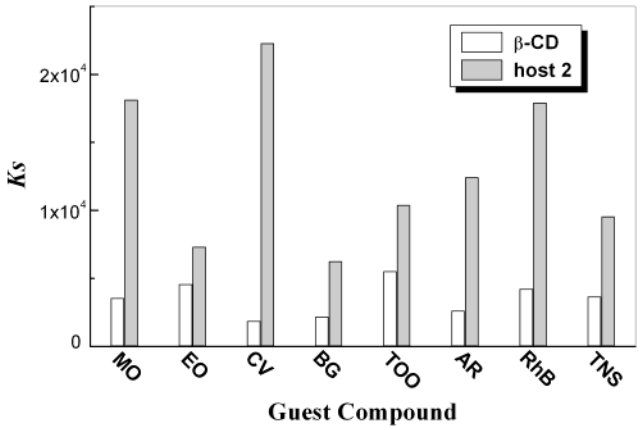

Figure 5. Complex stability constants (K_s) upon inclusion complexation of various dye guests with β-cyclodextrin and triad host **2** in aqueous buffer solution.

The K_s values obtained by the curve-fitting analysis are listed in Table 2, along with the free energy change of complex formation ($-\Delta G°$). The K_s values obtained for complexation of various dye guests with β-cyclodextrin and bis(β-cyclodextrin) **2** are comparatively plotted in Figure 5.

Molecular Binding Ability and Selectivity. Possessing a hydrophobic cavity in common, cyclodextrin and calixarene can bind analogous guest molecules, but the detailed molecular recognition mode, mechanism, and guest selectivity should appreciably differ from each other. Native and simple modified cyclodextrins afford only weak to moderate binding affinities toward various guest molecules probably due to the relatively weak hydrophobic interactions. However, once calixarene or another cyclodextrin moiety is introduced to the parent cyclodextrin, the resulting cyclodextrin−calixarene and bis(cyclodextrin) dyad hosts exhibit much higher affinities toward the above guests through cooperative binding by two adjacent hydrophobic cavities. As shown in Table 2, the K_s values for the complexation of **1** and **2** with AR in DMSO−H_2O solution are much larger than that of native β-cyclodextrin by factors of 55 and 33, respectively. The higher affinities shown by **1** may be attributed to the preferential binding of charged AR guest by the calixarene moiety.

Further examinations, using TNS as a fluorescent probe, proved the cooperative binding models. As can be seen from Table 1, calixarene-bridged bis(β-cyclodextrin) **2** affords 28 times higher K_s than that for native β-cyclodextrin, which is attributable to the cooperative binding by the closely located two cyclodextrin cavities. The much greater enhancement of up to 90 times obtained with cyclodextrin−calix[4]arene **1** indicates the more positive contribution of the calixarene moiety to the cooperative binding, probably as a result of the strict matching in size and shape as well as the interaction of the charged guest TNS with the calixarene moiety of **1**. It is also interesting to note that the cooperative enhancement effect, measured by the complexation free energy difference ($\Delta\Delta G°$), critically depends on the host−guest combination; i.e., the $\Delta\Delta G°$ value is larger for TNS (11.1 kJ mol^{-1}) than for AR (9.9 kJ mol^{-1}) upon complexation with **1**, but is larger for AR (8.7 kJ mol^{-1}) than for TNS (8.2 kJ mol^{-1}) upon complexation with **2**. These results mean that not only the enhancement of both binding ability and selectivity, but also the switching of guest selectivity can be materialized by choosing the right cooperative supramolecular host.

Using a variety of representative dye guests, the inclusion complexation behavior of triad **2** was further investigated in aqueous solution and compared with that of native β-cyclodextrin. As can be seen from Table 2 and Figure 5, the stability constants obtained for **2** are much higher than those for native β-cyclodextrin, which may be accounted for in terms of the inherent advantage of triad host **2** upon cooperative multiple binding of relatively large guest molecules. In contrast to bis(β-cyclodextrin)s which display stronger binding for linear, rather than bent, guests as a consequence of the exact size/shape fit between the host and guest,[7,12,18−20] host **2** displays the largest K_s for triangular CV (which is 12 times larger than the original value obtained with native β-cyclodextrin) and the highest molecular selectivity of up to 4.2 for CV/BG pair among the guest dye pairs examined. This phenomenon may be attributed to the cooperative binding of three dimethylanilino groups in CV by the

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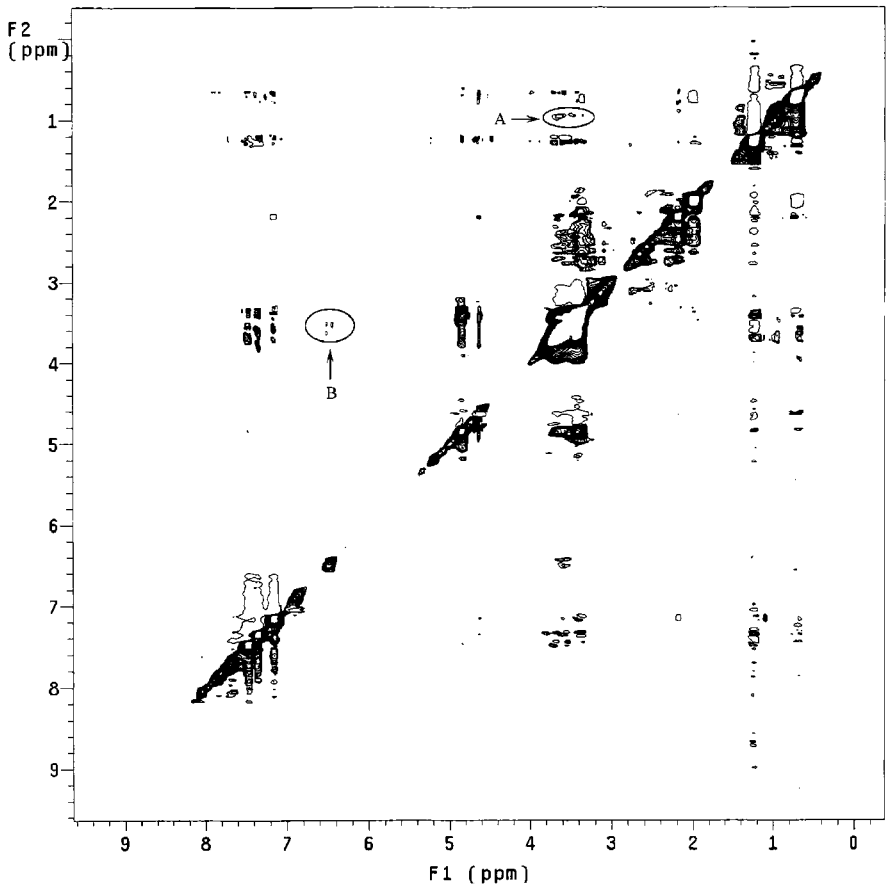

Figure 6. ^{1}H NOESY spectrum (300 MHz) of a mixture of **2** with RhB ([**2**] = [RhB] = 5.0×10^{-4} M) in D_2O at 298 K with a mixing time of 800 ms.

triple hydrophobic cavities (two cyclodextrin and one calixarene) in host **2**, leading to the strongest hydrophobic interaction between the host cavities and guest moieties. The lack of dialkylamino group on one of the phenyls in BG inevitably reduces the hydrophobic interactions upon complexation with host **2**, leading to the poor binding of BG with host **2**.

As can be seen in Table 2 and Figure 5, triad host **2** enhances the original binding ability of native β-cyclodextrin toward several azo dyes by a factor of 1.6−5.1 with a K_s sequence of methyl orange > tropaeolin OO > ethyl orange. These enhanced affinities may be ascribed primarily to the quasi-linear shape and size of these dyes fitted to the β-cyclodextrin cavities upon cooperative complexation, and additionally to the presence of a sulfonate group that can interact with the secondary hydroxyl groups of cyclodextrin through the electrostatic and/or hydrogen-bonding interactions, all of which jointly benefit the association of these dye guests with triad host **2**.

Using quasi-linear guest AR and T-shaped rhodamine B (RhB) as fluorescent probe, the inclusion complexation behavior of triad host **2** was further compared quantitatively with that of β-cyclodextrin and bis(β-cyclodextrin)s with a simple tether. Similar to the above-mentioned results, triad host **2** forms more stable inclusion complex with T-shaped RhB than with quasi-linear AR, which is contrary to the advantage of simple-tethered bis(β-cyclodextrin) upon association with linear substrate through the cooperative binding of two oriented β-cyclodextrin cavities. Comparative study on the structure of these two guests indicates that T-shaped RhB benefits its inclusion complexation with three hydrophobic (two cyclodextrin and one calixarene) cavities in host **2**, while quasi-linear conformation of AR only enables its association with two β-cyclodextrin cavities of host **2**. These factors jointly result in the uncommon stability tendency of triad host **2** with linear and T-shaped (or triangular) guests. This will in turn provide us with an efficient approach to the design and synthesis of functional "multisite" "hereto"-supramolecular hosts, along with the elucidation of the detailed cooperative binding mechanism.

NOESY Spectrum. Two-dimensional NMR spectroscopy has recently become an indispensable method to study the host−guest inclusion complexation behavior, since one can conclude that two protons are closely located in space if an NOE cross-peak is detected between the relevant protons in the NOESY or ROESY spectrum. To get further information about the geometry of the inclusion complexation between the calixarene−cyclodextrin triad host and guest molecule, 2-D NMR measurements were performed on a Varian INOVA 300 spectrometer. As shown in Figure 6, the NOESY spectrum of an equimolar mixture of triad host **2** with T-shaped guest RhB (0.5 mM each) displays clear NOE cross-peaks between the methyl protons of the diethylamino moiety in RhB and the H-3 and H-5 of cyclodextrin (peaks A), as well as the cross-peaks between the H-3 and H-5 and the aromatic protons of diethylaminophenyl in RhB (peaks B). This information indicates that the diethylaminophenyl groups of RhB are penetrating into

the cavities from the primary side of cyclodextrin to form a sandwich complex.

Cooperative Enhancement Effect in Different Solvent. From the data in Table 2 , one can find that, although both native β-cyclodextrin and cyclodextrin−calixarene triad **2** display lower K_s values in DMSO−H_2O solution than in aqueous solution for AR and TNS, host **2** shows much larger enhancement of binding constants than parent β-cyclodextrin in DMSO−H_2O solution through cooperative binding of two cyclodextrin units. One possible explanation is that there exists a competition between DMSO and guest molecule upon inclusion complexation with the hydrophobic cavities of host, and the inclusion of DMSO in cyclodextrin cavity disturbs the binding of guest molecule to some extent, affording relatively weak host−guest association. Therefore, the contribution of the second hydrophobic cyclodextrin cavity in **2** is much more pronounced to enhance K_s by a factor of 28−33 in DMSO−H_2O, as compared with the 2.6−4.7 times enhancement in aqueous solution.

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