Solution Conformation of αD(1–3)- and αD(1–6)-Linked Oligomannosides Using Proton Nuclear Magnetic Resonance†

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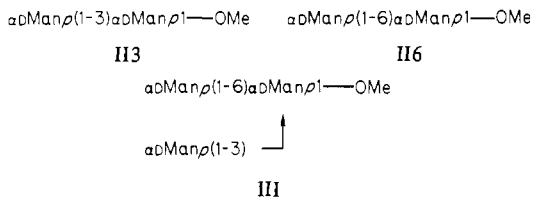
ABSTRACT: The solution conformations of synthetic methyl mannobiosides and a methyl mannotrioside containing αD(1–3) and αD(1–6) linkages have been determined through the use of ^1H nuclear magnetic resonance techniques, namely, the nuclear Overhauser effect and proton relaxation time measurements. $^3J(C,H)$ coupling constants, obtained from a compound enriched with ^{13}C, were also used. The allowed conformations were found to be in agreement with those determined from potential energy calculations and crystal structures. The methyl mannotrioside is an analogue of a mannotriose unit which occurs naturally in the "core" of asparagine-linked glycopeptides and in an "arm" of high mannose N-linked glycopeptides.

In the last 10 years the chemistry and biochemistry of glycoproteins have acquired an importance as great as that of other natural products, such as proteins and nucleic acids. One of the reasons for this surge of interest in these compounds is that glycoproteins are now recognized as playing key roles in biological recognition processes (Hughes & Sharon, 1978; Barondes, 1981). The interactions of N-linked sugars with proteins have been characterized primarily in terms of their primary structure (Goldstein & Hayes, 1978) since little was known about the three-dimensional structure of the carbohydrate chains. In order to gain some insight into the latter problem, we sought to determine the solution conformation of a series of synthetic methyl oligomannosides which are analogues of the trimannosyl core common to N-linked glycoproteins:



III

In a previous report the complete ^1H NMR assignment of the oligomannosides has been presented (Winnik et al., 1982a). Some chemical shift perturbations were noted and interpreted as being caused by interactions arising from the relative orientation of the sugars with respect to each other (J.-R. Brisson et al., unpublished experiments). Partial assignment of the ^1H and ^{13}C NMR spectra of similar compounds had previously been given by Ogawa & Sasajima (1981).

In this paper, ^1H nuclear magnetic resonance parameters which exhibit conformational dependency, namely, nuclear Overhauser enhancements (NOE), relaxation times, and coupling constants were used to determine the three-dimensional structure of these αD(1–3)- and αD(1–6)-linked oligomannosides. $^3J(C,H)$ coupling constants across the glycosidic bond, obtained from the methyl mannotrioside ^{13}C enriched at C1 of the αD(1–6) linkage, were also used in conjunction with the other methods. The allowed conformations of the methyl oligomannosides deduced from these experiments were compared to those determined from potential energy calculations and crystal structures, and the results from all three methods were found to be in excellent agreement.

These techniques have previously been applied to the determination of the three-dimensional structures of other oligosaccharides, namely, ABO and Lewis blood groups by Lemieux et al. (1980), and to lipopolysaccharides by Bock et al. (1982).

Experimental Procedures and Method of Calculation

Methyl-α-D-mannopyranose was obtained from the Sigma (St. Louis, Mo). The other oligomannosides were synthesized in our laboratory (Winnik et al., 1982a,b).

For ^1H NMR, the samples were dissolved in H_2O, passed through a Chelex column (Bio-Rad, Toronto) to remove heavy metals, lyophilized twice from 99.7% D_2O, and dissolved in 99.96% D_2O (Merck Sharp & Dohme, Montreal) under a nitrogen atmosphere. Argon or nitrogen was bubbled through the samples to reduce the presence of dissolved paramagnetic oxygen. Sample concentrations varied from 10 to 50 mM. Acetone was used as an internal chemical shift standard and was set at 2.225 ppm. ^1H NMR experiments were performed at 23 ± 1 °C on a 360-MHz Nicolet spectrometer located at the Toronto Biomedical NMR Centre (University of Toronto). Generally, spectra were accumulated by using 8K data points and a spectral width of ±500 Hz. Spin–lattice relaxation times were measured by the standard inversion–recovery method (Vold et al., 1968) utilizing 12–16 τ values. The T_1 values were determined from a three-parameter fit of the variation of the integrated intensity, or the change in height of the signals, as a function of the τ values (Levy & Peat, 1975). The NOE values were obtained by difference spectroscopy (Richarz & Wuthrich, 1978). Irradiation times and pulse intervals were greater than 5 times the longest T_1 for the signals of the sugar. Usually 512 difference FID's were accumulated and Fourier transformed with an exponential line broadening factor of 1 Hz in order to reduce noise.

The 90-MHz ^{13}C NMR spectra were also recorded at 23 ± 1 °C. The spectrometer conditions were 26 μs for a 90° pulse, 16K data points, and ±4 kHz spectral width. The samples were prepared in 99.7% D_2O at concentrations of 25 mM. The ^{13}C T_1's were measured, under conditions of total proton decoupling, by using the inversion recovery method with nine τ values ranging from 50 ms to 1.5 s and 2048 transients were accumulated per file. An exponential line broadening of 2 Hz was used to reduce noise.

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Atomic coordinates for αDMan*p*1-OMe were generated from neutron diffraction data (Jeffrey et al., 1977) and those for βDMan from the X-ray crystal structure of the trisaccharide: αDMan*p*(1–3)βDMan*p*(1–4)DGlc*p*NAc (Warin et al., 1979). The C1–O1 distances and the glycosidic bond angles for αD1-OMe and αD(1–3) linkages were chosen from the former and latter crystal structures, respectively. For an αD(1–6) linkage, the C1–O1 distance was fixed at 1.415 Å and the glycosidic bond angle at 111.5° (Arnott & Scott, 1972). The methyl group orientation in a βD1-OMe linkage was taken from Jeffrey & Takagi (1977). Hydrogen atoms in βDMan were oriented at 109.5° with respect to the two adjacent bonds on the pyranose ring, with C–H distances set at 1.10 Å (Arnott & Scott, 1972). Coordinates of the oligosaccharides were calculated from the coordinates of the above monosaccharides (Rees & Skerett, 1970). NOE values were calculated from (Noggle & Schirmer, 1971)

$$R_i f(i,k) + \sum_{j \neq i} \sigma_{ij} f(j,k) = \sigma_{ik} \qquad i \neq k \qquad (1)$$

where $f(i,k)$ = NOE of H_i on saturation of H_k. R_i = total dipolar relaxation rate of H_i, which is given by

$$R_i = \frac{\mu_0^2}{(4\pi)^2} \frac{\gamma^4 \hbar^2}{10} \left[\frac{3\tau_c}{1 + (\omega_0 \tau_c)^2} + \tau_c + \frac{6\tau_c}{1 + (2\omega_0 \tau_c)^2} \right] \sum_{j \neq i} r_{ij}^{-6} + R^s$$

σ_{ij} = cross relaxation rate between H_i and H_j, which is given by

$$\sigma_{ij} = \frac{\mu_0^2}{(4\pi)^2} \frac{\gamma^4 \hbar^2}{10} \left[\frac{6\tau_c}{1 + (2\omega_0 \tau_c)^2} - \tau_c \right] r_{ij}^{-6} \qquad j \neq i$$

where τ_c = isotropic rotational correlation time, r_{ij} = interproton distance, ω_0 = Larmor frequency, R^s = relaxation rate due to other mechanisms, μ_0 = magnetic permeability of free space, γ = proton gyromagnetic ratio, and $\hbar$ = Planck's constant divided by 2π. The simultaneous equations in (1) were solved by Gaussian elimination.

T_1 values were calculated from (Kalk & Berendsen, 1976; Sykes et al., 1978)

$$1/T_1 = R_i + \sum_{j \neq i} \sigma_{ij} \qquad (2)$$

When methyl groups are present, the internal rotation of the methyl group, characterized by a rotational correlation time, τ_r, must be taken into account. In this case, NOE and T_1 values were calculated by using the expressions derived by Heatley et al. (1980) and Woessner et al. (1969).

Steric maps were generated by using the minimum contact distances between nonbonded atoms determined by Ramachandran & Sasisekharen (1968). The hard sphere exoanomeric (HSEA) approach (Lemieux et al., 1980; Thorgersen et al., 1982) was used to calculate the potential energy of the oligosaccharides. This method consists of evaluating the conventional van der Waals interactions between nonbonded atoms (Venkatachalam & Ramachandran, 1967) and an additional term which takes into account the influence of the exoanomeric effect on conformational equilibria.

Molecular modeling, NOE calculations, steric maps, and potential energy calculations were performed on a VAX-11/780 (VMS operating system) located at the Ontario Cancer Institute in Toronto.

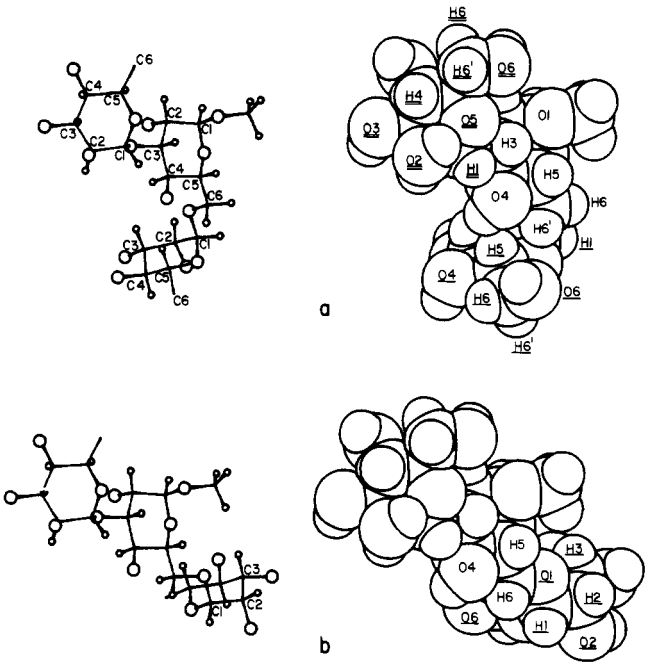

FIGURE 1: Projections of the preferred conformers for the methyl mannotrioside in solution for $\omega = 180°$ (a) and $\omega = -60°$ (b) at the αD(1–6) linkage. For the ball and stick models, the large sphere represents oxygen atoms and the smaller ones hydrogen atoms. The O and H atoms of terminal CH_2OH groups and the H atoms in O–H groups have been omitted for clarity. In the space filling model, selected atoms are labeled. The atoms of the αDMan*p*(1–6) residues are underlined and those for the αDMan*p*(1–3) are doubly underlined. $\omega = -60°$ for the hydroxymethyl group of the terminal mannopyranosyl units. The orientation of the O–H atoms is the same as in the crystal structure of αDMan*p*1-OMe (Jeffrey et al., 1977).

Results and Discussion

NOE measurements are dependent on a variety of experimental conditions: the concentration and nature of the sample, the presence of paramagnetic impurities, partial saturation of a signal, and instrumental conditions. Since the ratio of two NOE's, termed a relative NOE, is less sensitive to all these problems, conformational analysis was carried out in terms of relative NOE's.

Figure 1 depicts the structure of the methyl mannotrioside. The orientation of the glycosidically linked residues is determined by the torsion angles ϕ and ψ. The "linkage conformation" is given by ϕ = (H1,C1,O1,C*X'*) and ψ = (C1,O1,C*X'*,H*X'*), where C*X'* and H*X'* are the aglyconic atoms. For a (1–6) linkage ψ = (C1,O1,C6,C5) and ω, the torsion angle of the hydroxymethyl group, is defined as ω = (O6,C6,C5,H5) (Lemieux & Koto, 1974; Tvaroska et al., 1978). From the atomic coordinates generated from the solid state and the theory described above, NOE and T_1 values were calculated as a function of these torsion angles. When the computed values were compared with the observed values, the linkage conformation was determined (section C). However, since NOE and T_1 measurements are dependent on τ_c, the tumbling times of the molecules had to be determined (section A). In section B, the NOE and T_1 data for the methyl mannosides were analyzed to establish the validity of the methods and assumptions used to determine the oligomannoside conformations in section C.

(A) Determination of Rotational Correlation Times. The tumbling times of αDMan*p*1-OMe and the methyl mannotrioside were deduced from ^{13}C T_1 values (Doddrell et al., 1972). βDMan*p*1-OMe was assumed to have the same tumbling time as αDMan*p*1-OMe. The ring carbon atoms of

Table I: NOE and T_1 Values[a] for βDMan*p*1-OMe and αDMan*p*1-OMe

	H1	H2	H3	H4	H5	H6	H6′	HMe
				βDMan*p*1-OMe				
T_1 (s)								
obsd	1.1	1.9	1.6	2.9	1.3	0.70	0.70	1.4
calcd	1.2	2.3	1.6	3.2	1.3	0.65	0.68	1.4
absolute NOE, H1 saturated								
obsd	−1	0.15	0.08	0.02	0.15			0.05
calcd	−1	0.18	0.06	0.00	0.14			0.03
relative NOE, H2 saturated								
obsd	1	−14	1.6	0.3[b]	−0.6[b]	*b*	0.4[b]	
calcd	1	−10	1.5	0.1	−0.3		0.0	
relative NOE, H5 saturated								
obsd	1	−0.2	0.6	0.3	−8.3	0.3	0.06	
calcd	1	−0.3	0.9	0.4	−7.0	0.5	−0.09	
				αDMan*p*1-OMe				
T_1 (s)								
obsd	2.5	2.3	1.9	2.3[c]	2.3[c]	0.70	0.70	1.3
calcd	2.4	2.4	2.2	2.8	1.7	0.65	0.66	1.3
absolute NOE, H1 saturated								
obsd	−1	0.13						0.03
calcd	−1	0.17						0.03
relative NOE, H1 saturated								
obsd	1			0.38				−19
calcd	1			0.34				−13

[a] The average errors for T_1 and absolute NOE values are ±10% and ±20%, respectively. The NOE's were quantitated by setting the intensity of the saturated resonance for one proton in the NOE difference spectra at −1. For a relative NOE, the intensity of the reference is set at 1. The parameters involved in the calculations were $(\phi,\psi) = (50°,60°)$ for βDMan*p*1-OMe, $(\phi,\psi) = (-50°,40°)$ for αDMan*p*1-OMe, $\omega = -60°$, $\tau_c = 0.5 \times 10^{-10}$ s, $\tau_r = 1 \times 10^{-13}$ s, and $R^s = 0.03$ s^{-1} (see the text). [b] The contribution from partial saturation of H6′ has not been taken into account. [c] Strongly coupled signals.

αDMan*p*1-OMe and the mannotrioside had ^{13}C T_1 values of 1.1 ± 0.1 and 0.34 ± 0.05 s, respectively, which correspond to tumbling times of (0.50 ± 0.05) × 10^{-10} and (1.5 ± 0.2) × 10^{-10} s. From these values of τ_c, a solvent relaxation rate (R^s in eq 2) of 0.03 s^{-1} was then needed to account for the ^1H T_1 values in Tables I and II. Those ^1H T_1 values which were susceptible to variations in ϕ and ψ were not used to estimate R^s.

The tumbling time of the mannobiosides could not be evaluated from ^{13}C T_1 due to insufficient quantities of material. Instead they were estimated from ^1H T_1 measurements by selecting a value which gave the best fit to the T_1 data in Table II. The ^1H T_1 values for protons in the vicinity of the glycosidic linkage, which were found to be highly dependent on the linkage conformation, were not used in the estimation of τ_c. The resulting values were (1.2 ± 0.1) × 10^{-10} and (1.1 ± 0.1) × 10^{-10} s, respectively, for the mannobiosides II3 and II6.

From the ^{13}C T_1 of the methyl group in αDMan*p*1-OMe (1.8 ± 0.1 s), τ_r was found to be (5 ± 1) × 10^{-12} s (Rowan & Sykes, 1975). However, from the ^1H T_1 values of H1 and the methyl group (Tables I and II) a value of $\tau_r = 1 \times 10^{-13}$ s was found to give the best fit to the data, for any orientation of the methyl group. This discrepancy might arise from the absence, in both treatments, of the possible additional motion of the methyl group about the C1–O1 bond.

The CH_2OH group is also expected to have some degree of motion about the C5–C6 bond (Gagnaire et al., 1973). Indeed, the NT_1 values for the C_6 of the unsubstituted hydroxymethyl groups of the monosaccharide and trisaccharide were longer than those of the ring carbon atoms. However, when the CH_2OH was substituted, the C6 had the same NT_1 value as the ring carbon atoms, indicating that a C6 substitution decreased the rate of motion about the C5–C6 bond. In both cases, the rotation of the hydroxymethyl group did not affect the agreement between observed and predicted T_1 values for H6 and H6′ (Tables I and II).

Table II: T_1 Values for the Oligomannosides[a]

compd	residue[b]					
II3	M^M	signal	H1	H2	HMe	
		T_1(obsd) (s)	1.1	0.81	0.69	
		T_1(calcd) (s)	1.2	0.83	0.67	
	M^3	signal	H1	H2		
		T_1(obsd) (s)	1.0	1.2		
		T_1(calcd) (s)	0.96	1.2		
II6	M^M	signal	H1	H2	HMe	
		T_1(obsd) (s)	1.3	1.3	0.78	
		T_1(calcd) (s)	1.3	1.3	0.70	
	M^6	signal	H1	H3	H4	H6
		T_1(obsd) (s)	1.0	1.1	1.4	0.40
		T_1(calcd) (s)	1.0	1.2	1.5	0.34
III	M^M	signal	H1	H2	H6′	HMe
		T_1(obsd) (s)	1.1	0.72	0.26	0.65
		T_1(calcd) (s)	1.1	0.73	0.23	0.59
	M^3	signal	H1	H2		
		T_1(obsd) (s)	0.85	1.1		
		T_1(calcd) (s)	0.85	1.1		
	M^6	signal	H1	H2		
		T_1(obsd) (s)	0.99	0.96		
		T_1(calcd) (s)	0.92	1.1		

[a] The average error for the T_1 values is ±10%. For the calculations $(\phi,\psi) = (-50°,40°)$ for αD1-OMe, $(-45°,-15°)$ for αD(1–3), $(-60°,-150°)$ for αD(1–6) of II6, $(-60°,180°)$ for αD(1–6) of III. For all the residues $\omega = -60°$. The values of τ_c used for II3, II6, and III were 1.2 × 10^{-10}, 1.1 × 10^{-10}, and 1.5 × 10^{-10} s, respectively. $\tau_r = 1.0 \times 10^{-13}$ s and $R^s = 0.03$ s^{-1}. [b] See Figure 2 for nomenclature.

(*B*) *Monosaccharide Models.* The pyranose rings in these oligomannosides were assumed to have the same geometry as in the crystal state, and they were also assumed to be rigid in solution. These assumptions are justified since the magnitudes of the proton vicinal coupling constants observed for these compounds (Winnik et al., 1982a) were consistent with the existence of a normal $^4C_1$ chair conformation as observed for the crystal structures of oligomannosides. To further establish the validity of these assumptions and the methods used to deduce the three-dimensional structure of these compounds, the NOE and T_1 data for βDMan*p*1-OMe and αDMan*p*1-OMe were analyzed.

In Table I, the NOE and T_1 data for the monosaccharides are compared to the computed values by using interproton distances derived from their crystal structures. Since the NOE and T_1 values are dependent on r^{-6}, as well as being highly dependent on the geometry of the ring, they will also be affected by small variations in internuclear distances. For example, a change in torsion angle of 6° about the pyranose ring bonds (which is frequently observed upon comparison of crystal structures for different pyranose rings) can lead to changes in interproton distances of 0.05 Å, or 2% for r = 2.5 Å. Such a variation would alter the NOE and T_1 values by 12%. Since any particular proton may relax through interactions with more than one other proton, even greater differences in the computed values of the NOE or T_1, in some cases as great as 20 or 30%, may occur. Since the agreement between the observed and calculated values is within such a range, it can be concluded that the internuclear distances in solution and the solid state

must agree to within approximately 0.05 Å.

Another indication that the geometry of the ring is similar in solution to that of the solid state was the observation of a negative NOE on H2 on saturation of H5 in βDMan*p*1-OMe. The converse effect was observed upon saturation of H2 (Table I). These negative NOE's are due to the "three spin effect" (Noggle & Schirmer, 1971). They are highly dependent on the relative proximity of the protons involved. As can be observed in Table I, the computed NOE's accounted for these effects, supporting the validity of the methods that will be used to deduce the conformation of the oligosaccharides.

The computed NOE between H1 and H4 of βDMan*p*1-OMe is 0.00; however, a value of 0.02 is observed. This NOE probably arises because the transitions of H3 and H4 are strongly coupled ($J/\delta = 0.38$) and H3 experiences a strong NOE (Rowan & Sykes, 1975). Another indication of strong coupling effects is the presence of different T_1's for each component in the multiplet pattern of a proton (Rowan & Sykes, 1975; Vold & Vold, 1978). For example, the low-field doublet of the H3 multiplet of βDMan*p*1-OMe had a T_1 of 1.5 s and the high-field doublet had a T_1 of 1.7 s. The average of 1.6 s is given in Table I. Thus, for sugars, where strong coupling is the rule rather than the exception, strong coupling effects must be taken into account (section C).

For mannopyranosides in solution the hydroxymethyl group can exist in two stable conformations corresponding to $\omega = 180°$ and $\omega = -60°$, since the $\omega = 60°$ rotamer is unfavored (Marchessault & Perez, 1979; Gagnaire et al., 1973; De Bruyn & Anteunis, 1976). The spectral parameters will thus reflect the population distribution between these rotamers. For $\omega = 180°$, equal NOE's are expected on H6 and H6′ on saturation of H5, since H6 and H6′ are equidistant from H5. For $\omega = -60°$, however, a bigger NOE is expected on H6 (Figure 1a). Since, in βDMan*p*1-OMe, the NOE on H6 is 5 times that observed on H6′ (Table I), the rotamer corresponding to $\omega = -60°$ is favored in solution. Similarly, for $\omega = 180°$, both methylene protons are remote from H4 and the H4 of T_1 is calculated to be 4.9 s, while for $\omega = -60°$, the H4 is close to H6′ (Figure 1a) and H4 thus receives a significant relaxation contribution from H6′ (predicted $T_1 = 3.2$ s). Since the observed T_1 of H4 was found to be 2.9 ± 0.2 s, the rotamer with $\omega = -60°$ must be the most highly populated. Finally, for $\omega = 180°$, $J_{5,6'}$ should be 1–2 Hz, while for $\omega = -60°$, $J_{5,6'}$ should be near 8 Hz (J.-R. Brisson and J. P. Carver, unpublished experiments), yet the observed value of this coupling constant is 6 Hz. Thus, the $\omega = 180°$ rotamer must be present, although as a minor population. Because the preferred rotamer in solution is $\omega = -60°$, this value was used for unsubstituted hydroxymethyl groups in all the calculations quoted below. However, the calculations were also carried out with $\omega = 180°$ in order to determine the possible influence of the presence of this rotamer. For the oligomannosides the orientation of an unsubstituted hydroxymethyl group had no major effect on the results deduced below.

(*C*) *Oligomannoside Conformations.* The linkage conformation for two contiguous residues was determined from the ratio of interresidue to intraresidue NOE measurements and from those proton T_1 values which are sensitive to variations in ϕ and ψ. For each observed parameter, contour maps were generated which displayed the angles for which the theoretical value fell within the error bounds of the observed values. Thus, a series of bands were generated whose intersection represented the ϕ and ψ angles consistent with all the experimental observations.

The computed NOE and T_1 values are based on the assumption that the molecules are rigid. However, for sugars, some degree of motion is expected about the glycosidic and the C5–C6 bonds. The observed values will then depend on the population distribution of the conformers in solution (Noggle & Schirmer, 1971). Thus, the linkage conformation that is deduced from these measurements will reflect the weighted average of these conformers. To determine whether the conformation deduced from NOE and T_1 values represented averaging about a single stable conformer or averaging between several conformers distributed over a wide range of (ϕ,ψ) values, the experimentally deduced conformation was compared to the one observed in the solid state for a similar linkage. Agreement between the solution and solid state conformations can only imply that in solution a stable conformer is preferred and that the internal motion about the glycosidic bond is restricted to a narrow range about this conformer. Similar comparisons can be made with the results of potential energy calculations. Although barrier heights and population distribution among conformers usually are not reliable because of the neglect of solvent interactions, potential energy calculations generally give an accurate prediction of the energetically favored conformations. If the solution conformation deduced from NMR methods coincides with one of the latter, it is reasonable to conclude that this is the major conformer present in solution. Motion about such a linkage would then be expected, once again, to be confined to a narrow range about that conformer (Lemieux et al., 1980; Bock et al., 1982; Hayes et al., 1982).

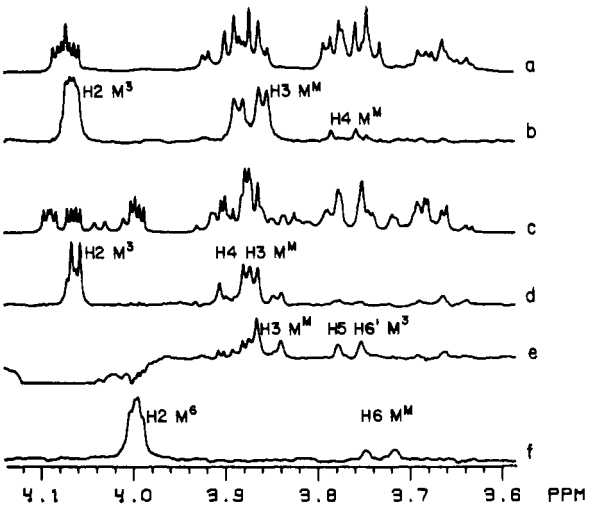

FIGURE 2: Spectra for a methyl mannobioside and a methyl mannotrioside. (a) Partial ^{1}H NMR spectrum of the αD(1–3)-linked methyl mannobioside, II3; the H1 and methyl resonances are not shown. (b) NOE difference spectrum for saturation of H1 M^3 in II3. (c) Partial ^{1}H NMR spectrum for the methyl mannotrioside. The other spectra are NOE difference spectra for the methyl mannotrioside: (d) saturation of H1 M^3, (e) saturation of H2 M^M and H2 M^3, and (f) saturation of H1 M^6. These spectra do not have the same vertical scale. The NOE's are labeled and quantitated in Table III. In the text, M^M refers to the αDMan*p*1-OMe residue, M^3 to the αDMan*p*-(1–3) residue, and M^6 to the αDMan*p*(1–6) residue.

The NOE values which were used to determine the linkage conformation between mannose residues are shown in Figure 2 and Table III. Since the complete assignment for the oligomannosides had previously been determined (Winnik et al., 1982a), all the signals which experienced a NOE could be assigned without any ambiguity.

αD(*1–3*) *Linkage.* Upon saturation of H1 M^3 (see Figure 2 for nomenclature), a large interresidue NOE is observed on H3 M^M and a smaller one on H4 M^M (Figure 2b). The small (1%) NOE on H4 M^M might arise from strong coupling ef-

Table III: NOE Values Used To Determine (ϕ,ψ) for αD(1-3) and αD(1-6) Linkages[a]

saturated resonance[b]		compd	resi-due	detected reso-nance	absolute NOE, obsd	relative NOE obsd	relative NOE calcd
H1	M^3	II3	M^3	H2	0.11	1.0	1.0
			M^M	H2	0.0	0.0	−0.1
				H3	0.11	1.0	1.0
				H4	0.01	0.1	0.04
H1	M^3	III	M^3	H2	0.09	1.0	1.0
			M^M	H2	0.0	0.0	−0.1
				H3-H4	0.10	1.1	1.0
H2	M^M	II3	M^M	H1	0.065	1.0	1.0
		III		H3	0.06	0.9	0.74
		c	M^3	H5	0.04	0.6	0.76
H1	M^6	III	M^6	H2	0.10	1.0	1.0
			M^M	H6	0.035	0.35	0.32
				H6′	0.00	0.00	0.00
H1	M^6	II6	M^6	H2	0.11	1.0	1.0
			M^M	H6	0.068	0.60	0.50
				H6′	0.00	0.00	−0.1

[a] The estimated errors are the same as in Table I. The parameters used in these calculations are the same as in Table II. [b] See Figure 2 for nomenclature. [c] Average values of the two compounds.

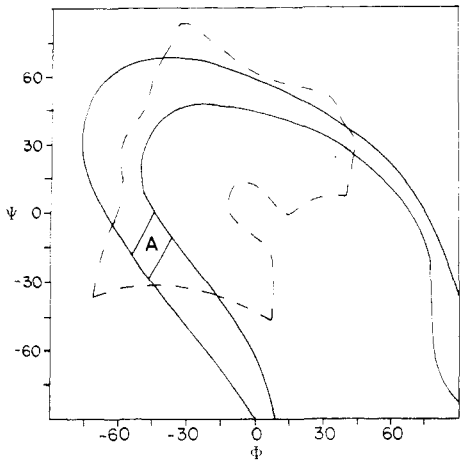

FIGURE 3: Contour map showing the values of (ϕ,ψ) for which the computed NOE or T_1 values are within error bounds of the observed values for an αD(1-3) linkage (Tables II and III). The band which spans the complete range of ϕ and ψ represents the relative NOE (H3 M^M/H2 M^3) of 1.0 ± 30% on saturation of H1 M^3 in II3. Area A represents the allowed (ϕ,ψ) values resulting from the additional constraints: the relative NOE (H5 M^3/H1 M^M) of 0.5 ± 30% on saturation of H2 M^M in II3 and III, the T_1 of H1 M^3 in III of 0.85 s ± 20% and the T_1 of H2 in III of 0.72 s ± 20%. The area enclosed by the dashed line represents the values of ϕ and ψ for the αD(1-3) linkage for which the mannose residues in αDMan*p*(1-3)αDMan*p*1-OMe are not sterically hindered.

fects, as discussed earlier for βDMan*p*1-OMe. For the methyl mannotrioside (Figure 3d) equal enhancements are now observed on H3 M^M and H4 M^M due to stronger coupling between H3 and H4 (Rowan & Sykes, 1975). However, in Table III, the sums of the NOE values for H3 and H4 are similar for the two compounds, indicating that the linkage conformations are the same.

The contour map shown in Figure 3 displays the (ϕ,ψ) angles for which the computed NOE on H3 M^M (H1 M^3 saturated) fell within the error bounds of the observed value. A steric map, which depicts the region where the two residues are not sterically hindered, it superimposed. As can be observed, there is still a considerable range of (ϕ,ψ) values which satisfy both these constraints. However, the computed T_1 values for H1 M^3 and H2 M^M were found to be highly sensitive to conformational changes. Thus, the T_1 values for H1 M^3

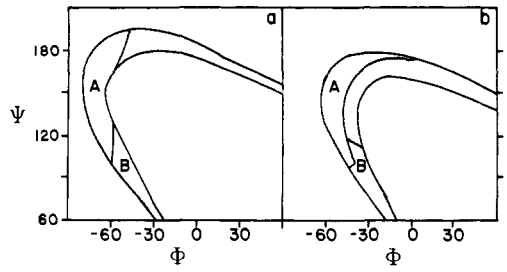

FIGURE 4: Contour map showing the values of ϕ and ψ for which the computed NOE and T_1 values are within error bounds of the observed values for an αD(1-6) linkage (Tables II and III). The band in (a) and (b) which spans the complete range of ϕ and ψ represents the relative NOE (H6 M^M/H2 M^6) of 0.35 ± 30% and 0.60 ± 30% in II6 and III, respectively, on saturation of H1 M^6. The intersection of that band in (a) and (b) with the band generated from the T_1 of H1 M^6 of 0.99 s ± 20% and 1.0 s ± 20%, respectively, is also shown. "A" represents the area where ω = −60°, and "A" and "B" the area where ω = 180° and 60°.

(0.85 s) and H2 M^M (0.72 s) of III (Table II) were used to generate additional contour maps. An error bound of ±20% was used to take into account the uncertainty in τ_c and in the measurements themselves. In Figure 3, the intersection of these three constraints (region "A") restricted the allowed (ϕ,ψ) values to a narrow range centered on ϕ = −45° and ψ = −15° with a width of 10–15°.

For this linkage conformation, H5 M^3 is found to be within 2.5 Å of H2 M^M (Figure 1a). In Figure 2e, it can be seen that an NOE exists on the complex signal arising from the strongly coupled H5 and H6′ resonances of M^3. This additional constraint did not restrict further the allowed (ϕ,ψ) values since the contour map generated from this NOE satisfied the bounds imposed from the previous measurements. However, the agreement between these four constraints suggests that motional averaging is confined to a narrow range about one stable conformation.

The computed NOE and T_1 values for an αD(1-3) linkage with torsion angles (−45°,−15°) are shown in Tables II and III. As can be seen, they account for the observed measurements, within experimental error, except for the relative NOE on H2 M^M when H1 M^3 is saturated. An absolute NOE of −0.01 is expected and no enhancement is observed. Since the expected NOE is so small, its absence could easily arise from slight differences between the solution and crystal ring geometries or slight conformational averaging about the glycosidic bonds.

Further evidence supporting the conclusion that a stable conformer exists in solution can be obtained from an examination of the the potential energy surface computed for the αD(1-3) linkage of II3. From HSEA calculations a predominant minimum is found near (−50°,−10°) and a shallower minimum, 0.4 kcal/mol above the latter, is found near (−40°,30°). Using semiempirical potential energy functions, Biswas and Rao (1981) found a predominant conformer for the same linkage near (−45°,−20°). Furthermore, in a crystal structure containing a αDMan*p*(1-3)βDMan unit, (ϕ,ψ) were found to be (−58°,−19°) (Warin et al., 1979). These observations strongly suggest that the structure determined from NMR parameters adequately describes the solution conformation of a terminal αD(1-3)-linked mannopyranosyl unit in these oligomannosides.

αD(1-6) Linkage. On saturation of H1 M^6 the only hydroxymethyl proton to experience an NOE was H6 M^M (Figure 2f). The calculated T_1 values for H1 M^6 (Table II) were also sensitive to the linkage conformation. The contour maps generated from both of these values are shown in Figure 4a for the methyl mannotrioside and in Figure 4b for the

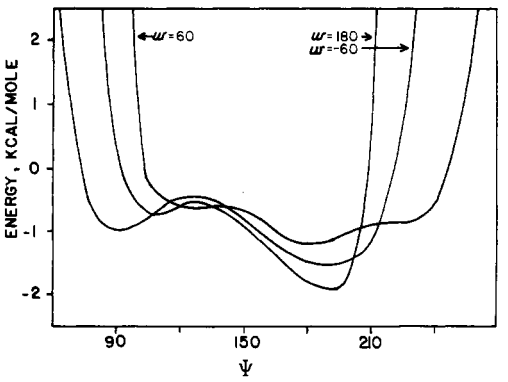

FIGURE 5: Potential energy in kilocalories per mole vs. the torsion angle ψ for an αD-Man$p$(1–6)αD-Man$p$1-OMe. The curves shown are for $\phi = -50°$ and $\omega = 180°$, $-60°$, and 60°, at which minima occur in the potential energy surface.

methyl mannobioside II6. The value of ϕ is restricted to −50 ± 20°, which is consistent with the exoanomeric effect (Lemieux et al., 1980; Thogersen et al., 1982). In contrast, a wide range of ψ values (90–200°) satisfy the conditions imposed by these two measurements. HSEA calculations (Figure 5) for the αD-Man$p$(1–6)Man unit with $\phi = -50°$ show little variation in the predicted potential energy over a similar range of ψ values. Thus, on the basis of the above results, the possibility of conformational averaging over this range of values cannot be eliminated.

However, in the methyl mannotrioside, ψ can be determined from the coupling constants ^3J(C,H) across the αD(1–6) glycosidic bond (Gagnaire et al., 1977; Hamer et al., 1978; Hayes et al., 1982) measured from a compound 90% enriched with ^{13}C at the C1 position of the M^6 residue. In a partially relaxed spectrum, the hydroxymethyl protons of the substituted residue could be clearly observed and ^3J(C,H) was estimated to be 1.0 ± 0.5 Hz for H6 M^M and 2.5 ± 0.1 Hz for H6′ M^M. These coupling constants are susceptible to variations of ψ and are expected to reflect the average orientation about the C6–O1 bond since their magnitude depends on the dihedral angle between the carbon atom and the protons experiencing the coupling. From the observed relationship between ^3J(C,H) and the dihedral angle for C–O–C–H linkages (Hamer et al., 1978), J(C1,H6) is expected to be greater than or equal to J(C1,H6′) for ψ values from 90° to 180°. Since the reverse is in fact observed, conformational averaging must be restricted to values of $\psi = 190 \pm 20°$ which corresponds to the shallow minimum in the potential energy curve. With $\psi = 190 \pm 20°$, the values of ϕ that are within the area of allowed (ϕ,ψ) angles in Figure 4a are from −50° to −70°. Within this range, the linkage conformation (−60°,180°) was found to give the best fit to the T_1 and NOE values for the methyl mannotrioside (Tables II and III).

For the methyl mannobioside II6, the area of allowed (ϕ,ψ) angles has shifted toward smaller values of ψ (Figure 4b), and a linkage conformation of (−60°,150°) accounts for the observed T_1 and NOE values (Tables II and III). Unfortunately, this change in the value of ψ could not be confirmed from the coupling constant, ^3J(C,H), across the glycosidic bond because the H6 M^M and H6′ M^M resonances were too severely broadened as a result of strong and virtual coupling. It would appear, therefore, that the linkage conformation about the αD(1–6) bond depends on the overall structure of the molecule. The reason for this difference between these two compounds is unclear. The presence or absence of the terminal αD(1–3)-linked mannopyranosyl unit in the structure had no effect on the shape of the conformational energy curve for an αD-(1–6) linkage shown in Figure 5. However, from an analysis of the chemical shifts of these compounds (J.-R. Brisson et al., unpublished experiments), it has been suggested that a reorientation of the C4–OH group, which is located between the αD(1–3) and the αD(1–6) linkages in the methyl mannotrioside, could be the cause. Such effects are not accounted for in the HSEA calculations.

The linkage conformations obtained above agree with the potential energy calculations for αD(1–6)-linked glycans carried out by Tvaroska et al. (1978). Of the many possible conformations for these structures, one corresponded to (ϕ,ψ) = (−60°,180°), in agreement with the above values. These authors also surveyed several crystal structures of oligosaccharides having the same linkage. They found ϕ values ranging from −36° to −61° and ψ values ranging from 144° to 190°. However, in contrast with the above results, in a crystal of dextran containing αD-Glc$p$(1–6) linkages, (ϕ,ψ) values near (−55°,70°) were found (Guizard, 1981). The latter torsional angles are incompatible with the results reported above and can only be interpreted as demonstrating that the stabilization of αD(1–6)-linked glucans in the solid state must differ significantly from that of oligomannosides in solution.

The value of the torsion angle ω could not be determined from the measurements presented in Tables II and III, because they were not sensitive to variation in ω for (ϕ,ψ) near (−50°,180°) (Figure 4). However, for the methyl mannotrioside, the values for $J_{5,6}$ and $J_{5,6'}$ (1.8 and 4.5 Hz, respectively) suggest that the $\omega = 180°$ and $\omega = -60°$ rotamers have similar probabilities of occurrence at the αD(1–6) linkage. The rate of interconversion between these conformers must be much slower than the overall tumbling rate of the molecule because the analysis of the ^{13}C T_1 measurements was consistent with a single correlation time.

Conclusion

The above data suggest that in solution the methyl mannotrioside exists as two preferred conformers with conformational variability occurring mainly at the C5–C6 bond of the αD(1–6) linkage. Projections for these two conformers are shown in Figure 1. The methyl mannotrioside is a linear structure for $\omega = -60°$ and a bent structure for $\omega = 180°$ at the αD(1–6) linkage.

The methyl oligomannoside is an analogue of the trisaccharide present in the core of Asn-linked glycopeptides (Montreuil, 1980). An understanding of the conformational properties of the mannotriose unit is important since the different classes of N-linked glycopeptides are characterized by the pattern of substitution on this core. Also in the recently determined biosynthetic pathway for these structures, a very specific sequential chain of enzymatic addition and removal of sugars on the core mannotrioside is responsible for determining the class of N-linked glycoproteins that are eventually synthesized (Kornfeld & Tabas, 1978; Harpaz & Schachter, 1980). The sequential action of these enzymes may well be specified by the three-dimensional structures of the substrates.

The results obtained with the synthetic methyl mannotrioside have been used in interpreting NOE experiments on a variety of higher molecular weight glycopeptides of natural origin and in estimating the effects of various substitutions on the conformational properties of the core mannotrioside (J.-R. Brisson and J. P. Carver, unpublished experimets). These studies have revealed alterations in the distribution of rotamers about the αD(1–6) bond in different classes of glycopeptides while substitution on the αD(1–3) arm has little effect on their conformation. As has been suggested by Montreuil (1980),

this double character of rigidity and flexibility may be central to the biological role of these structures.

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Registry No. βDMan*p*1-OMe, 22277-65-2; αDMan*p*1-OMe, 617-04-9; II3, 72028-62-7; II6, 78962-39-7; III, 68601-74-1.

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