

A conformational model of per-*O*-acetyl-cyclomaltoheptaose (- β -cyclodextrin) in solution: detection of partial inversion of glucopyranose units by NMR spectroscopy

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Abstract

The stereochemical features of per-*O*-acetyl-cyclomaltoheptaose (- β -cyclodextrin) have been investigated in solution by NMR spectroscopy, and the deviation of functionalised glucopyranose rings from 4C_1 chairs to skew-type conformations has been detected. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Cyclomaltooligosaccharides; Cyclodextrins; NMR; Stereochemistry; Conformational distortions

1. Introduction

Cyclomaltooligosaccharides, commonly known as cyclodextrins, are macrocycles constituted by α -(1 \rightarrow 4)-linked glucose units in a 4C_1 conformation. The ordered network of hydrogen bonds between the secondary hydroxyl groups of adjacent rings determines a global rigid truncated cone shape, where the hydroxyls are bent at the external surface making it hydrophilic, whereas the glycosidic oxygens and some methine groups, pointing at its inside, generate the hydrophobic environment inside the cavity. Thus, native cyclodextrins are soluble in aqueous solvents, but the included water molecules are easily displaced by apolar substrates, favouring inclusive complexation processes.^{1–3}

The presence of several hydroxyl functions, three for every glucopyranose ring, with strongly differentiated reactivities, has led to the development of many kinds of selectively or exhaustively functionalised cyclodextrins.^{1–3} The resulting changes of their chemical–physical and complexing properties reflect not only the stereoelectronic features of the new functional groups, but also, and in some cases mainly, the degree and the

nature of the conformational distortions introduced. More flexible structures can be obtained, due to the loosening or break of the hydrogen-bond belt. Consequently the glucopyranose rings can rotate about the glycosidic linkages, as well as some isolated units convert from the usual 4C_1 chair conformation to skew or quite completely inverted 1C_4 conformers.^{4–7} Furthermore, the functional groups may converge themselves towards the inner cavity producing bowl-shaped structures, where the external surface and the functional groups departing from it become more exposed to complexation phenomena than groups inside of the cavity.^{6,7}

Due to the remarkably wide variations among derivatized cyclodextrins, their stereochemical features have been the subject of many investigations in an attempt to correlate stereochemistry to their behaviour in several chemical, analytical or pharmaceutical applications. Such studies have been carried out in the solid state by X-ray analyses,⁸ but also the conformational preference between fast-exchanging conformers in solution has been considered by exploitation of the powerful methods offered by NMR spectroscopy.^{9–16} By using these tools, the conformations of methylated,^{4–6} benzylated and benzoylated^{4,7} cyclodextrins have been investigated.

In view of the relevance of peracetylated derivatives of cyclodextrins as effective slow-release carriers for

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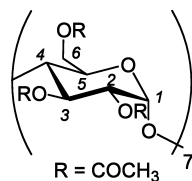


Fig. 1. Structure of peracetylated β -cyclodextrin.

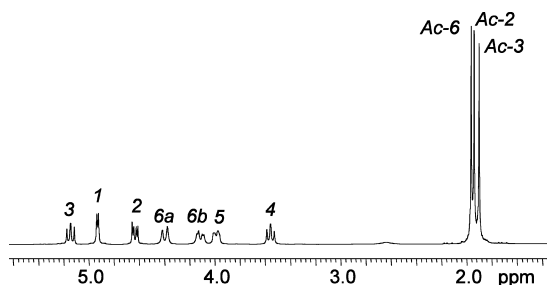


Fig. 2. ^1H NMR spectrum (600 MHz, CDCl_3 , 25 $^\circ\text{C}$) of peracetylated β -cyclodextrin.

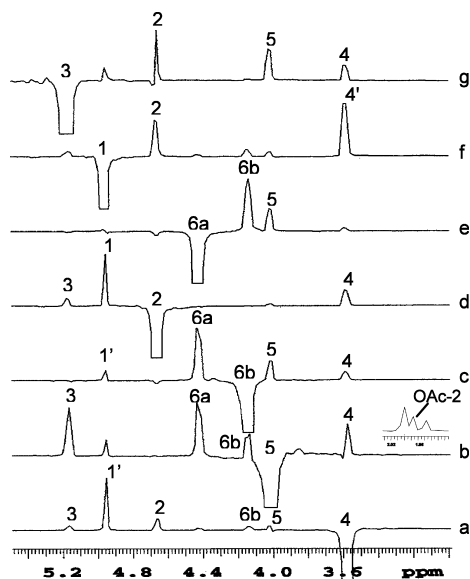


Fig. 3. 2D ROESY (600 MHz, CDCl_3 , 25 $^\circ\text{C}$, $\tau_m = 0.3$ s) analysis of peracetylated β -cyclodextrin. Traces corresponding to: (a) H_4 ; (b) H_5 ; (c) H_{6b} ; (d) H_2 ; (e) H_{6a} ; (f) H_1 ; (g) H_3 .

drugs,¹⁷ their solid-state structures have been recently described as elliptically distorted macrocycles where glucose units undergo significant rotations about the glycosidic connections and deformation to twisted conformers.¹⁸ In order to gain more insight on their solution stereochemistry, which could give a complementary contribution to the rationalization of their complexing capabilities, we have carried out an NMR investigation, involving the use of 2D ROESY¹⁹ and proton mono- and biselective relaxation methods,²⁰ on the heptakis(2,3,6-tri-*O*-acetyl)- β -cyclodextrin (Fig. 1) in two solvents: CDCl_3 and CD_3OD . The first constitutes an interesting means for analytical applications,

while the latter better fit the experimental conditions for drug-release experiments, where water–ethanol mixtures are employed.

2. Results and discussion

The proximity constraints between the protons of peracetylated β -cyclodextrin have been obtained by detecting their dipolar interactions by 2D ROESY analysis in CDCl_3 and CD_3OD . The proton spectrum of the cyclodextrin in CDCl_3 shows well-separated signals in the region between 1.80 and 5.30 ppm (Fig. 2). The three sharp singlets at 2.00, 1.97 and 1.93 ppm have been attributed to the methyl groups on the 6-, 2- and 3-sites, respectively, by detecting the long-range H–C connectivities in the HMBC map: the assigned H-3, H-2 and H-6a6b protons correlating to carbonyl groups on the same sites and these last to the respective methyl protons.

The *J*-coupling patterns of the protons between 3.5 and 5.3 ppm are very similar to those of the underivatized systems, thus allowing the immediate assignment of the cyclodextrin protons.

The analysis of the traces of the 2D ROESY map (Fig. 3) reveals the presence of NOEs, which can be interpreted as originated by protons lying on adjacent rings, giving their relative positions; other effects reflect the conformation of the glucopyranose rings and, hence, the degree and the nature of the deviation from the expected usual $^4\text{C}_1$ chair conformation. Finally, the pattern of dipolar interactions due to protons lying on the 5 and 6 sites allows us to define the conformational preference about the C-5–C-6 bond. To begin with this last aspect, the trace of the proton H-5 (Fig. 3b) shows a more intense dipolar interaction with the high-frequency H-6a proton relatively to the effect detected on the low-frequency H-6b. An interesting difference concerning the two diastereotopic methylene protons regards their interNOEs with H-4 and H-1', respectively, lying on the same unit and on the adjacent one. In fact, the proton H-6b (Fig. 3c) shows equivalent effects on them, whereas the H-6a–H-4 and H-6a–H-1' NOEs (Fig. 3e) are negligible.

Therefore, the conformation of this fragment can be described by the gauche Newman projection of Fig. 4 having the proton H-6a, closer to H-5, far away from H-4 and H-1' and the proton H-6b bent toward them.

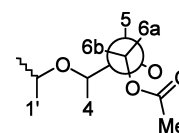


Fig. 4. Schematic representation of the conformation in CDCl_3 of the C-5–C-6 fragment.

On proceeding to discuss the inter-unit dipolar interactions, the most valuable information is drawn from the trace of the proton H-1 (Fig. 3f), which gives rise to a less intense NOE on proton H-2 of the same ring with respect to the effect measured on H-4' of the adjacent one, to indicate a low degree of rotation of the glucopyranose rings about the glycosidic linkages (Fig. 5).

It is noteworthy that also for underivatized cyclodextrins and, at least in solution, for methylated systems, the bonds C-1–H-1 and C-4'–H-4' of linked units are nearly coplanar,^{4–6} bringing the two respective protons at a distance that is shorter with respect to the intraring distance H-1–H-2. By contrast, in the cases of cyclodextrins bearing bulk benzyl or benzoyl groups on the secondary sites, a relevant clockwise or anticlockwise rotation of the linked units was detected.⁴

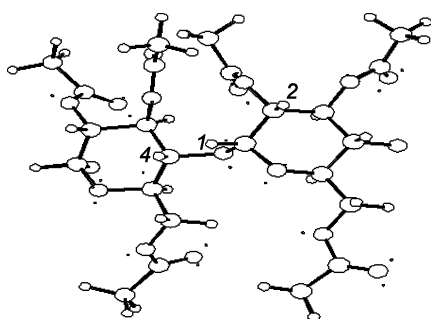


Fig. 5. Schematic representation of two adjacent glucose units of cyclodextrin.

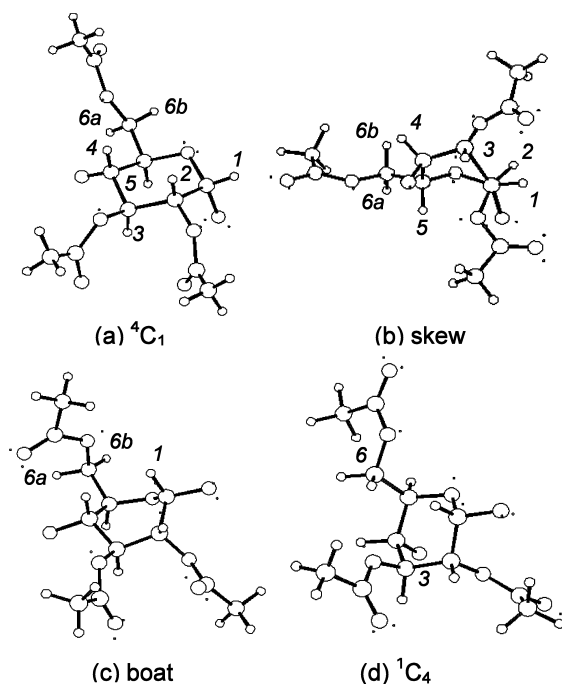


Fig. 6. Schematic representation of a glucopyranose unit of the peracetylated cyclodextrin in the 4C_1 chair (a), skew (b), boat (c) and 1C_4 chair (d) conformation.

Traces of H-2, H-3, H-4 and H-5 (Fig. 3) are the basis for the discussion of the conformational aspects of the glucopyranose rings. H-2 (Fig. 3d) shows not only the usual 1,3-dipolar interaction with the external proton H-4, but also a surprising NOE with internal H-3. The first effect is the expected one for the 4C_1 chair conformation (Fig. 6a), whereas the latter can be explained only through the skew-type conformation shown in Fig. 6b, which has H-2, H-3 and H-4 in inverted positions, i.e., equatorial. As a matter of fact, H-3 (Fig. 3g) produces the NOE at the frequency of H-5, which is due to the chair conformation, as well as NOEs at the frequencies of H-2 and H-4. On the basis of the above effects, some kinds of chair deformations can be excluded: no boat situations of Fig. 6c can occur as these should rather shorten the average value of the distance H-1–H-6a6b due to a relevant intra-ring contribution. The complete inversion to the 1C_4 chair (Fig. 6d) seems to be unlikely to occur, as this conformation should bring the acetyl on the three secondary sites in close proximity of the methylene protons, and the correspondingly intense dipolar interactions between the two groups are not detected. Finally, H-5 generates a detectable interNOE at 1.97 ppm (Fig. 3b), corresponding to the acetyl on the 2-site. This effect is strongly indicative of the fact that in the distorted glucopyranose ring(s) the acetyl on the 2-site must be in proximity of H-5 and hence pseudoaxial, bringing H-2 pseudoequatorial on the same site.

The extent of average distortion in the derivatized cyclodextrin has been also efficiently revealed by proton-selective relaxation-rate measurements,²⁰ already successfully employed to detect the conformational distortions occurring in alkylated, benzylated and benzoylated cyclodextrins.⁴ These parameters are determined by selective inversion of the spin i , leaving unperturbed the other protons, and following its recovery in the time (monoselective experiment, affording the monoselective relaxation rate of the proton i , R^i). When the same experiment is performed by inverting simultaneously the proton pair i – j and following the recovery of the magnetization of i , the biselective relaxation rate of i is determined, indicated as R^i_{ij} . The differences between the biselective and monoselective relaxation rates give the cross-relaxation parameter for the proton pair ij , σ_{ij} .

$$\sigma_{ij} = R^i_{ij} - R^i \quad (1)$$

In the initial rate approximation,²¹ the cross-relaxation terms describe the magnetization transfer between the proton pair, which is a very simple function of the reorientational correlation time τ_c of the vector connecting the two spins and of their distance r_{ij} .

$$\sigma_{ij} = 0.1\gamma^4\hbar^2 r_{ij}^{-6} \tau_c \left[\frac{6}{1 + 4\omega^2 \tau_c^2} - 1 \right] \quad (2)$$

Table 1

Measured mono- (R^i , s⁻¹), biselective (R_{ij}^i , s⁻¹) and cross-relaxation (σ_{ij} , s⁻¹) rates for the protons of peracetyl- β -cyclodextrin in CDCl₃ and calculated interproton distances (r_{ij} , Å)

ij	R^i	R_{ij}^i	σ_{ij}	r_{ij}
6a6b	2.03 ± 0.01	1.51 ± 0.01	-0.52	1.78 ²²
12	0.88 ± 0.01	0.77 ± 0.01	-0.11	2.31
23	0.67 ± 0.01	0.63 ± 0.01	-0.04	2.73
34	0.61 ± 0.01	0.56 ± 0.01	-0.05	2.63
24	0.67 ± 0.01	0.61 ± 0.01	-0.06	2.55
35	0.61 ± 0.01	0.54 ± 0.01	-0.07	2.49
56a	1.18 ± 0.01	1.07 ± 0.01	-0.11	2.31
56b	1.18 ± 0.01	1.12 ± 0.01	-0.06	2.55
14'	0.88 ± 0.01	0.70 ± 0.01	-0.18	2.12

where γ is the proton gyromagnetic ratio, ω is the proton Larmor frequency and \hbar is the reduced Planck's constant.

In the fast and slow motion regimes ($\omega^2\tau^2 \ll 1$ and $\omega^2\tau^2 \gg 1$, respectively) the above equation can be approximated as:

$$\text{fast motion } \sigma_{ij} = 0.5\gamma^4\hbar^2r_{ij}^{-6}\tau_c \quad (3)$$

$$\text{slow motion } \sigma_{ij} = -0.1\gamma^4\hbar^2r_{ij}^{-6}\tau_c \quad (4)$$

In the hypothesis of isotropic motion, the ratios of the cross-relaxation terms are very simply correlated to the ratios of the interproton distances:

$$\frac{\sigma_{ij}}{\sigma_{ik}} = \frac{r_{ik}^6}{r_{ij}^6} \quad (5)$$

Thus, we determined the monoselective relaxation rate of H-6a or H-6b and their respective biselective relaxation rates, which are indicated in Table 1. From their difference we calculated the cross-relaxation parameter corresponding to the fixed interproton distance of 1.78 Å.²² It is to be noted that the cross-relaxation rate is negative, according to the slowing down of the molecular motion of the cyclodextrin due to the introduction of three acetyl groups on every unit. Then we measured the monoselective relaxation rates of selected cyclodextrin protons and some biselective parameters, which are included in Table 1.

The calculated cross-relaxation rates for the different proton pairs have been used to determine the corresponding average proton distances (Table 1) by using Eq. (5), and by putting in it the cross-relaxation value σ_{6a6b} and the fixed distance r_{6a6b} . Of these distance values, the r_{23} and r_{34} , corresponding to vicinal protons, are 2.73 and 2.63 Å, respectively, and, hence, shorter than they are for the ⁴C₁ chair conformation. Therefore, these values must reflect the contribution arising from the skew units having the above vicinal protons in equatorial positions, as already discussed on the basis of NOE data.

On assuming that the distance r_{23} for the ⁴C₁ chair can be estimated as about 2.90 Å and that one for 1,2-diequatorial protons in the skew conformation as about 2.35 Å, from the Eq. (6), we can estimate the presence of about two distorted units.

$$r_{ij} = P_{\text{chair}}r_{ij}^{\text{chair}} + P_{\text{skew}}r_{ij}^{\text{skew}} \quad (6)$$

where P_{chair} and P_{skew} are the populations of the undistorted (chair) and distorted (skew) units, with $P_{\text{chair}} + P_{\text{skew}} = 1$ and r_{ij}^{chair} and r_{ij}^{skew} are the typical values for the ij interproton distances in the undistorted (chair) and distorted (skew) units.

The distance r_{12} of 2.31 Å corresponds to the mean value for two protons in an axial-equatorial spatial relationship, and it is unaffected by the presence of distorted units.

A different interpretation must be given to the r_{24} and r_{35} values, which are 2.55 and 2.49 Å, very close to the values for the 1,3-diaxial protons in the ⁴C₁ units. On the other hand, only the undistorted units can contribute to the distance values calculated by cross-relaxation data. Indeed, due to the nonlinear dependence of σ by the distance (Eq. (5)), any distortion leading the H-3-H-5 or H-2-H-4 protons in a relative conformation different from the diaxial ones (as in the case of the skew units) should generate interproton distances greater than 3.5 Å, which are not detectable by this kind of measurements.

The two distances r_{56a} and r_{56b} , 2.31 and 2.55 Å, respectively, are according to the gauche Newman projection indicated in Fig. 4 and to the relative intensities of the NOEs detected in the 2D ROESY map (Fig. 3b).

Finally, the distance of 2.12 Å calculated for the proton pair 1-4' on adjacent units, which is the average mean of all the units, reflects the low degree of tilting of the glucopyranose rings.

The same kind of analysis performed in CD₃OD gave very similar results, being clearly detected the presence of glucopyranose rings both in the ⁴C₁ chair and skew-type conformations.

In conclusion, peracetylation produces significant deformations of β -cyclodextrin, both in the solid state¹⁸ and in solution. These are determined by the deviation of some glucose units from the ⁴C₁ chair to skew-type rings, as clearly evidenced in solution on the basis of the proximity constraints imposed by NOE data and interproton distances obtained by relaxation methods. In this regard, it is worth noting that cross-relaxation parameters constitute a very efficient way to detect the contribution arising from conformers having low populations but characterized by short interproton distances. The stereochemical distortions evidenced in solution are similar to the symmetry perturbations detected for cyclodextrins bearing benzoyl, at least on the secondary sites.⁷ Owing to the loss of the strong hydrogen-bond network between the hydroxyl functions on the sec-

ondary sites of the adjacent units and the need of minimizing the repulsive steric interactions between the bulk substituents, the glucopyranose units assume preferential conformations, which are intermediate between the two chairs. These ring deformations must concern only some units as the undistorted 4C_1 chairs are also simultaneously detected. Consequently, the truncated cone shape is heavily perturbed, and the polarity differences between the outside and the inside of the cavity can be affected significantly. As a matter of fact, not only is the solubility of derivatized cyclodextrins strongly affected, but also their complexing properties. Moreover, the formation of supramolecular adducts depends on the functional groups introduced rather than the nature of the cavity.⁷ Finally, exhaustive functionalization makes the conformation of the cyclodextrin quite insensitive to the nature of the solvent employed, as for the two cases examined, CD_3OD and $CDCl_3$, very similar conformations have been found.

3. Experimental

3.1. General methods

NMR measurements were performed on a spectrometer operating at 600 and 150 MHz for 1H and ${}^{13}C$, respectively. The temperature was controlled to ± 0.1 °C. 1H NMR chemical shifts are referenced to Me_4Si as external standard. The 2D NMR spectra were obtained by using standard sequences. The ROESY (Rotating-frame Overhauser Enhancement Spectroscopy) spectra were recorded in the phase-sensitive mode, by employing a mixing time of 0.3 s. The spectral width used was the minimum required in both dimensions. The pulse delay was maintained at 5 s; 512 hypercomplex increments of 8 scans and 2K data points each were collected. The data matrix was zero-filled to $2K \times 1K$, and a Gaussian function was applied for processing in both dimensions. The gradient HMBC (Heteronuclear Multiple Bond Correlation) experiment was optimized for a long-range 1H – ${}^{13}C$ coupling constant of 8 Hz. The spectrum was acquired with 512 time increments, 32 scans per t_1 increment and a 3.5 ms delay period for suppression of one-bond correlation signals. The spectrum was acquired using no decoupling during acquisition. The selective relaxation rates were measured in the initial rate approximation²¹ by employing a selective π -pulse at the selected frequency. After the delay τ , a non-selective $\pi/2$ pulse was employed to detect the longitudinal magnetization. For the biselective measurements, the two protons were inverted consecutively. Each selective relaxation rate experiment was repeated at least four times.

3.2. Materials

Heptakis(2,3,6-tri-*O*-acetyl)- β -cyclodextrin was purchased from Aldrich Chemical Co.

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