J Incl Phenom Macrocycl Chem (2009) 64:135–141
DOI 10.1007/s10847-009-9545-6

ORIGINAL ARTICLE

NMR spectroscopic characterization of inclusion complexes of hydroxy-substituted naphthalenes with native and modified β-cyclodextrins

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Received: 21 November 2008 / Accepted: 17 January 2009 / Published online: 4 February 2009

Abstract The equilibrium constants (K) for the inclusion complexation of three kinds of β-cyclodextrins (β-CDs: native β-CD, heptakis(2,6-di-*O*-methyl)-β-CD, and 6-*O*-α-D-glucosyl-β-CD) with OH-substituted naphthalenes (2-naphthol, 2,3-dihydroxynaphthalene, and 2,6-dihydroxynaphthalene) were determined from the induced chemical shifts of NMR measurements for inclusion complexes: $K = 188$–$1{,}250\ \mathrm{mol}^{-1}\ \mathrm{dm}^3$. The modified β-CDs form stable 1:1 inclusion complexes with OH-substituted naphthalenes, and the high stability of inclusion complexes of 2,6-dihydroxynaphthalene having a hydrophobic body and hydrophilic ends is shown. In addition, the structures of inclusion complexes were characterized by 2D ROESY NMR measurements. The differences in the structure of the inclusion complexes were observed for three kinds of naphthol guest molecules. Based on the results, the inclusion abilities enhanced by methylation of the OH groups at the CD rim or the side chain of branched β-CD are discussed.

Keywords Cyclodextrin · Inclusion complex · NMR · ROESY · Naphthols

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that possess hydrophobic cavities capable of forming guest–host inclusion complexes with a variety of organic molecules in aqueous solution. Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) and 6-*O*-α-D-glucosyl-β-cyclodextrin (G-β-CD) are chemically modified β-CDs arising from substitution of O(2)–H and O(6)–H groups on the end of β-CD and maintain inclusion capabilities similar to native β-CD [1–3]. Modification of the β-CD's rim would lead to a change in water solubility, stability, and structure of the inclusion complexes [4, 5]. Thus, it is considered to be a more practical pharmaceutical carrier molecule for drugs that are unstable at ambient conditions or have poor water solubility.

As driving forces for inclusion complex formation with CD, several intermolecular interactions have been proposed: (1) hydrophobic interaction, (2) van der Waals interaction, (3) hydrogen bonding, (4) relief of high-energy water from the CD cavity upon inclusion of a guest, etc. [6]. The two rims of CD are occupied by hydroxy groups so that the CDs are hydrophilic at the periphery and hydrophobic in the central cavity, which can encapsulate guest molecules of suitable size. From a structural view, suitable guest molecules for CD inclusion should have a hydrophobic body and hydrophilic ends that can form hydrogen bonds.

In this study, we have established an inclusion behavior of native and modified β-CDs by means of 1D and 2D NMR experiments. For detailed studies on the modified β-CD inclusion complexation behavior, three kinds of OH-substituted naphthalenes having both hydrophilic and hydrophobic moieties were chosen as guest molecules. We found a characteristic inclusion complexation of OH-substituted naphthalenes by modified β-CDs. By comparing the inclusion complexation by modified β-CDs with that by native β-CD, the difference in the structure of the inclusion complexes and the dominant factor for the

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inclusion complex formation by modified β-CDs are discussed.

Experimental

Materials and measurements

The three kinds of β-CDs used as host molecules are shown in Fig. 1: β-cyclodextrin (β-CD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD), and 6-*O*-α-D-glucosyl-β-cyclodextrin (G-β-CD). The three kinds of OH-substituted naphthalenes used as guest molecules were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and were used as received: 2-naphthol, 2,3-dihydroxynaphthalene (2,3-DHN), and 2,6-dihydroxynaphthalene (2,6-DHN). Water was purified by distillation before use and was used as a solvent.

The ^{1}H-NMR spectra were measured in D_2O with a Varian Mercury 300 (300 MHz) at room temperature. Chemical shifts were reported as δ values relative to HOD (δ 4.79) as an internal standard [7]. Using the induced chemical shifts, the association constants for the inclusion complex formation were determined from the average of three replicate experiments and reproduced within 5% error. 2D ROESY-NMR experiments were recorded at 600 MHz in D_2O on a Varian Inova AS600 NMR spectrometer at 303 K. The mixing time for each ROESY experiment was 200 ms.

Results and discussion

Estimation of association constants

^{1}H NMR measurements have been shown to be informative in elucidating the guest–host molecular position in an inclusion complex because the induced chemical shifts of the guest protons indicate an inclusion of the proton moiety in the CD cavity [8, 9]. Figure 2a shows the ^{1}H NMR spectrum of DM-β-CD in D_2O. The assignments of the DM-β-CD protons were made according to the report by Miyake et al. [10]. When 2,3-DHN was added to the DM-β-CD solution, large shifts of the H-3,5 and C^6OCH_3 protons of DM-β-CD were observed, indicating the encapsulation of 2,3-DHN into the CD cavity. To confirm the stoichiometry of the DM-β-CD/2,3-DHN complex, we conducted NMR measurements by varying the mole fraction of the guest and host molecules in solution using the continuous variation method (Job's method) [11]. Job's diagram for the C^1–H proton of 2,3-DHN shows the minimum at a guest/(host + guest) ratio of 0.5 (Fig. 2c), suggesting a 1:1 inclusion complex formation. Similar results were obtained for the inclusion complexations of other CD/naphthol systems.

Figure 3 shows the chemical shifts for 2,3-DHN protons as a function of DM-β-CD concentration. Large chemical shifts of the $C^{6,7}$–H protons of 2,3-DHN are observed compared with those of the $C^{1,4,5,8}$–H protons, indicating a

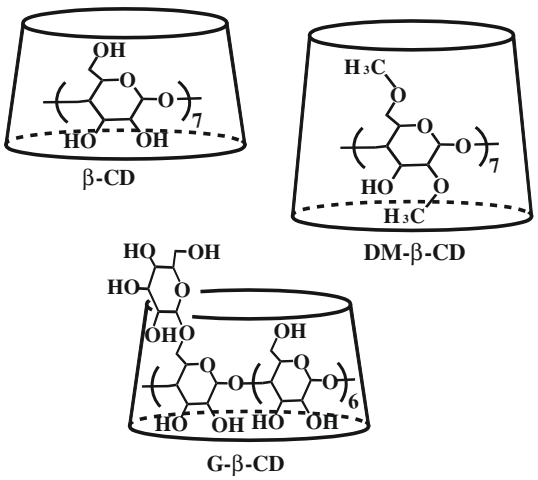

Fig. 1 Structures of native and modified β-CDs

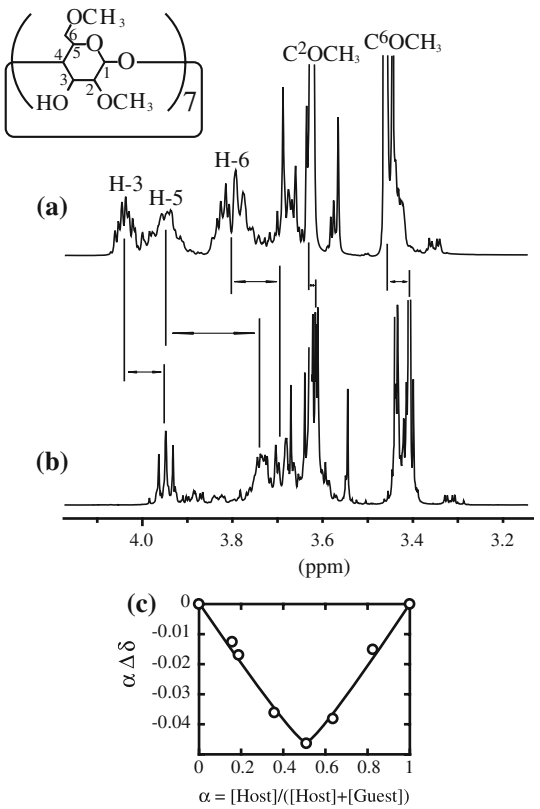

Fig. 2 ^{1}H NMR spectra of (**a**) DM-β-CD and (**b**) inclusion complex of 2,3-DHN with DM-β-CD in D_2O. **a** [DM-β-CD] = 7.19 × 10^{-3} mol dm^{-3}. **b** [DM-β-CD] = 7.19 × 10^{-3} mol dm^{-3} and [2,3-DHN] = 7.47 × 10^{-3} mol dm^{-3}. **c** Continuous variation plots (Job's plot) derived from the ^{1}H NMR data (C^1–H in 2,3-DHN)

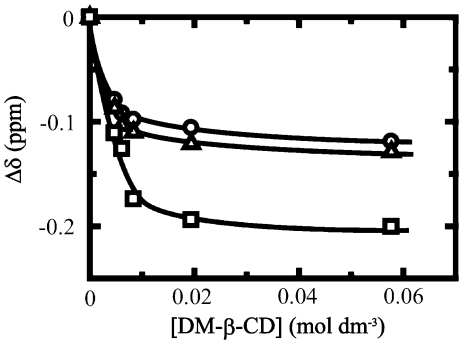

Fig. 3 Changes in chemical shifts of 2,3-DHN protons ([2,3-DHN] = 1.08×10^{-2} mol dm^{-3}) at various DM-β-CD concentrations ([DM-β-CD] = 4.73×10^{-3}–5.77×10^{-2} mol dm^{-3}); ○: $C^{1,4}$–H, Δ: $C^{5,8}$–H, □: $C^{6,7}$–H

high degree of hydrophobic interaction between the CD wall and the guest proton moiety. The induced chemical shifts of NMR signals upon the 1:1 complexation can be given by [12]:

$$G + H \overset{K}{\rightleftharpoons} C \tag{1}$$

$$\Delta\delta = \frac{\Delta\delta_{sat}}{2[G]_0}\left(\beta - \sqrt{\beta^2 - 4[G]_0[H]_0}\right) \tag{2}$$

$$\beta = \frac{1}{K} + [G]_0 + [H]_0 \tag{3}$$

where $[G]_0$ and $[H]_0$ denote the initial concentrations of guest and host molecules, respectively. K is an association constant for the inclusion complex formation and $\Delta\delta_{sat}$ is the saturated value of induced chemical shifts. Figure 3 shows the saturation curve for the induced chemical shifts. To obtain the reliable values of $\Delta\delta_{sat}$, we have conducted curve fitting to the NMR titration curve of Fig. 3: $\Delta\delta = (a[CD] + b)/(c[CD] + d)$ as a function of CD concentration. Using the $\Delta\delta_{sat}$ values, nonlinear least-square fits of the induced chemical shifts to Eq. 2 enable us to determine the 1:1 inclusion constants K of OH-substituted naphthalenes with β-CDs. The obtained association constants are listed in Table 1. The K values increase in the order of β-CD < G-β-CD < DM-β-CD. The large association constant with DM-β-CD reflects the high degree of stability of the inclusion complex. In the three kinds of guest molecules, 2,6-DHN forms the most stable inclusion complex.

Table 1 Equilibrium constants and free-energy changes for inclusion complex formation with β-CDs at 298 K

Guest	Host	K (mol^{-1} dm^3)	$-\Delta G°$ (kJ mol^{-1})
2-Naphthol	β-CD	311 ± 11	14.2
2-Naphthol	G-β-CD	631 ± 25	16.0
2-Naphthol	DM-β-CD	643 ± 20	16.0
2,3-DHN	β-CD	188 ± 9	13.0
2,3-DHN	G-β-CD	282 ± 13	14.0
2,3-DHN	DM-β-CD	864 ± 16	16.8
2,6-DHN	β-CD	640 ± 20	16.0
2,6-DHN	G-β-CD	930 ± 37	16.9
2,6-DHN	DM-β-CD	1,250 ± 52	17.7

Structure of the inclusion complex

The 2D ROESY-NMR experiments are instructive for elucidating the position of the guest molecule in the CD cavity. Figures 4 and 5 show the representative ROESY-NMR spectra of the inclusion complexes of 2-naphthol and 2,3-DHN with the β-CDs. The cross peaks were detected between the inner protons (H-3 and H-5) of the CD cavity and naphthol-guest molecules; there is no correlation with outer protons H-2 and H-4 of CD. These observations indicate that naphthol-guests are encapsulated into the CD cavity. The relative intensities of the cross peaks are listed in Table 2. The results can be summarized as follows: (1) In 2-naphthol/β-CD, the H-3,5 protons of β-CD interact strongly with the $C^{4,8}$–H protons in 2-naphthol. Cross peaks between the H-5 proton of β-CD and the $C^{6,7}$–H protons of 2-naphthol were not detected, while those between the H-3 proton of β-CD and the $C^{6,7}$–H protons appeared, as indicated by the arrows in Fig. 4a. These show that the OH group of 2-naphthol is located at the primary OH group side of β-CD. (2) In 2,3-DHN/β-CD, the cross peaks arising from the H-3,5 protons of β-CD and the $C^{1,4,5,8}$–H protons of 2,3-DHN were observed. However, those from the H-3,5 protons of β-CD and the $C^{6,7}$–H protons of 2,3-DHN did not appear. Further, as indicated by an arrow in Fig. 5a, the H-6 proton interacts with the $C^{1,4}$–H protons of 2,3-DHN, indicating that the two OH groups of 2,3-DHN are located at the CD's rim of the primary OH group side. (3) In 2-naphthol/DM-β-CD, the cross peaks between the $C^{1,3}$–H protons of 2-naphthol and the C^6–OCH_3 protons of DM-β-CD are seen in Fig. 4b but those between the $C^{6,7}$–H protons of 2-naphthol and the DM-β-CD protons are not seen, suggesting that the OH group of 2-naphthol is located at the narrow end of DM-β-CD and that the $C^{6,7}$–H moiety protrudes from the wider rim of the CD cavity. (4) In 2,3-DHN/DM-β-CD, the cross peaks arising from the H-3,5 protons of DM-β-CD and the $C^{1,4,5,6,7,8}$–H protons are observed. This suggests that the 2,3-DHN guest is deeply included in the CD cavity. The $C^{6,7}$–H protons and $C^{1,4}$–H protons of 2,3-DHN interact with C^6–OCH_3 and C^2–OCH_3 of DM-β-CD, respectively, which give evidence of the presence of the two OH groups moieties of 2,3-DHN at the wider end of the CD cavity. (5)

Table 2 Cross peaks observed in the ROESY-NMR spectra of inclusion complexes in D_2O at 303 K

Host	Guest	Guest protons	Host protons					
			H-3	H-5	H-6	H-3′	C^2OCH_3	C^6OCH_3
β-CD	2-Naphthol	$C^{1,3}$–H	−	++	++			
		C^4–H	++	++	+			
		$C^{5,8}$–H	++	+	−			
		$C^{6,7}$–H	+	−	−			
β-CD	2,3-DHN	$C^{1,4}$–H	++	++	++			
		$C^{5,8}$–H	++	++	−			
		$C^{6,7}$–H	−	−	−			
DM-β-CD	2-Naphthol	C^1–H	++	++			−	++
		C^3–H	−	++			−	++
		$C^{4,5}$–H	+	+			+	−
		$C^{6,7}$–H	−	−			−	−
		C^8–H	+	+			+	−
DM-β-CD	2,3-DHN	$C^{1,4}$–H	++	++			+	−
		$C^{5,8}$–H	++	++			−	++
		$C^{6,7}$–H	+	++			−	++
G-β-CD	2-naphthol	$C^{1,4,8}$–H	++	++		−		
		C^3–H	+	++		−		
		C^5–H	−	−		++		
		$C^{6,7}$–H	−	−		+		
G-β-CD	2,3-DHN	$C^{1,4}$–H	+	−	−	−		
		$C^{5,8}$–H	+	+	−	+		
		$C^{6,7}$–H	++	++	++	++		
G-β-CD	2,6-DHN	$C^{1,5}$–H	++	++	+	−		
		$C^{3,7}$–H	−	+	−	−		
		$C^{4,8}$–H	++	++	+	+		

In 2-naphthol/G-β-CD, as reported previously [13], the cross peaks arising from the H-3′ proton of G-β-CD and the $C^{5,6,7}$–H protons of 2-naphthol indicate interaction between the 6-*O*-α-D-glucosyl side chain of G-β-CD and the guest molecule. Since the interaction between the H-3,5 protons of G-β-CD and the $C^{1,3,4,8}$–H protons of 2-naphthol can be seen, 2-naphthol is deeply encapsulated into the CD cavity, and the OH group of the guest molecule may be placed at the wider end (secondary OH group side) of G-β-CD. (6) In 2,3-DHN/G-β-CD, the cross peaks between the $C^{1,4}$–H protons of 2,3-DHN and the H-5 proton of G-β-CD were not detected, while those between the $C^{1,4}$–H protons and the H-3 proton can be seen in Fig. 5b. Further, the cross peak arising from the $C^{6,7}$–H protons of 2,3-DHN and the H-3′ proton in the glucosyl side chain can be seen. This suggests that the two OH groups of 2,3-DHN are placed at the wider CD end. In the inclusions of DM-β-CD and G-β-CD, the inclusion complexes having a reversed orientation of 2,3-DHN are formed compared with those of the native β-CD complexes, which is responsible for the enhanced hydrophobic interaction by the methylation of the OH groups or the glucosyl side chain. (7) In 2,6-DHN/G-β-CD, the cross peaks between the 3,5-H of CD and the $C^{1,4,5,8}$–H protons of 2,6-DHN appear, suggesting that the OH group of the guest protrudes from the CD cavity to place it in the aqueous phase. Similar structures of the inclusion complexes explain the induced chemical shifts of NMR measurements for the inclusion complexes of 2,6-DHN with other β-CDs. In G-β-CD, the cross peak between the H-3′ proton and the C^8–H proton indicates the interaction of the 2,6-DHN guest molecule with the glucosyl side chain of G-β-CD. The deduced orientations of the OH-substituted naphthalenes in the CD cavities are shown in Fig. 6.

The free energy change of the inclusion complexation of OH-substituted naphthalenes can be given by:

$$\Delta G^\circ = -RT\ln K \tag{4}$$

The ΔG° values, which express the stability of the inclusion complexes, are listed on the right of Table 1. The

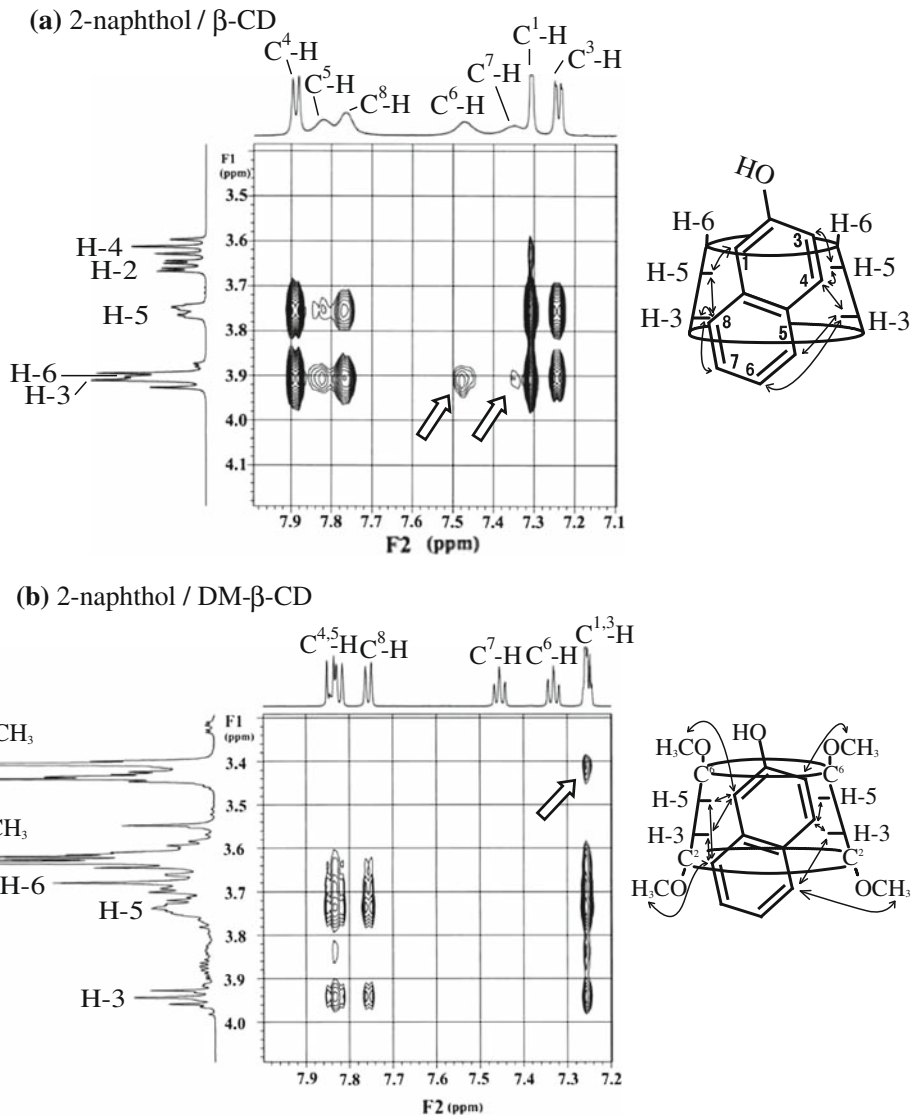

Fig. 4 2D ROESY-NMR spectra for the inclusion complexes of 2-naphthol at 303 K in D_2O: **a** [2-naphthol] $= 1.39 \times 10^{-3}$ mol dm^{-3} and [β-CD] $= 3.35 \times 10^{-3}$ mol dm^{-3}. **b** [2-naphthol] $= 4.55 \times 10^{-3}$ mol dm^{-3} and [DM-β-CD] $= 7.00 \times 10^{-3}$ mol dm^{-3}

absolute values are large compared with those for the inclusion complexation of phenol with native β-CD and modified DM-β-CD [2, 14]: $K = 94$ mol^{-1} dm^3 and $-\Delta G^\circ = 11.3$ kJ mol^{-1} for β-CD at 298 K, and $K = 221$ mol^{-1} dm^3 and $-\Delta G^\circ = 13.4$ kJ mol^{-1} for DM-β-CD at 298 K. We believe that the magnitude of hydrophobic interaction is related to the contact area of a guest molecule for the internal wall of the CD. The large $-\Delta G^\circ$ values for OH-substituted naphthalenes are attributed to the increase in the contact area of the large hydrophobic body of the naphthalene moiety in the CD cavity. The $-\Delta G^\circ$ values for G-β-CD and DM-β-CD are larger than are those for native β-CD. In DM-β-CD, the methylation of the $C^{2,6}$–OH groups of the CD rim lengthens the CD cavity without any significant distortion of the ring and enhances the inclusion ability. In G-β-CD, as suggested previously, the 6-*O*-α-D-glucosyl side chain of CD interacts with the guest molecules, capping the cavity by hydrophobic moiety which effectively enlarges the hydrophobic environment around the cavity. This is in accord with the induced fit-type inclusion complexation of branched cyclodextrins [15]. These high stabilities of the inclusion complexes for G-β-CD and DM-β-CD are ascribed to the enhanced hydrophobicity of the CD cavity. In particular, from inspection of the inclusion complex structure with DM-β-CD, we believe that the methylation of the O(6)–H group in the CD rim is effective for the host–guest hydrophobic interaction, resulting in the reversed orientation of the 2,3-DHN inclusion.

It is of note that the $-\Delta G^\circ$ value for the 2,6-DHN inclusion is remarkably large in comparison with those of 2-naphthol and 2,3-DHN. The two OH groups of 2,6-DHN protrude from the CD cavity and might be located at the vicinity of the OH (or OCH_3) groups of the CD's rim. We

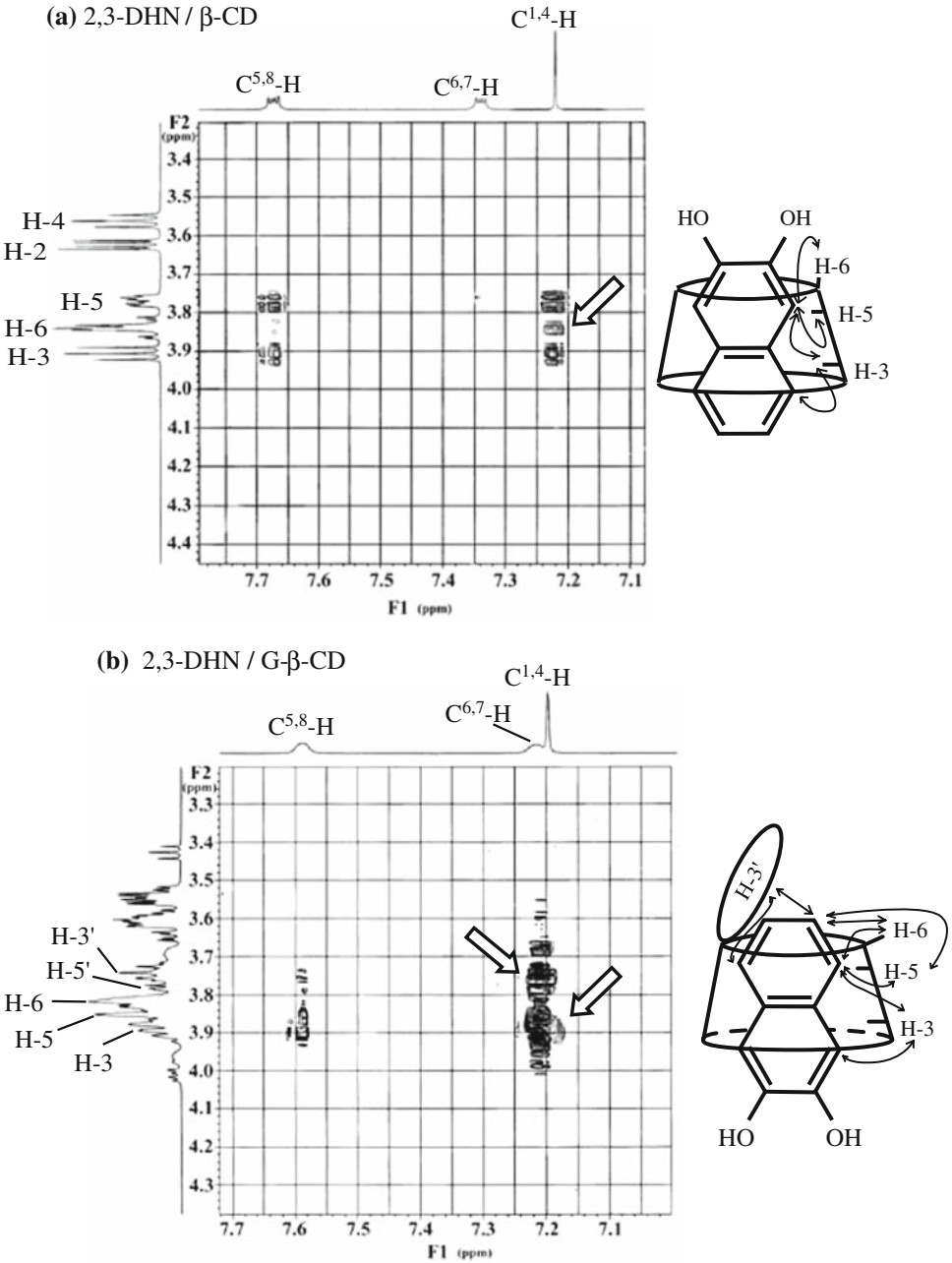

Fig. 5 2D ROESY-NMR spectra for the inclusion complexes of 2,3-DHN at 303 K in D_2O: **a** [2,3-DHN] = 2.98×10^{-3} mol dm^{-3} and [β-CD] = 6.78×10^{-3} mol dm^{-3}. **b** [2,3-DHN] = 8.52×10^{-3} mol dm^{-3} and [G-β-CD] = 1.50×10^{-2} mol dm^{-3}

believe that in the 2,6-DHN inclusion, the extra interaction by hydrogen bonding at the OH groups of the CD rim is operative, which is responsible for the high stability of the inclusion complex.

In summary, we have demonstrated that OH-substituted naphthalenes form 1:1 inclusion complexes with modified β-CDs. The dipole interactions between the guest and host protons can be seen in the ROESY-NMR spectra of the inclusion complexes and analyses of the cross peaks has confirmed the characteristic disposition of the guest molecules inside the CD cavities. The above results show that the enhanced hydrophobic interaction by methylation of the OH group at the CD's rim or the branched hydrophobic moiety changes the inclusion complex structure (the orientation of guest molecules). The hydrophobic interaction for the inclusion complex formation by modified β-CD is an important factor in the stability of inclusion complex.

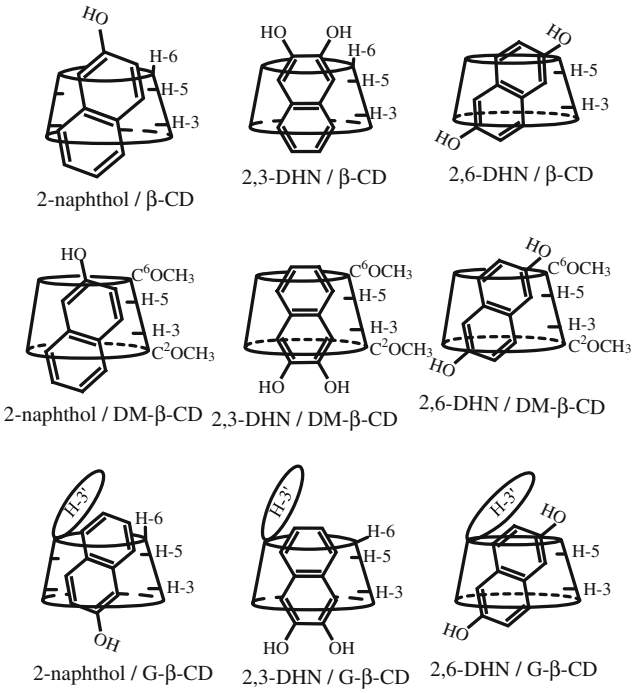

Fig. 6 Plausible structures of inclusion complexes of OH-substituted naphthalenes with native and modified β-CDs

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