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NUCLEAR OVERHAUSER EFFECTS FOR METHYL β-MALTOSIDE AND THE CONFORMATIONAL STATES OF MALTOSE IN AQUEOUS SOLUTION

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ABSTRACT

A nuclear Overhauser enhancement in methyl β-maltoside, resulting from pre-irradiation of H-1′ of the non-reducing glucose residue, has been measured and calculated theoretically. Comparison of these data reveals a complicated conformational equilibrium in aqueous solutions of maltose derivatives.

INTRODUCTION

We now report ^{1}H- and ^{13}C-n.m.r. data and theoretical studies of conformational states for methyl β-maltoside in aqueous solution. The $^{3}J_{C,H}$ values for maltose and its derivatives have been determined[1], whereas data on n.O.e. are lacking (some preliminary data have been reported[2]). Comparison of the observed and calculated data allows the conformational equilibrium in aqueous solutions of maltose to be assessed; we have carried out similar studies on α-cellobiose 1-phosphate[3].

RESULTS AND DISCUSSION

The different configurations of the anomeric centres of the glucose residues of methyl β-maltoside result in resolution of the ^{1}H-n.m.r. spectrum which is sufficient for the determination of the n.O.e.'s arising by pre-irradiation of H-1′. The key signals in the ^{1}H-n.m.r. spectrum were assigned using selective homonuclear resonance in both usual and difference versions (Fig. 1, Table I). The assignments of the signals agreed with those for maltose[4,5]. Pre-irradiation of H-1′ led to enhancement of the signals for H-2′ of the non-reducing unit and H-4 and H-3 of the reducing unit (Table II), with the n.O.e. for H-4 being 6 times as great as that for H-3. Overlapping of the signals for H-5 and H-2′ precluded determination of the n.O.e. for H-5.

The vicinal coupling constants for the fragments H-1′–C-1′–O–C-4 and C-1′–O–C-4–H-4 of methyl β-maltoside were also determined. The conformation of these fragments is determined by the rotation angles ϕ (C-1′–O-1′) and ψ

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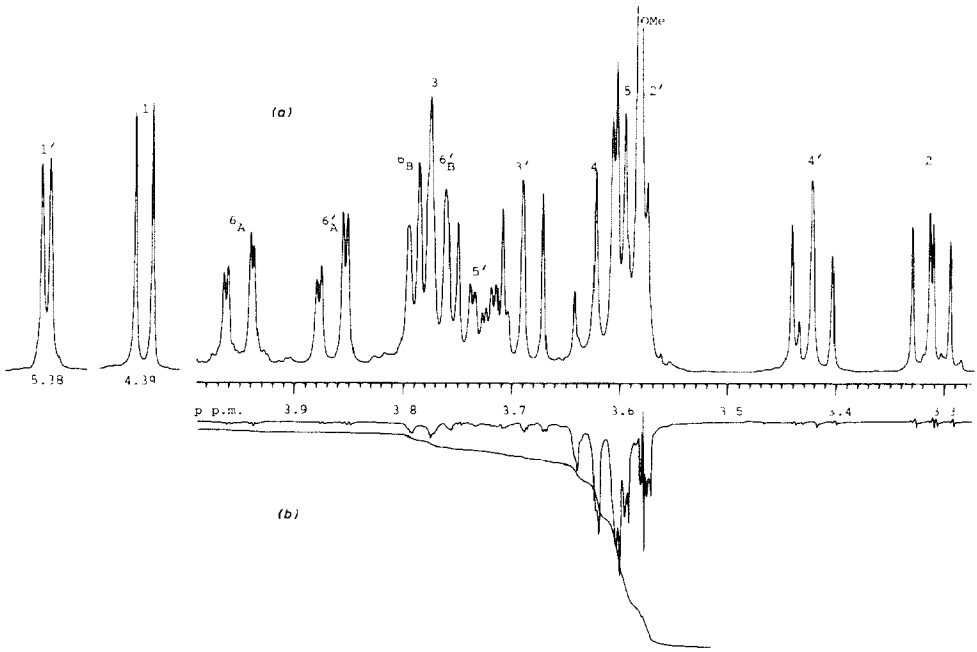

Fig. 1. 500-MHz ^{1}H-n.m.r. spectrum of methyl β-maltoside (*a*), and n.O.e.'s on pre-irradiation of H-1′ (*b*).

TABLE I

^{1}H-N.M.R. DATA FOR METHYL β-MALTOSIDE

Proton	*Chemical shift*		*Visible multiplicity*		*Coupling constant*			
	β-D-Glc	*α-D-Glc*	*β-D-Glc*	*α-D-Glc*	*β-D-Glc*		*α-D-Glc*	
H-1	4.39	5.38	d	d	$J_{1,2}$	7.6	$J_{1,2}$	3.7
H-2	3.31	3.59	dd	dd	$J_{2,3}$	9.2	$J_{2,3}$	9.6
H-3	3.78	3.69	t	dd	$J_{3,4}$	9.2	$J_{3,4}$	8.7
H-4	3.62	3.43		dd	$J_{4,5}$	9.2	$J_{4,5}$	9.6
H-5	3.59	3.72		ddd	$J_{5,6A}$	2.0	$J_{5,6A}$	2.0
H-6A	3.95	3.87	dd	dd	$J_{5,6B}$	5.0	$J_{5,6B}$	5.0
H-6B	3.78	3.77	dd	dd	$J_{6A,6B}$	12.2	$J_{6A,6B}$	12.0
CH_3	3.58							

TABLE II

OBSERVED N.O.E.'S ON PRE-IRRADIATION OF H-1′, AND $^3J_{C,H}$ FOR METHYL β-MALTOSIDE

Observed proton	*N.O.e. (%)*	*Coupling constant*	*Value (Hz)*
H-3	1.0	$^3J_{H\text{-}1'\text{-}C\text{-}1'\text{-}O\text{-}C\text{-}4}$ (J^{ϕ})	3.5 ±0.3
H-4	5.9	$^3J_{C\text{-}1'\text{-}O\text{-}C\text{-}4\text{-}H\text{-}4}$ (J^{ψ})	3.9 ±0.2
H-2′	7.9		

(O-1′–C-4)*. The corresponding $^3J_{C,H}$ ($^3J_{C\text{-}4,H\text{-}1'}$ or J^{ϕ}, and $^3J_{C\text{-}1',H\text{-}4}$ or J^{ψ}) values were similar, namely, 3.5 ±0.3 and 3.8 ±0.2 Hz, respectively (Table II). J^{ψ} was determined directly from the gated-decoupling spectrum since, for C-1′, there were no other large couplings with protons. J^{ϕ} was determined by the difference in separation between the extreme lines in the multiplet components of the signal for C-4 in the usual gated-decoupled spectrum, on the one hand, and in that obtained on pre-irradiation of H-1′ at the instant of data acquisition. Perlin and co-workers[1] reported J^{ϕ} ~3.5 and J^{ψ} ~3.5 Hz for maltose, and J^{ϕ} ~3.0 and J^{ψ} ~2.5 Hz for methyl β-maltoside, and Gidley and Bociek[6] reported J^{ϕ} ~4.3 and J^{ψ} ~5.5 Hz for maltoheptaose.

In calculating the conformations of maltose, account was taken of non-bonded interactions estimated using the functions of Scott and Scheraga[7]. The torsional barrier for the rotation around the glycosidic bonds was taken[8] as 0.9 kcal/mol, and other energy increments, *e.g.*, intramolecular hydrogen-bonding, the *exo*-anomeric effect, and electrostatic interactions, were neglected because, in aqueous solution, these factors are of no significance[3,9,10].

Conformational analysis for maltose was performed with two sets of data on the spatial structure of the glucose residues. In one analysis, the neutron diffraction data for methyl α-D-glucopyranoside[11] were taken for the non-reducing unit, and the X-ray data for methyl β-D-glucopyranoside[12] were taken for the reducing unit, *i.e.*, the calculation was performed for methyl β-maltoside. In the second analysis for methyl α-maltoside, performed with a view to determining the sensitivity of the final results to the geometry of the units (*cf.* ref. 13), the structural data[11] for both units were used. The glycosidic bond angle was assigned[14] a value of 117° and the C–H bond lengths were taken as 1.1 Å. The hydroxymethyl groups at C-5 were fixed in the gt orientation established in structural studies of methyl glycosides[11,12].

The character of the conformational map for maltose is sensitive to the position of the hydroxymethyl group of the reducing unit. Of two feasible orientations for the hydroxymethyl group[15], namely, gt and gg, the conformational freedom of the disaccharide unit was notably higher for gt. Thus, the molecule can be restricted to the predominant orientation of the hydroxymethyl group since there were no n.O.e.'s for H-6A,6B (Table I).

For a given conformation, the n.O.e. for a proton *d* on pre-irradiation of a proton *s* (f_s^d) was calculated using the formula by Shirmer and co-workers[16], taking into account the influence of neighbouring protons on the n.O.e. for the proton *d*. Consideration of the whole potential surface for the disaccharide, together with the Boltzmann probabilities of the conformers, allowed the mean n.O.e. values, $\langle f_s^d \rangle$.$\langle J^{\phi} \rangle$ and $\langle J^{\psi} \rangle$, for the given ϕ and ψ to be determined from the experimental dependence[17] of $^3J_{C,H}$ on the rotation angles.

In order to analyse the conformational equilibrium of methyl β-maltoside in

*ϕ is zero for the *cis* orientation of C-1′–H-1′ and O-1′–C-4; ψ is zero for the *cis* orientation of C-1′–O-1′ and C-4–H-4.

aqueous solution in more detail, use was made of the data on the linkage optical rotation Λ, which is +46° for the disaccharide[18]. The mean value $\langle\Lambda\rangle$ can be calculated using the equation of Rees[19], which relates Λ to the rotation angles ϕ and ψ.

Four local minima corresponding to the conformers A–D (Table III) were found on the conformational map ϕ–ψ for methyl β-maltoside (Fig. 4 of ref. 9). Conformer A with ϕ −70° and ψ −40° proved to be energetically the most favourable. However, the contribution of conformer A, as estimated from the statistical sum of the appropriate region of the conformational map, amounted to only 40%. Conformer B (ϕ −30°, ψ −20°), despite being energetically less favourable, had greater statistical weight (45%) due to its entropy predominance. This conclusion followed from a comparison of the areas inside the 1 kcal/mol contour lines for the regions A and B of the conformational map. Conformer C, which has an almost *trans* orientation of the aglycon, appeared to be unlikely even when only non-bonded interactions were taken into account, its statistical weight being <12%. Hence, there is no need to consider the *exo*-anomeric effect[20] in empirical calculations using the approach outlined in this paper and in ref. 9. Conformer D (ψ −160°) has non-bonded interactions between the hydroxymethyl group at C-5 and O-2′, in addition to the H-1′/H-3 and H-1′/H-5 interactions, and was virtually prohibited for maltose. In contrast, the cellobiose conformer with ψ 180° occurs in aqueous solution[3].

Thus, the conformers A and B are the most feasible spatial forms of maltose. Conformer A has a typical folded form involving the van der Waals contacts of H-3′ and H-5′ of the non-reducing unit with H-4 and H-6,6 (Fig. 2). The presence of the structure stabilised by hydrophobic interactions in aqueous solutions of maltose was first reported by Neel and Goring[21] and by Rees[19]. It is noteworthy

TABLE III

TORSIONAL ANGLES (°), ENERGIES U (KCAL/MOL), N O.E `S (%), COUPLING CONSTANTS (Hz), AND LINKAGE ROTATION (°) FOR OPTIMAL CONFORMATIONS OF METHYL β-MALTOSIDE

Parameter	*Conformer*			
	A	*B*	*C*	*D*
ϕ,ψ	−70, −40	−30, −20	20, 20	−30, −160
U	−4.0	−3.5	−3.0	−2 0
$f^{H\text{-}3}_{H\text{-}1'}$	6	0	−1	32
$f^{H\text{-}3}_{H\text{-}1'}$	5	22	21	−1
$f^{H\text{-}5}_{H\text{-}1'}$	0	−1	0	29
$f^{H\text{-}6,H\text{-}6}_{H\text{-}1'}$	0	0	0	0
J^{ϕ}	1.0	4.3	4.8	4.3
J^{ψ}	3.5	4.8	4.8	5.0
Λ	+85	−4	−187	−4
Statistical weights (%)[a]	40	45	12	3

[a]Calculated from the free energies of the conformers.

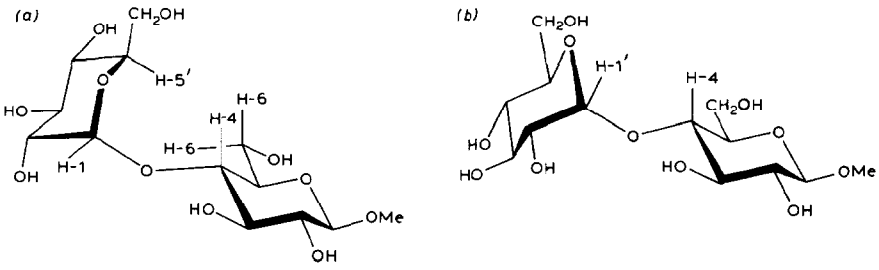

Fig. 2. Molecular models of optimal conformations of methyl β-maltoside with rotation angles ϕ,ψ −70, −40° (*a*), and −30, −20° (*b*).

that rotation angles ϕ and ψ close to those in the conformer A were found in 6-iodophenyl α-maltoside[22] (ϕ −52°, ψ −34°). The extended conformer B (Fig. 2), with H-1′ and H-4 being in close proximity (2.2 Å), is closer to the structures of maltose and its derivatives found in X-ray studies[23,24].

Four local minima were found for methyl α-maltoside, but with somewhat different energy relationships (Table IV). Conformer A had the lowest energy, and its statistical weight increased up to 60% (Table IV). At the same time, the statistical weight of conformer B decreased to 30%, and the probabilities for conformers C and D proved to be low (7 and 3%, respectively). Thus, the results calculated for maltose were dependent on the spatial structure of the monosaccharide units. This relates mainly to conformer A, for which even small displacements of atoms of the two units may lead to energy differences because of short van der Waals contacts (*cf.* the relative energies for the conformers A and B in Tables III and IV).

The calculated values of n.O.e., J^{ϕ}, J^{ψ}, and Λ are given in Table V. For qualitative considerations, the same data for the 4 optimal conformations of methyl β-maltoside are presented in Table III. Since measurements of n.O.e. depend on the experimental conditions[16], the analysis involves relative n.O.e.'s.

In the optimal conformer A, H-1′ was located ~3 Å from both H-4 and H-3. Hence, the calculated $f_{H\text{-}1'}^{H\text{-}4}$ and $f_{H\text{-}1'}^{H\text{-}3}$ were similar (5 and 6%, respectively; Table III). For conformer B, H-1′ was 2.2 Å from H-4; hence, there should be a

TABLE IV

CONFORMATIONAL DISTRIBUTION FOR METHYL α-MALTOSIDE[a]

Parameter	*Conformer*			
	A	*B*	*C*	*D*
ϕ,ψ	−70, −40	−20, −20	20, 30	−30, −160
U	−4.2	−3.1	−2.4	−2.6
Statistical weight (%)	60	30	7	3

[a]See footnote in Table III.

TABLE V

AVERAGE VALUES OF ROTATION ANGLES ϕ,ψ (°), N.O.E.'S (%), J^{ϕ}, J^{ψ} (Hz), AND Λ (°) FOR TWO CALCULATIONS OF MALTOSE DERIVATIVES

Parameter	*Methyl β-maltoside*	*Methyl α-maltoside*
$\langle\phi\rangle$,$\langle\psi\rangle$	+36, −20	−50, −38
$\langle f_{H\text{-}1'}^{H\text{-}3}\rangle$	2.5 (1.0)[a]	6.5 (1.0)
$\langle f_{H\text{-}1'}^{H\text{-}4}\rangle$	17 (6.8)	10.0 (1.5)
$\langle f_{H\text{-}1'}^{H\text{-}5}\rangle$	0.6 (0.2)	0.7 (0.1)
$\langle f_{H\text{-}1'}^{H\text{-}2}\rangle$	19.5 (7.8)	20.0 (3.1)
$\langle J^{\phi}\rangle$	3.5	2.5
$\langle J^{\psi}\rangle$	4.3	4.0
$\langle\Lambda\rangle$	+3	+39

[a]Relative n.O.e.'s in brackets.

considerable n.O.e. only on H-4 ($f_{H\text{-}1'}^{H\text{-}4}$, 22%). The same considerations apply to conformer C. In contrast, for conformer D, $f_{H\text{-}1'}^{H\text{-}4}$ vanished, whereas $f_{H\text{-}1'}^{H\text{-}3}$ and $f_{H\text{-}1'}^{H\text{-}5}$ were large (30%). In all the conformers, $f_{H\text{-}1'}^{H\text{-}6,H\text{-}6}$ was zero.

According to the experimental data, the largest n.O.e. was observed for H-4, which was 6 times as great as that on H-3 (Table II). Thus, the conformers B or C must be present in aqueous solutions of methyl β-maltoside (*cf.* the data in Table III). In order to decide which conformer preponderated, the data on linkage rotation Λ was used. Since Λ observed for solutions of maltose and its derivatives was positive[19], whereas the Λ values for conformers C and B are negative (−187° and −4°, respectively; Table III), conformer B was preponderant. This conclusion agreed with the calculated data, from which it followed that the statistical weight of conformer B was 4 times as great as that for conformer C (Table III).

Since the H-3 signal was enhanced (Table II), it followed that both conformers A and D may contribute to the equilibrium (Table III). However, further considerations showed that the n.O.e. on H-3 was determined mainly by conformer A. This inference followed from the fact that A was the only conformer having a positive linkage rotation (+85°, Table III), and the observed Λ for methyl β-maltoside[18] was +46°. Hence, the contribution of conformer A to the overall equilibrium is essential. Further, H-1′ and H-3 in conformer A were too remote (3 Å) for mutual disturbance of their electronic states, whereas, in conformer D, such a disturbance was quite possible because H-1′ was only 2 Å from H-3. Such "*γ-gauche*" interactions of protons usually lead to additional upfield shifts of the signals of the carbons carrying such protons[25]. Thus, for cellobiose, with a contribution[3] of conformer D of 10–15%, the β-effect of glycosidation on C-3 was[26] −1.5 p.p.m. However, for methyl β-maltoside, the β-effect was positive (+0.5 p.p.m.), showing that there was no "*γ-gauche*" interaction of H-1′ and H-3, and, hence, that there was no conformer D in aqueous solution.

Thus, comparison of the calculated and observed $f_{H\text{-}1'}^{H\text{-}4}$ and $f_{H\text{-}1'}^{H\text{-}3}$ for the optimal conformers of methyl β-maltoside (Table III) indicated that there was an

equilibrium in aqueous solution involving conformers A and B. The contribution of these forms was roughly equal since the observed Λ (+46°) was practically the mean of the linkage rotations of conformers A and B (Table III).

The calculated mean n.O.e.'s, as well as J^{ϕ}, J^{ψ}, and Λ, are cited in Table V. The conformational distribution for methyl β-maltoside (40, 45, 12, and 3%, respectively, for conformers A–D) reproduced the observed data on n.O.e.'s quite satisfactorily. The calculated ratio $\langle f^{H\text{-}3}_{H\text{-}1'}\rangle/\langle f^{H\text{-}4}_{H\text{-}1'}\rangle$ was 1:7, whereas that observed was 1:6 (Table II). The calculated coupling constants ($\langle J^{\phi}\rangle$ 3.5, $\langle J^{\psi}\rangle$ 4.3 Hz, Table V) were also close to those observed (~3.5 and ~3.8 Hz, respectively, Table II). The mean $\langle\Lambda\rangle$ value of +3° was an underestimate which indicated that the contribution of conformer A, having a positive rotation, was also underestimated, whereas that of conformer C (large negative Λ) was overestimated (Table III).

In contrast, for methyl α-maltoside, with a conformational distribution for A–D of 60, 30, 7, and 3%, respectively (Table III), the $\langle\Lambda\rangle$ value of +39° (Table V) was equal to the observed linkage rotation[19] for methyl α-maltoside. However, the value of $\langle f^{H\text{-}3}_{H\text{-}1'}\rangle$ was overestimated in this case (Table V).

Thus, the real contribution of the conformers of maltose and its derivatives to the equilibrium in aqueous solution was roughly an average of those predicted by the two calculations, *i.e.*, the statistical weights of A, B, and C + D are 50, 40, and 10%, respectively.

Hence, the analysis of n.O.e. data showed that the structure of maltose cannot be explained by only one conformer with ϕ −30°, ψ −20° (found by the HSEA calculations[20]) as proposed[2,27]. Although this structure leads to a considerable n.O.e. on H-4, it does not account for all of the observed data. Thus, for example, $f^{H\text{-}3}_{H\text{-}1'}$ for these ϕ and ψ values is zero (*cf.* the data for B in Table III), whereas a n.O.e. on H-3 was actually observed (Table II). The corresponding J^{ϕ} and J^{ψ} (4.3 and 4.8 Hz, respectively) exceed the observed values for methyl β-maltoside (Table II). Moreover, this conformation has a negative Λ, whereas the observed value[18] is +46°.

Thus, the conformational equilibrium of maltose and its derivatives in aqueous solution is determined by the whole form of the potential surface of non-bonded interactions for the disaccharide. A similar conclusion has been reached in a study of cellobiose[3].

EXPERIMENTAL

^{1}H-N.m.r. spectra were recorded with a Bruker WM-500 instrument for 3% solutions of the disaccharide in D_2O (internal sodium 4,4-dimethyl-4-silapentane-sulfonate). N.O.e.'s were measured on pre-irradiation of H-1′ within the t.O.e. technique[28]. The relaxation delay D_1 was 0.5 s and the build-up n.O.e. D_2 was 0.8 s. N.O.e.'s were expressed as a ratio of integrated intensities of the observed and presaturated proton in the difference n.O.e. spectrum.

REFERENCES

1 A. PARFONDRY, N. CYR, AND A. S. PERLIN, *Carbohydr. Res.*, 59 (1977) 299–309.
2 E. ALVARADO, O. HINDSGAUL, R. U. LEMIEUX, K. BOCK, AND H. PEDERSEN, *Abstr. Int. Carbohydr. Symp., XIIth, Utrecht*, 1984, p. 480.
3 G. M. LIPKIND, A. S. SHASHKOV, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 141 (1985) 191–197.
4 G. A. MORRIS AND L. D. HALL, *Can. J. Chem.*, 60 (1982) 2431–2441
5 A. DE BRUYN, M. ANTEUNIS, AND G. VERHEGGE, *Bull Soc. Chim. Belg.*, 84 (1975) 721–734
6 M. J. GIDLEY AND S. M. BOCIEK, *J. Chem. Soc., Chem. Commun.*, (1985) 220–222.
7 R. A. SCOTT AND H. A. SCHERAGA, *J. Chem. Phys.*, 44 (1966) 3054–3068.
8 I. TVAROŠKA AND T. BLEHA, *Tetrahedron Lett.*, (1975) 249–252.
9 G. M. LIPKIND, V. E. VEROVSKY, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 133 (1984) 1–13.
10 G. M. LIPKIND AND N. K. KOCHETKOV, *Bioorg. Khim.*, 10 (1984) 1229–1241.
11 G. A. JEFFREY, R. K. MCMULLAN, AND S. TAKAGI, *Acta Crystallogr., Sect. B*, 33 (1977) 728–737.
12 G. A. JEFFREY AND S. TAKAGI, *Acta Crystallogr., Sect. B*, 33 (1977) 738–742.
13 R. U. LEMIEUX AND K. BOCK, *Arch. Biochem. Biophys.*, 221 (1983) 125–134.
14 S. ARNOTT AND W. E. SCOTT, *J. Chem. Soc., Perkin Trans. 2*, (1972) 324–335.
15 A. DE BRUYN AND M. ANTEUNIS, *Carbohydr. Res.*, 47 (1976) 311–314.
16 R. E. SHIRMER, J. H. NOGGLE, J. P. DAVIS, AND P. A. HART, *J. Am. Chem. Soc.*, 92 (1970) 3266–3273.
17 G. K. HAMER, F. BALZA, N. CYR, AND A. S. PERLIN, *Can. J. Chem.*, 56 (1978) 3109–3116.
18 D. A. REES AND D. THOM, *J. Chem. Soc., Perkin Trans. 2*, (1977) 191–201.
19 D. A. REES, *J. Chem. Soc., B*, (1970) 877–884.
20 H. THØGERSEN, R. U. LEMIEUX, K. BOCK, AND B. MEYER, *Can. J. Chem.*, 60 (1982) 44–57.
21 J. L. NEEL AND D. A. I. GORING, *Can. J. Chem.*, 48 (1970) 3745–3747.
22 I. TANAKA, N. TANAKA, T. ASHIDA, AND M. KAKUDO, *Acta Crystallogr., Sect. B*, 32 (1976) 155–160.
23 S. S. C. CHU AND G. A. JEFFREY, *Acta Crystallogr., Sect. B*, 23 (1967) 1038–1049.
24 C. J. QUIGLEY, A. SARKO, AND R. H. MARCHESSAULT, *J. Am. Chem. Soc*, 92 (1970) 5834–5839.
25 D. M. GRANT AND B. V. CHENEY, *J. Am. Chem. Soc.*, 89 (1967) 5315–5318.
26 A. S. SHASHKOV, A. I. USOV, YU. A. KNIREL, B. A. DMITRIEV, AND N. K. KOCHETKOV, *Bioorg. Khim.*, 7 (1981) 1364–1371.
27 K. BOCK AND H. PEDERSEN, *Carbohydr. Res.*, 132 (1984) 142–149.
28 G. WAGNER AND K. WUTHRICH, *J. Magn. Reson.*, 33 (1979) 675–680.