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NMR Spectral Assignment of Per-substituted Key-Intermediates of β-Cyclodextrin and Implications in the Structures of the Derivatives

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Abstract

The compounds, 6-per-*O*-(*t*-butyldimethylsilyl)-β-cyclodextrin (**1**), 2,3-per-*O*-benzyl-6-per-*O*-(*t*-butyldimethylsilyl)-β-cyclodextrin (**2**), 2,3-per-*O*-benzyl-β-cyclodextrin (**3**), 2,3,6-per-*O*-benzyl-β-cyclodextrin (**4**), 2,3,6-per-*O*-benzoyl-β-cyclodextrin (**5**), are used as key intermediates in the synthesis of selectively substituted β-CD derivatives. Simple and assignable ^{1}H and ^{13}C NMR spectra (chemical shifts and coupling constants) were obtained for compounds **1–4** indicating C_7 symmetry, $^4C_1$ glucose conformation and major *gg* arrangement of H6, H6′ atoms at the primary side. The derivative **5**, however, gave very broad peaks at room temperature. The peaks could partially be assigned at 270 K, but the broadening was still present at 220 K. This implies that there exist several conformers of similar energy and C_1 symmetry that continuously interchange, since there is not a single type of stabilizing interaction that predominates. We attributed this phenomenon to the presence of the carbonyl group, which probably disfavors π-π stacking and induces random arrangements of the aromatic rings.

Introduction

Several key intermediates widely used in the synthesis of regioselectively substituted cyclodextrins, frequently exhibit conformational flexibility, especially in cases where the secondary hydroxyls are substituted. Such behavior has been observed in per-2,3-*O*-benzoyl-α-CDs and per-2,3-*O*-aroyl-α-CDs, molecules specifically characterized as having a C_3 molecular symmetry, instead of the anticipated C_6. This was attributed to the tilting of alternative constituent units to form intra-glucose H-bonds [1, 2]. In a later assessment [3], the C_3 symmetry of per-2,3-*O*-benzoyl-α-CD was attributed to exchange between two types of glucose units, one normal and one distorted (twisted) chair, where the establishment of C=O$\cdots$H—O hydrogen bonds (between the substituent and the primary OH groups) was invoked as the driving force to distortion. On the other hand, per-2,3,6-*O*-benzoyl-α-CD shows spectra originating from a C_6 symmetry [3] whereas per-2,3,6-*O*-benzoyl-β-CD is reported as being fluxional [4, 5] with no further elaboration. Similarly, per-6-*O*-aroyl-2,3-*O*-benzyl-β-CD derivatives exhibit conformational isomerism, with exchange between C_1 and C_7 conformers [4]. In all the above cases, the notion of "cavity" space is probably meaningless, since the conformational mobility totally destroys the cavity shape and flipping of the glucose units results in self-filling of the internal space of the macrocycles. Previous investigation in this laboratory of NMR data in aqueous solution of per-2,3,6-*O*-methyl-α-, -β-, and -γ-CD, also pointed to increased flexibility [6] as the cavity size increased.

In this report, we compare NMR data for a series of per-substituted β-CDs in an attempt to rationalize the occurrence of room temperature conformational exchange, in typical β-CD derivatives. The compounds used, are key intermediates in the synthesis of selectively substituted sites of β-CD.

Experimental

6-Per-*O*-(*t*-butyldimethylsilyl)-β-cyclodextrin (**1**) [7–9], 2,3-per-*O*-benzyl-6-per-*O*-(*t*-butyl-dimethylsilyl)-β-cyclodextrin (**2**) [5], 2,3-per-*O*-benzyl-β-cyclodextrin (**3**) [5], 2,3,6-per-*O*-benzyl-β-cyclodextrin (**4**) [10], 2,3,6-per-*O*-benzoyl-β-cyclodextrin (**5**) [2], were synthesized and purified by silica gel column chromatography using the corresponding literature methods. They were identified by their melting points and their NMR spectral data. The NMR spectra were acquired on a Bruker DRX Avance 500 MHz (11.7 Tesla) instrument at 300 K, unless stated otherwise, in deuterated chloroform or toluene, as indicated. The 2D spectra (COSY, TOCSY, HSQC, HMBC, ROESY) were acquired using pulse sequences provided in the software's library. For COSY, TOCSY and HSQC spectra, gradient selection was employed, while the others were run with phase cycling. The mixing times were 80 ms in the TOCSY

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Table 1. Chemical shifts ($CDCl_3$, δ_{1_H} at 7.24 ppm) and coupling constants of derivatives **1–4*** at 300 K.

#	δ (ppm)						Coupling constants J (Hz)						
	H1	H2	H3	H4	H5	H6 H6′	H1H2	H2H3	H3H4	H4H5	H5H6	H5H6′	H6H6′
1	4.870	3.623	4.019	3.542	3.605	3.888 3.698	3.4	9.2	9.2	9.2	3.0	1.5	−11.6
2	5.297	3.374	3.986	4.038	3.722	4.245 3.691	3.4	9.4	9.2	9.2	1.8	<1	−11.6
3	4.984	3.440	3.942	3.578	3.940	4.027 3.700	3.7	9.2	9.4	7.8	<1	Broad	−11.6
4	5.143	3.432	3.994	3.936	3.920	3.973	3.7	9.2	8.4	∼3	**	∼0	−10.4

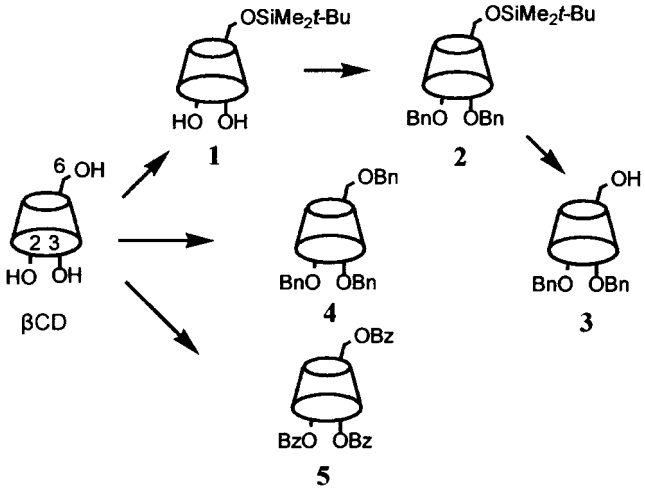
* The peaks of **5** were very broad; ** cannot be measured.

and 350 ms in the ROESY spectra. The HMBC experiment was optimized for observation of long range couplings using a delay (d6) of 65 ms.

Results and discussion

The compounds under study were prepared using published literature methods [2, 5, 7–10]. (Scheme 1). Their ^{1}H and ^{13}C NMR spectral assignments, carried out using information combined from several 2D NMR experiments (see experimental), are shown in Figure 1. The values of their ^{1}H and ^{13}C chemical shifts and ^{1}H coupling constants are summarized in Tables 1 and 2. The appearance of one resonance for each type of proton and carbon atom for the seven glucose residues (Scheme 2) reflects the C_7 symmetry of the derivatives **1-4**. The heptasilyl derivative **1** [7–9], despite the steric crowding that the bulky substituents impose on the primary side, seems to be very symmetrical suggesting that the H-bonds between O2H and O3H groups [11] maintain the overall conical shape. Benzylation of both O2H and O3H causes deshielding of the protons located at the glucose bridging points, H1 and H4, by more than 0.3 ppm (Table 1) and shielding of the corresponding carbon atoms (Table 2), indicating departure from the previous shape, and alteration of the inter-glucosidic torsion angles φ and ψ [6]. The spread of the diastereotopic hydrogen atoms H6 and H6′ also increases. Upon deprotection (Scheme 1, **2** → **3**) and complete benzylation (**4**), the so-far observed C_7 symmetry is maintained. However, the order of appearance of the ^{13}C resonances, from **1** to **2** (and **3**, **4**) is changed, i.e., C1 > C4 > C2 ≥ C3 > C5 > C6, to C1 > C3 > C2 ≅ C4 > C5 > C6. The drastic deshielding of C3 could probably be due to the benzyl group on C3 pointing toward the cavity (whereas that of C2 pointing outside) by analogy to per-2,3,6-*O*-methyl-β-CD [12]. The values of the coupling constants of the ring protons of **1–4**, indicate normal $^4C_1$ chair conformation (H1 equatorial, H2 axial and so on).

The values of J_{H5-H6} and $J_{H6-H6'}$ are smaller than any of the limiting values proposed for conformations of hydroxymethyl group in carbohydrates [13] suggesting vast prevalence of the *gg* conformer (-OR_1 groups away from the cavity). The picture changes drastically, when the per-2,3,6-*O*-benzoyl-β-CD derivative is examined, either in chloroform or in the aromatic (and hopefully interfering)

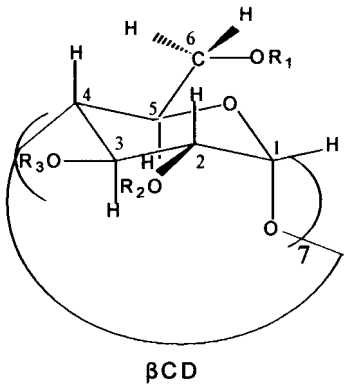


Scheme 1.

Table 2. ^{13}C Chemical shifts of β-CD derivatives **1–5** in $CDCl_3$, (δ_{13_C} at 77.0 ppm, 300 K).

#	C1	C2	C3	C4	C5	C6
1	102.06	73.66	73.43	81.82	72.60	61.66
2	97.98	79.33	80.91	77.78	72.56	62.42
3	98.24	78.70	80.66	79.25	73.15	62.10
4	98.43	78.83	80.89	78.69	75.39	69.31
5	97.5*	71.4*	71.4*	76.5*	70.1*	63.5*

*The peaks were broad, and except for C1 and C6 all others cannot be accurately assigned, due to the very broad ^{1}H spectra at this temperature.



Scheme 2.

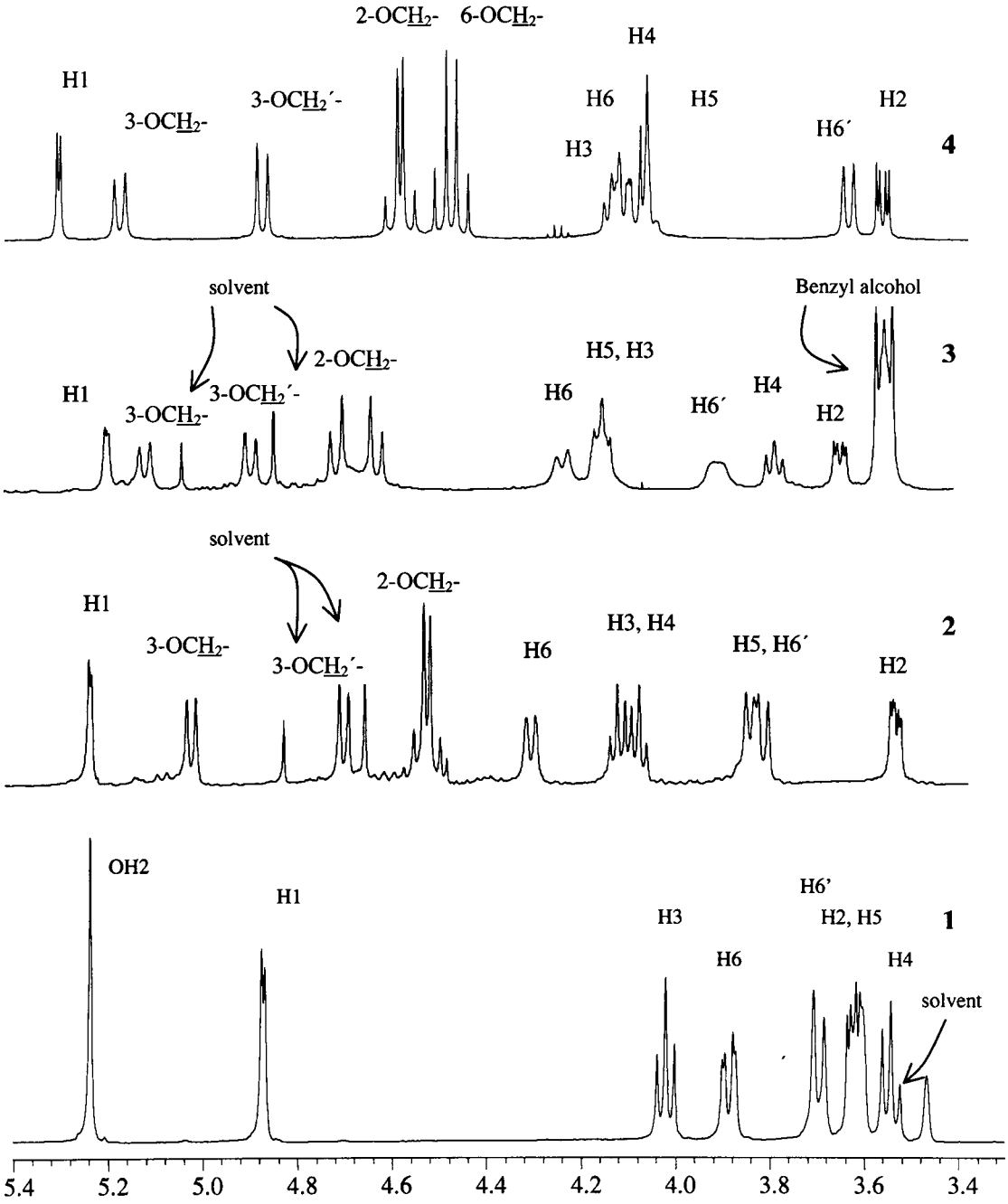


Figure 1. ^{1}H NMR spectra and assignments of **1–4** in $CDCl_3$ at 300 K.

solvent toluene-d_8. Very broad peaks are observed in the ^{1}H NMR spectra (Figure 2), whereas the ^{13}C lines (Figure 3) seem to have just passed coalescence. Lowering the temperature to 270 K (−3 °C) revealed a somewhat resolved spectrum, the lines of which could partially be assigned through the COSY, TOCSY (Figure 4) and HSQC (Figure 5) spectra. Although the chemical shifts of C1 are observed at ~100 ppm, the corresponding H1 protons span ~1.5 ppm (4.9 to 6.5 ppm). Similarly, six C6-type carbons are observed (Figure 3), whereas their attached protons range from 3.0 to 5.9 ppm. These observations are consistent with reduced symmetry (C_1) of the macrocycle. Further lowering of the temperature, continued to reshape the ^{1}H peaks and even at 220 K some exchange was still present. The corresponding ^{13}C peaks became entirely broad at 220 K. This implies that there exist several C_1 conformers of similar energy that continuously interchange with low $\Delta G^{\neq}$, since there is not a single type of stabilizing interaction that predominates. This observation rather excludes $^{4}C_1 \leftrightarrow {}^{1}C_4$ interconversion, as well as glucose flipping [3] since both events are expected to require rather high $\Delta G^{\neq}$. A 2D ROESY spectrum at 270 K showed only a few and weak interactions of some aromatic protons with CD protons, suggesting that there is no particular self-inclusion of phenyl rings in these conformers. The dispersion of the chemical shifts, on the other hand, of H1 and H6,6′ but also of the aromatic hydrogens up to ~9 ppm, indicates that the Bz groups are arranged in such way that they will cause serious deshielding of all these pro-

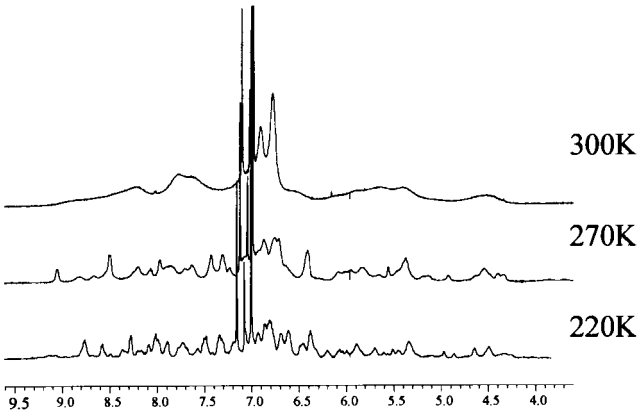


Figure 2. Variable temperature ^{1}H NMR spectrum of **5** in toluene-d_8.

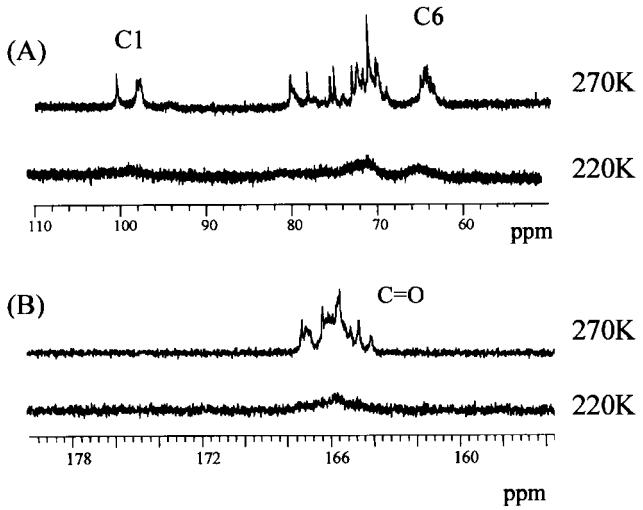


Figure 3. Variable temperature ^{13}C NMR spectrum of **5** in toluene-d_8. (A) β-CD region. (B) C=O region.

tons. Similar exchange between conformers is not observed for Bn derivatives such as **4** and 2,3,6-per-*O*-benzoyl-α-CD [3]. This could be explained by the fact that the C=O group, reduces the electron density of the phenyl ring and thus π-π ordering of the pendant groups is not efficient, allowing the aromatic rings to adopt random positions, and distort the inter-glucose torsion angles. On the other hand, "regular disorders" (e.g., every second unit) do not result in some symmetrical form due to the odd number of the glucose units in β-CD. Efforts to crystallize **4** and **5** are in progress.

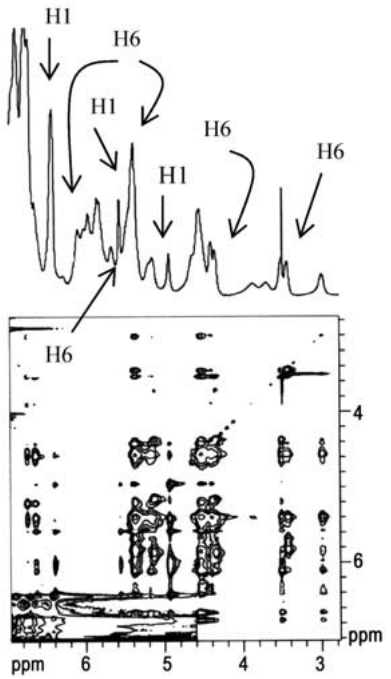


Figure 4. 2D TOCSY spectrum of **5** at 270 K, toluene-d_8.

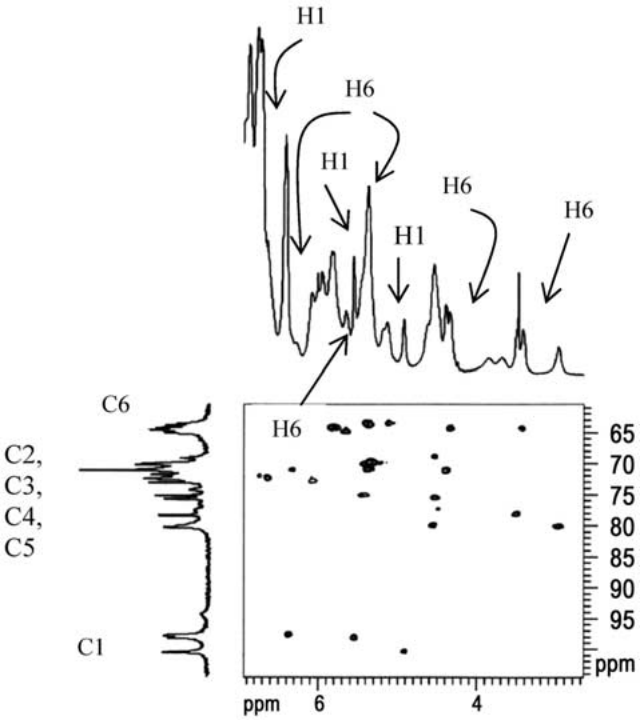


Figure 5. 2D HSQC spectrum of **5** at 270 K, toluene-d_6.

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