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From Capped to Three-Dimensional Cyclodextrins: the First Example of a New Class of Receptors by Trehalose Capping of a β-Cyclodextrin

VINCENZO CUCINOTTA[1],* GIULIA GRASSO[2] and GRAZIELLA VECCHIO[3]
[1] *Dipartimento di Scienza degli Alimenti, Università di Napoli "Federico II", via Università 100, 80055 Portici, Napoli, Italy*
[2] *Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico, CNR, V.le A.Doria 8, 95125 Catania, Italy*
[3] *Dipartimento di Scienze Chimiche, Università di Catania, V.le A.Doria 8, 95125 Catania, Italy*

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Abstract. A new capped derivative of cyclomaltoheptaose (CDTHCM) was synthesized by the reaction of 6,6′-dideoxy-6,6′-di(S-cysteamine)-α,α'-trehalose with 6A,6D-dideoxy-6A,6D-diiodo-cyclomaltoheptaose. The CDTHCM obtained was characterized, together with its behaviour towards protonation. The CDTHCM/ACS (anthraquinone-2-sulfonic acid sodium salt) system was investigated by ^{1}H NMR spectroscopy and by i.c.d. (induced circular dichroism) at two different pHs. The deep inclusion of ACS within the CDTHCM cavity, with an association constant about six times larger compared to the β-CD/ACS system was found at pH 6.

Key words: cyclodextrins, capped cyclodextrins, inclusion, trehalose

1. Introduction

The functionalization of cyclodextrins (CDs) can modify and improve various features of this class of molecules, such as solubility, stability and selectivity in inclusion complex formation. By replacing one or more OH groups at a desired position and with appropriate substitution groups, multisite recognition systems have been obtained [1–5]. Capped cyclodextrins have also been synthesized and their properties have been investigated. Among the capping groups, porphyrins [6, 7], cyclopeptides [8], azobenzenes [9] crown ethers [10] and other moieties have been used [11–14]. The expansion of the hydrophobic region in comparison with the parent CD has been underlined. The increase in hydrophobicity has resulted in larger association constants with different guests in comparison to the CD parents [9-11]. However, in all capped cyclodextrins reported so far, the carbohydrate cavity has been coupled with capping moieties with completely different features in comparison to CDs, as can also be seen from the short list reported above.

* Author for correspondence.

In this paper, we report the synthesis of the new capped 6A,6D-dideoxy-6A,6D-[6,6′-dideoxy-6,6′-di(S-cysteamine)- α,α'-trehalose]-β-cyclodextrin (CDTHCM). The CDTHCM may be considered as the first example of a three-dimensional cyclodextrin by virtue of the presence of a disaccharide unit. This extends the carbohydrate system to a plane perpendicular to the main cavity, with presumably an increased inclusion ability as regards the parent CD. Furthermore, the presence of heteroatoms (N and S) in the two bridges between CD and trehalose (TH) could increase the selectivity of this class of compounds as well as their ability to coordinate metal ions.

2. Experimental

2.1. Materials

Commercially available reagents were used unless otherwise noted. Pyridine and anhydrous N,N-dimethylformamide (Aldrich) were stored over molecular sieves. TLC was carried out on silica gel plates 60F-254 (Merck). CD derivatives were detected with UV light and anisaldehyde reagent. Merck Lichroprep RP-8 (40–63 μm) was used for reverse phase column chromatography.

2.2. Synthesis

Synthesis of 6,6′-O-(p-Tosyl)-Trehalose (THDTos). A solution of p-toluenesulfonyl chloride (7.9 g, 46 mmol) in anydrous pyridine (30 mL) was added to a solution of D(+) trehalose (4.5 g, 13 mmol) in anhydrous pyridine (10 mL) at -35 °C under stirring. After 3h the pyridine was distilled off in vacuo at 40 °C and the THDTos was isolated using reverse-phase column chromatography (35 $\times$ 360 mm) first by elution with a gradient of methanol in water (35–65%) (total volume, 3 L) and then with another linear gradient of methanol in water (65–73%) (4 L). Appropriate fractions were collected to give pure THDTos (R_f = 0.52, PrOH-AcOEt-H_2O-NH_3 5 : 3 : 2 : 2) (30% yield). FAB MS 649 m/z (M-1). ^{1}H NMR δ (200 MHz, CD_3OD): 7.79 (d, 4H, aromatic ring), 7.44 (d, 4H, aromatic ring), 4.74 (d, 2H, H-1), 4.21 (m, 4H, H-6), 3.92 (m, 2H, H-5), 3.67 (t, 2H, H-3), 3.22 (t, 2H, H-4), 2.45 (s, 6H, CH_3); ^{13}C NMR δ (50.33, CD_3OD): 148 (aromatic C-1), 136 (aromatic C-4), 132 (aromatic C-2), 130 (aromatic C-3), 96 (C-1 of TH), 76-70 (C-5, 3, 4, 2), 49 (C-6), 22 (CH_3).

Synthesis of 6,6′-dideoxy-6,6′-di(S-cysteamine)-α,α'-trehalose (THDCM). 2-Mercaptoethylamine hydrochloride (1.8 g, 15.4 mmol) in water solution (15 mL) containing NaOH (17 mmol) was added to THDTos (1g, 1.54 mmol) in DMF (2 mL). The reaction was carried out under stirring at 60 °C under nitrogen. After 7h the DMF was evaporated in vacuo at 40 °C and the obtained solid dissolved in water was applied on a column (3 $\times$ 500 mm) of CM-Sephadex C-25 resin (in NH_4^+ form). A gradient (0–0.35 M) of NH_4HCO_3 aqueous solution (6 L, total

volume) was used as eluent. The appropriate fractions, R_f = 0.45 (PrOH-H_2O-AcOEt-NH_3 5 : 3 : 1 : 1), were combined and evaporated to dryness at 40 °C in vacuo to decompose NH_4HCO_3. The product THDCM was then isolated (yield, 40% based on THDTos).

^{1}H NMR δ (200 MHz, D_2O): 5.15 (d, 2H, H-1), 3.92–3.74 (m, 4H, H-5,-3), 3.67-3.57 (dd, 2H, H-2), 3.33 (m, 2H, H-4), 3.00 (m, 2H, H-6), 2.90 (m, 4H, CH_2-S), 2.90-2.60 (m, 6H, CH_2-N and H-6′).

^{13}C NMR δ (50.33, D_2O): 95.8 (C-1), 75.1 (C-3,-4), 73.7 (C-2,5), 43.3 (C-6), 41.4 (CH_2-N), 35.0 (CH_2-S).

Synthesis of CDTHCM. A solution of 6A,6D-dideoxy-6A,6D-diiodo-β-cyclodextrin ($ADCDI_2$) [15] (1.1 g, 0.87 mmol) and 6,6′-dideoxy-6,6′-di(S-cysteamine)-α,α'-trehalose (0.4 g, 0.87 mmol) in DMF (150 mL) was stirred at 75 °C under nitrogen. After 4 days the DMF was distilled off in vacuo at 40 °C and the solid obtained was purified by chromatography using a column (30 × 500 mm) of CM-Sephadex C-25 (NH_4^+ form). The column was eluted with a linear gradient (0 → 0.25 M) of NH_4HCO_3 aqueous solution (2 L, total volume). The CDTHCM was isolated as pure. R_f = 0.31; eluent PrOH-H_2O-AcOEt-NH_3 (5 : 3 : 1 : 1); yield 21%. The purity of the product was checked by HPLC, using a Lichrosorb-NH_2 column (5 × 250 mm, 10 μm) and a mixture of CH_3CN and water (1 : 1) as eluent. FAB MS, m/z 1559 (M+1).

^{1}H NMR δ 5.28 (d, 1H, 1-H of TH) 5.25 (d, 1H, 1′-H of TH); 5.11 (d, 7H, 1-H of CD); 4.00-3.83 (m, 28H, 3,5,6-H of CD; 3,5-H of TH); 3.83-3.57 (m, 14H, 2,4-H of CD, 2-H of TH); 3.57–3.37 (m, 8H, cysteamine N-CH_2, 4-A,D, 4-H TH); 3.26 (m, 2H, 6_a-A,D); 3.15–2.76 (m, 10H, 6-H TH, cysteamine S-CH_2, 6_b-A,D).

^{13}C NMR δ: 104.6–101.9 (1-C CD); 95.3 (1-C TH); 86.4-83.5 (4-C CD), 75.7–74.0 (2,3,5-C CD and 2,3,4,5-C TH), 73.1 (5-C A,D), 63.0 (6-C CD), 52.0–50.9 (6-C A,D and cysteamine N-CH_2), 35.9–34.7 (6-C TH and cysteamine S-CH_2).

2.3. NMR SPECTROSCOPY

^{1}H NMR spectra (400 MHz) were recorded on a Varian Unity 400 spectrometer and ^{13}C NMR spectra (50.33 MHz) on a Bruker AC-200 spectrometer in D_2O solutions without a reference compound. Since most of the usual reference compounds interact with the β-CD cavity, DSS (2,2-dimethyl-2-silapentane-5-sulfonate, sodium salt) was used as external reference compound.

2.4. CD SPECTROSCOPY

Circular dichroism spectra were recorded on a JASCO J-600 spectropolarimeter at 25 °C, on freshly prepared aqueous solutions in phosphate buffer (0.015 mol dm^{-3}), pH = 6. Quartz cuvettes of 0.1 cm pathlength were used. The ACS concentration was kept constant (4.0×10^{-4} mol dm^{-3}), whereas the CDTHCM concentration

was varied so as to obtain 1 : 1.5, 1 : 2.5, 1 : 4.5, 1 : 8, 1 : 15, 1 : 25 ACS/CDTHCM molar ratios respectively. C.d. titrations of ACS by CDTHCM at pH = 9.5 and by β-CD were carried out in the same way by using an ammonia buffer (0.015 mol dm^{-3}). Results are reported in terms of $\Delta\epsilon$ (molar CD coefficient) in dm^3 mol^{-1} cm^{-1}.

2.5. CALCULATIONS

A graphical method was used for the determination of the association constants. The following equation was used, not neglecting the analytical concentration of the observed component (ACS in our case) in comparison with that of the other component [16] being taken into account as follows:

$$\frac{c_{0H}\, c_{0G}\, d}{(c_{0H} + c_{0G})\Delta A} = \frac{1}{K\Delta\varepsilon}\frac{1}{c_{0H} + c_{0G}} + \frac{1}{\Delta\varepsilon}$$

where c_{0H} and c_{0G} are the initial concentrations of the host (CDTHCM) and the guest (ACS) respectively. The other symbols have the usual meaning.

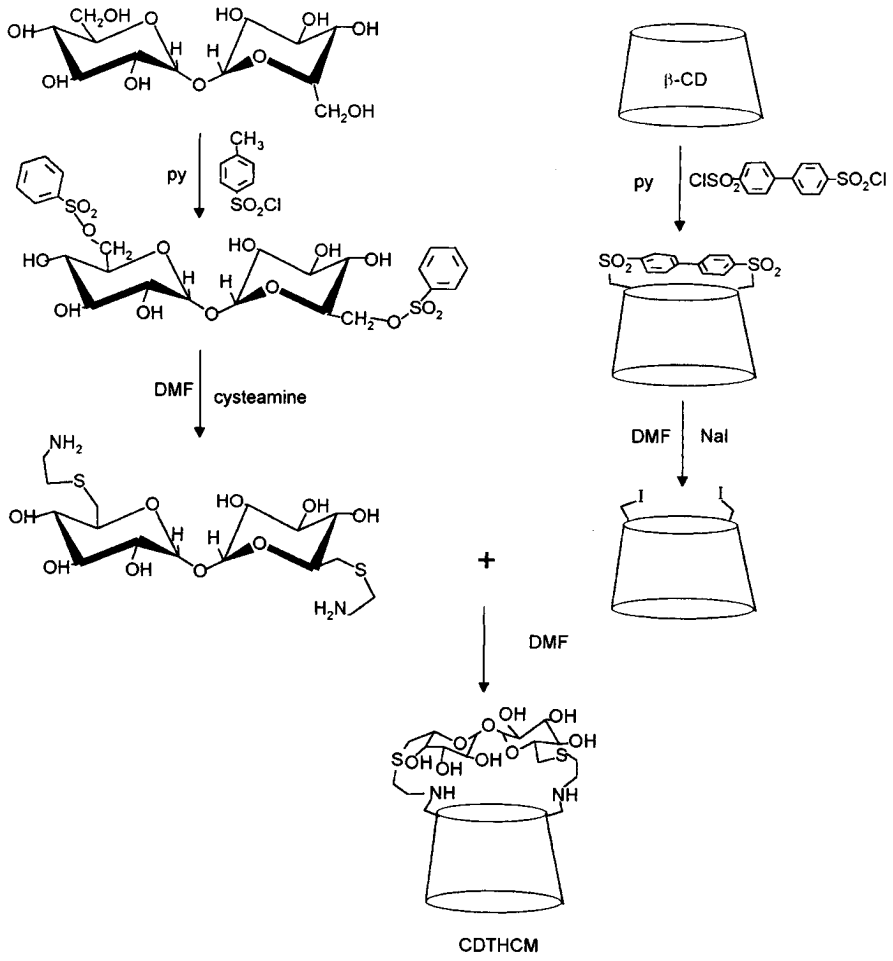
3. Results

Synthesis and characterization of intermediate products. The THDTos intermediate was synthesized according to a slightly modified procedure compared to that reported in the literature [17]. The TH was tosylated without protecting groups at $-35\,^{\circ}C$, exploiting the greater reactivity of the primary OH groups. Careful purification by reverse phase chromatography allows the isolation of pure THDTos. On the NMR spectra, the esterification of the two 6-OH groups induces a downfield shift of the 6-H and 5-H (δ = 4.2 and 2.9 ppm respectively). The aromatic proton peaks are present at 7.79 and 7.44 δ.

The new compound DTHCM was synthesized by nucleophilic substitution of the tosyl group with cysteamine. The large nucleophilicity of S allows the synthesis of the product in aqueous solution, which was isolated as pure by chromatography. The substitution was confirmed by NMR. On the ^{1}H NMR spectra, in addition to the peaks due to the protons of TH, the protons of the ethylenic chains are present. The substitution of two 6-OHs induces a shift of the signals in comparison with the TH spectra. In particular, the 6-OHs which are more affected by substitution are diastereotopic and are present at 2.7 and 3 ppm, upfield in comparison to the TH spectra. The 5-H peak is present at 3.85 ppm.

Synthesis and characterization of CDTHCM. The new compound CDTHCM was synthesized by the reaction of $ADCDI_2$ with the new compound THDCM (Scheme 1). In order to minimize the formation of dimers, THDCM was slowly added to a diluted $ADCDI_2$ solution.

The ^{1}H NMR spectrum of CDTHCM is reported in Figure 1a, together with its assignment, obtained with a COSY spectrum (Figure 2). It may be observed that



Scheme 1.

the trehalose substitution has a slight overall effect on the cavity protons, with the obvious exception of the methylene protons of the substituted rings (6A, 6D, 6′A and 6′D). Similarly, large shifts are observed on the 6-H of TH as well as on all the protons of the two cysteamine bridges. In the H-1 proton region only one peak is shown for all seven glucopyranosinic rings of β-CD, while the two H-1 protons of TH appear at a lower field as two separated doublets, thus showing a loss of equivalence in this symmetric molecule following the reaction with the cavity. This asymmetry is also seen in the TH H-6 protons. Besides the usual diastereotopicity between the two protons of each methylene, these show a subtle chemical shift difference between the analogous protons of the two different methylenes.

The CDTHCM has two secondary amino groups and can form a mono- and a di-protonated species. The CDTHCM was titrated by DCl and the variation

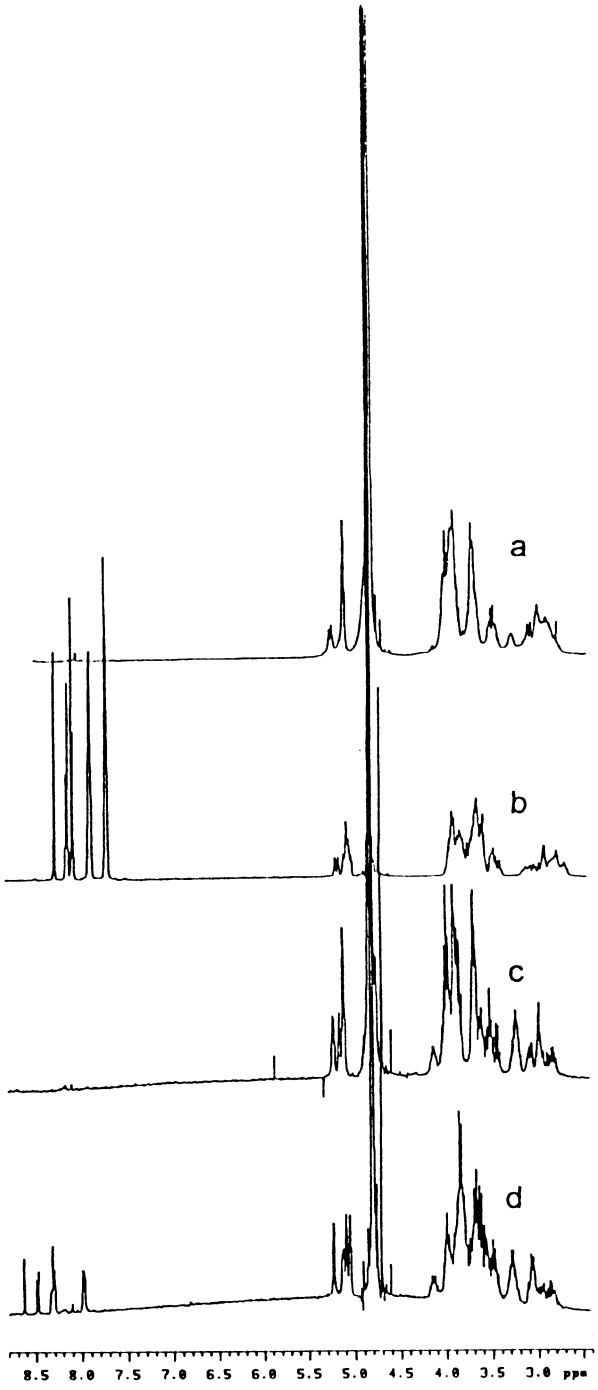


Figure 1. ^{1}H NMR Spectra of CDTHCM: (a) at pH 9.5 (unprotonated species), reported for comparison; (b) at pH 9.5 in the presence of ACS; (c) at pH 6 (diprotonated species); (d), at pH 6 in the presence of ACS.

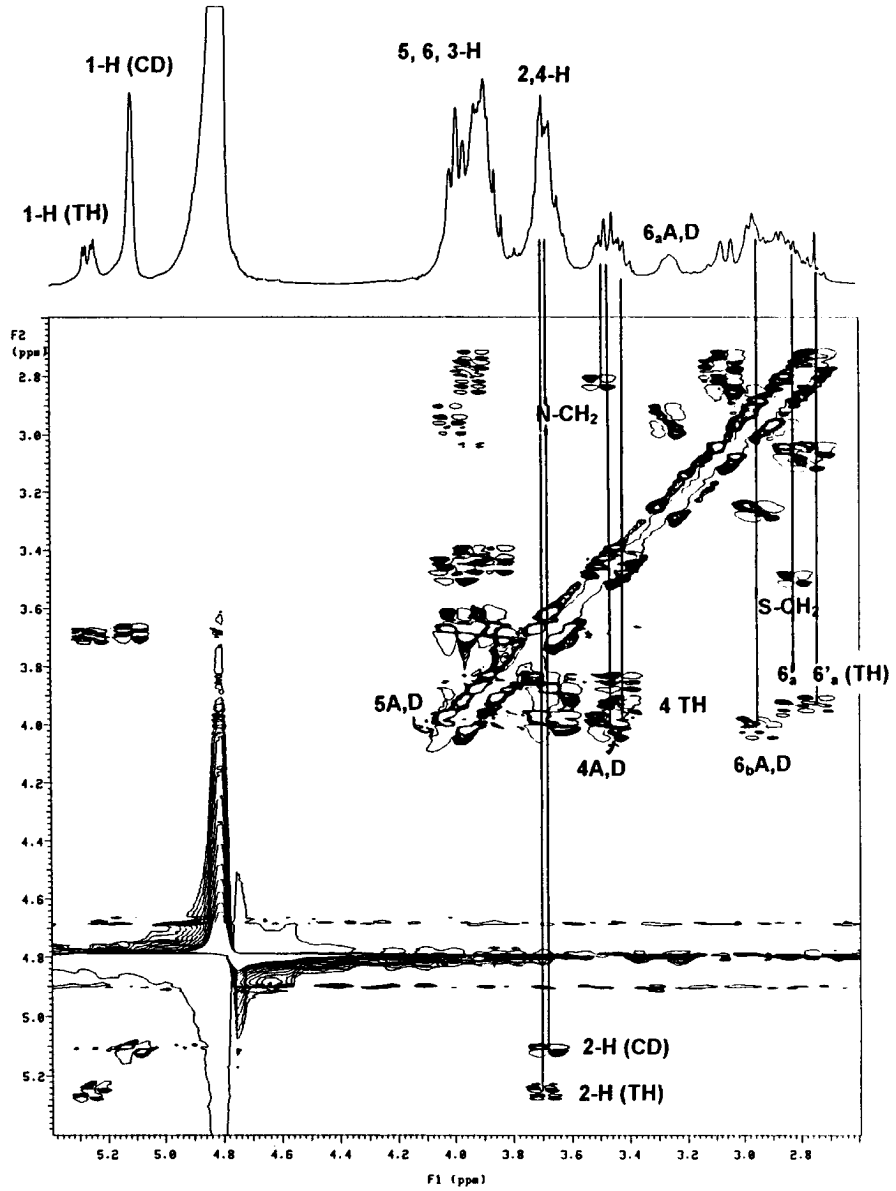


Figure 2. The DQF COSY contour plot for CDTHCM (400 MHz, D_2O).

was followed by ^{1}H NMR spectroscopy. These experiments show that the two protonation constants are very similar and quite low for an amino group (log K is about 7.5), as typically observed for this kind of derivative [18, 19].

The ^{1}H NMR spectrum of the diprotonated species (pH 6), reported in Figure 1c, appears as being more complex than that of the unprotonated species, in com-

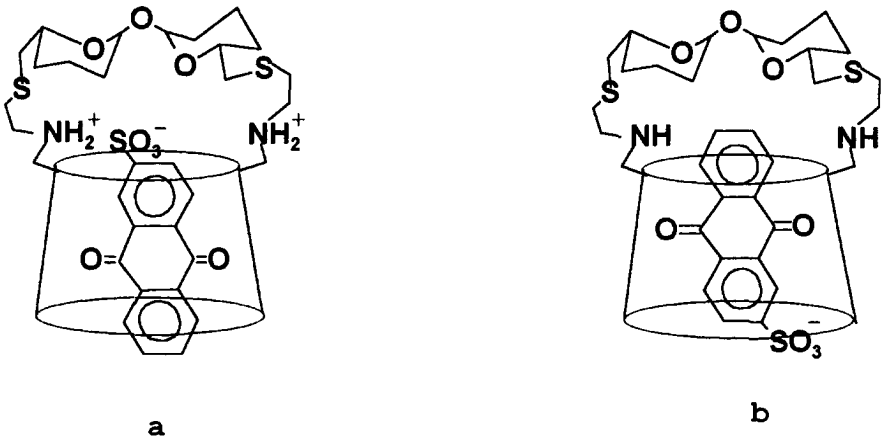


Figure 3. Schematic representation of the CDTHCM/ACS complexes at: (a) pH = 6 and (b) pH = 9.5.

parison with the other investigated systems, as is clearly observed for the 1-protons. The spectrum shows the increased asymmetry of the cavity, probably due to the formation of hydrogen bonds between the protonated nitrogens of cysteamine moieties and upper rim hydroxyl groups. As expected, the larger upfield shifts are observed for the methylene protons adjacent to protonation sites (nitrogen atoms) as well as for the 5-A and 5-D protons, which is usual for this kind of compound [18, 19].

Investigation of the CDTHCM-ACS system. The ^{1}H NMR spectrum of the CDTHCM-ACS (anthraquinone-2-sulfonic acid) system at 400 MHz in D_2O (no acid added) (Figure 1b) shows the dramatic effect that ACS inclusion has on host proton chemical shifts. As expected, the 3- and 5- protons of β-CD are greatly affected. Interestingly, TH proton chemical shifts are also influenced, suggesting the deep inclusion of ACS within the CDTHCM cavity. The ROESY spectrum shows correlations between the inner protons (3,5) of the cavity with the protons of the ACS unsubstituted benzene ring, suggesting that this ring is deeply included in the CD cavity. The sulphonic group seems to be near to the CD secondary OH rim, outside the cavity, as sketched in Figure 3a.

The ^{1}H NMR spectrum of the diprotonated species (pH 6, phosphate buffer) in the presence of ACS shows the spreading of the proton chemical shifts, clearly observed in the 1-H region (Figure 1d), where each 1-H has a chemical shift different from the other eight. This suggests that the inclusion of ACS, besides producing a ring current effect, induces a distortion of the cavity in addition to the protonation effect. The correlation observed in the ROESY spectrum between the inner protons (3 and 5 of CD) of the cavity and the protons of the substituted ring of ACS suggests that, at this pH, the ACS disposition is upside-down compared to

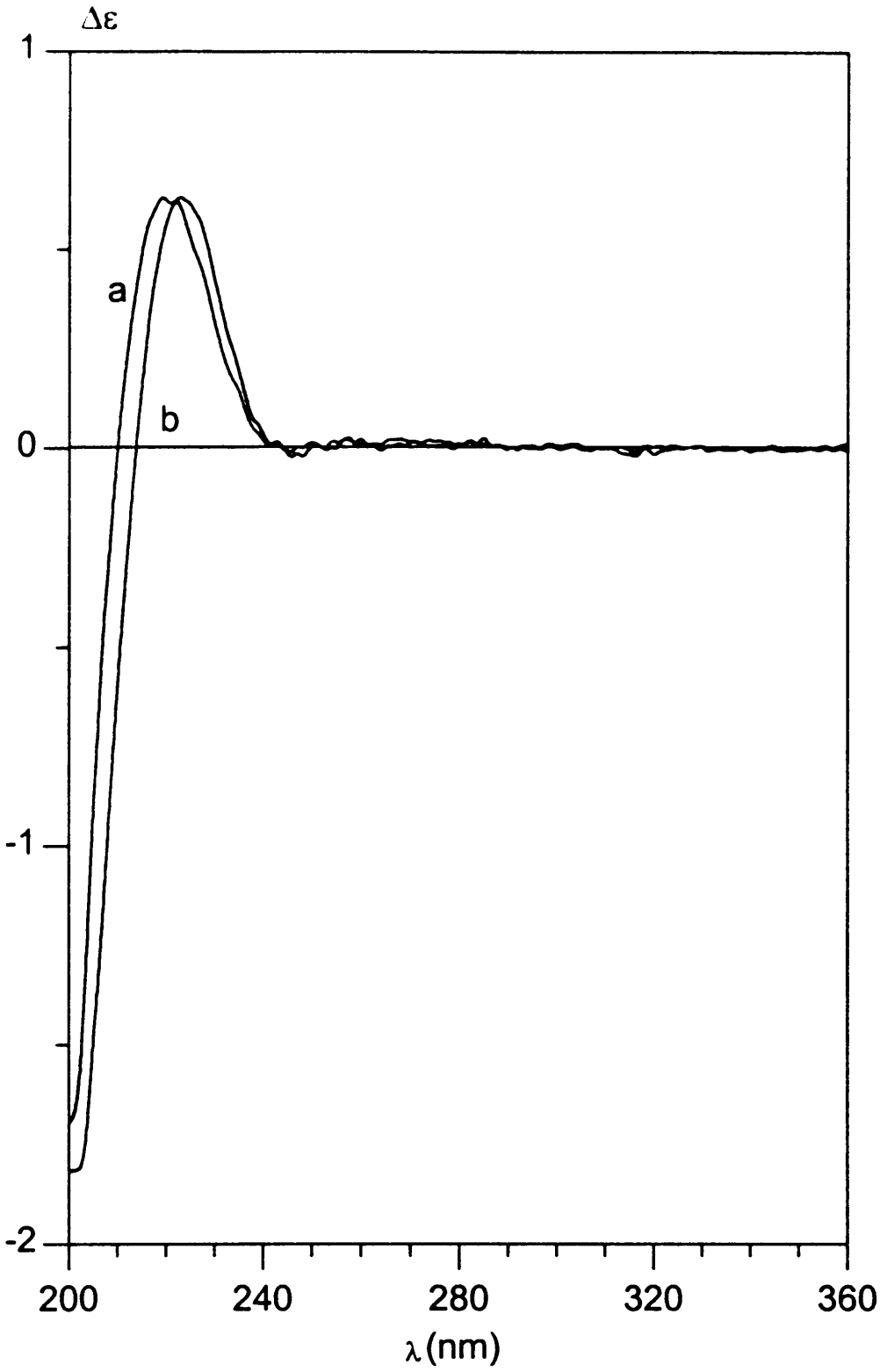


Figure 4. C.d. spectrum of CDTHCM: (a) pH = 6, (b) pH = 9.5.

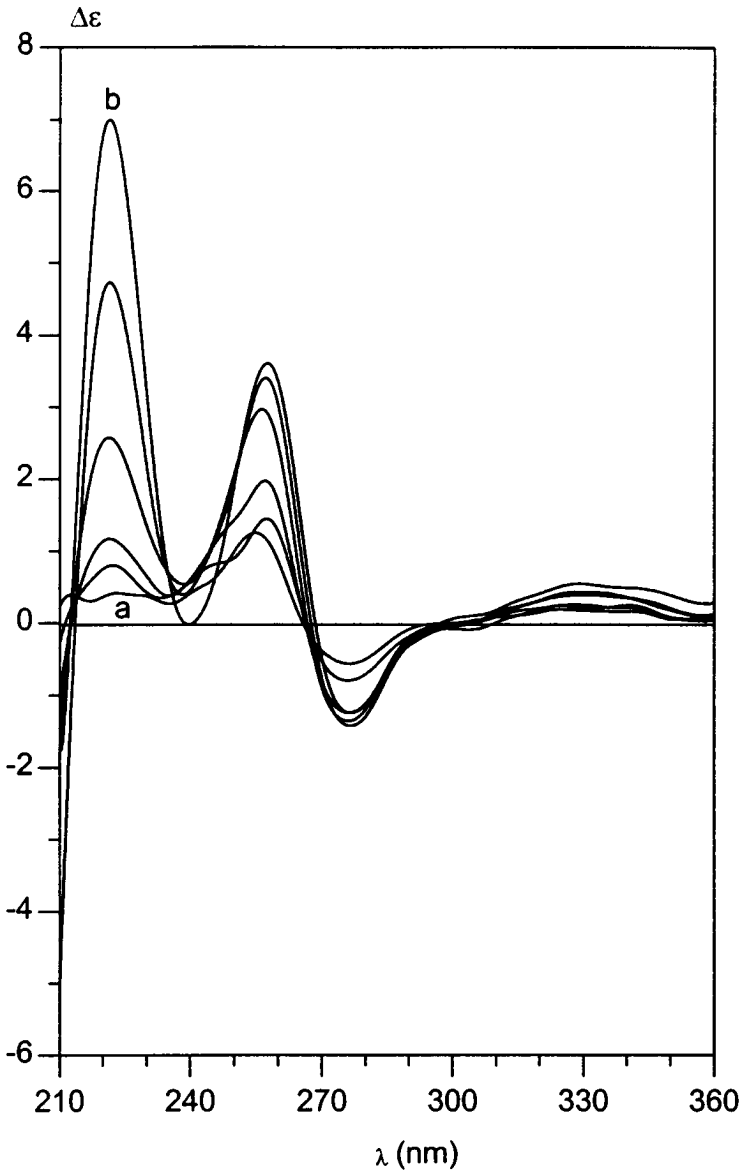


Figure 5. The i.c.d. spectra of ACS obtained by titration by CDTHCM: pH = 9.5. 1 : 1.5, 1 : 2.5, 1 : 4.5, 1 : 8, 1 : 15, 1 : 25 ACS/CDTHCM molar ratios (read from curve a to curve b).

what was observed at basic pH. This is presumably in order to permit an electrostatic interaction between the sulphonic group and one of the protonated amino groups. The proposed model is sketched in Figure 3b.

The c.d. spectrum of CDTHCM and that of its protonated species, reported in Figure 4, are very similar. The band at 220 nm in particular shows a positive Cotton

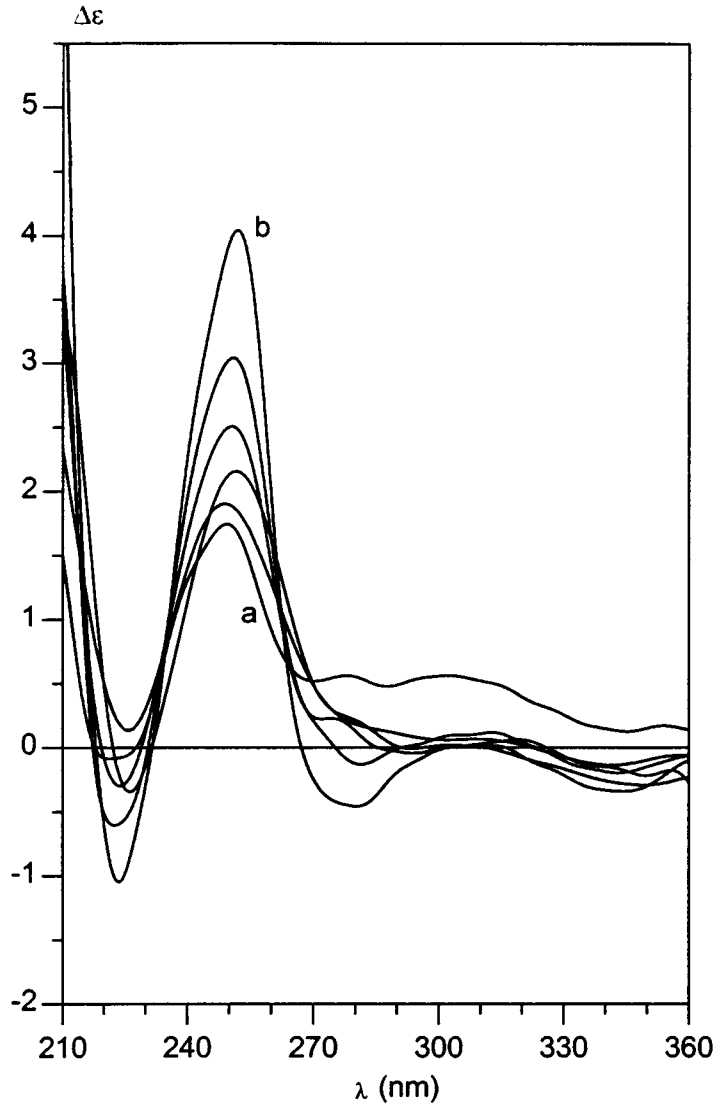


Figure 6. The i.c.d. spectra of ACS obtained by titration with CDTHCM at pH = 6. 1 : 1.5, 1 : 2.5, 1 : 4.5, 1 : 8, 1 : 15, 1 : 25 ACS/COTHCM molar ratios (read from curve a to b).

effect and is has undergone a slight shift due to a change in pH. The corresponding electronic transition involves the sulfur atoms of the achiral capping moiety. The observed Cotton effect is clearly induced by the moiety's interaction with the β-CD cavity to which it is bonded.

Figures 5 and 6 report the c.d. spectra of the CDTHCM/ACS system at basic and acidic pH values. Comparison with the c.d. spectrum of the host (Figure 4) and with the UV spectrum of ACS (not reported), shows that, while the band at

a shorter wavelength (about 220 nm) corresponds to a CDTHCM transition, the other two bands at about 250 and 280 nm, correspond to ACS transitions. This indicates that the achiral guest gives rise to an i.c.d. spectrum by inclusion in the CDTHCM cavity.

The inversion of the sign for the Cotton effect of the 220 nm transition, observed in the CDTHCM/ACS system at acidic pH, may be explained by recalling the structures of the inclusion complex. This is borne out by the ROESY results and can be seen in Figure 3. At acidic pH, the protonated nitrogens come as close as possible to the negative charge of the sulfonate, and force the capping moiety into a new conformation. This determines a different angular disposition between its average plane and the cavity.

As far as the electronic transitions of ACS at 250 and 280 nm are concerned, the sign for the Cotton effects (positive and negative respectively) at both the pH values investigated (Figures 5 and 6) confirms its longitudinal disposition inside the cavity (Figure 3), as is confirmed in the ROESY results. The titrations carried out at both pH values allowed determination of the CDTHCM-ACS association constant [16], by using the values observed at 252 nm where the Cotton effect is stronger, corrected for the slight CDTHCM contribution. The value obtained at pH = 6 (phosphate buffer) (K_{ass} = 3589 M^{-1}) is higher both compared to that obtained at basic pH (K_{ass} = 819 M^{-1}) and to that of β-CD (K_{ass} = 598 M^{-1}) [20], and shows the importance of the electrostatic interaction as well as the capping of the CD in order to increase the strength of the host-guest interaction.

4. Conclusion

The new compound CDTHCM shows improved inclusion properties in comparison with the parent CD. The NMR investigation at pH = 6 suggests that the electrostatic interaction between the NH protonated group and the sulfonic group assists the inclusion of the guest, promoting a specific disposition which directs it within the cavity. At basic pH the significant increase in the stability of the inclusion complex compared with that of unsubstituted β-CD is ascribable to the capping unit.

This host can be considered the prototype of a three-dimensional cyclodextrin. By varying the choice of "bridge" groups it is possible to tune the strength and selectivity of these receptors towards specific substrates as well as their coordinating ability towards metal ions. Work is in progress in our laboratories in order to synthesize other compounds of this class.

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