Molecular Recognition Studies on Supramolecular Systems. 32.[1] Molecular Recognition of Dyes by Organoselenium-Bridged Bis(*β*-cyclodextrin)s

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A series of novel bis(*β*-cyclodextrin)s tethered with organoselenium linkers, i.e., 6,6′-(*o*-phenylenediseleno)-bridged bis(*β*-cyclodextrin) (**2**), 6,6′-[2,2′-diselenobis(benzoyloxy)]-bridged bis(*β*-cyclodextrin) (**3**), and 6,6′-[2,2′-diselenobis[2-(benzoylamino)ethylamino]]-bridged bis(*β*-cyclodextrin) (**4**), were synthesized from *β*-cyclodextrin (**1**). The inclusion complexation behavior of **1**−**4** with some dyes, such as 8-anilinonaphthalenesulfonate (ANS), Brilliant Green, Crystal Violet, Tropaeolin OO, Auramine O, and Methyl Orange, was investigated in aqueous phosphate buffer solution (pH 7.20) at 25 °C by UV−vis, fluorescence, and circular dichroism spectrometry, as well as fluorescence lifetime measurements. The complex stability constants (K_S) and Gibbs free energy changes ($\Delta G°$) for the stoichiometric 1:1 inclusion complexation of **1**−**4** with the dyes were obtained by the spectrophotometric or spectropolarimetric titrations. The bis(*β*-cyclodextrin)s **2**−**4** showed much higher affinities toward these guest dyes than native *β*-cyclodextrin **1** with fairly good molecular selectivities. The cooperative binding abilities of these bis(*β*-cyclodextrin)s are discussed from the viewpoints of size/shape-fit interaction, induced-fit concept, and multiple recognition mechanism.

Introduction

Extensive studies on molecular recognition by native and modified cyclodextrins as molecular receptors have documented that the truncated corn-shaped hydrophobic cavity of cyclodextrins can selectively bind diverse substrates to form host−guest complexes or supramolecular species,[2−4] which provide an excellent model system mimicking the substrate-specific interaction of enzymes.[5] Possessing a good structural variety, dyes have been widely investigated as typical guest molecules, which can form stable inclusion complexes with cyclodextrins.[6−9] As a new family of cyclodextrin derivatives, bridged cyclodextrin dimers are known to greatly enhance the original molecular binding ability of native cyclodextrins through the cooperative binding of dual hydrophobic cavities located in a close vicinity. Consequently, a number of cyclodextrin dimers have been synthesized to investigate the inclusion complexation behavior which is significantly different from that of the monocyclodextrin counterparts. Such studies have helped us understanding the multiple recognition mechanism and the induced-fit interactions between the host bis(cyclodextrin)s and guest molecules.[10−17] Recently, the molecular recognition behavior of cyclodextrin dimers has been reviewed comprehensively by Breslow and co-workers.[18] However, little attention has been paid to the molecular recognition by organoselenium-bridged bis(cyclodextrin)s as synthetic receptors. Recently, we have reported a study on the molecular recognition of some dyes by several organoselenium bridged bis(*β*-cyclodextrin)s and their platinum(IV) complexes, which has provided some insights into the cooperation of several weak interactions working between host and guest.[19,20]

We now wish to report our study on the syntheses and molecular recognition behavior of organoselenium-bridged *β*-cyclodextrin dimers (**2**−**4**), shown in Chart 1. A simple

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Chart 1

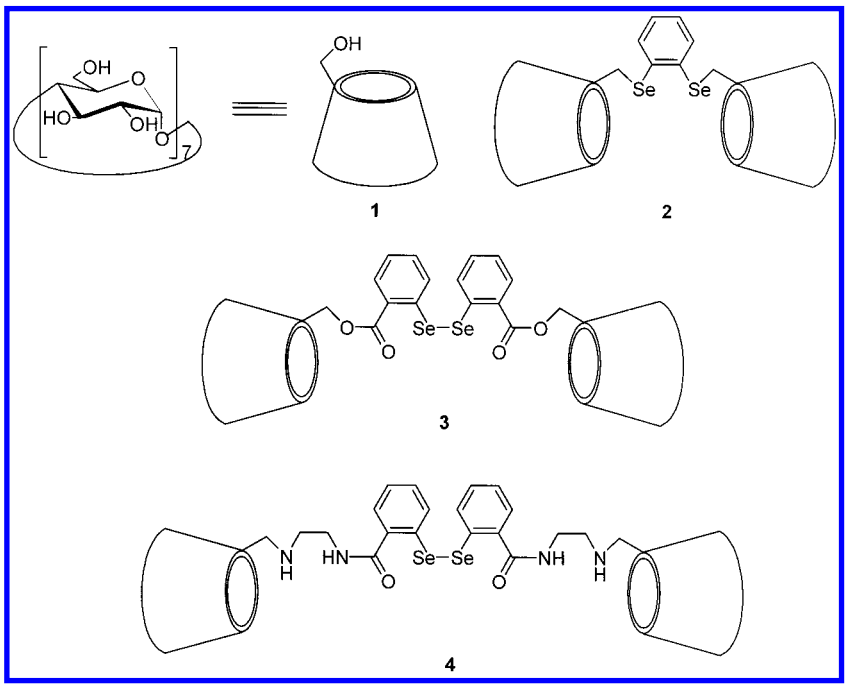
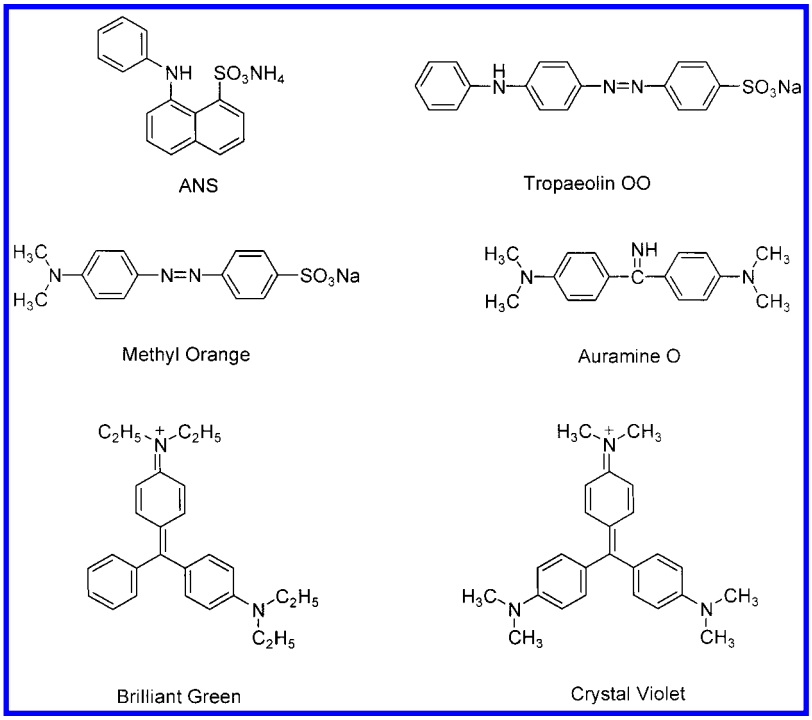


Chart 2



reason for choosing the (di)selenium tether in bis(*β*-cyclodextrin)s is that selenium, possessing a larger radius and lower electronegativity than carbon, can provide an Se−Se bond that is longer and more flexible than a C−C bond. A more serious reason is that selenium compounds are known to function as mimics of glutathione peroxidase. Therefore, the molecular recognition studies on selenium-bridged bis(cyclodextrin)s will be essential for understanding the interaction between the cyclodextrin-based selenium-containing enzyme model and substrates. The inclusion complexation behavior has been investigated at 25 °C in aqueous phosphate buffer solution (pH 7.20) by means of spectrophotometry, spectrofluorimetry, and spectropolarimetry as well as fluorescence lifetime measurement. The complex stability constants (log K_S) and Gibbs free energy changes ($\Delta G°$) obtained for some structurally related dye molecules, shown in Chart 2, are discussed from the viewpoints of the cooperative binding and the induced-fit interaction between the guest and dimeric host.

Experimental Section

Instruments. Combustion analyses were performed on a Perkin-Elmer-240 instrument. ^{1}H NMR spectra were recorded in dimethyl sulfoxide-d_6 on a Bruker AM200 instrument operated at 200 MHz. FT-IR spectra were obtained on a Nicolet 560 E.S.P. FT-IR spectrometer. Circular dichroism (CD) spectra were measured in a conventional quartz cell (10 × 10

× 45 mm) on a JASCO J-720W spectropolarimeter equipped with a PTC-348WI temperature controller to keep the temperature at 25 °C. UV−vis spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) at 25 °C on a JASCO UV-550 spectrometer.

Fluorescence lifetimes were determined by the time-correlated single-photon-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 10 000 were collected in each measurement. The accumulated signals were then processed and the lifetime determined by deconvolution with nonlinear least-squares fit.

Materials. 8-Anilinonaphthalenesulfonate (ANS), Brilliant Green, Crystal Violet, Tropaeolin OO, Auramine O, and Methyl Orange were purchased from Wako. All chemicals were reagent grade and used without further purification unless noted otherwise. β-Cyclodextrin of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried in vacuo at 95 °C for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under reduced pressure prior to use. 6,6′-*o*-Phenylenediseleno-bridged bis(β-cyclodextrin) (**2**) was prepared according to the reported procedures.[19] Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.10 M phosphate buffer solution of pH 7.20, which was used in spectral measurements.

Synthesis of 6,6′-[2,2′-Diselenobis(benzoyloxyl)]-Bridged Bis(β-cyclodextrin) (3). To a solution of DMF (100 mL) containing 2,2′-diselenobis(benzoic acid)[21] (0.4 g) and dicyclohexylcarbodiimide (DCC) (0.33 g) was added 2.5 g of β-cyclodextrin and 25 mL of dry pyridine in the presence of molecular sieves 4 Å. The resultant mixture was stirred for 12 h in an ice bath and another 18 h at room temperature and then allowed to stand for 3 days until no more precipitation deposited. The precipitate was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in a minimum amount of hot water and then poured into 150 mL of acetone. The precipitate formed was collected by filtration to obtain a white powder, which was purified on a column of Sephadex G-25 to give 0.5 g (19% yield) of **3** as a light yellow solid. FAB-MS: *m*/*z* 2636 ($M + H^+$). ^{1}H NMR (DMSO-d_6, TMS): δ 2.9−4.1 (m, 80H), 4.2−4.6 (m, 4H), 4.8−5.1 (m, 14H), 7.3−8.0 (m, Ar 8H). IR (KBr): ν 3405.7, 2925.8, 2150.3, 1638.8, 1415.1, 1369.3, 1336.2, 1302.1, 1257.1, 1200.9, 1156.9, 1079.8, 1028.9, 972.4, 938.0, 860.3, 755.9, 707.5, 609.0, 579.0, 530.5 cm^{-1}. Anal. Calcd for $C_{98}H_{146}O_{72}Se_2 \cdot 7H_2O$: C, 40.78; H, 6.03. Found: C, 40.83; H, 6.32.

Synthesis of 6,6′-[2,2′-Diselenobis[2-(benzoylamino)-ethyleneamino]]-Bridged Bis(β-cyclodextrin) (4). β-Cyclodextrin dimer **4** was prepared in 25% yield from 2,2′-diselenobis(benzoic acid)[21] and mono[6-(2-aminoethyleneamino)-6-deoxy]-β-cyclodextrin,[22] according to the similar procedures described above. FAB-MS *m*/*z*: 2722 ($M + H^+$). ^{1}H NMR (DMSO-d_6, TMS): δ 2.9−4.1 (m, 88H), 4.2−4.6 (m, 4H), 4.8−5.1 (m, 14H), 7.3−8.0 (m, Ar 8H). IR (KBr): ν 3384.7, 2928.5, 1635.2, 1541.0, 1403.3, 1335.0, 1302.8, 1258.0, 1200.1, 1155.7, 1079.2, 1028.3, 946.2, 856.6, 753.5, 706.8, 578.9, 530.2 cm^{-1}. Anal. Calcd for $C_{102}H_{158}O_{70}N_4Se_2 \cdot 8H_2O$: C, 42.79; H, 6.08; N, 1.96. Found: C, 42.98; H, 6.20; N, 2.04.

Results and Discussion

Induced Circular Dichroism Spectra of Cyclodextrin Dimers (2−4) and Their Inclusion Complexes.

It has been amply demonstrated that inclusion of chromophoric achiral guest in a chiral host such as cyclodextrin produces induced circular dichroism (ICD) signals at the wavelengths absorbed by the guest chromophore.[23−25] The sign and magnitude of the ICD signals are critical functions of the relative orientation of the chromophore against the host cavity. An empirical rule that interprets the ICD observed for a guest positioned inside or outside of the cyclodextrin cavity has been proposed by Kajtár et al.,[26] Harata and Uedaira,[27] and Kodaka.[28] This widely accepted sector rule predicts that, when a guest molecule is accommodated in the cavity, the transition dipole moment of the guest chromophore, which is parallel to the host axis, produces a positive ICD band, whereas the transition moment perpendicular to the axis gives a negative ICD.[27] In contrast, a guest located outside the cavity is known to show the completely opposite ICD behavior. Thus, the transition dipole moment parallel to the host axis affords a negative ICD signal, while the perpendicular transition gives the opposite signal.[28] Our previous study[29,30] has shown that this rule can be applied successfully to the analysis the conformation of the chromophore attached to cyclodextrin, although little effort has been devoted to the conformational analysis of cyclodextrin dimers. Here we try to explain the ICD signals of dimeric hosts **2**−**4** using the above rule.

As can be seen from Figure 1, hosts **2**−**4** display substantially different CD spectra in the absence of guest, indicating that there exist significant, but different degrees of, interactions between the aromatic tether and the two chiral cavities of cyclodextrin dimer. As we reported previously,[19] the CD spectrum of dimeric host **2** in aqueous solution shows a strong negative Cotton effect peak at 259 nm, which is ascribed to the substituent effect of the diseleno moiety and also to the cooperative binding by dual cyclodextrin cavities. It is interesting to compare the CD spectral behavior of hosts **3** and **4**. Host **3** displays two negative Cotton effect peaks at 233 nm ($\Delta\epsilon = -4.03$) and 266 nm ($\Delta\epsilon = -0.32$) and a weak positive peak at 325 nm ($\Delta\epsilon = 0.35$). As a higher homologue of **3**, β-cyclodextrin dimer **4** gives the opposite Cotton effects; i.e., a positive Cotton effect peak at 232 nm ($\Delta\epsilon = 27.6$) with a shoulder at 260 nm ($\Delta\epsilon = 8.64$) and a weak negative peak at 318 nm ($\Delta\epsilon = -1.43$). According to the sector rule,[26−28] we can deduce that the benzene ring in the tether of host **3** is shallowly self-included in the cavity, where both of the transition moments of the ^1L_a and ^1L_b bands at 233 and 266 nm are nearly perpendicular to the axis of cyclodextrin, resulting in the two negative Cotton effect peaks. On the other hand, the Se−Se moiety is located outside the cavity, but situated between the two primary rims of dual cyclodextrin cavities. Hence, the transition dipole of the Se−Se bond is perpendicular to the host axis, giving the

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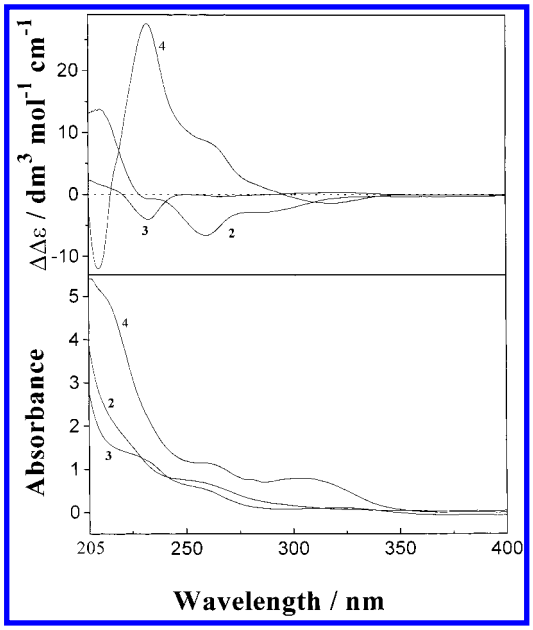

Figure 1. (a) Circular dichroism and (b) UV−vis absorption spectra of 6,6′-(*o*-phenylenediseleno)-bridged bis(*β*-cyclodextrin) **2**, 6,6′-[2,2′-diselenobis(benzoyloxy)]-bridged bis(*β*-cyclodextrin) **3**, and 6,6′-[2,2′-diselenobis[2-(benzoylamino)ethylamino]]-bridged bis(*β*-cyclodextrin) **4** (0.1 mM) in phosphate buffer solution (pH 7.2) at 25 °C.

positive Cotton effect at 325 nm. However, it is difficult to be self-included into the cavity for the benzene ring of host **4**, which is tethered to each cyclodextrin with a longer linker. Consequently, the transition moments of the ^1L_a and ^1L_b bands become perpendicular to the axis of cyclodextrin, affording two strong positive Cotton effects. On the contrary, the transition dipole moment of the Se−Se bond in host **4** is inferred to be parallel to the host axis, as the corresponding transition at 318 nm gives a negative Cotton effect.

Using the CD spectrometric titration technique, the inclusion complexation of *β*-cyclodextrin derivatives with some azo dye guests has been investigated.[28,31] We have also reported that native cyclodextrin and some cyclodextrin dimers give distinctly different ICD signals upon inclusion complexation of Methyl Orange.[20] In this study, we further investigate the inclusion behavior of Methyl Orange with the other cyclodextrin dimers linked by different organoselenium tethers. As reported previously,[20] native *β*-cyclodextrin **1** can induce an appreciable positive CD signal at 420 nm, which corresponds to the $\pi\rightarrow\pi^*$ transition band of the azo group of Methyl Orange (Figure 2, trace b), while no CD signals were observed in the absence of cyclodextrin (Figure 2, trace a). The positive CD signal observed indicates that Methyl Orange molecule penetrates into the cavities in the longitudinal direction,[26,28] where the $\pi\rightarrow\pi^*$ transition moment of the azo chromophore in Methyl Orange is parallel to the host axis, exhibiting a positive ICD signal at 420 nm.

Compared with native *β*-cyclodextrin **1**, the bridged bis(*β*-cyclodextrin)s **2**−**4** possessing dual hydrophobic cavities, displayed completely different ICD spectral behavior upon complexation of dyes, giving two ICD extreme with the opposite sign for each host−guest combination. As shown in Figure 2 (trace c), the ICD spectrum of Methyl

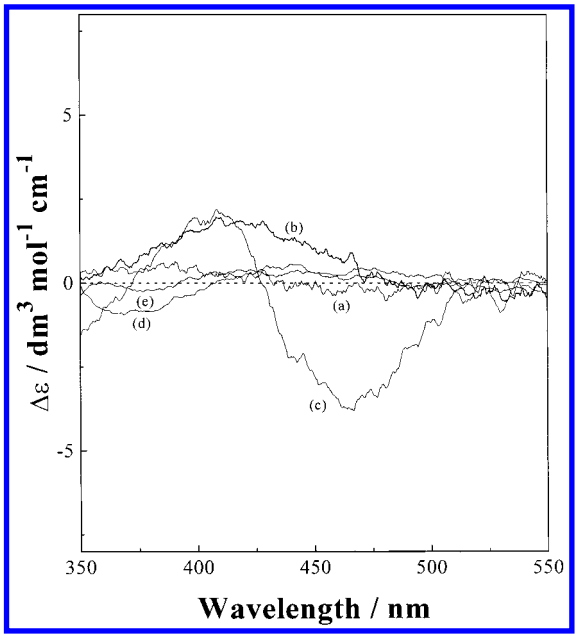

Figure 2. Induced circular dichroism spectra of Methyl Orange (10.7 *μ*M) (a) in the absence and in the presence of (b) *β*-cyclodextrin **1** (1.5 mM) and (c) 6,6′-(*o*-phenylenediseleno)-bridged bis(*β*-cyclodextrin) **2**, 6,6′-[2,2′-diselenobis(benzoyloxy)]-bridged bis(*β*-cyclodextrin) **3**, and 6,6′-[2,2′-diselenobis-[2-(benzoylamino)ethylamino]]-bridged bis(*β*-cyclodextrin) **4** (0.25 mM) in aqueous buffer solution at pH 7.20.

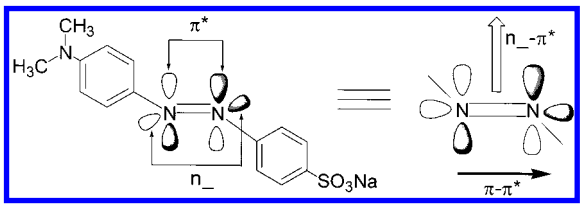

Figure 3. Illustration of the transition dipole moment for the $\pi\rightarrow\pi^*$ (solid arrow) and $n\rightarrow\pi^*$ (open arrow) electronic transitions of the azo chromophore of Methyl Orange.

Orange (11 *μ*M) caused by host **2** (50.6 *μ*M) shows a strong negative Cotton effect at 467 nm ($\Delta\epsilon = -3.82$) and a moderate positive peak at 408 nm ($\Delta\epsilon = 2.19$). However, host **3** (0.3 mM) induced a weak positive Cotton effect at 440 nm ($\Delta\epsilon = 0.56$) and a weak negative Cotton effect at 369 nm ($\Delta\epsilon = -0.94$) (Figure 2, trace d), while host **4** (0.3 mM) also induced a weak positive Cotton effect at 423 nm ($\Delta\epsilon = 0.40$) and a weak negative peak at 377 nm ($\Delta\epsilon = -0.26$) (Figure 2, trace e). Although the complete and comprehensive rationalization of these spectral features would be difficult, one possible explanation is that the broad absorption band of Methyl Orange at 350−500 nm is composed of two allowed transitions and their transition dipole moments are perpendicular to each other. Indeed, the transition moment of the $\pi\rightarrow\pi^*$ (parallel to the N=N bond)[28] and $n\rightarrow\pi^*$ (perpendicular to the N=N bond)[31] of azo chromophore are perpendicular to each other, as illustrated in Figure 3. The inverted sign of the split ICD spectra observed for host **2** and hosts **3** and **4** and the substantially weak ICD intensities for the latter two hosts would also be accounted for in terms of the depth of guest penetration, or the host−guest distance. Possessing the shortest tether, host **2** can include most part of the azo chromophore in the cavities, giving rise to the strong ICDs for both $\pi\rightarrow\pi^*$ (parallel to the

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Table 1. Fluorescence Lifetime (τ) and Relative Quantum Yield (Φ) of Ammonium 8-Anilino-1-naphthalenesulfonate (ANS) in the Presence and Absence of Native β-Cyclodextrin and Bis(β-cyclodextrin)s 1−4

ANS/μM	host	equiv	τ_S/ns	Φ_S/%	τ_L/ns	Φ_L/%	χ^2	ref
10	none		0.4	100			1.42	*a*
500	none		0.4	100			1.46	*b*
10	**1**	40	0.5	96.5	3.1	3.5	1.00	*a*
250	**1**	10	1.5	67.6	3.2	32.4	1.24	*b*
10	**2**	40	2.9	45.6	9.9	54.4	1.11	*b*
10	**3**	20	0.6	88.1	7.4	11.9	1.04	*a*
10	**4**	20	1.2	75.2	10.1	24.8	1.46	*a*

[a] This work. Measured in the presence of host in aqueous buffer solution of pH 7.20 at 25.0 °C; the fluorescence decay profile could be successfully deconvoluted by double exponential function, giving short and long lifetimes, denoted by subscripts S and L, respectively, along with the fitting parameter χ^2. [b] Reference 20.

host axis) and n→π* (perpendicular to the host axis) with the signs predicted for the chromophore inside the cavity from the sector rule. On the contrary, hosts **3** and **4** are linked by longer tethers and therefore most part of the azo chromophore cannot be fully accommodated in the cavities, resulting in the weak ICDs with the opposite signs, which are anticipated for the chromophore outside the cavity.

Fluorescence Lifetime. Bright[32] and Reinsborough[33] have demonstrated that the fluorescent dyes accommodated in the hydrophobic cavity of cyclodextrin leads to fluorescence enhancement and peak shifts[19] as well as significantly elongated fluorescence lifetimes. Recently, we have reported the fluorescence spectra and lifetimes of ANS in the presence of native cyclodextrin and some cyclodextrin dimers, which enabled us to elucidate the complex structure.[19,20] In the present study, we performed the nanosecond time-resolved fluorescence measurements with ANS in aqueous buffer solution (pH 7.20) in the presence or absence of β-cyclodextrin **1** and bis(β-cyclodextrin)s **2**−**4** in order to assess the microenvironmental polarity around the included ANS and to understand the inclusion complexation behavior of host compounds in further detail.

Since the rates of complexation/decomplexation are much slower than that of the fluorescence decay, the decay profile of fluorescence intensity ($F(t)$) can be described as a sum of unimolecular decays for all fluorescing species present in the solution

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

where A_i and τ_i represent the initial abundance and lifetime of the ith species. In the absence of host, the fluorescence decay curve observed for ANS in aqueous buffer solution was perfectly fitted to a single-exponential function, giving a lifetime of 0.4 ns. On the contrary, the decay profile of ANS in the presence of β-cyclodextrin or bis(β-cyclodextrin)s could be analyzed only by a linear combination of two exponential functions. The short and long fluorescence lifetimes (τ_S and τ_L) and relative quantum yields (Φ) observed for ANS in the presence of **1**−**4** are summarized in Table 1. The longer lifetime of ANS (3.1−3.2 ns) in the presence of **1** clearly indicated

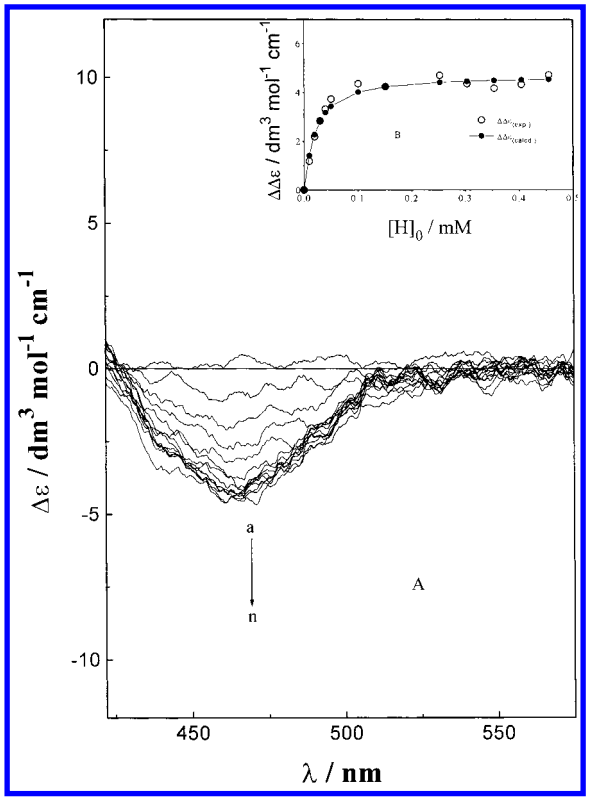

Figure 4. (A) ICD spectral changes of phosphate buffer solution of Methyl Orange (10.7 μM) in the presence of 6,6′-(*o*-phenylenediseleno)-bridged bis(β-cyclodextrin) **2**. The concentration of host **2** (from a to n): 0, 10, 20, 30, 40, 50.6, 101.1, 151.7, 202.2, 252.8, 303.4, 353.9, 404.4, and 455 μM, respectively. (B) Least-squares curve-fitting analysis for the complexations of Methyl Orange with **2**.

that the ANS molecule is located in a more hydrophobic environment rather than in the bulk water, forming a 1:1 inclusion complex with β-cyclodextrin. Interestingly, the shorter lifetime of ANS (τ_S = 2.9 ns) in the presence of **2** is in good agreement with the longer lifetime (τ_L = 3.1−3.2 ns) in the presence of **1**. The significantly long τ_S (2.9 ns) and much longer τ_L (9.9 ns) of ANS in the presence of **2** may indicate the coexistence of "intramolecular" 1:1 and 2:1 host−guest complexes, in which the ANS molecule is accommodated in one and two cavities of host **2**, respectively.[20] Similarly, the τ_S's of ANS (0.6−1.2 ns) in the presence of hosts **3** and **4** are appreciably longer than the original τ of ANS (0.4 ns) of ANS in water but shorter than that (2.9 ns) obtained with **2**, while the τ_L's of ANS (7.4−10.1 ns) in the presence of **3** and **4** are comparable to that in the presence of **2** (9.9 ns). As higher hydrophobicity around the fluorophore leads in general to a longer lifetime (τ_L), we may deduce that host **4** with a longer tether gives the most hydrophobic environment around ANS upon "sandwich" complexation. The smaller relative fluorescence intensities (Φ_L/Φ_S) for **3** and **4** than for **1** may indicate that the longer tethers in **3** and **4** are unfavorable in general for the sandwich complexation.

Spectropolarimetric Titrations. The quantitative ICD spectral study enables us not only to elucidate the conformation of the chromophore attached to the host but also to determinate the binding constants for various guests. Thus, the addition of hosts **1**−**4** to an aqueous dye solution induced substantial CD spectral changes at wavelengths that are absorbed by the dye. As can be seen from Figure 4A, the stepwise addition of host **2** of up to

(32) Bright, F. V.; Catena, G. C. *Anal. Chem.* **1989**, *61*, 905.
(33) Jobe, D. J.; Verrall, R. E.; Paleu, R.; Reinsborough, V. C. *J. Phys. Chem.* **1988**, *92*, 3582.

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 Bis(β-cyclodextrin)s (1−4) in Aqueous Buffer Solution (pH 7.20) at 25.0 °C

host	guest	K_S/M^{-1}	$K_S(\mathbf{X})/K_S(\mathbf{1})^a$	log K_s	$-\Delta G°$/kJ mol^{-1}	α	method[b]	ref
1	Brilliant Green	2190	≡1	3.34	19.1	13520	UV	*c*
	Crystal Violet	1870	≡1	3.27	18.7	13570	UV	*c*
	Methyl Orange	3560	≡1	3.55	20.3		CD	*d*
	Auramine O	819	≡1	2.91	16.6	4770	UV	*c*
	Tropaeolin OO	5530	≡1	3.74	21.4	1770	UV	*c*
	ANS	103	≡1	2.01	11.5	634450	FL	*e*
2	Brilliant Green	12700	5.8	4.10	23.4	8250	UV	*c*
	Crystal Violet	33100	17.7	4.52	25.8	17460	UV	*c*
	Methyl Orange	63900	17.9	4.81	27.4	426960	CD	*c*
	Auramine O	13700	16.7	4.14	23.6	3000	UV	*c*
	ANS	1280	12.4	3.11	17.7	883570	FL	*e*
3	Brilliant Green	3060	1.4	3.49	19.9	7910	UV	*c*
	Crystal Violet	20400	10.9	4.31	24.6	21150	UV	*c*
	Methyl Orange	27300	7.7	4.44	25.3	52950	CD	*c*
	Auramine O	3330	4.1	3.52	20.1	3170	UV	*c*
4	Brilliant Green	13300	6.1	4.12	23.5	28150	UV	*c*
	Crystal Violet	15300	8.2	4.18	23.9	15080	UV	*c*
	Methyl Orange	14800	4.2	4.17	23.8	52060	CD	*c*
	Tropaeolin OO	14800	2.7	4.17	23.8	1090	UV	*c*

[a] Relative selectivity for each dye, where host **X** = **1**, **2**, **3**, or **4**. [b] Method employed: CD, circular dichroism spectrometry; UV, UV−vis spectrophotometry; FL, fluorimetry. [c] This work. [d] Reference 20. [e] Reference 19.

0.45 mM to a dilute aqueous buffer solution of Methyl Orange (11 μM) causes a gradual increase of the ICD intensity over the wavelength range 420−520 nm. These ICD spectral changes can be used for the quantitative analysis to determine complex stability constant (K_S).

Assuming the 1:1 host/guest stoichiometry, the complexation of guest (G) with cyclodextrin host (H) is expressed by eq 1

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

The stability constant (K_S) can be determined using a nonlinear least-squares method according to the curve fitting eq 2[29]

$$\Delta\Delta\epsilon = [\alpha([H]_0 + [G]_0 + 1/K_S) \pm \sqrt{\alpha^2([H]_0 + [G]_0 + 1/K_S)^2 - 4\alpha^2[H]_0[G]_0}]/2 \qquad (2)$$

where $[G]_0$ and $[H]_0$ refer to the total concentrations of dyes and β-cyclodextrin derivatives, respectively, α is the proportionality coefficient for the effective CD intensity change induced by host complexation, which may be taken as a sensitivity factor for the CD change, and $\Delta\Delta\epsilon$ denotes the change in CD intensity upon stepwise addition of host. For each host examined, the plot of $\Delta\Delta\epsilon$ as a function of $[H]_0$ gave an excellent fit to the theoretical curve, verifying the validity of the 1:1 complex stoichiometry assumed above. As shown in Figure 4B, the observed $\Delta\Delta\epsilon$ value (open circle) are plotted against $[H]_0$ to give an excellent fit without serious deviations from the calculated values (small dots). In the repeated measurements, the K_S values were reproducible within an error of ±5%. The K_S and α values obtained by the curve fitting are listed in Table 2, along with the free energy changes of complex formation ($-\Delta G°$).

Spectrophotometric Titrations. To further investigate the molecular binding abilities and selectivities of dimeric hosts, complexation behavior of some azo and tri/diphenylmethane dyes possessing related structures, i.e., Tropaeolin OO, Auramine O, Brilliant Green, and Crystal Violet, was examined by differential UV−vis spectral titrations.

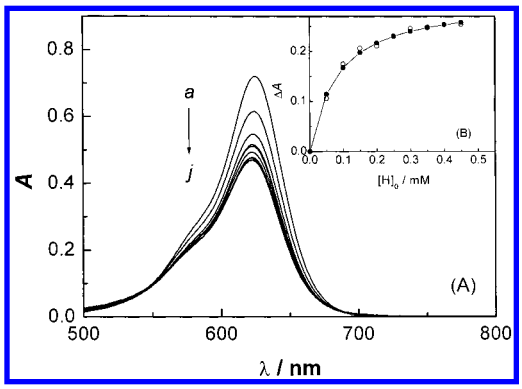

Figure 5. (A) UV−vis spectral changes of phosphate buffer solution of Brilliant Green (11 μM) in the presence of 6,6′-[2,2′-diselenobis(benzoylaminoethyleneamino)]-bridged bis(β-cyclodextrin) **4**. The concentration of host **4** (from a to j): 0, 50, 100, 150, 200, 250, 300, 350, 400, and 450 μM, respectively. (B) Least-squares curve-fitting analyses for complexations of Brilliant Green with **4**.

In the spectrophotometric titration experiments, the absorption of dyes gradually decreased in intensity upon addition of varying amounts of hosts **1**−**4**. Typical UV−vis spectral changes upon addition of host **4** to Brilliant Green solution are shown in Figure 5A. The absorbance at ca. 625 nm decreases with increasing concentration of **4**, indicating the formation of inclusion complex with dye. With the assumption of the 1:1 stoichiometry, the inclusion complexation can also be expressed by eq 1. The stability constant (K_S) may be determined using a nonlinear least-squares method according to the curve fitting eq 3[29]

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

where all the abbreviations are the same as eq 2, except for *A* which denotes the change in absorbance upon stepwise addition of host. For each host−guest combination examined, the plot of observed *A* (open circle) as a function of the initial host concentration $[H]_0$ gave an excellent fit to the theoretical value (small dots), as shown

in Figure 5B. In the repeated measurements, the K_S values were reproducible within an error of ±5%. The K_S and α values obtained by the curve fitting are also listed in Table 2, along with the free energy change of complex formation ($-\Delta G°$).

Molecular Binding Ability and Molecular Selectivity. It has been demonstrated in the preceding studies on molecular recognition by cyclodextrin dimers[10−18] that, possessing dual hydrophobic cavities, tethered bis(cyclodextrin)s exhibit greatly enhanced molecular binding ability through the cooperative binding and multiple recognition mechanism. In the present study on the inclusion complexation of several dyes, we obtained much higher stability constants (K_S) for dimeric hosts **2**−**4** than for native β-cyclodextrin **1**. Thus, the K_S values for the dimeric hosts are enhanced by factors of 5.8−18 for **2**, of 1.4−11 for **3**, and of 2.7−8.2 for **4**, respectively. There is a fairly general tendency of K_S, decreasing with increasing tether length. Thus the binding constants for Crystal Violet, Methyl Orange, and Auramine O gradually decrease in the order **2** > **3** > **4**, although Brilliant Green is exceptional, giving the lowest K_S with **3**. This tendency is entropic in origin and is attributable to the gradually declining cooperative effect upon extending the distance between two cyclodextrin moieties.

It is not appropriate or even hazardous in general to ascribe the observed inclusion complexation behavior to a single cause, since the binding ability and selectivity are affected by several factors, including size/shape matching, hydrophobicity, electrostatic interaction, and hydrogen-bonding ability. However, the guest dyes employed in the present study share some structural and functional similarities, possessing an anilino moiety in common and a quasi-linear or triangular shape with an anionic sulfonate or cationic iminonium/ammonium group. All of these guest features are suitable for investigating interaction with the ditopic hosts. We therefore discuss the inclusion behavior of bis(cyclodextrin)s with the structurally related guests.

As can be seen from Table 2, native β-cyclodextrin displays a selectivity sequence: Tropaeolin OO > Methyl Orange > Brilliant Green > Crystal Violet > Auramine O > ANS. The strongest binding of Tropaeolin OO and Methyl Orange may be ascribed primarily to the quasi-linear shape and size fitted to the β-cyclodextrin cavity and additionally to the presence of a sulfonate group that could interact with the secondary hydroxyl groups of cyclodextrin through the electrostatic and/or hydrogen-bonding interactions. It would be somewhat unexpected that β-cyclodextrin show the lowest binding ability toward ANS. As we reported,[20] examinations with CPK molecular models indicate that ANS can only partially penetrate into the cavity to form a weak inclusion complex as a result of the steric hindrance arising from the bent structure. The global selectivity sequence is affected also by the hydrophobicity of the guest molecules, or the numbers of hydrophobic benzene ring and of hydrophilic polar/charged group, which may explain the selectivity sequences within the two families of dyes: Tropaeolin OO > Methyl Orange and Brilliant Green > Crystal Violet > Auramine O.

Host **2** displayed the highest binding ability among the three dimeric cyclodextrins for all of the dyes examined, for which the shortest and least flexible tether is most probably responsible. The selectivity sequence shows an interesting difference from that observed for **1**. Thus, the K_S value obtained with **2** decreases in the order: Methyl Orange > Crystal Violet > Auramine O > Brilliant Green > ANS. It is noted that particularly Brilliant Green is not fully benefited from the cooperative binding. This is evident from the comparison of the relative binding constants, $K_S(\mathbf{2})/K_S(\mathbf{1}) = 5.8$, for Brilliant Green with the corresponding values of 12.4−17.9 for the other dyes (Table 2). This dramatic change in selectivity sequence and the much decreased cooperative effect observed for Brilliant Green may be ascribed to the different inclusion complexation behavior of native cyclodextrin and cyclodextrin dimers. Harata's studies[34] on the inclusion complexation behavior of native cyclodextrins clearly demonstrate that guest molecule usually penetrates into cyclodextrin cavity from the wider secondary side. However, 6,6′-bridged bis(cyclodextrin)s are considered to bind guest in a cooperative manner from the primary side of dual cyclodextrin moieties. Since one of the major structural differences between Brilliant Green and Crystal Violet or Auramine O is the alkyl group attached to the anilino nitrogen, it is inferred that the larger diethylamino substituent in Brilliant Green causes steric hindrance upon penetration into the cavities from the narrower primary side, preventing effective operation of the cooperative binding by dual cyclodextrins.

Host **3** with a tether of moderate length also binds all the examined dyes stronger than native cyclodextrin **1**, but gives appreciably lower K_S values with the same selectivity sequence as host **2**, i.e., Methyl Orange > Crystal Violet > Auramine O > Brilliant Green. The efficiency of cooperative binding as measured by $K_S(\mathbf{3})/K_S(\mathbf{1})$ is certainly lower than that observed for **2** in each case, but is much more widely spread from 1.4 for Brilliant Green to 10.9 for Crystal Violet. Consequently, the molecular selectivity for Methyl Orange over Brilliant Green is dramatically enhanced from 1.6 for host **1** to 5.0 for host **2** and then to 8.9 for host **3**. This global improvement of molecular selectivity observed for **3**, as a result of the guest-specific enhancement of K_S, probably originates from the moderate conformational freedom of **3**, which enables the tailoring of host conformation to fit to the shape of specific guest dyes. Both the weak ICD intensity observed for Methyl Orange (Figure 2) and the shortest τ_L (7.4 ns) for ANS (Table 1) in the presence of **3** also indicate that not the core but the peripheral part of aromatic chromophore of dyes is accommodated in the host cavities, which is consistent with the complexation behavior of dyad hosts.

It is intriguing that host **4** gives moderately enhanced (by a factor of 2.7−8.2) but mutually very close binding constants (K_S = 13300−15300 M^{-1}) for all of the azo and triphenylmethane dyes examined. The very flat selectivity profile observed for **4** is not anticipated or understood at all from a simple extension of the complexation behavior of its lower homologues **2** and **3**, although the rather limited enhancement of K_S seems reasonable in view of the entropic disadvantage of the longer tether which requires more extensive conformational changes, and greater reduction of entropy, upon guest inclusion. However, it is not an easy task to invent a mechanism that can rationalize the comparable affinities of **4** toward the four dyes, as the degree of affinity enhancement differs from guest to guest. Then, we may tentatively

(34) (a) Harata, K.; Uedaira, H.; Tanaka, J. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1627. (b) Harata, K. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2782.

conclude that the very close K_S's observed are just an incidental coincidence as a result of the larger enhancement (6.1−8.2 times) for the triphenylmethane dyes and the smaller enhancement (2.7−4.2 times) for the azo dyes.

In conclusion, we have demonstrated that several factors greatly and extensively affect the inclusion complexation of guest dyes with cyclodextrin dimers, including not only the shape/charge/substituent of guest and the tether of host but also the critical changes in host−guest interactions such as hydrophobic, van der Waals, and hydrogen-bonding interactions as well as the size/shape-fit, induced-fit, multiple recognition, and cooperative binding mechanisms. The inclusion complexation behavior of dimeric hosts mainly depend on the conformation, length, and flexibility of the organoselenium tether, which may control how the dual cyclodextrin cavities adjust their orientations and conformation to cooperatively bind guest molecule. Simultaneously, the hydrophobicity and substituent effect of guest determine the stability of host−guest complex through hydrophobic, van der Waals, and hydrogen-bonding interactions. Finally, we wish to emphasize that the size/shape-fit relationship and induced-fit concept as well as multiple recognition mechanism working between host and guest also play crucial roles in the molecular binding ability/selectivity of bis(cyclodextrin)s, inducing the ultimate conformation of host adjusted to the guest accommodated in the dual cyclodextrins.

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