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Synthesis of Bridged and Metallobridged Bis(β-cyclodextrin)s Containing Fluorescent Oxamidobisbenzoyl Linkers and Their Selective Binding towards Bile Salts

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Abstract: A series of β-cyclodextrin (β-CD) dimers containing fluorescent 2,2′-oxamidobisbenzoyl and 4,4′-oxamidobisbenzoyl linkers—that is, 6,6′-[2,2′-oxamidobis(benzoylamino)]ethyleneamino-6,6′-deoxy-bis(β-CD) (**2**), 6,6′-[2,2′-oxamidobis(benzoylamino)]diethylenediamino-6,6′-deoxy-bis(β-CD) (**3**), 6,6′-[4,4′-oxamidobis(benzoylamino)]ethyleneamino-6,6′-deoxy-bis(β-CD) (**4**), and 6,6′-[4,4′-oxamidobis(benzoylamino)]-diethylenediamino-6,6′-deoxy- bis(β-CD) (**5**)—were synthesized from the corresponding oxamidobis(benzoic acid)s through treatment with mono[6-aminoethyleneamino-6-deoxy]-β-CD or mono[6-diethylenetriamino-6-deoxy]-β-CD. Further treatment of **2**–**5** with copper perchlorate gave their Cu^{II} complexes **6**–**9** in satisfactory yields. The conformation and binding behavior of **2**–**9** towards two bile salt guests—sodium cholate (CA) and sodium deoxycholate (DCA)—was comprehensively investigated by circular dichroism, 2D NMR spectroscopy, and fluorescence spectroscopy in Tris-HCl buffer solution (pH 7.2) at 25 °C. Thanks to the cooperative host–linker–guest binding mode, the stoichiometric 1:1 complexes formed by bis(β-CD)s **2**–**5** with bile salts gave high stability constants (K_S values) of up to 10^3–$10^4\,M^{-1}$. Significantly, benefiting from the intramolecular 1:2 or 2:4 binding stoichiometry, the resulting complexes of metallobis(β-CD)s **6**–**9** with bile salts gave much higher K_S values of up to 10^6–$10^7\,M^{-2}$. The enhanced binding abilities of bis(β-CD)s and metallobridged bis(β-CD)s are discussed from the viewpoints of induced-fit interactions and multiple recognition between host and guest.

Keywords: bile salts · cyclodextrins · fluorescence spectroscopy · molecular recognition

Introduction

Cyclodextrins (CDs), a class of macrocyclic oligosaccharides consisting of six, seven, or eight glucose units linked by α-1,4-glucose bonds, are capable of encapsulating various organic, inorganic, and biological molecules within their truncated cone-shaped hydrophobic cavities to form stable host–guest inclusion complexes or supramolecular species and have thus been extensively used as molecular receptors and chiral selectors in many fields of chemistry and biology.[1–5] However, the binding abilities of native CDs are generally limited, which is unfavorable to their application as enzyme mimics and to a greater extent as antibody mimics. To improve the original binding abilities and molecular selectivities of native CDs, a great deal of effort has been devoted to the design and synthesis of functional CD derivatives[6–7] and to investigations into their molecular recognition behavior with various guest molecules.[8–13] Besides functional substituents, metal ions have also been introduced into CD systems as additional recognition sites to enhance the binding abilities and molecular selectivities of CDs further.[14–16]

Bile salts, on the other hand, are surfactant-like molecules that act to assist in the digestion of fats through the formation of micelles and micellar aggregates. Moreover, they can also interact with drugs used for the treatment of hyperlipidemia, such as neomycin, clidamycin, kanamycin, and lincomycin.[17] Because of the attractive properties of both CDs and bile salts, molecular binding between bile salts and CDs has been widely studied. Choi et al.[18] studied inclusion complex formation by β-CD and its dimers with cholesterol by Monte Carlo docking simulations, molecular dynamics calculations, and non-equilibrium molecular dynamics simulations. Tato and co-workers[19] studied the complex geometry of β-CD and its derivatives in D_2O by ROESY experiments, observing different binding modes upon inclusion complexation with bile salts. Cramer et al.[20] reported the first kinetic

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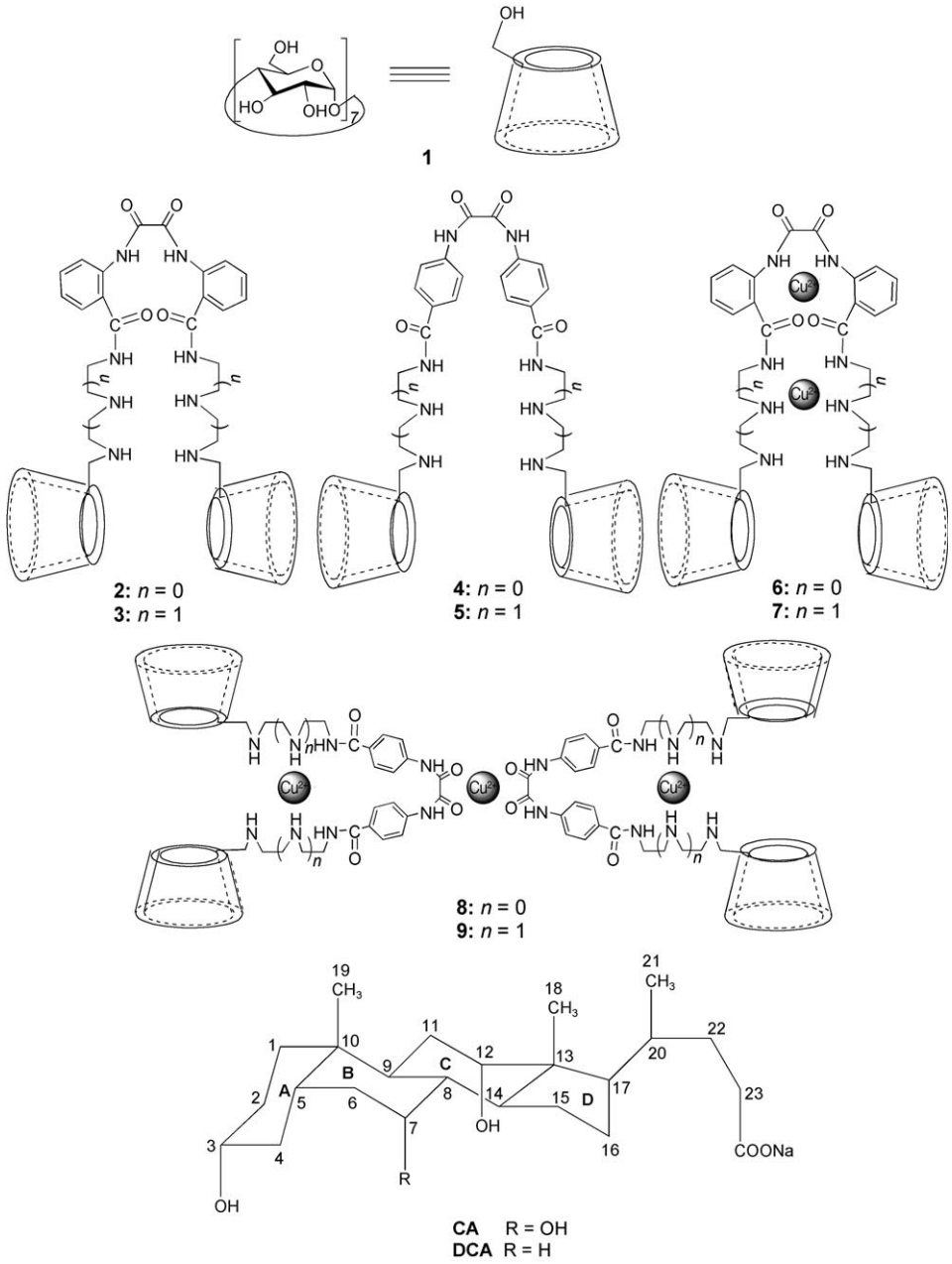
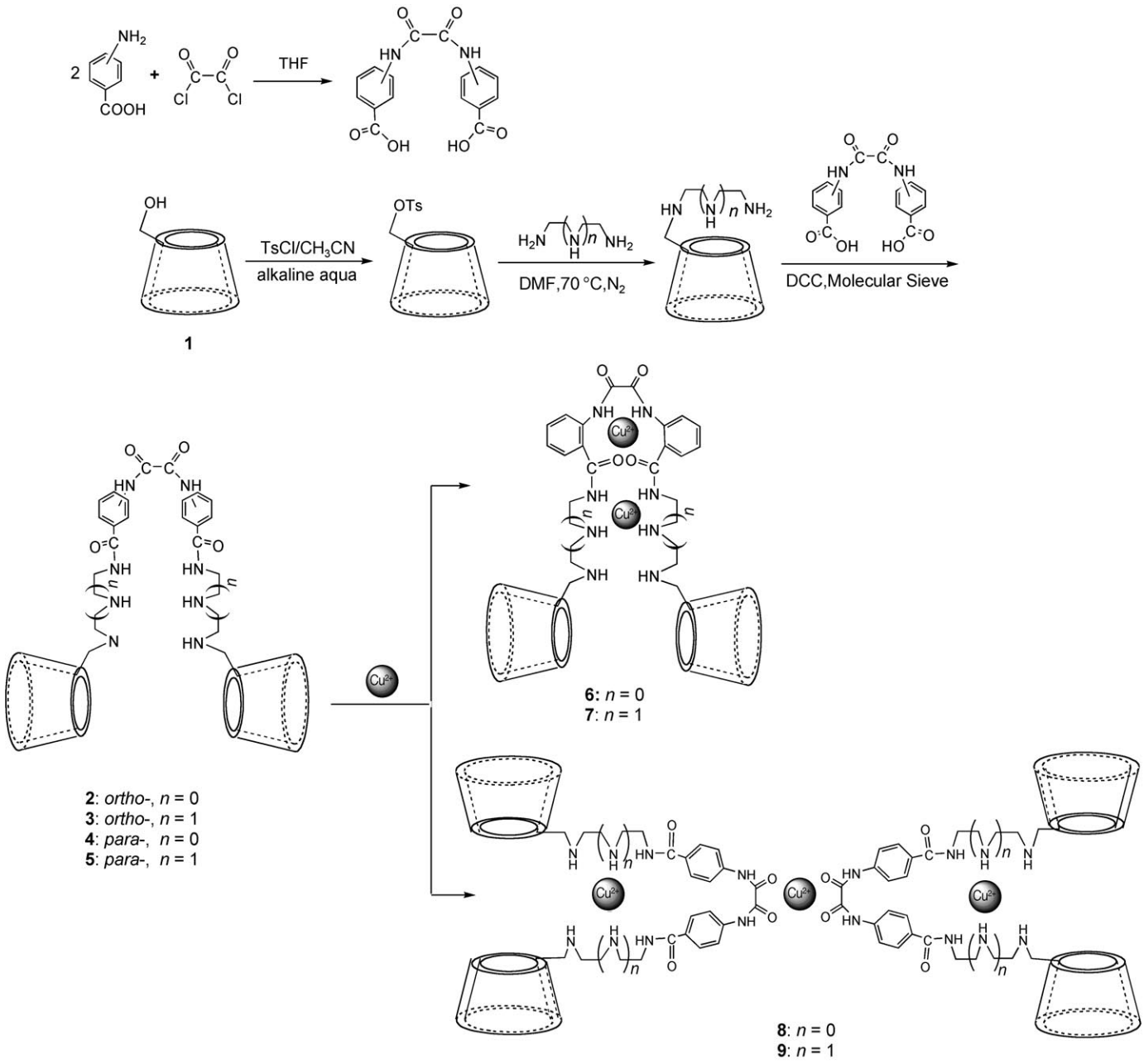
measurements performed on a series of naphthylazobenzene-β-CD/bile salt complexes, while Reinhoudt et al.[21] reported a microcalorimetric study on the cooperative binding of bile salts by CD dimers in a basic environment, showing enhanced binding abilities towards cholate and deoxycholate. We have recently also reported some significant results on the fluorescence sensing and binding modes of bridged bis(β-CD)s towards bile salts, demonstrating that bis(β-CD)s can form stoichiometric inclusion complexes (either 1:1 or 1:2) with bile salts.[22] An in-depth comparative study on the molecular binding behavior of bis(β-CD)s and metallo-bridged bis(β-CD)s with bile salts has yet to be reported, however, though such studies would be very important for elucidation of the molecular recognition mechanism and control of the binding behavior of synthetic receptors. In this work we have synthesized a series of bis(β-CD)s with fluorescent oxamidobisbenzoyl linkers and their Cu^{II} complexes, and have comparatively investigated their selective binding behavior towards some optically inert bile salt guest molecules. Here, the oxamidobisbenzoyl linker can act not only as a sensing site for a guest but also as a coordinating site for metal ions. First, the fluorescent oxamidobisbenzoyl linkers may undergo substantial conformational changes upon complexation with optically inert bile salts, producing appreciable fluorescence changes. Second, both the oligo(ethylenediamine) moieties and the carbonyl groups in the linker have the ability to coordinate to transition-metal ions, therefore enabling us to modify and potentially to switch the original binding ability through the metal coordination and the cooperative multiple recognition. Our particular interest is in exploration of the factors governing the molecular multiple recognition mechanism, especially of how metal coordination affects the binding behavior of bis(β-CD)s. This approach should serve to further our understanding of this developing but little investigated area in the field of supramolecular chemistry.



Results and Discussion

Synthesis: As illustrated in Scheme 1, the oxamidobisbenzoyl-linked bis(β-CD)s **2–5** were synthesized in moderate yields from oxamido-linked bis(benzoic acid)s and mono[6-aminoethyleneamino-6-deoxy]-β-CD or mono[6-diethylenetriamino-6-deoxy]-β-CD. Care should be taken to keep the mixture anhydrous and at a low temperature during these reactions, particularly at the initial stage, to achieve smooth and clean transformations without undesirable products. Further treatment of bis(β-CD)s **2–5** with Cu^{II} perchlorate gave their Cu^{II} complexes **6–9** in satisfactory yields (over 50%). The quantities of reactants used were consistent with the reaction stoichiometry, as determined by spectrophoto-



Scheme 1. Synthesis of CD derivatives.

metric titration. The compositions of the products were verified by elemental analysis.

Metal coordination behavior and stoichiometry: To investigate the coordination behavior of bis(β-CD)s in the presence of Cu^{II} ions, spectrophotometric titrations were performed at 25 °C in aqueous solution. A typical titration curve obtained by titration of bis(β-CD) **3** with Cu^{II} ions is shown in Figure 1. The absorbance intensity of bis(β-CD) **3** at 305 nm gradually decreases, while the absorbance intensities at 250 and 350 nm gradually increase with the addition of varying amounts of Cu^{II} perchlorate. In the control experiments, the UV/Vis spectrum of Cu^{II} ion displayed no appreciable change (within measurement concentration range) at 200–400 nm under comparable experimental conditions. These observations indicate that Cu^{II} ion coordinates to the bis(β-CD) to form a metallobridged bis(β-CD). Moreover, Job's experiments were also performed to explore the coordination stoichiometry of bis(β-CD)/Cu^{II} complexes in aqueous solution. The adsorption wavelengths used were 306 nm for **2**/Cu^{II}, 305 nm for **3**/Cu^{II}, 284 nm for **4**/Cu^{II}, and 286 nm for **5**/Cu^{II} systems. We had previously demonstrated that the coordination of bis(β-CD) **2** with Cu^{II} perchlorate has a 1:2 stoichiometry.[23] Here, this 1:2 stoichiometry was also observed in the case of the **3**/Cu^{II} complex, which indicates that one bis(β-CD) **3** moiety can bind two Cu^{II} ions. When,

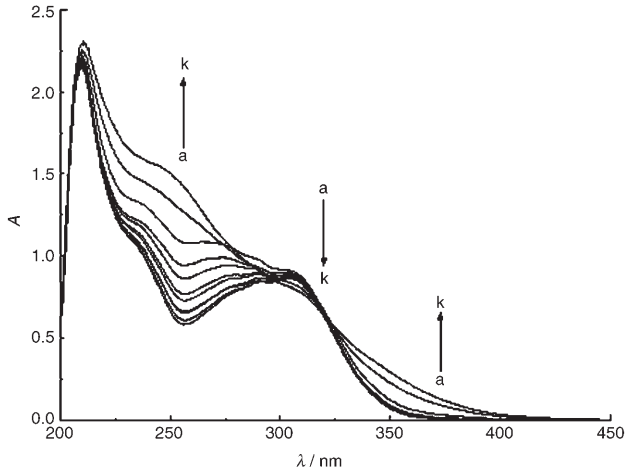


Figure 1. UV/Vis spectral changes of **3** upon addition of Cu^{II} perchlorate in aqueous solution ([**3**] = 8×10^{-5} mol dm^{-3}; [Cu^{II}] from a to k = 0, 0.082, 0.164, 0.205, 0.3075, 0.410, 0.615, 0.820, 1.025, 1.435, and 1.845×10^{-4} mol dm^{-3}).

however, the 2,2′-oxamidobis(benzoylamino) linkers in the bis(β-CD)s **2** and **3** were exchanged for 4,4′-oxamidobis(benzoylamino) linkers, the resultant bis(β-CD)s **4** and **5** each adopted a different coordination stoichiometry with Cu^{II} ion. The Job's plots for either **4**/Cu^{II} or **5**/Cu^{II} complexes each display a maximum at 0.4 corresponding to a 2:3 bis(β-CD)/Cu^{II} coordination stoichiometry, which indicates that—besides each bis(β-CD) unit associating with one Cu^{II} ion—two bis(β-CD) components also participate in the binding of the third Cu^{II} ion. These bis(β-CD)/Cu^{II} coordination stoichiometries were also verified by elemental analysis results.

Conformations of bis(β-CD)s and metallobridged bis(β-CD)s: It is well known that the inclusion of a chromophoric achiral guest/moiety in a chiral host such as a CD may produce induced circular dichroism (ICD) signals at wavelengths at which the guest chromophore has absorbance.[24] In a control experiment, an aqueous solution of an oxamido-linked bis(benzoic acid) gave neither a circular dichroism signal nor a rotatory signal, confirming that the oxamido-linked bisbenzoyl group is an achiral chromophore in aqueous solution. Therefore, in order to examine the conformations of hosts **2**–**9**, their circular dichroism spectra were measured at 25 °C in Tris-HCl buffer solution (pH 7.2). As can be seen from Figure 2a, bis(β-CD)s **2**–**5** have quite similar circular dichroism spectra in the absence of guest molecule, but the intensities of the ICD signals are clearly different, indicating different degrees of interactions between the aromatic linker and two chiral cavities in the bis(β-CD). The circular dichroism spectrum of **2** displays two positive Cotton effect peaks around 233 nm ($\Delta\varepsilon$ = 0.52 dm^{-3} mol^{-1} cm^{-1}) and 311 nm ($\Delta\varepsilon$ = 0.12 dm^{-3} mol^{-1} cm^{-1}), attributable to the ^1L_a and ^1L_b transitions, respectively, of the phenyl moieties in the linker group. As a higher-order homologue of **2**, bis(β-CD) **3** gives the strongest ICD signals, displaying a strong positive

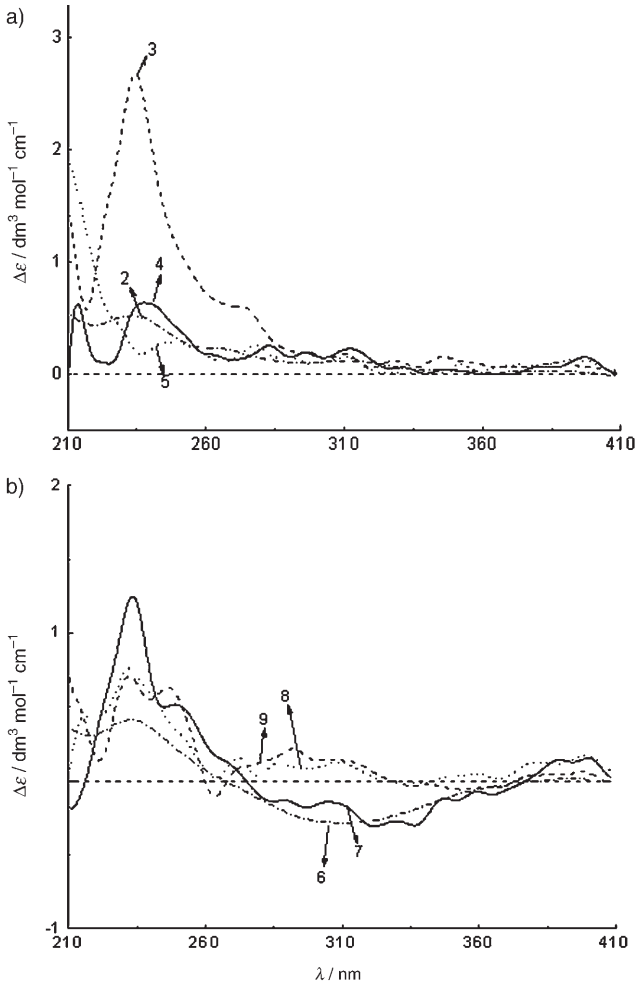


Figure 2. Circular dichroism spectra of hosts **2**–**9** (1.0×10^{-4} mol dm^{-3}) in aqueous buffer solution (pH 7.2) at 25 °C.

Cotton effect peak around 235 nm ($\Delta\varepsilon$ = 2.68 dm^{-3} mol^{-1} cm^{-1}) and a moderate positive Cotton effect peak around 272 nm ($\Delta\varepsilon$ = 0.60 dm^{-3} mol^{-1} cm^{-1}) for the ^1L_a and ^1L_b transitions, respectively. When the 2,2′-oxamidobis(benzoylamino) linker in bis(β-CD) **2** is exchanged for the 4,4′-oxamidobis(benzoylamino) linker, the resultant bis(β-CD) **4** displays ICD signals similar to but stronger than those of **2**, giving two positive Cotton effect peaks around 238 ($\Delta\varepsilon$ = 0.67 dm^{-3} mol^{-1} cm^{-1}) and 312 nm ($\Delta\varepsilon$ = 0.23 dm^{-3} mol^{-1} cm^{-1}) for the ^1L_a and ^1L_b transitions. Bis(β-CD) **5**, possessing the longer linker, displays relatively weak ICD signals, giving two positive Cotton effect peaks around 246 nm ($\Delta\varepsilon$ = 0.28 dm^{-3} mol^{-1} cm^{-1}) and 278 nm ($\Delta\varepsilon$ = 0.26 dm^{-3} mol^{-1} cm^{-1}) for the ^1L_a and ^1L_b transitions. From a generally accepted empirical rule[25–27] for the signs of ICD signals induced by CDs and from our previous report on the conformation of bis(β-CD) **2**,[23] we can deduce that the oxamidobisbenzoyl linkers in the bis(β-CD)s **2**, **4**, and **5** are only shallowly included in the β-CD cavities with an acclivous orientation to form self-included complexes, whilst that in **3** is deeply buried in the CD cavity.

Since self-inclusion of linkers often weakens the binding abilities of bis(β-CD)s toward guests, appropriate adjustment of the linker conformation is necessary in the design of functional bis(β-CD)s. In this work we tried to introduce metal centers into the linker groups of the bis(β-CD)s to alter their conformation and thus to abolish the disadvantageous self-inclusion. As can be seen in Figure 2b, the circular dichroism spectra of **6** or **7** each show both a positive and a negative Cotton effect peak, clearly different from the circular dichroism spectrum of **2** or **3**, each showing two positive Cotton effect peaks. This result indicates that, after the metal coordination, the linker group of **6** or **7** is now located outside the CD cavity, producing the opposite ICD signal to that of **2** or **3** around 310 nm. On the other hand, the circular dichroism spectra of **8** and **9** still resemble those of **4** and **5**, which indicates that **4** and **5** each retain their original conformation after the metal coordination.

2D NMR spectroscopy has become an essential method for the study of the conformations of CDs and their complexes, since it may be concluded that two protons are closely located in space—0.4 nm apart at most—if an NOE cross-peak is detectable between the relevant protons in the NOESY or ROESY spectrum. It is therefore possible to estimate the orientation of a linker group in a CD cavity with the aid of the assigned NOE correlations. If the linker group is self-included in the CD cavity, NOE correlations between the linker group protons and the H3/H5/H6 protons of the CD should be observed. Figure 3 illustrates a representative ROESY spectrum of **4** in a pH 7.2 buffer solution, showing three appreciable cross-peaks between the linker group protons and the CD H3/H5/H6 protons. As can be seen in Figure 3, the cross-peaks A and B are attributable to NOE correlations between the CD H3/H5/H6 protons and the H_a/H_b protons of the 4,4′-oxamidobisbenzoyl linker. These results unambiguously show that the linker group is embedded in the CD cavity, which is in excellent agreement with the results obtained in the circular dichroism experiments. Moreover, cross-peak C, attributable to NOE correlations between the ethyleneamino protons of the linker group, and the H5/H6 protons of CD are also observed. Since the H5/H6 protons are located near to the narrow opening of the CD cavity, while the H3 protons are near to the wide opening, we can conclude that the linker group of **4** is self-included in the CD cavity from the narrow opening, and a possible conformation of **4** based on these results is shown in Figure 4. ROESY spectra of other bis(β-CD)s (**2**, **3**, and **5**) were also measured under identical conditions, giving the same results as deduced from the ICD experiments. Unfortunately, the conformations of metallobridged bis(β-CD)s **6–9** could not be estimated by 2D NMR, due to the paramagnetic disturbance of Cu^{II}.

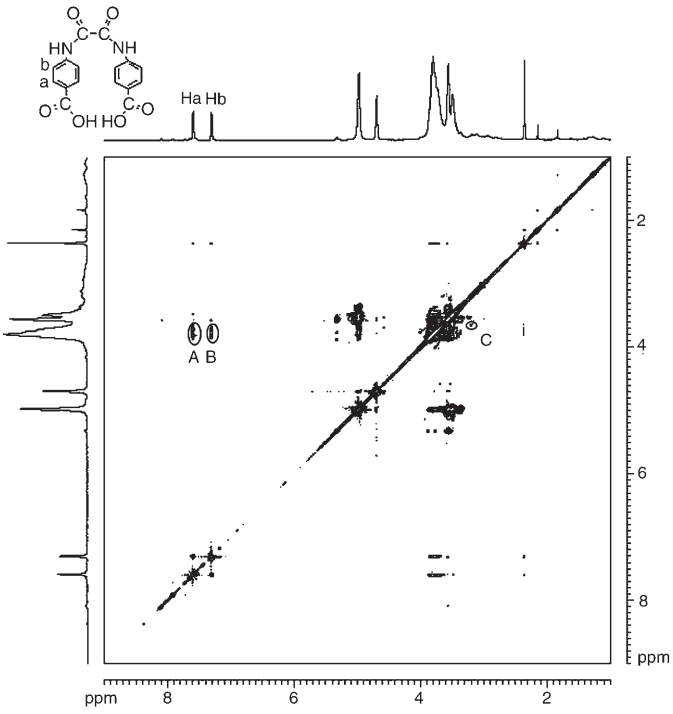


Figure 3. ^{1}H ROESY spectrum of **4** (2.6×10^{-3} mol dm^{-3}) in pH 7.2 buffer solution at 25 °C with a mixing time of 400 ms.

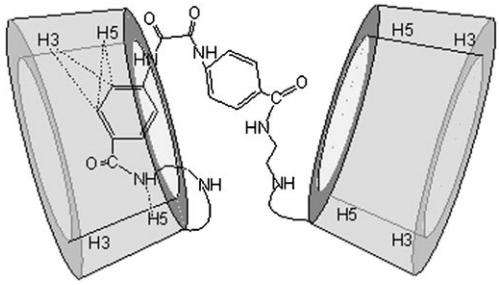


Figure 4. Possible conformation of **4**.

Spectral titration: Many investigations have demonstrated that fluorophore-attached CDs may undergo substantial conformational changes upon guest inclusion and thus give rise to relevant fluorescence changes. Moreover, the introduction of guest molecules certainly changes the microenvironmental hydrophobicity around the fluorophore, which also affects the fluorophore emission to some extent. In this work, the fluorescence intensities both of bis(β-CD)s and of metallobridged bis(β-CD)s gradually increased with the addition of bile salts (Figure 5). This property might allow their application as fluorescence sensors for the molecular recognition of bile salts.

The stoichiometries for the inclusion complexation of hosts **2–9** with representative guests were determined by Job's experiments by fluorescence spectroscopy. The emission wavelengths used were 408 nm for **2**/bile salt, 402 nm for **3**/bile salt, 417 nm for **4**/bile salt, 415 nm for **5**/bile salt, 407 nm for **6**/bile salt, 401 nm for **7**/bile salt, 414 nm for **8**/bile salt, and 411 nm for **9**/bile salt systems. Within the examined concentration range, each of the Job's plots for the inclusion complexation of bis(β-CD)s **2–5** with bile salts shows a maximum at a bis(β-CD) molar fraction of 0.5, con-

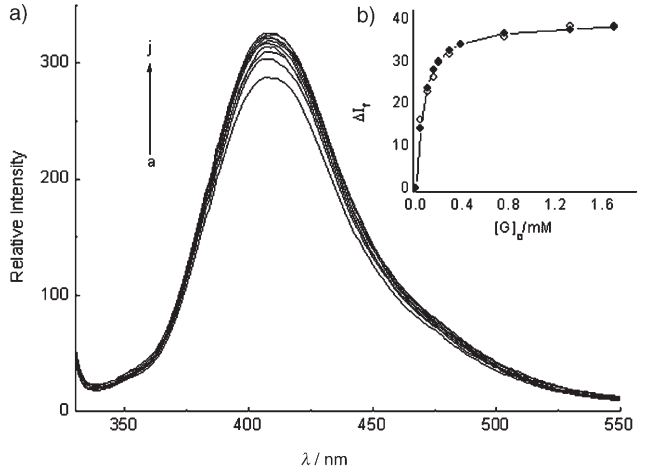


Figure 5. a) Fluorescence spectral changes of bis(β-CD) **2** (1.91×10^{-5} mol dm^{-3}) upon addition of CA (from a to j = 0, 0.0378, 0.0945, 0.1512, 0.189, 0.2835, 0.378, 0.756, 1.323, and 1.70×10^{-3} mol dm^{-3}) in Tris-HCl buffer solution (pH 7.2) at 25 °C (λ_{ex} = 315 nm). b) Nonlinear least-squares analysis of the differential intensity (ΔF) to calculate the complex formation constant (K_S).

firming the 1:1 binding stoichiometry between host and guest (Figure 6a). For the inclusion complexation of metallobridged bis(β-CD)s **6**–**9** with bile salts, however, each of the Job's plots shows a maximum at a bis(β-CD) unit molar fraction of 0.33 (Figure 6b), which indicates a 1:2 stoichiometry between each bis(β-CD) unit and guest. The metallobridged bis(β-CD) **6** or **7**, each possessing one bis(β-CD) unit, may only bind two bile salts to form a stoichiometric 1:2 inclusion complex. On the other hand, the metallobridged bis(β-CD) **8** or **9**, each possessing two bis(β-CD) units, each of which can form a stoichiometric 1:2 inclusion complex with two guest molecules, may adopt a intramolecular 2:4 stoichiometry upon inclusion complexation with bile salts.

With the 1:1 host/guest stoichiometry, the complexation of bile salts (guest) with bis(β-CD)s (host) can be expressed by Equation (1).

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

The stability constant (K_S) for the host–guest inclusion complexation can be obtained from analysis of sequential changes of fluorescence intensity (ΔF) at various guest concentrations, by use of a nonlinear least-squares curve-fitting method.[14c,22c] For each host examined, the plot of ΔF as a function of $[G]_0$ gives an excellent fit, verifying the validity of the 1:1 inclusion complexation stoichiometry. Figure 5 (insert) shows a typical curve-fitting plot for the fluorescence titration of CA with bis(β-CD) **2**. We find that there are no serious differences between the experimentally measured and the calculated data. When repeated measurements are made, the K_S values are reproducible within error limits of 5%. The K_S values obtained are listed in Table 1, along with the free energy changes of complex formation ($-\Delta G°$).

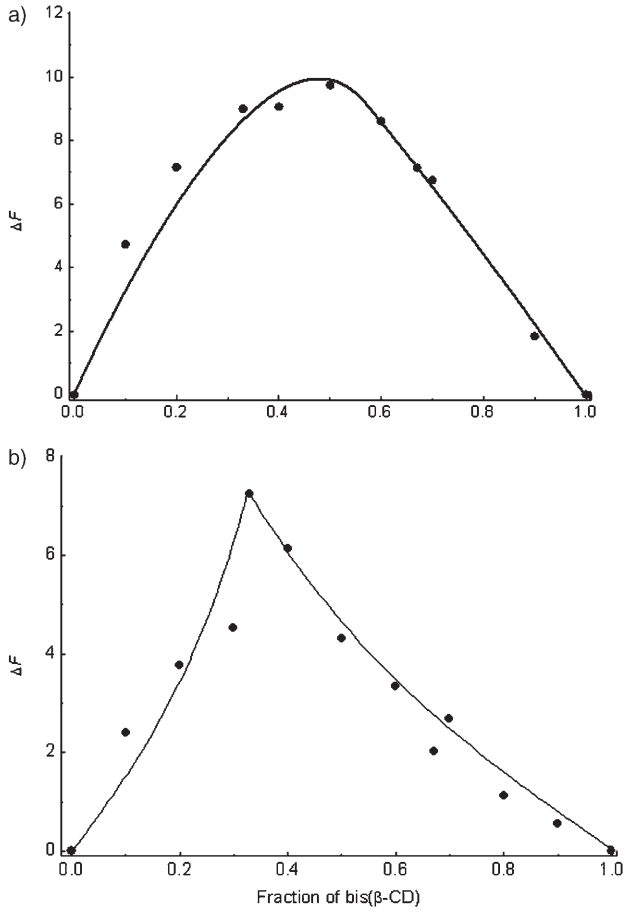


Figure 6. a) Job's plot of **4**/CA system at 417 nm ([**4**] + [CA] = 1.0×10^{-4} mol dm^{-3}). b) Job's plot of **9**/CA system at 411 nm ([bis(β-CD) unit] + [CA] = 1.5×10^{-5} mol dm^{-3}).

Table 1. Stability constants (K_S) and Gibbs free energy changes ($-\Delta G°$) for the inclusion complexation of bis(β-CD)s **2**–**5** with bile salts in Tris-HCl buffer solution (pH 7.2) at 25 °C.

Host	Guest	K_S [M^{-1}]	log K_S	$-\Delta G°$[kJ mol^{-1}]
2	CA	18500	4.27	24.34
	DCA	12200	4.09	23.31
3	CA	8130	3.91	22.31
	DCA	[a]	–	–
4	CA	11900	4.08	23.25
	DCA	11500	4.06	23.17
5	CA	8820	3.95	22.51
	DCA	1870	3.27	18.67

[a] The guest-induced variations in the fluorescence intensities are too small for the K_S value to be determined.

By treating one bis(β-CD) moiety in **6**–**9** as a host unit, the stoichiometric 1:2 inclusion complexation of two bile salts (G) with a host unit (H) can be expressed by Equation (2), and the complex stability constant (K_S) is given by Equation (3), where [H], [G], and [H·2G] represent the equilibrium concentrations of host unit, guest, and complex, respectively.

$$H + 2G \overset{K_S}{\rightleftharpoons} H \cdot 2G \quad (2)$$

$$K_S = [H \cdot 2G]/[H][G]^2 \quad (3)$$

The fluorescence intensity (F) is proportional to the concentration of fluorophore (c) in dilute solution [Eq. (4)].

$$F = \varepsilon_F c \quad (4)$$

From Equation (4) we can obtain Equations (5) and (6). (In this case the guest is nonfluorescent), where $[H]_0$ signifies the initial concentration of host unit and ε_F and ε_F' represent the molar fluorescence intensities of free host unit and inclusion complex.

$$F_0 = \varepsilon_F [H]_0 \quad (5)$$

$$F = \varepsilon_F[H] + \varepsilon_F'[H \cdot 2G] = \varepsilon_F([H]_0 - [H \cdot 2G]) + \varepsilon_F'[H \cdot 2G] = \varepsilon_F[H]_0 + (\varepsilon_F' - \varepsilon_F)[H \cdot 2G] \quad (6)$$

By subtracting Equation (5) from Equation (6), we obtain Equation (7), where ΔF and $\Delta\varepsilon_F$ denote the changes in the fluorescence intensity and molar fluorescence intensity of host unit upon complexation with guest molecules.

$$\Delta F = F - F_0 = (\varepsilon_F - \varepsilon_F')[H \cdot 2G] = \Delta\varepsilon_F[H \cdot 2G] \quad (7)$$

We also have Equations (8) and (9).

$$[H] = [H]_0 - [H \cdot 2G] \quad (8)$$

$$[G] = [G]_0 - 2[H \cdot 2G] \quad (9)$$

By combining Equations (3), (7), (8), and (9), and by neglecting the terms $[H{\cdot}2G]^2$ and $[H{\cdot}2G]^3$ in a similar way to Tamaki[28] and Bender[29] for the spectral changes on the complex formation, we can derive Equation (10).

$$\frac{[H]_0[G]_0^2}{\Delta F} = \frac{1}{\Delta\varepsilon_F K_S} + \frac{[G]_0([G]_0 + 4[H]_0)}{\Delta\varepsilon_F} \quad (10)$$

The complex stability constant (K_S) for each host–guest combination can be determined from the approximate linear plot of $[H]_0[G]_0^2/\Delta F$ against $[G]_0([G]_0 + 4[H]_0)$. Figure 7 illustrates typical fluorescence titration curves for the inclusion complexation of metallobridged bis(β-CD) **7** with CA and the curve-fitting result. The complex stability constants (K_S) and the free energy changes calculated from the slope and intercept are listed in Table 2. When repeated measurements were made the K_S values were reproducible within error limits of 5%.

Binding mode: As can be seen from the compound structures above, the bile salts CA and DCA have similar frameworks, each containing four rings (A–D) and a carboxylate side chain, but with a small difference in the C-7 substituent (R): a hydroxy group in CA and a hydrogen atom in DCA. Upon inclusion complexation with a CD, the A-ring or the carboxylate group of a bile salt can enter the CD cavity from either the narrow or the wide opening of the CD, which should result in dramatically different binding behavior between host and guest. It is therefore very important to investigate the binding modes between these bis(β-CD)s or metallobridged bis(β-CD)s and bile salts for elucidation of the mechanism of molecular recognition. Figure 8 and Figure 9 illustrate some typical ^{1}H ROESY spectra for inclusion complexation of bis(β-CD)s with bile salts. The notations used are H*n* for CD protons and *Pn* for bile salt protons, where *n* is the carbon number indicated in the compound structures above. In the control experiments the ROESY spectra of bile salts show the intramolecular NOE correlations between the bile salt protons, which should help us easily recognize the NOE correlations between host and guest from the ROESY spectra of bis(β-CD)/bile salt complexes.[22b] As can be seen in Figure 8, the ^{1}H ROESY spectrum for the resulting complex of **3**/CA in pH 7.2 buffer solution displays complicated NOE cross-peaks, which come not only from the intermolecular correlations between β-CD and the bile salt, but also from the intramolecular correlations of **3** or CA. Among them, peak A corresponds to the NOE correlations between the ethyleneamino protons of the linker group and the CD's H5/H6 protons, and peak C

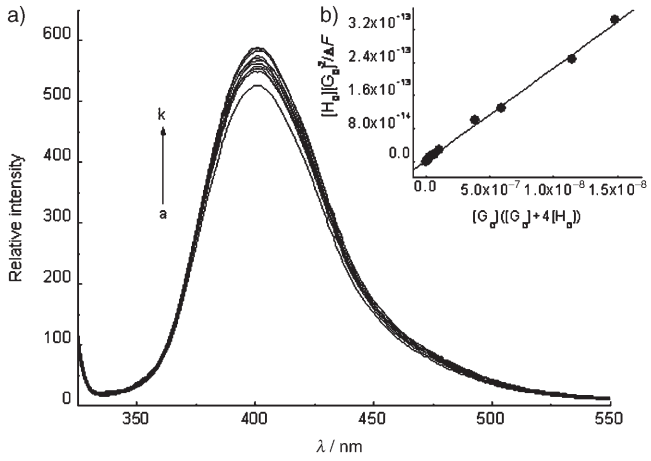


Figure 7. a) Fluorescence spectral changes of host **7** (1.36×10^{-5} mol dm^{-3}) upon addition of CA (from a to k = 0, 0.0298, 0.0745, 0.1192, 0.149, 0.2235, 0.298, 0.596, 0.745, 1.043, 1.192×10^{-3} mol dm^{-3}) in Tris-HCl buffer solution (pH 7.2) at 25 °C (λ_{ex} = 315 nm). b) Linear least-squares analysis plot to calculate the complex formation constant (K_S).

Table 2. Complex formation constant (K_S) and Gibbs free energy change ($-\Delta G°$) for the inclusion complexation of metallobridged bis(β-CD)s **6–9** with bile salts in Tris-HCl buffer solution (pH 7.2) at 25 °C.

Host	Guest	K_S [M^{-2}]	log K_S	$-\Delta G°$ [kJ mol^{-1}]
6	CA	5.73×10^7	7.76	44.26
	DCA	2.03×10^7	7.31	41.70
7	CA	9.93×10^7	8.00	45.62
	DCA	3.47×10^7	7.54	43.02
8	CA	3.96×10^7	7.60	43.35
	DCA	3.78×10^7	7.58	43.23
9	CA	2.95×10^7	7.47	42.62
	DCA	6.2×10^6	6.79	38.76

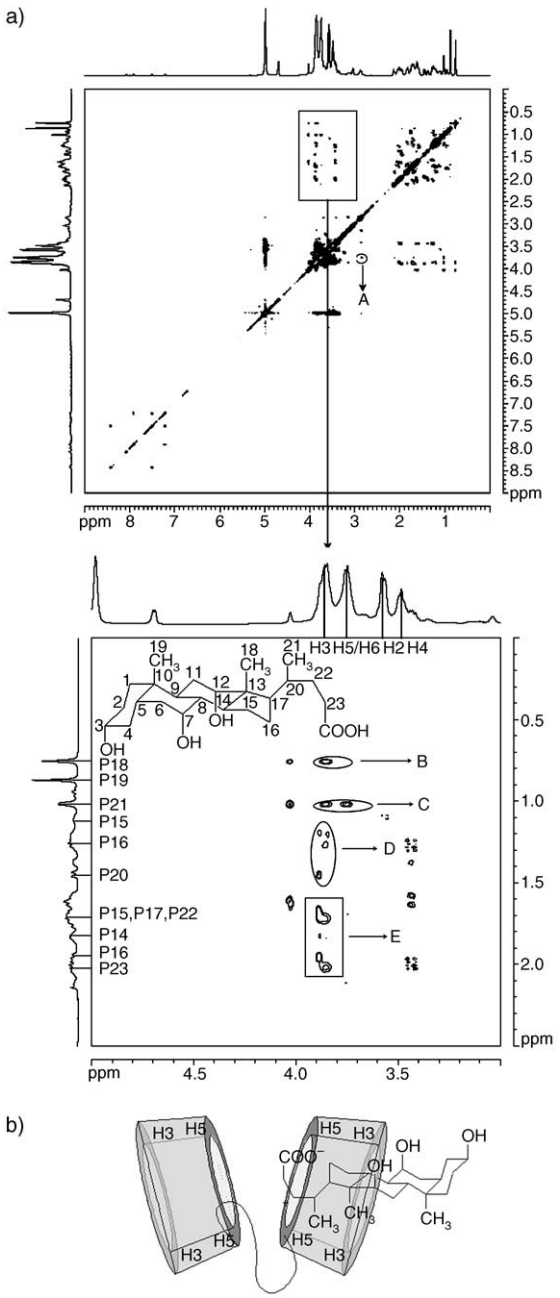


Figure 8. a) ROESY spectrum of the **3**/CA complex with a mixing time of 200 ms at 298 K. b) Possible binding mode of **3** with CA.

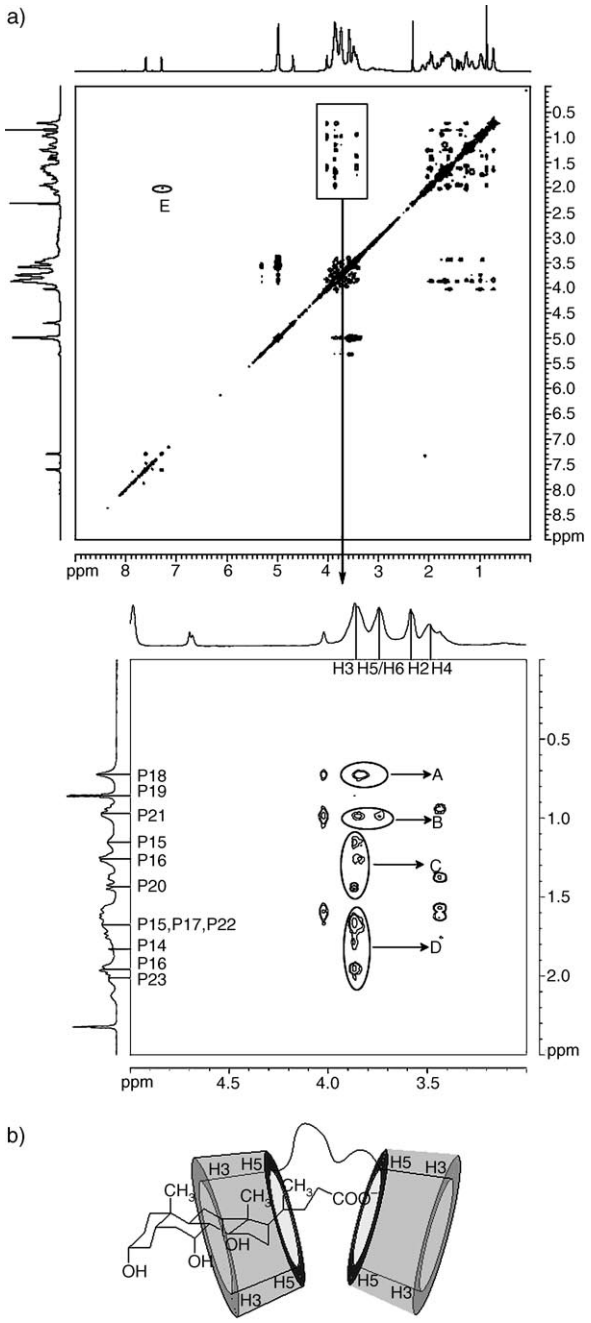


Figure 9. a) ROESY spectrum of the **4**/CA complex with a mixing time of 200 ms at 298 K. b) Possible binding mode of **4** with CA.

corresponds to the NOE correlations between the CA's P21 protons and the CD's H3/H5/H6 protons. Meanwhile, cross-peaks B, D, and E correspond to the NOE correlations between the protons in the D-ring of CA and the CD's H3 protons. These NOE correlations, along with the 1:1 binding stoichiometry, jointly indicate a "host–linker–guest" binding mode[22a,c] between **3** and CA. That is, upon inclusion complexation with bis(β-CD), the carboxylate tail and the D-ring of CA enter into one CD cavity of **3** from the wide opening, while the linker group of **3** is partially self-included in the other CD cavity, as illustrated in Figure 8b. There is an inherent advantage for this binding mode. Under our experimental conditions, the carboxylate group of CA is not protonated and should exist as a carboxylate anion, while the −NH− fragments in the linker group of bis(β-CD) should be partly protonated. Therefore, the electrostatic interactions between the protonated amino groups ($-NH_2^+-$) in the linker and the anionic carboxylate tail of CA may to some extent favor the inclusion complexation of **3** with CA. A similar binding mode is also observed for the inclusion complexation of **3** with DCA by analysis of the ROESY spectrum of the **3**/DCA complex.

With a shallowly self-included conformation, bis(β-CD)s **2**, **4**, and **5** show a binding mode different from that of **3**—which possesses a deeply self-included conformation—upon inclusion complexation with bile salts. In the ^{1}H ROESY spectrum of the **4**/CA complex (Figure 9a), peaks A, C, and D correspond to the NOE correlations between the D-ring protons of CA and the CD's H3 protons, whilst peak B corresponds to NOE correlations between the CA's P21 protons and the CD's H3/H5/H6 protons. Moreover, a clear cross-peak (peak E) corresponding to NOE correlation between the CA's P23 protons and the aromatic protons in the linker group of **4** is observed, while the NOE correlations between the ethyleneamino protons of the linker group and the CD's H5/H6 protons become very weak. From these ROESY data and the 1:1 binding stoichiometry of **4** with CA, we can deduce a possible binding mode of **4**/CA complex as illustrated in Figure 9b. That is, the carboxylate tail and D-ring of CA enter the CD cavity from the wide opening, and the carboxylate tail is located close to the linker group. On the other hand, the linker group is mostly moved out from the CD cavity after complexation with CA. Close examination of the ROESY spectra of **2**/CA, **2**/DCA, **4**/DCA, **5**/CA, and **5**/DCA complexes consistently demonstrates a similar binding mode to the **4**/CA complex.

Although the paramagnetic disturbance caused by the coordinated Cu^{II} ions makes it impossible to measure the 2D NMR spectra of metallobridged bis(β-CD)s, we can deduce their binding modes through their structures and binding stoichiometries. We have demonstrated that, for the inclusion complexation of bile salts with bis(β-CD)s **2**–**5**, the carboxylate tails of bile salts enter the CD cavity through the wide opening, due to the electrostatic attraction from the protonated amino groups ($-NH_2^+-$) in the linker. In the cases of the metallobridged bis(β-CD)s **6**–**9**, the strong electrostatic attraction from the coordinated Cu^{II} ions in the linker group may also favor the penetration of the carboxylate tail of bile salt into the CD cavity through the wide opening. Moreover, the 1:2 or 2:4 binding stoichiometry indicates that each CD cavity of a metallobridged bis(β-CD) is occupied by a bile salt, so we can deduce the possible binding modes of **6**–**9** as illustrated in Figure 10.

We have demonstrated that, upon inclusion complexation with bile salts, bis(β-CD)s **2**–**5** adopt "host–linker–guest" binding modes, while the metallobridged bis(β-CD)s **6**–**9** adopt either the 1:2 or the 2:4 binding modes. These results

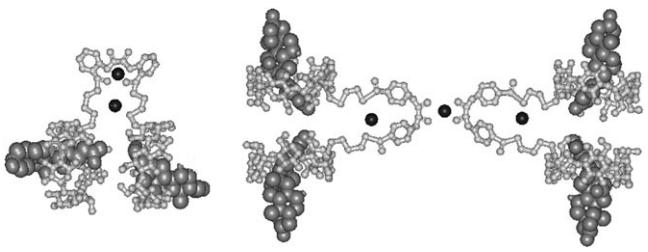


Figure 10. Possible binding modes of metallobridged bis(β-CD)s **6**–**9** with bile salts.

may consequently explain the enhanced fluorescence of bis(β-CD)s induced by the guest. That is, in these binding modes, the bile salt is located near to the oxamidobisbenzoyl linker, so the fluorophore in the linker may be efficiently protected from the deactivating water attack by steric shielding by the closely located bulk bile salt guests. In addition, the introduction of a hydrophobic bile salt guest should certainly increase the microenviromental hydrophobicity around the fluorophore, which should also contribute to the enhanced fluorescence of the bis(β-CD)s.

Binding ability: Many investigations have demonstrated that several weak noncovalent forces, including van der Waals forces, hydrophobic interactions, hydrogen bonding, and dipole–dipole interactions, contribute cooperatively to inclusion complexation by CDs, and a good match of the size and shape of the host to those of the guest should greatly favor host–guest inclusion complexation, since the strength of these interactions is closely related to the distance and contact surface area between host and guest. Native and modified monomeric CDs display relatively limited binding ability towards guest molecules, probably because of weak hydrophobic interactions. However, bis(β-CD)s and metallobridged bis(β-CD)s have greatly enhanced binding abilities in relation to the parent CDs, owing to a multiple recognition mechanism. As can be seen in Table 1, the K_S values for the inclusion complexation of CA and DCA with bis(β-CD)s **2**–**5** are in the range of 10^3 to $10^4\,M^{-1}$, higher than the K_S values (10^1 to $10^3\,M^{-1}$) reported for the inclusion complexation of these bile salts with native or monomodified β-CDs.[12,22b] These enhanced binding abilities highlight the inherent advantage of the cooperative "host–linker–guest" binding mode of bis(β-CD)s **2**–**5**. In addition to the association of the CD cavity with a guest molecule, the linker group provides some additional binding interactions towards the accommodated guest. These factors jointly contribute to the stronger binding abilities achieved by bis(β-CD)s in relation to monomeric CDs.

It is also interesting to compare the "host selectivity" sequence for each bile salt. The order of K_S values for the inclusion complexation of bis(β-CD)s **2**–**5** with each bile salt is **2** > **4** > **5** > **3**. That is, the bile salts CA and DCA are better bound by bis(β-CD) **2**, which possesses the shortest linker group, than by the long-linked bis(β-CD)s. This may be attributable to the strict size-fit between these bile salts and the short-linked bis(β-CD) **2**, which consequently gives strong van der Waals and hydrophobic interactions between host and guest.

Significantly, metallobridged bis(β-CD)s **6**–**9** show greatly enhanced binding abilities with regard to the bis(β-CD)s **2**–**5**. As can be seen in Table 2, the Gibbs free energy changes ($-\Delta G°$) for the inclusion complexation of **6**–**9** with bile salts (38.76–45.62 kJ mol^{-1}) are almost twice as high as those for the inclusion complexation of **2**–**5** (18.67–24.34 kJ mol^{-1}) and are even higher than our previously reported values (23.5–35.6 kJ mol^{-1}) for metallo biquinolino-bridged bis(β-CD)s.[22c] These significant enhancements in the binding abil-

ities may be attributable to a more complicated multiple recognition mechanism involving the cooperative binding of several CD cavities, conformation adjustment by the metal coordination, and additional binding interactions between the metal-coordinated linker group and the accommodated guest molecules. Upon inclusion complexation with guest molecules, each metal-coordinated bis(β-CD) unit associates with two bile salts, and the coordinated Cu^{II} ions enter into electrostatic interactions with the anionic carboxylate tails of the accommodated bile salts. In addition, the coordination of Cu^{II} ions shortens the effective length of linker group to some extent and thus improves the size relationship between the host and the guest. As a cumulative result of these factors, metallobridged bis(β-CD)s **6–9** show much high binding abilities than monomeric and dimeric CDs.

The importance of the multiple recognition mechanism is more clearly demonstrated by comparing the binding abilities of these bis(β-CD)s and metallobridged bis(β-CD)s for CA and DCA. As can be seen in Table 1 and Table 2, all of the hosts examined (except for **3**) display higher binding abilities for CA than for DCA. One possible reason for the stronger affinities for CA may involve hydrogen bond interactions between the 7-hydroxy group of CA and the 2- and 3-hydroxy groups of CD. We have demonstrated that the carboxylate tail and the D-ring of CA enter into the CD cavity through the wide opening. In this binding mode, the 7-hydroxy group of CA is located outside the CD cavity and near to the wide opening of CD, and so can easily interact with the 2- and 3-hydroxy groups of CD through hydrogen bond interactions, which subsequently strengthen the host–guest association.

Conclusion

In summary, we have successfully synthesized a series of bis(β-CD)s bearing fluorescent oxamidobisbenzoyl linkers and their Cu^{II} complexes as fluorescence sensors for the molecular recognition of bile salts. Significantly, the introduction of metal ions into bis(β-CD)s can not only alter the original conformations of the bis(β-CD)s but can also affect the binding behavior of bis(β-CD)s and even change their binding stoichiometries. These results provide a convenient and powerful method for controlling the binding behavior of dimeric receptors in aqueous solution, which should be useful for the design and synthesis of new supramolecular systems and further improve understanding of molecular multiple recognition mechanism in supramolecular systems.

Experimental Section

Materials: All chemicals were reagent grade and were used without further purification unless noted otherwise. β-CD of reagent grade 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 two days and was then distilled under reduced pressure prior to use. Mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD (6-OTs-β-CD) was prepared by treatment of *p*-toluenesulfonyl chloride with β-CD in alkaline aqueous solution.[30] 6-OTs-β-CD was then converted into mono(6-aminoethylamino-6-deoxy)-β-CD or mono(6-diethylenetriamino-6-deoxy)-β-CD in ca. 70% yield upon heating in excess ethylenediamine or diethylenetriamine at 70 °C for 7 h.[31,32] Sodium cholate (CA) and deoxycholate (DCA) were purchased from Acros and were used as received. 6,6′-[2,2′-Oxamidobis-(benzoylaminoethyleneamino)]-6,6′-deoxy-bis(β-CD) (**2**) and its Cu^{II} complex (**6**) were prepared by our recently reported procedures.[23] A Tris-HCl buffer solution (pH 7.2) was used as solvent throughout the spectral measurements.

Apparatus: Elemental analyses were performed on a Perkin–Elmer-2400C instrument. NMR spectra were recorded on a Varian Mercury VX300 instrument. Circular dichroism and UV/Vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a JASCO J-715S spectropolarimeter and a Shimadzu UV-2401PC spectrophotometer fitted with a PTC-348WI temperature controller to keep the temperature at 25 °C, respectively. Fluorescence spectra were measured in a conventional quartz cell (10×10×45 mm) at 25 °C on a JASCO FP-750 spectrometer with a constant-temperature water bath, with excitation and emission slits of 10 nm.

6,6′-[2,2′-Oxamidobis(benzoylaminodiethylenediamino)]- 6,6′-deoxy-bis-(β-CD) (3): Mono(6-diethylenetriamino-6-deoxy)-β-CD (1 mmol) was dissolved in DMF (30 mL) in the presence of a small amount of 4 Å molecular sieves, and DCC (1 mmol) and 2,2′-oxamidobis(benzoic acid)[33] (0.5 mmol) were then added. The mixture was stirred for two days in an ice bath and for another two days at room temperature, and was then allowed to stand for 5 h 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 minimal amount of hot water and was then poured into acetone (150 mL), and the precipitate formed was collected by filtration. This procedure was repeated twice. The crude product obtained was purified on a column of Sephadex G-25 with water as eluent. After drying in vacuo, a pure sample of **3** was obtained in 27% yield. ^{1}H NMR (300 MHz, D_2O, TMS): δ = 2.63–2.93 (m, 16H), 3.25–3.83 (m, 84H), 4.86 (m, 14H), 7.10–7.16(m, 2H), 7.34–7.42 (m, 2H), 7.81–7.84 (m, 2H), 8.38–8.42 ppm (m, 2H); UV/Vis (water): λ_{max} (ε) = 305 nm (10470 $M^{-1}cm^{-1}$); elemental analysis (%) calcd for $C_{108}H_{170}O_{72}N_8 \cdot 19H_2O$: C 42.19, H 6.82, N 3.64; found: C 42.29, H 6.85, N 3.19.

6,6′-[4,4′-Oxamidobis(benzoylaminoethyleneamino)]-6,6′-deoxy-bis(β-CD) (4): Compound **4** was prepared in 25% yield from 4,4′-oxamidobis-(benzoic acid)[33] and mono(6-aminoethylamino-6-deoxy)-β-CD by a procedure similar to that employed in the synthesis of **3**. ^{1}H NMR (300 MHz, D_2O, TMS): δ = 2.55–3.22 (m, 8H), 3.41–3.74 (m, 84H), 4.89 (s, 14H), 7.21–7.23 (m, Ar 4H), 7.50–7.53 ppm (m, Ar 4H); UV/Vis (water): λ_{max} (ε) = 284 nm (8130 $M^{-1}cm^{-1}$); elemental analysis (%) calcd for $C_{104}H_{160}O_{72}N_6 \cdot 16H_2O$: C 42.57, H 6.59, N 2.86; found: C 42.58, H 6.64, N 2.87.

6,6′-[4,4′-Oxamidobis(benzoylaminodiethylenediamino)]-6,6′-deoxy-bis(β-CD) (5): Compound **5** was prepared in 20% yield from 4,4′-oxamidobis-(benzoic acid)[33] and mono(6-diethylenetriamino-6-deoxy)-β-CD by a procedure similar to that employed in the synthesis of **3**. ^{1}H NMR (300 MHz, D_2O, TMS): δ = 2.69–2.87 (m, 16H), 3.34–3.74 (m, 84H), 4.85 (s, 14H), 7.56 (m, Ar 4H), 7.74–7.77 ppm (m, Ar 4H); UV/Vis (water): $\lambda_{max}(\varepsilon)$ = 286 nm (7240 $M^{-1}cm^{-1}$); elemental analysis (%) calcd for $C_{108}H_{170}O_{72}N_8 \cdot 10H_2O$: C 44.54, H 6.58, N 3.85; found: C 44.38, H 6.47, N 4.07.

Bis(β-CD)-Cu^{II} complex 7: Bis(β-CD) **3** was added dropwise to a dilute aqueous solution of a slight excess of Cu^{II} perchlorate in an ice/water bath. Several drops of chloroform were further added, and the resultant solution was kept at 5 °C for 2 days. The precipitate formed was collected by centrifugation, washed successively with a small amount of ethanol and diethyl ether, and then dried in vacuo to give complex **7** in 68% yield as a blue solid. UV/Vis (water): λ_{max} (ε) = 310 nm (6170 $M^{-1}cm^{-1}$); elemental analysis (%) calcd for $C_{108}H_{170}O_{72}N_8 \cdot 2Cu(ClO_4)_2 \cdot 24H_2O$: C 35.16, H 5.96, N 3.04; found: C 35.14, H 5.64, N 2.74.

Bis(β-CD)-Cu^{II} Complex 8: Bis(β-CD)-Cu^{II} complex **8** was prepared in 58% yield as a green solid by a procedure similar to that used in the syn-

thesis of **7**. UV/Vis (water): λ_{max} (ε) = 279 nm (8510 $M^{-1}cm^{-1}$); elemental analysis (%) calcd for $C_{104}H_{160}O_{72}N_6 \cdot 1.5\,Cu(ClO_4)_2 \cdot 14\,H_2O$: C 36.87, H 5.59, N 2.48; found: C 36.59, H 5.32, N 2.41.

Bis(β-CD)-Cu^{II} complex 9: Bis(β-CD)-Cu^{II} complex **9** was prepared in 50% yield as a green solid by a procedure similar to that used in the synthesis of **7**. UV/Vis (water): λ_{max} (ε) = 280 nm (5500 $M^{-1}cm^{-1}$); elemental analysis (%) calcd for $C_{108}H_{170}O_{72}N_8 \cdot 1.5\,Cu(ClO_4)_2 \cdot 12\,H_2O$: C 38.81, H 5.85, N 3.35; found: C 38.45, H 5.83, N 3.41.

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[1] a) S. Monti, S. Sortino, *Chem. Soc. Rev.* **2002**, *31*, 287–300; b) W. Saenger, J. Jacob, K. Gessler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S. M. Smith, T. Takaha, *Chem. Rev.* **1998**, *98*, 1787–1802.
[2] a) J. Szejtli in *Cyclodextrins and Their Inclusion Complexes*, Akademia Kiado, Budapest, **1982**; b) J. Szejtli in *Cyclodextrin Technology*, Kluwer-Academic, Dordrecht, **1988**.
[3] a) F. Venema, H. F. M. Nelissen, P. Berthault, A. E. R. Birlirakis, M. C. Feiters, R. J. M. Nolte, *Chem. Eur. J.* **1998**, *4*, 2237–2250; b) H. F. M. Nelissen, M. C. Feiters, R. J. M. Nolte, *J. Org. Chem.* **2002**, *67*, 5901–5906.
[4] G. Wenz, *Angew. Chem.* **1994**, *106*, 851–870; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 803–822.
[5] J. Szejtli, T. Osa in *Comprehensive Supramolecular Chemistry, Vol. 3* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle), Elsevier, Oxford, U. K., **1996**.
[6] A. P. Croft, R. A. Bartsch, *Tetrahedron* **1983**, *39*, 1417–1474.
[7] A. R. Khan, P. Forgo, K. J. Stine, V. T. D'Souza, *Chem. Rev.* **1998**, *98*, 1977–1996.
[8] R. Breslow, S. Chung, *J. Am. Chem. Soc.* **1990**, *112*, 9659–9660.
[9] B. Zhang, R. Breslow, *J. Am. Chem. Soc.* **1993**, *115*, 9353–9354.
[10] W.-Q. Tong, J. L. Lach, T. F. Chin, J. K. Guillory, *J. Pharm. Biomed. Anal.* **1991**, *9*, 1139–1146.
[11] Y. Inoue, Y. Liu, L.-H. Tong, B.-J. Shen, D.-S. Jin, *J. Am. Chem. Soc.* **1993**, *115*, 10637–10644.
[12] H. Ikeda, M. Nakamura, N. Ise, N. Oguma, A. Nakamura, T. Ikeda, F. Toda, A. Ueno, *J. Am. Chem. Soc.* **1996**, *118*, 10980–10988.
[13] A. Matsushita, T. Kuwabara, A. Nakamura, H. Ikeda, A. Ueno, *J. Chem. Soc. Perkin Trans. 2* **1997**, 1705–1710.
[14] a) Y. Liu, C.-C. You, T. Wada, Y. Inoue, *Tetrahedron Lett.* **2000**, *41*, 6869–6873; b) Y. Liu, C.-C. You, B. Li, *Chem. Eur. J.* **2001**, *7*, 1281–1288; c) Y. Liu, Y. Chen, L. Li, H.-Y. Zhang, S.-X. Liu, X.-D. Guan, *J. Org. Chem.* **2001**, *66*, 8518–8627; d) Y. Liu, L. Li, H.-Y. Zhang, Y. Song, *J. Org. Chem.* **2003**, *68*, 527–536.
[15] a) E. Rizzarelli, G. Vecchio, *Coord. Chem. Rev.* **1999**, *188*, 343–364; b) B. Zhang, R. Breslow, *J. Am. Chem. Soc.* **1997**, *119*, 1676–1681.
[16] H. F. M. Nelissen, A. F. J. Schut, F. Venema, M. C. Feiters, R. J. M. Nolte, *Chem. Commun.* **2000**, 577–578.
[17] K. D. R. Setchell, J . *M. Street, J. Sjovall in The Bile Acids*, Vol. *4* (Eds.: K. D. R. Setchell, D. Kritcheveky, P. P. Nair), Plenum Press, New York, London, **1988**, Chapter 12, pp. 543.
[18] Y. H. Choi, C. H. Yang, H. W. Kim, S. Jung, *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *39*, 71–76.
[19] a) P. Ramos Cabrer, E. Alvarez-Parrilla, F. Meijide, J. A. Seijas, E. Rodriguez Nunez, J. Vazquez Tato, *Langmuir* **1985**, *1*, 5489–5495; b) A. P. Singh, P. Ramos Cabrer, E. Alvarez-Parrilla, F. Meijide, J. Vazquez Tato, *J. Inclusion Phenom. Macrocyclic Chem.* **1999**, *35*, 335–348; c) P. Ramos Cabrer, E. Alvarez-Parrilla, W. Al-Soufi, F. Meijide, E. Rodriguez Nunez, J. Vazquez Tato, *Supramol. Chem.* **2003**, *15*, 33–43.
[20] F. Cramer, W. Saenger, H.-Ch. Spatz, *J. Am. Chem. Soc.* **1967**, *89*, 14–20.
[21] M. R. de Jong, J. F. J. Engbersen, J. Huskens, D. N. Reinhoudt, *Chem. Eur. J.* **2000**, *6*, 4034–4040.
[22] a) Y. Liu, Y. Song, H. Wang, H.-Y. Zhang, T. Wada, Y. Inoue, *J. Org. Chem.* **2003**, *68*, 3687–3690; b) Y. Liu, Y.-W. Yang, E.-C. Yang, X.-D. Gaun, *J. Org. Chem.* **2004**, *69*, 6590–6602; c) Y. Liu, Y. Song, Y. Chen, X.-Q. Li, F. Ding, R.-Q. Zhong, *Chem. Eur. J.* **2004**, *10*, 3685–3696; d) Y. Liu, L. Li, Y. Chen, L. Yu, Z. Fan, F. Ding, *J. Phys. Chem. B* **2005**, *109*, 4129–4134.
[23] Y. Liu, Y. -L. Zhao, Y. Chen, F. Ding, G.-S. Chen, *Bioconjugate Chem.* **2004**, *15*, 1236–1245.
[24] a) K. A. Connors, *Chem. Rev.* **1997**, *97*, 1325–1357; b) Y. A. Zhdanov, Y. E. Alekseev, E. V. Kompantseva, E. N. Vergeichik, *Russ. Chem. Rev.* **1992**, *61*, 563–575.
[25] M. Kajtar, T. C. Horvath, E. Kuthi, J. Szejtli, *Acta Chim. Acad. Sci. Hung.* **1982**, *110*, 327–355.
[26] M. Kodaka, *J. Am. Chem. Soc.* **1993**, *115*, 3702–3705.
[27] F. V. Bright, G. C. Catena, *Anal. Chem.* **1989**, *61*, 905–909.
[28] T. Tamaki, T. Kokubu, K. Ichimura, *Tetrahedron* **1987**, *43*, 1485–1494.
[29] R. L. Vanetten, J. F. Sebastian, G. A. Clowes, M. L. Bender, *J. Am. Chem. Soc.* **1967**, *89*, 3242.
[30] R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel, F.-T. Lin, *J. Am. Chem. Soc.* **1990**, *112*, 3860–3868.
[31] I. Tabushi, N. Shimizu, T. Sugimoto, M. Shiozuka, K. Yamamura, *J. Am. Chem. Soc.* **1977**, *99*, 7100–7102.
[32] B. L. May, S. D. Kean, C. J. Easton, S. F. Lincoln, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3157–3160.
[33] K. Nakatani, J. Y. Carriat, Y. Journaux, O. Kahn, F. Lloret, J. P. Renard, Y. Pei, J. Sletten, M. Verdaguer, *J. Am. Chem. Soc.* **1989**, *111*, 5739–5748.

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