JOC Notes

Selective Binding of Steroids by 2,2′-Biquinoline-4,4′-dicarboxamide-Bridged Bis(β-cyclodextrin): Fluorescence Enhancement by Guest Inclusion

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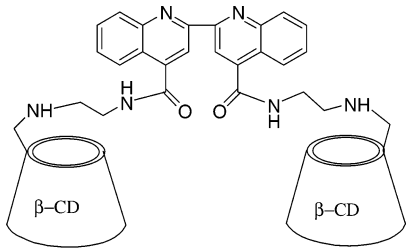
Abstract: A novel bis(β-cyclodextrin) was synthesized, and its binding behavior with steroids was investigated to demonstrate that the cooperative co-inclusion of guest and tether by two cyclodextrin moieties is operative to afford the highest molecular selectivity of up to 3.6 for deoxycholate over taurocholate.

Introduction of a chromogenic group to a cyclodextrin (CD) host not only alters the original binding ability and selectivity but also provides us with a spectral probe for investigating the inclusion complexation behavior with optically silent guest molecules, as the chromogenic aromatic group originally accommodated in the CD cavity suffers substantial conformational changes upon guest inclusion, accompanying appreciable spectral changes. Hence, the binding ability of modified CDs can be quantitatively assessed by analyzing the spectral changes induced by guest inclusion. Indeed, a variety of modified CDs with fluorescent aromatic groups have been synthesized, and their inclusion complexation behavior has been investigated with chiral steroids and alcohols by fluorometric titration to give relatively good molecular selectivities.[1−6] Furthermore, bis-CDs tethered by a simple spacer are known to exhibit greatly enhanced binding abilities, compared to those shown by native and modified mono-CDs, through the cooperative binding of one guest molecule with two hydrophobic CD cavities located in a close vicinity.[7−14] Nevertheless, dimeric CDs linked by a chromogenic bridge have rarely been reported so far.[15]

In this work, we report the synthesis of bis-β-CD with a fluorescent 2,2′-biquinoline-4,4′-dicarboxamide tether (**1**) and its inclusion complexation behavior with chiral steroids. The binding constants (K_S) obtained for a series of structurally related steroids (Chart 1) are discussed in terms of the cooperative binding and induced-fit interactions between the steroid guests and dimeric host **1**. It is also of our particular interest to examine the role of originally self-included tether group of **1** upon complexation with the steroid guests.



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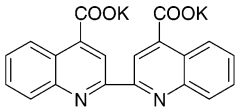

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N,*N*′-Bis(2-aminoethyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis-β-CD **1** was synthesized as follows. 2,2′-biquinoline-4,4′-dicarboxylic dichloride[16,17] (0.30 g, 0.8 mmol) was dissolved in dry DMF (30 mL) containing dicyclohexylcarbodiimide (0.7 g, 34 mmol), to which were added dry 6-(2-aminoethylamino)-6-deoxy-β-CD[18] (3.0 g, 2.55 mmol) and dry pyridine (25 mL). The resultant mixture was stirred for 20 h in an ice bath and for an additional 2 days at room temperature, and then the precipitate formed was removed by filtration and the

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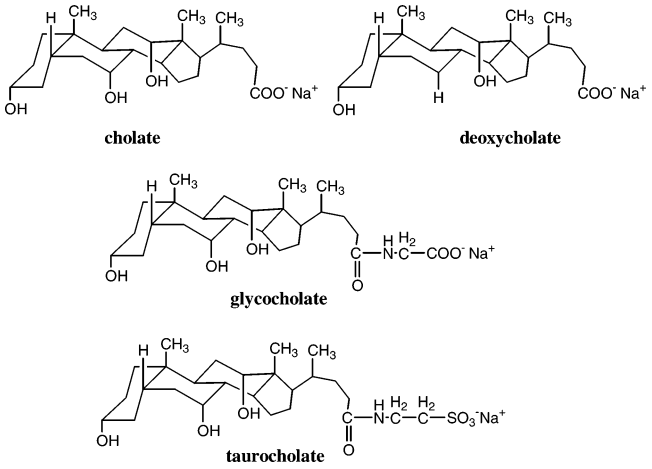
CHART 1. Molecular Structures of Guests



filtrate was evaporated under a reduced pressure to dryness. The residue was dissolved in water, and the aqueous solution was poured into acetone (200 mL) to give a red precipitate. The crude product obtained was dried and purified by column chromatography over Sephadex G-25 with distilled deionized water as an eluent to give pure bis-β-CD **1** in 35% yield as a red solid. ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 1.0−2.0 (m 8H), 4.4−4.7 (m 12H), 4.8−5.2 (m 14H), 5.4−6.2 (m 28H), 7.6−9.4 (m Ar 10H). IR (KBr): ν/cm^{-1} 3343, 2929, 2056, 1708, 1661, 1642, 1592, 1549, 1427, 1331, 1238, 1202, 1153, 1078, 1031, 944, 850, 757, 706, 577. UV−vis (water): λ_{max}/nm (ϵ/M^{-1} cm^{-1}) 264.6 (37280), 339.0 (15480). Anal. Calcd for $C_{108}H_{164}O_{72}N_6 \cdot 8H_2O$: C, 46.22; H, 6.32; N, 2.99. Found: C, 46.35; H, 6.31; N, 3.10.

To elucidate the conformation of **1**, circular dichroism and ^{1}H NMR spectral studies were performed by using a JASCO-750S and a Varian Mercury VX300 spectrometer, respectively. It is well-known that there is a balance between inclusion and dissociation of modified cyclodextrins in solution, so the cross-peaks arising from space correlations between host and guest present both inside protons (H-3/H-5)and outside protons (H-2/H-4) of the CD in the NOESY spectra. In the present observation, we mainly deduce self- and co-inclusion modes with guests from 2D NMR spectra. As shown in Figure 1, the 2D NOESY spectrum of **1** in D_2O shows six cross-peaks between the spacer's aromatic protons and the CD's interior protons. The cross-peaks A, B, and C, which are assigned to the NOE with the CD's H-5 protons, unambiguously indicate inclusion of the spacer moiety into the CD cavity. Since the H-5 protons are located at the primary side of the cavity, we may conclude that the biquinoline spacer penetrates into the CD cavity from the narrower opening. However, no cross-peaks are found between the biquinoline's 3,3′- or 5,5′-protons and the CD protons. Hence, it is likely that the unsubstituted rings of biquinoline penetrate into the CD cavities only shallowly from the primary side.

The self-inclusion behavior of spacer group in **1** can also be verified by the circular dichroism spectral studies, as the inclusion of achiral chromophoric guest/moiety leads to the induced circular dichroism.[19] The circular

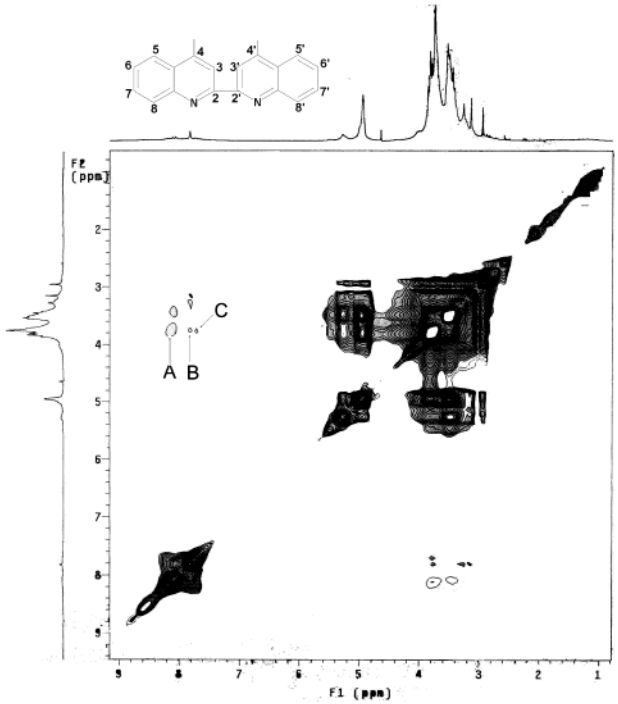


FIGURE 1. ^{1}H NOESY spectrum (300 MHz) of dimer **1** (1.0×10^{-3} M) in D_2O at 298 K with a mixing time of 800 ms.

dichroism spectrum of **1** exhibited two negative Cotton effect peaks of moderate intensities at 225 nm ($\Delta\epsilon$ −3.83 M^{-1}cm^{-1}) and 283 nm ($\Delta\epsilon$ −2.22 M^{-1}cm^{-1}), which may be assigned to the ^1L_a and ^1L_b bands, respectively. The sign of induced Cotton effect enables us to elucidate the conformation of biquinoline unit in **1**. The negative Cotton effects observed for the ^1L_a and ^1L_b bands indicate that the biquinoline unit is shallowly included into the two CD cavities of **1** in the longitudinal direction,[20−22] which is in good agreement with the 2D NMR spectral study.

Fluorescence spectral study provides further supports for this complex mode. Dipotassium 2,2′-biquinoline-4,4′-dicarboxylate (**2**), as a reference fluorophore, gave only weak emission as shown in Figure 2 (trace a), but the fluorescence intensity of **2** was significantly enhanced upon addition of mono[6-(2-aminoethylamino)-6-deoxy]-β-CD (trace b in Figure 2), most probably through the inclusion of biquinolinedicarboxylate **2** in the CD cavity. Notably, much stronger fluorescence was observed for free **1** under the identical conditions (trace c in Figure 2), which is ascribed to the cooperative binding of the biquinoline spacer by the dual hydrophobic cavities**,** leading to more effective shielding of the fluorophore from the deactivating water attack. Unexpectedly, the fluorescence intensity was not reduced but significantly enhanced upon addition of steroid guest (trace d in Figure 2). It is well documented that the emission of fluorophore-appended mono-CDs is quenched upon guest inclusion (including steroids),[1−6] as a consequence of decomplexation of the initially self-included fluorophore moiety.

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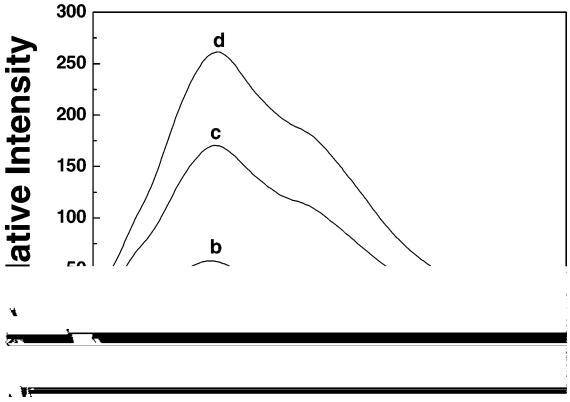

FIGURE 2. Fluorescence spectra of (a) **2**, (b) **2** + mono[6-(2-aminoethylamino)-6-deoxy]-β-cyclodextrin, (c) **1**, and (d) **1** + deoxycholate at the same concentration of 5×10^{-6} M in phosphate buffer solution (pH 7.20) at 25 °C; excitation wavelength (λ_{ex}) 330 nm.

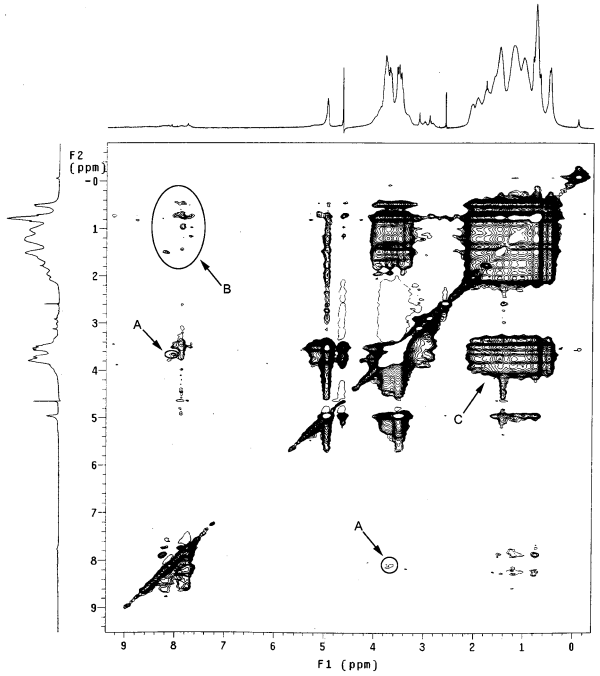

FIGURE 3. ^{1}H NOESY spectrum (300 MHz) of **1** with deoxycholate (5.0×10^{-4} M each) in D_2O at 25 °C with a mixing time of 600 ms.

Hence, we need another mechanism to explain the enhanced fluorescence intensity upon addition of steroid guests observed specifically with dimeric host **1**. The increased fluorescence intensity can be rationalized by the increased microenvironmental hydrophobicity and/or steric shielding around the fluorophore arising from the cooperative guest−tether−host interactions.

A 2D NMR study is expected to reveal the cooperative binding behavior in more detail. As illustrated in Figure 3, the NOESY spectrum of an equimolar mixture of dimer **1** with deoxycholate (0.5 mM each) displayed clear NOE cross-peaks between CD's H-5 and biquinoline's aromatic protons (peak A), indicating that the biquinoline moiety is not driven out of the CD cavity even after the guest inclusion. Furthermore, the protons of deoxycholate gave not only the cross-peak B with biquinoline's protons but also the cross-peak C with CD's H-5, confirming the cooperative co-inclusion of fluorophore tether and steroid guest in the two CD cavities.

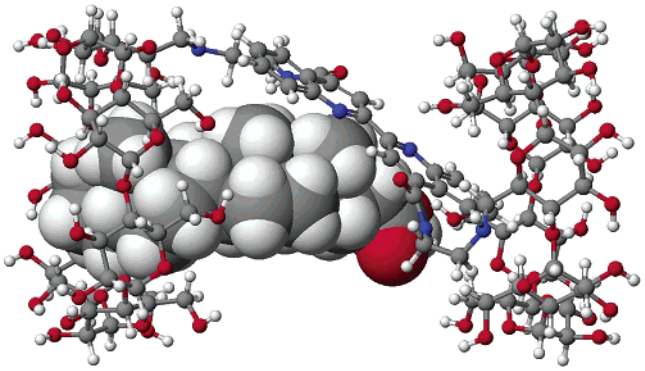

FIGURE 4. MM2-optimized structure of **1**−deoxycholate complex compatible with the Job plot, 2D-NMR, and fluorescence spectral studies.

To elucidate a plausible complex structure for the **1**−deoxycholate complex (Figure 4), we performed the molecular modeling study using the CAChe 3.2 program (Oxford Molecular Co., 1999). The initial geometry of β-cyclodextrin was taken from the crystal structure in the literature[23] and its energy was minimized by using the MM2 force field. It should be noted that the structure is a plausible illustration of the geometry that is compatible with our experimental results, i.e., fluorescence enhancement and 2D-NMR, but is not taken as "evidence" for the complex structure.[24]

Quantitative investigations of the complexation behavior of **1** were performed with selected steroids in phosphate buffer solution (pH 7.20) at 25 °C, by means of titration fluorimetry using a JASCO FP-750 instrument, to give the complex stability constants (K_S) and Gibbs free energy changes ($-\Delta G°$). In the fluorimetric titration experiments, the fluorescence intensity of **1** gradually increased with increasing steroid concentration (Figure 5). A representative Job plot for the inclusion complexation of host **1** with deoxycholate shown in Figure 6, confirms the formation of 1:1 sandwhich complex of steroid guest with **1**.

Assuming the 1:1 stoichiometry for all the steroids employed, the K_S values are calculated by using the nonlinear least-squares method,[25] and the results are listed in Table 1, along with the $-\Delta G°$ values.

As can be readily recognized from Table 1, dimeric host **1** displays distinctly different binding ability, and hence significant discrimination, toward these homologous steroids of differing skeletons and anionic groups. The K_S values obtained for deoxycholate and cholate, i.e., 21 730 and 11 300 M^{-1}, respectively, are 20−30 times larger than those reported for modified mono-CDs by Ueno et al.[2−6] under practically the same experimental conditions. This enhancement is probably due to the cooperative binding of the steroids by **1**. The complex stability decreases in the order: deoxycholate > cholate

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> glycocholate > taurocholate; the largest difference in free energy gain ($-\Delta\Delta G°$) amounts to 3.18 kJ mol^{-1}. The highest affinity for deoxycholate is likely to arise from its more hydrophobic steroid skeleton, lacking the 7-hydroxyl group. It is interesting to note that host **1** shows comparable affinities toward cholate and glycocholate, which share the same skeleton and anionic tail (COO^-), whereas taurocholate, possessing a highly polar anionic tail (SO_3^-), gives the lowest K_S. Thus, the highest molecular selectivity of up to 3.6 was observed for deoxycholate against taurocholate, for which the more hydrophobic steroid skeleton and the less polar carboxylic tail are jointly responsible.

In the present investigation, we have demonstrated that the fluorophoric tether introduced to bis-CDs functions not only as a spectral probe for elucidating the complex structure but also as a convenient and powerful tool for enhancing the guest binding ability and selectivity. Furthermore, the cooperative guest−tether−host interactions observed may find some similarities with the biological molecular recognition involving the multicomponent, induced-fit receptor−substrate interactions.

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