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Chapter 12

Thermodynamics of Resulting Complexes Between Cyclodextrins and Bile Salts

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Additional information is available at the end of the chapter

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1. Introduction

Cyclodextrins (CDs), a class of macrocyclic oligosaccharides consisting of six, seven, or eight glucose units linked by α-1,4-glucose bonds, have been widely used as receptors in molecular recognition in the field of supramolecular chemistry because they are able to form inclusion complexes with hydrophobic guests in aqueous solution owing to their hydrophilic outer surface and their lipophilic cavity [1–3]. Therefore, much effort has been devoted to the design and synthesis of a wide variety of cyclodextrin (CD) derivatives to explore their binding behaviors for model substrates [4]. In order to further explore their inclusion complexation mechanism, most of these studies have been focused on the binding modes and complexation thermodynamics based on CDs and their derivatives in recent years [5]. Among the numerous guests researched, bile salts attracted much more attention because they are one kind of important surfactant-like biological amphipathic compounds possessing a steroid skeleton, which have distinctive detergent properties and play an important role in the metabolism and excretion of cholesterol in mammals [6]. For example: the thermodynamics and structure of inclusion compounds of glyco- and tauro-conjugated bile salts with CDs and their derivatives have been studied by Holm et al. during the last years [7–11]; the interactions of different kinds of bile salts with β-CD dimers linked through their secondary faces have been investigated by Reinhoudt and Vargas-Berenguel et al. [12–14]. It has been demonstrated that the formation of inclusion complexes between CDs and guest molecules is cooperatively governed by several weak forces, such as van der Waals interactions, hydrophobic interactions, hydrogen bonding, electrostatic interactions, and every weak force does its contribution to the complexation. In this chapter, the related investigations concerned on the binding modes, binding abilities, molecular selectivities and their thermodynamic origins of CDs and their derivatives with four typical bile salts (Cholate (CA), Deoxycholate (DCA), Glycocholate (GCA), and Taurocholate (TCA)) (Figure

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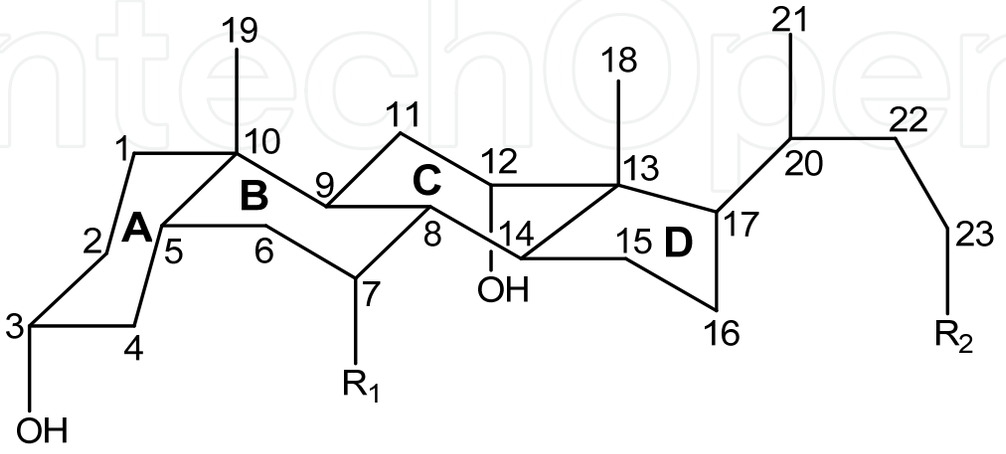
1) have been summarized, which will be discussed from the aspect of the types of host molecules: (1) natural CD series; (2) modified CD series; (3) bridged CD series. This summary is helpful to improve understanding of the correlation between the structural features and molecular-recognition mechanism from thermodynamic viewpoints, and further guide its biological, medicinal and pharmaceutical applications in the future.



Guests	R_1	R_2
Cholate (CA)	OH	COONa
Deoxycholate (DCA)	H	COONa
Glycocholate (GCA)	OH	$CONHCH_2COONa$
Taurocholate (TCA)	OH	$CONHCH_2CH_2SO_3Na$

Figure 1. Molecular structures of four typical bile salts

2. Natural CD series

2.1. Binding modes for bile salts and natural CD series

Since two protons located closely in space (the corresponding internuclear distance is smaller than 3–4 Å) can produce NOE (Nuclear Overhauser Effect) cross-peaks between the relevant protons in NOESY (Nuclear Overhauser Effect Spectroscopy) or ROESY (Rotating Frame Overhauser Effect Spectroscopy) spectra, 2D NMR spectroscopy has become an important method for the investigation of the interaction between different kinds of CDs and guest molecules. It is well-known that only H3, H5, and H6 of CDs can give cross-peaks for analyzing host–guest interactions, as H2 and H4 are not facing to the inner cavity and H1 is affected by D_2O. For example, the ROESY study on the resulting complex of natural β-CD **1** (Figure 2) with CA has been reported by Tato et al. [15,16]. The results successfully indicated that in the 1:1 complex between **1** and CA the steroid body entered forward into the inner cavity of **1** by the side of the secondary hydroxyl groups, with the side chain folded toward the steroid body, i.e., rings D and C are totally and partially included, respectively. Therefore, the binding modes of bile salts with different kinds of CDs have been widely deduced by 2D NMR spectroscopy during the last years.

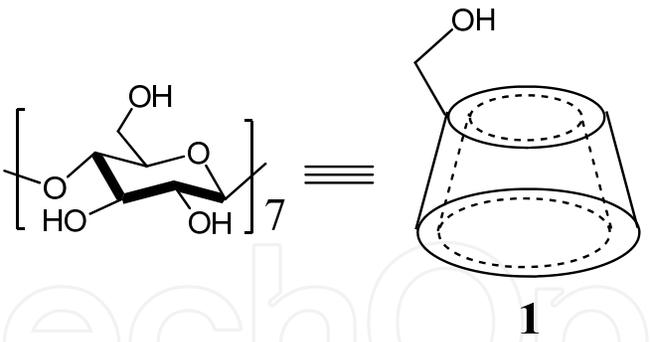


Figure 2. Structure of natural β-CD

2.2. Complexation thermodynamics for bile salts and natural CD series

The microcalorimetric titrations can be used to simultaneously determine the enthalpy and equilibrium constant from a single titration curve. Titrations were performed below the critical micelle concentration of bile salts. In each run, a solution of the host (guest) molecules in syringe was sequentially injected into the calorimeter sample cell containing a solution of guests (hosts). Each addition of hosts (guests) into the sample cell gave rise to a heat of reaction, caused by the formation of inclusion complexes between hosts and guests. The heats of reaction decrease after each injection because less and less molecules in cell are available to form inclusion complexes. A control experiment was performed to determine the heat of dilution by injecting a host (guest) solution into a pure solution containing no guest (host) molecules. The dilution enthalpy was subtracted from the apparent enthalpy obtained in each titration run, and the net reaction enthalpy was analyzed by using the "one set of binding sites" model. This model will work for any number of sites N if all sites have the same Ks and $\Delta H°$. In this case, the total heat Q was fitted via a nonlinear least-squares minimization method to the total host concentration in cell (M_t) using the following equation:

$$Q = (NX_t\Delta HV_o/2)\{1 + M_t/(NX_t) + 1/(NK_sX_t) - \{[1 + M_t/(NX_t) + 1/(NK_sX_t)]^2 - 4M_t/(NX_t)\}^{1/2}\} \quad (1)$$

where N is the number of binding sites of host, X_t is the total concentration of guests in cell and V_o is the cell volume. The value of Q above can be calculated (for any designated values of N, K, and ΔH) at the end of the ith injection and designated $Q(i)$. Then the correct expression for the heat released, $\Delta Q(i)$, from the ith injection is

$$\Delta Q(i) = Q(i) + dV_i/(<ital> V_o)\{[Q(i) + Q(i-1)]/2\} - Q(i-1) \quad (2)$$

where dV_i is the volume of titrant added to the solution. Along with obtaining of Ks and $\Delta H°$ in this fitting program, the N value in eq 1 can also be obtained, which represents the numbers of guests bound to one host molecule.

The ORIGIN software (Microcal), used for the calculation of the binding constant (Ks) and standard molar reaction enthalpy ($\Delta H°$) from the titration curve, gave the relevant standard derivation on the basis of the scatter of data points in a single titration experiment. The binding

stoichiometry was also given as a parameter when fitting the binding isotherm. Knowledge of the binding constant (Ks) and molar reaction enthalpy ($\Delta H°$) enabled the calculation of the standard free energy of binding ($\Delta G°$) and entropy change ($\Delta S°$) according to

$$\Delta G° = -RT \ln K_S = \Delta H° - T\Delta S° \quad (3)$$

where R is the gas constant and T is the absolute temperature.

The microcalorimetric experiments of natural β-CD **1** with bile salts (CA, DCA, GCA, and TCA) showed typical titration curves of 1:1 complex formation [17]. The stoichiometric ratios observed from curve-fitting results of the binding isotherm fell within the range of 0.9–1.1. This clearly indicated that the majority of the inclusion complexes had a 1:1 stoichiometry of bile salts and **1**.

Thermodynamically, the binding behaviors of bile salts by **1** were entirely driven by favorable enthalpy changes accompanied by small unfavorable entropy changes, which are attributed to the predominant contribution of the van der Waals interactions arising from the size/shape fit and geometrical complement between host and guest and to the accompanying decreases in translational and structural freedoms upon complexation.

As can be seen from Table 1, the enthalpy change for the complexation of **1** with DCA is more favorable than that with CA, which directly contributes to the increased complex stability. It is reasonable that DCA possesses a more hydrophobic structure due to the absence of C-7 hydroxyl group as compared with CA, as a result, it is easier to bind into the cavity of **1**, which leads to more favorable hydrophobic and van der Waals interactions and gives larger enthalpy and entropy changes. However, the enhanced favorable entropy gain by the desolvation effect may be canceled by the unfavorable entropy change caused by the structural freezing of the resulting complexes of **1** and DCA. Therefore, the stronger interaction between **1** and DCA only shows the larger negative enthalpy change, directly contributing the relatively larger complex stability constant. Meanwhile, **1** shows a lower binding ability upon complexation with GCA and TCA. Compared with **1** and CA, the complexation of **1** with GCA and TCA exhibit similar enthalpy changes but much more unfavorable entropy changes. The more polar side chains of GCA and TCA may be the reason for it.

3. Modified CD series

3.1. Binding modes for bile salts and modified CD series

3.1.1. Aminated β-CDs

The ROESY study on the resulting complex of **2** (Figure 3) with CA has been reported by Tato et al. [15]. The results exhibited different interactions of the side chain of CA with H5 and H6 of **2** from natural β-CD **1**. The facts indicated that the side chain was unfolded, with the negative carboxylate group moving toward the positive protonated amino group, and the side-chain elongation produced a deeper penetration of the steroid body in the inner cavity of **2**.

The ROESY experiments of modified *β*-CD **3** in the presence of CA or DCA have been performed in D_2O by Liu et al. [17]. The results indicate that the D-ring of CA is accommodated shallowly in the cavity and CA enters **3** from the second side of CD with the side chain and D-ring. At the same time, the side chain with the negative carboxylate group of CA moves toward the positive protonated amino group of **3**. For the resulting complex of DCA–**3**, the ROESY spectrum exhibits entirely different NOE cross-peaks and the D-ring of DCA is included within the cavity of CD from the primary side of CD. Meanwhile, the ethide protons of chiral tether interact with H6 of CD.

2D ROESY NMR experiment of **5** and CA has also been performed by Liu et al. in D_2O to investigate the binding mode between bile salt and CD [18]. The results show that steroid body enters the CD cavity from the second side with its tail and D-ring parts.

3.1.2. Nucleobase-modified β-CDs

In host **8**, the adenine group is deeply inserted into the *β*-CD cavity with an orientation parallel to the C7 axis of *β*-CD while the thymine and uracil groups are shallowly inserted in the *β*-CD cavity with an orientation perpendicular to the C7 axis of *β*-CD [19]. As a result, upon complexation with DCA guest, the deeply included adenine group in host **8** should be expelled from the cavity upon complexation with DCA guest, however, the shallowly included thymine and uracil groups in hosts **9** and **10** are hardly influenced by the inclusion of DCA guest.

3.1.3. Tryptophan- and Tyrosine-modified β-CDs

The binding modes of L/D-Trp-*β*-CD (**11** and **12**) with bile salts have been examined by Liu et al. by 2D ROESY NMR experiments [20]. For L-Trp-*β*-CD (**11**), the results show that in the absence of guest, L-Trp residue is only shallowly included or perching on the rim of the CD cavity. However, in the presence of DCA, the D-ring of DCA is close to the wide end of CD cavity, and the D-ring of DCA and the side chain is co-included in the same cavity from the primary side of **11**. For D-Trp-*β*-CD (**12**), the 2D NMR results indicate that the D-Trp residue attached to *β*-CD is more deeply self-included than the corresponding L-Trp residue in the absence of guest. However, in the presence of DCA, the carboxylate side chain and D-ring of DCA penetrate into the CD cavity from the secondary side shallowly.

The binding modes of L/D-Tyr-*β*-CD (**13** and **14**) with bile salts have further been examined by Liu et al. by 2D ROESY NMR experiments [21]. The results show that the L-tyrosine moiety was self-included in the *β*-CD cavity from the narrow opening. The DCA guest entered the *β*-CD cavity from the wide opening with the tail and the D ring and coexisted with the L-tyrosine substituent in the *β*-CD cavity to form a cooperative inclusion manner. For D-tyrosine-modified *β*-CD (**14**), the D-tyrosine substituent was deeply self-included in the *β*-CD cavity and might be located in the center of the *β*-CD cavity. Upon complexation with DCA, the D-tyrosine substituent of **14** would partially move out of the *β*-CD cavity. Compared with DCA + **13** complex, DCA penetrated into the *β*-CD cavity of **14** more deeply (Figure 4).

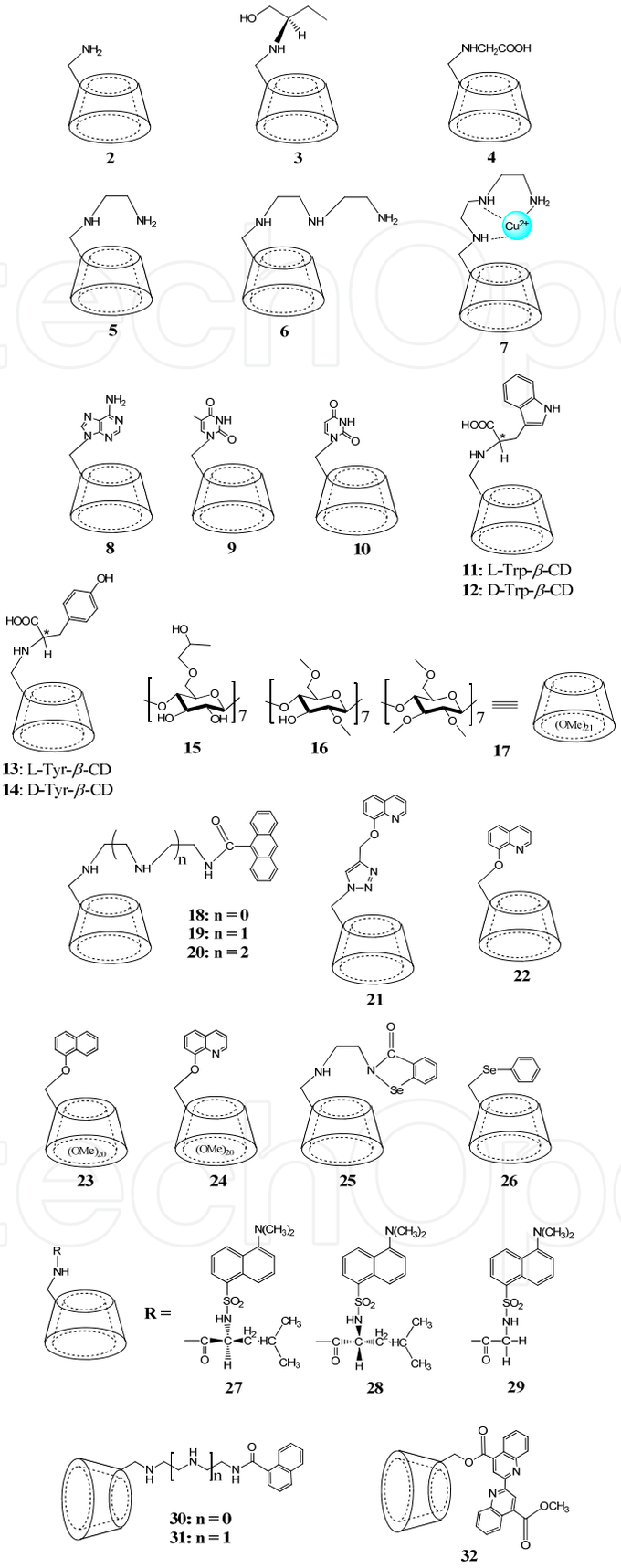


Figure 3. Structures of mono-modified β-CD derivatives

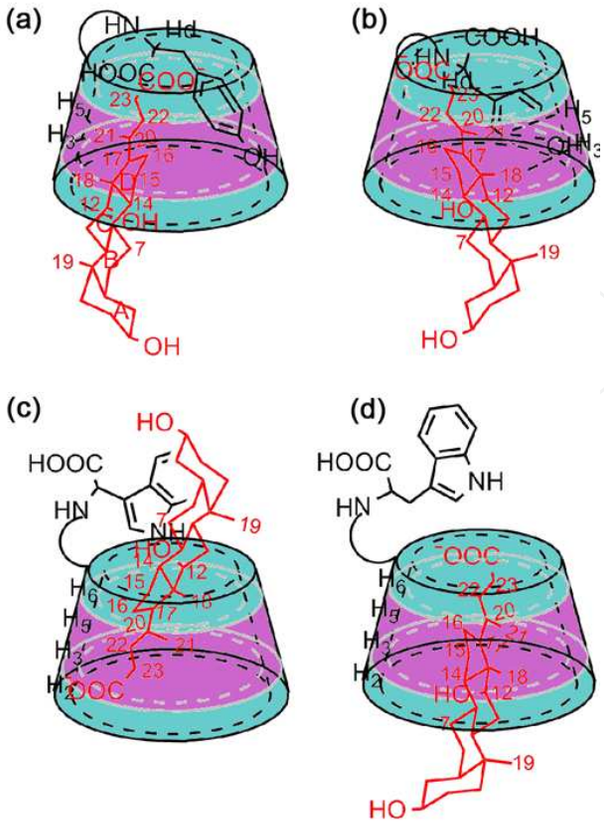


Figure 4. The possible binding modes of **11–14** (**11** (c); **12** (d); **13** (a); **14** (b)) with DCA

3.2. Complexation thermodynamics for bile salts and modified CD series

3.2.1. Aminated β-CDs

The microcalorimetric experiments of aminated *β*-CDs with bile salts clearly indicate that the majority of the inclusion complexes had a 1:1 stoichiometry [17]. Thermodynamically, the binding constants of **4** upon inclusion complexation with DCA, GCA, and TCA are less than that with natural *β*-CD **1**. It is reasonable that modified *β*-CD **4** decreased the microenvironment hydrophobicity of natural *β*-CD cavity due to the hydrophilic carboxylic group in the sidearm, and at the same time there is electrostatic repulsion between the anionic carboxylate at the sidearm of **4** and anionic carboxylate or sulfonate of bile salts. Unexpectedly, the resulting complex stability of aminated *β*-CD **4** with CA is higher than that of native *β*-CD **1**, which is mainly attributed to the more favorable enthalpy change. The possible reason may be the enhanced cooperative van der Waals, hydrogen-bonding, and electrostatic interactions exceeding the decreased hydrophobicity of the interior of *β*-CD **4**.

Positively charged monoamino-modified *β*-CD **2** and modified *β*-CD **3** possessing an additional binding site in the chiral arm evidently enhance the molecular binding ability and selectivity towards CA and DCA compared to those for native *β*-CD **1**, which is mainly attributed to the more favorable enthalpy change accompanied with unfavorable entropy change [17]. The more favorable enthalpy change most likely originates from effective electrostatic interactions and the additional binding site of hydroxyl group. In addition, the

unfavorable entropy change is likely to originate from the conformation fixation of host and guest and the rigid complex formation upon complexation. β-CD derivatives **2** and **3** give a lower binding ability upon complexation with GCA and TCA as compared to the complexation with CA and DCA, which is similar to that for the complexation of β-CD **1** and derivative **4**. For the same reason, the more polar side chains at C23 for GCA and TCA remarkably affect their binding thermodynamics.

A study of ^{13}C chemical shifts as a function of concentration at different pH values has been performed by Tato et al., which shows a different behavior of complexation for CA and DCA with **5** resulting in 1:1 and 1:2 inclusion complexes [22]. However, the complexation phenomena do not depend on the pH of the solution. ^{13}C NMR chemical shifts of the host and guest molecules change on passing from the free to the complexed state. The side chains in **5** at position C-6 have a significant effect on the complexation process with the bile salts. The ROESY experiments confirm the overlap of the CA molecule with **5** resulting a 1:1 inclusion complex, while in the case of DCA molecule, the first molecule of **5** encapsulates the bile salt to a larger extent than the second molecule of **5**, resulting a 1:2 inclusion complex. Hence the most important factors for the formation of a stable inclusion complex are the relative size of **5** and the bile salt molecules, the nonpolar cavity of **5**, the hydrophobicity of the bile salts, and the presence of an electrostatic environment outside the toroidal cavity.

3.2.2. Nucleobase-modified β-CDs

The nucleobase-modified β-CDs **8–10** exhibit distinguishable binding abilities toward bile salts compared with parent β-CD **1** [19]. Host **10** shows increased binding of TCA/GCA. Host **9** exhibits increased binding of GCA while hosts **8–10** show less binding of the other bile salts. The inclusion complexation of hosts **8–10** is driven by favorable enthalpy changes, accompanied with unfavorable entropy changes. The driven forces are hydrogen-bonding and van der Waals interactions, simultaneously producing marked geometric configuration change. Host **8** displays weaker binding ability for every bile salt than hosts **9** and **10** owing to expelling adenine group from β-CD cavity to accommodate bile guests in hosts **8**, which is unfavorable to the host–guest complexation.

3.2.3. Tryptophan- and tyrosine-modified β-CDs

The microcalorimetric titrations of L/D-Trp-modified β-CD (**11** and **12**) with a series of bile acids, i.e., CA, DCA, GCA, and TCA, showed typical titration curves, which can be nicely analyzed by assuming the 1:1 complex stoichiometry [20]. Modified β-CDs **11** and **12** exhibited appreciably smaller binding abilities for GCA and TCA guests than those of native β-CD **1** since GCA and TCA, possessing a strongly hydrophilic and hydrated sulfonate tail, are not expected to deeply penetrate into the CD cavity by removing the originally included L/D-Trp group out of the hydrophobic cavity. In contrast, DCA and CA, possessing a less hydrophilic/hydrated carboxylate tail, showed comparable or even stronger binding and higher selectivities for host's chirality than TCA and GCA.

The ITC experiments of hosts **13** and **14** with bile salts (CA, DCA, GCA, and TCA) also showed the typical titration curves of the 1:1 complex formation [21]. The stoichiometric ratio "*N*" observed from the curve-fitting results was within the range 0.9 to 1.1, which clearly indicated that the majority of the inclusion complexes had a 1:1 binding mode. Thermodynamically, the binding of all CDs with the bile salts was entirely driven by the favorable enthalpy changes accompanied by the unfavorable entropy changes. **14** gave the higher bind ability toward CA and DCA than **1** and **12** due to the introduction of D-tyrosine substituent and the conformational difference between **12** and **14**. In addition, the bind constant of **14** for DCA was slightly bigger than that for CA. Possessing a more hydrophobic structure due to the absence of the C-7 hydroxyl group as compared with CA, DCA was easier to bind to the *β*-CD cavity than CA, which consequently led to the more favorable hydrophobic interactions between hosts and guests. Host **14** exhibited the obviously smaller binding abilities for GCA and TCA guests than **1** and **12**. Thermodynamically, the decreased binding affinities of host **14** toward GCA and TCA arose from the entropy change rather than the enthalpy change due to the weakened hydrophobic interactions and the relatively poor size-fit between host and guest. Compared with **1** and **11**, **13** showed clearly decreased binding abilities toward all four of the bile salts, especially for GCA and TCA. Thermodynamically, the inclusion complexation of **13** with four bile salts exhibited the favorable enthalpy changes and unfavorable entropy changes. The favorable enthalpy gain of **13** was slightly higher than those of **1** and **11**, but the entropy loss of **13** was much more than those of **1** and **11** toward corresponding guests.

3.2.4. Methyl-β-CD and 2-hydroxypropyl-β-CD

The interactions of CA, DCA, GCA, and TCA with **15** and **16** have been studied by Ollila et al. by means of isothermal titration calorimetry [23]. The results show that both CA and DCA bound to **15** and **16** with a 1:1 stoichiometry. The binding constant was significantly higher for DCA to **15** and **16** compared to CA. This difference in binding affinity is likely explained by the more hydrophobic nature of DCA due to the absence of the C-7 hydroxyl group, which is present in CA. The binding affinity was somewhat lower for CA binding to **15** compared to **16**, while DCA showed a markedly lower affinity for **15** compared to **16**. GCA and TCA have lower affinities to **15** and **16** compared to CA and DCA. TCA bound with lower affinity to **15** compared to GCA. Both GCA and TCA gave the same 1:1 stoichiometry for binding to **15** and **16** as did CA and DCA.

For **17**, all the hydroxyls are methylated, and the loss of hydrogen bonds for the resulting complexes is inevitable [24]. Therefore, host **17** only shows weak complex stability constants to bile salts, which are much lower than those of **1** and **16**. In addition, 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 cases of **17** because almost no water molecule resides in the cavity of **17**. Besides that, **17** should need some conformational adjustment to accommodate bile guests, which is entropy-unfavorable for the inclusion complexation.

3.3. Binding modes for bile salts and chromophore-modified CD series

3.3.1. Anthryl-modified β-CDs

^{1}H ROESY experiment has been performed by Liu et al. to confirm the binding model of host **19** with CA [25]. The results indicate that CA molecule is included into the hydrophobic cavity from the secondary side of *β*-CD, with the side chain folded towards the steroid skeleton, and the anthracene group is excluded outside the cavity of *β*-CD. CA molecule and the tether of *β*-CD can be co-included into the cavity through the induced-fit interaction between host and guest.

3.3.2. Quinolinyl- and naphthyl-modified β-CDs

2D ROESY NMR experiments accompanied with molecular modeling studies have been performed by Liu et al. to investigate the binding modes of DCA with **21** and **22** [26]. The results show that the side chain and D-ring of bile salts were encapsulated in the *β*-CD cavity from the wide opening (Figure 5).

2D ROESY NMR experiment of complex of **23** with CA has also been performed to investigate the binding geometry between permethylated *β*-CDs and bile salts [24]. The results show that CA is deeply included into the cavity of host **23** with its ring A in the region of the narrow side and ring D in the region of the broad side. However, upon complexation with CA guest, the appended naphthalene group in **23** is not entirely expelled out of the cavity of permethylated *β*-CD but is removed from the central cavity to the region of the narrow torus rim. The cooperative inclusion manner of both guest molecule and substituent sidearm into the cavity is mainly benefited from the extended framework of permethylated *β*-CD.

3.4. Complexation thermodynamics for bile salts and chromophore-modified CD series

3.4.1. Anthryl-modified β-CDs

The stoichiometric ratios gotten from curve-fitting results of the binding isotherm fell within the range of 0.9–1.1, indicating that the resulting complexes of bile salts and CDs (**18**–**20**) are 1:1 [25]. As compared with parent *β*-CD **1**, modified *β*-CDs **18**–**20** with different chain length not only enhanced molecular binding ability but also significant molecular selectivity upon inclusion complexation with homologous steroids, except for resulting complex of **20** with TCA. The stability constants for the inclusion complexation of hosts and the each steroid molecule decreased in the following order: DCA > CA > GCA > TCA. The hydroxyl group at the C7 carbon atom of CA, GCA and TCA guests prevented deeper inclusion of the steroids in the *β*-CD cavity than that of DCA guest. On the other hand, the tether length of the host and induced-fit interactions also played crucial roles in the selective molecular binding process of modified *β*-CD **18**–**20** with guests. Host **19** possessing suitable tether length could

encapsulate more tightly the steroid guests than the other, through the size/shape-matching and the induced-fit interactions between the host and guest.

Thermodynamically, the inclusion complexation of **18–20** with steroid guests is entirely driven by favorable enthalpy contribution with negative or minor positive entropy change [25]. The strong interaction between host and guest leads to the more favorable negative enthalpy change, which is counteracted by the relative more unfavorable negative entropy change. The introduction of anthracene group with different chain length, and additional binding site to CD rim can significantly enhance the binding ability of parent CD toward steroid guests.

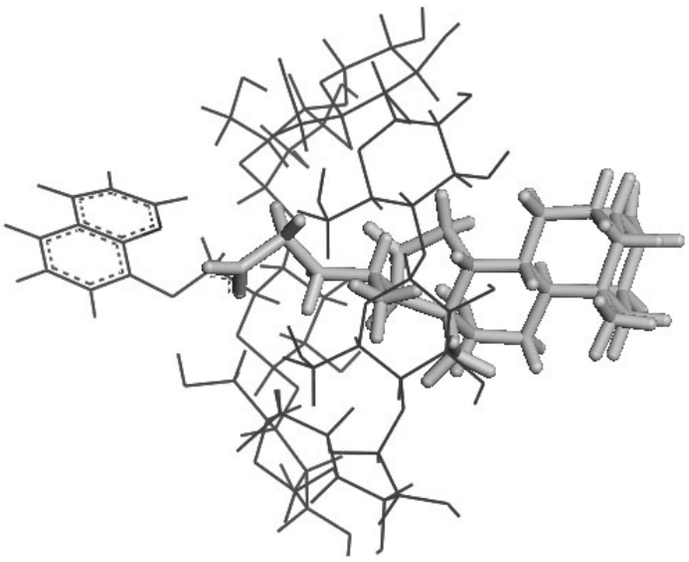


Figure 5. The possible binding mode of **22** with DCA

3.4.2. Quinolinyl- and naphthyl-modified β-CDs

The binding behaviors of two *β*-CD derivatives bearing 8-hydroxyquinolino and triazolylquinolino groups (**21** and **22**) with bile salts have been studied in aqueous buffer solution by means of microcalorimetrical titration [26]. The results showed that the host–guest binding behaviors were mainly driven by the favorable enthalpy changes, accompanied by the unfavorable entropy changes, and the hydrogen-bonding interactions and van der Waals interactions were the main driven forces governing the host–guest binding.

The binding stoichiometry of the permethylated *β*-CD derivatives **23** and **24** with bile salts has been determined by the Job's plot method, which showed that hosts and guests formed 1:1 complexes [24]. Thermodynamically, hosts **23** and **24** show much higher binding ability to bile salts than permethylated *β*-CD **17** when the naphthalene (or quinoline) sidearm is appended on it. The pronounced enhancement of complex stabilities for hosts **23** and **24** can be attributed to the cooperative complex interactions of both the cavity of permethylated *β*-CD and the chromophore sidearms. Furthermore, it should be mentioned that host **24** always forms more stable complexes with bile guests than host **23**, which indicates that the N atom on the quinoline ring plays a crucial role during the course of recognition of bile guests.

Hosts	Guests	pH	*Ks*	$\Delta H°$	$T\Delta S°$	Methods	Refs.
1	CA	7.2 (PBS)	4068	–22.98	–2.38	ITC	17
	DCA	7.2 (PBS)	4844	–25.79	–4.76	ITC	17
	GCA	7.2 (PBS)	2394	–22.99	–3.7	ITC	17
	TCA	7.2 (PBS)	2293	–23.77	–4.59	ITC	17
2	CA	7.2 (PBS)	11160	–25.53	–2.43	ITC	17
	DCA	7.2 (PBS)	7705	–32.16	–9.98	ITC	17
	GCA	7.2 (PBS)	2075	–25.90	–6.97	ITC	17
	TCA	7.2 (PBS)	2309	–26.89	–7.69	ITC	17
3	CA	7.2 (PBS)	16920	–28.11	–3.98	ITC	17
	DCA	7.2 (PBS)	9382	–35.78	–13.11	ITC	17
	GCA	7.2 (PBS)	3904	–24.74	–4.24	ITC	17
	TCA	7.2 (PBS)	2796	–20.37	–0.7	ITC	17
4	CA	7.2 (PBS)	4832	–24.90	–3.87	ITC	17
	DCA	7.2 (PBS)	4034	–38.91	–18.33	ITC	17
	GCA	7.2 (PBS)	2221	–19.75	–0.65	ITC	17
	TCA	7.2 (PBS)	1322	–32.75	–14.93	ITC	17
5	CA	7.2 (PBS)	11060	–36.44	–13.36	ITC	18
	DCA	7.2 (PBS)	11350	–41.15	–18.01	ITC	18
	GCA	7.2 (PBS)	3050	–25.48	–5.59	ITC	18
	TCA	7.2 (PBS)	3061	–18.43	1.47	ITC	18
6	CA	7.2 (PBS)	25315	–34.26	–9.13	ITC	18
	DCA	7.2 (PBS)	30300	–38.13	–12.55	ITC	18
	GCA	7.2 (PBS)	3098	–25.82	–5.89	ITC	18
	TCA	7.2 (PBS)	4659	–14.86	6.08	ITC	18
7	CA	7.2 (PBS)	25850	–23.53	1.65	ITC	18
	DCA	7.2 (PBS)	24785	–27.59	–2.51	ITC	18
	GCA	7.2 (PBS)	4722	–21.22	–0.25	ITC	18
	TCA	7.2 (PBS)	3022	–24.29	–4.43	ITC	18
8	CA	7.2 (PBS)	1726	–31.0	–13.3	ITC	19
	DCA	7.2 (PBS)	2839	–34.8	–14.9	ITC	19
	GCA	7.2 (PBS)	1032	–25.7	–8.5	ITC	19
	TCA	7.2 (PBS)	1003	–26.6	–9.5	ITC	19
9	CA	7.2 (PBS)	2567	–29.3	–9.9	ITC	19
	DCA	7.2 (PBS)	3137	–34.0	–14.0	ITC	19
	GCA	7.2 (PBS)	2898	–31.2	–11.4	ITC	19
	TCA	7.2 (PBS)	2284	–30.0	–10.8	ITC	19
10	CA	7.2 (PBS)	2605	–28.6	–9.1	ITC	19
	DCA	7.2 (PBS)	3813	–33.7	–13.3	ITC	19
	GCA	7.2 (PBS)	3140	–29.6	–9.7	ITC	19
	TCA	7.2 (PBS)	2402	–28.8	–9.5	ITC	19

Hosts	Guests	pH	*Ks*	$\Delta H°$	$T\Delta S°$	Methods	Refs.
11	CA	7.2 (PBS)	2020	–23.2	–4.3	ITC	20
	DCA	7.2 (PBS)	2310	–32.1	–12.9	ITC	20
	GCA	7.2 (PBS)	1110	–23.4	–6.0	ITC	20
	TCA	7.2 (PBS)	1060	–23.1	–5.8	ITC	20
12	CA	7.2 (PBS)	6680	–37.9	–14.5	ITC	20
	DCA	7.2 (PBS)	6770	–46.0	–24.1	ITC	20
	GCA	7.2 (PBS)	1760	–24.9	–6.4	ITC	20
	TCA	7.2 (PBS)	1470	–24.3	–6.2	ITC	20
13	CA	7.2 (PBS)	871	–26.7	–9.9	ITC	21
	DCA	7.2 (PBS)	1087	–33.1	–15.8	ITC	21
	GCA	7.2 (PBS)	428	–28.3	–13.3	ITC	21
	TCA	7.2 (PBS)	391	–25.7	–10.9	ITC	21
14	CA	7.2 (PBS)	8689	–41.7	–19.2	ITC	21
	DCA	7.2 (PBS)	9962	–50.5	–27.9	ITC	21
	GCA	7.2 (PBS)	1105	–30.5	–13.1	ITC	21
	TCA	7.2 (PBS)	809	–26.7	–10.1	ITC	21
15	CA	7.4 (Tris–NaCl)	2510	–7.9	38.6	ITC	23
	DCA	7.4 (Tris–NaCl)	4429	–10.65	34.0	ITC	23
	GCA	7.4 (Tris–NaCl)	1764	–8.2	34.5	ITC	23
	TCA	7.4 (Tris–NaCl)	1399	–8.75	31.0	ITC	23
16	CA	7.4 (Tris–NaCl)	2693	–5.7	46.6	ITC	23
	DCA	7.4 (Tris–NaCl)	6276	–6.8	49.9	ITC	23
	GCA	7.4 (Tris–NaCl)	1958	–7.9	36.6	ITC	23
	TCA	7.4 (Tris–NaCl)	2148	–7.2	39.6	ITC	23
17	CA	7.2 (PBS)	61			ITC	24
	DCA	7.2 (PBS)	774			ITC	24
	GCA	7.2 (PBS)	228			ITC	24
	TCA	7.2 (PBS)	162			ITC	24
18	CA	7.2 (PBS)	11760	–42.70	–19.47	ITC	25
	DCA	7.2 (PBS)	15030	–42.72	–18.87	ITC	25
	GCA	7.2 (PBS)	3870	–25.23	–4.75	ITC	25
	TCA	7.2 (PBS)	2647	–20.99	–1.47	ITC	25

Hosts	Guests	pH	*Ks*	Δ*H*°	*T*Δ*S*°	Methods	Refs.
19	CA	7.2 (PBS)	18965	–32.37	–7.95	ITC	25
	DCA	7.2 (PBS)	22485	–36.48	–11.46	ITC	25
	GCA	7.2 (PBS)	4888	–21.61	–0.56	ITC	25
	TCA	7.2 (PBS)	3755	–19.15	0.7	ITC	25
20	CA	7.2 (PBS)	11850	–33.23	–9.98	ITC	25
	DCA	7.2 (PBS)	13365	–39.57	–16.20	ITC	25
	GCA	7.2 (PBS)	4254	–20.07	0.65	ITC	25
	TCA	7.2 (PBS)	1833	–26.58	–7.96	ITC	25
21	CA	7.2 (PBS)	2216	–25.04	–5.94	ITC	26
	DCA	7.2 (PBS)	2007	–51.92	–33.07	ITC	26
	GCA	7.2 (PBS)	2434	–31.07	–11.74	ITC	26
	TCA	7.2 (PBS)	3478	–23.98	–3.76	ITC	26
22	CA	7.2 (PBS)	2443	–35.60	–16.25	ITC	26
	DCA	7.2 (PBS)	3177	–33.89	–13.90	ITC	26
	GCA	7.2 (PBS)	2811	–34.94	–15.24	ITC	26
	TCA	7.2 (PBS)	2809	–30.37	–10.68	ITC	26
23	CA	7.2 (Tris–HCl)	910			Fluorescence	24
	DCA	7.2 (Tris–HCl)	4320			Fluorescence	24
	GCA	7.2 (Tris–HCl)	4340			Fluorescence	24
	TCA	7.2 (Tris–HCl)	3820			Fluorescence	24
24	CA	7.2 (Tris–HCl)	3290			Fluorescence	24
	DCA	7.2 (Tris–HCl)	7460			Fluorescence	24
	GCA	7.2 (Tris–HCl)	10690			Fluorescence	24
	TCA	7.2 (Tris–HCl)	8710			Fluorescence	24
25	CA	7.4 (Tris–NaCl)	7400	–22.3	–0.2	ITC	27
	DCA	7.4 (Tris–NaCl)	6700	–32.1	–10.2	ITC	27
26	CA	7.4 (Tris–NaCl)	1280	–28.3	–10.5	ITC	27
	DCA	7.4 (Tris–NaCl)	2570	–33.3	–13.8	ITC	27

Hosts	Guests	pH	*Ks*	Δ*H*°	*T*Δ*S*°	Methods	Refs.
27	CA	7.0 (PBS)	1650			Fluorescence	38
	DCA	7.0 (PBS)	2660			Fluorescence	38
28	CA	7.0 (PBS)	588			Fluorescence	38
	DCA	7.0 (PBS)	1520			Fluorescence	38
29	CA	7.0 (PBS)	60.4			Fluorescence	38
	DCA	7.0 (PBS)	1030			Fluorescence	38
30	CA	aqueous solution	–			Fluorescence	36
	DCA	aqueous solution	–			Fluorescence	36
	GCA	aqueous solution	–			Fluorescence	36
31	CA	aqueous solution	–			Fluorescence	36
	DCA	aqueous solution	–			Fluorescence	36
	GCA	aqueous solution	–			Fluorescence	36
32	CA	aqueous solution	–			Fluorescence	36
	DCA	aqueous solution	–			Fluorescence	36
	GCA	aqueous solution	–			Fluorescence	36

PBS: Phosphate Buffer Solution; ITC: Isothermal Titration Calorimetry;

Tris: Tris(hydroxymethyl)aminomethane;

–: The guest-induced variations in the excimer emission are too small for these values to be determined.

Table 1. Complex stability constants (*Ks*/M^{-1}), enthalpy (Δ*H*°/(kJ·mol^{-1})), and entropy changes (*T*Δ*S*°/(kJ·mol^{-1})) for intermolecular complexation of bile salts with natural *β*-CD and its mono-modified derivatives in aqueous solution

All the permethylated *β*-CD derivatives (**17**, **23** and **24**) present the weakest binding ability to CA guest because the cavity of permethylated *β*-CD possesses a broader hydrophobic region in comparison with **1**, and then permethylated *β*-CD is more suitable to include bile guests with longer tails (GCA and TCA) than **1** [24]. 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 **17**, **23** and **24** than CA. 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 permethylated *β*-CDs.

4. Bridged CD series

4.1. Binding modes for bile salts and bridged CD series

4.1.1. Diseleno- and bipyridine-bridged β-CDs

ROESY experiments for the complexes of CDs (**25**, **26**, **33**, and **35**) and DCA have been performed to illustrate the binding modes between the CDs and bile salts [27]. The results show that the bridge linker does not interact with DCA and the bile salt molecule is not

cooperatively bound by the two cavities of one dimer molecule. DCA is not included in the cavity of the dimer from the primary side (narrow open), but penetrates slightly into the cavity from the secondary side (wide open) using the side chain and D-ring moiety. For **33** (Figure 6), the A-ring moiety of DCA is simultaneously shallowly included in one of the cavities of another CD to form a liner structure. For monomer **25**, the D-ring moiety of DCA penetrates deep into the cavity of **25** from the secondary side. However, for monomer **26**, DCA is included in the cavity of **26** from the secondary side by its A-ring moiety, differing from other CDs (by D-ring moiety).

To further obtain the information about the binding modes of bile salts with diseleno- and bipyridine-bridged *β*-CDs, 2D ROESY spectra for typical host–guest pairs have also been determined by Liu et al. [28]. For dimer **35** and CA, the results indicate that the carboxylate side chain and D-ring of CA may penetrate into the CD cavity from the secondary side shallowly and two CA molecules are bound separately into two cavities of **35** from the secondary side, which is consistent with the 1:2 binding stoichiometry (Figure 7a). For dimer **39** and DCA, the results are quite different and show a 1:1 cooperative binding mode. The A-ring of DCA penetrates deeply into one CD cavity of **39**, attributing to the less steric hindrance and higher hydrophobicity of the substituent group on the C-7 position of DCA (Figure 7b). Under the same experiment using DCA as guest, host **38** adopts a different binding mode from **39**. The carboxylate side chain of two DCA molecules deeply penetrates into the CD cavity of **38** from the secondary side separately.

4.1.2. Oligoethylenediamino-bridged β-CDs

To obtain the information about the binding modes between bile salts and oligoethylenediamino-bridged *β*-CD dimers (**42**–**44**), 2D ROESY spectra for typical host–guest pairs have been determined by Liu et al. [29]. The results of ROESY experiments indicated that the D ring and side-chain of bile salt guest enter one *β*-CD cavity from the wide opening, and the linker group is partially self-included in the other *β*-CD cavity (Figure 8).

4.1.3. Aromatic diamino- and sulfonyldianiline-bridged β-CDs

From ROESY experiments, Zhao et al. found that the D-ring of CA is wholly included in the CD cavity of **45** from the wide opening, while the side-chain is located near the narrow opening of CD cavity and folded toward the steroid body and the phenyl moiety is not driven out of the CD cavity even after the guest inclusion [30]. Similar binding mode is also observed in other cases of **45**/bile salts complexes.

The binding modes between the aromatic diamino-bridged *β*-CDs **46**–**48** and bile salts have also been investigated by Zhao et al. via 2D ROESY experiments and the results show that the D-ring of CA is wholly included in the CD cavity with the wide opening, while the side chain is located near the narrow opening of the CD cavity and is folded toward the steroid body [31]. The phenyl moiety is not driven out of the CD cavity even after the guest inclusion.

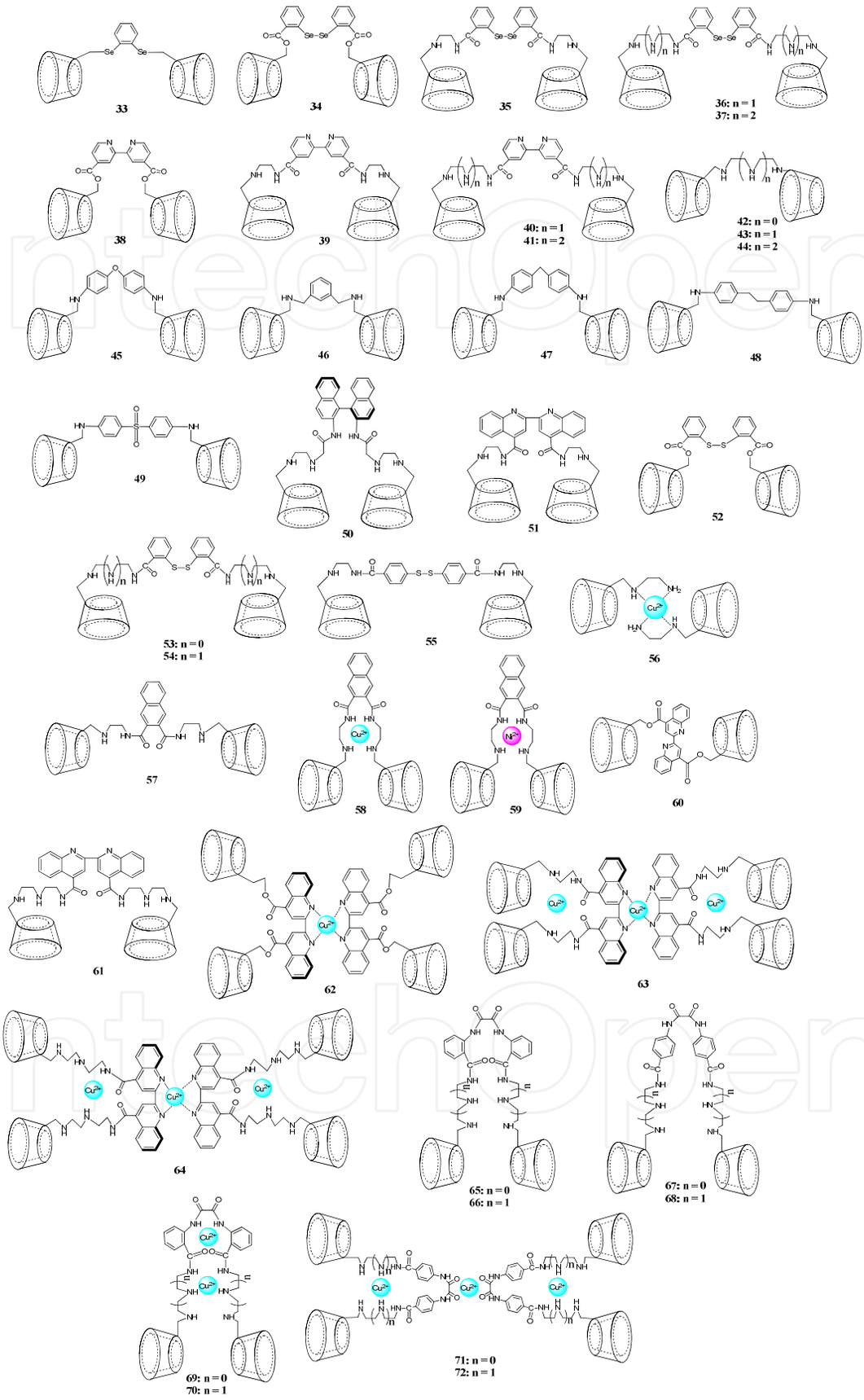


Figure 6. Structures of bridged β-CDs

To obtain the information about the binding modes between bile salts and sulfonyldianiline-bridged *β*-CD **49**, 2D ROESY spectra for typical host–guest pairs have further been determined by Zhao et al. [32]. The correlation signals, along with the 1:1 binding stoichiometry, jointly indicate a host-linker-guest binding mode between **49** and CA. That is, upon complexation with **49**, the carboxylate tail and the D ring of CA penetrate into one CD cavity of **49** from the wide opening deeply, while the phenyl moiety of the CD linker is partially self-included in the other *β*-CD cavity. Similar binding modes are also observed in other cases of **49**/bile salt complexes.

4.1.4. Binaphthyl-, biquinoline- and dithio-bridged β-CDs

The binding modes of binaphthyl-, biquinoline- and dithio-bridged *β*-CDs (**50**–**55**) and bile salts have been investigated by 2D ROESY experiments in aqueous solution [33]. The results show that CA enters the CD cavity of **53** from the second side of CD with the side chain and D-ring. The side chain with the negative carboxylate group of CA moves toward the positive protonated amino group of **53**. The other binaphthyl-, biquinoline- and dithio-bridged *β*-CDs/bile salts complexes show a similar binding mode as the complex **53**/CA, with only a slight degree of difference in the depth of guest insertion.

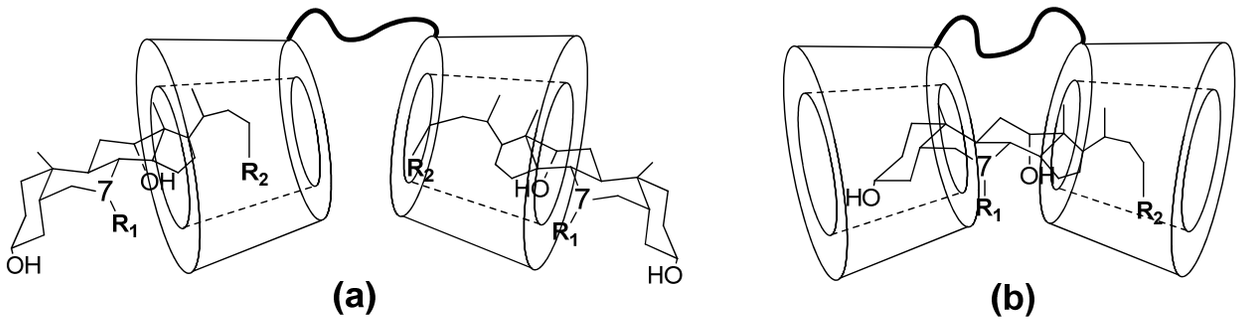


Figure 7. The possible binding modes of **35** with CA (a) and **39** with DCA (b)

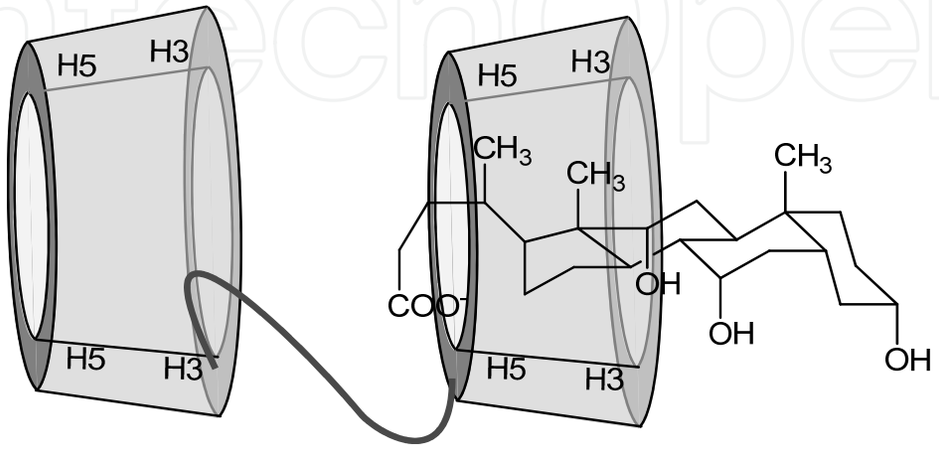


Figure 8. The possible binding mode of **42**–**44** with CA

4.2. Complexation thermodynamics for bile salts and bridged CD series

4.2.1. Diseleno- and bipyridine-bridged β-CDs

To elucidate the difference in binding behavior between the CD dimer and monomer, two CD dimers (**33** and **35**) and their monomer analogs (**25** and **26**) have been used for titration microcalorimetry with CA and DCA [27]. It is interesting that the results of the thermodynamic measurements show a 1:1 binding stoichiometry for hosts **25**, **26** and **33**, but 1:2 stoichiometry for host **35**. In addition, although the stability constants for the complexation between dimer **33** and the bile salts are much larger than those for monomer **26**, the long-linked dimer **35** unusually displays a lower cavity binding ability than its corresponding monomer **25** upon complexation with both guests CA and DCA. The enhancement of the binding ability of dimer **33** compared to monomer **26** could be ascribed not only to the cooperative binding but also partly to the peculiar self-inclusion conformation of **26** that leads to more unfavorable entropy changes, especially for the **26**–CA pair. For **35**, the two guest molecules are separately and independently included in the two cavities of **35** because the longer linker, especially the ethylenediamino moiety of dimer **35**, makes it possess a relatively large conformational freedom. As the considerable entropy loss cancels the advantage of enthalpy gain, dimer **35** displays relatively weak binding abilities. Both hosts **35** and **25** show similar binding ability for DCA and CA. The reason is that either binding with host **35** or host **25**, the two guest bile salts are included into the cavity of CDs by its D-ring and side-chain moiety, which reduces the influence of the substituent in C7. However, while binding with hosts **33** and **26**, the A-ring moiety participates in the binding process, so the more hydrophobic C7 substituent of DCA makes it bind more strongly with the host CDs, giving the higher binding constants than with CA, especially for host **26**.

Either for diseleno-bridged β-CDs (**34**–**37**) or for bipyridine-bridged β-CDs (**38**–**41**), the host–guest stoichiometry changes in the same order, that is, from 1:2 to 1:1 with the increase of spacer length [28]. For diseleno-bridged β-CDs, only **36** and **37** adopt the 1:1 binding mode. However, for bipyridine-bridged β-CDs, only host **38** adopts the 1:2 binding mode; the others all show the 1:1 cooperative binding mode. The thermodynamic results reveal that, with the longest spacer, **37** gives the largest stability constants in all diseleno-bridged β-CDs, while the largest stability constants of bipyridine-bridged β-CDs toward each guest molecule is obtained by the dimers **39** and **40** with the moderate spacer lengths, which suggests that only the CD dimers possessing the proper spacer length can give the perfect cooperative binding toward guests.

For the dimers adopting 1:1 cooperative binding mode, the enthalpy changes are not only the main contribution to the binding process but also the determining factor for the binding abilities [28]. Comparing the diseleno-bridged β-CDs with bipyridine-bridged β-CDs, all of the bipyridine-bridged β-CDs display much stronger binding abilities toward bile salts than corresponding diseleno-bridged β-CDs, which indicate that the presence of rigid spacer favors formation of a relatively fixed binding mode and results in the close contact between

two CD cavities and guest molecule, leading to the stronger binding abilities. On the other hand, due to the presence of the bipyridine fragment**,** the hydrogen bond between the hydroxyl group of the bile salt and the nitrogen atom of bipyridine might also be taken as a plausible explanation for the strong binding abilities of bipyridine-bridged *β*-CDs as compared with diseleno-bridged *β*-CDs. Upon complexation with CA and DCA, all dimer hosts adopting a 1:1 binding mode show higher binding abilities than native *β*-CD **1** due to more favorable enthalpy changes, which perfectly confirms the advantage of cooperative binding of guests by two CD cavities.

4.2.2. Oligoethylenediamino-Bridged β-CDs

1:1 binding stoichiometry is observed for all the complexes between bile salts and oligoethylenediamino-bridged *β*-CDs (**42**–**44**) [29]. The inclusion complexation of bile salts with **42**–**44** is driven by favorable enthalpy changes, accompanied by slight to moderate entropy loss. Interestingly, the enthalpy changes for the inclusion complexation of **42**–**44** increased, while the entropic changes decreased, with the elongation of the linker group, giving a binding constant **42** > **43** > **44**. The stronger binding of bile salts by the short-linked *β*-CD dimer is not thermodynamically accomplished by an increase of the originally favorable enthalpy gain, but by a reduction of the unfavorable entropy loss. The short-linked *β*-CD dimer, with a better size and hydrophobicity match to bile salts, may experience more extensive desolvation upon complexation, and thus exhibits the less unfavorable entropy loss. With the elongation of linker group, the protonated amino group in the linker is located distant from the anionic carboxylate (or sulfonate) tail of bile salt, which consequently weakens the electrostatic interactions between the linker group and bile salt. Moreover, the increase of the number of -NH- fragments in the linker group decrease the hydrophobicity of *β*-CD dimer to some extent, which is also unfavorable to the hydrophobic interactions between host and guest.

The stability constants of the complexes formed by *β*-CD dimers **42**–**44** with bile salts are larger than those of the complexes formed by native *β*-CD **1** [29]. These enhanced binding abilities of *β*-CD dimers may be mainly attributed to the cooperative host-linker-guest binding mode between host and guest. The electrostatic interactions between the protonated amino groups in the linker and the anionic carboxylate (or sulfonate) tail of bile salt may strengthen the inclusion complexations of these *β*-CD dimers with bile salts. Moreover, the hydrogen bond interactions of the hydroxyl groups of *β*-CD and the -NH- fragments of the oligo(ethylenediamino) linker with the carboxylate (or sulfonate) tail of bile salt also contribute to the enhanced binding abilities of *β*-CD dimers **42**–**44**.

Compared with CA, GCA and TCA, DCA possesses a more hydrophobic structure due to the absence of C-7 hydroxyl group, which consequently leads to stronger hydrophobic interactions between host and guest. Therefore, DCA gives the highest binding abilities among the bile salts examined upon complexation with most CDs [29]. Possess more polar side-chains, GCA and TCA show weak binding abilities upon inclusion complexation

complexation with β-CD dimers due to the relatively poor hydrophobic interactions between host and guest.

4.2.3. Aromatic diamino- and sulfonyldianiline-bridged β-CDs

The stoichiometry for the inclusion complexation of **45** with bile salts were determined by the continuous variation method and the results showed a 1:1 inclusion complexation between **45** and bile salts [30]. The stability constants for the inclusion complexation of **45** with bile salts are much higher than those values for the native β-CD **1**. These enhanced binding abilities of **45** may be mainly attributed to the cooperative host-linker-guest binding mode between host and guest because the linker group provides some additional binding interactions towards the accommodate guest. Host **45** displays higher binding ability for CA than for DCA due to the hydrogen bond interactions between the 7-hydroxy group of CA and the 2- and 3-hydroxy group of CD. Host **45** shows the weaker binding abilities upon inclusion complexation with GCA and TCA than that of CA and DCA because GCA and TCA are unfavorable to insert into the cavity from the second side of CD cavity with their D ring attributing to the more hydrophilic tail attached to the end of the D ring.

The stoichiometries for inclusion complexation of aromatic diamino-bridged β-CDs **46–48** with bile salts were further determined by the continuous variation method and the results show that all the hosts and guests form 1:1 complexes [31]. β-CD dimers **46–48** also show enhanced binding ability toward bile salts as compared with β-CD **1**. The enhanced binding abilities of aromatic diamino-bridged β-CDs may be mainly attributed to the cooperative host-linker-guest binding mode between host and guest. 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.

Unlike the β-CD **1**, the bridged β-CDs **46–48** show larger binding constants for CA than for DCA [31]. Among them, the host **47** gave the highest stability constant for inclusion complexation with CA. One possible reason for the stronger affinity for CA may involve hydrogen-bond interactions between the 7-hydroxy group of CA and the 2- and 3-hydroxy groups of CD, which subsequently strengthen the host–guest association. Moreover, all the hosts show lower binding ability for complexation with GCA and TCA as compared with complexation with CA and DCA. The highest binding constants towards GCA and TCA are with host **47**. The universally decreased binding ability toward GCA and TCA must be related to structure differences between CA and DCA. Attributing to the more hydrophilic tail, which is attached to the end of the D ring, GCA and TCA are unfavorable for insertion into the cavity from the second side of the β-CD cavity with their D ring.

The binding constants for the complexation of each bile salt by hosts **46–48** increases in the following order: **47** > **48** > **46** [31]. That is, host **47** with a tether of moderate length and rigidity among the β-CD dimers studied is the most suitable for inclusion complexation with bile salts. This may be attributable to the strict size fit between these bile salts and the moderate length-tethered β-CD dimer **47**, which consequently exhibits strong van der Waals and hydrophobic interactions between host and guest.

The stoichiometry for the inclusion complexation of sulfonyldianiline-bridged β-CD **49** with bile salts has also been determined by the "continuous variation" method and the results indicate that all the bile salts can form 1:1 complexes with **49** [32]. Thermodynamically, the binding constants of **49** with bile salts are larger than those of native β-CD **1**. The enhanced binding abilities of **49** may be also mainly attributed to the cooperative host-linker-guest binding mode between host and guest. In addition to the association of the CD cavity with a guest molecule, the linker group provides some additional binding interactions towards the accommodate guest. Distinctly, the binding constant is significantly higher for DCA compared to CA by native β-CD **1**. However, different from native β-CD **1**, sulfonyldianiline-bridged β-CD **49** reverses this binding selectivity, showing larger binding constants for CA than DCA. One possible reason for the stronger affinity for CA may involve H-bond interactions between CA and CD, which subsequently strengthen the host-guest association. Moreover, all the hosts show a weaker binding ability upon complexation with GCA and TCA than with CA and DCA. The universal decreased binding ability toward GCA and TCA must relate to the structure differences from CA and DCA. Attributing to the more hydrophilic tail, which is attached to the end of the D ring, GCA and TCA are unfavorable to insert into the cavity from the second side of β-CD cavity with their D ring. It is worthy to note that the binding ability of **49** is significantly larger for TCA than for GCA, which leads to a relatively strong molecular selectivity.

4.2.4. Binaphthyl-, biquinoline- and dithio-bridged β-CDs

The stoichiometric ratios from the binding patterns for the titrations of steroids with binaphthyl-, biquinoline- and dithio-bridged β-CDs **50**–**55** fell within the range of 1.8-2.1, which clearly indicates that the majority of the inclusion complexes have a 1:2 stoichiometry of steroids and bridged β-CDs [33]. Thermodynamically, bridged β-CD **52**, possessing a relatively short and rigid tether without amino groups, still gives an enhanced binding ability upon complexation with steroids, except TCA, when compared its one single unit of cavity with that of native β-CD **1**. The enthalpy changes for the inclusion complexation of bridged β-CD **52** with DCA and CA are more negative than that of native β-CD **1**, resulting in the relatively stronger binding. On the other hand, the enthalpy change for the complexation of **52** with DCA is higher than that with CA, which directly contributes to the increased complex stability. It is reasonable that, possessing the more hydrophobic structure due to the absence of C-7 hydroxyl group as compared with CA, DCA is easier to bind into the β-CD cavity than CA, which should lead to the more favorable van der Waals interactions.

All the complexation of aminated bridged β-CDs (**50**, **51**, and **53**–**55**) toward DCA and CA give more negative enthalpy changes as compared with that of neutral bridged β-CD **52**, validating the contribution of the attractive electrostatic interactions between positively charged protonated amino group of β-CD tethers and negatively charged carboxylate group of DCA and CA [33]. Accompanied with the more exothermic reaction enthalpies, the inclusion complexation of DCA and CA by aminated bridged β-CDs (**50**, **51**, and **53**–**55**) exhibits more unfavorable entropy changes compared to that for neutral bridged bis(β-CD)

52, which possibly originates from the conformation fixation of host and guest and the rigid complex formation upon complexation.

Mostly, bridged *β*-CDs **50**, **51**, and **53–55** give the lower binding ability upon complexation with GCA and TCA as compared with the complexation with CA and DCA, which is similar as the complexation of *β*-CD **1** and bridged *β*-CD **52** [33]. The universal decreased binding ability toward GCA and TCA must relate to the structure differences from CA and DCA. The more polar side chains at C23 for GCA and TCA remarkably affect their binding thermodynamics.

4.3. Binding modes for bile salts and metallobridged CD series

4.3.1. Metallobridged β-CDs with naphthalenecarboxyl linkers

2D ROESY NMR and circular dichroism spectroscopy experiments for the complexes of bile salts with bridged and metallobridged CDs with naphthalenecarboxyl linkers have been performed by Liu et al. to investigate the binding modes between host and guests [34]. The result of **57**/DCA complex showed that the guest DCA was included in the *β*-CD cavity with the D-ring and the carboxylic tail located near the narrow opening but the B-ring located near the wide opening and the naphthyl group was excluded from the *β*-CD cavity upon inclusion complexation. Moreover, the result of 2D ROESY NMR showed that the ethylenediamino moiety of the linker group was also partially self-included in the *β*-CD cavity from the narrow opening. Similar results were also found in other ROESY experiments of hosts **57** and **59** with bile salts.

4.3.2. Metallobridged β-CDs with biquinoline linkers

2D NMR experiments in D_2O and molecular modeling studies for the complexes of bridged and metallobridged *β*-CDs with biquinoline linkers and bile salts have been performed by Liu et al. to deduce the binding modes between the bile salts and *β*-CD dimers [35,36]. The results show that a cooperative "host-tether-guest" binding mode is operative in the association of *β*-CD dimers with a guest molecule; upon complexation with *β*-CD dimers, the guest steroid is embedded into one hydrophobic *β*-CD cavity from the primary side, while the tether group is partly self-included in the other cavity. In the metallobridged *β*-CDs, the tether group is entirely excluded from the *β*-CD cavities as a result of metal coordination. This arrangement allows two side groups of the guest molecule to be embedded into the hydrophobic *β*-CD cavities from the primary side of the *β*-CD to form a sandwich host–guest inclusion complex.

4.3.3. Metallobridged β-CDs with oxamidobisbenzoyl linkers

^{1}H ROESY experiments have been performed in D_2O to investigate the binding modes between bridged and metallobridged *β*-CDs with oxamidobisbenzoyl linkers and bile salts [37]. The results show a "host-linker-guest" binding mode between **66** and CA. That is, upon inclusion complexation with *β*-CD dimer, the carboxylate tail and the D-ring of CA enter

into one CD cavity of **66** from the wide opening, while the linker group of **66** is partially self-included in the other CD cavity (Figure 9a). A similar binding mode is also observed for the inclusion complexation of **66** with DCA.

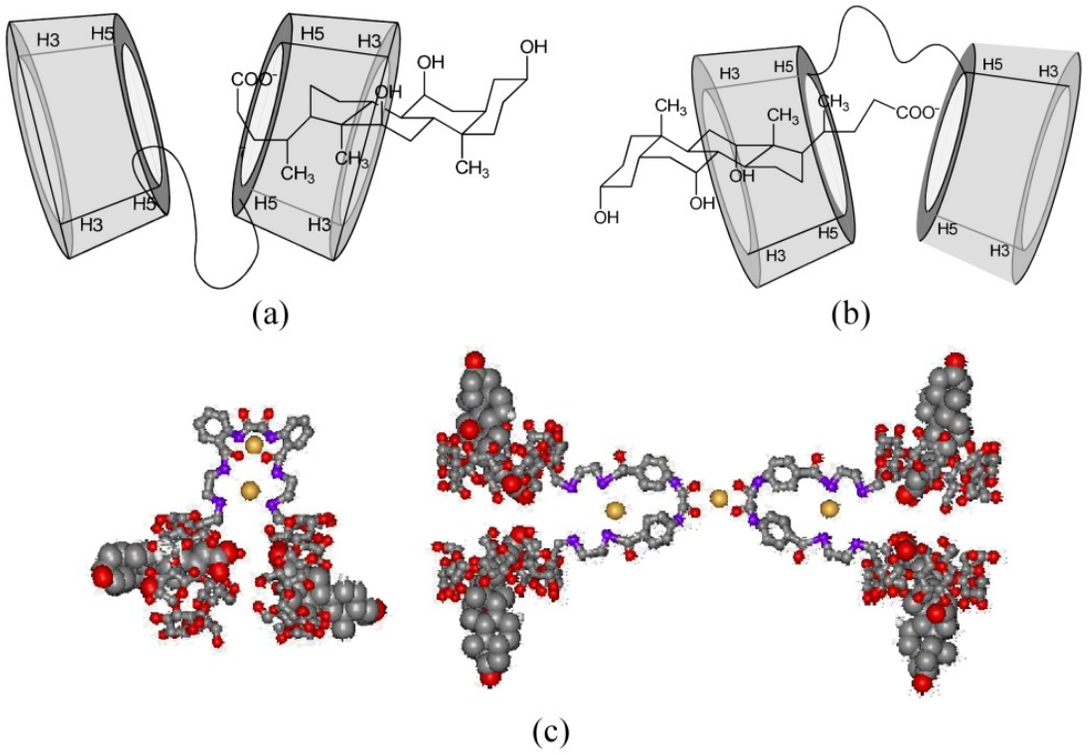


Figure 9. The possible binding modes of **66** (a) and **67** (b) with CA, and the possible binding modes of **69–72** with bile salts

With a shallowly self-included conformation, *β*-CD dimers **65**, **67**, and **68** show a binding mode different from that of **66**. For example, for **67**/CA complex, 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 (Figure 9b). **65**/CA, **65**/DCA, **67**/DCA, **68**/CA, and **68**/DCA complexes show a similar binding mode to the **67**/CA complex.

In the cases of the metallobridged *β*-CDs **69–72**, 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 *β*-CD is occupied by a bile salt (Figure 9c).

4.4. Complexation thermodynamics for bile salts and metallobridged CD series

4.4.1. Metallobridged β-CDs with naphthalenecarboxyl linkers

The interactions between hosts (**27–29**, **57–59**) and bile salts have been studied by Liu and Ueno et al. by the method of fluorescence [34,38]. The results show that all the hosts (**27–29**,

57–59) can form 1:1 complexes with bile salts CA and DCA. Thermodynamically, bridged β-CDs possess much stronger binding abilities compared with mono-modified β-CDs. These enhanced binding abilities should be attributed to cooperative binding of the β-CD cavity and the linker group towards the guest molecule, leading to greatly strengthened van der Waals and hydrophobic interactions between host and guest when compared with mono-modified β-CDs. Furthermore, after metal coordination, the metallobridged bis(β-CD)s **58** and **59** significantly enhance the original binding ability of native β-CD **1**, mono-naphthyl-modified β-CDs **27–29** and even parent bridged β-CD **57**. This enhancement may be subjected to a multiple recognition mechanism of metallobridged β-CDs towards model substrates. On one hand, the coordination of a metal ion to the linker group shortens the effective distance of two β-CD cavities to some extent and thus improves the size-fit degree between host and guest. On the other hand, the electrostatic attraction between the anionic carboxyl group of guest bile salt and the coordinated metal ion of metallobridged β-CD may also favour the host–guest binding to some extent.

4.4.2. Metallobridged β-CDs with biquinoline linkers

The interactions between host **51** and bile salts have been investigated by Liu et al. by the method of fluorescence [35]. The results show that all the bile salts can form 1:1 complexes with **51**. Thermodynamically, the binding constants obtained for CA and DCA are much larger than those reported for mono-modified β-CDs by Ueno et al. under practically the same experimental conditions [38]. This enhancement is probably due to the cooperative binding of the steroids by **51**. The complex stability decreases in the order: DCA > CA > GCA > TCA. The highest affinity for DCA is likely to arise from its more hydrophobic steroid skeleton. Host **51** shows comparable affinities toward CA and GCA, whereas TCA, possessing a highly polar anionic tail gives the lowest binding constant.

The stoichiometry for the inclusion complexation of hosts **60–64** with bile salts has also been determined by Job's method [36]. The results show that the stoichiometry of the inclusion complex formed by the **63**/CA system is likely to be 2:2, with intramolecular complexation. Stoichiometries of 1:1 (for bridged β-CD) or 2:2 (for metallobridged β-CD) were obtained in other similar cases of host–guest inclusion complexation. Thermodynamically, the stability constants of the complexes of bridged β-CDs **51**, **60** and **61** with bile salts are larger than those of the complexes formed by mono-modified β-CDs **27–29** by a factor of about 1.1 to around 200 benefitting from cooperative binding. In addition to inclusion complexation of the guest molecule within one hydrophobic CD cavity, the tether group located near the accommodated guest provides some additional interactions with the guest. In control experiments, the changes in the fluorescence spectra of **30–32** upon addition of guest steroids were too small to allow calculation of the stability constants, which may be attributed to strong self-inclusion of the substituted group preventing penetration of the guest into the CD cavity. Except **60**, the mono- and bridged-β-CDs display higher binding affinities for DCA than for CA. This stronger affinity for DCA is likely to arise from the

more hydrophobic steroid skeleton of this compound compared with that of CA. The abilities of both the short-tethered compound **60** and the long-tethered host **61** to bind CA and DCA are unexpectedly limited compared to the binding abilities of mono-modified CDs **27–29** due to the self-inclusion of the tether group for the short-tethered *β*-CD dimer **60** and the steric hinderance from the relatively large 5-amino-3-azapentyl-2-quinoline-4-carboxyamide fragment on the exterior of the CD cavity for the long-tethered *β*-CD dimer **61**, respectively.

The metal-ligated oligomeric *β*-CDs **62–64** have significantly enhanced (around 50–4100 higher) binding affinities for the tested guest molecules compared with those of the mono-modified *β*-CDs [36]. These results can be explained by considering a mechanism involving an uncommon multiple recognition behavior of metallobridged *β*-CDs. A metallobridged *β*-CD affords four hydrophobic binding sites (four CD cavities) and one (or three) metal coordination center(s), which jointly contribute to the cooperative binding of the oligomeric host with the guest molecule upon inclusion complexation. In addition, ligation of a Cu^{II} ion shortens the effective length of the tether to some extent and thus improves the size fit of the host with the guest. The cumulative result of these factors is that the metal-ligated *β*-CD oligomers have binding abilities around 6–200 times higher than those of their parent bridged *β*-CDs.

4.4.3. Metallobridged β-CDs with oxamidobisbenzoyl linkers

The stoichiometry for the inclusion complexation of hosts **65–72** with bile salts has been determined by Job's method [37]. The results indicate that each of the Job's plots for the inclusion complexation of **65–68** with bile salts shows a maximum at a *β*-CD dimer molar fraction of 0.5, confirming the 1:1 binding stoichiometry between host and guest. For the inclusion complexation of metallobridged *β*-CDs **69–72** with bile salts, however, each of the Job's plots shows a maximum at a bridged *β*-CD unit molar fraction of 0.33, which indicates 1:2 stoichiometry between each bridged *β*-CD unit and guest. The metallobridged *β*-CDs **69** and **70** may only bind two bile salts to form a stoichiometric 1:2 inclusion complex. However, the metallobridged *β*-CDs **71** and **72** may adopt intramolecular 2:4 stoichiometry upon inclusion complexation with bile salts. Thermodynamically, the binding constants for the inclusion complexation of CA and DCA with bridged *β*-CDs **65–68** are higher than the *K*s values reported for the inclusion complexation of these bile salts with native or mono-modified *β*-CDs [33,38]. These enhanced binding abilities highlight the inherent advantage of the cooperative "host-linker-guest" binding mode of bridged *β*-CDs **65–68**. 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.

The bile salts CA and DCA are better bound by bridged *β*-CD **65**, which possesses the shortest linker group, than by the long-linked bridged *β*-CDs [37]. This may be attributable to the strict size-fit between these bile salts and the short-linked bridged *β*-CD **65**, which

consequently gives strong van der Waals and hydrophobic interactions between host and guest.

Significantly, metallobridged *β*-CDs **69–72** show greatly enhanced binding abilities with regard to the bridged *β*-CDs **65–68** [37]. These significant enhancements in the binding abilities 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.

Except for **66**, all of the hosts examined display higher binding abilities for CA than for DCA [37]. 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.

4.4.4. Metallobridged β-CDs with aminated linkers

The microcalorimetric experiments of *β*-CD **1** and modified *β*-CDs **2**, **5**, **6**, **7** with bile acids showed typical titration curves of 1:1 complex formation [18]. However, metallobridged *β*-CD **56** displays a 1:2 host–guest binding stoichiometry. Thermodynamically, as compared with native *β*-CD **1**, most oligo(ethylenediamino)-*β*-CDs **2**, **5 6**, and their Cu^{II} complexes **7** and **56** show enhanced molecular binding abilities and guest selectivities towards bile acids. The inclusion complexation of bile acids with native *β*-CD **1** and their derivatives (**2**, **5**, **6**, **7**, and **56**) is absolutely driven by favorable enthalpy changes accompanying with moderate unfavorable or slightly favorable entropy changes. The favorable enthalpy change is attributed to the dominant contribution of the hydrophobic interactions. Meanwhile, the unfavorable entropy given by most of the complexes is due to the decrease of rotational and structural freedom upon complex construction.

As compared with native *β*-CD **1**, **5** shows increased binding abilities toward negatively charged bile acids guest molecules, which should be mainly due to the additional electrostatic interactions between the amino tether moiety of hosts and anionic carboxylate or sulfonate tail of guests [18]. Moreover, *β*-CD dimer **56** shows a larger binding constant upon inclusion complexation with CA and DCA than **5**. This may be attributed to that the coordination of copper ion onto the amino tether of CD affords a more positive charged environment as compared with its precursor **5**. Compared with **5**, host **6** also shows stronger binding abilities toward guest molecules. However, the introduction of copper actually decreases the original binding ability of **6** towards DCA and gives comparable stability constant upon complexation with CA. All the hosts, including native *β*-CD **1** and modified *β*-CDs **2**, **5**, **6**, **7**, and **56**, show the weaker binding abilities upon inclusion complexation with GCA and TCA than those of CA and DCA. It is also found that complexes stabilities enhance with the extended length of spacer for the same guest except for **2**/CA to **5**/CA. It is reasonable to believe that the increased stability is due to the enlarged hydrogen binding interactions.

Hosts	Guests	pH	*Ks*	Δ*H*°	*T*Δ*S*°	Methods	Refs.
33	CA	7.4 (Tris–NaCl)	6860	–30.5	–8.6	ITC	27
	DCA	7.4 (Tris–NaCl)	9700	–37.0	–14.3	ITC	27
35	CA	7.4 (Tris–NaCl)	2700	–27.1	–7.5	ITC	27
	DCA	7.4 (Tris–NaCl)	3300	–35.7	–15.7	ITC	27
36	CA	7.4 (Tris–NaCl)	4100	–24.9	–4.3	ITC	28
	DCA	7.4 (Tris–NaCl)	5400	–35.0	–13.7	ITC	28
37	CA	7.4 (Tris–NaCl)	5030	–29.1	–8.0	ITC	28
	DCA	7.4 (Tris–NaCl)	6100	–40.2	–18.6	ITC	28
39	CA	7.4 (Tris–NaCl)	12700	–32.4	–9.0	ITC	28
	DCA	7.4 (Tris–NaCl)	12400	–45.4	–22.0	ITC	28
40	CA	7.4 (Tris–NaCl)	12400	–25.5	–2.2	ITC	28
	DCA	7.4 (Tris–NaCl)	13100	–31.9	–8.3	ITC	28
41	CA	7.4 (Tris–NaCl)	6800	–25.4	–3.5	ITC	28
	DCA	7.4 (Tris–NaCl)	7500	–35.2	–13.1	ITC	28
42	CA	7.2 (PBS)	21065	–32.8	–8.1	ITC	29
	DCA	7.2 (PBS)	22780	–42.7	–17.9	ITC	29
	GCA	7.2 (PBS)	9707	–23.0	–0.3	ITC	29
	TCA	7.2 (PBS)	6848	–22.4	–0.5	ITC	29
43	CA	7.2 (PBS)	5868	–39.3	–17.7	ITC	29
	DCA	7.2 (PBS)	7017	–47.4	–25.5	ITC	29
	GCA	7.2 (PBS)	4031	–25.8	–5.2	ITC	29
	TCA	7.2 (PBS)	2947	–26.9	–7.1	ITC	29
44	CA	7.2 (PBS)	5606	–41.0	–19.6	ITC	29
	DCA	7.2 (PBS)	5511	–52.1	–30.7	ITC	29
	GCA	7.2 (PBS)	2847	–26.5	–6.9	ITC	29
	TCA	7.2 (PBS)	1877	–29.0	–10.3	ITC	29
45	CA	7.2 (PBS)	27050			Fluorescence	30
	DCA	7.2 (PBS)	22930			Fluorescence	30

Hosts	Guests	pH	*Ks*	$\Delta H°$	$T\Delta S°$	Methods	Refs.
	GCA	7.2 (PBS)	7200			Fluorescence	30
	TCA	7.2 (PBS)	17610			Fluorescence	30
46	CA	7.2 (PBS)	15310			Fluorescence	31
	DCA	7.2 (PBS)	8790			Fluorescence	31
	GCA	7.2 (PBS)	3040			Fluorescence	31
	TCA	7.2 (PBS)	4100			Fluorescence	31
47	CA	7.2 (PBS)	39900			Fluorescence	31
	DCA	7.2 (PBS)	31880			Fluorescence	31
	GCA	7.2 (PBS)	10400			Fluorescence	31
	TCA	7.2 (PBS)	5360			Fluorescence	31
48	CA	7.2 (PBS)	25930			Fluorescence	31
	DCA	7.2 (PBS)	14330			Fluorescence	31
	GCA	7.2 (PBS)	7950			Fluorescence	31
	TCA	7.2 (PBS)	4590			Fluorescence	31
49	CA	7.2 (PBS)	26200			Fluorescence	32
	DCA	7.2 (PBS)	10140			Fluorescence	32
	GCA	7.2 (PBS)	3150			Fluorescence	32
	TCA	7.2 (PBS)	7730			Fluorescence	32
50	CA	7.2 (PBS)	7351	–33.0	–10.9	ITC	33
	DCA	7.2 (PBS)	5504	–42.7	–21.4	ITC	33
	GCA	7.2 (PBS)	5936	–15.1	6.4	ITC	33
	TCA	7.2 (PBS)	3058	–24.5	–4.6	ITC	33
51	CA	7.2 (PBS)	5559	–49.3	–27.9	ITC	33
	CA	7.2 (PBS)	11300			Fluorescence	35
	DCA	7.2 (PBS)	8372	–48.1	–25.7	ITC	33
	DCA	7.2 (PBS)	21730			Fluorescence	35
	GCA	7.2 (PBS)	2979	–18.1	4.2	ITC	33
	GCA	7.2 (PBS)	11040			Fluorescence	35
	TCA	7.2 (PBS)	4441	–19.7	1.1	ITC	33
	TCA	7.2 (PBS)	6040			Fluorescence	35
52	CA	7.2 (PBS)	5039	–28.2	–7.1	ITC	33
	DCA	7.2 (PBS)	7900	–31.6	–9.4	ITC	33
	GCA	7.2 (PBS)	4262	–21.5	–0.8	ITC	33
	TCA	7.2 (PBS)	1975	–22.0	–3.2	ITC	33
53	CA	7.2 (PBS)	10700	–30.6	–7.6	ITC	33
	DCA	7.2 (PBS)	8912	–38.1	–15.6	ITC	33
	GCA	7.2 (PBS)	5689	–22.7	–1.3	ITC	33
	TCA	7.2 (PBS)	2762	–37.3	–17.6	ITC	33
54	CA	7.2 (PBS)	9899	–37.5	–14.7	ITC	33
	DCA	7.2 (PBS)	11150	–39.9	–16.8	ITC	33
	GCA	7.2 (PBS)	4061	–23.5	–2.9	ITC	33
	TCA	7.2 (PBS)	2502	–20.2	0.8	ITC	33

Hosts	Guests	pH	*Ks*	Δ*H*°	*T*Δ*S*°	Methods	Refs.
55	CA	7.2 (PBS)	6196	–39.3	–17.6	ITC	33
	DCA	7.2 (PBS)	10325	–39.4	–16.5	ITC	33
	GCA	7.2 (PBS)	2891	–23.3	–3.5	ITC	33
	TCA	7.2 (PBS)	2189	–20.0	–0.9	ITC	33
56	CA	7.2 (PBS)	13330	–29.77	–6.23	ITC	18
	DCA	7.2 (PBS)	12065	–34.02	–10.72	ITC	18
	GCA	7.2 (PBS)	2925	–23.36	–3.58	ITC	18
	TCA	7.2 (PBS)	2478	–21.46	–2.09	ITC	18
57	CA	7.4 (Tris–HCl)	10540			Fluorescence	34
	DCA	7.4 (Tris–HCl)	12400			Fluorescence	34
58	CA	7.4 (Tris–HCl)	15500			Fluorescence	34
	DCA	7.4 (Tris–HCl)	15700			Fluorescence	34
59	CA	7.4 (Tris–HCl)	31400			Fluorescence	34
	DCA	7.4 (Tris–HCl)	95900			Fluorescence	34
60	CA	aqueous solution	5380			Fluorescence	36
	DCA	aqueous solution	2790			Fluorescence	36
	GCA	aqueous solution	–			Fluorescence	36
61	CA	aqueous solution	3380			Fluorescence	36
	DCA	aqueous solution	3710			Fluorescence	36
	GCA	aqueous solution	–			Fluorescence	36
62	CA	aqueous solution	30500			Fluorescence	36
	DCA	aqueous solution	529000			Fluorescence	36
	GCA	aqueous solution	1745000			Fluorescence	36
63	CA	aqueous solution	196000			Fluorescence	36
	DCA	aqueous solution	283700			Fluorescence	36
	GCA	aqueous solution	13000			Fluorescence	36
64	CA	aqueous solution	246000			Fluorescence	36
	DCA	aqueous solution	54000			Fluorescence	36
	GCA	aqueous solution	891000			Fluorescence	36
65	CA	7.2 (Tris–HCl)	18500			Fluorescence	37
	DCA	7.2 (Tris–HCl)	12200			Fluorescence	37
66	CA	7.2 (Tris–HCl)	8130			Fluorescence	37
	DCA	7.2	–			Fluorescence	37

Hosts	Guests	pH	*K*s	Δ*H*°	*T*Δ*S*°	Methods	Refs.
		(Tris–HCl)					
67	CA	7.2 (Tris–HCl)	11900			Fluorescence	37
	DCA	7.2 (Tris–HCl)	11500			Fluorescence	37
68	CA	7.2 (Tris–HCl)	8820			Fluorescence	37
	DCA	7.2 (Tris–HCl)	1870			Fluorescence	37
69	CA[a]	7.2 (Tris–HCl)	5.73×10^7			Fluorescence	37
	DCA[a]	7.2 (Tris–HCl)	2.03×10^7			Fluorescence	37
70	CA[a]	7.2 (Tris–HCl)	9.93×10^7			Fluorescence	37
	DCA[a]	7.2 (Tris–HCl)	3.47×10^7			Fluorescence	37
71	CA[a]	7.2 (Tris–HCl)	3.96×10^7			Fluorescence	37
	DCA[a]	7.2 (Tris–HCl)	3.78×10^7			Fluorescence	37
72	CA[a]	7.2 (Tris–HCl)	2.95×10^7			Fluorescence	37
	DCA[a]	7.2 (Tris–HCl)	6.2×10^6			Fluorescence	37

PBS: Phosphate Buffer Solution; ITC: Isothermal Titration Calorimetry;

Tris: Tris(hydroxymethyl)aminomethane;

–: The guest-induced variations in the fluorescence intensities are too small for these values to be determined.

[a]: Unit of *K*s is in M^{-2}.

Table 2. Complex stability constants (*K*s/M^{-1}), enthalpy (Δ*H*°/(kJ·mol^{-1})), and entropy changes (*T*Δ*S*°/(kJ·mol^{-1})) for intermolecular complexation of bile salts with bridged *β*-CDs in aqueous solution

5. Conclusion

In conclusion, the binding modes, binding abilities, and molecular selectivities of four typical bile salts (CA, DCA, GCA, and TCA) upon complexation with CDs and their derivatives are summarized in this chapter from thermodynamic viewpoints. Generally, native and mono-modified CDs display relatively limited binding ability towards guest molecules, probably because of weak interactions between hosts and guests, which would result in a relative small negative enthalpy change, and then, a relative weak binding. However, bridged and metallobridged CDs have greatly enhanced the binding abilities in

relation to the parent CDs, owing to a multiple recognition mechanism, which would lead to a relative large negative enthalpy change, and then a strong binding. This summary of the binding modes and thermodynamic data for the complexation of bile salts with CDs and their derivatives is quite important to improve the understanding of molecular recognition mechanism in supramolecular systems and further guide the design and synthesis of new supramolecular systems based on different kinds of CDs in the future.

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