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Novel Permethylated β-Cyclodextrin Derivatives Appended with Chromophores as Efficient Fluorescent Sensors for the Molecular Recognition of Bile Salts

Yu Liu,* Jun Shi, and Dong-Sheng Guo

Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin, 300071, People's Republic of China

yuliu@nankai.edu.cn

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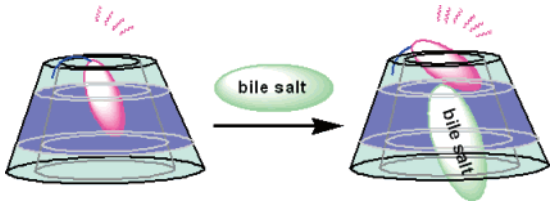

Two novel permethylated β-cyclodextrin (PM-β-CD) derivatives, i.e., 6^{I}-*O*-(1-naphtholxy)-2^{I},3^{1}-di-*O*-methylhexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-β-cyclodextrin (**1**) and 6^{I}-*O*-(8-hydroxyquinoline)-2^{I},3^{1}-di-*O*-methylhexakis(2^{II-VII},3^{II-VII},6^{II-VII}- tri-*O*-methyl)-β-cyclodextrin (**2**), were synthesized in satisfactory yields, and their inclusion modes, complex-induced fluorescent behaviors, binding ability, and selectivity for bile salts of biological relevance (cholic acid sodium salt, CA; deoxycholic acid sodium salt, DCA; glycochoic acid sodium salt, GCA; taurocholic acid sodium salt, TCA) were investigated by the circular dichroism, 2D NMR, steady-state, and time-resolved fluorescent spectra. The results obtained from induced circular dichroism and ROESY spectra show that the chromophore groups of **1** and **2** reside in the central cavity of PM-β-CD, and are expelled to the region of narrow torus rim upon complexation with bile guests, which presents the binding mode of cooperative inclusion. The transfer of the chromophore groups from the central cavity to the more hydrophobic torus rim leads to the remarkable increase of fluorescent intensities and longer fluorescent lifetimes of hosts **1** and **2** upon gradual addition of bile salts, which is importantly distinct from the molecular recognition of the chromophore-modified β-CD species with bile salts. Interestingly, hosts **1** and **2** present much stronger binding ability for bile guests than PM-β-CD. Differing from native β-CD, all the PM-β-CDs are more prone to include bile salts with longer tails, such as GCA and TCA. Their corresponding binding ability and molecular selectivity are closely discussed from the viewpoints of difference of cavity size/shape between β-CD and PM-β-CD, effect of substituent groups, and structures of bile guests, respectively.

Introduction

In recent years, molecular recognition based on cyclodextrins (CDs) and their derivatives has been extensively studied as a significant topic in both chemistry and biology.[1] Consequently, much effort has been devoted to the synthesis of a wide variety of CD derivatives to explore further their binding behaviors for model substrates.[2,3] In this context, methylated CDs, including dimethylated CD, permethylated CD, random methylated CD, and so on represent a particular family of CD derivatives that are not only water-soluble but also ester-soluble.[4,5] The multiple modifications induce the cavity of CD to be more patulous and

(1) (a) Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743−1753. (b) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917. (c) Takahashi, K. *Chem. Rev.* **1998**, *98*, 2013−2033. (d) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997−2011. (e) Liu, Y.; Chen, Y. *Acc. Chem. Res.* **2006**, *39*, 681−691.

(2) (a) Kuwabara, T.; Nakamura, A.; Ueno, A.; Toda, F. *J. Phys. Chem.* **1994**, *98*, 6297−6303. (b) Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartor, G. *J. Org. Chem.* **1997**, *62*, 6283−6289. (c) Nowakowska, M.; Loukine, N.; Gravett, D. M.; Burke, N. A. D.; Guillet, J. E. *J. Am. Chem. Soc.* **1997**, *119*, 4364−4368. (d) McAlpine, S. R.; Garcia-Garibay, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 4269−4275. (e) Rekharskey, M.; Yamamura, H.; Kawai, M.; Inoue, Y. *J. Am. Chem. Soc.* **2001**, *123*, 5360−5361. (f) Kuwabara, T.; Aoyagi, T.; Takamura, M.; Matsushita, A.; Nakamura, A.; Ueno, A. *J. Org. Chem.* **2002**, *67*, 720−725. (g) Park, J. W.; Song, H. E.; Lee, S. Y. *J. Phys. Chem. B* **2002**, *106*, 5177−5183.

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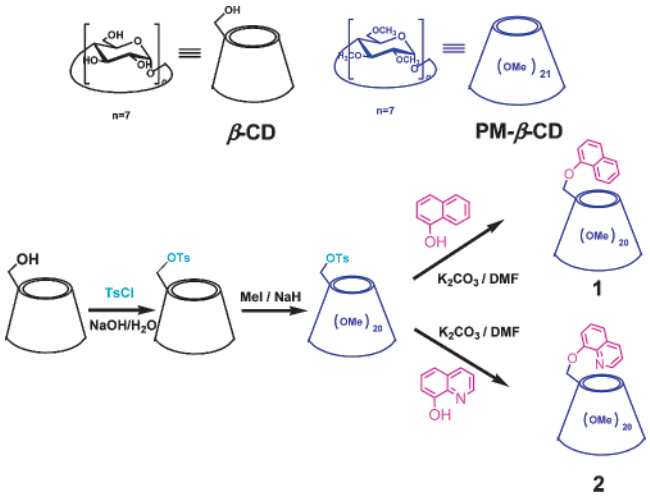
flexible,[6] and thereby endow methylated CDs many intrinsic characteristics which cannot be owned by native CDs. As a result, methylated CDs present much potential in applications of chiral molecules even enantiomer separation and drug delivery with more advantages than native CDs.[7] Particularly, once all the hydroxyl groups of CDs are substituted by methyloxyls, which are called permethylated cyclodexytins (PMCDs), not only the further alteration about cavity size and shape appears, but also the most cavity hydrophobic region inverses from the center to two torus rims of the cavity compared with other methylated CDs and native CDs.[6] Therefore, PMCDs promise to exhibit much distinguishable inclusion complexation behaviors for some special substrates during the course of molecular recognition and separation.[8−10] For example, PM-β-CDs can afford binding constants up to 10^{-5} to 10^{-6} M^{-1} with charged porphyrins in aqueous[9d] or aqueous/organic media.[9b] However, investigations on the inclusion complexation behaviors of the PMCDs, especially their functional derivatives, are still very immature relative to those of CDs. Gelb and co-workers[8] compared the binding abilities between native β-CD and methylated β-CDs for a series of adamantanylammonium derivatives. Kano and co-workers[11] synthesized some PM-β-CD derivatives and examined the inclusion mode and thermodynamic parameters for complexation behavior of charged porphyrins with these receptors.

On the other hand, fluorescent spectra are a powerful tool for studying the host−guest inclusion phenomena, which have been widely employed in molecular recognition of CDs.[12−14] It is well-known that the introduction of fluorophore sidearms to the CD framework not only provides additional binding sites but the sidearms are also capable of acting as fluorescent sensors with some organic guest molecules.

SCHEME 1. Schematic Representation of the Formation of Hosts 1 and 2



In the present work, we synthesized two novel PM-β-CD derivatives (**1** and **2**) appended with naphthalene and quinoline fluorophores, and further investigated their molecular binding ability and selectivity for bile salts. Bile salts are classical surfactant-like biological amphipathic compounds containing a steroid skeleton which have distinctive detergent properties and play a significant role in the metabolism and excretion of cholesterol in mammals.[15] Previously, research about molecular recognitions of bile salts based on substituted CDs has been widely performed.[16,17] Possessing potential existent vivo adaptability, the studies on the inclusion behaviors of PM-β-CDs with bile salts seem to be more attractive. However, to the best of our knowledge, the molecular recognition of bile salts by PM-β-CD and its corresponding derivatives has never been studied before. It is our special interest to compare the binding mechanisms between β-CD and PM-β-CD with bile salts, and further discuss how the substituted fluorescent groups affect the inclusion behaviors of PM-β-CD derivatives and explore closely their photophysical/photochemical behaviors upon complexation with guests.

Results and Discussion

Conformations of Hosts and Binding Manners. The CD cavity can provide a chiral microenvironment where achiral

(3) (a) Liu, Y.; Zhang, Y.-M.; Qi, A. D.; Chen, R.-T.; Yamamoto, K.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1997**, *62*, 1826−1830. (b) Liu, Y.; You, C.-C.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1999**, *64*, 3630−3634. (c) Liu, Y.; You, C.-C.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1999**, *64*, 7781−7787. (d) Liu, Y.; Li, B.; Wada, T.; Inoue, Y. *Supramol. Chem.* **1999**, *10*, 173−184. (e) Liu, Y.; Li, L.; Li, X.-Y.; Zhang, H.-Y.; Wada, T.; Inoue, Y. *J. Org. Chem.* **2003**, *68*, 3646−3657.

(4) Steiner, T.; Saenger, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 3404−3407.

(5) Harada, K. *Chem. Rev.* **1998**, *98*, 1803−1827.

(6) (a) Stefan, I.; Frieder, W. L, D. *Starch/Stärke* **1996**, *48*, 225−232. (b) Mino, R. C.; Susan, A. B.; Welcome, T. M.; Pamela, M. D. *Chem. Commun.* **2004**, 2216−2217.

(7) (a) Janusz, Z.; Danuta, S. *J. Chromatogr. A* **1988**, *436*, 381−390. (b) Wilhelm, K.; Angela, K.; Wilhelm, M. *J. High Resolut. Chromatogr.* **1991**, *14*, 507−529. (c) Takashi, A.; Shinji, T.; Minoru, T. *Anal. Chim. Acta* **2000**, *410*, 37−45. (d) Sigurdsson, H. H.; Stefánsson, E.; Gudmundsdóttir, E.; Eysteinsson, T.; Thorsteinsdóttir, M.; Loftsson, T. *J. Controlled Release* **2005**, *102*, 255−262.

(8) Gelb, R. I.; Schwartz, L. M. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1989**, *7*, 537−543.

(9) (a) Carofiglioa, T.; Fomasierb, R.; Lucchinic, V.; Rossob, C.; Tonellatob, U. *Tetrahedron Lett.* **1996**, *37*, 8019−8022. (b) Kano, K.; Nishiyabu, R.; Asada, T.; Kuroda, Y. *J. Am. Chem. Soc.* **2002**, *124*, 9937−9944. (c) Kano, K.; Kitagishi, H.; Tamura, S.; Yamada, A. *J. Am. Chem. Soc.* **2004**, *126*, 15202−15210. (d) Kano, K.; Nishiyabu, R.; Doi, R. *J. Org. Chem.* **2005**, *70*, 3667−3673.

(10) Takashi, A.; Yudai, K.; Shinji, T.; Minoru, T. *J. Chromatogr. A* **1999**, *845*, 455−462.

(11) (a) Sasaki, K.; Nakagawa, H.; Zhang, X.; Sakurai, S.; Kano, K.; Kuroda, Y. *Chem. Commun.* **2004**, 408−409. (b) Kano, K.; Nishiyabu, R.; Yamazaki, T.; Yamazaki, I. *J. Am. Chem. Soc.* **2003**, *125*, 10625−10634. (c) Kano, K.; Kitagishi, H.; Dagallier, C.; Kodera, M.; Matsuo, T.; Hayashi, T.; Hisaeda, Y.; Hirota, S. *Inorg. Chem.* **2006**, *45*, 4448−4460.

(12) (a) Hamasaki, K.; Ikeda, H.; Nakamura, A.; Ueno, A.; Toda, F.; Suzuki, I.; Osa. T. *J. Am. Chem. Soc.* **1993**, *115*, 5035−5040. (b) Ikeda, H.; Nakamura, M.; Ise, N.; Oguma, N.; Nakamura, A.; Ikeda, T.; Toda, F.; Ueno, A. *J. Am. Chem. Soc.* **1996**, *118*, 10980−10988. (c) Ueno, A.; Ikeda, A.; Ikeda, H.; Ikeda, T.; Toda, F. *J. Org. Chem.* **1999**, *64*, 382−387. (d) Nakashima, H.; Yoshida, N. *Org. Lett.* **2006**, *8*, 4997−5000.

(13) (a) Ken, S.; Masahiko, N.; Yasuhisa, K. *Chem. Commun.* **2001**, 2630−2631. (b) Ikeda, H.; Lidaka, Y.; Ueno, A. *Org. Lett.* **2003**, *5*, 1625−1627.

(14) (a) Liu, Y.; Han, B.-H.; Sun, S.-X.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1999**, *64*, 1487−1493. (b) Liu, Y.; Han, B.-H.; Chen, Y.-T. *J. Phys. Chem. B* **2002**, *106*, 4678−4687.

(15) Danielsson, H.; Sjövall, J. *Sterols and Bile Acids*; Elsevier: Amsterdam, The Netherlands, 1985; Chapter 13.

(16) (a) Tan, Z. J.; Zhu, X. X.; Brown, G. R. *Langmuir* **1994**, *10*, 1034−1039. (b) Yim, C. T.; Zhu, X. X.; Brown, G. R. *J. Phys. Chem. B* **1999**, *103*, 597−602. (c) Singh, A. P.; Cabrer, P. R.; Alvarez-Parrilla, E.; Meijide, F.; Vázquez Tato, J. *J. Inclusion Phenom. Macrocyclic Chem.* **1999**, *35*, 335−348. (d) Alvarez-Parrilla, E.; Cabrer, P. R.; Singh, A. P.; Al-Soufi, W.; Meijide, F.; Rodríguez Núñez, E.; Vázquez Tato, J. *Supramol. Chem.* **2002**, *14*, 397−404. (e) Cabrer, P. R.; Alvarez-Parrilla, E.; Al-Soufi, W.; Meijide, F.; Rodríguez, Núñez, E.; Vázquez Tato, J. *Supramol. Chem.* **2003**, *15*, 33−43.

(17) (a) Liu, Y.; Yang, Y.-W.; Cao, R.; Song, S.-H.; Zhang, H.-X.; Wang, L.-H. *J. Phys. Chem. B* **2003**, *107*, 14130−14139. (b) Wang, H.; Cao, R.; Ke, C.-F.; Liu, Y.; Wada, T.; Inoue, Y. *J. Org. Chem.* **2005**, *70*, 8703−8711.

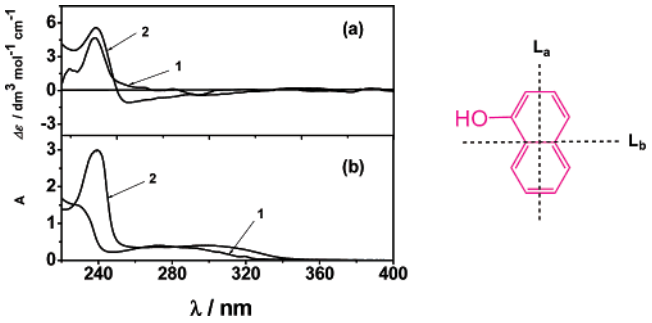


FIGURE 1. Circular dichroism spectra (a) and UV/vis absorption spectra (b) of hosts **1** and **2** (2.0×10^{-4} mol·dm^{-3}) in aqueous solution at 25 °C.

groups complexed by CDs show the induced circular dichroism (ICD) signal(s) in corresponding transition band(s), and thereby, circular dichroism spectrometry has become a convenient method for the elucidation of the structure features of CD derivatives and complexes.[18,19] The circular dichroism spectra and corresponding UV/vis absorption spectra of hosts **1** and **2** are shown in Figure 1. As can be seen, hosts **1** and **2** present similar ICD signals, which implies that the conformations of hosts **1** and **2** should be in a similar form. The circular dichroism spectra of both hosts **1** and **2** show the strong positive cotton effect peaks around 238 nm (host **1**: $\Delta\epsilon = 4.70$ dm^3·mol^{-1}·cm^{-1}; host **2**: $\Delta\epsilon = 5.56$ dm^3·mol^{-1}·cm^{-1}), which should be attributed to the L_a transition band of naphthalene and quinoline chromophores. Moreover, a weak negative cotton effect peak is found around 293 nm ($\Delta\epsilon = -0.40$ dm^3·mol^{-1}·cm^{-1}) in the spectrum of host **1**, which reflects the L_b transition band of the naphthalene chromophore. Similarly, a weak negative ICD signal is also presented in the spectrum of host **2**. According to the empirical rules on the ICD phenomena of CD complexes,[18a,b] the positive signal of the L_a transition band and the negative signal of the L_b transition band indicate that the substituent chromophores of hosts **1** and **2** are located inside their cavities with the L_a transition band parallel to the CD axis and the L_b transition band upright to the CD axis.

The conformation of host **1** was further determined by the 2D ROESY NMR experiment. ROESY cross-peaks are indicative of specific proximity relationships between adjacent protons (the distance between protons is generally in the region of 4−5 Å).[20] The obtained ROESY spectrum of host **1** is shown in Figure S4 (Supporting Information). Although the spectrum appears complicated and the peaks of protons of PM-β-CD overlap each other, the cross-peaks between the inner PM-β-CD protons and the naphthalene protons can be observed, which also indicates that the appended naphthalene is self-included into the PM-β-CD cavity. Therefore, combining the results of circular dichroism and 2D NMR experiments, the possible

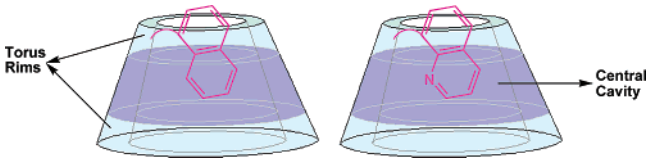


FIGURE 2. Possible conformations of hosts **1** and **2** deduced from the circular dichroism and 2D NMR spectra.

conformations of hosts **1** and **2** are illustrated in Figure 2. Different from the native CD, PM-β-CD possesses many methyloxyls instead of hydroxyls and a more extended cavity. Its cavity is divided into two regions: the central cavity and the additional torus rims. According to the aforementioned ICD signals, the substituent chromophores (naphthalene and quinoline) should be immersed into the chiral microenvironment of the central cavity.

Furthermore, the 2D ROESY NMR experiment of complex of **1** with CA was performed to investigate the binding geometry between PM-β-CDs and bile salts. There are four hydrophobic rings (A, B, C, and D) in each bile molecule. As reported before,[17,21] native CD and their derivatives can include bile guests from their primary or secondary side, and either ring A or ring D can be preferred to be included into the cavity. That is to say, there are several possible binding manners during the course of complexation between CDs and bile salts, which can be well identified by 2D NMR spectroscopy. Moreover, for the PM-β-CD cases, besides the CD protons of the inner cavity (H3 and H5), the substituent methyl groups (CH_3-2, CH_3-3, and CH_3-6) can also provide valuable information about the complex structures by analyzing the correlations between the protons of methyls and guests. As shown in Figure 3, several cross-peaks between PM-β-CD and CA protons appear in the 2D NMR spectrum, suggesting that the CA guest exists in the cavity of host **1**. Peak C represents the correlation signals between CH_3-2 protons of PM-β-CD and those of CA including the H21 of the side chain and some protons of the ring D. Peak D shows the weak correlation signals between H19 of CA and H5, H6, or H3 of PM-β-CD. Furthermore, peak E exhibits the correlation of H12 of CA and H3 of PM-β-CD as well as the correlation signal between H7 of CA and H6 of PM-β-CD. It can be inferred from these three cross-peaks that CA is deeply included into the cavity of host **1** with its ring A in the region of the narrow side and ring D in the region of the broad side. On the other hand, the competitive inclusion of guests generally drives the substituent group out of the cavity of CDs, especially for the guest molecules with larger size/shape such as bile salts.[17b] However, upon complexation with CA guest, the appended naphthalene group in **1** is not entirely expelled out of the cavity of PM-β-CD but is removed from the central cavity to the region of the narrow torus rim. It can be clearly illustrated from Figure 3 (a1) that peak A shows the correlation signals between He, Hf of naphthalene and H6, H6′ of PM-β-CD. Considering the superposition of H3 and H6, H6′ peaks, the correlations between H3 of PM-β-CD and naphthalene protons are also possible, which indicates that the naphthalene group still resides in the central cavity of host **1**. However, the correlation signals between other inner cavity proton H5 and any naphthalene protons are not observed. Therefore, the hypothesis of the naphthalene group in the central cavity should be excluded. All

(18) (a) Harata, K.; Uedaira, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 375−378. (b) Kajtár, M.; Horvath-Toro, C.; Kuthi, E.; Szejtli, J. *Acta Chim. Acad. Sci. Hung.* **1982**, *110*, 327−355. (c) Kodaka, M. *J. Am. Chem. Soc.* **1993**, *115*, 3702−3705.

(19) (a) Yang, S.-Y.; Green, M. M.; Schultz, G.; Jha, S. K.; Muller, A. H. E. *J. Am. Chem. Soc.* **1997**, *119*, 12404−12405. (b) McAlpine, S. R.; Garcia Garibay, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 4269−4275. (c) Inoue, Y.; Wada, T.; Sugahara, N.; Yamamoto, K.; Kimura, K.; Tong, L.-H.; Gao, X.-M.; Hou, Z.-J.; Liu, Y. *J. Org. Chem.* **2000**, *65*, 8041−8050. (d) Nakashima, H.; Takenaka, Y.; Higashi, M.; Yoshida, N. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2096−2103. (e) Takenaka, Y.; Higashi, M.; Yoshida, N. *J. Chem. Soc., Perkin Trans. 2* **2002**, 615−620.

(20) Schneider, H.-J.; Hacket, F.; Rüdiger, V. *Chem. Rev.* **1998**, *98*, 1755−1785.

(21) Ollila, F.; Pentikäinen, O. T.; Forss, S.; Johnson, M. S.; Slotte, J. P. *Langmuir* **2001**, *17*, 7017−7111.

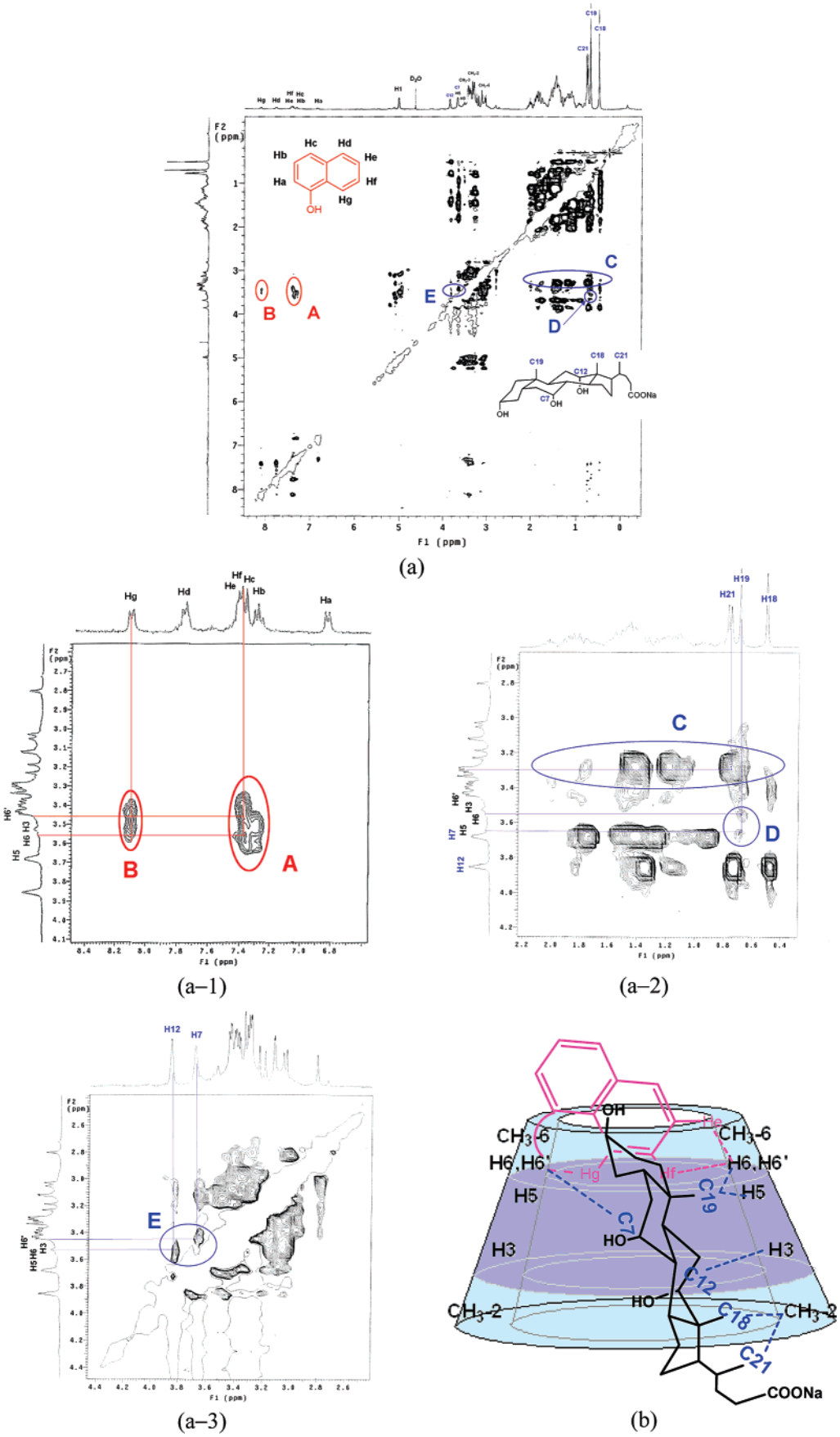
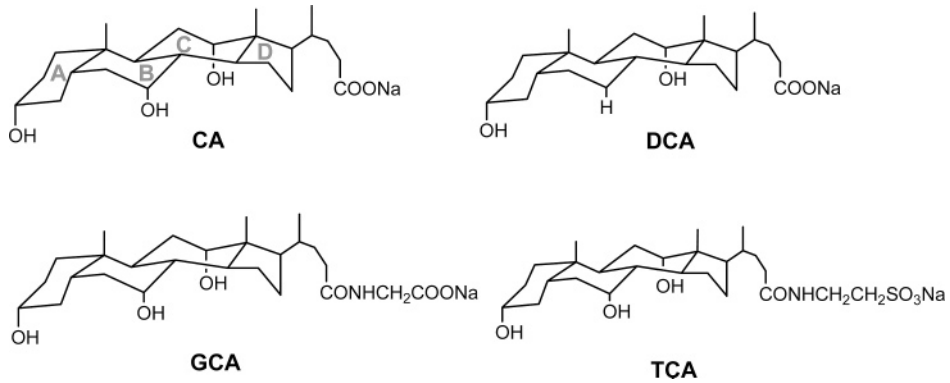


FIGURE 3. (a) ROESY spectrum of complex of **1** (1.2×10^{-3} mol·dm^{-3}) with CA (2.3×10^{-3} mol·dm^{-3}) in D_2O at 25 °C with a mixing time of 300 ms and (b) possible binding mode of complex **1**+CA.

CHART 1. Structures of the Guest Molecules Employed



the above information reveals that the CA guest is deeply included into the PM-*β*-CD cavity from the secondary side with the hydrophobic ring A, which is in accord with the binding structure of methylated CD with bile salts reported before.[21] Figure 3b shows the possible structure of the complex of host **1** with CA. The cooperative inclusion manner of both guest molecule and substituent sidearm into the cavity is mainly benefited from the extended framework of PM-*β*-CD.

Fluorescent Behaviors and Spectral Titrations. For a qualitative assessment of the inclusion complexation behaviors of naphthaline or quinoline modified PM-*β*-CD derivatives, the spectral titrations of hosts **1** and **2** with CA, DCA, GCA, and TCA are performed at 25 °C in Tris-HCl aqueous buffer solution at pH 7.2 by fluorescence spectroscopy. Figures 4 and 5 show the typical spectral changes of **1** and **2** upon gradual addition of GCA, respectively. As can be seen, the relative fluorescent intensities of **1** and **2** present a continuous enhancement upon the addition of GCA, which are distinct from most cases of CDs appended with fluorescent groups.[12a,b,14a] In general, the fluorescent intensities of sidearms of CD derivatives undergo an obvious decrease when suitable guests are added because of the competitive inclusion. That is, the preferred binding of guests into the cavity of CDs excludes their own fluorescent sidearms out of the cavity. And then the transfer of the fluorescent groups from hydrophobic region to hydrophilic region makes their fluorescence rationally be quenched. For the present PM-*β*-CD cases, the naphthaline or quinoline group is not entirely out of the cavity but moves from the central cavity to the more hydrophobic region of the narrow torus rim, and thereby the relative fluorescent intensities of hosts **1** and **2** are enhanced dramatically upon complexation with bile salts. In other words, PM-*β*-CDs can simultaneously accommodate the fluorescent sidearms and bile salt guest into their extended cavities, which is promised to favor multipoint cooperative binding.

The complexed fluorescent phenomena were further validated by the measurements of fluorescent lifetime experiments for host **1** in the absence and presence of CA or DCA (shown in the Supporting Information). It implies that host **1** presents two different lifetimes (τ_1 = 13.09 ns; τ_2 = 17.44 ns), which indicates that the naphthaline group is located in two sorts of environment of distinct hydrophobicity. As mentioned above, the appended naphthaline chromophore can be self-included into the cavity of PM-*β*-CD, and there should be a balance of coexistence of both included and free naphthaline groups for host **1**. Thus, the shorter lifetime (τ_1) is reasonably assigned to the naphthaline chromophore that is exposed to the bulk aqueous solution, and the longer lifetime (τ_2) is assigned to the naphthaline chromophore that is located in the hydrophobic cavity.[17b] Upon complexation with CA or DCA, the shorter lifetime (τ_1) almost maintains its original value, but the longer lifetime (τ_2 = 20.13, 20.18 ns) is enhanced to a larger extent than for host **1** itself. This result of fluorescent lifetime also shows that the naphthaline sidearm in host **1** undergoes a transfer to a more hydrophobic environment upon complexation with bile salts.

The binding stoichiometry of the PM-*β*-CD derivatives **1** and **2** with bile salts was determined by the Job's plot method. As shown in Figure 6, the plot maximum point appears at a PM-*β*-CD's molar fraction of 0.5, which obviously indicates that a 1:1 inclusion complex is formed between host **1** and CA guest. The same results were obtained in the other cases of the inclusion complexation of PM-*β*-CDs with bile salts, which is consistent with previous reports that native *β*-CD and their monomodified derivatives always form a 1:1 inclusion complex with bile salts.[17]

Using 1:1 host/guest stoichiometry as elucidated before, we could express the complexation of host (H) with the bile salt guest (G) as eq 1, where K_S represents complex formation constant.

$$H + G \overset{K_S}{\rightleftharpoons} H\cdot G \tag{1}$$

In our fluorescence titration experiments, the host concentration was fixed, and the fluorescent intensity increased upon the addition of guest molecule. Therefore, we defined ΔF as the relative fluorescence intensity change, where $\Delta F = F$(with guest molecule) $- F$(without guest molecule), which is assumed to be proportional to the concentration of inclusion complex (H·G), i.e.

$$\Delta F = \alpha[H\cdot G] \tag{2}$$

The proportionality coefficient α is taken as a sensitivity factor for the fluorescence change upon inclusion complexation. Thus, the effective complex formation constant (K_S) can be expressed by eq 3.

$$K_S = \frac{[H\cdot G]}{[H]\cdot[G]} \tag{3}$$

Colligated the eq 2 and eq 3, ΔF could be expressed as eq 4.

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

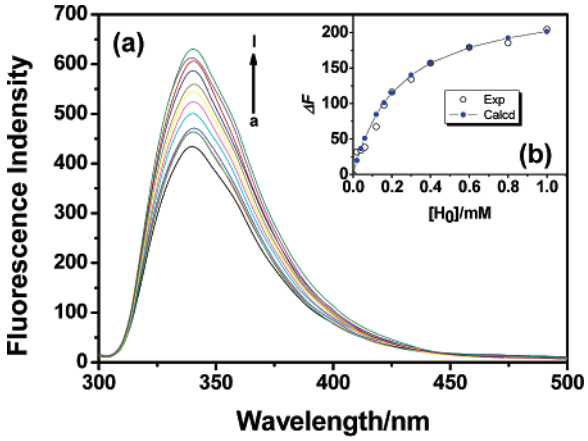


FIGURE 4. (a) Fluorescence spectral changes of host **1** (2.0×10^{-6} mol·dm^{-3}) upon addition of GCA ($0-1.0 \times 10^{-3}$ mol·dm^{-3} from a to l) in 0.1 M tris buffer solution (pH 7.2) at 25 °C. (b) the nonlinear least-squares analysis of the differential intensity (ΔF) to calculate the complex formation constant (K_S). ($\lambda_{ex} = 282.0$ nm, $\lambda_{em} = 339.0$ nm.)

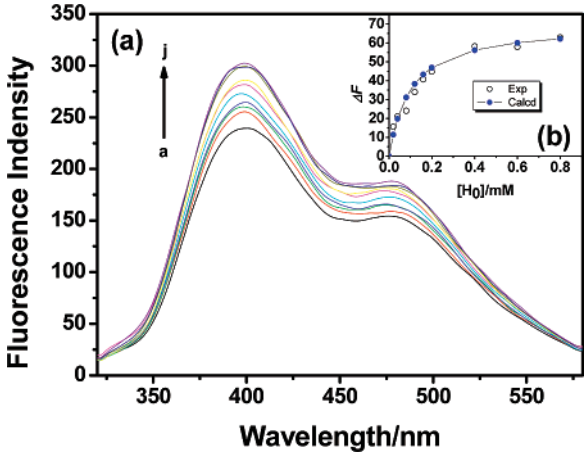


FIGURE 5. (a) Fluorescence spectral changes of host **2** (1.0×10^{-5} mol·dm^{-3}) upon addition of GCA ($0-1.0 \times 10^{-3}$ mol·dm^{-3} from a to j) in 0.1 M tris buffer solution (pH 7.2) at 25 °C. (b) The nonlinear least-squares analysis of the differential intensity (ΔF) to calculate the complex formation constant (K_S). ($\lambda_{ex} = 300.0$ nm, $\lambda_{em} = 398.0$ nm.)

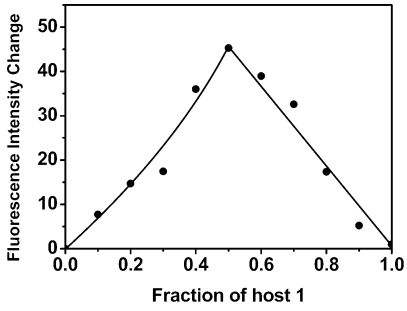


FIGURE 6. Job's plot of host **1**/CA system at 339 nm. ([host **1**]·[CA] = 1×10^{-5} mol·dm^{-1}).

We obtained the complexation stability constant for each host−guest complexation as shown in Table 1 using the nonlinear least-squares curve-fitting method. Typical fitting curve plots (Figures 4b and 5b) for titration of CA with host **1** and GCA with host **2** show excellent fits between the experimental and calculated data. All the obtained R values of the curve fitting plot are above 0.98, which supports the reliability of the stability constants obtained as well as the 1:1 stoichiometry between host and guest molecules. When we repeated the measurements, the K_S values were reproducible within an error of ±5%.

TABLE 1. Complex Stability Constants (K_S) for the Inclusion Complexation of Hosts 1 and 2 with Bile Salt Guests in Tris-HCl Aqueous Buffer Solution (pH 7.2) at 25 °C

	K_S(HG)/M^{-1}				
	β-CD	2,6-mβ-CD	PM-β-CD	**1**	**2**
CA	4844[a]	2693[b]	61 ± 2[c]	910 ± 45[d]	3290 ± 175[d]
DCA	4086[a]	6276[b]	774 ± 38[c]	4320 ± 210[d]	7460 ± 200[d]
GCA	2394[a]	1958[b]	228 ± 10[c]	4340 ± 190[d]	10690 ± 340[d]
TCA	2293[a]	2148[b]	162 ± 9[c]	3820 ± 170[d]	8710 ± 270[d]

[a] Reference 17a. [b] Reference 21. [c] Tested by the ITC method. [d] Tested by the fluorescence method.

Binding Ability and Selectivity. As discussed extensively before, the hydrophobic and van de Waals interactions play a large role in the inclusion complexation of cyclodextrins with guests.[1b,17] Moreover, the hydrogen bonds offered by the (narrow and wide) rims' hydroxyls of CDs with guests also help stabilize the complexes to some extent.[22] It was also well-documented that the host−guest hydrogen-bonding interactions contribute to stabilize the complexes during the course of complexation of β-CD with bile salts besides the hydrophobic and van de Waals interactions.[17,23] For the PM-β-CD cases, all the hydroxyls are methylated, and the loss of hydrogen bonds for the resulting complexes is inevitable. Therefore, PM-β-CD only shows weak complex stability constants (K_S) to bile salts, which are much lower than those of β-CD and 2,6-m-β-CD. In addition, the 1−2 orders of magnitude weaker binding ability of PM-β-CD to bile salts than those of β-CD can also be reflected from two other viewpoints. One is that the release of higher energy water molecules in the cavity of β-CD upon complexation with guests makes the inclusion complexation more favorable, which cannot be obtained in the PM-β-CD cases because almost no water molecule resides in the cavity of PM-β-CD.[24] The other is that, differing from β-CD, the shape of PM-β-CD's cavity is pinched to be elliptical, and so PM-β-CD possesses much stronger binding ability to the planer-shape porphyrin guests as mentioned above. However, bile salts are tridimensional guests with some columnar shape, and thereby PM-β-CD should need some conformational adjustment to accommodate bile guests, which is entropy-unfavorable for the inclusion complexation.

Interestingly, hosts **1** and **2** show much higher binding ability to bile salts than PM-β-CD when the naphthalene (or quinoline) sidearm is appended on it. As can be seen from Table 1, the K_S value for TCA is enhanced 23 times from PM-β-CD to host **1**, and it is enhanced 53 times from PM-β-CD to host **2**. The pronounced enhancement of complex stabilities for hosts **1** and **2** can be attributed to the cooperative complex interactions of both the cavity of PM-β-CD and the chromophore sidearms. According to the binding geometry deduced from the 2D NMR experiment, the appended chromophore (naphthaline or quinoline) and bile guest are simultaneously accommodated into the

(22) (a) Tormo, L.; Organero, J. A.; Douhal, A. *J. Phys. Chem. B* **2005**, *109*, 17848−17854. (b) Douhal, A. *Acc. Chem. Res.* **2004**, *37*, 349−355.
(23) (a) Narita, M.; Koshizaka, S.; Hamada, F. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1999**, *35*, 605−619. (b) Liu, Y.; Li, L.; Chen, Y.; Yu, L.; Fan, Z.; Ding, F. *J. Phys. Chem. B* **2005**, *109*, 4129−4134.
(24) (a) Grandeury, A.; Petit, S.; Gouhier, G.; Agassea, V.; Coquerela, G. *Tetrahedron*: *Asymmetry* **2003**, *14*, 2143−2152. (b) Tsorteki, F.; Bethanis, K.; Mentzafos, D. *Carbohydr. Res.* **2004**, *339*, 233−240.

cavity of PM-β-CD. The nearness between chromophore and bile guest makes it possible that the appended sidearm provides additional binding sites. Besides the hydrophobic and van de Waals interactions between PM-β-CD's cavity and bile salts, there should be other noncovalent interactions between the appended sidearm and bile guests to reinforce the complex stabilities, such as C−H···π, nonconventional hydrogen bonds, and so on. Upon complexation with bile guests, the naphthaline (or quinoline) chromophore of host **1** (or **2**) is not expelled out of the cavity thoroughly, but is located at the narrow rim of PM-β-CD. Therefore, hosts **1** and **2** appear more like a barrel with a bottom, which can accommodate guest molecules more immovable than PM-β-CD. Furthermore, it should be mentioned that host **2** always forms more stable complexes with bile guests than host **1**. For example, the host selectivity of the **1**/**2** pair for the CA guest is up to 3.6 times. This implies that the N atom on the quinoline ring plays a crucial role during the course of recognition of bile guests. The N atom possesses a lone electron pair pointing out of the aromatic ring, which can act as a suitable hydrogen-bonding acceptor to form hydrogen bonds with bile guests. So, in comparison with host **1**, the particular host−guest interaction supplied by the N atom in host **2** makes it present a stronger binding ability to bile guests.

As also can be seen from Table 1, β-CD presents the strongest binding ability to CA guest among the four selected bile salts, and the molecular selectivity for CA/GCA pairs is 2.0 times. However, much different from β-CD, all the PM-β-CD derivatives, PM-β-CD, hosts **1** and **2**, present the weakest binding ability to CA guest. It is ascribed that the cavity of PM-β-CD possesses a broader hydrophobic region in comparison with β-CD, and then PM-β-CD is more suitable to include bile guests with longer tails (GCA and TCA) than β-CD. The molecular selectivity for hosts **1** and **2** is reversed from that of β-CD, such as, for host **1**, $K_S^{GCA}/K_S^{CA} = 4.8$, and for host **2**, $K_S^{GCA}/K_S^{CA} = 3.3$. As a result, host **2** affords the highest stability constant up to 10 690 M^{-1} upon complexation with GCA guest. Moreover, there are similar structures between CA and DCA except for the difference of one hydroxyl in ring B. It is attractive that DCA can be included more tightly by PM-β-CD, hosts **1** and **2** than CA. Particularly, the molecular selectivity of PM-β-CD for the DCA/CA pair is high, up to 12.7 times, although their K_S values are not very high. One reasonable explanation is that the absence of one hydroxyl in ring B makes the whole framework of DCA more hydrophobic than CA, and thereby DCA is more suitable to be immersed into the cavity of PM-β-CDs. It also demonstrates that high complex stability constant does not always imply high molecular selectivity.

Conclusion

In summary, the binding behaviors of PM-β-CD and its derivatives modified with fluorescent probes (**1** and **2**) with some bile salts have been determined. According to the ICD and ROESY experiments, the appended naphthaline (quinoline) chromophore in host **1** (**2**) is proved to be self-included into the central cavity of PM-β-CD. Upon complexation with bile guests, the chromophores are not thoroughly expelled out of the cavity but transfer from the original central cavity to the region of the narrow torus rim. Therefore, both hosts **1** and **2** present the enhancement of complex-induced fluorescence because the torus rims are more hydrophobic regions than the central cavity for PM-β-CD. Furthermore, PM-β-CD only shows weak binding ability for bile salts, while hosts **1** and **2** display much stronger binding ability than PM-β-CD, with the augmentation of 1−2 orders of magnitude. This mainly originates from the cooperative binding manners of **1** (**2**) with bile guests. The chromophore sidearms in **1** and **2** not only act as the fluorescent probes but also play a great role as an additional binding site together with the cavity of PM-β-CD during the course of inclusion complexation. Finally, it is also well-demonstrated that the family of PM-β-CD and its derivatives with the extended cavity of two additional torus rims always presents distinguishable binding modes, binding ability, and molecular selectivity for special guest molecules in comparison with native CD species.

Experimental Section

Materials. Both 1-naphthol and 8-hydroxylquinoline were commercially available without further purification. Reagent Grade β-CD was recrystallized twice from water and then dried in vacuum at 95 °C for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was distilled under reduced pressure below 50 °C after being dried over calcium dihydride for 2 days before use. All the other chemicals used in reactions were reagent grade and were used without further purification. Tris(hydroxymethyl)aminomethane and hydrochloric acid were dissolved in deionized water to make a 0.1 M Tris-HCl aqueous buffer solution of pH 7.2, which was measured by pH electrode for fluorescence spectral analysis.

All the bile salts including Cholic acid sodium salt (CA), deoxycholic acid sodium salt (DCA), glycochoic acid sodium salt (GCA), and taurocholic acid sodium salt (TCA) were commercial grade and were used without further purification.

Instruments. Circular dichroism and UV/vis spectra were recorded in a conventional quartz cell (light path 10 mm) in aqueous solution at 25 °C. Fluorescence spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm^3) in 0.1 M Tris-HCl buffer solution (pH 7.2) at 25 °C on a fluorescence spectrometer with excitation and emission slits of 10 nm width. Fluorescence lifetimes were recorded on a combined steady state and lifetime spectrometer with a time resolution of 0.19 ns in 0.1 M Tris-HCl buffer solution (pH 7.2) at 25 °C. A Nanosecond pulsed flash lamp filled with hydrogen gas was employed as a pulsed light source. Maximum counts of up to 10 000 were collected for each measurement.

As the controlling experiments, the complex stability constants (K_S) of PM-β-CD with bile guests are determined by the method of isothermal titration calorimetry (ITC) because there is no chromophore in PM-β-CD to be detected by spectroscopy. The ITC experiments were performed at atmospheric pressure in aqueous phosphate buffer solution (pH 7.2) at 25 °C. The instrument is calibrated chemically as we described before.[17] All solutions were degassed and thermostated before each titration run, and the titrations were performed below the critical micelle concentration (cmc) of four bile acid salts.[21] In each run, a phosphate buffer solution of host charged in a 250 μL syringe was sequentially injected with stirring at 300 rpm into a buffer solution of bile acid guest in the calorimeter's sample cell. The sample volume was 1.4227 mL in all experiments. Each titration experiment was composed of 29 successive injections (10 μL per injection). A detailed experimental description and typical graphs of ITC titration and fitting curve are provided in the Supporting Information.

Synthesis of 6^{I}-*O*-(1-Naphtholxy)-2^{I},3^{1}-di-*O*-methylhexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-β-cyclodextrin (1). 1-Naphthol (28.8 mg, 0.2mmol) dissolved in DMF (5 mL) and potassium carbonate (320 mg, 2.0 mmol) was stirred under nitrogen atmosphere for 1 h, then 6-OTs-PM-β-CD (160 mg, 0.1 mmol) was added into the mixture. The reactant mixture was heated to 80 °C and kept stirring under nitrogen atmosphere for 30 h. The precipitate was removed by filtration, and the filtrate was evaporated to dryness

under reduced pressure. The crude product obtained was dissolved in 2 mL of ethyl acetate and purified by chromatography over silica gel, using ethyl acetate−methanol (8:1 v:v) as eluent. A pure sample was obtained in a yield of 39%. UV/vis λ_{max} (H_2O)/nm 280 nm; ^{1}H NMR (400 MHz, CD_3Cl, ppm) δ 8.24 (d, Ar−H, 1H), 7.78 (d, Ar−H, 1H), 7.56−7.39 (m, Ar−H, 4H), 6.83 (d, Ar−H, 1H), 5.13 (s, 7H, 1-H), 4.02−3.11 (m, 102H), 3.64 (21H, 3-OMe), 3.51 (21H, 2-OMe), 3.37 (18H, 6-OMe). ESI-MS *m*/*z* 1563.97 [M + Na^+]. Anal. Calcd for $C_{72}H_{116}O_{35}$: C 56.09, H 7.58. Found: C 55.84, H 7.82.

Synthesis of 6^{I}-*O*-(8-Hydroxyquinoline)-2^{I},3^{I}-di-*O*-methylhexakis-(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-β-cyclodextrin (2). This synthesis procedure was similar to that of compound **1**. The crude product obtained was dissolved in 2 mL of ethyl acetate and purified by chromatography over silica gel, using ethyl acetate−methanol (7:1 v:v) as eluent. A pure sample was acquired in a yield of 56%. UV/vis λ_{max} (H_2O)/nm 238 nm, 293 nm; ^{1}H NMR (400 MHz, CD_3-Cl, ppm) δ 8.87 (d, Ar−H, 1H), 8.12 (d, Ar−H, 1H), 7.91 (s, Ar−H, 1H), 7.46−7.37 (m, Ar−H, 2H), 7.17 (d, Ar−H, 1H), 5.13 (s, 7H, 1-H), 4.02−3.11 (m, 102H), 3.64 (s, 21H, 3-OMe), 3.51 (s, 21H, 2-OMe), 3.37 (m, 18H, 6-OMe). ESI-MS *m*/*z* 1542.55 [M^+]. Anal. Calcd for $C_{71}H_{115}O_{35}N$: C 55.28, H 7.51, N 0.91 Found: C 56.25, H 7.43, N 0.88.

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Supporting Information Available: General synthesis, ^{1}H NMR spectra for new compounds **1** and **2** and intermediate compound 6-OTs-PM-β-CD, 2D ROESY NMR spectrum of host **1** in D_2O, typical fluorescence lifetime spectra and experimental data for host **1** in the absence and presence of CA or DCA, and detailed ITC experimental description and typical graphic of titration and fitting curve. This material is available free of charge via the Internet at http://pubs.acs.org.

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