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Synthesis and X-ray crystallographic investigation of *N*-(3-deoxy-3-acetamido-β-D-glycopyranosyl)alkanamides as analogs of *N*-glycoprotein linkage region

Manoharan Mathiselvam · Babu Varghese · Duraikkannu Loganathan

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Abstract As part of our ongoing program aimed at understanding the structural significance of GlcNAcβAsn linkage conserved in all eukaryotic *N*-glycoproteins, the present study reports on the synthesis and X-ray crystal structures of *N*-(3-deoxy-3-acetamido-β-D-glycopyranosyl)acetamide (Glc3NAcβNHAc) and the corresponding propionamide (Glc3NAcβNHPr). Comparative analysis of these structures with those of the corresponding GlcNAc (C2 acetamido) compounds showed that the bifurcated anti-parallel pattern involving N–H…O and C–H…O hydrogen bonds, the hallmark feature of the *N*-glycoprotein models, GlcNAcβNHAc and GlcNAcβAsn, is absent in both the C3 acetamido analogs. The extended (*anti*) conformation of the amido aglycon moiety as defined by χ_2 seen in the case of C2 acetamido derivative, GlcNAcβNHPr, turns into *gauche* for the C3 acetamido analog (Glc3NAcβNHPr). This observation is consistent with the earlier work on the critical role of the C2-NHAc group of GlcNAcβAsn in controlling χ_2 at the linkage region of *N*-glycoproteins.

Keywords *N*-glycoprotein linkage region analogs · Glc3NAc · Synthesis · Trifurcated hydrogen bond · X-ray

Dedicated to late Prof. Nathan Sharon

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Abbreviations

Glc3NAcβNHAc	*N*-(3-deoxy-3-acetamido-β-D-glycopyranosyl)acetamide
Glc3NAcβNHPr	*N*-(3-deoxy-3-acetamido-β-D-glycopyranosyl)propionamide
NMR	Nuclear Magnetic Resonance
ESI-MS	Electrospray Ionization Mass Spectrometry
ORTEP	Oak Ridge Thermal Ellipsoid Plot

Introduction

Glycosylation is a complex, co- or post-translational protein modification that serves to expand the diversity of the proteome. *N*-Glycosylation of the side chain of asparagine (Asn) residues present in the consensus sequence Asn-Xaa-Ser/Thr is the most common carbohydrate modification of proteins [1]. The resultant *N*-glycan chains of glycoproteins play key roles in many biological processes as both recognition determinants as well as modulators of the intrinsic properties including folding of proteins [2]. The linkage sugar, 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and amino acid, Asn, are conserved in the *N*-glycoproteins of all eukaryotes. Elucidation of the conformation of the linkage region, GlcNAcβAsn (Fig. 1), of *N*-glycoproteins is of fundamental importance as the same can influence the presentation of the glycan chains on the cell surface.

A systematic X-ray crystallographic study of models GlcNAcβAsn [3] and GlcNAcβNHAc(**1**) [4] (Fig. 2) of the *N*-glycoprotein linkage region and many of their analogs has shown that the key torsion, ϕ_N, is influenced to a greater

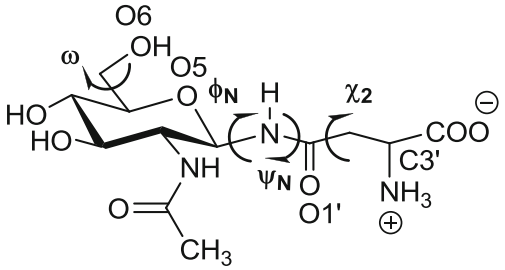


Fig. 1 Schematic representation of the linkage region (GlcNAcβAsn) of the *N*-glycoproteins with the depiction of torsion angles, ω= O5–C5–C6–O6, ϕ_N=O5–C1–N1–C1', ψ_N=C1–N1–C1'–C2' and χ_2=N1–C1'–C2'–C3'

extent by the structural variation of the glycan part than that of the aglycon group [5].

A comprehensive analysis of molecular packing involving the regular hydrogen bonds and the C–H…O/N interactions in the crystal structures of several *N*-glycoprotein models and analogs showed a cooperative anti-parallel network of bifurcated hydrogen bonds consisting of N–H…O and C–H…O interactions seen uniquely for the model, GlcNAcβNHAc (**1**), and not for any analog including the propionamide derivative, GlcNAcβNHPr (**2**). [6, 7]. The combined application of X-ray crystallography and *ab initio* quantum chemical calculations of the model, GlcNAcβNHAc (**1**), and several *N*-(β-D-glycopyranosyl)alkanamides and haloacetamides has shown that the *N*-acetyl group at C2 controls χ_2 at the linkage region and retains the extended aglycon conformation [8]. In the present study, *N*-(3-deoxy-3-acetamido-β-D-glycopyranosyl)alkanamides (Fig. 2) have been chosen as newer analogs to find out how the conformation of the *N*-glycosidic linkage would be affected if the *N*-acetyl group is moved from C2 to C3 position. Herein we report the synthesis and X-ray crystallographic investigation of *N*-(3-deoxy-3-acetamido-β-D-glycopyranosyl) alkanamides, **3** and **4**.

Materials and methods

Materials

Thin-layer chromatograms were performed on 25 mm E. Merck silica gel plates (60F-254). Detection was done by spraying the plates with 10% sulfuric acid in ethanol and heating on a hot plate. Optical rotations were measured at 30°C on a JASCO- DIP 200 digital polarimeter using a cell of 10 mm length. NMR spectra were recorded on a Bruker AV400 spectrometer. ESI-MS spectra were measured on a Micromass Q-Tof mass spectrometer.

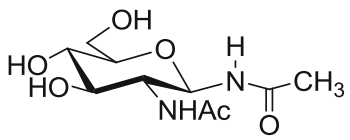
Methods

Synthesis of 1,2:5,6-di-O-isopropylidene-3-deoxy-3-acetamido-α-D-glucofuranose (6)

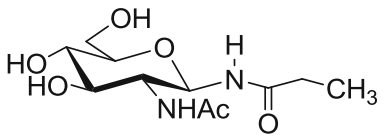
1,2,5,6-Di-*O*-isopropylidene-3-deoxy-3-azido-α-D-glucofuranose (**5**) [9] (0.7 g, 2.46 mmol) and Pd/C (70 mg) were taken in CH_2Cl_2 (3 mL) and the mixture was stirred at 30°C under H_2 atmosphere for 3 h. Pyridine (0.79 mL) and acetic anhydride (0.46 mL, 4.92 mmol) were then added at 0°C and stirring was continued overnight. The mixture was filtered through celite and washed with MeOH. Excess of pyridine and acetic anhydride was removed under vacuum. The residue obtained was recrystallized from ethyl acetate/hexane (1:1) to give title product in 68% yield.

Crystalline solid; mp 92–94°C (Lit [10] 94-96°C); $[\alpha]_D^{30}$ -43.8 (c 1.2, MeOH) (Lit [10] $[\alpha]_D^{30}$ -44.1° (c 1.2, $CHCl_3$)); IR (KBr, cm^{-1}): 3559, 3427, 3289, 3062, 2991, 2932, 2899, 1656, 1551, 1449, 1426, 1378, 1210, 1259, 1215, 1162, 1079, 1018, 951, 874, 847, 756, 629, 607; ^1H NMR ($CDCl_3$, 400 MHz): δ 6.48 (d, 1H, *J*=6.4 Hz, NH), 5.86 (d, 1H, *J*=3.6 Hz, H-1), 4.63 (d, 1H, *J*=3.6 Hz, H-2), 4.43-4.34 (m, 2H), 4.19 (dd, *J*=3.6 & 6.0 Hz, H-4), 4.13

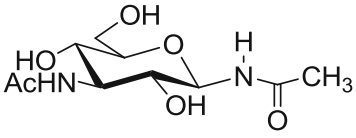
Fig. 2 *N*-Glycoprotein linkage region model (**1**) and analogs (**2**–**4**)



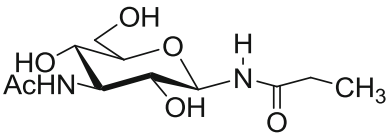
GlcNAcβNHAc (1)



GlcNAcβNHPr (2)

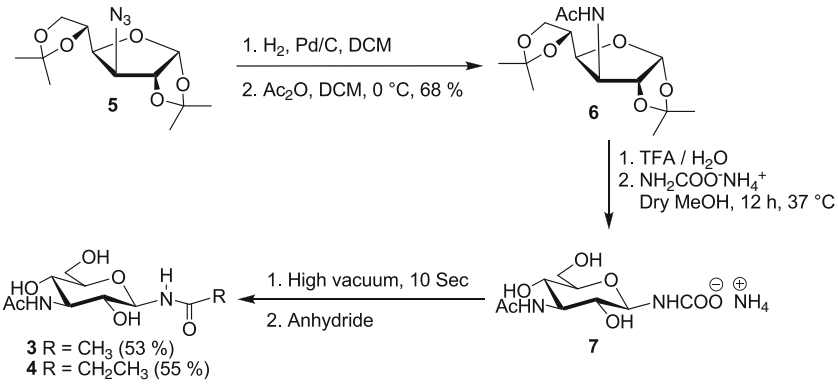


Glc3NAcβNHAc (3)



Glc3NAcβNHPr (4)

Scheme 1 Synthesis of C3 acetamido derivatives **3** and **4**



(dd, 1H, *J*=6.4 & 8.0 Hz), 3.84 (dd, 1H, *J*=6.8 & 8.0 Hz), 2.00 (s, 3H, -NHCOC$\underline{H}_3$), 1.51, 1.45, 1.38, 1.30 (4 s, 12H, 4 x -C$\underline{H}_3$).

General procedure for preparation of N-(3-deoxy-3-acetamido-β-D-glycopyranosyl) alkanamides

A solution of compound **6** (0.2 g, 0.65 mmol) in trifluoroacetic acid/H_2O (2:1, 3 mL) was stirred for 10 min after which the solvent was removed on a rotoevaporator. The resulting solid was dissolved in methanol (2 mL) and to this solution, ammonium carbamate (78 mg, 4 equiv) was added. The reaction mixture was stirred for 16 h at 37°C and then at 0°C for 1 h. The fluffy white precipitate, glycosylammonium carbamate, formed was filtered, washed twice with cold methanol and subjected to high vacuum for no more than 10 s. The glycosylamine thus obtained as a colorless solid was dissolved in dry MeOH (2 mL) and cooled to 0°C. Anhydride (0.98 mmol) was then added and the mixture was stirred overnight at RT. The reaction mixture was concentrated on a rotavaporator to dryness to obtain the alkanamides **3** and **4** in 53% and 55% of yield, respectively.

N-(3-Deoxy-3-acetamido-β-D-glycopyranosyl)acetamide (3) Crystalline solid; mp 225–227°C; $[\alpha]_D^{30}$ 15.5 (c 1,

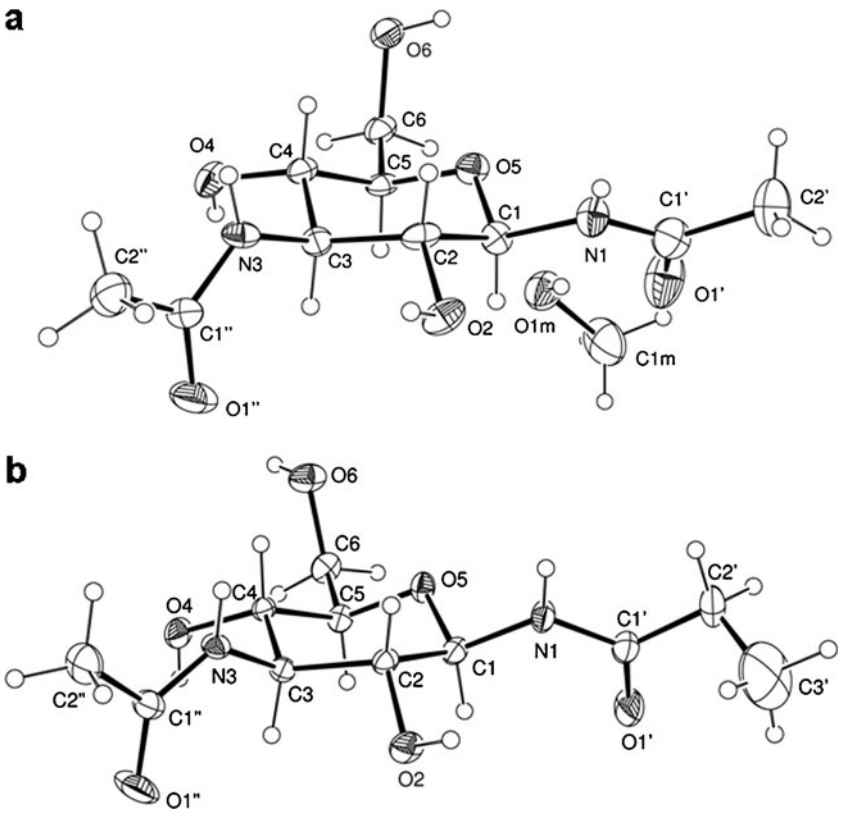


Fig. 3 ORTEP representation with atom numbering of **3** (a) and **4** (b). The ellipsoids are drawn at the 30% probability level

Table 1 Selected bond lengths (Å) and bond angles (°) of Glc3NAcβNHAc (**3**) and Glc3NAcβNHPr (**4**)

Parameter	Glc3NAcβNHAc (**3**)	Glc3NAcβNHPr (**4**)
C1–O5	1.407(8)	1.428(3)
C5–O5	1.441(8)	1.437(3)
C6–O6	1.441(9)	1.413(3)
C1–N1	1.415(10)	1.432(3)
C3–N3	1.432(9)	1.453(3)
C1′–N1	1.333(11)	1.339(3)
C1" –N3	1.349(10)	1.315(3)
C1′–O1′	1.229(10)	1.230(3)
C4–C5–C6	118.5(6)	114.5(2)
O5–C5–C6	107.5(6)	108.5(2)
O5–C1–N1	108.1(6)	107.6(2)
C2–C1–N1	110.4(6)	109.1(2)
N1–C1′–O1′	122.8(8)	121.6(3)

MeOH); IR (KBr, cm^{-1}): 3457, 3337, 3293, 2925, 2857, 1652, 1556, 1445, 1376, 1307, 1198, 1173, 1126, 1068, 1035, 902, 793, 718, 684, 599; ^{1}H NMR (D_2O, 400 MHz): δ 4.98 (d, 1H, *J*=9.2 Hz, H-1), 3.95-3.79 (m, 2H), 3.70 (dd, 1H, *J*=4.8 & 12.0 Hz), 3.62-3.51 (m, 1H), 3.47-3.34 (m, 2H), 2.03 (s, 6H, 2 x -NHCOC$\underline{H}_3$); ^{13}C NMR (D_2O, 100 MHz): δ 175.3, 174.9, 79.9 (C-1), 78.4, 70.1, 67.5, 60.4 (C-6), 58.0 (C-3), 22.2, 22.0; ESI-MS: calcd for $C_{10}H_{19}N_2O_6$: 263.1243. $[M+H]^+$: Found: 263.1248.

N-(3-Deoxy-3-acetamido-β-D-glycopyranosyl)propionamide (4) Crystalline solid; mp 91–93°C; $[\alpha]_D^{30}$ 15.0 (c 1, H_2O); IR (KBr, cm^{-1}): 3277, 2965, 2921, 2869, 1659, 1563, 1435, 1380, 1336, 1279, 1038, 918, 886, 769, 734, 683, 612, 567; ^{1}H NMR (D_2O, 400 MHz): δ 4.97 (d, 1H, *J*=9.2 Hz, H-1), 3.93-3.78 (m, 2H), 3.67 (dd, 1H, *J*=5.2 & 12.4 Hz), 3.60-3.51 (m, 1H), 3.45-3.33 (m, 2H), 2.27 (m, 2H, -C$\underline{H}_2$-), 2.0 (s, 3H, -NHCOC$\underline{H}_3$), 1.06 (t, *J*=7.6 Hz, -C$\underline{H}_3$); ^{13}C NMR (D_2O, 100 MHz): δ 179.2, 174.9, 79.9 (C-1), 78.5, 70.1, 67.5, 60.5 (C-6), 58.1 (C-3), 29.0 (−C$\underline{H}_2$-) 22.2 (−CO$\underline{C}H_3$), 9.0 (−$\underline{C}H_3$); ESI-MS: calcd for $C_{11}H_{21}N_2O_6$: 277.1400. $[M+H]^+$: Found: 277.1413.

Crystal structures solution and refinement: Single crystals of compound **3** and **4** were obtained from methanol: water mixture at room temperature by the slow evaporation method. X-ray data collection was performed with Bruker AXS Kappa Apex II CCD diffractometer equipped with graphite monochromated Mo (Kα) (λ=0.7107Å) radiation. Crystal fixed at the tip of the glass fiber was mounted on the goniometer head with the aid of video microscope. The automatic cell determination routine, with 32 frames at three different orientations of the detector was employed to collect reflections and the program APEX2-SAINT (Bruker, 2004) was used for finding the unit cell parameters. Four-fold redundancy per reflection was utilized for achieving good absorption correction using multi-scan procedure. Besides absorption, Lorentz polarization and decay correction were applied during data reduction. The program SADABS [11] was used for absorption correction using multi-scan procedure. The structures were solved by direct methods using SIR92 (WinGX) and refined by full-matrix least squares techniques using SHELXL-97 [12] computer programs.

All hydrogen atoms except the nitrogen hydrogen were fixed at chemically meaningful positions and riding model refinement was applied. The nitrogen hydrogen was located through difference Fourier map and refined with isotropic thermal parameters. Molecular graphics were drawn using ORTEP32 [13] and Mercury programs [14].

Results and discussion

Synthesis of *N*-(3-deoxy-3-acetamido-β-D-glucopyranosyl) alkanamides (3–4)

The *N*-(3-deoxy-3-acetamido-β-D-glycopyranosyl)alkanamides, **3**–**4**, were planned to be synthesized starting from the commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. The reported literature procedure [9] was employed for converting 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose to the C3 azido sugar (**5**) (Scheme 1). Reduction of **5** using Pd/C under hydrogen atmosphere followed by *N*-acetylation of the resultant amine using acetic anhydride/pyridine gave the C3 acetamide (**6**). Treatment of **6** with aq. triflouroacetic acid furnished the deprotected C3 acetamide, which was then reacted with ammonium carba-

Table 2 Selected torsion angles of Glc3NAcβNHAc (**3**) and Glc3NAcβNHPr (**4**)

Torsion angle	ϕ_N	ψ_N	χ_2	ω	Reference
GlcNAcβNHAc (**1**)	−89.8(1)	174.2(2)	-	−66.4(2)	[4]
Glc3NAcβNHAc (**3**)	−78.4(9)	177.1(8)	-	−61.6(8)	This work
GlcNAcβAsn.3H_2O	−98.9	180.0	−172.2	−60.42	[3]
GlcNAcβNHPr (**2**)	−91.0(5)	172.5(5)	172.4(6)	−66.0(4)	[5]
Glc3NAcβNHPr (**4**)	−99.4(3)	−176.6(3)	107.5(4)	−67.8(3)	This work
GlcβNHPr	−89.3(6)	166.5(6)	114.7(8)	−68.6(5)	[5]

Table 3 Regular hydrogen bond parameters for Glc3NAcβNHAc (**3**) and Glc3NAcβNHPr (**4**)

D–H…A	H…A (Å)	D…A (Å)	D–H…A(°)	Symmetry
Glc3NAcβNHAc (**3**)				
N1–H1N…O4	2.16(2)	2.990(9)	166(7)	-x+2, y+1/2, -z+3/2
N3–H3N…O1”	2.085(18)	2.929(10)	171(9)	x+1, y, z
O2–H2O…O6	1.88	2.669(7)	162.6	-x+2, y+1/2, -z+3/2
O4–H4O…O2	1.91	2.727(7)	172.4	-x+1, y-1/2, -z+3/2
O6–H6O…O1M	1.91	2.713(8)	167.3	x+1, y, z
O1M–H4M…O1’	2.05	2.811(9)	153.4	x, y, z
Glc3NAcβNHPr (**4**)				
N1–H1N…O2	2.21(3)	3.060(3)	163(3)	1+x, y, z
N3–H3N…O1”	1.97(3)	2.724(3)	152(3)	1+x, y, z
O2–H2O…O1’	1.87	2.677(3)	168.6	-x, 1/2+y, 1/2-z
O4–H4O…O6	1.90	2.698(3)	164.6	−1+x, y, z
O6–H6O…O4	1.91	2.714(2)	165.5	-x, 1/2+y, -z+1/2

mate to give the intermediate glycosylammonium carbamate salt (**7**). The free glycosylamine, obtained by decomposition of the salt, was reacted with appropriate acid anhydride to afford the newer analogs **3** and **4** in 53 & 55% overall yield (starting from **6**), respectively. These two alkanamides were fully characterized based on physical and spectral methods including 2 dimensional NMR and ESI-MS. The β-anomeric configuration of **3** and **4** was evident from the H-1 proton coupling constant value of 9.2 Hz observed in both cases.

Structure description

Alkanamides **3** and **4** were crystallized from aqueous methanol. Analog **3** crystallized with one molecule of methanol whereas **4** crystallized in the anhydrous state. Both compounds crystallize in the $P2_12_12_1$ space group. ORTEP representations of the structures with atom numbering are shown in Fig. 3. Selected list of bond lengths and bond angles is provided in Table 1. Comparison of pyranose ring C–O bond lengths shows that C1–O5 bond length is shorter than the C5–O5, which was also observed in other β-1-*N*-alkanamido sugar derivatives including GlcNAcβAsn [3] & GlcβAsn [15]. The C1–N1 and C3–N3 bond distances are in the range of 1.41–1.45 Å. The C–N bond lengths (C1’–N1 and C1”–N3) of amide groups are in the range of 1.31-1.34 Å due to partial double bond character. Alkanamides **3** and **4** adopt $^4C_1$ conformation. The hydoxymethyl group in both **3** and **4** takes up gauche-gauche (gg) [16] conformation, which is commonly observed in the solid state structure of D-glucose derivatives.

Linkage region conformation

The conformation of the *N*-glycosidic linkage is defined by the torsion angles, ϕ_N ψ_N and χ_2 (Fig. 1). Selected torsion angles of compounds **1**–**4** are presented in Table 2. The ϕ_N values of C3 acetamido analogs **3** and **4** are determined to be −78.3°, −99.4°, respectively, and the difference of 21° is noteworthy. This is in contrast to the negligible difference in the ϕ_N values of the corresponding C2 acetamido sugars **1** and **2** (−89.8° and −91.0°, respectively). The larger rotational freedom around C1-N1 bond (ϕ_N) available in **3** and **4** in view of the absence of C2 acetamido group could account for the these observations. The ψ_N values of **3** and **4** are comparable and taken together with the ϕ_N values reveal the

Table 4 C-H···O hydrogen bonding parameters for Glc3NAcβNHAc (**3**) and Glc3NAcβNHPr (**4**)

D–H…A	H…A (Å)	D…A (Å)	D–H…A(°)	Symmetry
Glc3NAcβNHAc (**3**)				
C2’–H2’A…O1”	2.69	3.54	149	1-x, 1/2+y, 1.5-z
C2–H2…O4	2.67	3.33	125	2-x, 1/2+y, 1.5-z
C5–H5…O6	2.56	3.39	143	−1+x, y, z
C6–H6B…O2	2.66	3.40	134	1-x, -1/2+y, 1.5-z
Glc3NAcβNHPr (**4**)				
C2–H2…O1”	2.54	3.27	131	1+x, y, z
C4–H4… O1”	2.62	3.35	131	1+x, y, z

Table 5 Data collection and refinement statistics for Glc3NAcβNHAc (**3**) Glc3NAcβNHPr (**4**)

Parameter	Glc3NAcβNHAc (**3**)	Glc3NAcβNHPr (**4**)
Empirical Formula	C_{11} H_{22} N_2 O_7	$C_{11}H_{20}$ N_2O_6
Formula weight	294.31	276.29
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell Dimensions	a=4.8986(5) Å	a=4.9033(4) Å
	b=12.3614(17) Å	b=9.7021(10) Å
	c=24.068(3) Å	c=28.911(3) Å
Volume ($Å^3$)	1457.4(3)	1375.4(2)
Z, calculated density (Mg/m^3)	4, 1.341	4, 1.334
Absorption coefficient (mm^{-1})	0.112	0.109
F(000)	632	592
Crystal size (mm)	0.25×0.22×0.20	0.25×0.22×0.10
Theta range (°)	1.69 to 20.08	2.21 to 29.14
Index ranges	−3<=h<=4	−6<=h<=4
	−11<=k<=11	−12<=k<=12
	−20<=l<=23	−33<=l<=38
Reflections collected/unique	6106/3566	5887/2759
	[R(int)=0.0179]	[R(int)=0.0371]
Data/restraints/parameters	1370/0/186	2759/2/197
Goodness-of-fit on F^2	1.158	0.936
Final R indices [I>2sigma (I)]	R1=0.0663	R1=0.0486
	wR2=0.1891	wR2=0.0866
R indices (all data)	R1=0.0810	R1=0.0852
	wR2=0.2047	wR2=0.0997

Z-anti conformation of the amide aglycon moiety. The Z-*anti* conformation of the amide linkage has been observed in **1, 2** and GlcNAcβAsn [3].

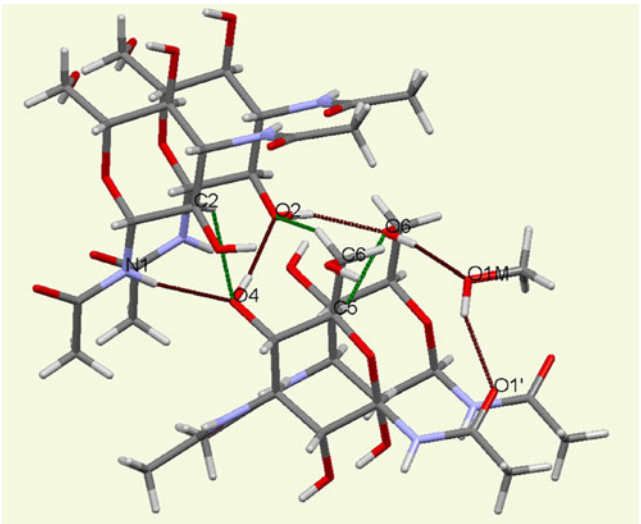


Fig. 4 Finite chain consisting of regular hydrogen bonds and C–H…O interactions observed in **3**

The χ_2 value of the C3 acetamido analog **4** with a free C2-OH group turns out to be 107.5°, which corresponds to a *gauche* conformation of the propionamido moiety, deviating significantly from the values of 174.4° and −172.2° reported earlier for the C2 acetamido compounds GlcNAcβNHPr **(2)** [5] and GlcNAcβAsn, respectively (Table 2). Satisfyingly, the reported χ_2 value (114.7°) of *N*-(β-D-glucopyranosyl)

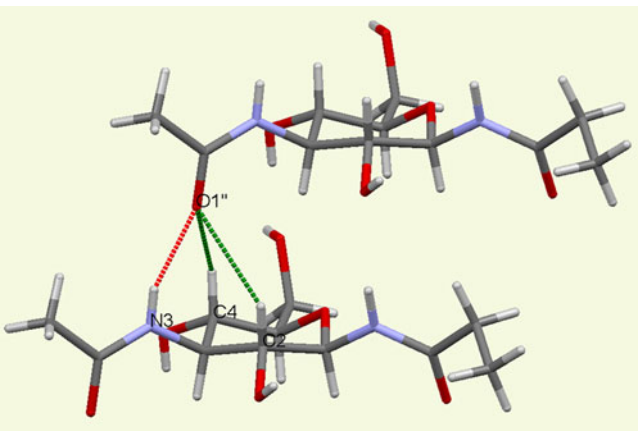


Fig. 5 Molecular packing showing a trifurcated hydrogen bond in **4**

propionamide (GlcβNHPr), which also lacks a C2 acetamido group, compares well with that of the C3 acetamido analog **4**. These observations lend further credence to our earlier finding that the role of C2-acetamido group in controlling the χ_2 and maintaining the extended (*anti*) conformation of the amido aglycon moiety.

Molecular packing

Detailed analysis of molecular packing in the crystal structures of the title compounds was then undertaken. The various hydrogen bonding parameters of compounds **3** and **4** are listed in the Tables 3 and 4. As mentioned earlier, the characteristic molecular assembly feature of the linkage region models GlcNAcβAsn and GlcNAcβNHAc (**1**) is the anti-parallel double-pillared network of bifurcated hydrogen bonds. This network consists of N1 & C2' acting as hydrogen donors for O1' in one direction and C1 & N2 acting as hydrogen donors for O1" (C2 amide carbonyl oxygen) in the opposite direction. Such a double-pillared network is missing in both the C3 acetamido analogs **3** and **4** due to lack of direct H-bonding between N1 and O1'. In the case of analog **3**, a finite chain of hydrogen bonds starts with N1 acting as donor, runs through O4, O2, O6 and O1M and ends with O1' as the acceptor. Branching of this chain occurs through C–H…O hydrogen interactions at O4, O2 and O6 with C2–H, C6–H6B and C5–H, respectively, rendering these three oxygen atoms tri-coordinated (Table 5) (Fig. 4).

The finite chain in the case of **4** is much shorter with O2 connecting N1–H to O1'. The infinite chain involving O4 and O6 as both donors and acceptors forms a homodromic cycle that stabilizes the molecular packing. On the other hand, N3–H and O1" of the C3 acetamido group in both **3** and **4** are directly bonded resulting in a single-pillared network. Further stabilization of this network is achieved by O1" serving as a bifurcated acceptor (C2'–H···O1``···H–N3) in **3** and more interestingly as a trifurcated acceptor for N3–H, C2–H and C4–H in **4** (Fig. 5).

Conclusions

Comparison of the crystal structures of C3 acetamido analogs **3** and **4** solved during the present study with those of C2 acetamido alkanamides **1** and **2** reported earlier has brought to light interesting differences in both their *N*-glycosidic linkage conformation and molecular packing. The difference of 21° noted between the ϕ_N values of the β-1-*N*-acetamido- and propionamido derivatives (**3** and **4**) of C3 acetamido sugar, as compared to the negligible difference seen for the corresponding derivatives of GlcNAc (C2 acetamido), suggests greater rotational freedom around C1–N1 bond in the C3 acetamido sugars. The χ_2 value of the C3 acetamido analog **4** with a free C2-OH group turns out to be 107.5°. This value corresponds to a *gauche* conformation of the propionamido moiety and comparable to the reported χ_2 value (114.7°) of *N*-(β-D-glucopyranosyl)propionamide (GlcβNHPr), which also lacks a C2 acetamido group. These observations strengthen our earlier finding [5] on the role of C2 acetamido group of GlcNAcβAsn in controlling the χ_2 and maintaining the extended (*anti*) conformation of the amido aglycon moiety. The occurrence of GlcNAc with an additional NHAc group at C3 as the proximal sugar in the linkage region of *N*-glycoproteins of certain bacteria has recently been reported [17]. The structural knowledge gained from the present work would thus be valuable for the modeling of such rare glycoconjugates.

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Supplementary Data Complete structural data of the Glc3NAcβNHAc (**3**) and Glc3NAcβNHPr (**4**) have been deposited at the Cambridge Crystallographic Data Centre (CCDC # 840488 – 840489, respectively), and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif (or from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; or email: deposit@ccdc.cam.ac.uk).

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