Crystal Structure of Hydrated $6^A,6^C,6^E$-Tri-*O*-methylcyclohexaamylose† and its Conformation in Solution

Viviane Durier,[a] Georges Buisson,*[,b] Emile Duée,[b] Hugues Driguez*[,a] and François-R. Taravel[a]

[a] *Centre de Recherches sur les Macromolécules Végétales, CNRS, B.P. 53 X, 38041 Grenoble cedex, France*

[b] *LCM IBS, 41 Avenue des Martyrs, F38027 Grenoble cedex 1, France*

The crystal structure of uncomplexed $6^A,6^C,6^E$-tri-*O*-methylcyclohexaamylose† (CD-3OMe) has been determined by X-ray analysis. The structure was solved by molecular replacement methods and refined by the full matrix least-squares method to the *R*-value of 0.074. Cyclodextrin molecules are arranged in a herring-bone cage-type packing. Inside the cavity only two water molecules are found close to the primary hydroxyl rim. One *O*-methyl group of a molecule is inserted into a neighbouring host molecule from the secondary hydroxy side. The conformation of CD-3OMe was also studied in solution by NMR spectroscopy. In particular heteronuclear $^3J_{^{13}C-^1H}$ coupling constants through the glycosidic linkages were measured. The results obtained in deuteriated water and pyridine could be intepreted in terms of an averaged flexible conformation.

Cyclodextrin glycosyltransferase (CGTase EC 2.4.1.19) transforms starch oligomers and polymers into cyclic maltooligosaccharides consisting of six, seven or eight units (α, β- and γ-CD). Several papers on the three-dimensional structure of this enzyme have recently been published and in order to visualize the active site,[1-3] it is usually necessary to soak crystals of protein in a substrate solution. This enzyme has been used for the first enzymatic synthesis of a regioselectively modified CD.[4] The high yield obtained for the synthesis of $6^A,6^C,6^E$-tri-*O*-methylcyclohexamylose suggests a preferential binding of the modified maltose in the active site. With the aim of confirming this hypothesis by docking experiments, we have established the X-ray structure of this modified CD and its conformation in solution using NMR techniques.

Results and Discussion

Crystallographic Results.—The numbering of the structure is shown in Fig. 1. Each pyranose ring is in the $^4C_1$ conformation. Average values of bond lengths and angles for the glucosyl residues are given in Fig. 2. These values are in good agreement with the corresponding values of native cyclomaltohexaose[5-7] and methylated cyclomaltohexaose.[8,9]

Examination of the structure of the glucose units reveals that all the C(6)–O(6) bonds are *gauche–gauche* except in G1. In this case a *trans–gauche* conformation is observed. The methyl group is disordered in the G2 and G4 units and stabilized in G6; the C(7)–O(6) bonds are oriented perpendicularly and away from the C3 rotation axis of the macrocycle for the G4 and G6 units whereas in G2 this bond is aligned parallel to this rotation axis. Although the methylation does not have any great effect on the overall structure, it does have a significant effect on the interglycosidic torsional angles (Table 1).

In the macrocyclic structure the six O(4) atoms are coplanar within 0.097 Å, and these atoms form a nearly regular hexagon with a side length of 4.141–4.260 Å and a mean angle of 119.83°. The tilt angle (Table 1) which is defined as the angle made by the plane through the six O(4) atoms and the plane through C(1*n*), C(4*n*), O(4*n*) and O(4*n* + 1), has a mean value of 10.0° for five glucose units giving an overall conic shape to the molecule. The

† IUPAC-recommended name: $6^1,6^3,6^5$-tri-*O*-methylcyclomaltohexaose.

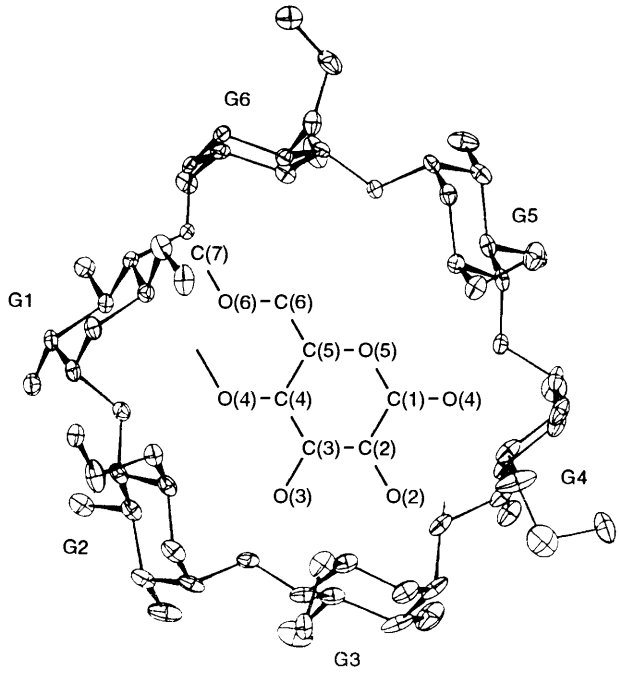

Fig. 1 Thermal ellipsoid plot of the molecule showing the atom labelling scheme

G5 unit is more tilted towards the centre of the cavity with a tilt angle of 29.18°. The average distances of 2.875 Å between O(2) of G*n* and O(3) of G*n* + 1 permit the formation of strong intramolecular hydrogen bonds; they involve HO(3) for G2–G5, HO(2) for G6 and either HO(2) or HO(3) for G1. These hydrogen bonds stabilize a regular macrocyclic conformation. Furthermore the short distance of O(4)G*n*–O(4)G*n* + 1 (4.217 Å) and the large glycosidic angle (119.9°) can be correlated with the values found for form III of native α-CD which has the most regular macrocycle.[5] It is noteworthy that this form of α-CD is crystallized in the presence of $CaCl_2$ and that CD-3OMe is crystallized in the presence of $BaCl_2$. This suggests that the presence of divalent cations favours a regular macrocyclic conformation.

In CD-3OMe, three water molecules W1, W2 and W3 are in fully occupied position and the 3.7 others are distributed over 7

Table 1 Geometrical data and torsional angles of methylated and unmethylated residues of CD-3OMe

	Residue					
	G1	*G2	G3	*G4	G5	*G6
d^a	−0.187	0.148	−0.007	−0.099	0.066	0.078
t^b	17.18	14.20	9.16	4.59	29.28	3.95
C(1*n*)–O(4*n* + 1)–C(4*n* + 1)(°)	118.7	119.9	120.0	120.7	120.0	120.1
O(4*n* − 1)···O(4*n*)···O(4*n* + 1)(°)	121.1	119.6	119.6	118.5	122.9	117.4
O(4*n*)···O(4*n* + 1)(Å)	4.26	4.22	4.24	4.17	4.26	4.14
O(2*n*)···O(3*n* + 1)(Å)	2.83	2.88	2.82	2.51	2.26	2.95
φ[O(5*n*)–C(1*n*)–O(4*n* + 1)–C(4*n* + 1)](°)	111.3	107.0	111.8	110.1	113.9	99.9
ψ[C(1*n* − 1)–O(4*n*)–C(4*n*)–C(5*n*)](°)	−119.9	−110.2	−107.9	−108.1	−123.6	−91.6

^{a} *d* (Å) is the r.m.s. deviation of each O(4) atom from the least squares plane of the six O(4) atoms. ^{b} The tilt-angle *t* (°) is defined as the angle made by the plane through the six O(4) atoms and a plane through C(1*n*), C(4*n*), O(4*n*) and O(4*n* + 1) of each residue. * Methylated residues.

Table 2 ^{1}H Chemical shifts (ppm) of CD-3OMe in various deuteriated solvents

Solvent	Unit	1-H	2-H	3-H	4-H	5-H	6_a-H	6_b-H
D_2O	Unmethylated	5.01	3.61[a]	3.95[b]	3.55[c]	3.79	3.84–3.90	3.84–3.90
	Methylated	5.00	3.60[a]	3.92[b]	3.54[c]	3.85	3.81	3.75
[$^2H_6$]DMSO	Unmethylated	4.79	3.26	3.77[d]	3.41	3.60–3.68	3.53–3.75	3.53–3.75
	Methylated	4.77	3.20	3.75[d]	3.30	3.60–3.68	3.53–3.75	3.53–3.75
[$^2H_5$]Pyridine	Unmethylated	5.42	4.08	4.67	4.15	4.36	4.30–4.41	4.30–4.41
	Methylated	5.52	4.08	4.65	4.13	4.41	3.83	4.00

[a,b,c,d] Within a column and for each solvent, ^{1}H assignments for different D-glucosyl residues may be exchanged.

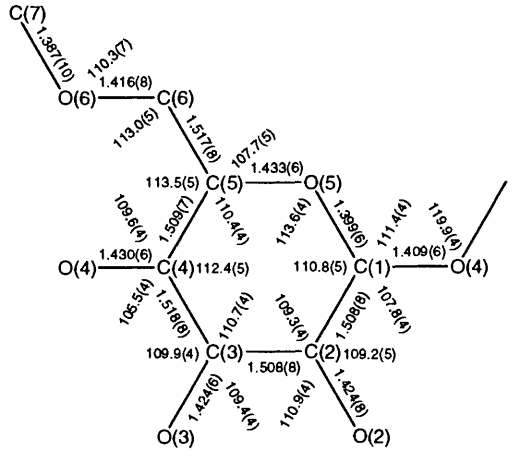

Fig. 2 Average bond distances (Å) and angles (°) for CD-3OMe residues

sites with an occupancy factor of 0.7 for W4, W5, W6 and W7 and 0.3 for W8, W9 and W10.[10,11]

The CD-3OMe molecules are stacked along the crystallographic *c* axis in a herring bone cage-type packing[12,13] (Fig. 3). In this packing, the molecules are inclined towards each other with an angle of 56° between the O(4) planes. Both sides of the cavity are covered by neighbouring molecules. One methyl group of a molecule enters into the secondary hydroxy side of the cavity of the neighbouring molecule. Two ordered water molecules (W2 and W3) are located in the annular cavity on the O(6) side of the torus, the other water molecules (W1 and the 3.7 disordered ones) are distributed in the intermolecular space between CD-3OMe molecules. The packing is stabilized by eight intermolecular hydrogen bonds.

Proton NMR Analysis of CD-3OMe.—The ^{1}H spectra of CD-3OMe were performed in three deuteriated solvents: D_2O, [$^2H_6$]DMSO and [$^2H_5$]pyridine. The respective spectra (Fig. 4) are similar to what would be expected for the threefold C_3 symmetry of trisubstituted cyclodextrins. This means that the signals of the methylated (prime) and of the unmethylated (unprimed) units were distinguishable. However, even at 400 MHz, the signals were not well separated. The best separation of the signals was obtained in pyridine. Assignments were performed by means of COSY and NOE experiments (see Table 2).

Each type (primed and unprimed) of proton resonated very close to each other with the exception of the protons H(6′)$_a$ and H(6′)$_b$ and also H(5′) in water and pyridine. This could indicate the influence of the methyl groups on their nearest environment. In DMSO and pyridine the protons H(1) and H(1′) could be clearly distinguished as separate doublets. This was also the case for H(4) and H(4′) in pyridine. The coupling constants $J_{5,6}$ and $J_{5'6'}$, which could give an estimation of the rotameric population around the C(5)–C(6), and C(5′)–C(6′) bonds could not be measured accurately, because of signal overlapping, even with resolution enhancement. Use of a high field spectrometer overcame this problem. In the solid state it was found for the parent cyclomaltohexaose, α-CD, that only *gauche–gauche* ('away' from the cavity) HO(6) conformations were present.[14] Furthermore molecular models showed[15] that the rotation around the C(5)–C(6) bond of the glucopyranosyl residues was restricted by steric hindrance due to the OMe group. The relative chemical shifts observed for H(1) and H(1′), H(4) and H(4′) in the three solvents could be an indication, firstly on the influence of solvation, and secondly on the conformation of the glycosidic linkages. In the solid state, it was shown that the most plausible source of the difference in ^{13}C-shifts for C(1) and C(4) was the diversity of conformations about the glycosidic linkage.[16] Also de Bruyn has shown how by consideration of a combination of effects through bonds and through space, the ^{1}H NMR chemical shifts in a series of glucobioses and methyl glucobiosides could yield generalizations related to the conformation of the glycosidic bond.[17] In our case two conformations of the glycosidic bonds averaged by the rapid interconversion of the conformers could be expected.

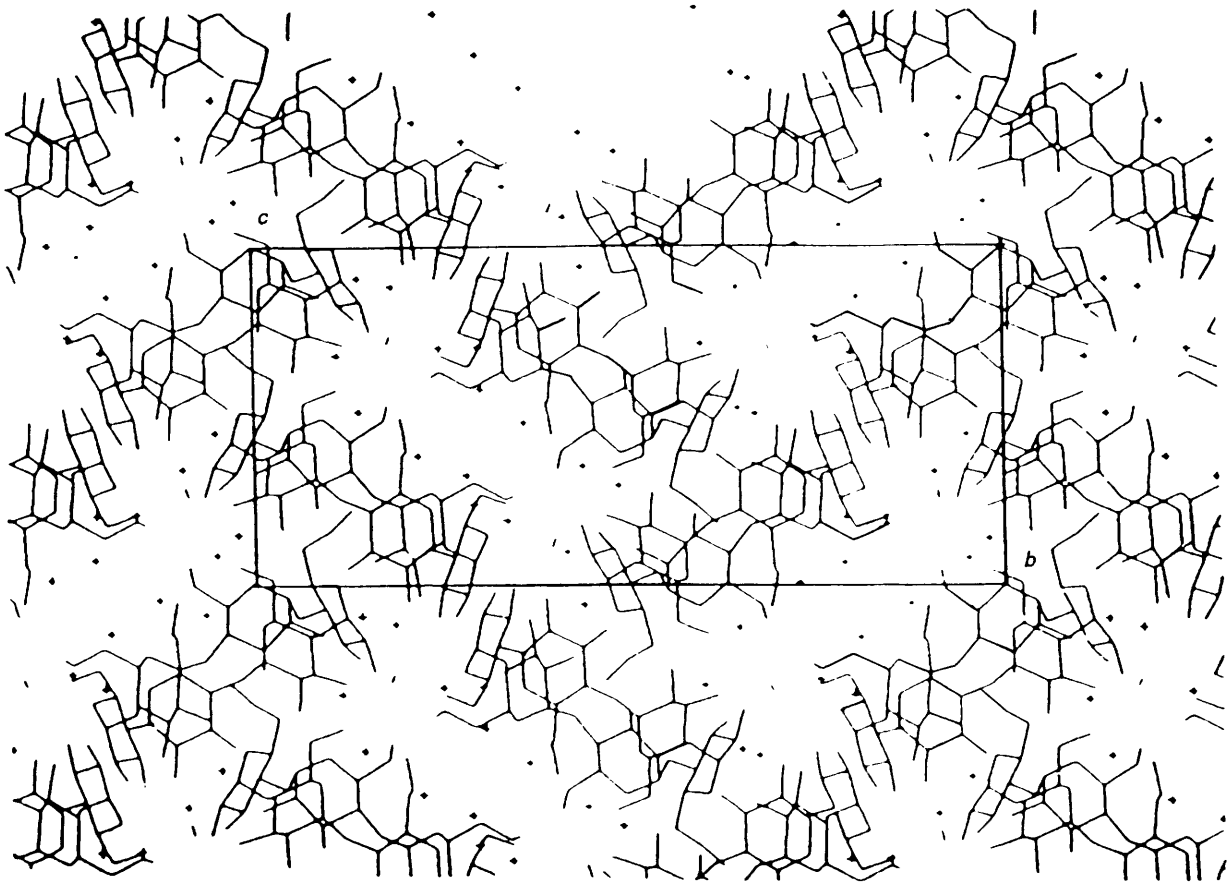

Fig. 3 Packing diagram projected down *a*

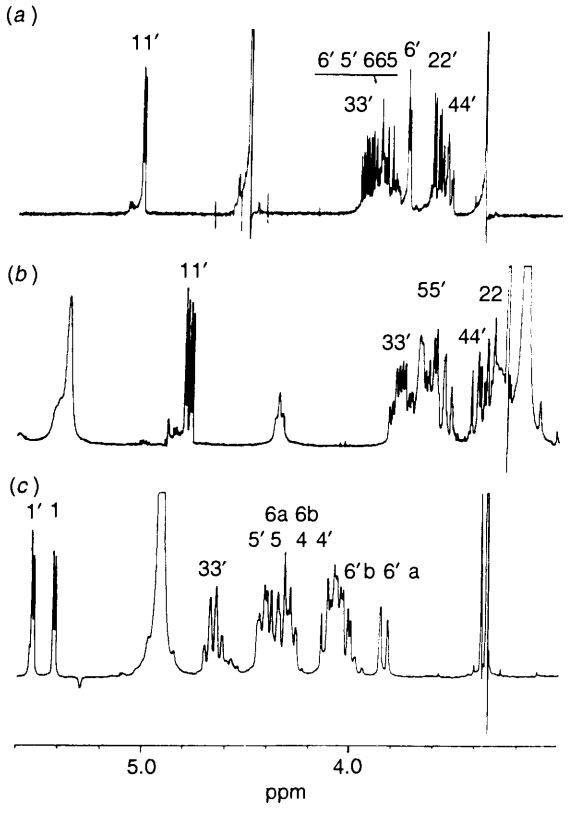

Fig. 4 Spectra (300 MHz) of CD-3OMe in D_2O at 303 K (*a*), in [$^2H_6$]DMSO at 333 K (*b*) and in [$^2H_5$]pyridine at 303 K (*c*)

Heteronuclear Coupling Constants.—The potential value of long-range $^3J_{^{13}C,^1H}$ coupling constants as a tool for stereochemical and conformational analysis of carbohydrates and related compounds is well known.[18-21] In the field of carbohydrates the most important couplings are those through the glycosidic linkage, involving a heteroatom X. In general vicinal coupling constants depend on several factors (substitution, hybridization, electronegativity, bond and angle changes). In this respect, a Karplus-type relationship between the magnitude of the $^3J_{^{13}C,^1H}$ coupling constant and the dihedral angle φ for the C–X–C–H system has been derived.[22,23] Bax and Freeman[24] were the first to propose a 2D-experiment specially designed for the detection and assignment of long-range carbon–proton coupling constants. Although the sensitivity of this method is not very high, it has been successfully used as such or under a modified form involving, for example, a DANTE sequence.[25] Other methods rely on carbon and proton detected proton–carbon chemical shift correlation experiments adjusted at low J values.[26,27] Methodologies based on inverse detection have also been presented.[28,29]

In this paper a modified version[25] of the Bax and Freeman experiment was used to measure the $^3J_{^{13}C,^1H}$ coupling constants between C(1) and H(4'), C(1') and H(4), C(4) and H(1') and C(4') and H(1) in order to estimate the following torsion angles in water and pyridine:

$$\psi'H = C(1)\text{–}O(4')\text{–}C(4')\text{–}H(4')$$

$$\psi H = C(1')\text{–}O(4)\text{–}C(4)\text{–}H(4)$$

$$\varphi'H = C(4)\text{–}O(4)\text{–}C(1')\text{–}H(1')$$

$$\varphi H = C(4')\text{–}O(4')\text{–}C(1)\text{–}H(1)$$

Table 3 ^{13}C Chemical shifts (ppm) of CD-3OMe in water and in pyridine at 303 K. Primed numbers and last column refer to atoms of the methylated residues

Solvent	C(1)	C(1′)	C(2)–C(2′)	C(3)–C(3′)	C(4)–C(4′)	C(5)	C(5′)	C(6)	C(6′)	CH_3
D_2O	102.51	102.33	72.73	74.37	82.31	73.07	71.75	61.43	71.84	59.54
[$^2H_5$]Pyridine	102.83	102.62	73.09	73.99	82.20	73.58	71.39	60.42	70.82	58.07

Table 4 $^3J_{^{13}C,^1H}$ Coupling constants (Hz) measured in water and in pyridine for CD-3OMe. The coupling constants calculated from the crystal structure are also given. Primed numbers refer to atoms in the methylated residues

$^3J_{^{13}C,^1H}$	D_2O	[$^2H_5$]Pyridine	Crystal structure
H(1)–C(4′)	6.0	4.0	5.5
H(1′)–C(4)	5.3	5.1	5.1
H(4)–C(1′)	5.6	5.2	5.6
H(4′)–C(1)	5.4	5.0	5.2

^{13}C-Assignments were made by 2D ^{13}C–^1H heteronuclear shift-correlation using polarization transfer from ^1H to ^{13}C *via* $^1J_{^{13}C,^1H}$ (Table 3). The heteronuclear coupling constants ($^3J_{^{13}C,^1H}$) are shown in Table 4 for two solvents. The values of the coupling constants calculated for the crystal structure by means of a Karplus-type equation are also given. This equation was established for a series of conformationally rigid carbohydrate derivatives having known X-ray structures.[23] In water and in the solid state the respective values were very close. In solution the values measured were representative of a virtual conformation for which the averaged torsion angles were all in the region 0 to ± 12°. The influence of the 6-OMe was clear on its near environment as well as on the corresponding torsion angles which had slightly different values to those of unmethylated residues. These values were in general agreement with those obtained for the native cyclomaltoheptaose.[22] In fact it seems that like the parent cyclomaltohexaose, CD-3OMe adopts a regular conformation in water. However in pyridine the values found could correspond to a more twisted conformation with for example $\langle\varphi H\rangle$ ± 33°. This was also confirmed by a NOESY experiment showing a stronger correlation (so a shorter distance in first approximation) for H(1′)/H(4) than for H(1)/H(4′). In all cases substitution and solvation of the cyclodextrin are linked to the concepts of flexibility and of adaptability.

Experimental

Crystallographic Analysis of CD-3OMe.—Crystal data. $C_{39}H_{66}O_{30}$ 6.7 H_2O, M = 1014.9. Orthorhombic, a = 13.975(1), b = 29.162(2), c = 12.965(1) Å, V = 5283.9(6) Å^3; Cu-Kα radiation; space group $P2_12_12_1$, Z = 4; D_x = 1.447 g cm^{-3}. Colourless crystal of approximate dimensions 0.46 × 0.42 × 0.33 mm^3 was sealed in a quartz capillary in equilibrium with its mother liquor; the cell parameters were determined by least squares free refinements of the setting angle of 18 reflections within 16.4° < θ < 27.7°, μ = 11.3 cm^{-1}, T = 291.5 K.

Data Collection.—Intensity data were collected using an Enraf-Nonius CAD4 diffractometer with graphite monochromated Cu-Kα radiation λ = 1.5418 Å. $F(000)$ = 2480. 5634 h, k, l intensities were collected with index range of h: 0–17, k: 0–35, l, 0–16 by θ/θ scan mode to θ_{max} = 70°. Absorption correction was applied: I_{max}/I_{min} = 1.35 and μ = 11.3 cm^{-1}. Five standard reflections (701, 269, 2211, 222, 522) showed a decrease of intensity of 16.3%; a linear decay correction was applied.

Structure Analysis and Refinement.—The structure was solved by molecular replacement method[30] with a hydrated α-CD (form III)[5] as a model and refined on F with the full matrix least squares technique using the SHELX program.[31] The positions of glucosidic hydrogen atoms were first predicted geometrically, then every hydrogen atomic coordinates and a common thermal isotropic parameter were refined. Positional and anisotropic thermal parameters were refined for all non-H atoms. The function minimized was $\Sigma w(F_o - F_c)^2$ with $w = 1/[\sigma^2(F) + g(F)^2]$ and a final g value of: 0.001307. Min and max heights in the final $\Delta\rho$ were −0.570 and 0.599 e A^{-3}. The values of R and wR were R = 0.0736 and wR = 0.0823 (max shift/e.s.d. = 0.9) for 5183 reflections with $I > 3\sigma(I)$. All calculations were performed on a VAX 3200 computer. The final atomic coordinates and equivalent isotropic thermal parameters of all non hydrogen atoms have been deposited at the Cambridge Crystallographic Data Centre.*

NMR Study.—NMR spectra were obtained on either an AC 300, AM 300 or an AM 400 Bruker spectrometer, all equipped with a process controller, an Aspect 3000 computer and a variable temperature system. Samples of CD-3OMe were dissolved in D_2O, [$^2H_6$]DMSO and [$^2H_5$]pyridine (1% solution for ^1H, 2% solution for ^{13}C). Reference values of the shifts were taken from standard tables.

^1H and ^{13}C assignments were made with 2D-COSY and 2D ^{13}C–^1H correlation spectroscopy respectively.

COSY spectra were recorded using a 2048 × 512 data matrix and processed with zero-filling in the F_1 dimension. The spectral width was 900 Hz in both dimensions. A sinebell window function was used prior to Fourier transformation. The ^1H–^{13}C shift correlation spectra were recorded using a 2048 × 256 data matrix and were zero filled in the F_1 dimension. Spectral widths were 5000 Hz in F_2 and 900 Hz in F_1. Gaussian multiplication was performed prior to Fourier transformation.

Selective 2D-J heteronuclear experiments[24,25] were run to measure $^3J_{^{13}C,^1H}$ coupling constants. To minimize pulse imperfection, EXORCYCLE phase cycling was used for ^{13}C pulses. The ^1H 180 pulse was created using a DANTE sequence.[32] 4K Data points were used in the F_2 dimension. After Fourier transformation in the F_2 dimension, only the slices corresponding to ^{13}C lines of interest were treated in the F_1 dimension in which no apodization function was used.

Phase-sensitive NOESY[33] was acquired with a mixing time of 0.5 s. A 20 ms random delay was introduced to cancel scalar correlation effects. A total of 512 × 1K data matrix was used and zero-filled to 1K × 1K. Prior to Fourier transformation sine-squared weighing functions were applied in both directions. NOESY crosspeak intensities were evaluated by integration after symmetrization of the matrix.

Acknowledgements

We wish to thank Dr. A. Grand (*DRFMC, CEN-Grenoble*) for preliminary X-ray investigations and Dr. S. Cottaz (*CERMAV*) for the synthesis of the modified cyclodextrin. We are grateful to Dr. W. Warnock (*CERMAV*) for careful review of this manuscript.

* For details of the CCDC deposition scheme, see 'Instructions for Authors,' *J. Chem. Soc., Perkin Trans. 2*, 1993, issue 1.

References

1 C. L. Lawson, J. Bergsma, P. M. Bruinenberg, G. de Vries, L. Dijkhuizen and B. W. Dijkstra, *J. Mol. Biol.*, 1990, **214**, 807.
2 C. Klein, J. Hollender, H. Bender and G. Schulz, *Biochemistry*, 1992, **31**, 8740.
3 M. Kubota, B. Mikami, Y. Tsujisaka and Y. Moreta, *J. Biochem. (Tokyo)*, 1988, **104**, 12.
4 S. Cottaz, C. Apparu and H. Driguez, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2235.
5 K. K. Chacko and W. Saenger, *J. Am. Chem. Soc.*, 1981, **103**, 1708.
6 B. Klar, B. Hingerty and W. Saenger, *Acta Crystallogr., Sect B*, 1980, **36**, 1154.
7 K. Lindner and W. Saenger, *Acta Crystallogr., Sect B*, 1982, **38**, 203.
8 K. Harata, K. Uekama, M. Otagiri and F. Hirayama, *J. Incl. Phenom.*, 1984, **1**, 279.
9 K. Harata, K. Uekama, M. Otagiri and F. Hirayama, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 497.
10 D. W. Griffiths and M. L. Bender, *Adv. Catal.*, 1973, **23**, 209.
11 B. Lesyng and W. Saenger, *Biochem. Biophys. Acta*, 1981, **678**, 408.
12 W. Saenger, *Isr. J. Chem.*, 1985, **25**, 43.
13 W. Saenger, *J. Incl. Phenom.*, 1984, **2**, 445.
14 I. Furo, I. Pocsik, K. Tompa, R. Teeäär and E. Lippmaa, *Carbohydr. Res.*, 1987, **166**, 27.
15 Y. Yamamoto, M. Onda, Y. Takahashi, Y. Inoue and R. Chûjô, *Carbohydr. Res.*, 1987, **170**, 229.
16 Y. Inoue, T. Okuda and R. Chûjô, *Carbohydr. Res.*, 1985, **141**, 179.
17 A. de Bruyn, *J. Carbohydr. Chem.*, 1991, **10**, 159.
18 R. U. Lemieux, T. L. Nagabhustan and B. Paul, *Can. J. Chem.*, 1972, **50**, 773.
19 J. A. Schwarcz and A. S. Perlin, *Can. J. Chem.*, 1972, **50**, 3667.
20 K. Bock and C. Pedersen, *Acta Chem. Scand., Ser. B*, 1977, **31**, 354.
21 D. Y. Gagnaire, R. Nardin, F. R. Taravel and M. R. Vignon, *Nouv. J. Chim.*, 1977, **1**, 423.
22 B. Mulloy, T. A. Frenkiel and D. B. Davies, *Carbohydr. Res.*, 1988, **184**, 39.
23 I. Tvaroska, M. Hricovini and E. Petrakova, *Carbohydr. Res.*, 1989, **189**, 359.
24 A. Bax and R. Freeman, *J. Am. Chem. Soc.*, 1982, **104**, 1099.
25 C. Morat, F. R. Taravel and M. R. Vignon, *Carbohydr. Res.*, 1987, **163**, 265.
26 G. E. Martin and A. Zektzer, *Magn. Reson. Chem.*, 1988, **26**, 631.
27 J. J. Titman, D. Neuhaus and J. Keeler, *J. Magn. Reson.*, 1989, **85**, 111.
28 M. Ochs and S. Berger, *Magn. Reson. Chem.*, 1990, **28**, 994.
29 L. Poppe and H. van Halbeek, *J. Magn. Reson.*, 1991, **93**, 214.
30 P. M. D. Fitzgerald, *J. Appl. Cryst.*, 1988, **21**, 273.
31 G. M. Sheldrick, SHELX-76, Program for Crystal Structure Determinations, University of Cambridge, 1976.
32 G. Bodenhausen, R. Freeman and G. A. Morris, *J. Magn. Reson.*, 1976, **23**, 171.
33 G. Bodenhausen, H. Kogler and R. R. Ernst, *J. Magn. Reson.*, 1984, **58**, 370.

Paper 3/01748F
Received 26*th March* 1993
Accepted 24*th May* 1993