Structural Mapping of an Unsymmetrical Chemically Modified Cyclodextrin by High-field Nuclear Magnetic Resonance Spectroscopy

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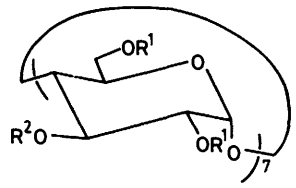
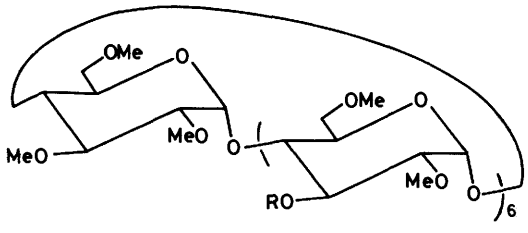
Samples of 2,6-per-*O*-methyl-β-cyclodextrin (DMβCD), prepared according to literature procedures, have been shown to be less than 70% pure. The major impurity has been identified as the unsymmetrical over-methylated cyclodextrin derivative (DM+1)βCD. Samples of pure DMβCD and (DM+1)βCD have been prepared by (i) benzoylating a chromatographically homogeneous mixture obtained after methylating βCD with dimethyl sulphate, (ii) separating chromatographically the resulting DMβCD heptabenzoate (DMβCD-B_7) and (DM+1)βCD hexabenzoate [(DM+1)βCD-B_6], and then (iii) subjecting the pure perbenzoates to de-*O*-benzoylation. Both DMβCD-B_7 and (DM+1)βCD-B_6 have been fully characterised. High-resolution ^{1}H and ^{13}C n.m.r. spectroscopy, with use of modern pulse techniques (homonuclear double resonance difference spectroscopy, COSY, *J*-resolved, JMOD, XHCORRD, and CHORTLE) for signal assignment and n.O.e. difference spectroscopy for residue sequencing, has been used to assign individually (i) 41 out of the 49 heterotopic protons and (ii) 29 out of the 42 heterotopic carbon atoms of the unsymmetrical (DM+1)βCD-B_6 (nuclei associated with *O*-methyl and *O*-benzoyl groups were excluded from consideration). The complete spectroscopic characterisation of unsymmetrical chemically modified cyclodextrins is important in the investigation of these compounds as potential enzyme models. The necessity of preparing pure chemically modified cyclodextrin derivatives cannot be over-emphasised.

Cyclodextrins behave as molecular receptors towards a wide variety of guest species in aqueous solution as well as in the solid state.[1] The complexes which result have many potential, as well as demonstrated, applications.[2] It is the involvement of cyclodextrins as second-sphere ligands for transition metal complexes that has aroused our interest[3] in this unique class of naturally occurring compounds. To date, we have examined[4] the suitabilities of α- and β-cyclodextrins (αCD and βCD) as second-sphere ligands. However, recently we have turned our attention towards the investigation of chemically modified derivatives. As well as being soluble in organic solvents, 2,6-per-*O*-methyl-β-cyclodextrin (DMβCD) displays[1] a greatly increased aqueous solubility compared with that of the parent βCD. Notably, DMβCD appears to offer many advantages over βCD, especially in the field of pharmaceutical formulations.[5]

Our initial preparation of DMβCD followed literature procedures[6] and the first product we isolated appeared to be a pure compound in terms of all the criteria previously employed to assess its homogeneity. However, a more rigorous n.m.r. spectroscopic examination proved that we had isolated a mixture of compounds consisting of, predominantly, the expected symmetrical DMβCD derivative and the over-methylated homologue, 2,6-per-*O*-methyl-3^A-*O*-methyl-β-cyclodextrin [(DM+1)βCD]. Subsequently, we have observed that all the samples of so-called pure DMβCD that were available to us commercially were of a similar dubious quality, *i.e.* they contained only *ca.* 65% DMβCD. Sample homogeneity implied by chromatography on silica gel belies the fact that the product first isolated is a mixture. In order to separate this mixture, it was necessary to prepare derivatives. Treatment of the methylated cyclodextrins with benzoyl chloride in pyridine afforded a mixture from which the 2,6-per-*O*-methyl-β-cyclodextrin 3-per-*O*-benzoate (DMβCD-B_7) and the 2,6-per-*O*-methyl-3^A-*O*-methyl-β-cyclodextrin 3^B,3^C,3^D,3^E,3^F,3^G-hexa-*O*-benzoate [(DM+1)βCD-B_6] were isolated and characterised in *ca.* 40 and 15% yields, respectively, after chromatography on silica gel. De-*O*-benzoylation of these compounds afforded the pure DMβCD and (DM+1)βCD derivatives, respectively.



	R^1	R^2
βCD	H	H
DMβCD	Me	H
DMβCD-B_7	Me	COPh
TMβCD	Me	Me



(DM+1)βCD	R = H
(DM+1)βCD-B_6	R = COPh

Amidst the large amount of literature[7] on chemically modified cyclodextrins, there are very few reports[8] describing pure, fully characterised symmetrical compounds and none, to our knowledge, relating to unsymmetrical derivatives. Here, we present the first account of the complete characterisation of a

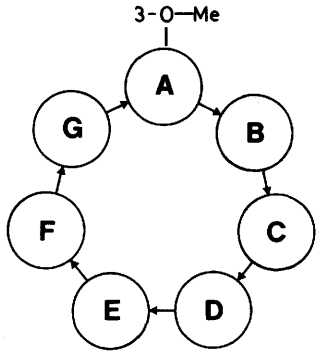


Figure 1. Schematic diagram of (DM + 1)βCD viewed from the secondary face, illustrating the ring-labelling sequence (the arrows correspond to α-1,4-glucosidic linkages)

new, unsymmetrical chemically modified cyclodextrin, (DM + 1)βCD-B_6.

The compound descriptors we have adopted in this paper are based on a nomenclature system for cyclodextrins introduced by Tabushi *et al.*[9] The 2,3,6-tri-*O*-methyl-D-glucopyranose unit in (DM + 1)βCD has been arbitrarily designated as residue A and the remaining constitutionally heterotopic 2,6-di-*O*-methyl-D-glucopyranose units have been labelled B, C, D, E, F, and G sequentially in a clockwise direction (Figure 1), when the cyclodextrin torus is viewed from the face originally bearing the secondary hydroxy groups.

Experimental

β-Cyclodextrin (Aldrich) was used as received. Dimethylformamide, dimethyl sulphoxide, and benzoyl chloride were distilled before use. Pyridine was used from a freshly opened bottle. Methanol was dried according to a standard procedure and distilled before use. Column chromatography was performed on Silica Gel 60 (Merck 9385). M.p.s were determined with a Reichert hot-stage apparatus. Microanalyses were carried out by the University of Sheffield Microanalytical Service. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Fast atom bombardment (FAB) mass spectrometry (carrier gas, Argon) was performed with a Kratos MS 80 instrument. ^{1}H N.m.r. (250 MHz) spectra were recorded with a Bruker AM 250 spectrometer; the solvent peak was used as reference, with respect to Me_4Si.

Methylated Cyclodextrins.—A mixture of β-cyclodextrin hydrate (1.2 g, 1 mmol), dimethyl sulphate (2 g, 15 mmol), barium oxide (2 g, 14 mmol), barium hydroxide octahydrate (2 g, 7 mmol), dimethylformamide (6 ml), and dimethyl sulphoxide (6 ml) was stirred vigorously at 0 °C under nitrogen for 48 h. Concentrated ammonia solution (2 ml) was added and the mixture was stirred for a further 2 h at room temperature. The solvents were removed under high vacuum and the resulting solid cake was extracted with chloroform (3 × 50 ml). The combined extracts were washed with saturated aqueous sodium chloride (4 × 50 ml) and water (2 × 50 ml), and then dried (Na_2SO_4). Evaporation to a small volume and addition of hexane gave a white precipitate, which was filtered off and washed with hexane. Chromatography of this crude product on silica gel with 2% v/v methanol in chloroform as eluant afforded a mixture (1.1 g, 83%) of 2,6-*per*-O-*methyl*-β-*cyclodextrin* (DMβCD) and 2,6-*per*-O-*methyl*-3-*mono*-O-*methyl*-β-*cyclodextrin* [(DM + 1)βCD]; *m/z* (negative ion FAB), 1 329 ($[M - H]^-$ for DMβCD) and 1 343 ($[M - H]^-$ for (DM + 1)βCD); δ_H (250 MHz; $CDCl_3$) 3.28 (7 H, dd, $J_{1,2}$ 4, $J_{2,3}$ 10 Hz, H-2), 3.37—3.55 (28 H, m, H-4 and 6-*O*-Me), 3.55—3.85 (42 H, m, H-5, H-6a, H-6b, and 2-*O*-Me), 3.94 (7 H, dd, $J_{2,3}$ 10, $J_{3,4}$ 10 Hz, H-3), 4.98 (7 H, d, $J_{1,2}$ 4 Hz, H-1), and 5.09 (7 H, s, OH); δ_H (250 MHz; C_6D_6) 2.95—3.10 (m, impurity), 3.10—3.42 (m, H-2, 6-*O*-Me, and impurity), 3.42—3.56 (m, 2-*O*-Me and impurity), 3.56—3.95 (m, H-4, H-6a, H-5b, and impurity), 4.02—4.24 (m, H-5 and impurity), 4.35—4.55 (m, H-3 and impurity), 4.93 [d (with shoulders), $J_{1,2}$ 4 Hz, H-1 and impurity], 4.99 (d, *J* 4 Hz, impurity), and 5.15—5.52 (m, OH and impurity). The comment 'impurity' refers to signals for (DM + 1)βCD for the most part.

Methylated Cyclodextrin Benzoates.—Distilled benzoyl chloride (10 ml) was added to a solution of the mixture (0.67 g, 0.5 mmol) of methylated cyclodextrins [DMβCD and (DM + 1)βCD] in dry pyridine (15 ml). The dark red solution was stirred at 40—50 °C under nitrogen for 4 days and the resulting dark brown solution was evaporated to dryness under high vacuum. The black tar obtained was dissolved (with cooling) in methanol and the solution was stirred for 1 h at room temperature before being evaporated to dryness under high vacuum. The residue was dissolved in chloforom and the solution was washed with water, dried, and evaporated to dryness to yield a dark brown solid. Chromatography on silica gel with 5% v/v methanol in chloroform as eluant afforded, in order of elution from the column, 2,6-*per*-O-*methyl*-3-*mono*-O-*methyl*-β-*cyclodextrin hexabenzoate* [(DM + 1)βCD-B_6] (0.15 g, 15%), m.p. 126—129 °C (Found: C, 60.5; H, 6.7. $C_{99}H_{124}O_{41}$ requires C, 60.4; H, 6.3%); $[\alpha]_D$ −34° (*c* 1.0 in $CHCl_3$); *m/z* (positive ion FAB) 1 992 ($[M + Na]^+$); and 2,6-*per*-O-*methyl*-β-*cyclodextrin heptabenzoate* (DMβCD-B_7) (0.42 g, 40%), m.p. 134—136 °C (Found: C, 61.5; H, 6.4. $C_{105}H_{126}O_{42}$ requires C, 61.2; H, 6.2%); $[\alpha]_D$ −93° (*c* 1.0 in $CHCl_3$); *m/z* (positive ion FAB) 2 082 ($[M + Na]^+$).

2,6-*Per*-O-*methyl*-β-*cyclodextrin.*—Sodium metal (0.01 g) was added to a solution of 2,6-per-*O*-methyl-β-cyclodextrin heptabenzoate (DMβCD-B_7) (0.2 g, 0.1 mmol) in dry methanol (10 ml) and the solution was stirred at room temperature under nitrogen for 2 days. The solution was then neutralised with dilute hydrochloric acid and evaporated. Methyl benzoate was removed under high vacuum and the residue was dissolved in chloroform; the solution was washed with water, dried, and evaporated to dryness. The resulting solid was recrystallised from hot water to afford pure 2,6-*per*-O-*methyl*-β-*cyclodextrin* (DMβCD) (0.12 g, 90%) (Found: C, 50.3; H, 7.3. $C_{56}H_{98}O_{35}$ requires C, 50.5; H, 7.4%); δ_H (250 MHz; C_6D_6) 3.21 (7 H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 9.4 Hz, H-2), 3.29 (21 H, s, 6-*O*-Me), 3.51 (21 H, s, 2-*O*-Me), 3.61 (7 H, dd, $J_{3,4}$ 9.1, $J_{4,5}$ 10.0 Hz, H-4), 3.75 (7 H, dd, $J_{5,6a}$ 1.7, $J_{6a,6b}$ 10.6 Hz, H-6a), 3.85 (7 H, dd, $J_{5,6b}$ 4.6, $J_{6b,6a}$ 10.6 Hz, H-6b), 4.10 (7 H, m, $J_{4,5}$ 10.0 Hz, H-5), 4.45 (7 H, dd, $J_{2,3}$ 9.4, $J_{3,4}$ 9.1 Hz, H-3), 4.93 (7 H, d, $J_{1,2}$ 3.7 Hz, H-1), and 5.43 (7 H, s, OH); δ_C (62 MHz; C_6D_6) 102.3 (C-1), 84.7 (C-4), 82.9 (C-2), 74.4 (C-3), 71.8 (C-6), 71.1 (C-5), 60.4 (2-*O*-Me), and 58.7 (6-*O*-Me).

2,6-*Per*-O-*methyl*-3-*mono*-O-*methyl*-β-*cyclodextrin.*—Methanolysis of 2,6-per-*O*-methyl-3-mono-*O*-methyl-β-cyclodextrin hexabenzoate [(DM + 1)βCD-B_6] according to the procedure just described afforded 2,6-*per*-O-*methyl*-3-*mono*-O-*methyl*-β-*cyclodextrin* (90%) (Found: C, 50.6; H, 7.2. $C_{57}H_{100}O_{35}$ requires C, 50.9; H, 7.4%); δ_H (250 MHz; C_6D_6) 2.5—5.5 (m, all H).

N.m.r. Spectroscopy.—N.m.r. spectra were recorded on a Bruker WH 400 spectrometer equipped with an Aspect 2000 computer using DISNMRP program version DISN861 operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C. All the measurements were carried out for solutions in deuteriochloroform and, in order to reduce signal broadening as a result of high viscosity, all the experiments were performed at 57 °C.

The probe temperature was measured with a Comark thermocouple in an n.m.r. tube containing chloroform before and after each series of experiments. The same sample of (DM + 1)βCD-B_6 (60 mg) was used throughout, and the volume of $CDCl_3$ (0.5 ml) was kept constant in order to maintain the concentration (0.061M). The concentration of DMβCD-B_7 (60 mg in 0.5 ml; 0.058M) was maintained in a similar fashion for all measurements.

Decoupling difference proton spectra[10] were recorded with decoupling power of 24 dB below 0.2 W so as to perturb as narrow a band as possible since most of the signals overlapped. N.O.e. experiments were recorded with irradiation power of 50 dB below 0.2 W for the same reason. Two-dimensional proton homonuclear correlation[11] spectra (COSY) were obtained using the pulse sequence D1–90°–D0–90°–FID where D1 is a relaxation delay and D0 is the incremental delay. The parameters used were D1 = 1.5 s, ΔD0 = 1.334 ms, SW1 = ±375 Hz, NE = TD1 = 256, SW = 750 Hz, TD = SI = 2 K, NS = 64; the F1 data field was zero-filled to 1 K. The digitisation was 0.366 Hz per point for (DM + 1)βCD-B_6. For DMβCD-B_7, the parameters were chosen so as to give a digitisation of 8 Hz per point. The data were processed with a sine-bell window in both dimensions and symmetrised about the diagonal. Two-dimensional proton homonuclear *J*-resolved[12] spectra were acquired using the pulse sequence D1–90°–D0–180°–D0–FID. The parameters used were D1 = 1.5 s, NS = 64, SW1 = ±23 Hz, SI = TD = 8 K, TD1 = NE = 64, SI1 = 128 W. The digitisation was 0.385 Hz per point in both F1 and F2. The data were processed with a sine-bell window function in both dimensions, tilted, and symmetrised about the centre of the F1 axis.

^{13}C N.m.r. spectra were acquired using the *J*-modulation (JMOD) pulse sequence[13] D1–90°–D3–180°–D3–FID with broad-band (BB) proton decoupling from the 180° pulse through to the acquisition of the FID. BB decoupling during D1, the relaxation delay (usually at lower power to avoid heating), builds up the n.O.e. With D3 = $1/J_{CH}$, the spectra are recorded with CH and CH_3 signals displayed in one direction and quaternary carbon (C) and CH_2 signals in the other.

JMOD ^{13}C n.m.r. spectroscopy showed that the C-6 methylene resonances overlapped badly with a set of CH resonances. In order to separate these two sets of signals, a pair of modified JMOD experiments was performed. In the first experiment, BB decoupling was turned on after the 180° pulse; in the second experiment it was turned on after the 90° pulse, and in both cases the n.O.e. was suppressed by turning off the decoupler during D1. The first sequence generated a spectrum in which the CH and CH_3 signals appear in a negative direction and C and CH_2 signals in a positive direction. The second sequence generated a spectrum with all the carbon signals in a positive direction, having been run under conditions as similar as possible to the first. A total of 1 400 scans was taken for each spectrum but in groups of 200 interleaved to average out the effect of fluctuations in field or temperature. The FIDs were added together and the absolute intensity was set equal to 1 in order to give normalised spectra. Fourier transformation afforded a spectrum including only CH_2 signals (and those of quaternary carbon). Subtraction of the FIDs and subsequent Fourier transformation gave the spectrum containing only CH and CH_3 signals.

One-dimensional C–H correlation spectroscopy was performed using the CHORTLE technique,[14] for which the pulse sequence is as follows:

^{1}H: D1–90°–(D2 + *t*/2)–180°–(D2 − *t*/2)–90°– –//BB//
^{13}C: –180°– –90°–D3–180°–D3–FID

D1 is a relaxation delay, D2 and D3 are delays chosen to optimise polarisation transfer to ^{13}C from directly bonded protons, and *t* is a variable time delay. The technique involves the acquisition of a series of ^{13}C spectra with different values of the proton evolution time *t* and the intensity of each ^{13}C signal is modulated according to the phase angle α, where α = 2πF*t*, F being the resonance offset of the proton(s) bonded to the carbon. Through proper choice of the phase of the ^{1}H polarisation transfer pulse, the contribution to the intensity of the ^{13}C signal from each directly bonded ^{1}H can be made proportional to either sinα or cosα and the CHORTLE program generates a 'sine' and a 'cosine' spectrum for each value of *t* used. A non-linear least-squares program then calculates the chemical shifts of the directly bonded protons. The times (*t*) used were 0.16, 1.0, 2.4, 2.8, and 3.2 ms. The shortest time (0.16 ms) was chosen such that, with the decoupler at δ 3, all the signals in the region of interest (δ 0—6) in the 'cosine' spectrum were positive. These data were used for setting the phase and also for a rough determination of the ^{1}H chemical shifts. The decoupler was set at exactly 3.000 p.p.m. from Me_4Si and the chemical shifts were calculated relative to the decoupler frequency, and then finally referenced to Me_4Si.

To confirm that the CH signals overlapping the C-6 methylene signals were in fact the C-5 signals, they were located separately using a modification of the CHORTLE pulse sequence. Selection of the C-5 resonances by suppression of the C-6 resonances was achieved through the generation of only one 'cosine' spectrum. The decoupler frequency O2 was set at the average position of the H-5 signals and *t* was chosen so as to give a null point midway between the chemical shifts of the geminal protons of the C-6 signals. The intensities of the C-6 signals are thus reduced to zero as one geminal proton gives a positive value which is cancelled by the negative value of the same magnitude generated from the other geminal proton.

Two-dimensional C–H correlation spectroscopy was performed using the XHCORRD pulse sequence:[15]

^{1}H: D0–90°–D0–90°–D3–180°–D3–90°–D0–D3–90°– –BB
^{13}C: D1– –180°– –90°–D4–FID

D1 is a relaxation delay, D0 the incremental delay, D3 = $1/2J_{CH}$, and D4 = $1/2J_{CH}$ for augmenting only CH signals. It was not possible to perform a single experiment to cover the entire region of interest because the sweep width and resolution required with such closely spaced resonances placed impossible demands upon the disc capacity of the computer. Three separate 2D C–H correlation experiments were therefore performed. The first covered the C-1/H-1 region and digitisations of 0.34 Hz per point in the proton domain and 1.5 Hz per point in the carbon domain were achieved. The second covered the C-5/H-5 region (0.948 Hz per point for H and 1.5 Hz per point for C) and the final experiment correlated the remaining carbon atoms and protons of interest (1.8 Hz per point for H and 1.9 Hz per point for C). Other parameters used were D1 = 1.5 s, D3 = D4 (for CH resonances only) = $1/2J_{CH}$ = 3.125 ms. In order to observe the correlations, it was necessary to apply a Lorentz-Gaussian function before Fourier transformation, typically LB = 3, GB = 0 for carbon and LB = −2, GB = 0.2 for protons.

Results and Discussion

As part of our investigation into the second-sphere coordination of transition metal complexes by cyclodextrins, we prepared a sample of DMβCD according to routine literature procedures.[6] Several reports of investigations into the complexing properties of this compound have appeared in the recent literature[16] and the purity of the material used has always been implied, often without any n.m.r. spectroscopic

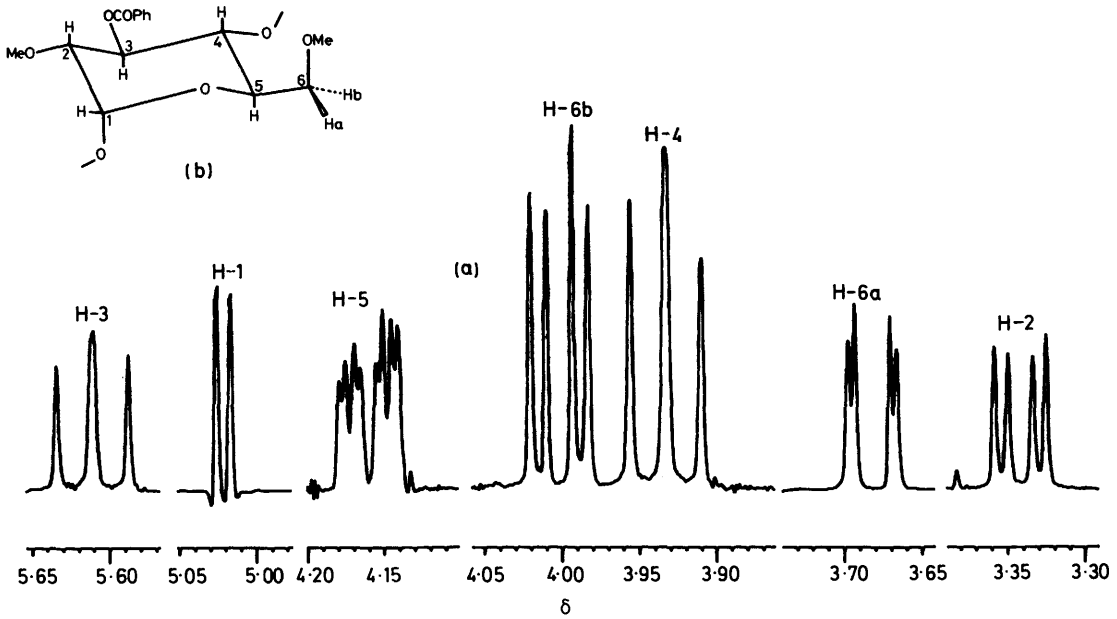


Figure 2 (a) Partial ^{1}H n.m.r. spectrum of DMβCD-B_7 (400 MHz; $CDCl_3$; 57 °C; ref. Me_4Si); (b) Formula for one residue of DMβCD-B_7 showing notation used, particularly for H-6. Only the *gauche–gauche* conformation of the C(5)–C(6) bond is depicted. The H-6a, H-6b notation adopted is that according to Sadler and his co-workers (ref. 8) and Saenger and his co-workers (D. J. Wood, F. E. Hruska, and W. Saenger, *J. Am. Chem. Soc.*, 1977, **99**, 1735)

data being quoted. Initial attempts to determine association constants for complexation of cyclodextrins with suitable guest species in solution relied upon[4] measurements of the concentration dependence of the ^{1}H n.m.r. chemical shifts of suitable probe protons in the host and/or in the guest. Experience has led us to believe that this is a reliable technique and we were rather surprised, therefore, when we could not obtain sensible quantitative results for the complexation of DMβCD with a particular guest which we had found qualitatively by ^{1}H n.m.r. spectroscopy to exist as a complex in aqueous solution. After eliminating other possible explanations for these anomalous results, we decided to investigate further the purity of the DMβCD, although h.p.l.c. analysis and ^{1}H and ^{13}C n.m.r. spectra (in $CDCl_3$ and in D_2O) of our sample implied that it was a pure homogeneous compound. However, when the ^{1}H n.m.r. spectrum was recorded for a solution in [$^2H_6$]-benzene, it revealed the presence of at least one other compound. Tentatively, the impurity was identified as an unsymmetrically methylated cyclodextrin derivative. Fast atom bombardment mass spectrometry with negative ion detection showed a major peak at *m/z* 1 329, corresponding to the $[M - H]^-$ ion for DMβCD and a large additional peak at *m/z* 1 343 (*i.e.* DMβCD + 14 mass units). This result led us to believe that the impurity was the cyclodextrin derivative with all the 2- and 6-hydroxy groups methylated, and only one of the 3-hydroxy groups methylated, *i.e.* the derivative (DM + 1)βCD. We were unable to separate the two compounds by any direct methods, including h.p.l.c. which could not distinguish between them under a wide range of experimental conditions. We therefore resorted to an indirect means of separation. In order to magnify the difference between the two compounds, the remaining hydroxy groups were benzoylated according to a procedure[6] previously used by Lehn and his co-workers to prepare the 2,3,6-per-*O*-benzoyl cyclodextrin derivatives. The product of the benzoylation was analysed by h.p.l.c.* and was found to contain two major products together with a number of by-products. The major components were separated by chromatography on silica gel and were characterised as the

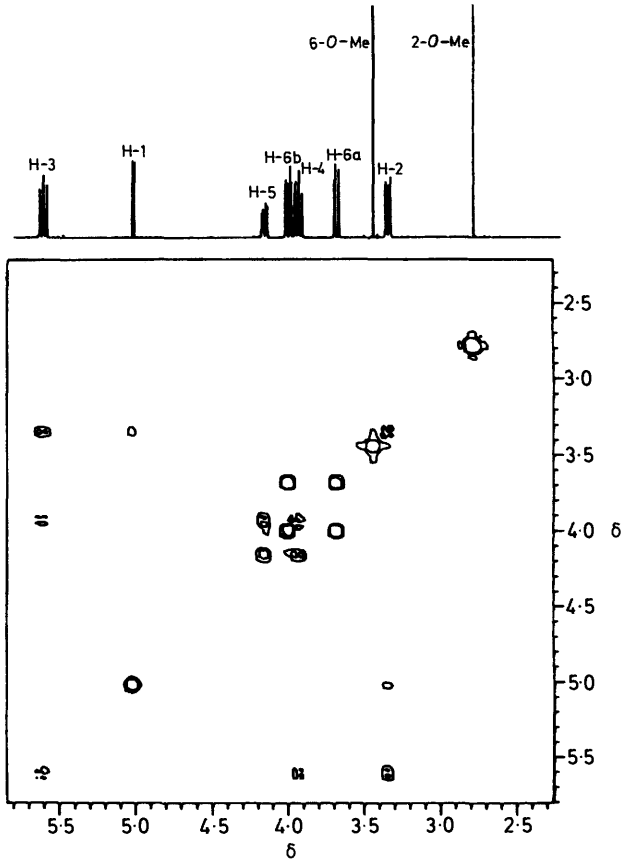


Figure 3. Partial 2D ^{1}H COSY contour plot for DMβCD-B_7 (400 MHz; $CDCl_3$; 57 °C; ref. Me_4Si)

* Conditions: Hypersil column (5 μ; 250 × 4 mm) was eluted with acetonitrile (2.0 ml min^{-1}); both refractive index and u.v. (280 nm) detection were employed.

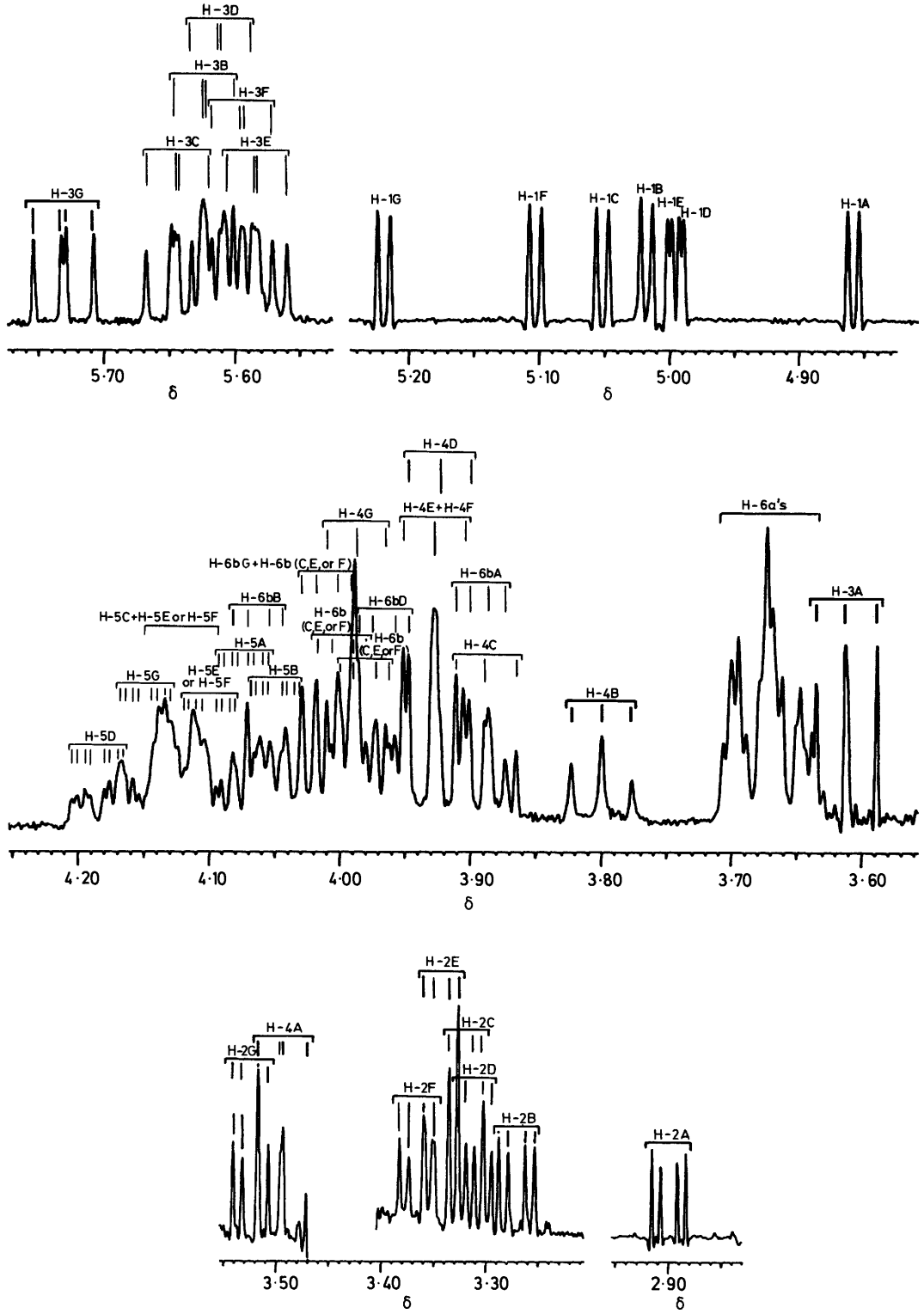


Figure 4. Partial ^{1}H n.m.r. spectrum of (DM + 1)βCD-B_6 (400 MHz; $CDCl_3$; 57 °C; ref. Me_4Si). The assigned signals are numbered according to the usual convention shown in Figure 2(b) and the letters refer to the residue sequence defined in Figure 1

symmetrical DMβCD heptabenzoate (DMβCD-B_7) and the unsymmetrical (DM + 1)βCD hexabenzoate [(DM + 1)βCD-B_6]. Quantitative h.p.l.c. analysis of the crude product revealed that the ratio of DMβCD-B_7 to (DM + 1)βCD-B_6 was *ca.* 65:35. This result implied that the original DMβCD was only 65% pure at best. ^{1}H N.m.r. spectroscopic analysis of commercially available DMβCD revealed that it was of a similar quality. Removal of the benzoyl groups by treatment of DMβCD-B_7 and (DM + 1)βCD-B_6 with catalytic quantities of sodium methoxide in methanol afforded the pure methylated derivatives, DMβCD and (DM + 1)βCD, respectively. The n.m.r. spectroscopic characterisation of the unsymmetrical

Table 1. ^{1}H N.m.r. chemical shifts (p.p.m.) for (DM + 1)βCD-B_6, DMβCD-B_7 (400 MHz; $CDCl_3$; 57 °C), and TMβCD[a]

	(DM + 1)βCD-B_6 residue[b]								
Proton	A	B	C	D	E	F	G	DMβCD-B_7	TMβCD
H-1	4.855	5.017	5.052	4.992	4.995	5.104	5.219	5.021	5.12
H-2	2.900	3.269	3.317	3.308	3.339	3.363	3.523	3.341	3.18
H-3	3.611	5.624	5.643	5.611	5.583	5.595	5.730	5.609	3.50
H-4	3.494	3.799	3.887	3.920	3.928	3.929	3.988	3.934	3.60
H-5	4.073	4.048	4.120	4.186	4.130 or 4.102[c]	4.130 or 4.102[c]	4.147	4.159	3.79
H-6a[d]	3.659	3.645	3.660 or 3.683[c]	3.673	3.660 or 3.683[c]	3.660 or 3.683[c]	3.693	3.681	3.56
H-6b[d]	3.893	4.060	4.012 or 3.995 or 3.980[c]	3.963	4.012 or 3.995 or 3.980[c]	4.012 or 3.995 or 3.980[c]	4.012	4.001	3.84

[a] Values obtained from ref. 8. [b] Residues labelled according to Figure 1. [c] Individual assignments could not be made for these resonances; the proton is located at one of the δ values. [d] The H-6a/H-6b notation is explained in Figure 2(b).

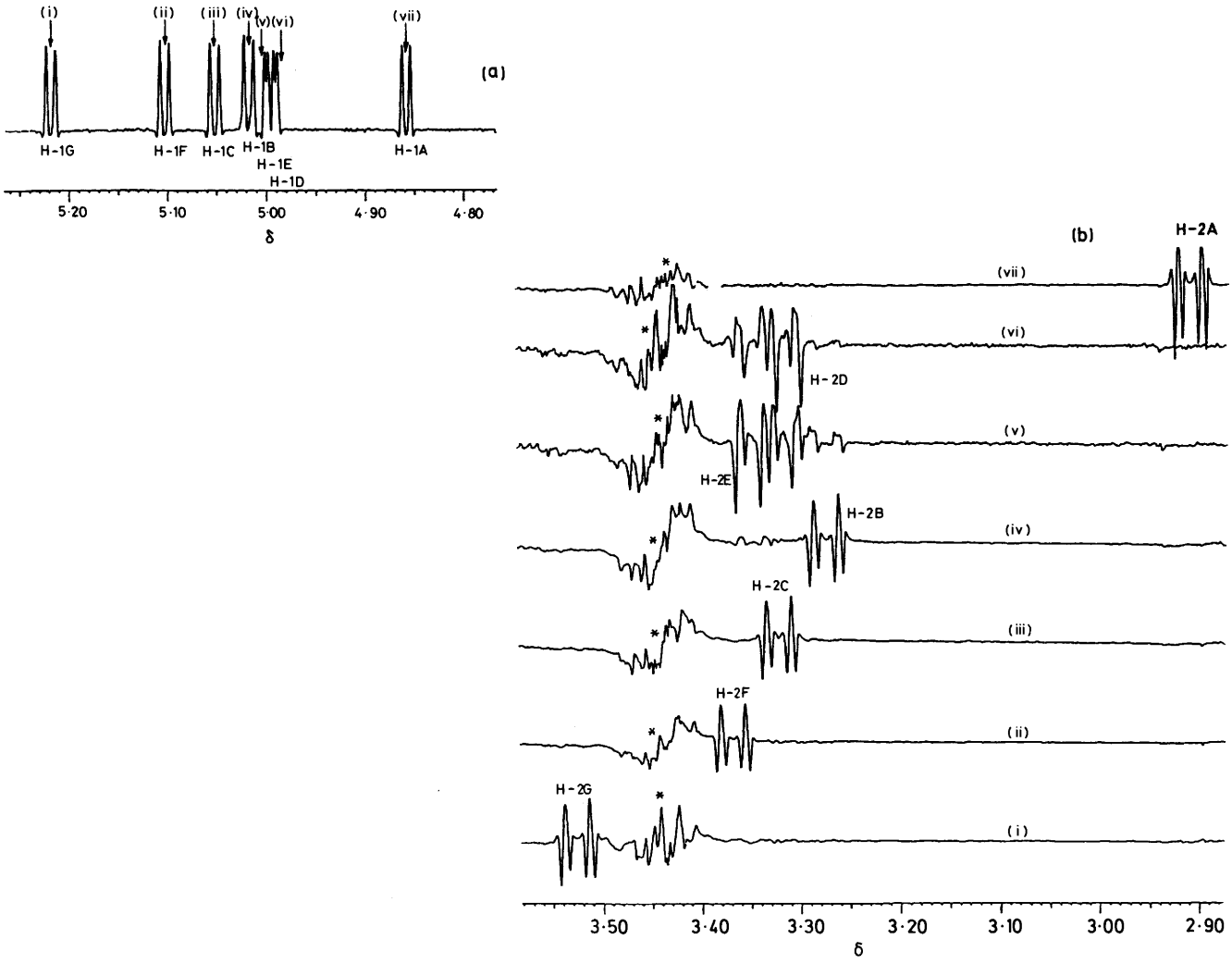


Figure 5 (a) Irradiations (i)—(vii) of the H-1 resonances for decoupling difference spectroscopy; (b) decoupling difference spectra for irradiations (i)—(vii) at the H-1 resonances and observing the signals in the H-2 region of the spectrum (* residual signal from OMe)

(DM + 1)βCD derivative is at present being undertaken. The analysis of the intermediate unsymmetrical (DM + 1)βCD-B_6 derivative is described in this paper.

The partial ^{1}H n.m.r. spectrum (400 MHz; $CDCl_3$; 57 °C) of DMβCD-B_7 is shown (methoxy and aromatic proton resonances omitted) in Figure 2. The assignments were made on the basis of a two-dimensional (2D) homonuclear proton correlation spectroscopy (COSY) experiment, a contour plot of

Table 2. Coupling constants (Hz) for pairs of vicinal (and geminal H-6) protons on the substituted D-glucopyranose residues of (DM + 1)βCD-B_6, DMβCD-B_7 (400 MHz; $CDCl_3$; 57 °C), and TMβCD[a]

	(DM + 1)βCD-B_6 residue[b]								
$J_{H,H}$	A	B	C	D	E	F	G	DMβCD-B_7	TMβCD
$J_{1,2}$	3.3	3.7	3.7	3.4	3.4	3.7	3.7	3.5	3.6
$J_{2,3}$	10.0	10.2	10.2	10.2	10.2	10.2	10.2	9.7	9.7
$J_{3,4}$	8.8	8.6	8.6	8.2	8.2	8.2	8.6	9.1	8.6
$J_{4,5}$	9.8						9.3	9.7	9.5
$J_{5,6a}$[c]				1.5				1.7	1.3
$J_{5,6b}$[c]	4.2		4.0	4.0	4.0	4.0	4.0	4.0	4.0
$J_{6a,6b}$[c]	11.0	11.0		11.0				10.8	10.3

[a] Values obtained from ref. 8. [b] Residues are labelled according to Figure 1. [c] The H-6a/H-6b notation is explained in Figure 2(b).

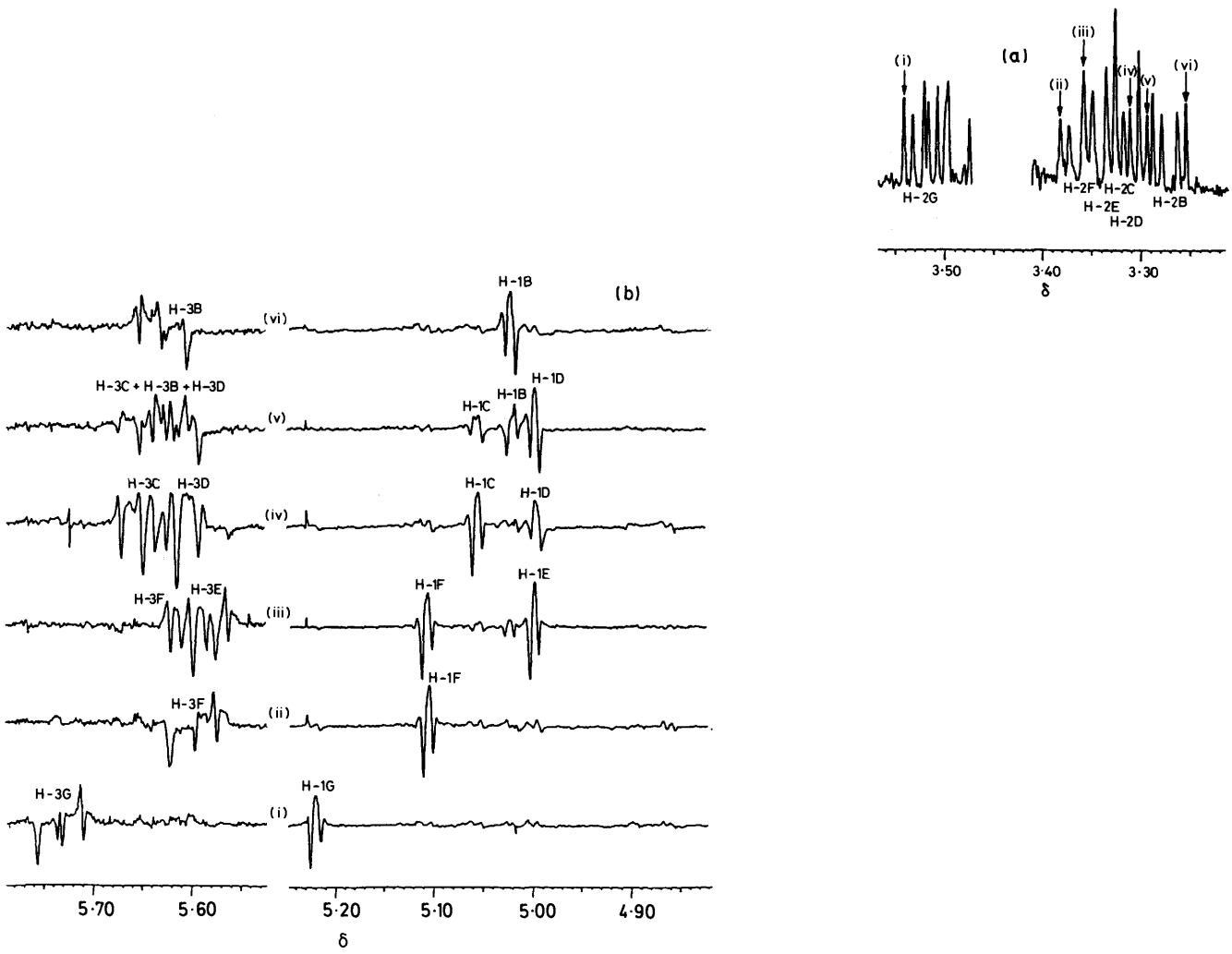


Figure 6 (a) Irradiations (i)—(vi) of the H-2 resonances for decoupling difference spectroscopy; (b) decoupling difference spectra for irradiations (i)—(vi) at the H-2 resonances and observing the signals in the H-3 and H-1 regions

which is shown in Figure 3. The chemical shifts are listed in Table 1 and the coupling constants in Table 2. The partial ^1H n.m.r. spectrum (400 MHz; $CDCl_3$; 57 °C) of (DM + 1)βCD-B_6 is shown (methoxy and aromatic proton resonances omitted) in Figure 4 with the known assignments marked on the spectrum. By comparison with the spectrum of DMβCD-B_7, the resonances at δ 4.85—5.25 (Figure 4) were assigned to the protons at the 1-position (*i.e.* the H-1 resonances) and it was possible to identify a separate doublet for each of the seven heterotopic anomeric protons. At the start of the investigation, the protons were designated arbitrarily and then subsequently labelled according to the schematic diagram in Figure 1 when the residue order was eventually established. However, in order to avoid confusion in this discussion, the final labelling system according to Figure 1 will be adopted from the outset. All the H-2 protons coupled to the H-1 protons were assigned by double resonance decoupling difference spectroscopy. Irradiations (i)—(vii) of each of the H-1 resonances at the positions

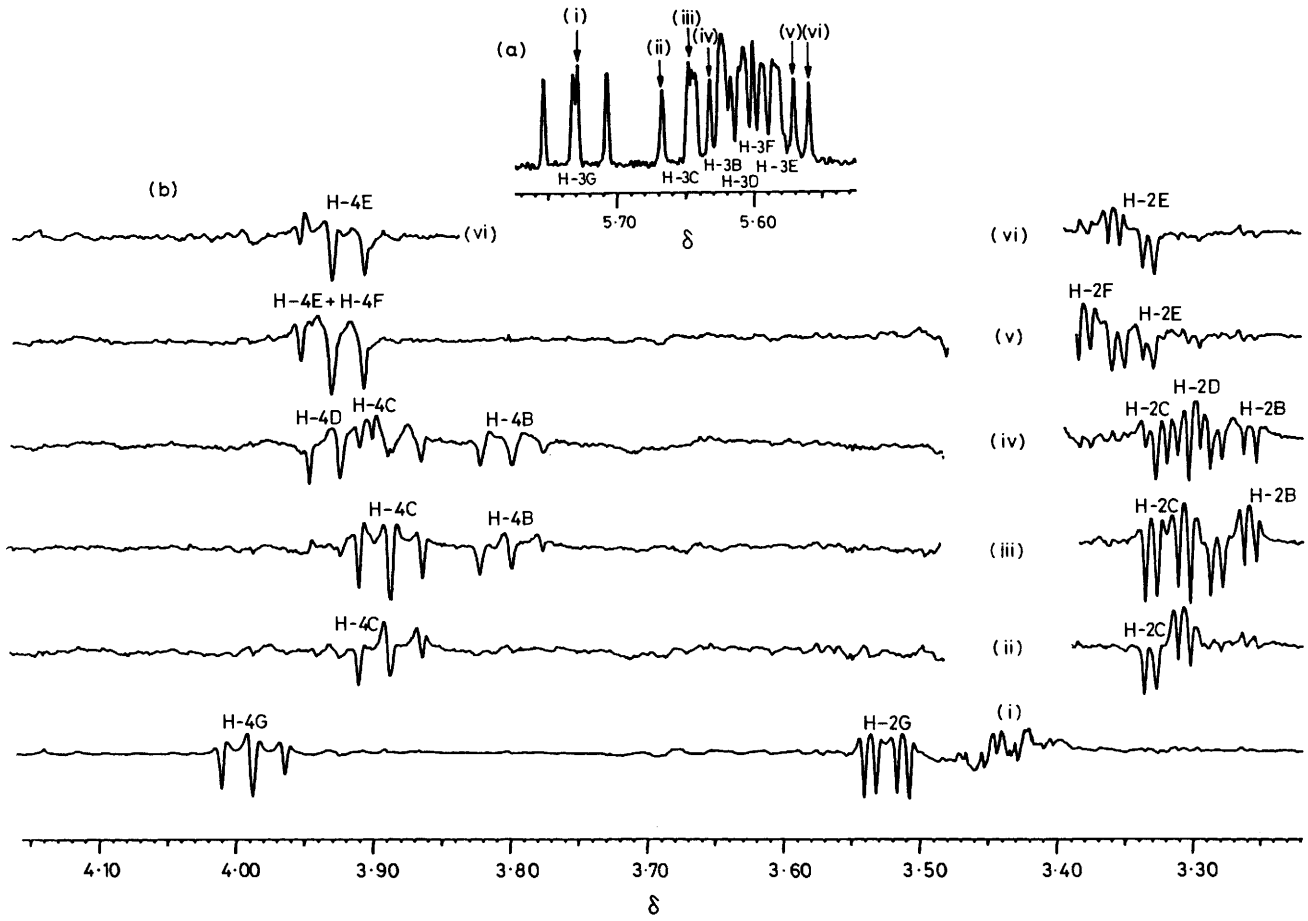


Figure 7. (a) Irradiations (i)—(vi) of the H-3 resonances for decoupling difference spectroscopy; (b) decoupling difference spectra for irradiations (i)—(vi) at the H-3 resonances and observing the signals in the H-4 and H-2 regions

Table 3. Results of n.O.e. experiments to determine residue sequence[a]

Proton(s) resonance irradiated	Resonance at which n.O.e. observed[b]
H-1G	H-4A
H-1A	H-4B
H-1D and H-1E	H-4E and H-4F
H-1B	H-4C
H-1C	H-4D
H-1F	H-4G

[a] Residues are labelled according to Figure 1. [b] See Figure 8.

indicated in Figure 5(a) identified each coupled H-2 resonance as a doublet of doublets. This is shown clearly by the difference spectra in Figure 5(b). The H-3 signals were identified similarly by irradiation at the frequencies of the H-2 resonances. As a consequence of the considerable overlap of signals, irradiation of only one line of each doublet of doublets for the H-2 protons was necessary. Even this precaution did not prevent irradiation of more than one resonance at a time. Figures 6(a) and (b) show respectively the irradiation points for the H-2 resonances and the corresponding difference spectra for the H-3 protons. Analysis of the H-1 region of the spectrum confirmed the assignments. The signal for H-3A at δ 3.611, located by irradiation of the resonance for H-2A at δ 2.90, is not shown. Irradiation (i) of the H-2G resonance clearly identified the H-3G signal. The H-3F resonance was easily located by irradiating (ii) only the highest frequency line for the H-2F signal. In a subsequent experiment, the resonances for H-2E and H-2F were irradiated simultaneously (iii), allowing the resonances for both H-3E and H-3F to be identified. In the knowledge of the chemical shift for H-3F from the previous experiment, an assignment could be made to H-3E. The fourth irradiation (iv) of the H-2C and H-2D signals pin-pointed both the H-3C and H-3D resonances while the fifth irradiation (v) of the H-2B, H-2C, and H-2D signals influenced the resonances for H-3D, H-3B, and, to a lesser extent, H-3C. In the final experiment, irradiation (vi) of the signal for H-2B unambiguously located the H-3B resonance. This made it possible to eliminate it from the trio identified in the previous irradiation (v). Furthermore, it allowed the assignment of the H-3C and H-3D signals on the basis of a comparison of the relative intensities observed for them during the fourth (iv) and fifth (v) irradiations. Figure 6 shows that the H-3D resonance was most influenced by irradiation (v) while the H-3C signal was only slightly perturbed; by contrast, during irradiation (iv) both were similarly affected. Inspection of the positions of irradiation of the previously assigned H-2 resonances permitted the higher-frequency resonance during irradiations (iv) and (v) to be assigned to H-3C, and the lower-frequency signal to H-3D. These results were corroborated (Figure 6) by the H-1 difference spectra.

At this point in the analysis residue A was identified as the one bearing the 3-*O*-methyl substituent on the basis of a comparison with the ^{1}H n.m.r. spectrum[8] of the fully 3-*O*-methylated derivative, 2,3,6-per-*O*-methyl-β-cyclodextrin (TMβCD), the protons of which have recently been assigned fully to the signals in the spectrum. The chemical shift (δ 3.6) of H-3A is close to the

Table 4. ^{13}C N.m.r. chemical shifts and corresponding ^{1}H chemical shifts (from CHORTLE sequence) together with assignments[a] for DMβCD-B_7 and (DM + 1)βCD-B_6 (400 MHz; $CDCl_3$; 57 °C)

δ_C (p.p.m.)	δ_H (p.p.m.)[b] from CHORTLE	Assignment[b] from CHORTLE	Assignment from 2D C-H correlation
DMβCD-B_7			
99.81	5.020 ± 0.001	C-1	
79.57	3.343 ± 0.001	C-2	
73.83	5.607 ± 0.001	C-3	
78.69	3.934 ± 0.001	C-4	
71.41	4.152 ± 0.001	C-5	
71.63	3.685, 4.011 ± 0.001	C-6	
59.05	3.433 ± 0.001	6-OMe	
58.79	2.775 ± 0.001	2-OMe	
(DM + 1)βCD-B_6			
99.92	5.025 ± 0.004	C-1B	C-1B
99.88	5.058 ± 0.005	C-1C	C-1C
99.74			C-1D, C-1E[d]
99.68			C-1A, C-1F[e]
99.62	5.221 ± 0.009	C-1G	
82.73	2.897 ± 0.005	C-2A	C-2A
81.89	3.602 ± 0.002	C-3A	C-3A
80.66	3.489 ± 0.003	C-4A	C-4A
79.79	3.269 ± 0.006	C-2B	C-2B
79.72			C-2G + C-2C, C-2D or C-2F[c]
79.62	3.334 ± 0.005	C-2E	
79.42[g]			Two of C-2C, C-2D or C-2F[c]
79.36	3.793 ± 0.002	C-4B	C-4B
78.93	3.932 ± 0.010	*c*	C-4D, C-4E or C-4F[c]
78.86	3.922 ± 0.005	*c*	C-4D, C-4E or C-4F[c]
78.76	3.892 ± 0.005	C-4C	
78.56	3.973 ± 0.007	C-4G	
78.38	3.922 ± 0.008	*c*	C-4D, C-4E or C-4F[c]
73.88	5.615 ± 0.010	C-3D	
73.80	5.586 ± 0.008	C-3E	
73.61	5.592 ± 0.006	C-3F	
73.39	5.647 ± 0.008	C-3C	
73.26	5.720 ± 0.007	C-3G	
73.06	5.628 ± 0.003	C-3B	
71.59		C-5s	C-5B
71.54			C-5E or C-5F[f]
71.43			C-5E or C-5F[f]
71.28			C-5C
71.22			C-5D
71.10			C-5A
71.08			C-5G
72.01	3.652, 3.900 ± 0.007	C-6A	
71.68	3.665, 3.966 ± 0.007	C-6D	
71.57		C-6s	
71.51[g]			
71.47			
71.17			

[a] Residues are labelled according to Figure 1. [b] Where no assignment is made and proton δ is not given, this is as a result of overlapping carbon signals. [c] Assignment could not be made as a result of close proximity or coincidence of proton signals and lack of digitisation in 2D C–H correlation experiments. [d] Both carbon and proton signals are coincident. [e] Carbon signals are coincident but assignment has been made, see Figure 12(a). [f] Assignment could not be made as a result of the inability to assign fully the proton resonances. [g] Two coincident carbon signals.

value of δ 3.5 reported[8] for H-3 in TMβCD, while the remainder of the H-3 signals between δ 5.55 and 5.76 lie close to the value of δ 5.6 observed for H-3 in DMβCD-B_7. The resonances for H-4 were identified (Figure 7) in an analogous manner. The protons H-4G, H-4C, H-4E, and H-4A were assigned unambiguously to signals by irradiating [(i), (ii), and (vi), respectively; the irradiation of H-3A is not shown] the H-3G, H-3C, H-3E, and H-3A resonances (or parts of them) in turn. The signal for H-4A at δ 3.49 is not shown in Figure 7. The resonances for H-3B and H-3C were irradiated simultaneously (iii), as were (v) the resonances for H-3E and H-3F. With the chemical shifts of H-4C and H-4E [by irradiating H-3C and H-3E, (ii) and (vi), respectively] already established, the signals for H-4B and H-4F could be identified. Irradiation (iv) of the overlapping signals for H-3B, H-3C, and H-3D permitted the assignment to be made to the signal for H-4D, since the chemical shifts for H-4B and H-4C were known.

At this stage, although the resonances for H-1, H-2, H-3, and H-4 on each separate glucopyranosidic residue had been identified, the sequence of glucosidic linking between the residues was unknown. This was determined by observing the nuclear Overhauser effects (n.O.e.s) between the H-1 and H-4 protons across the seven α-1,4-glucosidic linkages. All the resonances for the H-1 protons were irradiated in turn and the n.O.e.-induced intensity changes on the signals for the H-4 protons were observed by difference spectroscopy. The spectra are illustrated in Figure 8 and the results are summarised in Table 3. The observed n.O.e.s allowed the residue sequence to be determined unambiguously and the residues were assigned according to the notation (Figure 1) already discussed. The sequence was confirmed by observing n.O.e.s in the reverse direction, *i.e.* irradiating the resonances for the H-4 protons and observing the n.O.e.s in the signals for the H-1 protons.

Since H-5 protons and H-6 protons resonate within 1 p.p.m. of each other, a 2D-COSY experiment was performed over this region of the spectrum. The resulting contour plot is shown in Figure 9. To avoid misinterpretation or ambiguity, all assignments were confirmed by decoupling difference spectroscopy. From H-4A resonating at δ 3.494, H-5A was traced to a signal centred on δ 4.073 and H-3A to a signal centred on δ 3.611. From the signal for H-5A, the H-6aA and H-6bA resonances were located at δ 3.659 and 3.893, respectively. The resonance for H-5B was found at δ 4.048 from H-4B resonating at δ 3.799 and, as a consequence, the H-6aB and H-6bB resonances were located at δ 3.645 and 4.060, respectively. As a result of the close proximity of the H-5B and H-6bB signals, confirmation of the assignment was obtained through a difference decoupling experiment. The signal for H-6a has a large coupling of 11 Hz to the geminal H-6b resonance and a small coupling of 1.5 Hz to the adjacent H-5 resonance. Irradiation of the signal for H-6aB gives rise to a decoupling difference spectrum typical of the perturbation of a large coupling at δ 4.060 and characteristic of the pattern observed for H-6a-decoupled H-6b signals. The presence of an H-6b resonance at δ 4.060 was later confirmed by *J*-resolved spectroscopy [see Figure 10(c)]. The resonance for H-5C was found at δ 4.120 from the signal for H-4C at δ 3.887. The resonance for H-5D at δ 4.186 was located from the signal for H-4D at δ 3.920 and, as a consequence, the H-6aD and H-6bD resonances were found at δ 3.673 and 3.963, respectively. Relying on the signals for H-4E and H-4F (at δ 3.928 and 3.929, respectively) led to the location of the resonances for H-5E and H-5F in the range δ 4.095—4.150. In view of the close proximity of the signals for these two H-4 protons, the two H-5 protons could not be assigned individually. Since the resonance for H-5C lies within the same spectral region, none of the H-6 (C, E, or F) protons could be assigned with any certainty, although chemical shifts for the H-6 protons were derived subsequently.

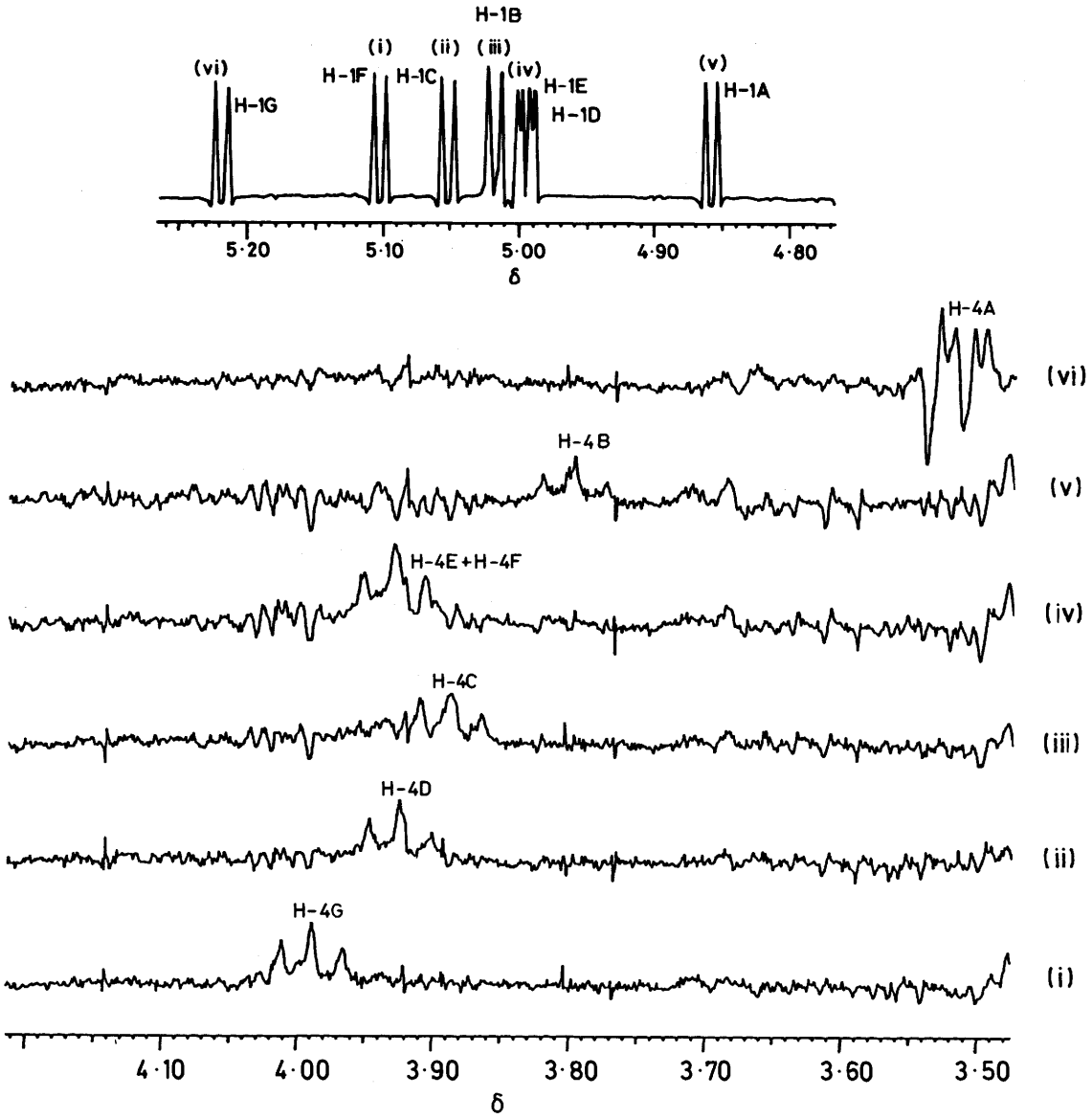


Figure 8. N.O.e. difference spectra in the H-4 region upon irradiations (i)—(vi) of the H-1 resonances

From inspection of the conventional ^{1}H n.m.r. spectrum (Figure 4), it was evident that a portion of another signal overlaps with the lower-frequency component of the multiplet for H-5D. Difference decoupling experiments on the resonances for H-6a ascertained that the H-6b protons did not account for this signal. On this basis, the overlapping signal was ascribed to another H-5 proton. Indeed, it was shown to arise from H-5G by a decoupling difference experiment which influenced the signal previously assigned to H-4G. The resonances for H-6aG and H-6bG were located at δ 3.693 and 4.012, respectively.

In order to identify more resonances with their protons and to gain more precise information on coupling constants, a series of *J*-resolved 2D n.m.r. experiments was performed. The resulting contour plot for the H-3 region is illustrated in Figure 10(a). The six resonances (H-3A is at lower frequency) can be clearly identified. Figure 10(b) shows the H-2 region (except for H-2A and H-2G resonances) while Figure 10(c) shows the contour plot for the remaining protons of interest (except for the H-2A and the H-1 resonances where this technique was considered unnecessary and for the H-5 resonances which were not detected under these conditions even at lower contour levels). From Figure 10(c), the chemical shifts of the remaining unidentified H-6b (C, E, and F) protons were found centred on δ 3.980, 3.995, and 4.012, but they could not be assigned uniquely. Similarly, the chemical shifts of the H-6a (C, E, and F) protons were found centred on δ 3.683 (2 signals) and 3.660. The chemical shifts of the other protons were confirmed by the *J*-resolved experiments and the chemical shifts of all the protons, that have been assigned unambiguously, are listed in Table 1. The coupling constants for H-1, H-2, H-3, and H-4, obtained from the conventional spectrum, were confirmed, but it was not possible to measure the $^3J_{5,6}$ and $^2J_{6a,6b}$ values with sufficient precision to warrant their inclusion in Table 2. All the accessible coupling constant data are given in this Table.

The partial *J*-modulation (JMOD) ^{13}C n.m.r. spectrum of the symmetrical DMβCD-B_7 is shown in Figure 11(a) with the resonances for the methoxy and benzoyl groups omitted. The assignments were made on the basis of one-dimensional (1D) carbon–proton correlation spectroscopy (CHORTLE, described in detail in the Experimental section). The ^{13}C chemical shifts, together with the corresponding ^{1}H chemical shifts, are

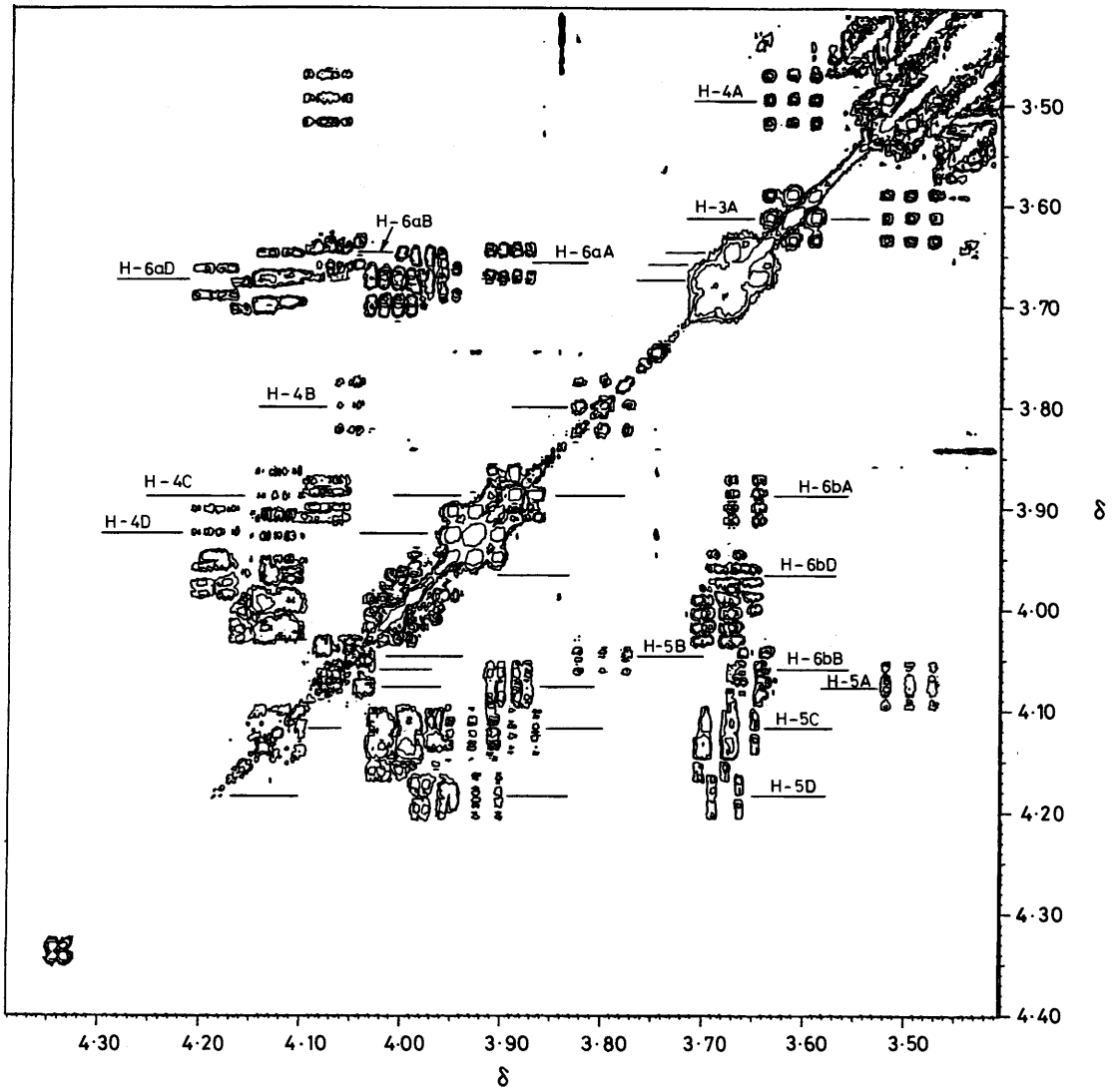


Figure 9. Partial 2D ^{1}H COSY contour plot for (DM + 1)βCD-B_6 in the δ 4.4—3.4 region (400 MHz; $CDCl_3$; 57 °C)

listed in Table 4. The partial JMOD ^{13}C n.m.r. spectrum of (DM + 1)βCD-B_6 is shown in Figure 11(b), with the C-5 and C-6 signals conveniently separated. This was achieved by addition and substraction of spectra in which the CH_2 signals have opposite signs. The procedure employed is described in detail in the Experimental section. Tentative assignments were made by comparison with the spectra for DMβCD-B_7 [Figure 11(a)] and the spectral data[8] for TMβCD. One-dimensional C–H correlation spectroscopy (CHORTLE) was used to assign individual carbon signals that did not overlap with each other. Confirmation of the tentative assignment made to the signals for C-5, which were severely overlapped by the C-6 signals, was provided by a modified version of the CHORTLE pulse sequence. The technique employed is described in detail in the Experimental section. Two-dimensional C–H correlation spectroscopy (XHCORRD), performed over selected regions of the spectrum, was used to confirm the CHORTLE assignments and to assign the overlapping carbon signals. All the assigned ^{13}C chemical shifts with the corresponding ^{1}H chemical shifts derived from the CHORTLE program are given in Table 4. The 2D C–H correlation contour plot for the C-1/H-1 region is illustrated in Figure 12(a). Only the C-1G/H-1G correlation was not detected. Figure 12(b) shows the contour plot for the C-5/H-5 region. This allowed the determination of the chemical shifts of H-5E and H-5F (not obtained from the COSY experiment), although individual assignments could not be made.

In an effort to assign more of the nuclei to the resonances, the n.m.r. spectra were also recorded for a solution in [$^2H_8$]-toluene. The resulting significant differences in the n.m.r. spectra can be attributed to the effect of the solvent entering the cavity of the cyclodextrin. The resonances for H-1, H-2, H-3, and H-4 were all readily assigned using the same techniques as before. The H-4 signals were all well resolved and n.O.e. experiments involving the H-4 and H-1 resonances confirmed the ring sequence. All the H-6a signals were resolvable but this fortuitous situation could not be exploited as the result of severe overlap between the resonances for H-5 and H-6b. Variation of the temperature up to 100 °C proved unsuccessful as a means to disperse the signals. In fact, the first set of experiments in $CDCl_3$ was more informative and so details of the spectra recorded for the solution in [$^2H_8$]toluene will not be presented.

Conclusion

The detailed investigation reported in this paper has demonstrated, that high-field n.m.r. spectroscopy, employing

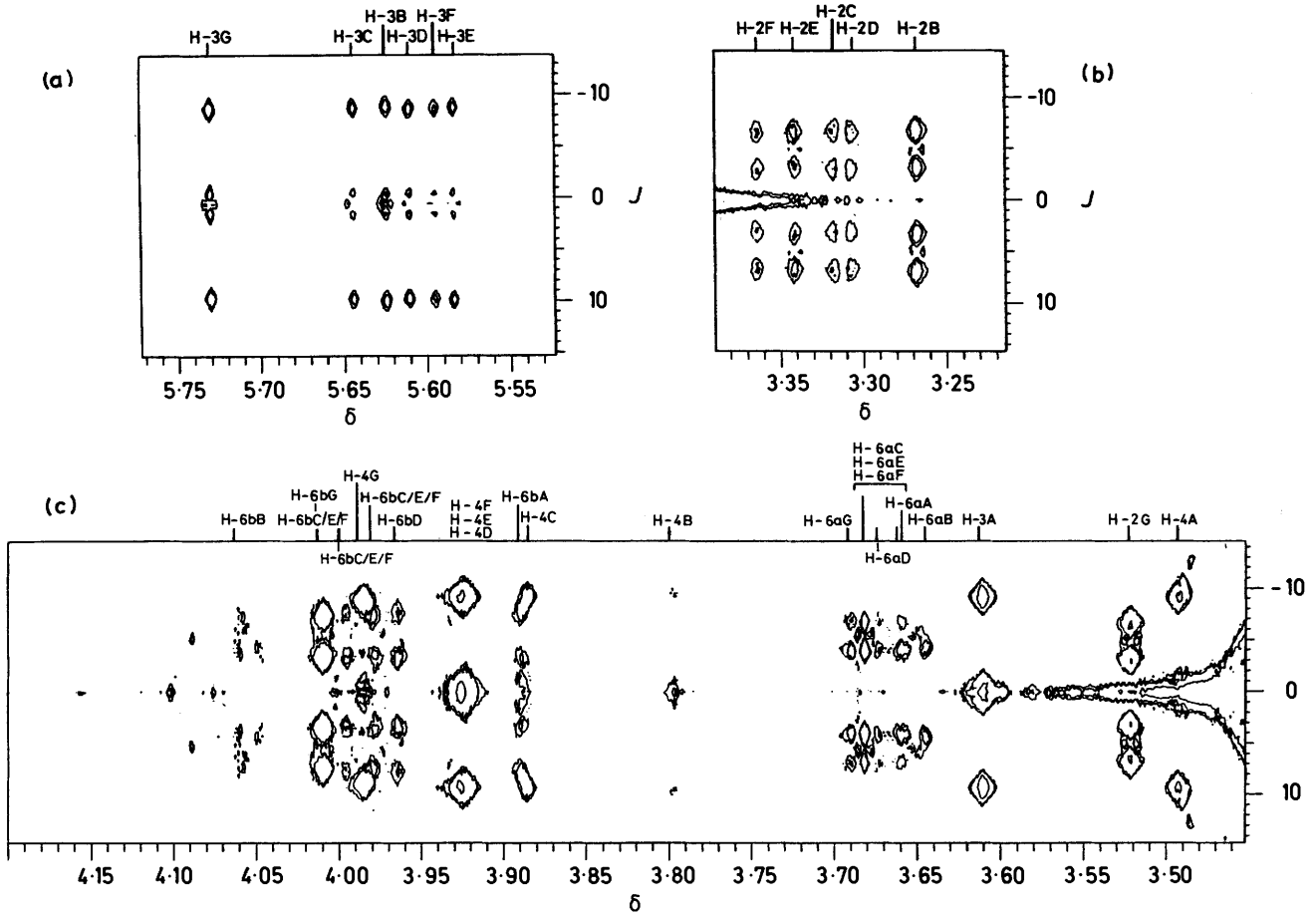


Figure 10. *J*-Resolved 2D contour plots for (DM + 1)βCD-B6 in (a) the H-3 region, (b) the H-2 region, and (c) for other protons (400 MHz; $CDCl_3$; 57 °C)

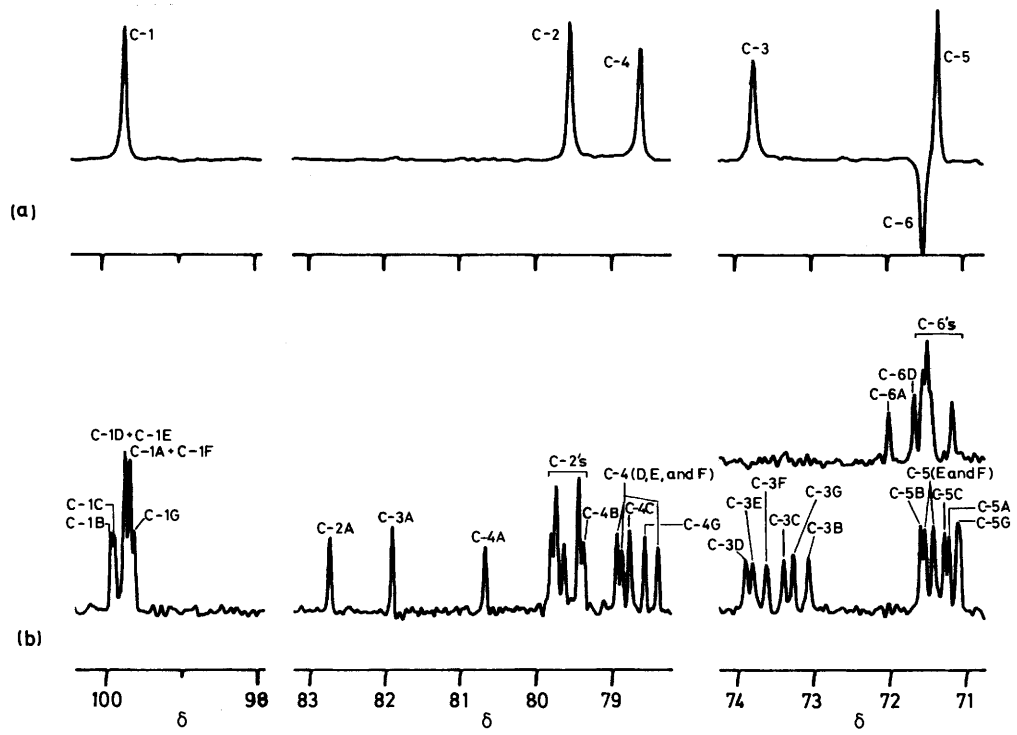


Figure 11. Partial JMOD ^{13}C n.m.r. spectra for (a) DMβCD-B_7 and (b) (DM + 1)βCD-B_6 (100 MHz; $CDCl_3$; 57 °C)

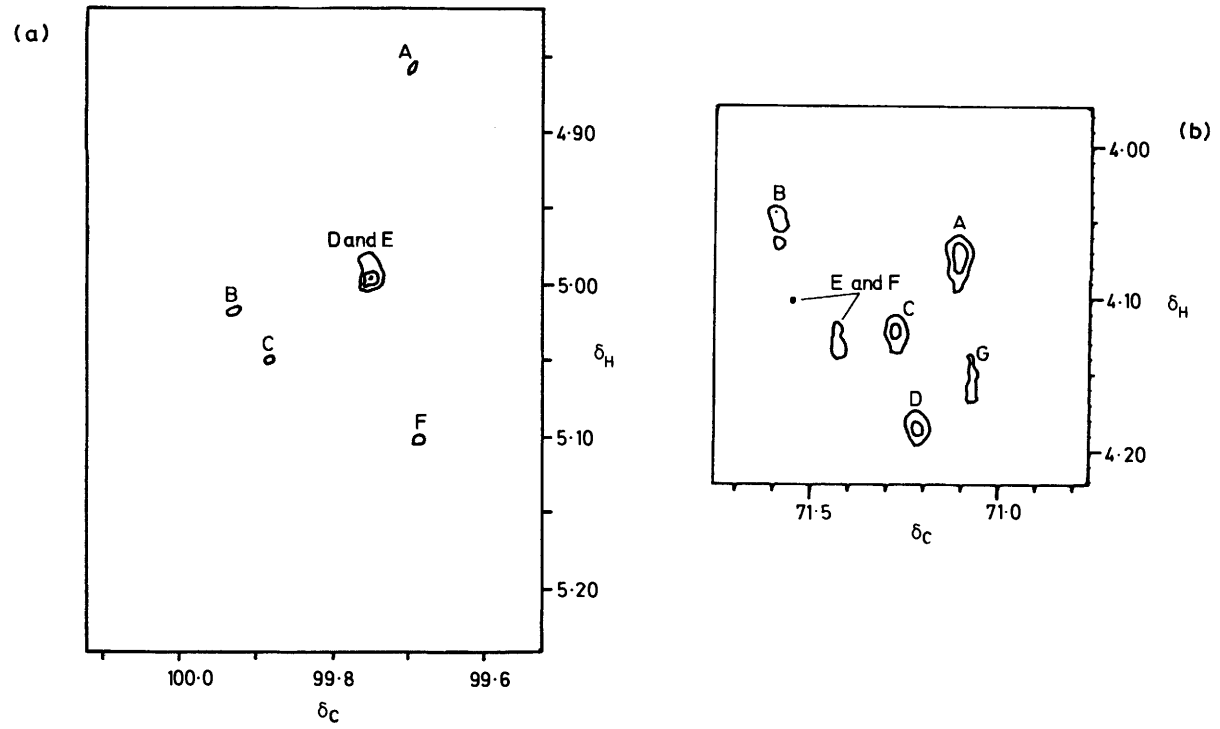


Figure 12. 2D C–H Correlation contour plots for (DM + 1)βCD-B_6 in (a) the C-1/H-1 region and (b) the C-5/H-5 region (400 MHz for ^1H; 100 MHz for ^{13}C; $CDCl_3$; 57 °C)

modern pulse sequence techniques, is an indispensible tool for elucidating the structural characteristics of complex dissymmetric molecules. If we exclude methoxy and benzoyl groups from the reckoning, the unsymmetrical chemically modified cyclodextrin derivative (DM + 1)βCD-B_6 contains 49 heterotopic protons and 42 heterotopic carbon atoms. Each one of these 91 nuclei has been located as a resonance in the n.m.r. spectra of the compound. However, it has only been possible with available hardware and software to assign individually and unambiguously, 41 out of the 49 protons and 29 out of the 42 carbon atoms. As one might expect, the residues (B and G) adjacent to the unique one (A) carrying three methoxy substituents display the most marked chemical-shift differences in their nuclei. By contrast, the residues (D and E) most remote from A experience more modest chemical-shift differences relative to those observed for nuclei in DMβCD-B_7. It is possible that these relatively large differences in chemical shifts involving protons and carbon atoms on glucose residues B and G could be attributed to conformational changes brought about by the extra methoxy substituent on glucose residue A. The observation that the specific rotations of (DM + 1)βCD-B_6 and DMβCD-B_7 are considerably different (−34° and −93°, respectively, for $[\alpha]_D$ in $CHCl_3$) lends support to this suggestion. Recently, the symmetrical α- and β-CDs have been shown[17] by ^{13}C CP-MAS n.m.r. spectroscopy to exhibit discrete and different ^{13}C n.m.r. chemical shifts in the solid state for the C-1 and C-4 probes in each glucose residue. This phenomenon has been ascribed to conformational features present in α- and β-CDs in the solid state as indicated by their *X*-ray crystal structures.[18]

This is the first report of a detailed and extensive investigation of the n.m.r. spectroscopic characteristics of an unsymmetrical chemically modified cyclodextrin. The ability to map out the molecular torus of these derivatives spectroscopically will enable structural studies to be undertaken which could lead to a much fuller understanding of the fundamental forces involved during complex formation with suitable substrates in solution. This type of investigation should complement the work at present being carried out[19] on the use of unsymmetrical chemically modified cyclodextrins as enzyme models. Indeed, without detailed evidence for the rigorous structural identification of such enzyme models, scepticism must surround the quantitative aspects of any claims made in the literature. The task of isolating and characterising unsymmetrical chemically modified cyclodextrins is not insurmountable and should not be avoided.

Acknowledgements

We thank The Leverhulme Trust (Research Fellowship to J. F. S.), Johnson Matthey Technology Centre (C.A.S.E. Award to R. Z.), and the S.E.R.C. for financial support.

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Received 8*th October* 1986; *Paper* 6/1978