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NMR Spectroscopic Properties of Heptakis(2,6-di-*O*-pentyl)-β-Cyclodextrin: Two-Dimensional NMR Spectra of a Key Intermediate in Preparing Chiral Stationary Phases for Enantioselective Capillary Gas Chromatography

Wolfram Meier-Augenstein and Barend V. Burger
Laboratory for Ecological Chemistry, Department of Chemistry, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

Hendrik S. C. Spies
NMR Laboratory, Department of Chemistry, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

The ^{1}H and ^{13}C NMR spectra of heptakis(2,6-di-*O*-pentyl)-β-cyclodextrin in deuteriochloroform have been fully and unambiguously assigned. Several methods, including homo- and heteronuclear spin decoupling, two-dimensional homo- and hetero-nuclear correlation NMR spectroscopy, spectral simulation and the measurement of relaxation times were used for chemical shift assignments.

KEY WORDS: Heptakis(2,6-di-*O*-pentyl)-β-cyclodextrin ^{13}C NMR ^{1}H 2D NMR ^{13}C spin-lattice relaxation times

INTRODUCTION

Over the last 2 years, considerable progress has been made in the development of chiral stationary phases for enantioselective capillary gas chromatography (GC). Chiral phases that have been investigated are based on suitably derivatized cyclodextrins (CDs).[1,2] Although the enantioselectivity of these phases is probably due to host–guest interactions, the nature of the substituent on the hydroxyl group in position 3 also plays an important role in chiral recognition. König *et al.*[3] demonstrated this influence by comparing the enantioselectivity of hexakis(2,3,6-tri-*O*-pentyl)-α-cyclodextrin and hexakis (3-*O*-acetyl-2,6-di-*O*-pentyl)-α-cyclodextrin towards lactones and trifluoracetylated hydroxy compounds, and of heptakis(2,3,6-tri-*O*-pentyl)-β-cyclodextrin (2,3,6TPβCD) and heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (3A2,6DPβCD) towards amines and amino compounds.

König *et al.*'s results prompted us to investigate the possible influence of the introduction of a moderately polar, electron-rich substituent such as the benzyl group on enantioselectivity. The results of these investigations will be reported elsewhere.* Several reports of investigations into the enantioselective properties of 2,3,6TPβCD and 3A2,6DPβCD have appeared in the recent literature and the preparation of the key intermediate, heptakis(2,6-di-*O*-pentyl)-β-cyclodextrin (2,6DPβCD) has often been briefly described, without any NMR spectroscopic data being quoted.[1] Although it must be admitted that it is difficult to detect impurities at levels lower than 5% by NMR spectroscopic methods, we believe that these data are essential to allow the rapid control of purity. An alternative method to prove the purity of derivatized CDs, based on reductive cleavage and subsequent GC–MS investigation, was reported recently by Mischnick-Lübbecke and Krebber.[4]

In this paper we report a modified method for the preparation of 2,6DPβCD and the interesting NMR spectroscopic properties of this key intermediate.

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EXPERIMENTAL

Heptakis (2,6-di-*O*-pentyl)-β-cyclodextrin

βCD was first pentylated according to the literature.[5,6] Although the reaction was already complete after 3 h, we modified this method as deep yellow by-products were formed which were not further investigated. βCD (1.32 mmol; 1.5 g) was dissolved in a mixture of 21 ml of dry dioxane and 21 ml of dry dimethyl sulphoxide (DMSO) at an oil-bath temperature of 50 °C. This solution was stirred and 41.6 mmol (1.7 g) of freshly powdered NaOH and 41.6 mmol (4.4 ml) of 1-bromopentane were added. After 1 h, a further 1.7 g of NaOH and 4.4 ml of 1-bromopentane were added. After a total reaction time of 12 h the mixture was

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poured into 300 ml of ice-cold water and the resulting mixture was extracted three times with diethyl ether. The combined organic layers were washed three times with water and then dried over Na_2SO_4. Evaporation of the solvent and additional drying at the oil pump yielded 2.57 g of a pale yellowish viscous oil, which consisted of more than 90% of the desired product [thin-layer chromatography (TLC)]. This oil was recrystallized from methanol to give white, waxy crystals. Melting point, 122.2–123.5 °C; TLC, $R_F = 0.62$ [toluene–ethyl acetate (5 : 1, v/v)].

NMR spectroscopy

NMR spectra were recorded on a Varian VXR 300 spectrometer operating at 299.095 MHz for ^1H and 75.42 MHz for ^{13}C using the switchable (^1H/broad-band) probe. Low-power (1 W) WALTZ decoupling was used to acquire ^1H-decoupled ^{13}C spectra. Digital resolutions of 1.25 and 0.125 Hz were used to acquire the 1D ^{13}C and ^1H spectra, respectively. T_1 values were recorded by means of the inversion–recovery sequence.[7] The concentration of 2,6DPβCD (70 mg in 0.7 ml of $CDCl_3$) was maintained for all measurements, and TMS was used as an internal reference.

Two-dimensional ^1H–^1H correlation spectra (COSY) were obtained using the pulse sequence D_1–90°–(t_1 + D_3)–45°–AQ,[8] where t_1 is the incremented delay (256 increments of 0.606 ms each were used). Typical parameters used were: relaxation delay $D_1 = 2$ s, fixed delay $D_3 = 0$ s to suppress long-range modulations or $D_3 =$ 0.25 s to enhance long-range effects and acquisition time AQ = 0.31 s to provide a digital resolution of 3.2 Hz per point along the F_2 axis. The data along the t_1 axis were zero-filled once to obtain the same resolution. The data in both dimensions were pseudo-echo-weighted and symmetrized.

Two-dimensional ^1H–^{13}C correlation spectra (HETCOR) were obtained using the HETCOR pulse sequence described by Wilde and Bolton.[9] The relaxation delay D_1 was 1 s. A value of $^1J(CH) = 140$ Hz was used for the calculation of the refocusing delay. For each of the 256 increments along the t_1 axis (^1H), 2K data points were used along the t_2 axis (^{13}C). F_1 data were zero-filled and the data were processed as above (COSY) without symmetrization. The digital resolution was 3.2 and 8.1 Hz per point for the ^1H axis and the ^{13}C axis, respectively.

RESULTS AND DISCUSSION

This paper presents the first complete NMR spectroscopic characterization of 2,6DPβCD. Complete chemical shift assignments were possible by employing 1D and 2D NMR techniques.

The ^1H NMR spectrum (300 MHz; $CDCl_3$; 25 °C) of 2,6DPβCD (**1**) together with the ^1H–^1H COSY contour plot, is shown in Fig. 1. Beginning with the resonances for 3-OH at δ 5.08 and for H-1 at δ 4.91 all the ring proton resonances could be assigned (Table 1). Coupling constants of the ring protons are presented in Table 2. As the resonances of the diastereotopic protons H-6a and H-6b form part of a spectrum of higher order, the spectrum was simulated to confirm their assignments. The data for the simulated ABCDE spectrum include

Table 1. ^1H NMR chemical shifts[a] for the sugar moiety of βCD, 2,6DPβCD and 2,6DMβCD

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	3-OH
βCD[b]	4.82	3.30	3.63	3.35	3.56	3.62		5.67
2,6DPβCD[c]	4.91	3.35	3.92	3.43	3.73	3.59	3.7	5.08
2,6DMβCD[d]	4.93	3.21	4.45	3.61	4.10	3.75	3.85	5.43
2,6DMβCD[e]	4.77	2.99	3.51	3.14	3.50	3.37		4.70

[a] In ppm from TMS.
[b] In $(CD_3)_2SO$ (300 MHz, 25 °C); values obtained from ^1H–^{13}C COSY.
[c] In $CDCl_3$ (300 MHz, 25 °C).
[d] Values given in Ref. 11 (250 MHz, C_6D_6, 57 °C).
[e] Values given in Ref. 12 [500 MHz, $(CD_3)_2SO$, 35 °C].

Table 2. Coupling constants (Hz) for the ring protons of βCD and 2,6DPβCD

Compound	$^3J(1,2)$	$^3J(2,3)$	$^3J(3,4)$	$^3J(4,5)$	$^3J(5,6a)$	$^3J(5,6b)$	$^2J(6a,6b)$
βCD[a]	3.5	9.9	9.4	9.4	[b]	[b]	[b]
2,6DPβCD[c]	3.7	9.6	9.2	9.5	0.9	3.6	−9.3

[a] In $(CD_3)_2SO$ (300 MHz, 25 °C).
[b] Values could not be determined as a result of the close proximity or coincidence of proton signals.
[c] In $CDCl_3$ (300 MHz, 25 °C).

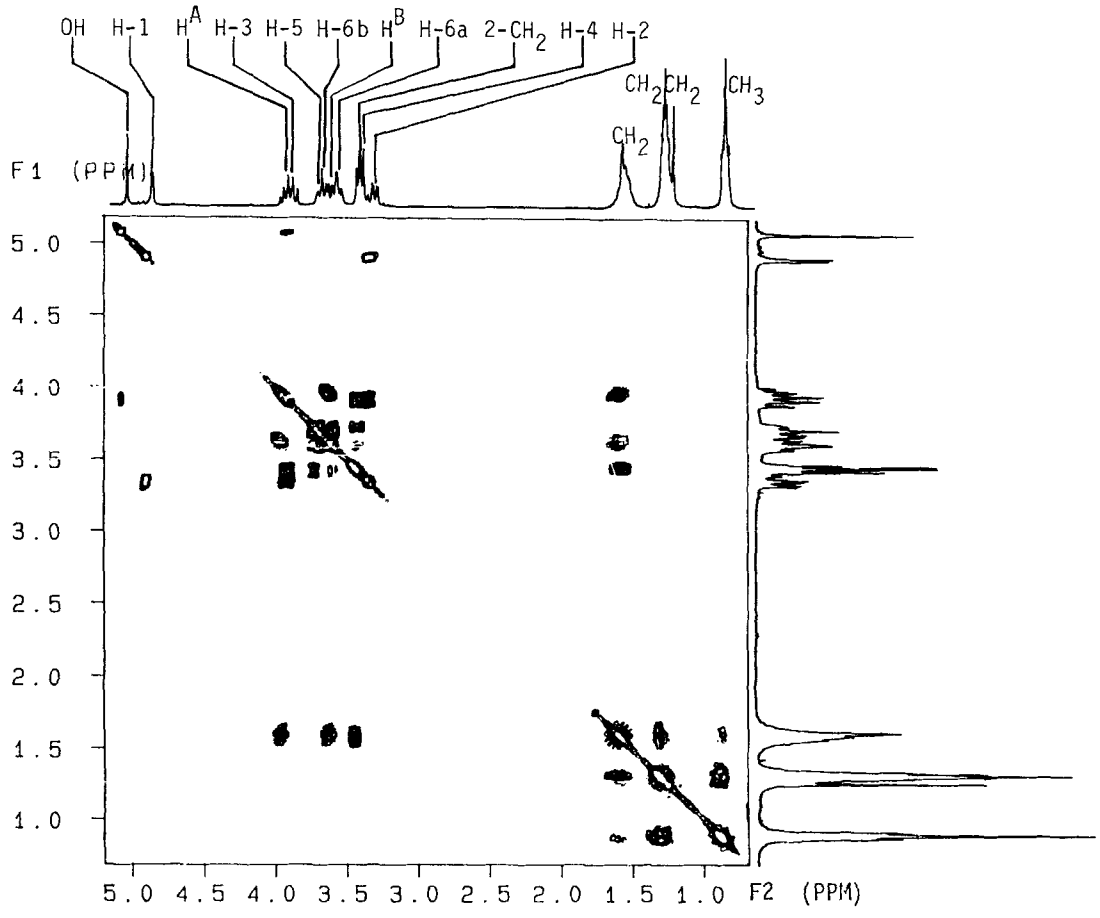
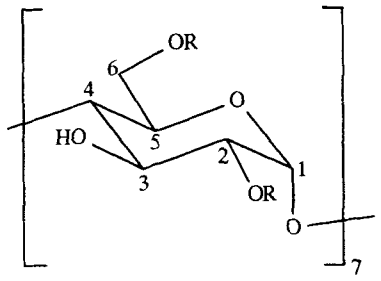


Figure 1. ^{1}H–^{1}H 45°COSY spectrum of 2,6DPβCD in $CDCl_3$ at 25 °C. The 300 MHz ^{1}H NMR spectra are shown along the axes.

observed chemical shifts and coupling constants of H-6a, H-6b, H-5, H-4 and H-3 [Fig. 2(A)].



R = $\overset{\alpha}{C}H_2\overset{\beta}{C}H_2\overset{\gamma}{C}H_2\overset{\delta}{C}H_2CH_3$

1

The behaviour of the α-methylene protons of the 6-*O*-pentyl groups was completely unexpected. Whereas the α-methylene protons of the 2-*O*-pentyl groups gave a triplet due to the coupling with the β-methylene protons, as expected for both chemically and magnetically equivalent protons, the 6-*O*-α-methylene protons formed an ABCC′ system with their vicinal protons. We refer to this set of geminal protons as an AB system, although it meets the condition for an AX system ($\Delta\nu/J \geqslant 10$ Hz). Chemical shifts and coupling constants are given in Tables 3 and 4, respectively.

As the proton resonances of both the β-methylene groups lie very close together, irradiation at an intermediate value of δ 1.59 was used to saturate both, in order to verify our assignments [Fig. 2(C)]. The triplet of the 2-*O*-α-methylene protons simplified to a singlet, whereas the multiplets due to the α-methylene protons of the 6-*O*-pentyl groups each collapsed to one doublet. The different coupling constants of the doublet of doublets for both the ACC′ and the BCC′ system [Fig. 2(B)] are given in Table 4. Hence, both the chemical and mag-

Table 3. ^{1}H and ^{13}C NMR chemical shifts[a] for the pentyl groups of 2,6DPβCD in $CDCl_3$ at 25 °C

Group	6-*O*-R (^{1}H)	2-*O*-R (^{1}H)	6-*O*-R (^{13}C)	2-*O*-R (^{13}C)	T_1[b] (^{13}C)	
αCH$_2$	3.97 H^A 3.63 H^B	3.45	73.03	71.58	0.29	0.29
βCH$_2$	1.61[c]	1.57[c]	29.37[d]	29.42[d]	0.53	0.56
γCH$_2$	1.30[e]	1.29[e]	27.93	28.31	1.04	1.15
δCH$_2$	1.31[d,e]	1.32[d,e]	22.56[d]	22.46[d]	1.61	1.80
CH$_3$	0.90[d]	0.89[d]	14.01[d]	14.08[d]	2.48	2.75

[a] In ppm from TMS.
[b] Relaxation times (s) for the side-chain carbons (75.42 MHz, 25 °C).
[c] Obtained from ^{1}H–^{1}H COSY.
[d] Assignments are interchangeable as a result of close proximity or insufficient digital resolution in 2D correlation experiments.
[e] Obtained from ^{1}H–^{13}C HETCOR.

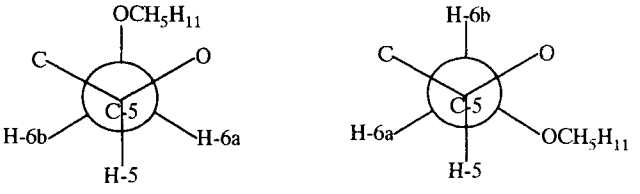
Table 4. Coupling constants (Hz) for the protons of the free hydroxyl groups of βCD and for the ABCC′ system of the 6-*O*-pentyl group of 2,6DPβCD

Compound	J(OH,2)	J(OH,3)	J(OH,6)	$J_{A,B}$	$J_{A,C}$	$J_{A,C'}$	$J_{B,C}$	$J_{B,C'}$
βCD[a]	6.9[b]	2.5[b]	5.6[b]					
2,6DPβCD[c]		[d]		9.2	7.6	6.8	7.5	6.1

[a] In $(CD_3)_2SO$ at 25 °C.
[b] The corresponding chemical shifts for 2-OH, 3-OH and 6-OH are 5.72, 5.67 and 4.46 ppm, respectively.
[c] In $CDCl_3$ at 25 °C.
[d] No coupling constant was observed.

netic non-equivalences of these geminal protons are indicative of a strongly restricted rotation around the 6-*O*—C-α bond. As two different sets of coupling constants with the β-methylene protons were observed, the rotation around the C-α–C-β bond must also be restricted. A possible explanation might be that the 6-*O*-pentyl groups are preferably oriented parallel to the upper rim of the cyclodextrin torus (i.e. its more narrow end), with their ends pointing towards the lipophilic inner side of the cyclodextrin cavity. Such an orientation would explain a more or less fixed 6-*O*—C-α bond.

Wood *et al.*[10] suggested that the *gauche–gauche* (**1a**) and *gauche–trans* (**1b**) conformations contribute significantly to the time averaged values for the J(5,6a) and J(5,6b) coupling constants. This would result in a small value for J(5,6a), as H-6a does not occupy a position *trans* to H-5 in either conformation. Based on this assumption, we suggest that in 2,6DPβCD the *gauche–trans* (**1b**) conformation is preferably occupied, as here the 6-*O*-pentyl groups would be oriented more or less parallel to the upper rim of the cyclodextrin torus, thus resulting in the observed chemical and magnetic non-equivalence of their α-methylene protons. If the *gauche–gauche* (**1a**) conformation is preferably occupied, the 6-*O*-pentyl groups would point away from the cylinder, taking an axial position as do H-2 and H-4. In such a position there would be no restricted rotation around the 6-*O*—C-α bond and a triplet should be observed as in the case of the α-methylene protons of the 2-*O*-pentyl groups.



(**1a**) (**1b**)

The ^{13}C resonances of the sugar moiety were first assigned based on the ^{13}C assignments for βCD from a broad-band proton decoupled ^{13}C NMR spectrum. A spectrum using the APT pulse sequence was recorded in order to determine the multiplicities. These assignments were verified and confirmed (Fig. 3) by carrying out

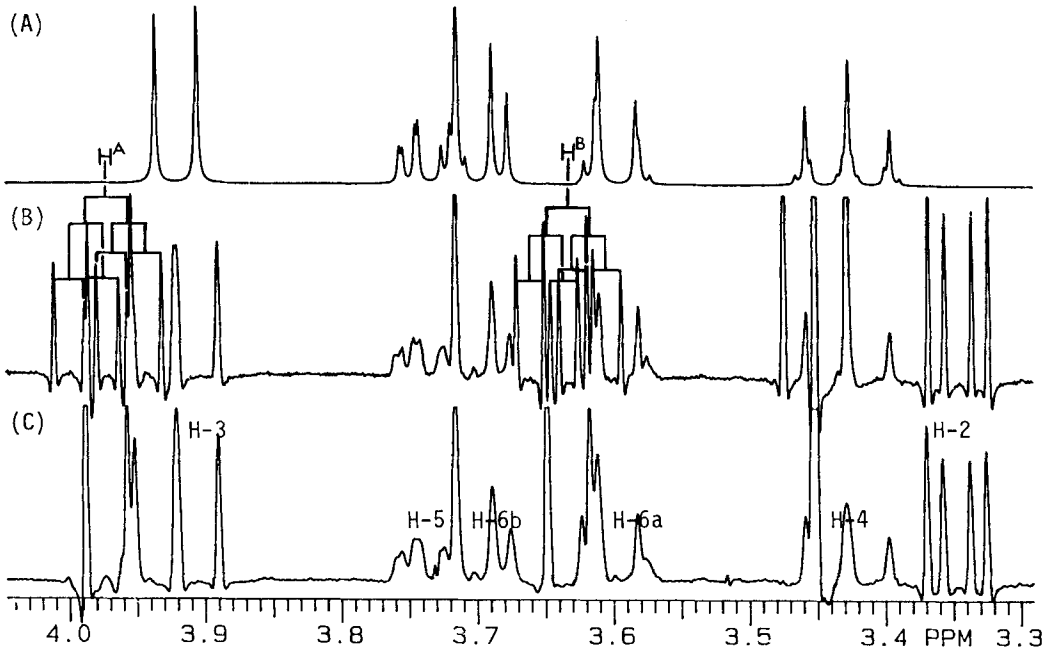


Figure 2. (A) Simulated ABCDE NMR spectrum for protons H-3, H-4, H-5, H-6a and H-6b. Resonances of H^A and H^B are omitted and for H-3 only the coupling constant with H-4 is considered. (B) Partial ^1H NMR spectrum of 2,6DPβCD (300 MHz, $CDCl_3$, 25 °C; reference TMS); (C) with irradiation at δ 1.59 to decouple the β-methylene from the α-methylene protons.

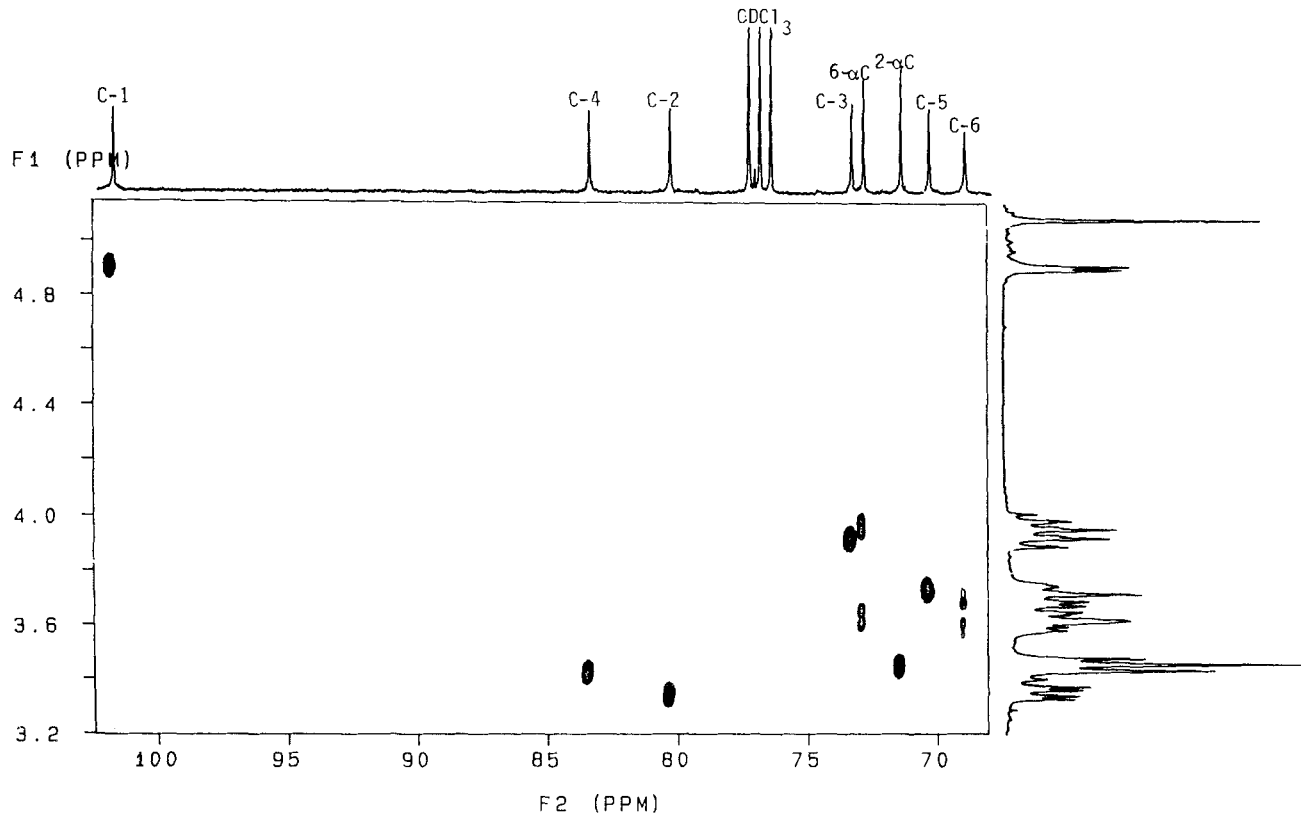


Figure 3. ^{1}H–^{13}C HETCOR spectrum (short-range) of 2,-6DPβCD in $CDCl_3$ at 25 °C. The ^{1}H and ^{13}C NMR spectra are shown along the F_1 and F_2 axes, respectively.

both short- and long-range 2D heterocorrelated experiments (^{1}H–^{13}C HETCOR). The assignments of the α-methylene carbon resonances were made from a fully proton coupled ^{13}C NMR spectrum and from a low-power selectively proton decoupled ^{13}C NMR spectrum, saturating the β-methylene protons at $\delta = 1.59$, thus allowing the determination of their different multiplicities due to three-bond proton coupling through oxygen. As the chemical shift difference between the two β-methylene as well as the two methyl carbon atoms of the side-chains is very small ($\Delta\nu = 4.5$ Hz), their assignments given in Table 3 are based on measured relaxation times (T_1). A gradual decrease in T_1 values was observed on going along the side-chain towards the oxygen. The carbon assignments for the side-chains were made assuming that the less mobile chain would give rise to smaller T_1 values. This assumption was substantiated by the observation of the shortest T_1 value for C-6, placing C-6 in the environment with the least segmental motion. Similarly to heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (2,6DMβCD), the C-2 and C-6 ring carbons show the strongest changes in chemical shift induced by the pentylation of the corresponding hydroxyl groups (Table 5).[11,12]

Table 5. ^{13}C NMR chemical shifts[a] for the sugar moiety of βCD, 2,6DPβCD and 2,6DMβCD

Compound	C-1	C-2	C-3	C-4	C-5	C-6
βCD[b]	101.72	72.19	72.83	81.32	71.82	59.71
2,6DPβCD[c]	101.89	80.47	73.49	83.58	70.49	69.13
2,6DMβCD[d]	102.3	82.9	74.4	84.7	71.1	71.8
2,6DMβCD[e]	100.5	82.2	73.2	83.2	70.3	71.2
T_1[f]	0.15	0.16	0.16	0.16	0.16	0.09

[a] In ppm from TMS.
[b] In $(CD_3)_2SO$ (75.42 MHz, 25 °C).
[c] In $CDCl_3$ (75.42 MHz, 25 °C).
[d] Values given in Ref. 11 (62 MHz, C_6D_6, 57 °C).
[e] Values given in Ref. 12 [$(CD_3)_2SO$, 35 °C].
[f] Relaxation times (s) for the ring carbons of 2,6DPβCD (75.42 Mhz, 25 °C).

CONCLUSION

The results demonstrate that NMR spectroscopy, using both homo- and hetero-nuclear 2D NMR methods, is a powerful tool for the elucidation of the structural characteristics of complex cyclodextrin derivatives. The findings of a preferably occupied conformation of the C-6-*O*-pentyl groups, presumably forced by lipophilic interaction of the alkyl chain with the inner side of the cyclodextrin cavity, are in good accordance with those reported for the C-6-*O*-methyl groups of methylated β-cyclodextrins.[11–13] Taking into account the observations made by König *et al.*,[3] it is conceivable that guest molecules enter the cyclodextrin cavity from the lower rim of the torus (i.e. its wide end).

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