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CRYSTAL AND MOLECULAR STRUCTURE OF METHYL *O*-α-D-MANNOPYRANOSYL-(1→2)-α-D-MANNOPYRANOSIDE

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ABSTRACT

The crystal and molecular structure of a synthetic mannosyl disaccharide, methyl *O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside, has been determined from X-ray diffractometer data by direct methods by use of the MULTAN programs. The crystals are monoclinic, space group $P2_1$ with unit cell dimensions, *a* 8.086(1), *b* 9.775(1), *c* 9.975(2) Å, β 104.58(1)°, *Z* 2, and D_m 1.54 g/cm³. The structure was refined to an *R*-value of 0.033 for 1359 reflections measured with Cu*Kα* radiation. The mannopyranose units have the chair conformations $^4C_1$(D) with C-5′ and C-2′ deviating from the best plane through the other four atoms of the ring by −0.68 and +0.53 Å in the nonreducing group, and C-3 and O-5 deviating from the mean plane through the other four atoms by +0.57 and −0.66 Å, respectively, in the "potentially" reducing residue. The ring-to-ring conformation can be described as (ϕ,ψ) = (−64.5, 105.5°). The conformation across the C-5–C-6 bond is *gauche–gauche* in both the sugars. The crystal structure is stabilized by a network of intermolecular O–H · · · O hydrogen bonds.

INTRODUCTION

Phosphorylation of the D-mannose units of lysosomal enzymes is essential for the transport and uptake of these enzymes to lysosomes[2,3]. The GlcNAc-*P*-transferase enzyme plays a major role in this process. In order to evaluate the role of the carbohydrate portion of lysosomal enzymes in the phosphorylation reaction, the acceptor specificity of Glc-NAc-*P*-transferases from rat liver microsomes and fibroblasts has been studied with the help of synthetic D-mannose disaccharides[4,5]. Four methyl D-mannobiosides have been studied, *i.e.*, α-D-Man*p*-(1→2)-, α-D-Man*p*-(1→3)-, α-D-Man*p*-(1→4)-, and α-D-Man*p*-(1→6)-α-D-Man*p*OMe. Among

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these four, α-D-Man*p*-(1→2)-α-D-Man*p*-OMe was found to be the best acceptor, whereas reducing α-D-Man*p*-(1→2)-D-Man was not as efficient, the Glc-NAc-*P*-transferase activity of both liver and normal fibroblast being less than half for the latter compound. The crystal structure study of these substrates are in progress in order to evaluate the acceptor specifications, and we report herein the crystal structure of methyl *O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside.

EXPERIMENTAL

Crystals were grown by slow evaporation of a water–methanol solution. A crystal of dimensions 0.5 × 0.1 × 0.05 mm was used for data collection on a CAD-4 diffractometer. Unit cell parameters were obtained from measurements of 25 reflections with θ in the range from 10 to 30°. The crystal data are given in Table I. Complete three dimensional data were measured with a $\omega/2\theta$ scan. Scan widths were calculated with the equation (A + B tan θ), where A and B had values of 0.60 and 0.14, respectively. Aperture widths were calculated with the equation (3.0 + 0.12 tan θ). The maximum time spent on a reflection measurement was 100 s, and faster scans were used for strong reflections. Background was measured for half the scan time. Orientation of the crystal was monitored after measurement of 100

TABLE I

CRYSTAL DATA FOR METHYL-α-D-MANNOPYRANOSYL-(1→2)-α-D-MANNOPYRANOSIDE

Formula	$C_{13}O_{11}H_{24}$
Mol. wt.	356.3
Z	2
Space group	$P2_1$
Cell dimensions (Å)	
a	8.086(1)
b	9.775(1)
c	9.975(2)
β	104.58(1)°
V (Å^3)	763.7
D_{obs}	1.54 g.cc^{-1}
D_{calc}	1.549 g.cc^{-1}
μ(Cu$K\alpha$)	11.3 cm^{-1}
CAD-4 data	1725 reflections ($1359 \geq 3\sigma$)
Function minimized in Full-matrix least squares	$w = (\|Fo\| - (1/k)\|Fc\|)^2$ where $w = 4\|Fo\|2/\sigma^2(\|Fo\|)^2$ $\sigma^2(\|Fo\|^2 = [\sigma^2(I) + p^2I^2]/LP\,p = 0.05\sigma(I)$, $\sigma(I)$ standard deviation of intensity I based on counting statistics and K is scale factor
S (goodness of fit)	2.16
Refined extinction parameter	$8.58 \cdot 10^{-6}$
Final $\|\Delta\rho\|$	0.14 e/Å^3
Final R factor	0.033
Weighted R factor	0.038

reflections. The intensities of three reflections were measured after every hour of X-ray exposure, and the variation in intensity during the entire data collection was <0.1%. Three reflections with χ ~90° were chosen and their intensities were measured for all values of ϕ from 0° to 360° in steps of 10°, and the resultant curve of transmission was used to correct for absorption effect. The maximum and minimum transmission was 0.99 and 0.80, respectively, with an average value of 0.91. The data were corrected for Lorentz and polarization effects; 1725 unique reflections were measured, out of which 1359 had intensities $\geq 3\sigma$. All the calculations were made on a PDP 11/34 instrument with the Enraf–Nonius package of programs for structure determination[6].

RESULTS AND DISCUSSION

Structure determination. — The structure was solved by the direct method[7]. A set of 216E values ($|E| \geq 1.4$) was used with the MULTAN program to establish the phase relationship. Of the possible 28 E maps, the map corresponding to the largest "combined figure of merit" of 2.983 revealed all the non-hydrogen atoms in the disaccharide molecule. These atomic coordinates were subjected to several cycles of least-squares refinements. At the end of the isotropic refinement, the *R* factor was 0.188. Further refinements were carried out with anisotropic temperature factors and the *R* factor was refined to 0.076. A difference-electron-density map computed at this stage revealed all the hydrogen atoms in the molecule. Further refinements were carried out with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms. The final *R* factor was 0.033 for the 1359 reflections with $I \geq 3\sigma$. The weighted *R* factor was 0.038 and the e.s.d. of an observation of unit weight was 2.16. In the final cycles of refinement, the maximum shift/e.s.d. was 0.16 and the average shift/e.s.d. was 0.03. The final difference-electron-density synthesis showed maximum and minimum electron densities of 0.14 and −0.11 e/Å^3, respectively.

Table II lists the positional parameters of all the atoms and their B_{eq} values. The anisotropic thermal vibration parameters and a Table of observed and calculated structure factors have been deposited*.

Molecular geometry. — The bond distances and angles in the molecule are given in Fig. 1, and the torsion angles in Table III. The average C–C bond length is 1.519(3) Å and the average C–O bond length 1.426(3) Å; endocyclic C-5–O-5 bonds are 1.430(3) and 1.448(3) Å. The anomeric bond length (C-1–O-1 to the methyl group) of 1.403 Å is in the normal range for an equatorial linkage[8]. The average internal C–C–C ring angle value is 111.3° (range 109.4–113.7°).

*The vibrational parameters V_{ij} of the heavy atoms and a list of Fo and Fc structure factors are deposited with, and can be obtained from: Elsevier Science Publishers, B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/403/*Carbohydr. Res.*, 186 (1989) 167–175.

TABLE II

FINAL POSITIONAL PARAMETERS[a]

Atom	*X*	*Y*	*Z*	B($Å^2$)
C-1	0.9811(4)	0.3113(4)	0.9865(3)	1.99(5)
C-2	0.8492(4)	0.2155(3)	1.0210(3)	1.78(5)
C-3	0.7162(4)	0.1788(3)	0.8888(3)	1.82(5)
C-4	0.6376(4)	0.3059(3)	0.8109(3)	1.93(5)
C-5	0.7711(4)	0.4115(3)	0.7968(3)	1.99(5)
C-6	0.6929(5)	0.5519(4)	0.7598(4)	2.97(7)
C-8	1.1992(4)	0.3140(4)	0.8683(3)	2.91(7)
C-1′	0.8100(3)	0.2410(4)	1.2481(3)	1.75(5)
C-2′	0.6897(4)	0.3062(3)	1.3237(3)	1.96(5)
C-3′	0.7221(4)	0.4591(3)	1.3427(3)	1.93(5)
C-4′	0.9089(4)	0.4858(3)	1.4121(3)	1.86(5)
C-5′	1.0218(4)	0.4165(3)	1.3319(3)	1.86(5)
C-6′	1.2104(4)	0.4175(4)	1.4057(3)	2.43(6)
O-2	0.7608(2)	0.2837(3)	1.1094(2)	1.96(4)
O-3	0.5890(3)	0.0898(3)	0.9167(2)	2.79(5)
O-4	0.5465(3)	0.2647†	0.6748(2)	2.33(4)
O-5	0.9025(3)	0.4318(2)	0.9212(2)	2.13(4)
O-6	0.6383(4)	0.6076(3)	0.8751(3)	3.72(6)
O-8	1.0661(2)	0.2375(3)	0.9041(2)	2.21(4)
O-2′	0.7232(3)	0.2371(3)	1.4541(2)	2.67(4)
O-3′	0.6151(3)	0.5111(3)	1.4234(2)	2.73(4)
O-4′	0.9363(3)	0.6303(3)	1.4104(2)	2.56(4)
O-5′	0.9823(2)	0.2724(3)	1.3114(2)	1.86(4)
O-6′	1.2416(3)	0.3617(3)	1.5417(3)	3.20(5)
H(C-1)	1.048(4)	0.343(4)	1.057(3)	1.8(7)[b]
H(C-2)	0.909(4)	0.133(4)	1.064(3)	0.0(6)[b]
H(C-3)	0.782(4)	0.133(4)	0.834(4)	1.0(6)[b]
H(C-4)	0.548(6)	0.352(6)	0.850(5)	5(1)[b]
H(C-5)	0.830(5)	0.384(5)	0.736(4)	4(1)[b]
H-1–C-8	1.170(7)	0.395(7)	0.830(5)	6(1)[b]
H-2–C-8	1.258(6)	0.256(6)	0.823(5)	5(1)[b]
H-3–C-8	1.30(1)	0.35(1)	0.981(9)	13(3)[b]
H–C-1′	0.800(4)	0.144(5)	1.254(3)	2.3(8)[b]
H–C-2′	0.559(5)	0.291(5)	1.263(3)	2.6(8)[b]
H–C-3′	0.697(4)	0.498(5)	1.255(4)	2.4(8)[b]
H–C-4′	0.932(4)	0.446(4)	0.506(3)	1.3(6)[b]
H–C-5′	1.020(4)	0.464(4)	1.246(3)	1.2(6)[b]
H-1–C-6	1.289(4)	0.364(3)	1.352(3)	0.3(5)[b]
H-2–C-6	1.250(4)	0.524(4)	1.403(3)	1.6(7)[b]
H–O-3	0.561(7)	0.146(9)	0.991(6)	7(2)[b]
H–O-4	0.422(7)	0.309(8)	0.623(5)	6(1)[b]
H–O-6	0.724(5)	0.601(5)	0.937(4)	3.2(9)[b]
H–O-2′	0.653(9)	0.27(1)	1.481(7)	10(2)[b]
H–O-3′	0.563(7)	0.592(7)	1.387(5)	6(1)[b]
H–O-4′	1.032(8)	0.632(8)	1.473(6)	7(2)[b]
H–O-6′	1.156(9)	0.258(9)	1.486(6)	8(2)[b]
H-1–C-6′	0.414(4)	0.048(4)	0.311(3)	2.1(7)[b]
H-2–C-6′	0.212(7)	0.118(8)	0.246(5)	7(2)[b]

[a]Estimated standard deviation in parentheses. [b]Data refined isotropically. Anisotropically refined data are given in the form of the isotropic equivalent displacement parameter defined as: $(4/3)*[A2*B(1,1) + B2*(2,2) + C2*B(3,3) + AB(\cos\gamma)*B(1,2) + AC(\cos\beta)*B(1,3) + BC(\cos\alpha)*B(2,3)]$.
†This atom was kept fixed for origin definition.

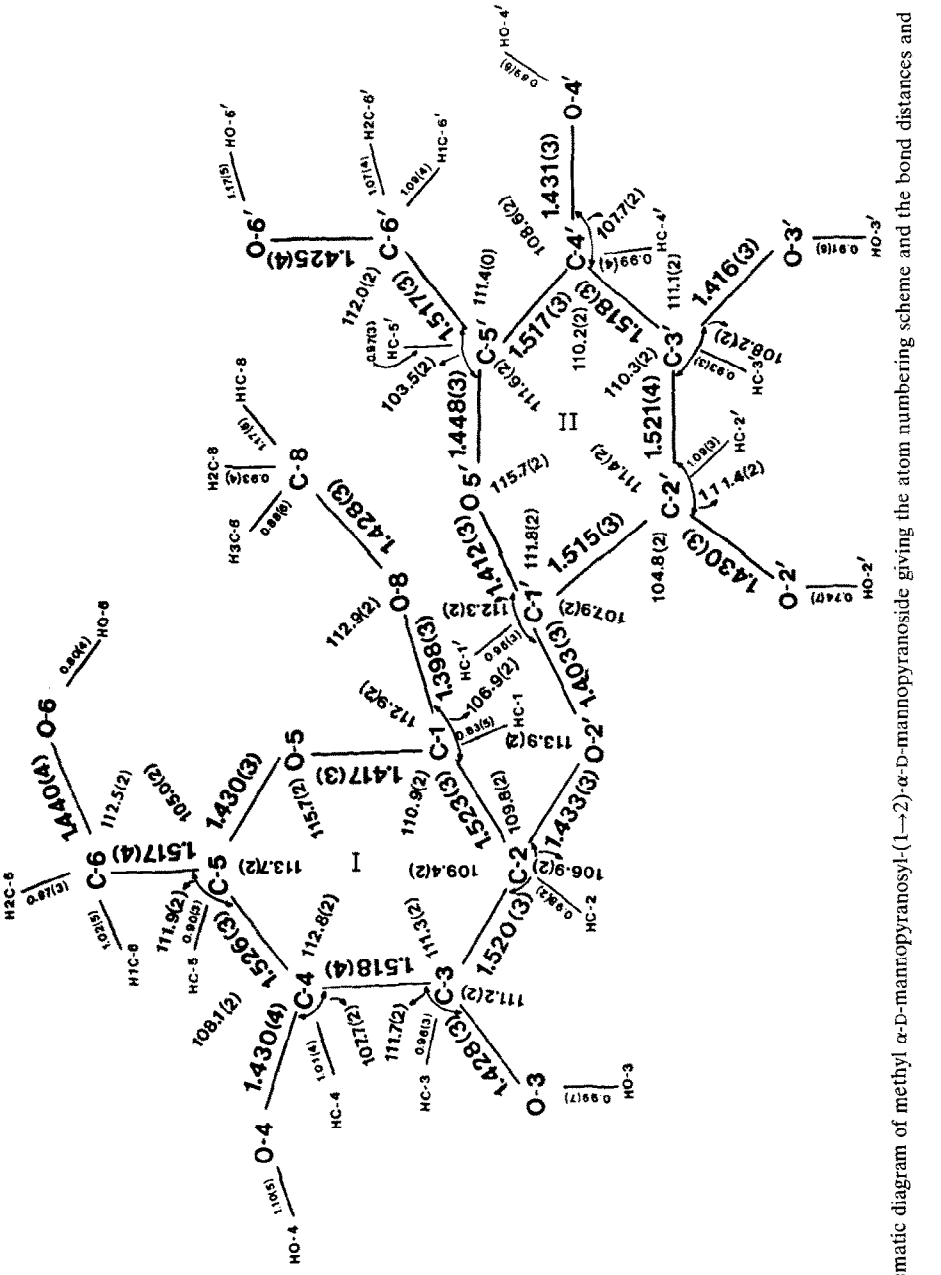

Fig. 1. A schematic diagram of methyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside giving the atom numbering scheme and the bond distances and angles.

TABLE III

TORSION ANGLES[a]

Angle		*Angle*	
O-5–C-1–C-2–C-3	−58.7(4)	O-5′–C-1′–C-2′–C-3′	−52.6(4)
C-1–C-2–C-3–C-4	54.2(2)	C-1′–C-2′–C-3′–C-4′	53.7(5)
C-2–C-3–C-4–C-5	−47.3(5)	C-2′–C-3′–C-4′–C-5′	−54.0(5)
C-3–C-4–C-5–O-5	43.8(4)	C-3′–C-4′–C-5′–O-5′	53.5(4)
C-4–C-5–O-5–C-1	−49.7(4)	C-4′–C-5′–O-5′–C-1′	−54.7(5)
C-5–O-5–C-1–C-2	57.4(4)	C-5′–O-5′–C-1′–C-2′	53.8(4)
C-1–O-5–C-5–C-6	−169.8(4)	C-1′–O-5′–C-5′–C-6′	−177.7(5)
C-3–C-4–C-5–C-6	162.3(5)	C-3′–C-4′–C-5′–C-6	170.4(5)
C-4–C-5–C-6–O-6	−52.9(5)	C-4′–C-5′–C-6′–O-6′	−53.9(5)
O-5–C-5–C-6–O-6	70.1(5)	O-5′–C-5′–C-6′–O-6′	67.5(4)
C-2–O-2–C-1′–O-5′	−64.5(5)°	C-5′–C-4′–O-4′–H-O4′	79(6)
C-1–C-2–O-2′–C-1′	105.5(4)	C-5′–C-6′–O-6′–H-O6′	−52(4)
Angles involving hydrogen atoms			
C-2–C-3–O-3–H-O3	−53(5)	C-1′–C-2′–O-2′–H-O2′	175(8)°
C-4–C-3–O-3–H-O3	72(6)	C-3′–C-2′–O-2′–H-O2′	−65(9)
C-3–C-4–O-4–H-O4	−140(5)	C-2′–C-3′–O-3′–H-O3′	136(4)
C-5–C-4–O-4–H-O4	98(6)	C-4′–C-3′–O-3′–H-O3′	−103(5)
C-5–C-6–O-6–H-O6	−64(4)	C-3′–C-4′–O-4′–H-O4′	−161(6)

[a]In degrees. The Creemer and Pople parameters Q and θ are 0.52 Å and 10.69°, respectively, in the primed residue, and 0.54 Å and 3.81°, respectively, in the unprimed residue.

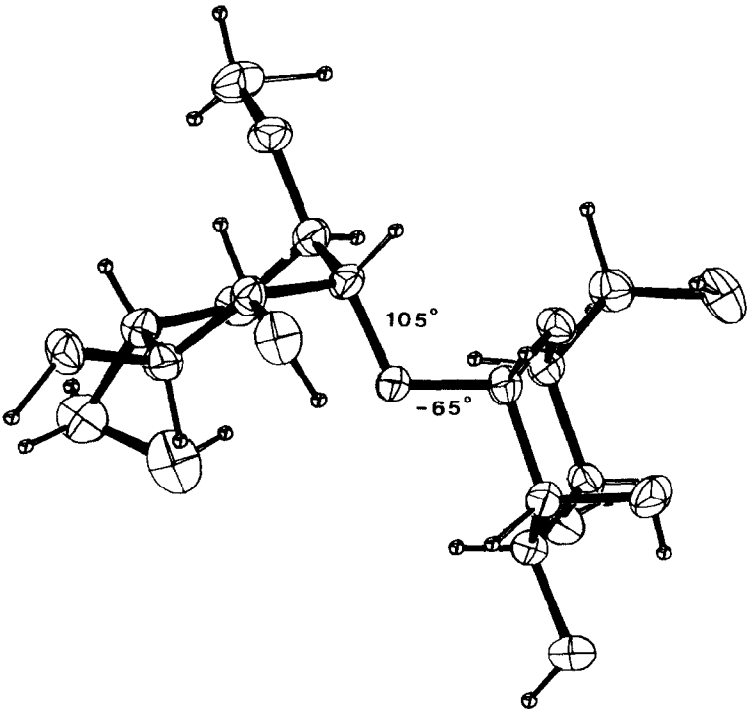

Fig. 2. An Ortep diagram showing the conformation of the two mannopyranose units.

TABLE IV
HYDROGEN BOND DISTANCES[a] AND ANGLES[b]

Donor	*Hydrogen*	*Acceptor*	*D-H*[a]	*H · · · A*[a]	*D · · · A*[a]	*D-H · · · A*[b]	*Data set*
O-2′	H-O2	O-4	0.74(7)	2.31(5)	2.924(2)	141(6)	$(x, y, 1 + z)$
O-4	H-O4	O-6	1.10(5)	1.57(4)	2.664(2)	176(4)	$(1 + x, y, z)$
O-6′	H-O6′	O-5	0.80(4)	2.23(4)	2.691(2)	117(3)	$(1 - x, y - 1/2, 1 - z)$
		O-8	0.80(4)	2.42(3)	3.087(3)	142(4)	$(x - 1, y, z - 1)$
O-4′	H-O4	O-2′	0.89(6)	2.10(5)	2.938(4)	145(4)	$(x - 1, 3/2 + y, 1 - z)$
O-3	H-O3	O-6′	1.00(7)	2.37(5)	3.106(3)	131(4)	$(x, y, z - 1)$
C-H · · · O interactions							
C-5′	H-2–C-6	O-5	1.02(5)	2.59(6)	3.606(5)	169(3)	$(x - 1, y, z - 1)$
C-3′	H–C-3	O-3′	0.93(3)	2.67(4)	3.378(4)	133(2)	$(1 - x, 1/2 + y, z)$

[a]In Angströms. [b]In degrees.

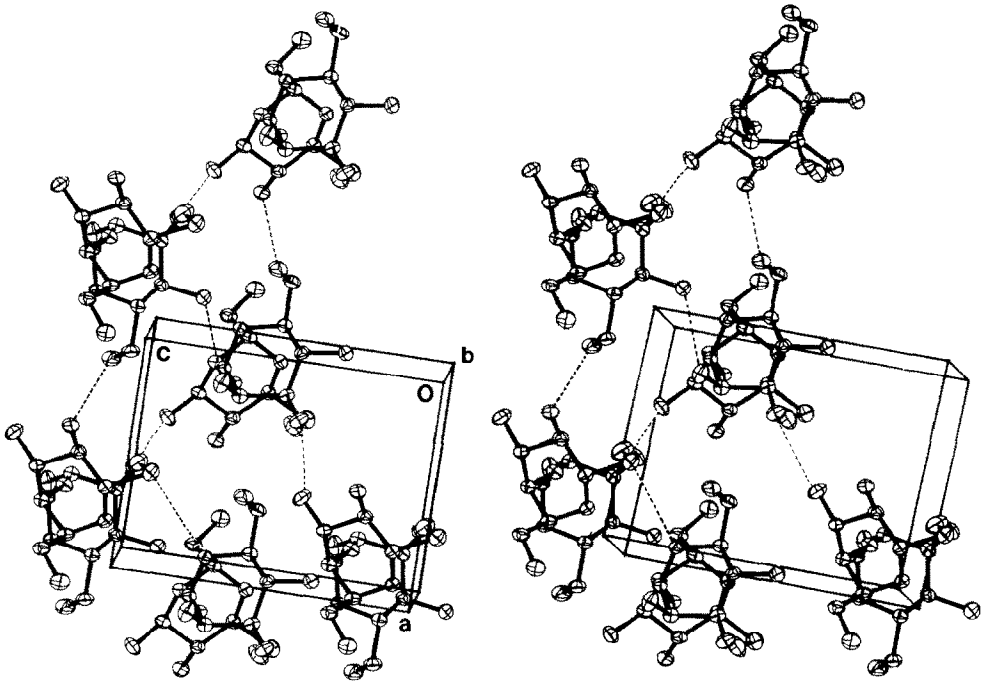

Fig. 3. Stereodiagram showing the packing of the molecules in the unit cell. Hydrogen bonds are indicated by dashed lines.

Both the sugar rings have the expected chair conformations. Fig. 2 gives an Ortep[9] diagram showing the conformations of the two sugar rings. C-5′ and C-2′ deviate by −0.68 and 0.53 Å, respectively, from the best mean plane through the remaining four atoms in the α-D-mannopyranosyl group(II), whereas in the potentially reducing α-D-mannopyranosyl residue (I), C-3 and O-5 deviate by −0.66 and 0.57 Å, respectively, from the best mean plane through the other four atoms of the ring. The glycosidic torsion angles ϕ and ψ, C-2–O-2–C-1′–O-5′ and C-1–C-2–O-2′–C-1′ have values of −64.5 and 105.5°, respectively, and are similar to the values found in other α-D anomers[8]. The Cremer and Pople[10] parameters Q and θ are 0.52(6) and 10.69(5)° in the residue I, and 0.54(6) Å and 3.81(4)° in the nonreducing group II. The conformation across C-5–C-6 is *gauche–gauche* for both sugars. An analysis of the crystal structures of other mannobiose compounds revealed $^4C_1$(D) as the preferred conformation for the mannobiose residues. α-D-Man*p*-(1→3)-β-D-Man*p*- (1→4)-α-D-GlcNAc[11], β-D-Man*p*-(1→4)-α-D-Man[12], and 3,4,6-tri-*O*-acetyl-β-D-mannose[13], all have $^4C_1$(D) conformations with different distortions from the ideal $\psi = 0$. In our case, the distortion in the residue I (10.7) is larger than that in the nonreducing group II (3.8).

Hydrogen bonding and packing. — The crystal structure is stabilized by a network of hydrogen bonds of O–H · · · O type. Table IV gives a list of hydrogen

bond distances and angles. There is a bifurcated O–H · · · O interaction involving O-6 of residue I as donor and O-5 and O-8 of symmetry related residue II. The covalent O–H distances vary from 0.74 to 1.10 Å and are distributed symmetrically about the normalized[8] (neutron) distance of 0.97 Å. Fig. 3 gives an Ortep drawing of the packing of the molecules in the unit cell, projected down the *b*-axis. Some of the hydrogen bonds are shown by thin broken lines in the packing diagram.

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