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# $^1$H NMR Studies of Maltose, Maltoheptaose, α-, β-, and γ-Cyclodextrins, and Complexes in Aqueous Solutions with Hydroxy Protons as Structural Probes

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The $^1$H NMR chemical shifts, coupling constants, temperature coefficients, and exchange rates have been measured for the hydroxy protons of aqueous solutions of α-, β-, and γ-cyclodextrins, maltose, and maltoheptaose. In cyclodextrins (CDs), the high chemical shift of the O(3)H signal and its small $^3J_{OH,CH}$ value suggest that O(3)H is involved in a hydrogen bond. The small temperature coefficients and rate of exchange values of O(2)H and O(3)H confirm the involvement of O(3)H in hydrogen bonding and indicate that O(2)H is the hydrogen bond partner. In maltose, two distinct NMR signals with two different vicinal coupling constants are found for O(2′)H. A cross-peak in the ROESY spectrum indicates chemical exchange between the O(2′)H and O(3)H protons. The existence of two distinct NMR signals with different *J* values for O(2′)H shows the influence of anomeric configuration on the O(2′)H−O(3)H interaction. The effect of complexation with methyl benzoate, adamantane-1-carboxylic acid, adamantane-1-ol, and L- and D-tryptophane on the NMR spectra of the hydroxy protons of α-, β-, and γ-cyclodextrins and of maltose has been investigated. No significant spectral changes were observed upon addition of methyl benzoate and adamantane-1-carboxylic acid. The addition of adamantane-1-ol resulted in an upfield shift and a strong broadening of the O(2)H signal from α-CD, and a small temperature coefficient was measured upon complexation. The O(2)H and O(3)H signals in β-CD were broadened and shifted downfield upon addition of L- and D-tryptophane.

## Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of at least six 1,4-linked α-D-glucopyranosyl residues. The most common ones are α-CD, β-CD, and γ-CD consisting of 6, 7, and 8 glucopyranose residues, respectively. The CDs have a hydrophobic cavity formed by two rings of CH groups and a ring of glycosidic oxygens. In CDs, the O(2)H group of a glucopyranose unit can form a hydrogen bond with the O(3)H group of the adjacent glucopyranose unit.[1] For instance, in β-CD, a complete secondary belt is formed by these hydrogen bonds, making the molecule rather rigid. This intramolecular hydrogen bond probably explains the lowest water solubility of β-CD among the three. In α-CD, the hydrogen bond belt is incomplete because one glucopyranose unit is in a distorted position.[1] Thus, instead of six possible hydrogen bonds, only four can be fully established. The γ-CD is a noncoplanar, more flexible structure, and is therefore the most soluble of the three. The primary O(6)-Hs placed at the smaller rim of the torus are not participating in intramolecular hydrogen bonds and can therefore rotate to partially block the cavity.

Many of these findings were first observed by X-ray in the solid state[1] and later confirmed by NMR spectroscopy on CDs in DMSO solutions.[2] A $^{13}$C NMR investigation[3] of deuterium-induced differential isotope shift for β-CD in water indicated that there are no directional intramolecular hydrogen bonds in water solution. However, $^{13}$C NMR studies would not give information about weak hydrogen bond interactions that are more likely to occur in aqueous systems. Since the best way of monitoring the existence of hydrogen bonds is to study the hydroxy protons,[4] and since hydrogen bonds that exist in DMSO solutions do not necessarily persist in water solutions,[4d] we have studied α-, β-, and γ-CDs in water by $^1$H NMR spectroscopy. The model compounds maltose, the disaccharide building block of CDs, and maltoheptaose, the open form of β-cyclodextrin, were also investigated. The presence of hydrogen bonds can be established in several different ways: (i) chemical shifts (δ), (ii) temperature coefficients (d$\delta$/d$T$), (iii) vicinal coupling constants ($^3J_{OH,CH}$), and (iv) exchange rates with the solvent ($k_{ex}$). Downfield shift, smaller temperature coefficient, slower rate of exchange, and coupling constant indicating restricted rotation are usually indicative of hydrogen bonds.

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The presence of a hydrophobic central cavity in CDs enables many molecules to be incorporated into the cavity. There are no covalent bonds formed or broken during the complex formation process, and the complexed molecules are in equilibrium with uncomplexed molecules in solution. As for intramolecular interactions, intermolecular hydrogen bonds are hardly thought to be formed in aqueous systems because of strong hydration to hydrogen-bonding sites of both host and guest molecules. Biological systems, however, clearly suggest that hydrogen bonds can be formed in water when the hydrogen-bonding sites are located in a microscopically hydrophobic environment and/or situated very close to each other. Although examples have been reported about inclusion complexes of cyclodextrins where hydrogen bonds could participate in complexation, no direct evidence for formation of hydrogen bonds in water has been obtained. Accordingly, we have investigated the interactions of α-, *β*-, and *γ*-CDs with several guest molecules. These substrates have previously been investigated by NMR spectroscopy or other techniques. Adamantane-1-carboxylic acid was chosen since a computational study[5] suggested that adamantane-1-carboxylate complexes with α-CD through a hydrogen bond with the O(2)H group of the CD. In the case of *β*-CD, adamantane-1-carboxylate was found to be fully immersed into the cavity.[5] L- and D-tryptophane were chosen since *β*-CDs have been shown to recognize the chirality of guest molecules.[1,6] Methyl benzoate was used as a negative probe since hydrogen bonds are not supposed to be an important factor in the complexation of uncharged benzene derivatives with cyclodextrins.[7] To assess the extent of hydroxy proton chemical shift changes upon complexation, maltose was used as a reference since none of the chosen guests are expected to have strong complexation with maltose.

## Results and Discussion

**I. α-, *β*-, and *γ*-CDs, Maltose, and Maltoheptaose: Assignment of Hydroxy Proton Resonances.** The assignment of the hydroxy proton signals in α-, *β*-, and *γ*-CDs, **1**−**3** (Table 1), was straightforward, and obtained from DQF-COSY spectra. The assignment of the resonances in maltose, **4**, and maltoheptaose, **5** (Table 2), was obtained from DQF-COSY, TOCSY, NOESY, ROESY, and HSQC-DEPT experiments (for structures **1**−**5** see Chart 1).

**TABLE 1. $^1$H NMR Chemical Shifts (*δ*), Chemical Shift Differences (Δ*δ*), $^3J_{OH,CH}$ Coupling Constants (*J*), Temperature Coefficients (d*δ*/d*T*), and Exchange Rates ($k_{ex}$) for the Hydroxy Protons of α-, *β*-, and *γ*-CD[a]**

| | | *δ* | Δ*δ*[b] | *J*[c] | d*δ*/d*T*[d] | $k_{ex}$[e] |
|---|---|---|---|---|---|---|
| α-CD (**1**) | O(2)H | 6.224 | −0.145 | 6.6 | −7.9 | 7.8 |
| | O(3)H | 6.572 | 0.172 | <3[f] | −8.2 | 8.0 |
| | O(6)H | 6.094 | 0.088 | 5.6 | −12.3 | 35.7 |
| *β*-CD (**2**) | O(2)H | 6.401 | 0.032 | 6.7 | −7.5 | 2.7 |
| | O(3)H | 6.717 | 0.317 | <3[f] | −8.7 | 3.0 |
| | O(6)H | 6.066 | 0.060 | 5.4 | −13.3 | 18.5 |
| *γ*-CD (**3**) | O(2)H | 6.435 | 0.066 | 7.2 | −7.5 | 6.4 |
| | O(3)H | 6.715 | 0.315 | <3[f] | −8.3 | 6.2 |
| | O(6)H | 6.020 | 0.014 | 5.2 | −12.9 | 47.9 |

[a] All measurements were performed on 25 mM, 85% $H_2O$/15% $(CD_3)_2CO$ sample solutions at −10 °C. [b] Δ*δ* values are calculated as the difference between hydroxy proton chemical shift in the CD and the corresponding methyl glycoside. A positive Δ*δ* indicates a downfield shift in the CD. [c] Coupling constants are given in Hz. [d] Temperature coefficients are given in ppb deg$^{-1}$. [e] Exchange rates are given in s$^{-1}$. [f] No splitting is observed despite a very strong Gaussian window function applied on FIDs, and the measured widths at the crowns of the peaks suggest that the couplings are smaller than 3 Hz.

Two peaks should be observed for all the CH and OH protons on the reducing residue and for all the protons on the nonreducing residues due to the two anomeric forms of the reducing rings. However, because the anomeric configuration has usually a negligible influence on the conformation, the proton chemical shifts of the nonreducing units are very similar, and due to limited spectral resolution, only the protons on the reducing residue are clearly differentiated. Thus, 12 hydroxy proton signals were expected for maltose, **4**: four for the α-form of the reducing unit present in 30%, four for the *β*-form present in 70%, and four for the nonreducing unit. The NMR spectra revealed the presence of 13 hydroxy proton signals. The DQF-COSY (Figure 1) shows the existence of two O(2′)H signals separated by 0.047 ppm giving a COSY cross-peak to two C(2′)H signals separated by 0.005 ppm. In the ROESY spectra, an exchange cross-peak was observed between the O(3)H signal of the reducing *β*-D-glucose and the signal at *δ* 6.412 ppm, allowing this signal to be assigned to O(2′)H of *β*-maltose [O(2′)H(*β*)]. The signal at *δ* 6.365 ppm was subsequently assigned to the O(2′)H of the sugar linked to the reducing α-D-glucose [O(2′)H(α)]. The corresponding ROE cross-peak was not observed for the α-anomer. The assignment of O(2′)H(α) and O(2′)H(*β*) in maltose, **4**, was supported by the assignment of the hydroxy proton signals from maltoheptaose, **5**. The O(2″)H of the sugar at the terminal nonreducing end has a chemical shift of *δ* 6.376 ppm, very similar to the shift measured for O(2′)Hα in maltose (*δ* 6.365 ppm). The O(2′)H of the residue 1,4-linked to the reducing end has a shift of *δ* 6.405 ppm, very similar to the shift measured for O(2′)H(*β*) in maltose (*δ* 6.412 ppm). The O(2′)H of the sugar 1,4-linked to the α-reducing form was not visible in the NMR spectra.

**Chemical Shift Differences (Δ*δ*).** Tables 1 and 2 show that the hydroxy proton signals in **1**−**5** have

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**TABLE 2. $^1$H NMR Chemical Shifts ($\delta$), Chemical Shift Differences ($\Delta\delta$), $^3J_{OH,CH}$ Coupling Constants ($J$), Temperature Coefficients (d$\delta$/d$T$), and Exchange Rates ($k_{ex}$) for the Hydroxy Protons of Maltose and Maltoheptaose[a]**

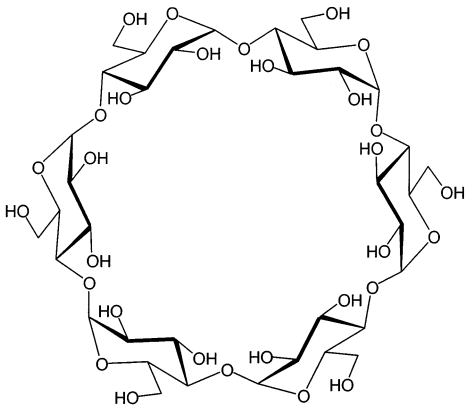
| | | $\delta$ | $\Delta\delta$[b] | $J$[c] | d$\delta$/d$T$[d] | $k_{ex}$[e] |
|---|---|---|---|---|---|---|
| maltose (**4**) | O(1)H($\alpha$) | 7.333 | 0.107 | 3.2 | −10.9 | 80.7 |
| | O(2)H($\alpha$) | 6.276 | 0.094 | 5.9 | −13.6 | 89.9 |
| | O(3)H($\alpha$) | 6.380 | 0.012 | 5.7 | −10.9 | *f* |
| | O(6)H($\alpha$) | 5.869 | −0.071 | 10.0[g] | −12.7 | *f* |
| | O(1)H($\beta$) | 8.034 | 0.057 | 6.2 | −11.3 | 63.4 |
| | O(2)H($\beta$) | 6.662 | 0.097 | 4.7 | −13.9 | 84.8 |
| | O(3)H($\beta$) | 6.485 | −0.020 | 5.1 | −12.0 | *f* |
| | O(6)H($\beta$) | 5.938 | −0.076 | 9.0[g] | −12.9 | 91.8 |
| | O(2′)H($\alpha$) | 6.365 | −0.004 | 8.0 | −10.0 | *f* |
| | O(2′)H($\beta$) | 6.412 | 0.043 | 6.6 | −11.6 | *f* |
| | O(3′)H | 6.456 | 0.056 | 4.4 | −13.6 | *f* |
| | O(4′)H | 6.490 | 0.076 | 5.9 | −12.9 | *f* |
| | O(6′)H | 6.035 | 0.029 | 10.2[g] | −13.7 | 90.3 |
| maltoheptaose (**5**) | O(1)H($\alpha$) | 7.299 | 0.073 | 3.6 | −8.7 | 142.2 |
| | O(2)H($\alpha$) | 6.225 | 0.043 | 5.5 | −11.2 | 166.6 |
| | O(3)H($\alpha$) | 6.357 | −0.011 | 6.4[h] | −8.2 | *f* |
| | O(6)H($\alpha$) | 5.842 | −0.098 | 9.7[g,h] | −10.8 | *f* |
| | O(1)H($\beta$) | 8.015 | 0.038 | 5.9 | −9.6 | 105.1 |
| | O(2)H($\beta$) | 6.634 | 0.069 | 4.3 | −11.6 | 137.8 |
| | O(3)H($\beta$) | 6.471 | −0.034 | 6.4[h] | −9.2 | *f* |
| | O(6)H($\beta$) | 5.909 | −0.105 | 12.4[g,h] | −9.6 | *f* |
| | O(2′)H | 6.405 | 0.036 | 11.6[h] | −8.1[i] | *f* |
| | O(3′)H | 6.414 | 0.014 | 9.2[h] | −8.1[i] | *f* |
| | O(6′)H | 5.935 | −0.071 | 13.4[g,h] | −10.5 | *f* |
| | O(2″)H | 6.376 | 0.007 | 6.8 | −9.3 | *f* |
| | O(3″)H | 6.408 | 0.008 | 6.5[h] | *f* | *f* |
| | O(4″)H | 6.475 | 0.061 | 7.0[h] | −9.4 | *f* |
| | O(6″)H | 6.015 | 0.009 | 11.5[g,h] | −11.8 | 92.0 |

[a] All measurements were performed on 12.5 mM, 85% $H_2O$/15% $(CD_3)_2CO$ sample solutions at −10 °C. A single prime designates the nonreducing sugar in **4** and the five glucose units in the middle that have the same chemical shifts in **5**. A double prime designates the nonreducing end sugar unit for **5**. [b] $\Delta\delta$ values are calculated as the difference between hydroxy proton chemical shift in the CD and the corresponding methyl glycoside. A positive $\Delta\delta$ indicates a downfield shift in the CD. [c] Coupling constants are given in Hz. [d] Temperature coefficients are given in ppb deg$^{-1}$. [e] Exchange rates are given in s$^{-1}$. [f] Severe spectral overlap impeded the extraction of these values. [g] These coupling constants are given as the sum of two couplings, $^3J_{O(6)H-H6a}$ and $^3J_{O(6)H-H6b}$. [h] Values from DQF-COSY spectrum (an overestimation of 2 Hz is obtained; for example, for O(2)H($\alpha$), 5.5 Hz is read from 1D, 7.5 from 2D DQF-COSY). [i] Average values that are measured together for both O(2′)H and O(3′)H moving together with respect to temperature change.

chemical shifts similar (small $\Delta\delta$) to those in the corresponding monosaccharide. The exception is found in $\alpha$-, $\beta$-, and $\gamma$-CDs for O(3)H which is deshielded, relative to the methyl glycoside, by 0.17 ppm in $\alpha$-CD, **1**, and ∼0.32 ppm in $\beta$-CD, **2**, and $\gamma$-CD, **3**, and for O(2)H in $\alpha$-CD, **1**, which is shielded by 0.14 ppm. The other hydroxy protons in **1**−**5** have $\Delta\delta$ < 0.10| ppm.

**Temperature Coefficients (d$\delta$/d$T$).** Protons involved in hydrogen bonds are expected to have smaller temperature coefficients than those that are freely exchanging with water.[4a] For saccharides in DMSO, temperature coefficients, d$\delta$/d$T$, lower than 3 ppb/°C are usually taken as reference for protons involved in strong hydrogen bonds.[4b] Temperature coefficients as low as 4 ppb/°C have been measured for trisaccharides[4i] in water solution. The data in Tables 1 and 2 show that all hydroxy protons in **1**−**5** have d$\delta$/d$T$ values larger than 7 ppb/°C. Whereas O(6)H has the same value (between −12.3 and −13.7 ppb/°C) in compounds **1**−**4**, O(2)H and O(3)H have smaller d$\delta$/d$T$ values in $\alpha$-, $\beta$-, and $\gamma$-CDs, **1**−**3** (∼8 ppb/°C), than

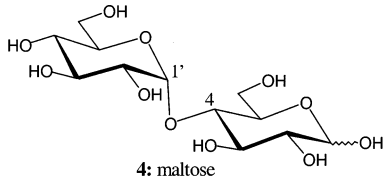
**CHART 1**



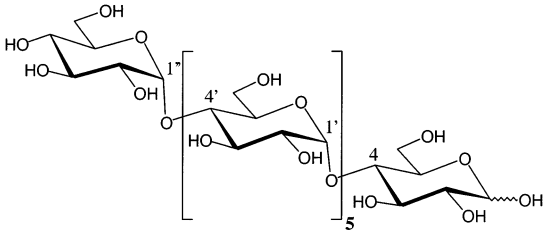
**1:** $\alpha$-cyclodextrin (6 glucose units)

**2:** $\beta$-cyclodextrin (7 glucose units)

**3:** $\gamma$-cyclodextrin (8 glucose units)



**4:** maltose



**5:** maltoheptaose

in maltose, **4** (between −10.0 and −13.9 ppb/°C). These smaller temperature coefficients suggest that O(2)H and O(3)H in CDs have reduced contact with water, as a result of weak hydrogen-bonding interactions reflected by the d$\delta$/dT's values larger than 3 ppb/°C. It can also be noted that O(3)H and O(2′)H in $\alpha$-maltose, **4**, have a slightly smaller temperature coefficient (−10.9 and −10.0 ppb/°C, respectively) than in $\beta$-maltose (−12.0 and −11.6 ppb/°C, respectively). In maltoheptaose, **5**, the O(2′)H and O(3′)H exhibit d$\delta$/d$T$ values similar to those for **1**−**3** (∼8 ppb/°C), indicating that the middle part of a maltoheptaose molecule structurally resembles the CDs to a larger extent than maltose, **4**. The comparison of d$\delta$/d$T$ values for O(6)H in **5** with those in **1**−**4** shows that the O(6)Hs in **5**, with the exception of the one at the nonreducing end, have smaller d$\delta$/d$T$ values than in the CDs, indicating that they are more prone to interresidue interactions and less exposed to water. This effect is probably increasing toward the midpoint of the molecule, but the value measured is an average value. The O(6″)H at the nonreducing end in **5** has a d$\delta$/d$T$ more similar to the ones in CDs.

**Coupling Constants ($^3J_{OH,CH}$).** The vicinal coupling constants, $^3J_{OH,CH}$, of hydroxy protons are sensitive to restricted rotation and thus to hydrogen bond formation. According to the Karplus equation derived for hydroxy

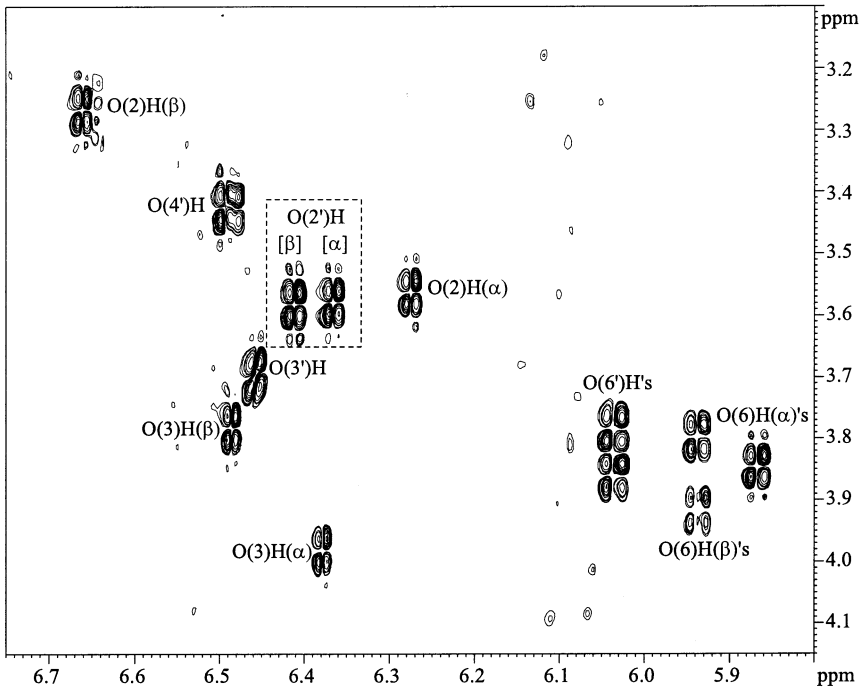



**FIGURE 1.** Expanded region of the 2D DQF-COSY spectrum (85% $H_2O$/15% $(CD_3)_2CO$, −10 °C) of maltose, **4**, showing the scalar connectivities ($^3J_{HO,CH}$) between OH and CH protons.

protons,[10] vicinal coupling constants of the order of 5.5 ± 0.5 Hz indicate a free rotation around the C−O bond. Most of the hydroxy protons in **1**−**5** have values representing conformational averaging, with the exception of O(3)H in α-, *β*-, and *γ*-CDs, **1**−**3**, for which $^3J_{OH,CH}$ values < 3 Hz are measured (Tables 1 and 2). These values should be compared to that of 5.1 Hz obtained for O(3)H in methyl α-D-glucopyranoside.[4h] They indicate a change in conformation relative to that of the monosaccharide, and a more restricted rotation around the C(3)−O(3) bonds. Even though the *J* values of 6.6 up to 7.2 Hz obtained for O(2)H are more similar to the values measured[4h] in the methyl α-D-glucopyranoside (6.0 Hz), these values are also appropriate for the O(2)H conformations necessary in order to be involved in the hydrogen-bonding interaction with O(3)H on the adjacent glycopyranosyl unit, showing restricted rotation ($^3J_{OH,CH}$ < 3 Hz) around C(3)−O(3). In maltose, **4**, the O(2′)H signal has a $^3J_{OH,CH}$ value of 8.0 Hz when linked to the reducing α-sugar and of 6.6 Hz when linked to the reducing *β*-sugar. In methyl α-D-glucopyranoside, a value of 6.0 Hz was measured for O(2)H. Therefore, a $^3J_{OH,CH}$ value of 8 Hz should indicate a change in conformation around the C(2′)−O(2′) bond.

**Exchange Rates ($k_{ex}$).** Hydroxy protons which are protected from contact with the solvent have slower exchange rates ($k_{ex}$). Due to spectral overlap, the exchange rates could be calculated only for some hydroxy protons in maltose, **4**, and maltoheptaose, **5**, and the data in Table 2 show that these hydroxy protons have similar $k_{ex}$ values. In α-, *β*-, and *γ*-CDs, **1**−**3** (Table 1), the exchange rates of the secondary hydroxy protons are considerably lower than that of the primary hydroxy proton, indicating that they are protected from exchange with water. The rates of exchange measured for *β*-CD, **2**, are much lower than those measured for α-, **1**, and *γ*-CDs, **3**, but the relative ratios between secondary and primary hydroxy protons are the same. Since exchange rates are very sensitive to pH, temperature, and catalysis by small traces of impurities, it is an indication that the conditions of measurement are not similar, and thus that rate of exchanges should not be compared between two different samples.

**NOEs and ROEs.** Both NOESY and ROESY spectra were acquired to discriminate between cross-peaks due to dipolar relaxation and cross-peaks due to chemical exchange. In α-, *β*-, and *γ*-CDs, **1**−**3**, a chemical exchange cross-peak is found between the O(2)H and O(3)H signals. As mentioned previously, a cross-peak due to chemical exchange was found between the O(3)H and the O(2′)H signals in *β*-maltose, **4**.

**Differences between DMSO and Water Solutions of Cyclodextrins.** In water, the smaller temperature coefficients and lower exchange rates measured for O(2)H and O(3)H in **1**−**3** indicate that they are involved in hydrogen bonding. The downfield shift of the O(3)H signal and the restricted rotation of O(3)H around the C(3)−O(3) bond, reflected by its small $^3J_{OH,CH}$ value, confirm the involvement of O(3)H in hydrogen bonding. Thus, the hydrogen bond between the O(2)H and O(3)H of two adjacent sugar units found for α-, *β*-, and *γ*-CDs in the solid state and by NMR in DMSO solutions also exists in water. In DMSO, both O(2)H and O(3)H were found[2] to be deshielded by 1 ppm. In water, only O(3)H is deshielded to a much smaller extent. This smaller deshielding effect can be due to weaker intramolecular

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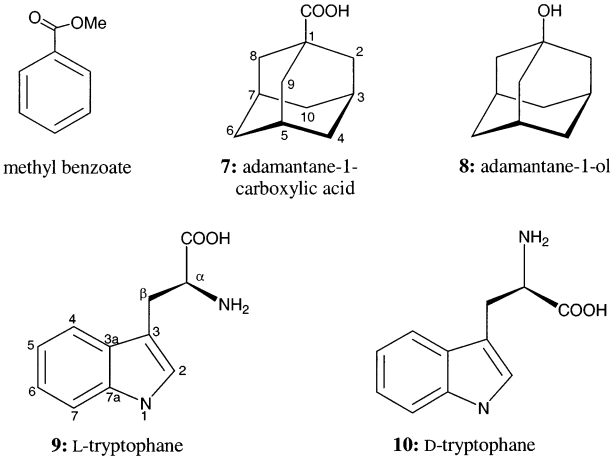
hydrogen-bonding interaction in water inducing strong solvation. The $^3J_{OH,CH}$ values are very similar in water and DMSO solutions. In the solid state,[11] O(3)H in CDs is usually found as a hydrogen bond donor and O(2) as the acceptor on adjacent sugar units. As mentioned in the coupling constants section, this agrees with the conformations suggested by the $^3J_{OH,CH}$ values for O(3)H (< 3 Hz) and O(2)H (between 6.6 and 7.2 Hz). In DMSO, the small $^3J_{OH,CH}$ value of O(3)H together with the $^2$H isotope effect on $^{13}$C and $^1$H shifts[2b] and with the temperature coefficients[2c] of the hydroxy proton chemical shifts suggest that this arrangement persists in DMSO solution. Contradictory results[1] about the relative strength of hydrogen bonds in α-, *β*-, and *γ*-CDs were obtained in DMSO from temperature coefficients and exchange rates. The latter suggested that *β*-CD has the strongest hydrogen bond network making it less flexible in comparison to α-CD and *γ*-CD, but the size of d$\delta$/d$T$ was found to increase in the order $\alpha < \beta < \gamma$. In water, it is difficult to differentiate the relative strengths of hydrogen bonds in α-, *β*-, and *γ*-CDs from the values of coupling constants, temperature coefficients, or exchange rates. Possibly, the fact that $\Delta\delta$ of O(3)H is larger in *β*- and *γ*-CDs than in α-CD (Table 1) indicates a stronger hydrogen bond network in *β*- and *γ*-CDs.

**Differences between DMSO and Water Solutions of Maltose.** In water, the existence of two distinct NMR signals with different *J*-coupling values for O(2′)H in maltose, **4**, and an exchange cross-peak between O(2′)H and O(3)H in *β*-maltose indicate that the anomeric configuration has an influence on the O(2′)H−O(3)H interaction. The large temperature coefficients and small $\Delta\delta$ values indicate that solvation is dominating the interaction, or that the interaction occurs through water. The larger $^3J_{OH,CH}$ value measured for O(2′)H in α-maltose and the slightly smaller temperature coefficients measured for O(3)H(α) and O(2′)H(α) could indicate a stronger interaction in α-maltose. It should be mentioned that in an NMR study[12] on sucrose, done under supercooled conditions, the observation of a chemical exchange cross-peak between O(2)Hg and O(1)Hf in the ROESY spectra was attributed to the existence of a weak and transient intramolecular hydrogen bond. Thus in maltose, the chemical exchange observed between O(2′)H and O(3)H in the ROESY spectra can also indicate the existence of a weak and transient hydrogen bond.

A recent study[13] on the hydrogen bond interactions of maltose in DMSO has shown that maltose is characterized by flip-flop hydrogen bonds between O(3)H and O(2′)H. Some significant differences between the data obtained in DMSO and our data should be noted. In DMSO, the chemical shift difference between the O(2′)H signal linked to the reducing α- and *β*-end was only 0.010 ppm (0.050 ppm in water), and the $^3J_{OH,CH}$ values for O(2′)H(α) and O(2′)H(*β*) were 6.2 and 6.3 Hz, values similar to those in the corresponding monosaccharides. From the $^3J_{OH,CH}$ value of 3.2 Hz measured for O(3)H, it was suggested that this proton could act as a hydrogen donor.[15] These data together with the downfield shift measured for the O(2′)H and O(3)H lead the authors to the conclusion that both O(2′)H and O(3)H act as hydrogen bond donors. The small value of $^3J_{OH,CH}$ for O(3)H suggested, however, a preponderance of the species with O(3)H as hydrogen bond donor to O(2′).[15] A crystallographic study[14] has likewise shown that the hydrogen bond between O(2′) and O(3)H is important in the solid state while another study[15] suggested that it is only weakly populated.

**CHART 2**




**6:** methyl benzoate **7:** adamantane-1-carboxylic acid **8:** adamantane-1-ol

**9:** L-tryptophane **10:** D-tryptophane

**Effect of Acetone-$d_6$ on the Conformation.** To slow the rate of exchange of hydroxy protons with water, it is usually necessary to lower the temperature below 0 °C. To perform this, 15% acetone-$d_6$ is added to prevent the solution from freezing down to −15 °C. Acetone-$d_6$ is chosen since it is a relatively weak hydrogen bond donor and acceptor,[16] but the question on the influence of acetone on the NMR data obtained for hydroxy protons has arisen. Until now, it has not been possible to study hydroxy protons of carbohydrates in pure water, except at very high concentrations[17] or in supercooled water.[18] For *β*-CD, **2**, we have been able to observe hydroxy proton signals from 95% $H_2O$/5% $D_2O$ at relatively low concentration (25 mM), and the chemical shifts are comparable in the two solvent systems, 95% $H_2O$/5% $D_2O$ and 85% $H_2O$/15% $(CD_3)_2CO$. It should also be mentioned that a recent study[19] on hydrogen bonding in dicarboxylic acids has shown that even in 90% $(CD_3)_2CO$/10% $H_2O$, the water is sufficient to allow full solvation of the intramolecularly hydrogen bonded species.

**II. Complexation Studies: Interactions with Maltose.** The changes in chemical shifts were measured for the hydroxy proton signals of maltose, **4**, upon addition of 1 equiv of methyl benzoate, **6**, adamantane-1-carboxylic acid, **7**, adamantane-1-ol, **8**, and L-, **9**, and D-tryptophane, **10** (see structures in Chart 2). The addition of 1 equiv of methyl benzoate and adamantane-1-ol does not have any effect (CIS < 0.005 ppm) on the chemical shifts of the

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**TABLE 3. $^1$H NMR Complexation Induced Chemical Shift Differences (CIS) for the Ring Protons of α-, *β*-, and *γ*-Cyclodextrins, 1−3, upon Complexation**

| | | methyl benzoate | adamantane-1-COOH | adamantane-1-ol | L-tryptophane | D-tryptophane |
|---|---|---|---|---|---|---|
| **1** | C(1)H | −0.031 | 0.003 | 0.000 | −0.004 | −0.007 |
| | C(2)H | −0.044 | 0.004 | −0.014 | 0.000 | −0.010 |
| | C(3)H | −0.182 | 0.010 | 0.050 | 0.000 | −0.011 |
| | C(4)H | −0.018 | 0.003 | 0.021 | −0.012 | −0.015 |
| | C(5)H | 0.037 | 0.002 | −0.016 | 0.007 | 0.004 |
| | C(6)H[a] | −0.005 | 0.003 | 0.005 | 0.000 | −0.011 |
| **2** | C(1)H | −0.012 | 0.000 | 0.004 | 0.008 | 0.007 |
| | C(2)H | 0.005 | 0.000 | 0.005 | 0.022 | 0.020 |
| | C(3)H | −0.039 | 0.007 | 0.005 | 0.032 | 0.030 |
| | C(4)H | −0.007 | 0.000 | 0.007 | 0.007 | 0.004 |
| | C(5)H | −0.124 | 0.012 | 0.015 | −0.032 | −0.029 |
| | C(6)H[a] | −0.008 | 0.000 | −0.006 | −0.018 | −0.017 |
| **3** | C(1)H | −0.030 | 0.000 | −0.006 | 0.000 | 0.000 |
| | C(2)H | −0.013 | 0.008 | 0.000 | −0.006 | −0.005 |
| | C(3)H | −0.080 | −0.003 | −0.025 | 0.008 | 0.002 |
| | C(4)H | −0.023 | 0.000 | −0.005 | −0.006 | −0.005 |
| | C(5)H | −0.104 | −0.011 | −0.005 | 0.010 | 0.005 |
| | C(6)H[a] | −0.022 | 0.000 | 0.000 | 0.000 | 0.000 |

[a] For each cyclodextrin molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H′ and C(6)H″ protons.

hydroxy protons in maltose. The addition of adamantane-1-carboxylic acid and of tryptophane (L and D) induces the same minor chemical shift changes (0.022−0.068 ppm) for a specific hydroxy proton signal regardless of the compounds (**7**, **9**, and **10**) added (see Supporting Information for the whole data). In addition this induced chemical shift difference effect was also observed for all the hydroxy protons of maltose, **4**. Since methyl benzoate, **6**, and adamantane-1-ol, **8**, have no effect on the chemical shifts, but adamantane-1-carboxylic acid, **7**, and tryptophane, **9** and **10**, which are not expected to bind more strongly to maltose have, it is possible that these chemical shift changes are caused by the pH adjustment introducing drastic change in the ionic strengths of the solutions.

**Cyclodextrins' Complexes.** NOEs and/or ROEs and the measurements of chemical shift changes of nonexchangeable protons are usually used for the study of cyclodextrin complexes in solution by NMR spectroscopy. Especially, the distinct shifts induced by guest molecules with strong shielding tensors such as aromatic systems are widely used. Thus, benzoic acid derivatives have been shown to form inclusion complexes with CDs from the complexation induced shifts (CIS) on the CDs ring protons H1 to H6. Table 3 shows that the H1, H2, H4, and H6 protons that are remote from the complexation site have small CIS, while the H3 proton in α-CD and the H5 proton in *β*- and *γ*-CD have a large shielding effect up to −0.18 ppm. These shifts prove the formation of inclusion complexes, and are in good agreement with previous studies.[1] The larger upfield shifts measured for H5 in *β*- and *γ*-CD suggest a deeper immersion into the wider CD cavity. The shifts induced by the aliphatic protons of adamantane-1-carboxylic acid and adamantane-1-ol are too weak for interpretation (Table 3), but a ROESY study[5] has indicated that adamantane-1-carboxylate is fully immersed into the *β*-CD cavity. The corresponding complex with α-CD showed contact only at the wider rim and a tilted conformation that allows

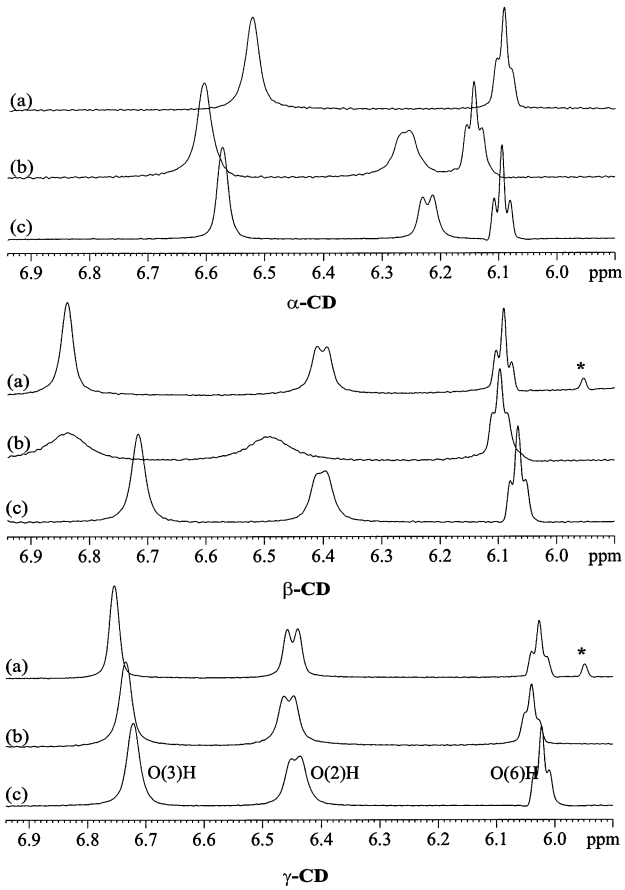


**FIGURE 2.** $^1$H NMR spectral changes observed at −10 °C for the hydroxy protons of α-, *β*-, and *γ*-CDs upon addition of 1 equiv of (a) adamantane-1-ol and (b) D-tryptophane. (c) $^1$H NMR reference spectra of CDs alone. The O(2)H signal of α-CD with adamantane-1-ol is too broad to be visible. An asterisk designates the adamantane-1-ol OH signals reading 5.955, 5.950 ppm for *β*- and *γ*-CD, respectively.

formation of the hydrogen bond between the guest molecule and the O(2)H of the CD.[5]

The changes in chemical shifts and the temperature coefficients measured for the hydroxy proton signals in α-, *β*-, and *γ*-CD, **1**−**3**, upon addition of 1 equiv of **6**−**10** are shown in Table 4. Relatively small changes (between 0.01 and 0.06 ppm) are measured when methyl benzoate, **6**, and adamantane-1-carboxylic acid, **7**, are added. The small CIS values measured with **6** suggest that hydrogen bonding is not an important factor in the interaction with CDs. A computational study[5] on the complexation of α-CD with adamantane-1-carboxylate showed a hydrogen bond between the carboxylate function on the guest and the O(2)H group of the CD. However, when adamantane-1-carboxylic acid is added to α-CD, no large CIS is measured for O(2)H (Table 4). It should be noticed that the study (ref 5) was done on the $COO^-$ form. It was recently shown[20] by NMR spectroscopy that the sodium salt of *p*-methyl benzoate (*p*-$CH_3$-$C_6H_4CO_2^-$) interacts with *β*-CD through hydrogen bonding in DMSO whereas the carboxylic acid form (*p*-$CH_3$-$C_6H_4COOH$) does not.

Figure 2 shows the $^1$H NMR spectral changes observed for the hydroxy proton signals in **1**−**3** upon addition of 1

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**TABLE 4. $^1$H NMR Complexation Induced Chemical Shift Differences (CIS) and Temperature Coefficients (d$\delta$/dT) for the Hydroxy Protons of α-, $\beta$-, and $\gamma$-Cyclodextrins, 1−3, upon Complexation[a]**

| | | methyl benzoate | | adamantane-1-COOH | | adamantane-1-ol | | L-tryptophane | | D-tryptophane | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CIS | d$\delta$/d$T$ | CIS | d$\delta$/d$T$ | CIS | d$\delta$/d$T$ | CIS | d$\delta$/d$T$ | CIS | d$\delta$/d$T$ |
| **1** | O(2)H | −0.063 | −6.5 | 0.010 | −8.5 | −0.226[b] | −3.8 | 0.039 | −7.8 | 0.038 | −7.9 |
| | O(3)H | 0.019 | −8.5 | 0.039 | −8.7 | −0.050 | −7.0 | 0.034 | −8.5 | 0.031 | −8.7 |
| | O(6)H | −0.001 | −12.0 | 0.034 | −12.8 | −0.002 | −12.0 | 0.049 | −12.1 | 0.048 | −12.6 |
| **2** | O(2)H | −0.023 | −6.4 | −0.015 | −6.9 | 0.001 | −7.0 | 0.108 | −8.3 | 0.088 | −8.6 |
| | O(3)H | 0.006 | −8.4 | 0.069 | −8.6 | 0.122 | −9.5 | 0.130 | −9.6 | 0.123 | −10.1 |
| | O(6)H | 0.015 | −12.5 | 0.022 | −12.5 | 0.024 | −12.7 | 0.035 | −12.5 | 0.032 | −12.6 |
| **3** | O(2)H | 0.022 | −7.4 | 0.009 | −7.5 | 0.005 | −7.0 | 0.015 | −7.4 | 0.013 | −7.7 |
| | O(3)H | 0.051 | −8.5 | 0.035 | −8.2 | 0.033 | −7.9 | 0.010 | −8.2 | 0.015 | −8.5 |
| | O(6)H | 0.006 | −12.0 | 0.016 | −12.6 | 0.005 | −12.2 | 0.023 | −12.3 | 0.018 | −12.6 |

[a] CIS values were calculated as the hydroxy proton chemical shifts in α-, $\beta$-, and $\gamma$-cyclodextrins, **1**−**3**, subtracted from the chemical shifts of the hydroxy proton signals in the 12.5 mM, 1:1 molar ratio complexes at −10 °C. [b] Value calculated from the fitted line of the temperature dependence of chemical shifts ($\delta$ (OH)$_{\text{adamantane-1-ol}}$ is 5.955, 5.950 ppm with $\beta$- and $\gamma$-CD, respectively, whereas the calculated $\delta$ value is 5.990 ppm with α-CD).

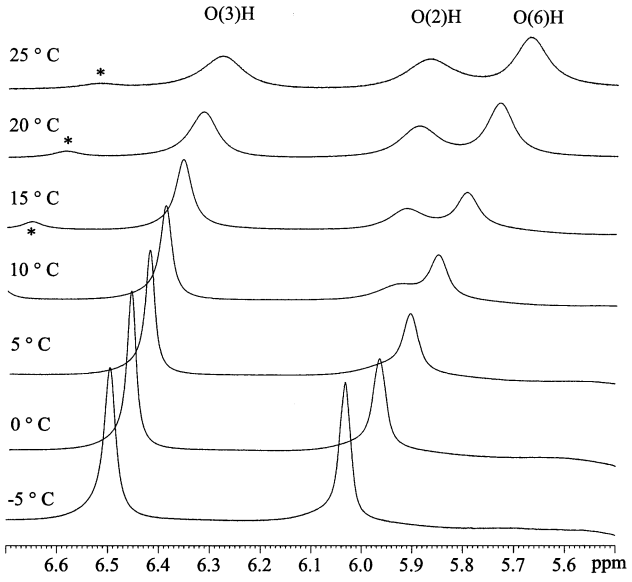


**FIGURE 3.** $^1$H NMR spectra showing the temperature dependence from 25 to −10 °C of the hydroxy protons in the α-CD/adamantane-1-ol (1:1) complex. OH signals of the hydrate form of acetone are designated by an asterisk.

equiv of adamantane-1-ol, **8**. The addition of **8** does not affect the chemical shift and the line shape of the O(3)H and O(6)H signals in α-CD, **1**. The O(2)H signal is shifted by −0.226 ppm (calculated value, see the footnote in Table 4) and is not observable in the NMR spectrum at −10 °C (Figure 2) due to extensive line broadening. Such broadening effect ($T_2$ shortening) usually occurs because of exchange processes or when the mobility is limited by the formation of an inclusion complex.

Hydroxy proton signals become usually sharper at lower temperature as a consequence of a slower exchange with water. In the α-CD/adamantane-1-ol complex, the opposite behavior is observed for the O(2)H signal, which becomes broader as the temperature is decreased from 25 to −10 °C (Figure 3). Thus, the broadening effect due to the formation of an inclusion complex with adamantane-1-ol is stronger than the sharpening effect due to slower proton exchange with water. On the other hand, the two hydroxyl resonances might also have coalesced into a broad single peak and furthermore become sharper over increasing rate of exchange between the complexing and free CD molecules due to increasing temperature (from −10 up to 25 °C). It is, however, not possible to check the cases where complexation rate would be small enough to show two distinct peaks (≪−10 °C), because of the sample facing the freezing problem at those temperatures. While the temperature coefficients for O(3)H and O(6)H are similar to those measured for α-CD alone, a temperature coefficient of −3.8 ppb/°C is measured for O(2)H. This low-temperature coefficient together with the shielding experienced by O(2)H indicate that the proton is located in an environment where the water accessibility is reduced. This more hydrophobic environment can be created by the inclusion of adamantane-1-ol into the CD cavity. The upfield shift observed for the O(2)H signal is in agreement with data from previous studies[4i,k−m] on carbohydrates showing that hydroxy protons located in sterically crowded regions are shielded due to a reduced hydration. Upon complex formation, the hydroxy proton signal of adamantane-1-ol is not visible in the NMR spectrum in the temperature range 25 to −10 °C, possibly due to strong broadening as for O(2)H. The nonexchangeable protons in adamantane-1-ol, as well as H3 of α-CD, are also strongly broadened upon complexation. The addition of adamantane-1-ol to $\beta$-CD leads to a shift by 0.122 ppm for the O(3)H signal, while the O(2)H and O(6)H signals have the same chemical shifts as in $\beta$-CD alone. No broadening of resonance lines was observed in the NMR spectra, and the temperature coefficients are slightly larger than those in $\beta$-CD alone.

The $^1$H NMR spectral changes observed for α-, $\beta$-, and $\gamma$-CDs upon addition of D-tryptophane are also shown in Figure 2. In α- and $\gamma$-CD, the hydroxy protons are all slightly deshielded (CIS < 0.05 ppm), and no broadening of resonance lines is observed. The CIS values are larger for α-CD (∼0.04 ppm) than for $\gamma$-CD (∼0.02 ppm), probably because the smaller size of the α-CD cavity allows more contact with the guest molecule. The small CIS values observed for α-CD in the presence of L- and D-tryptophane are in good agreement with a calorimetry and NMR study[21] that has shown that the contribution of hydrogen bonding was secondary in importance. In $\beta$-CD, the signals of the O(2)H and O(3)H protons are broadened and shifted by 0.10−0.12 ppm upon addition of 1 equiv of D-tryptophane or L-tryptophane, while O(6)H

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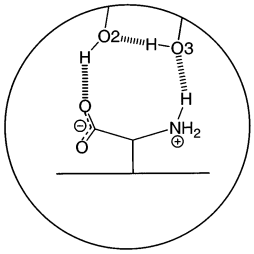


**FIGURE 4.** Schematic representation of a two-centered hydrogen bonds between the O(2)H and O(3) of $\beta$-CD and the $NH_3^+$ and $COO^-$ groups of tryptophane.

is unaffected. The downfield shifts and broadening experienced by the O(2)H and O(3)H signals are tentatively attributed to the formation of hydrogen bonds between $\beta$-CD and tryptophane. It is known[22] that cooperative hydrogen bonding causes significant stabilization of hydrogen-bonded complexes, and two-centered hydrogen bonds could be formed between the O(2)H and O(3)H of $\beta$-CD and the $NH_3^+$ and $COO^-$ groups of tryptophane (Figure 4). The CIS are slightly larger and the temperature coefficients slightly smaller in L-tryptophane than in D-tryptophane (Table 4). However, the differences are too small to draw conclusions about chirality recognition or relative strength of hydrogen bonding. NOEs from H3 of $\beta$-CD to the H$\alpha$ and H$\beta$ protons as well as to the aromatic protons of tryptophane were found for both the L and D forms. These NOEs confirm the inclusion of tryptophane into the CD cavity. The addition of the guests **8**−**10** has no effect on the NMR parameters (chemical shifts, temperature coefficients, and line shape) of the hydroxy proton signals in $\gamma$-CD (Table 4, Figure 2). Simple molecular models show that all guest molecules are completely immersed in $\gamma$-CD, and no short distance intermolecular contacts to the hydroxy protons exist. This lack of interaction is well represented by the small CIS values of $\gamma$-CD hydroxy protons (Table 4), by their d$\delta$/d$T$ values (Table 4) that are identical with the ones measured for $\gamma$-CD alone, and by their line shapes which are very similar to those for $\gamma$-CD alone (Figure 2).

## Conclusions

The O(2)H−O(3)H hydrogen bond interaction previously reported for $\alpha$-, $\beta$-, and $\gamma$-CDs in the solid state and in DMSO solutions exists also in aqueous solution. In maltose, a weak and transient O(2′)H−O(3)H hydrogen bond, probably water mediated, is present. Of particular interest is the existence of two different NMR signals for O(2′)H with two $J$ values, which indicates the influence of anomeric configuration on the O(2′)H−O(3)H interaction.

In this study, it is also shown that important structural information about the formation of inclusion complexes can be obtained from the NMR of hydroxy protons. The chemical shift, temperature coefficient, and line shape of the hydroxy proton signals have been used to monitor the formation of hydrophobic environment and intermolecular hydrogen bonds. Together with other NMR methods, the information obtained from hydroxy protons should be useful to understand the driving forces and binding modes of CD complexes.

## Experimental Section

The NMR experiments were performed on spectrometers operating at 400.13 and 600.13 MHz, respectively, for proton observation. Compounds **1**−**5** were dissolved in a mixture of 85% $H_2O$ and 15% $(CD_3)_2CO$. The sample concentration was 25 mM for **1**−**3** and 12.5 mM for **4** and **5**. Prior to NMR experiments the pH of all the sample solutions was adjusted in order to set a pH range between 6.4 and 6.8. The $^1$H NMR spectra were referenced by setting the residual acetone-$d_5$ signal to $\delta_H$ 2.204 ppm. The temperature coefficients were obtained from one-dimensional NMR spectra measured by variation of temperature from −10 to 20 °C in steps of 5 °C. The one- and two-dimensional $^1$H NMR spectra were acquired with use of the WATERGATE pulse sequence[8] for water suppression. DQF-COSY, TOCSY, NOESY, ROESY, and $^1$H-$^{13}$C HSQC-DEPT spectra were recorded with standard pulse sequences. The rates of exchange of the hydroxy protons with water were calculated from 2D phase-sensitive chemical exchange experiments[9] performed at −10 °C. Mixing times of 3−21 ms in steps of 3 ms were used. The samples of CD complexes were prepared to have 12.5 mM concentration and a 1:1 molar ratio for both host and guest molecules. The CIS values (difference between the chemical shift of hydroxy proton in the complex and the chemical shift of the same hydroxy proton in pure CD solution) were measured by using the 1D NMR spectra recorded at −10 °C. The temperature coefficients (d$\delta$/d$T$ values) were obtained as described above with the exception of the $\alpha$-CD/adamantane-1-ol complex for which a spectrum was also recorded at 25 °C.

**Acknowledgment.** This work was supported by grants from the Swedish Research Council.

**Supporting Information Available:** All the nonexchangeable chemical shifts of protons of $\alpha$-, $\beta$-, and $\gamma$-CD, maltose, maltoheptaose, adamantane-1-ol, adamantane-1-carboxylic acid, methyl benzoate, and D- and L-tryptophane; complexation-induced chemical shift differences (CIS) for the hydroxy protons of maltose; CIS for the protons of adamantane-1-ol, adamantane-1-carboxylic acid, methyl benzoate, and D- and L-tryptophane. This material is available free of charge via the Internet at http://pubs.acs.org.

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