On the Conformational Variability of the *N*-Acetylglucosamine β-(1 → 4) Linked Dimer. Crystal and Molecular Structure of β-*N*,*N*'-Diacetylchitobiose Trihydrate

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β-*N*,*N*'-Diacetylchitobiose crystallizes with three molecules of water in space group $P2_1$; $a = 11.569(3)$, $b = 8.920(2)$, $c = 11.086(4)$ Å, $\beta = 99.00(3)°$, $Z = 2$. The structure was solved by a tangent-refinement procedure, refinement by full-matrix least squares was based on 1940 F_0 corrected for absorption, and the final value of R and R_w is 0.035. The two $^4C_1$ chair rings of the molecule describe a small left-handed helical twist, $\psi_H = -15°$, as in methyl β-cellobioside. In both structures the $-CH_2OH$ group of the nonreducing moiety is oriented *gauche-trans* which allows O(6) to participate in a bifurcated intramolecular hydrogen bond with O(3'). Comparison with the structures of α-*N*,*N*'-diacetylchitobiose and the disaccharide in protein complexes provides further evidence of a remarkably large flexibility in the β-(1→4) glycosidic bridge. The geometric constraints imposed by an *inter*-ring hydrogen bond are rather slack, and the results obtained indicate that the intermolecular lattice energy is a more important factor in determining the conformation of these molecules. The general orientation of the equatorial 2-*N*-acetyl group in substituted gluco- and galactopyranosidic rings has the C−C bond *gauche-gauche* relative to the ring bonds C(1)−C(2) and C(3)−C(2). The crystal structure differs markedly from that of the α-anomer. Stacks of molecules along *b* are laced together through an extensive system of hydrogen bonds in which all three water molecules take part both as donors and acceptors.

Studies of polysaccharide conformation also include X-ray diffraction analyses of the solid-state structures. Reasonable polymer models are usually improved by computer manipulation of a few selected parameters characterizing the repeating unit and relative orientation of the chains.[1–5] A normal crystallographic refinement is not applicable since the number of parameters in general exceeds by far the number of observations. In practice, therefore, the analyses have been based on semi-rigid models of the mono- and dimer constituents as, *e.g.*, in the linked-atom least-squares approach,[6] and on considerations of molecular packing.[7] Systematic single-crystal X-ray investigations of these constituents and related compounds provide the required experimental information on bond lengths and angles and other geometrical features such as preferred conformations at the glycosidic bridge and the orientation of side groups.

N,*N*'-Diacetylchitobiose, or (GlcNAc)$_2$, is the repeating segment of the natural polysaccharide chitin. A crystallographic study of this molecule was carried through partly in order to obtain structure parameters possibly relevant to the polymer structure and to other β-(1→4) linked polysaccharides.

(GlcNAc)$_2$ is also an inhibitor of lysozyme[8] and forms stable complexes with this enzyme. From X-ray analyses of crystalline hen egg-white lysozyme-saccharide complexes structure details at a resolution of 2 Å have become available both for tetragonal lysozyme-(GlcNAc)$_3$[9] and the triclinic lysozyme-GlcNAc and -(GlcNAc)$_2$ complexes.[10] The crystal structure of α-(GlcNAc)$_2$ monohydrate was discussed previously;[11] in the present paper we report that of β-(GlcNAc)$_2$ trihydrate. Thus, data are now at hand for a study of possible conformational changes of this disaccharide when bound to the active site of the enzyme. More generally, observations of the dimer structure in four different

crystalline environments presumably relate some measure of its flexibility, in particular at the glycosidic bridge and in the orientation of the N-acetyl and primary hydroxyl groups.

EXPERIMENTAL

Thin, rodshaped crystals of β-(GlcNAc)$_2$ trihydrate were obtained from aqueous 2-methyl-2,4-pentanediol. Bisphenoidal crystals of the orthorhombic α-(GlcNAc)$_2$ monohydrate grow from the same solution.

Lattice constants of the β-anomeric crystals were determined from the setting angles of 15 reflections, cf. Table 1. The measurements were repeated several times during data collection. Changes in all parameters were within one standard deviation.

A crystal mounted with b tilted ca. 1° from the diffractometer ϕ axis was used for the X-ray measurements. Data set 1 comprising 2114 $hk \pm l$ reflections (including 80 measured twice) was collected without attenuators to a limit in 2θ of 129° with Ni-filtered CuKα radiation on a diffractometer; in addition a partial set of $-hk \pm l$ reflections (set 2) was collected. The scan mode was $\omega/2\theta$ at 2° min^{-1} in 2θ, basic scan width was 2.3° and backgrounds were measured for 20 s at each limit of the scan. Three standard reflections were monitored at intervals of 100 reflections. The intensities were scaled according to the average standard decay and corrected for coincidence loss and absorption. A recovery constant[12] $\tau = 6.0 \times 10^{-8}$ counts^{-1} was determined with this particular crystal, absorption coefficients were in the range 1.097 to 1.210.

Weighted averages of F^2 and $\sigma(F^2)$ were calculated for reflections measured twice within each

Table 1. Crystal data. β-N,N'-Diacetylchitobiose trihydrate, $C_{16}H_{28}O_{11}N_2 \cdot 3H_2O$.

F.W.	478.45
a (Å)	11.569(3)
b	8.920(2)
c	11.086(4)
β (°)	99.00(3)
V (Å^3)	1130
At temp. (°C)	20.0(5)
Space group	$P2_1$
λ (Å)	1.5418
Z	2
D_x (g cm^{-3})	1.406
μ (cm^{-1})	10.8
Size (mm^3)	$\sim 0.41 \times 0.10 \times 0.09$
Needle axis	b

data set, and the averaging was repeated for 1281 pairs of equivalent reflections in sets 1 and 2. Weights were assigned according to relations given previously,[12] the parameter S in the expression $\sigma^2(I_i) = \sigma^2_{i\,count} + (S\,I_{i\,net})^2$ was adjusted to 0.025. The agreement index $D = \sum|F_1^2 - F_2^2|/\sum F_{ave}^2$ for the 1281 pairs was 0.040. Of the 2030 unique reflections excluding extinctions, 87 had $F^2 \leq \sigma(F^2)$. They were given zero weight together with three low-angle reflections having very uneven backgrounds.

Programs used for analyses and corrections of the data are described elsewhere.[13] Other crystallographic programs were from the X-RAY 76 system.[14] Drawings of molecules were made with ORTEP.[15]

STRUCTURE DETERMINATION AND REFINEMENT

Structure solution was by the multisolution tangent-refinement program TANNY[16,17] using 177 reflections with $E \geq 1.60$. All non-H atom positions of the disaccharide were identified in the E map of the best phase model which included 233 E's ≥ 1.49. The ΔF map following one isotropic refinement cycle of these atoms showed three prominent maxima corresponding to the O atoms of three water molecules. All H atoms were located in subsequent ΔF maps, the one bonded axially to the anomeric C(1') of the reducing ring in a peak of density ~ 0.68 e Å^{-3}. Another peak of similar strong density at $x,y,z \sim 0.10, 0.50, 0.04$ did not correspond to a reasonable H position. Further analysis of this feature was deferred till the end of the refinement.

Several cases of α/β anomeric disorder in crystalline saccharides have been discussed in the literature, α-(GlcNAc)$_2$ being one such example, cf. Ref. 11 and references cited therein. In the present structure, the anomeric H refined to a C$-$H distance 1.13(2) Å and an isotropic $U \sim 0$. Very similar results were obtained in the study of the α-GlcNAc monomer,[12] and in analogy with this case, we consider the evidence for α/β cocrystallization in β-(GlcNAc)$_2$ not conclusive.

The temperature factors of a few H atoms increased to large values during the full-matrix least-squares refinement and it was necessary eventually to constrain all H parameters in water molecules W' and W'''' and those of H(O1'); only the coordinates of H(C8'2) were allowed to refine in the last cycles. Refinement based on 1940 F_o with weights $w = 1/\sigma^2(F_o)$ converged at a conventional

Table 2. Final atomic parameters. Thermal parameters, U_{ij}(Å^2 × 10^4) for C, O and N, and U(Å^2 × 10^3) for H are defined by: $\exp[-2\pi^2(U_{11}a^{*2}h^2 + \ldots + 2U_{12}a^*b^*hk + \ldots)]$ and $\exp[-8\pi^2 U(\sin^2\theta/\lambda^2)]$, respectively. E.s.d.'s appear in parentheses.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	0.4334(2)	0.4241(3)	0.2202(2)	385(15)	267(15)	291(13)	−9(13)	85(11)	−35(12)
C(2)	0.3192(2)	0.3798(4)	0.1405(2)	428(16)	303(15)	268(12)	−5(14)	16(11)	19(13)
C(3)	0.3170(3)	0.2116(3)	0.1126(2)	536(17)	260(16)	313(13)	−43(15)	−17(12)	6(13)
C(4)	0.4286(3)	0.1595(3)	0.0699(3)	681(20)	234(15)	314(14)	8(15)	49(13)	−28(12)
C(5)	0.5335(3)	0.2120(4)	0.1578(3)	552(18)	301(17)	370(15)	81(15)	87(13)	−27(14)
C(6)	0.6502(3)	0.1721(5)	0.1240(3)	579(20)	535(25)	537(19)	138(19)	48(15)	−208(19)
C(7)	0.1408(3)	0.5239(4)	0.1532(3)	537(18)	559(23)	379(15)	129(18)	−19(13)	−2(16)
C(8)	0.0424(4)	0.5465(7)	0.2242(5)	677(27)	835(38)	657(26)	292(27)	173(21)	−15(26)
O(1)	0.4418(2)	0.5793(2)	0.2267(2)	414(10)	225(10)	318(9)	−4(9)	−34(8)	−28(8)
O(3)	0.2168(2)	0.1817(3)	0.0238(2)	658(16)	388(14)	607(14)	−98(14)	−208(11)	−67(12)
O(4)	0.4273(3)	0.0000	0.0622(2)	1023(20)	245(11)	476(13)	21(13)	176(13)	−69(11)
O(5)	0.5293(2)	0.3726(3)	0.1642(2)	424(11)	301(11)	390(10)	12(10)	121(8)	−68(9)
O(6)	0.7361(2)	0.2334(5)	0.2156(3)	526(16)	1307(34)	1020(21)	215(20)	−21(15)	−626(23)
O(7)	0.1528(3)	0.6013(4)	0.0624(2)	900(23)	695(23)	597(18)	368(19)	190(14)	239(16)
N	0.2191(2)	0.4218(3)	0.1965(2)	386(13)	395(15)	334(13)	1(12)	45(10)	42(12)
C(1')	0.6575(2)	0.8894(4)	0.4279(2)	362(15)	325(16)	380(14)	−22(14)	6(11)	−36(14)
C(2')	0.7052(2)	0.7301(4)	0.4370(2)	366(15)	342(16)	300(13)	60(13)	44(11)	−17(13)
C(3')	0.6412(2)	0.6306(3)	0.3368(2)	390(15)	283(16)	278(13)	32(13)	29(11)	−15(11)
C(4')	0.5091(2)	0.6424(3)	0.3360(2)	395(15)	239(15)	274(13)	26(12)	23(11)	−20(12)
C(5')	0.4695(2)	0.8068(3)	0.3343(2)	451(16)	240(14)	315(13)	−3(13)	−10(11)	−23(12)
C(6')	0.3427(3)	0.8249(4)	0.3457(3)	417(17)	332(19)	564(19)	41(15)	1(14)	−75(16)
C(7')	0.9092(3)	0.7200(5)	0.5363(3)	411(16)	628(24)	456(16)	56(18)	12(13)	−116(18)
C(8')	1.0345(3)	0.7409(8)	0.5211(4)	390(19)	1343(51)	626(23)	11(27)	45(17)	−233(32)
O(1')	0.7125(2)	0.9688(3)	0.5286(2)	534(12)	351(13)	460(11)	−10(11)	−34(9)	−91(10)
O(3')	0.6846(2)	0.4822(3)	0.3633(2)	511(12)	317(12)	371(11)	110(10)	3(9)	−59(10)
O(5')	0.5352(2)	0.8864(2)	0.4340(2)	391(10)	293(10)	396(9)	7(9)	32(8)	−77(9)
O(6')	0.3146(2)	0.7661(3)	0.4559(2)	533(13)	338(13)	737(16)	−32(11)	246(12)	−70(12)
O(7')	0.8796(2)	0.6948(3)	0.6374(2)	476(11)	854(20)	428(11)	144(14)	−5(9)	2(13)
N'	0.8303(2)	0.7325(3)	0.4355(2)	353(12)	514(17)	337(12)	1(13)	41(10)	−34(13)
O(w')	0.1198(3)	0.9108(4)	0.0566(4)	1004(23)	577(21)	1686(31)	−69(20)	266(21)	96(23)
O(w'')	0.8441(4)	0.8427(4)	0.1945(3)	1328(29)	661(23)	514(16)	93(22)	99(18)	−3(17)
O(w''')	0.9522(2)	0.1293(5)	0.1781(2)	582(15)	1474(33)	658(15)	−83(21)	−44(12)	−243(20)

Atom	x	y	z	U	Atom	x	y	z	U
H(C1)	0.437(2)	0.382(4)	0.302(2)	31(7)	H(C5')	0.484(2)	0.846(4)	0.259(2)	40(8)
H(C2)	0.312(2)	0.427(4)	0.069(2)	48(9)	H(C6'1)	0.295(2)	0.778(4)	0.284(2)	40(8)
H(C3)	0.314(2)	0.162(3)	0.191(2)	31(7)	H(C6'2)	0.323(3)	0.935(5)	0.340(3)	53(9)
H(C4)	0.435(3)	0.208(4)	−0.014(3)	51(9)	H(C8'1)	1.050(3)	0.776(6)	0.439(3)	87(13)
H(C5)	0.530(2)	0.164(4)	0.239(3)	50(9)	H(C8'2)	1.061(6)	0.635(9)	0.534(6)	186
H(C61)	0.654(3)	0.072(5)	0.125(3)	55(11)	H(C8'3)	1.071(3)	0.801(6)	0.576(3)	83(14)
H(C62)	0.659(2)	0.220(4)	0.036(3)	54(9)	H(O1')	0.694	1.066	0.495	220
H(C81)	−0.029(5)	0.514(8)	0.167(5)	151(23)	H(O3')	0.681(3)	0.449(5)	0.303(3)	61(13)
H(C82)	0.023(5)	0.625(8)	0.229(5)	123(25)	H(O6')	0.336(5)	0.835(8)	0.511(5)	156(26)
H(C83)	0.064(3)	0.526(5)	0.307(3)	73(12)	H(N')	0.852(2)	0.753(4)	0.367(2)	46(9)
H(O3)	0.189(4)	0.109(6)	0.021(4)	87(18)	H(Ow'1)	0.079	0.834	0.050	230
H(O4)	0.437(4)	−0.025(6)	−0.001(4)	93(17)	H(Ow'2)	0.166	0.912	−0.019	300
H(O6)	0.799(4)	0.217(7)	0.201(4)	110(18)	H(Ow''1)	0.813(4)	0.812(7)	0.134(4)	93(17)
H(N)	0.213(3)	0.392(5)	0.259(3)	55(11)	H(Ow''2)	0.884(4)	0.919(7)	0.167(4)	119(22)
H(C1')	0.678(2)	0.952(3)	0.345(2)	1(5)	H(Ow'''1)	1.003	0.050	0.139	170
H(C2')	0.690(2)	0.698(3)	0.513(2)	29(7)	H(Ow'''2)	1.027	0.155	0.242	205
H(C3')	0.663(2)	0.660(3)	0.258(2)	19(6)	O(a)	0.104	0.503	0.041	40(4)
H(C4')	0.487(2)	0.589(4)	0.410(2)	43(8)					

$R[=\sum \|F_o| - K|F_c\|/\sum |F_o|]$ of 0.038. The residual density at this stage was everywhere in the range -0.19 to 0.15 e Å^{-3} except one maximum of density 0.41 e Å^{-3} at (0.10, 0.49, 0.04). A peak in this position present in all ΔF maps could indicate a pile-up of systematic errors in the data, however, a structural interpretation is also possible. The position is at a distance ~ 1.3 Å from C(7) in one of the N-acetyl groups and defines, together with N and C(8), part of a tetrahedral arrangement about C(7). This would be realized if the carbonyl of one of the N-acetyl groups of the disaccharide were partially replaced by the corresponding secondary alcohol. An O atom, O(a), was placed in the new position and population parameters for O(a) and O(7) were refined in alternating cycles together with the other variables. Positional parameters of O(a) were fixed in the last cycle. The refinement was terminated at $R=0.035$ and $R_w\{=[\sum w(|F_o| - K|F_c|)^2/\sum w F_o^2]^{\frac{1}{2}}\}$ $=0.035$ with weights as given above. It gave physically more plausible thermal parameters for O(7) and improved the geometry slightly in parts

of the molecule. Maximum parameter shifts in the last cycle were 0.1σ, except U of O(a) at 0.4σ.

The largest change in structure compared with the previous model was shortening of one C$-$H bond from 1.20 to 0.99 Å. No shifts exceeded 3σ, only three of 34 bond lengths and one of 65 bond angles involving H changed by as much as 2σ. Except for an increase of 0.009 Å (2σ) in the C(7)$-$O(7) bond length, only two of 30 bond lengths and three of 43 bond angles involving only C, O or N changed by 1σ. Final population parameters were 0.90(1) for O(7) and 0.11(1) for O(a) and the residual density was in the range -0.14 to 0.15 e Å^{-3}. Although the chemical implications of the refinement are not proved, we prefer the second model since it appears to be a physically better interpretation of the data. Because the common structure parameters of the two models are practically unchanged, only those of the latter will be discussed. Final atomic parameters from this refinement are given in Table 2. A list of structure factors is available from the author. Scattering factors were
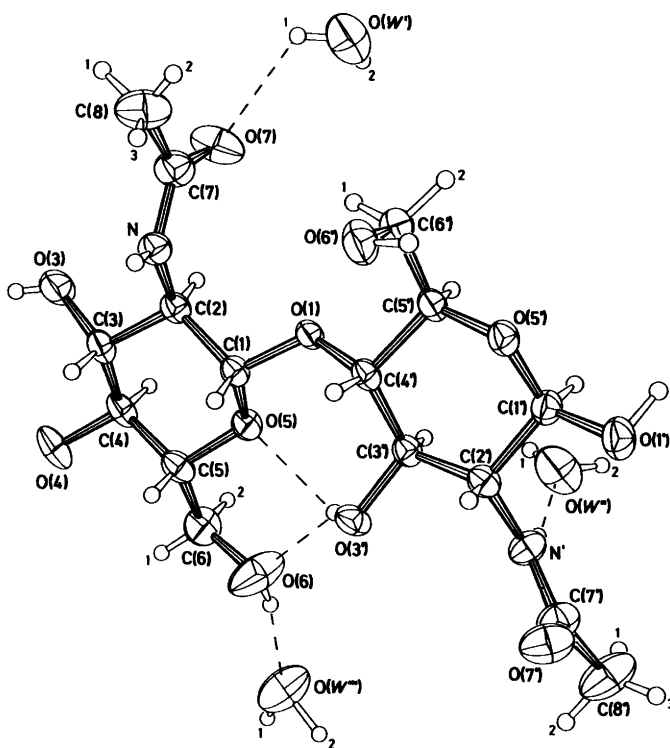

Fig. 1. Molecular structure and atomic labelling. Sequential numbering of H atoms is given only where necessary. Thermal ellipsoids of the heavier atoms correspond to a 40 % probability.

Table 3. Conformational parameters of the β-(1→4) bridge in β- and α-(GlcNAc)$_2$. Angles ϕ and ψ describe torsion[20] and pseudotorsion[11,21] between the rings in the sign convention of Klyne and Prelog.[24]

	Angle	β	α
ϕ_1	O(5)C(1)O(1)C(4')	−90.3°	−79.5°
ϕ_1'	C(2)C(1)O(1)C(4')	151.7	161.5
ϕ_2	C(1)O(1)C(4')C(3')	77.3	133.5
ϕ_2'	C(1)O(1)C(4')C(5')	−162.3	−106.8
ψ_1	O(5)C(1)C(4')C(3')	−13.5	49.1
ψ_1'	O(5)C(1)C(4')C(5')	122.7	−165.9
ψ_2	C(2)C(1)C(4')C(5')	−16.8	58.8
ψ_2'	C(2)C(1)C(4')C(3')	−152.9	−86.3
$\psi_H = \frac{1}{2}(\psi_1 + \psi_2)$		−15	54

those of Doyle and Turner[18] except for H.[19] Corrections for anomalous dispersion were not applied.

RESULTS AND DISCUSSION

The molecular conformation. Both pyranose rings of β-(GlcNAc)$_2$ are in the $^4C_1$ chair conformation (Fig. 1). Labelling of atoms in the nonreducing (unprimed) and reducing (primed) rings is according to the usual convention.

The conformation at the glycosidic bridge is a point of particular interest in studies of oligosaccharide structures. Table 3 gives for both anomers of (GlcNAc)$_2$ the torsion[20] and pseudotorsion[21] angles, and also a helicity parameter ψ_H, which is a measure of the *inter*-ring twist.[11] Clearly, the two anomers differ drastically in this respect; while the α-anomer has a strong right-handed helical twist of +54°, β-(GlcNAc)$_2$ has a twist in the opposite sense, $\psi_H = -15°$. This conformational difference of nearly 70° is illustrated in Fig. 2. As noted previously,[11] ψ_H of (GlcNAc)$_2$ in the triclinic lysozyme complex is approximately +22°, the twist between the B and C site rings of (GlcNAc)$_3$ in the tetragonal lysozyme complex appears to be in the
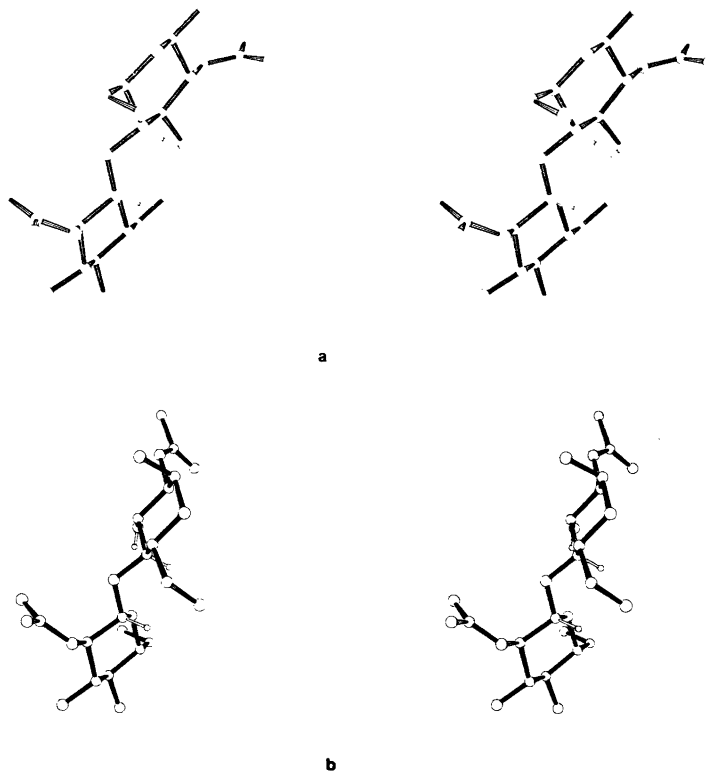

a

b

Fig. 2. Identical stereo views relative to the bridge atoms C(1)−O(1)−C(4') of β-(GlcNAc)$_2$ (a) and α-(GlcNAc)$_2$ (b). H(Cl), H(C4') and H(O3') are shown in addition to the nonhydrogen atoms.

Table 4. Endo- and exocyclic torsion angles (°) of β-(GlcNAc)$_2$ including differences ($\times$ 10) with the α-anomer. The full atomic sequence is given only for exocyclic angles; sign convention is that of Klyne and Prelog.[24]

Nonreducing ring			Reducing ring		
	β	$10(\beta-\alpha)$		β	$10(\beta-\alpha)$
Endocyclic					
C(1)C(2)	53.1	−69	C(1')C(2')	57.5	11
C(2)C(3)	−48.2	85	C(2')C(3')	−52.0	24
C(3)C(4)	50.9	−34	C(3')C(4')	50.4	−41
C(4)C(5)	−58.6	−38	C(4')C(5')	−54.8	9
C(5)O(5)	67.4	83	C(5')O(5')	61.8	15
O(5)C(1)	−64.5	−24	O(5')C(1')	−62.9	−20
Exocyclic					
O(1)C(1)C(2)N	−66.6	−82	O(1')C(1')C(2')N'	−60.9	
NC(2)C(3)O(3)	64.4	37	N'C(2')C(3')O(3')	62.9	9
O(3)C(3)C(4)O(4)	−66.7	−17	O(3')C(3')C(4')O(1)	−75.8	−77
O(4)C(4)C(5)C(6)	61.8	−2	O(1)C(4')C(5')C(6')	64.2	−40
C(5)O(5)C(1)O(1)	177.1	−30	C(5')O(5')C(1')O(1')	−179.9	
O(5)C(5)C(6)O(6)	58.6		O(5')C(5')C(6')O(6')	−60.6	
C(4)C(5)C(6)O(6)	178.8		C(4')C(5')C(6')O(6')	60.8	

range $+30$ to $+40°$. Thus, the uncomplexed and hydrated α- and β-anomers possess the extreme conformations at the bridge, intermediate torsion angles characterize the di- and trimers of the protein complexes. The wide range of ψ_H is evidence contrary to the view that the β-(1→4) glycosidic link of carbohydrates is a rigid one, in particular that of chitin oligomers, presumably because of steric hindrance by the bulky N-acetyl groups.[22]

The range of endocyclic torsion angles in the nonreducing ring is nearly 20° (48.2 to 67.4°). Comparison with the values of α-(GlcNAc)$_2$ (Table 4) shows that the conformations of these rings differ significantly in the two anomers. The puckering is more similar in the primed rings, the average difference between corresponding torsion angles there is 2.0°, *cf.* 5.6° for the unprimed rings.

The conformation about the exocyclic C(5)−C(6) bonds of β-(GlcNAc)$_2$ is *gauche-trans*[20] (*g-t*) in the unprimed and *gauche-gauche* (*g-g*) in the primed units, both conformations are favoured energetically over the *t-g* type, at least in the free molecules.[20] In the α-anomer[11] as also in the monomers α-GlcNAc[12] and *p*-nitrophenyl-β-GlcNAc,[23] the CH$_2$OH groups are oriented *g-g*. For α-chitin two probable orientations were deduced from a rigid-body refinement of the structure:[2] one nearly midway between *g-t* and the anticlinal[24] (*i.e.* ∼180° away from *g-g*) orientation proposed by Carl-

ström,[25] the other about 20° away from the *g-g* orientation which is near the one inferred from infra-red spectroscopy.[26] X-Ray studies of the structure of β-chitin has indicated *g-g* as the most probable orientation in this polymorphic form.[1.b] The hydroxymethyl groups of saccharides usually are important for hydrogen bonding. Therefore, the preference for any one of the conformations *g-t* and *g-g* that predominate in mono- and disaccharides is most likely determined by several interrelated geometric requirements associated with molecular conformation and packing. β-(GlcNAc)$_2$ has a bridge conformation very similar to that of methyl β-cellobioside[27] in which $\psi_H = -12.5°$.[11] It is interesting that in both molecules the unprimed hydroxymethyl group adopts the *g-t* orientation which allows O(6) to form an intramolecular hydrogen bond with O(3').

The orientation of the N-acetyl groups is given in Table 5 as torsion angles[24] about the vectors C(2)−C(7) (χ) and about the bonds C(2)−N (ζ_N). Torsion angles of the α-anomer and three related monosaccharides also appear in the table. In all structures, the C(7)−C(8) bond of the N-acetyl group is oriented *g-g* relative to C(1)−C(2) and C(3)−C(2) (pseudo *g-g*). This appears to be a characteristic feature of the equatorial 2-N-acetyl group in glycopyranosidic rings as it is seen also in molecular plots of the complexes of triclinic lyso-

Table 5. Conformational parameters (°) of the equatorial *N*-acetyl group in some 2-substituted gluco- and galactopyranosidic rings.[a]

	(I)	(I')	(II)	(II')	(III)	(IV)	(V)
N-acetyl orientation							
χ_1 C(1)C(2)C(7)C(8)	−80.9	−80.0	−81.1	−51.8	−67.3	−106.8	−73.6
χ_2 C(3)C(2)C(7)C(8)	66.9	67.8	65.7	92.8	77.8	33.0	75.6
ζ_N C(1)C(2)NC(7)	113.7	100.4	100.5	138.7	140.9	81.6	109.9
ζ'_N C(3)C(2)NC(7)	−122.5	−135.2	−137.0	−98.9	−96.8	−155.6	−126.9
N-acetyl nonplanarity[30]							
ω_1 C(8)C(7)NC(2)	178.4	−173.7	−173.9	−179.7	169.9	−179.6	−174.3
ω_2 O(7)C(7)NH(N)	167.0	177.5	179.2	−173.8	−169.6	177.9	164.9[b]
ω_3 O(7)C(7)NC(2)	−5.2	5.2	3.0	−2.1	−9.7[c]	1.6	6.6
ω_4 C(8)C(7)NH(N)	−9.4	−1.3	2.3	8.6	9.9	−3.3	−16.1[b]
$\tau = \frac{1}{2}(\omega_1 + \omega_2)$	172.7	−178.1	−177.3	−176.8	−179.9	179.2	175.3
$\chi_C = \omega_1 - \omega_3 + \pi$ } mod 2π	3.6	1.2	3.1	2.4	−0.4	−1.2	−0.9
$\chi_N = -\omega_1 + \omega_4 + \pi$	−7.8	−7.6	−3.8	8.3	20.0	−3.7	−21.8[b]

[a] References: (I) β-(GlcNAc)₂ nonreducing ring. (I') β-(GlcNAc)₂ reducing ring. (II) α-(GlcNAc)₂ nonreducing ring.[11] (II') α-(GlcNAc)₂ reducing ring.[11] (III) α-GlcNAc.[12] (IV) α-GalNAc.[29] (V) *p*-Nitrophenyl-β-GlcNAc.[23]   [b] H positions were not refined.   [c] Sign of ω_3 was incorrectly given as + in the original paper.[12]

zyme with GlcNAc and (GlcNAc)₂[10] and of tetragonal lysozyme with GlcNAc and (GlcNAc)₃.[28] A similar orientation was proposed both for the α-[2,25] and β-forms[1,b] of chitin, and ζ_N calculated from the published coordinates are 112 and 136°, respectively. The mean values of ζ_N and ζ'_N in Table 5 including the galactose derivative, α-GalNAc,[29] are 112.2 and −124.7°, respectively, with ranges of ±30° about the mean. The range and distribution of ζ_N angles are depicted in Fig. 3.

Table 5 also gives conformational parameters describing the nonplanarity of the *N*-acetyl
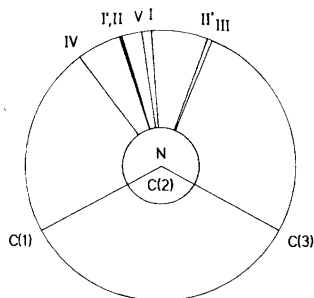

Fig. 3. Orientation of *N*-acetyl group in seven 2-acetamido-2-deoxy gluco- and galactopyranosidic rings given by the position of C(7) projected along the C(2) − N bond. Designation of rings is explained in Table 5.

groups,[30] τ is a measure of the twist about the N − C(7) bond, χ_C and χ_N give out-of-plane bending at C(7) and N, respectively. In all seven *N*-acetyl groups, χ_C deviates less than 4° from planarity. The contribution from χ_N to nonplanarity is generally larger since it involves displacement of the lighter H atom. A very large χ_N of 20° was found in α-GlcNAc. Table 5 shows that the *N*-acetyl group in GlcNAc mono- and oligomers has a rather high degree of rotational freedom about a pseudo *g-g* orientation, but comparable in magnitude to the conformational flexibility at the glycosidic bridge. Distortions from planarity at the N atom may also become significant.

Bond lengths and angles. Bond lengths and angles with their e.s.d.'s are given in Tables 6 and 7. Significant dissimilarities related to anomeric configuration are observed in corresponding C − O bonds in the reducing rings of the (GlcNAc)₂ anomers. As shown in Table 6, the two bonds are of equal length in the β-anomer, 1.428 Å; in the α-anomer C(1')−O(5') is significantly shorter than C(5')−O(5'). This is consonant with observations on a number of crystalline β-D- and α-D-pyranoses.[31] Relative bond lengths of the five-atom sequences C−O$_{ring}$−C$_{ano}$−O−H agree reasonably well for both anomers with quantum-chemical calculations of bond-length variations in model compounds of the β- and α-pyranose moieties.[32] The anomalous shortening of the α-anomeric C(1')−O(1') bond was

Table 6. Bond lengths (Å) with standard deviations of β-(GlcNAc)$_2$ including differences ($\times 10^3$) with the α-anomer.

Nonreducing ring			Reducing ring		
	β	$10^3 \times (\beta - \alpha)$		β	$10^3 \times (\beta - \alpha)$
C(1)−C(2)	1.523(4)	8	C(1')−C(2')	1.521(5)	−5
C(2)−C(3)	1.531(4)	13	C(2')−C(3')	1.521(4)	−6
C(3)−C(4)	1.515(5)	−2	C(3')−C(4')	1.530(4)	11
C(4)−C(5)	1.508(4)	−29	C(4')−C(5')	1.535(4)	16
C(5)−O(5)	1.436(4)	9	C(5')−O(5')	1.428(3)	−10
C(1)−O(5)	1.430(4)	16	C(1')−O(5')	1.428(3)	9
C(1)−O(1)	1.389(4)	−6	C(1')−O(1')	1.390(3)	29
C(3)−O(3)	1.424(4)	−7	C(3')−O(3')	1.430(4)	9
C(4)−O(4)	1.426(3)	4	C(4')−O(1)	1.448(3)	−1
C(5)−C(6)	1.500(5)	−17	C(5')−C(6')	1.500(4)	−11
C(6)−O(6)	1.415(5)	−8	C(6')−O(6')	1.413(5)	−7
C(2)−N	1.446(4)	−14	C(2')−N'	1.450(3)	1
N−C(7)	1.320(4)	3	N'−C(7')	1.332(4)	−14
C(7)−O(7)	1.246(5)	15	C(7')−O(7')	1.243(4)	13
C(7)−C(8)	1.496(6)	−10	C(7')−C(8')	1.497(5)	7
C(7)−O(a)	1.27(2)				

Endocyclic C−C bonds

	β	α		β	α
Mean	1.519	1.522	Mean	1.527	1.523
Range	0.023	0.022	Range	0.014	0.008

Exocyclic C−O bonds not involving O(1) and O(1')

Mean	1.422	1.425	Mean	1.422	1.421
Range	0.011	0.009	Range	0.017	0.001

ascribed to disorder problems.[11] There are larger disparities in pairs of bonds of the nonreducing rings; in fact, half of the endocyclic bonds differ by 3σ or more. The two β-anomeric rings have markedly distinct conformations (Table 4) which can be related to differences in the crystal environments, *cf. The crystal structure.* The unprimed C−O ring bonds of β-(GlcNAc)$_2$ are very similar and of normal length, in α-(GlcNAc)$_2$ the C(1)−O(5) bond is about 0.01 Å shorter than the mean value for β-pyranosidic rings.

The mean values of endocyclic C−C−X angles (X=C, O, N) are nearly the same in all rings of the two anomers, but ranges are larger in the β-compound, in particular in the distorted nonreducing ring (Table 7). As expected, the nonreducing rings also exhibit the most pronounced deviations in pairs of angles, maximum and average values are

3.1 and 1.5°, respectively. The largest difference between the pair of reducing rings, is 1.9° in C(5')−O(5')−C(1'). This discrepancy and also that of the O(5')−C(1')−O(1') angles are characteristic, however, for the α- and β-configurations and the two pairs of angle values are in very good agreement with previous observations on pyranose sugars.[6] As was noted before, differences in strain associated with conformational dissimilarities at the glycosidic bridge seem to be absorbed largely in the exocyclic angles at C(4'), those at C(1) being very little affected.[11] The bridge bonds and angle of β-(GlcNAc)$_2$ are within the range observed for other crystalline β-(1→4) linked disaccharides in the ψ_H range −15 to +54°.

It is clear that characteristic differences and similarities in structure dimensions associated with the α- and β-configurations are present in the

Table 7. Bond angles (°) with standard deviations of β-(GlcNAc)$_2$ including differences ($\times 10$) with the α-anomer.

Nonreducing ring			Reducing ring		
	β	$10(\beta-\alpha)$		β	$10(\beta-\alpha)$
O(5)C(1)C(2)	109.0(2)	−10	O(5')C(1')C(2')	109.4(2)	−2
C(1)C(2)C(3)	110.9(2)	16	C(1')C(2')C(3')	111.5(2)	15
C(2)C(3)C(4)	112.1(3)	31	C(2')C(3')C(4')	109.6(2)	−2
C(3)C(4)C(5)	110.0(2)	−3	C(3')C(4')C(5')	111.2(2)	5
C(4)C(5)O(5)	108.1(2)	−23	C(4')C(5')O(5')	110.1(2)	9
C(5)O(5)C(1)	112.2(2)	−7	C(5')O(5')C(1')	112.6(2)	−19
O(5)C(1)O(1)	106.9(2)	−8	O(5')C(1')O(1')	107.7(2)	−47
C(2)C(1)O(1)	109.7(2)	14	C(2')C(1')O(1')	107.9(2)	−11
C(1)C(2)N	111.3(2)	1	C(1')C(2')N'	109.8(3)	−2
C(3)C(2)N	110.6(3)	−13	C(3')C(2')N'	111.9(2)	2
C(2)C(3)O(3)	108.0(2)	−32	C(2')C(3')O(3')	106.0(2)	−15
C(4)C(3)O(3)	111.6(2)	36	C(4')C(3')O(3')	112.5(2)	13
C(3)C(4)O(4)	108.9(3)	−31	C(3')C(4')O(1)	112.6(2)	58
C(5)C(4)O(4)	110.4(3)	28	C(5')C(4')O(1)	103.9(2)	−51
C(4)C(5)C(6)	115.5(3)	24	C(4')C(5')C(6')	113.3(3)	−11
O(5)C(5)C(6)	106.8(3)	−10	O(5')C(5')C(6')	106.9(2)	7
C(5)C(6)O(6)	106.7(3)	−55	C(5')C(6')O(6')	113.2(3)	−12
C(2)NC(7)	125.0(3)	30	C(2')N'C(7')	123.0(2)	−18
NC(7)O(7)	121.2(3)	−26	N'C(7')O(7')	121.5(3)	−24
NC(7)C(8)	115.9(3)	0	N'C(7')C(8')	116.4(3)	11
O(7)C(7)C(8)	122.7(4)	25	O(7')C(7')C(8')	122.2(3)	14
NC(7)O(a)	111,4(11)		Bridge		
C(8)C(7)O(a)	111.5(9)		C(1)O(1)C(4')	117.1(2)	8

Endocyclic C − C − X angles ^{a}

	β	α		β	α
Mean	110.0	109.8	Mean	110.4	109.9
Range	4.0	1.4	Range	2.1	1.5

Angles involving H

Type ^{a}	Number		Range	Mean	σ_{ave}
XCH	38		103.0 − 113.5	109.1	1.7
CCH(methyl	6		98.4 − 116.9	110.0	3.2
COH	6		95.6 − 120.4	107.6	3.6
CNH	4		114.5 − 120.2	117.8	2.2
HCH	8		94.8 − 129.7	109	4
HOH	3		88.5 − 107.0	99	−

^{a} X = C, O, N.

(GlcNAc)$_2$ anomers. However, the largest disparities appear in the pair of β-anomeric nonreducing rings and in the conformation at the glycosidic bridge. These facts suggest that the crystalline environment has a decisive influence on the conformation of these flexible molecules. Recent theoretical calculations on other types of flexible molecules have also emphasized the importance of the intermolecular

lattice energy in determining intramolecular conformation.[33,34] It follows, that extrapolations from lower oligomers to obtain a model of the polysaccharide structure may be grossly inadequate if the molecular packing schemes are different.

The dimensions of the two N-acetyl groups in β-(GlcNAc)$_2$ are quite similar, the largest differences are in the C(2)$-$N$-$C(7) angles ($\Delta = 2.0°$) and in the N$-$C(7) bonds ($\Delta = 0.012$ Å). Compared with average values for the peptide unit,[35] both C(7)$-$O(7) bonds are longer by about 0.015 Å; however, part of the lengthening in the unprimed unit may be effected by the refinement of the disordered model. The apparent shortening of the C(7)$-$C(8) bonds is probably largely due to thermal motion.

Except for C(1')$-$H(Cl') at 1.13 Å, the pyranosidic C$-$H bond lengths vary from 0.89 to 1.08, the mean is 0.98 Å. The range of methyl C$-$H bonds is 0.74$-$1.00, mean 0.92 Å, and O$-$H bonds in the carbohydrate molecule range from 0.72 to 0.96 with a mean of 0.81 Å. A summary of valency angles involving H is given in Table 7.

The crystal structure. The crystal structure is illustrated in Fig. 4. Compared with the α-anomer, there are fewer ($\sim 30 \%$) intermolecular nonpolar contacts in the β-(GlcNAc)$_2$ crystal. About half the number of contacts to the unprimed unit involve atoms C(6), C(8) and O(4), while C(4), C(7), O(1), O(5) and O(6) apparently contribute negligibly to this type of interaction. Contacts are more regularly distributed over the outer atoms of the primed unit. Short distances are:* C(7')$\cdots$H(OW'''2)$_{11}$

---

* The subscript$_{11}$ denotes molecule at $2-x, \frac{1}{2}+y, 1-z$. The symmetry code is set out in Table 8.

2.52, O(1')$\cdots$H(N)$_9$ 2.47(3), O(7')$\cdots$H(N)$_9$ 2.44(4), H(OW''2)$\cdots$H(OW'''1)$_7$ 1.87 and H(O3)$\cdots$H(OW''2)$_6$ 1.82 Å.

The molecules are interconnected through an extensive system of hydrogen bonds indicated by broken lines in Fig. 4. In the absence of unbiased parameters for H, a more reliable interpretation of the geometry of hydrogen bonding can be made after shifting the H atoms along the parent covalent bonds to obtain neutron-diffraction values of the bond lengths. This type of correction has been applied in previous X-ray studies by us[12,36,37] and also by others.[38] Results for the present case appear in Table 8. All O and N atoms except O(1), O(5') and N participate in hydrogen bonding; bonding to O(a) as indicated in the table would stabilize a structure partially substituted at C(7).

The molecular packing of the α- and β-anomers differ substantially, cf. Fig. 2 of Ref. 11. The crystal structure of the β-anomer may be described as stacks of molecules along b. Molecules 7, 1 and 6 constitute part of one such stack, molecules 2 and 3 are part of another. A set of hydrogen bonds involving O(W') link molecules within one stack. In the disorder model, atoms O(a) of a screw-related stack could replace O(7) in hydrogen bonding with O(W'):

$$
\begin{array}{cc}
O(a)_3 & O(a)_2 \\
\downarrow & \downarrow \\
O(3)_7 \rightarrow O(W')_1 \rightarrow O(7)_1 \text{---} O(3)_1 \rightarrow O(W')_6 \rightarrow O(7)_6
\end{array}
$$

Stacks 6, 1, 7 and 4, 5 related by the screw axis in $\frac{1}{2},y,0$ are connected directly through a helical system of hydrogen bonds, the sense donor-acceptor advancing along $-b$: O(4)$_7 \rightarrow$O(5)$_5$---O(4)$_5 \rightarrow$O(5)$_1$
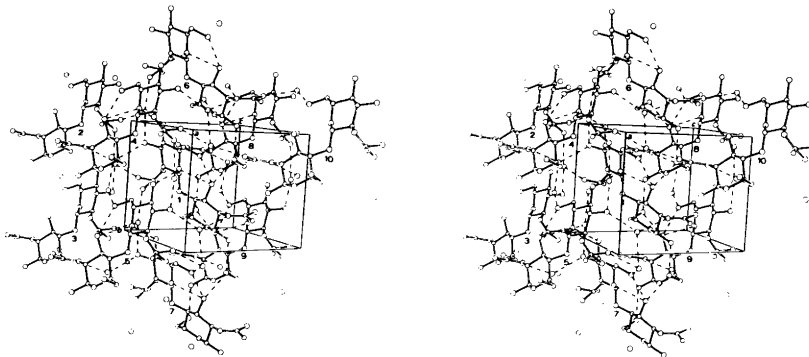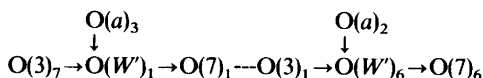

Fig. 4. Stereo drawing of the β-(GlcNAc)$_2$ molecular packing with hydrogen bonds shown as broken lines. Molecules are numbered according to the symmetry code in Table 8.

Table 8. The geometry of the hydrogen-bonding system.

Symmetry code for subscripts

(1)	$x,$	$y,$	z	(5)	$1-x,$	$\frac{1}{2}+y,$	$-z$	(9)	$1-x,$	$\frac{1}{2}+y,$	$1-z$
(2)	$-x,$	$-\frac{1}{2}+y,$	$-z$	(6)	$x,$	$-1+y,$	z	(10)	$2-x,$	$-\frac{1}{2}+y,$	$1-z$
(3)	$-x,$	$\frac{1}{2}+y,$	$-z$	(7)	$x,$	$1+y,$	z	(11)	$2-x,$	$\frac{1}{2}+y,$	$1-z$
(4)	$1-x,$	$-\frac{1}{2}+y,$	$-z$	(8)	$1-x,$	$-\frac{1}{2}+y,$	$1-z$	(12)	$1+x,$	$-1+y,$	z

$D-H\cdots A$	$D\cdots A$ Å	$H\cdots A$ Å	$(H\cdots A)_{corr}$ [a] Å	$(\angle D-H\cdots A)_{corr}$ [a] °
$O(3)-H(O3)\cdots O(W')_6$	2.712(5)	2.00(5)	1.76	164
$O(4)-H(O4)\cdots O(5)_4$	2.870(3)	2.12(5)	1.90	170
$O(6)-H(O6)\cdots O(W''')_1$	2.759(4)	1.99(5)	1.80	166
$O(1')-H(O1')\cdots O(6')_9$	2.679(3)	1.87	1.86	140
$O(3')-H(O3')\cdots O(5)_1$	2.796(3)	2.25(3)	2.08	128
$O(3')-H(O3')\cdots O(6)_1$	2.874(5)	2.29(4)	2.10	134
$O(6')-H(O6')\cdots O(3')_9$	2.781(3)	1.96(6)	1.86	155
$N'-H(N')\cdots O(W')_1$	2.875(4)	2.06(3)	1.92	157
$O(W')-H(OW'1)\cdots O(7)_1$	2.787(5)	2.24	2.16	120
$O(W'')-H(OW''1)\cdots O(3)_5$	2.807(4)	2.08(5)	1.88	156
$O(W'')-H(OW''2)\cdots O(W''')_7$	2.864(6)	2.03(6)	1.96	152
$O(W''')-H(OW'''1)\cdots O(a)_4$	$\begin{cases} 2.66(2) \\ 2.56\,^b \end{cases}$	$\begin{cases} 2.21 \\ 2.09\,^b \end{cases}$	2.11 [b]	106 [b]
$O(W''')-H(OW'''2)\cdots O(7')_{10}$	2.659(3)	1.62	1.69	168
$O(a)-H(Oa)\cdots O(W')_2$	$\begin{cases} 2.77 \\ 2.69\,^b \end{cases}$			
Intermolecular contacts				
$N-H(N)\cdots O(7')_8$	3.075(4)	2.44(4)	2.24	139
$O(W''')-H(OW'''1)\cdots O(W')_{12}$	3.192(5)	2.14	2.22	174
$O(W''')-H(OW'''1)\cdots O(7)_4$	2.763(4)	2.68	2.67	85

[a] Distances and angles involving H have been normalized (corr.) assuming lengths of 0.98 Å for the $O-H$ [40] and 1.015 Å for the $N(sp^2)-H$ bonds. [41] [b] Geometry is calculated with a $C(7)-O(a)$ bond length of 1.40 Å.

---$O(4)_1\rightarrow O(5)_4$. Two helical H-bond systems link the stacks 6, 1, 7 and 8, 9: $O(6')_6\rightarrow O(3')_8$---$O(1')_8\rightarrow O(6')_1\rightarrow O(3')_9$, and the screw-related $O(1')_6\rightarrow O(6')_8\rightarrow O(3')_1$---$O(1')_1\rightarrow O(6')_9\rightarrow O(3')_7$. The donor-acceptor sequence is along b. Water molecules W'' and W'''' are involved both as donors and acceptors in loops of hydrogen bonds lacing together the four stacks 4, 5 and 6, 1, 7 and 8, 9 and 10, 11, for example:

$$O(3)_4 \leftarrow O(W'')_6 \leftarrow N'_6$$
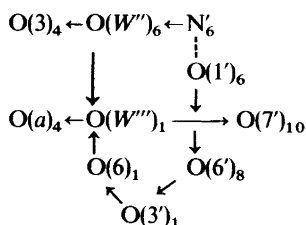
The geometries of the $O-H\cdots O$ contacts $O(W')_1\rightarrow O(7)_1$ and, in particular, $O(W''')_1\rightarrow O(a)_4$ indicate rather weak hydrogen bonding.

Atom $O(3')$ can form a bifurcated hydrogen bond with $O(5)$ and $O(6)$, the geometry being very similar to that of methyl β-cellobioside. [27] An intramolecular hydrogen bond was not observed in α-$(GlcNAc)_2$ [11] but was reported for β-lactose with $\psi_H=+39°$. [39] Thus, the geometrical requirements of the $O(3')-H\cdots O(5)$ bond are satisfied over a ψ_H range of at least 55° (-15 to $+39°$). This observation confirms the view taken in a previous paper [11] that the constraints imposed on molecular conformation by the formation of this hydrogen bond are relatively slack, which should be taken into account when considering structure models of the polymer.

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