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Note

Unusual conformational behavior of trisaccharides containing *N*-acetylglucosamine

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Abstract—Protected trisaccharides containing *N*-acetylglucosamine can adopt unexpected conformations through the formation of hydrogen bonds involving the amide group. This conformational behavior was observed by NMR spectroscopy when three protected trisaccharides were dissolved in deuterated chloroform and to a lesser extent in deuterated dichloromethane. In contrast, NMR spectra of the same analogues acquired in the hydrogen bond-accepting solvents deuterated acetonitrile and dimethylsulfoxide showed that the *N*-acetylglucosamine residues adopted the expected $^4C_1$ conformation.

Keywords: GlcNAc conformations; Non-hydrogen bonding solvents; Unusual conformations; GlcNAc-containing intermediates; Lewis A analogues

The natural occurrence of numerous glycosides of *N*-acetylglucosamine in biologically important oligo- and polysaccharides such as bacterial polysaccharides and blood group antigens[1–4] often necessitates the efficient chemical synthesis of 2-amino-2-deoxyglycopyranosides. Thus, we have recently reported[5,6] the preparation of rhamnosylated and fucosylated trisaccharides **1**–**3** as well as that of the *N*-methyl imidate **4** and the N,N-diacetylated analogue **5**. Having on hand additional trisaccharides containing an *N*-acetylglucosamine residue, we further investigated whether the unusual conformational behavior that we have communicated[5] for trisaccharide **1** was also observed in trisaccharides **2** and **3**.

Indeed the ^{1}H NMR spectra measured for **2** and **3** dissolved in $CDCl_3$ showed similar unusual features to those that we have reported[5] for **1**. The coupling

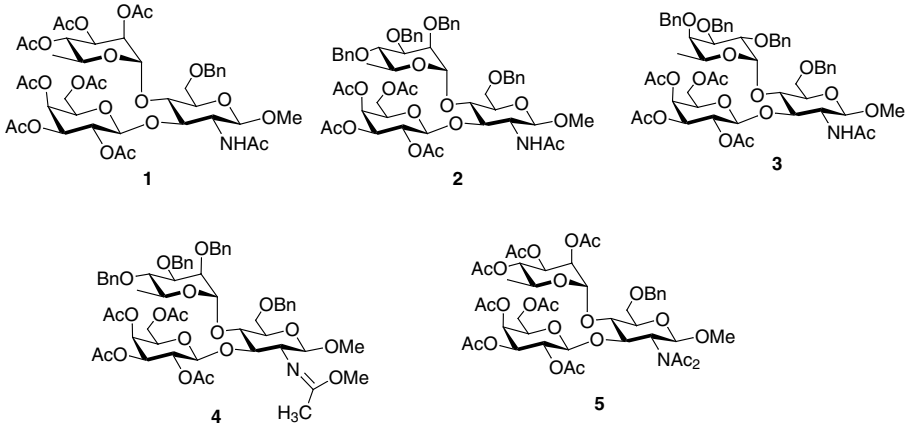

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Table 1. NMR data measured (400 MHz) at 298 K for **1**–**3** (10 mM) in deuterated solvents

Entry	Compound	Solvent	$J_{H\text{-}1,H\text{-}2}$ (Hz)	$J_{C\text{-}1,H\text{-}1}$ (Hz)	δ NH (ppm)
1	**1**	$CDCl_3$[a]	4.2/4.1[b]	166/166[b]	5.85/5.86[b]
2	**1**	CD_2Cl_2[c]	5.1	165	5.74
3	**1**	CD_3CN[d]	7.2	161	6.47
4	**1**	DMSO-d_6[e]	8.0	161	7.90
5	**2**	$CDCl_3$[a]	4.3/4.3[b]	166/166[b]	5.74/5.76[b]
6	**2**	CD_2Cl_2[c]	5.5	165	5.64
7	**2**	CD_3CN[d]	7.6	161	6.69
8	**2**	DMSO-d_6[e]	7.3	161	7.83
9	**3**	$CDCl_3$[a]	2.7/2.7[b]	167/167[b]	6.67/6.69[b]
10	**3**	CD_2Cl_2[c]	4.3	165	6.23
11	**3**	CD_3CN[d]	7.5	160	6.44
12	**3**	DMSO-d_6[e]	7.9	160	7.88

[a] Dielectric constant[23] ε_r 4.89.
[b] $CDCl_3$ passed on a column of basic alumina.
[c] Dielectric constant[13] ε_r 8.93.
[d] Dielectric constant[23] ε_r 35.94.
[e] Dielectric constant[23] ε_r 46.45.

constants $J_{H\text{-}1,H\text{-}2}$ and $J_{C\text{-}1,H\text{-}1}$ (Table 1, entries 1, 5, and 9) measured for the glucosamine ring in **1**, **2**, and **3** were, respectively, smaller (2.7–4.2 Hz) and bigger (166–167 Hz) than the values one would expect for a $^4C_1$ conformation of the β-glucosamine unit.[7] In contrast, ^1H NMR spectroscopy of trisaccharide methyl imidate **4** and N-diacetylated trisaccharide **5** in $CDCl_3$ showed $J_{H\text{-}1,H\text{-}2}$ coupling constants of 8.5 and 7.7 Hz, respectively, which were consistent with the expected $^4C_1$ chair conformation for the glucosamine ring. Therefore, it appears that the N-acetylated trisaccharides **1**–**3** have a different conformational behavior in $CDCl_3$ than the trisaccharides **4** and **5** not carrying an NH group at C-2 of the glucosamine. To assess whether residual traces of acid commonly found in $CDCl_3$ could be the origin of these unusual conformations, NMR spectra were recorded for 10 mM solutions of compounds **1**–**3** in $CDCl_3$ that had been previously passed through a small column of basic alumina. As can be seen in Table 1 (entries 1, 5, and 9) these measurements were virtually identical to those that were recorded in untreated $CDCl_3$ thus showing that the unusual conformations observed for **1**–**3** were not caused by residual traces of acid in the NMR solvent.

NMR spectra collected for concentrations of **1**, **2**, and **3** in $CDCl_3$ (Fig. 1) down to ~10 mM showed a linear positive concentration dependence of δ_{NH}, which is usually indicative of intermolecular H-bonds.[8–11] However, further decrease of the concentration from 10 mM down to 0.1 mM no longer showed variation in the δ_{NH} supporting the notion that intermolecular hydrogen bonds no longer existed at these concentrations. In contrast, the coupling constants measured between H-1 and H-2 remained the same within experimental error (±0.2 Hz) at all concentrations for each compounds **1**–**3**. These results support that the conformational distortion of the glucosamine ring in compounds **1**–**3** was not concentration dependent and therefore unlikely due to the formation of intermolecular hydrogen bonds.

NMR experiments at decreasing temperatures (down to −55 °C) were recorded for 10 mM solutions of compounds **1**–**3** in $CDCl_3$, a concentration at which we have shown (above) that intermolecular H-bonding did not take place. These NMR experiments showed a linear negative temperature dependence of the δ_{NH} chemical shift for all three compounds (Fig. 2), which suggests that at this concentration the NH group is involved in the formation of intramolecular hydrogen bonds.[8] For all three derivatives, the coupling constant measured between H-1 and H-2 became smaller with decreasing

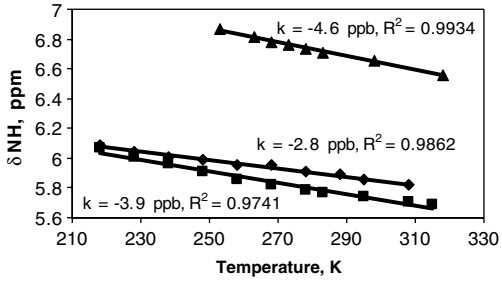

Figure 2. Temperature dependence of δ (NH) measured by ^1H NMR (400 MHz) for 10 mM solutions of **1** (♦), **2** (▲), and **3** (■) in $CDCl_3$.

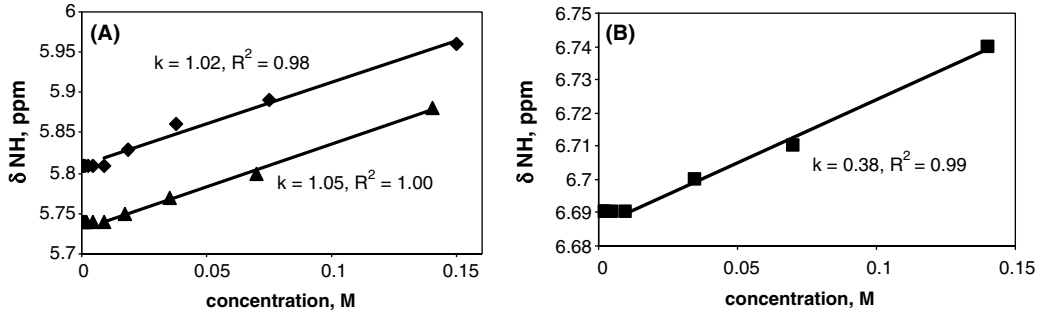

Figure 1. Concentration dependence of δ (NH) measured by ^1H NMR (400 MHz) for compound **1** (♦), **2** (▲) (A), and **3** (B, ■) in $CDCl_3$.

temperatures and signal broadening eventually prevented its accurate measurement. These observations suggest that compounds **1**–**3** did not adopt one single more stable conformation in $CDCl_3$ but rather multiple conformations in fast equilibrium with each other on the NMR time scale.

The ^1H NMR spectra of **1**, **2**, and **3** dissolved (10 mM) in the slightly more polar solvent CD_2Cl_2 showed larger $J_{H\text{-}1,H\text{-}2}$ and smaller $J_{C\text{-}1,H\text{-}1}$ coupling constants than those measured in $CDCl_3$ (Table 1, entries 2, 6, and 10). Nevertheless, these coupling constants were still smaller and larger, respectively, than one would expect for a $^4C_1$ conformation of the glucosamine ring. In contrast, ^1H NMR spectroscopy in hydrogen bond-accepting solvents CD_3CN (Table 1, entries 3, 7, and 11) and DMSO-d_6 (Table 1, entries 4, 8, and 12) gave $J_{H\text{-}1,H\text{-}2}$ and $J_{C\text{-}1,H\text{-}1}$ coupling constants that were consistent with a $^4C_1$ conformation of the glucosamine residue. These results further supported that the conformational behavior of the *N*-acetamido trisaccharides **1**, **2**, and **3** in $CDCl_3$ resulted from the formation of intramolecular hydrogen bonds involving the glucosamine NH group. These hydrogen bonds were disrupted when the compounds were dissolved in more polar solvents such as CD_2Cl_2 and transferred to the solvent when using a strong hydrogen bond-accepting solvent such as CD_3CN or DMSO-d_6. Because the NH chemical shift in $CDCl_3$ is found at lower field than when measured in CD_2Cl_2 for all three compounds, we can conclude that this hydrogen is more strongly H-bonded in $CDCl_3$ than in the more polar solvent CD_2Cl_2. For compounds **1** and **2**, the δ_{NH} measured in $CDCl_3$ or CD_2Cl_2 is found at higher field than when measured in CD_3CN or DMSO-d_6 suggesting that the NH group is more strongly hydrogen bonded by acetonitrile or dimethylsulfoxide than it is in the distorted conformations. In contrast, compound **3**, which is the most strongly distorted in chloroform (i.e., giving the smallest coupling constant $J_{H\text{-}1,H\text{-}2}$), showed a δ_{NH} at lower field in $CDCl_3$ than in CD_3CN. Therefore, the greater conformational distortion seen for this compound in chloroform may result from the NH group being involved in a stronger hydrogen bond than that formed in the distorted conformations formed by compounds **2** and **3** in $CDCl_3$. Additional ^1H and ^{13}C NMR data for compounds **1**–**3** in $CDCl_3$, CD_2Cl_2, CD_3CD, and DMSO-d_6 are listed in Tables 2 and 3.

It has been well established that the value of the coupling constant between two vicinal protons cannot be accurately calculated solely using the original Karplus[12] equation without considering the electronegativities of the substituents.[13–16] However, we can safely assume that the $J_{H\text{-}1,H\text{-}2}$ coupling constants measured in $CDCl_3$ for **1**, **2**, and **3** resulted from these trisaccharides adopting conformations in which the glucosamine residue is distorted leading to a dihedral angle $\phi_{H\text{-}1,H\text{-}2}$ smaller than the 180° expected for a $^4C_1$ chair. Measurement of the coupling constants between other vicinal glucosamine hydrogens was more difficult due to extensive signal overlap of the ring hydrogens (Table 2). However, when measurable, the $J_{H\text{-}2,H\text{-}3}$ also supported a dihedral angle between H-2 and H-3 smaller than 180° (Table 2, compounds **1** and **2** in $CDCl_3$). In contrast, the coupling constants that could be measured between H-3 and H-4 as well as between H-4 and H-5 for compound **1** in $CDCl_3$ supported a dihedral angle between these hydrogens that was closer to the expected 180°. Thus, in a first approximation mostly based on the coupling constants that were measured for compound **1**, we concluded that the glucosamine ring was distorted around the C1–C2 and C2–C3 bonds. In an attempt to better define these conformational distortions, 2D NOESY experiments (600 MHz) were acquired for each of the compounds **1**–**3** in $CDCl_3$ and CD_2Cl_2 at six mixing times (300–800 ms, 100 ms increments). Initial linear positive NOE build up rates were used to evaluate average inter proton distances. These distances where calculated using an internal reference NOE cross-peak corresponding to a known distance and using the isolated spin pair approximation: $r_A = (I_B/I_A)^{1/6} r_B$ where I_B and r_B are, respectively, the cross-peak intensity and internuclear distances of the reference pair of nuclei.[17] The values obtained were plotted against the mixing time and linear regression with extrapolation to zero gave the distances reported in Table 4 with an experimental error estimated to be less than 10%.[18] As can be seen from this table, the distance measured from NH to H-1 as well as NH to H-2 supported, as expected,[9] a preference for a *Z-anti* orientation of the NHAc moiety in both solvents. The intra-glucosamine distances obtained for trisaccharides **1** (Table 4, entries 1 and 2, first 5 columns) and **2** (Table 4, entries 3 and 4, first 5 columns) supported conformations around the glucosamine ring similar for both compounds. However, signal overlap that prevented accurate distance measurement did not allow conclusions to be made on the conformation of the glucosamine ring in compound **3**. Interestingly, the distance measured between NH and H-5 in compounds **1** and **2** (entries 1-4) was much shorter ($\sim$ 2.9 Å) than the distance one would expect for these two hydrogens in a *Z-anti* $^4C_1$ conformation (Table 4, entry 7) or in a *Z-anti* $^1C_4$ conformation (Table 4, entry 9). Thus, we concluded that these trisaccharides did not exist in conformational equilibrium between the $^4C_1$ and $^1C_4$ conformations around the glucosamine ring. Therefore, the conformational behavior of trisaccharides **1**–**3** does not follow that of the branched protected trisaccharides described by Bock et al.[19] In this work, it was reported that the central glucose residue in a series of 2,3-branched protected trisaccharides adopted a $^1C_4$ conformation in equilibrium with the expected $^4C_1$ conformation. This conformational behavior was shown[19] to result from the anomeric effect that prompted the

Table 2. ^{1}H NMR chemical shifts and coupling constant for compounds **1** and **2** solubilized in $CDCl_3$, CD_2Cl_2, CD_3CN, and DMSO-d_6

Proton, ppm[a]	Compound **1**				Compound **2**				Compound **3**			
J, Hz[b]	$CDCl_3$	CD_2Cl_2	CD_3CN	DMSO	$CDCl_3$	CD_2Cl_2	CD_3CN	DMSO	$CDCl_3$	CD_2Cl_2	CD_3CN	DMSO
β-D-GlcNAc												
H-1	4.62	4.56	4.29	4.24	4.52	4.49	4.28	4.23	4.55	4.47	4.22	4.20
H-2	3.81	3.78	3.83	3.69	3.80	3.75	3.80	3.70	4.01	3.90	3.83	3.70
$J_{2,3}$	3.7	5.0	8.7	9.2	4.3		7.9					
H-3	3.99	3.98	3.86	3.80	3.71	3.91(a)	3.76	3.69	3.87(b)	3.88(c)	3.78	3.70
$J_{3,4}$	6.6	8.0	8.7	9.2	4.8		8.6	9.0		5.5		9.1
H-4	4.17	4.08	3.77	3.60	3.96	3.63(a)	3.66	3.53	4.02(b)	3.97(c)	3.66	3.50
$J_{4,5}$	6.6	8.0		9.5	4.8		8.6	9.0				9.1
H-5	3.71	3.65	3.47	3.54	3.90	3.91	3.41	3.45	4.13	3.88	3.38	3.44
H-6	3.71	3.72	3.72	3.66	3.65	3.63.	3.69	3.63	3.73	3.69	3.65	3.58
H-6′	3.71	3.72	3.72	3.66	3.65	3.68	3.69	3.63	4.01	3.91	3.89	3.86
NH	5.84	5.73	6.47	7.88	5.74	5.64	6.48	7.84	6.66	6.23	6.44	7.89
β-D-Gal												
H-1	5.07	5.04	4.97	4.95	4.79	4.81	4.86	4.89	4.76	4.79	4.89	4.94
$J_{1,2}$			7.7		8.0	8.2	7.5		8.1	8.0	8.0	
H-2	5.17	5.10	5.11	4.95	5.15	5.10	5.04	4.87	5.16	5.12	5.07	4.94
$J_{2,3}$			10.5		10.5	10.5	10.5	10.3	10.5	10.5		
H-3	5.06	5.01	5.06	5.04	4.99	4.96	5.00	4.95	5.01	4.99	5.07	5.01
$J_{3,4}$	3.33	3.5	3.3	3.6	3.5		3.2	2.8	3.5	3.4	2.3	3.6
H-4	5.40	3.38	5.37	5.26	5.35	5.34	5.29	5.18	5.39	5.39	5.38	5.25
$J_{4,5}$		1.1	1.2	nd			1.2			0.8		
H-5	3.99	3.98	4.09	4.16	3.90	3.91	4.03	4.05	3.87	3.92	4.05	4.10
H-2	4.23	4.11	4.21	4.04	4.14	4.11	4.11	4.05	4.06	4.08	4.06	4.00
H-6′	4.09	4.20	4.21	4.14	4.14	4.18	4.26	4.16	4.09	4.08	4.15	4.00
α-L-Rha for **1** and **2**, α-L-Fuc for **3**												
H-1	5.06	4.90	4.93	4.83	4.90	4.95	4.98	4.90	5.24	5.17	5.01	4.91
$J_{1,2}$	1.0	1.2	1.2	1.4	1.5	2.0	1.5		3.9	3.8	2.8	
H-2	5.17	5.17	5.20	5.10	3.74	3.80	3.90	3.89	4.13	4.06	3.95	3.86
$J_{2,3}$				3.4	2.8	2.7	3.0	2.8	10.0	10.1		
H-3	5.17	5.13	5.18	5.02	3.66	3.75	3.83	3.73	3.82	3.85	3.85(d)	3.86
$J_{3,4}$			10	10.1	9.0	9.2	9.3	9.1	2.7	2.5		
H-4	5.11	5.05	5.05	4.91	3.62	3.56	3.51	3.41	3.71	3.75	3.95(d)	3.77
$J_{4,5}$			10	10.1	9.0	9.2	9.3					
H-5	4.30	4.45	4.76	4.75	3.93	4.11	4.46	4.43	4.02	4.25	4.80	4.76
$J_{5,6}$	6.1	6.2	6.2	6.2	6.2	6.2	6.1	6.1	6.5	6.4	6.4	6.5
H-6	1.29	1.25	1.25	1.17	1.33	1.30	1.29	1.19	1.18	1.22	1.17	1.29

(a)–(d): Assignments might be reversed.

[a] Chemical shifts assigned from 600 MHz NMR spectra.

[b] Only unambiguous coupling constants are listed.

β-D-glucose ring to achieve an axial orientation of the electronegative anomeric substituent by adopting a $^1C_4$ conformation.

Because our experimental data did not support a conformational equilibrium between the $^4C_1$ and $^1C_4$ conformations around the glucosamine ring, we embarked on a search of the conformations that a fully protected *N*-acetylglucosamine ring may adopt in the gas phase. Thus, using the MOE software suite,[20] we carried out a systematic conformational search on methyl 2-acetamido-3,4,6-trimethyl-2-deoxy-β-D-glucopyranoside. The resulting conformations were minimized with Amber94[21] and, arbitrarily, only those conformations within 10 kcal/mol of the global minimum were examined. Of the 339 conformations obtained only 111 had a dihedral angle between H-1 and H-2 of less than 155°. Amongst these conformations we retained only those 47 conformations in which the NH group was close to both H-5 and H-1 (distances <3 Å). Figure 3 shows that these 47 conformations fell into two major conformational families distorted around the C1–C2 and C2–C3 linkages. These two families are quite similar and can be described as $^1S_5$-like conformations (Fig. 3C) for Family A and $^3S_5$-like conformations (Fig. 3D) for Family B. As shown in Table 4 both families (entries 10 and 11) led to a distance between H-1 and H-3 longer than that found in $^4C_1$ conformations (entries 7 and 8). As reported in Table 5, the dihedral angles measured in both families between H-2 and H-3 differed by more than 60° from the trans-diaxial angle that is measured in a $^4C_1$ conformation while the H-3–H-4 and H-4–H-5 dihedral angles only deviate by 10–60° from a trans-diaxial arrangement. Therefore, both conformational families are in good agreement with the experimental

Table 3. ^{13}C NMR chemical shifts and coupling constant for compounds **1** and **2** dissolved in $CDCl_3$, CD_2Cl_2, CD_3CN, and DMSO-d_6

Carbon, ppm[a]	Compound **1**				Compound **2**				Compound **3**			
	$CDCl_3$	CD_2Cl_2	CD_3CN	DMSO	$CDCl_3$	CD_2Cl_2	CD_3CN	DMSO	$CDCl_3$	CD_2Cl_2	CD_3CN	DMSO
β-D-GlcNAc												
C-1	99.7	99.8	102.4	101.7	100.2	100.2	102.6	101.31	100.8	100.6	102.7	101.2
C-2	53.5	54.1	55.9	54.8	~52	53.8	55.7	54.2	49.8	51.4	55.6	54.1
C-3	77.3(a)	77.6(c)	78.6	77.7	74.8	76.5(f)	78.6	77.4	75.0(h)	75.7(i)	78.4	77.9
C-4	72.8	73.0	74.6	74.4	72.7	74.3(f)	75.2	74.0	69.4(h)	70.0(i)	73.3	72.3
C-5	73.4	73.2	75.1	74.2	75.5(e)	73.1(f)	75.8	74.3	76.1	71.6(i)	75.6	74.2
C-6	68.8	68.4	69.4	68.8	69.6	69.1	69.7	68.5	69.8	68.9		67.5
α-L-Rha for **1** and **2**, α-L-Fuc for **3**												
C-1	96.4	96.5	97.8	97.0	97.7	97.8	99.3	97.8	93.1	94.2	98.1	96.8
C-2	70.8(b)	70.4(d)	71.1	70.2	74.8	75.5	77.3	75.8	70.8	75.8	80.7(j)	79.3(l)
C-3	70.6(b)	69.8(d)	70.2	70.9	79.6	79.7	81.0	79.7	79.9	79.5	79.0	74.3(l)
C-4	70.0(b)	68.9(d)	71.7	71.0	80.1	80.0	81.1	79.7	77.1	77.5	76.7(j)	77.5
C-5	67.0	66.2	67.0	65.9	70.7(e)	68.2	68.8	67.3	66.9(h)	66.6	67.1	65.6
C-6	17.2	16.8	17.8	16.8	17.9	17.9	18.3	17.5	16.7	16.2	17.2	16.4
β-D-Gal												
C-1	99.0	99.2	101.1	100.16	99.4	99.4	101.1	99.7	99.5	99.5	101.4	99.8
C-2	69.1(b)	67.8	69.0	68.3	68.4	68.0	69.0	67.8(g)	68.3	67.8	71.8(k)	68.0
C-3	68.3(b)	70.6	71.7	69.2	70.9	70.6	71.8	70.6(g)	70.8	70.5	69.2(k)	70.6
C-4	66.7	66.8	68.4	67.9	66.8	66.5	68.0	67.0	66.9	66.4	68.0	66.9
C-5	70.8(a)	70.5(c)	71.6	70.4	68.8(e)	70.4(f)	71.2	69.6	70.6	70.2	71.0	69.3
C-6	60.5	60.5	62.3	61.6	60.9	60.8	62.4	61.3	60.6	60.4	61.7	60.4

(a)–(l): Chemical shifts may be exchanged.
[a] Chemical shifts assigned using HSQC experiments.

Table 4. Measured distances in compounds **1**–**3** from NOE build-up curves[a] in $CDCl_3$ and CD_2Cl_2 solvents (entries 1–6) and within calculated conformations (entries 7–10)

Entry	Compound/conformation	Solvent	Inter-proton distances (Å)						
			NH-1G	NH-2G	NH-5G	NH-3R/F	NH-1Ga	1G-2G	1G-3G
1	**1**	$CDCl_3$	2.6	2.8	2.9	3.8	—[b]	2.6	3.6
2	**1**	CD_2Cl_2	2.7	2.6	2.9	>4	3.2	2.7	—[c]
3	**2**	$CDCl_3$	2.6	2.7	2.7	2.9	—[b]	2.8	—[c]
4	**2**	CD_2Cl_2	2.7	2.7	—[c]	—[c]	3.1	2.8	—[c]
5	**3**	$CDCl_3$	2.5	—[c]	—[c]	3.1	>4	—[c]	—[c]
6	**3**	CD_2Cl_2	2.7	—[c]	—[c]	—[c]	3.3	—[c]	—[c]
7	*Z-anti* $^4C_1$	—[d]	2.7	3.0	4.5	—[d]	—[d]	3.0	2.7
8	*Z-syn* $^4C_1$	—[d]	3.6	2.2	5.3	—[d]	—[d]	3.0	2.7
9	*Z-anti* $^1C_4$	—[d]	2.6	2.9	3.7	—[d]	—[d]	2.5	4.2
10	Figure 3A	—[d]	2.5–2.7	2.9	2.0–2.6	—[d]	—[d]	2.4–2.5	4.1–4.2
11	Figure 3B	—[d]	2.1–3.0	2.9	2.1–2.6	—[d]	—[d]	2.6–2.8	4.0–4.2

[a] All NOEs were positive, accuracy of the measured distances was estimated[18] to be 10%.
[b] NOE was not observed.
[c] NOE cross-peak that could support a short distance between these hydrogens was seen, an accurate distance could not be measured due to signal overlap.
[d] Not applicable.

evidence collected for compounds **1** and **2** in $CDCl_3$ and CD_2Cl_2. Finally, as can be seen in Table 4, these conformations gave distances from NH to H-5 as well as H-1 to H-3 that were, respectively, slightly shorter and longer than those experimentally measured for compounds **1** in $CDCl_3$. This may be the result of the $^4C_1$ conformation being also, albeit poorly, populated in these conditions.

Based on the experimental data presented above, we propose that the glucosamine ring in compounds **1**, **2**, and **3** adopted *Z-anti* $^1S_5$- or $^3S_5$-like conformations in $CDCl_3$ and CD_2Cl_2 that were to some extent in equilibrium with the usual $^4C_1$ conformation. The fact that higher energy conformations were observed likely resulted from the formation of intramolecular hydrogen bonds involving the *N*-acetamido group. However, none of the experimental data collected allowed us to conclude confidently, which electronegative atom was the bonding partner in forming this hydrogen bond. From the 2D-NOESY data (Table 4) it is interesting to notice

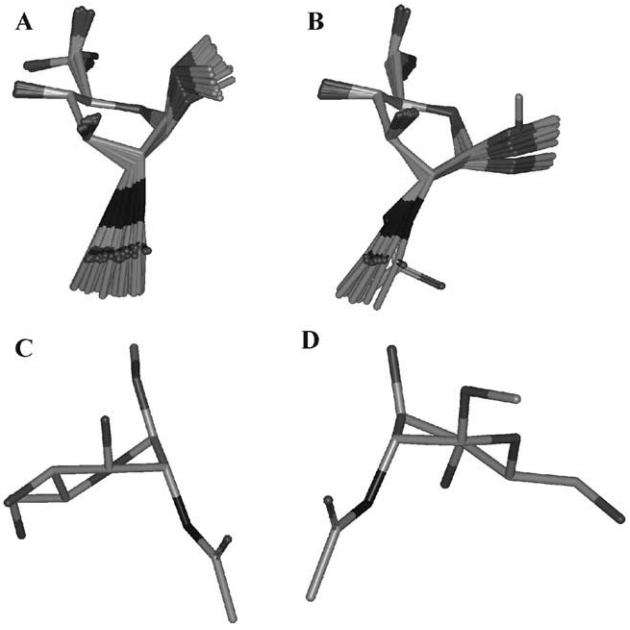

Figure 3. Conformations found by systematic search. **A**: Superposed conformations in Family A. **B**: Superposed conformations in Family B. **C**: $^{1}S_{5}$ conformation selected from Family A. **D**: $^{3}S_{5}$-like conformation selected from Family B.

Table 5. Torsional angles measured for conformational families A and B (Fig. 3) and for the *Z-anti* $^{4}C_{1}$ conformation

Entry	Torsional angle (deg)				
	NH, H-2	H-1, H-2	H-2, H-3	H-3, H-4	H-4, H-5
Figure 3A	133–154	59–68	87–112	141–163	176–180
Figure 3B	136–150	78–107	78–100	115–144	170–180
Z-anti $^{4}C_{1}$	170	175	178	173	170

the distance that was measured in $CDCl_3$ between NH and H-3″, whether this residue was a rhamnosyl residue (**1** and **2**) or a fucosyl unit (**3**). While in these cases this distance was shorter than 4 Å, it is usually larger than 6 Å in the conformation that deprotected Le^{a} analogues adopt in D_2O.[22] It is also interesting to notice that H-5″ (fucose or rhamnose) in compounds **1**–**3** are found at lower field ($\Delta\delta$ >0.45 ppm) in the hydrogen bonding solvents CD_3CN and DMSO than in $CDCl_3$ and CD_2Cl_2. This is to be correlated with the established[22] fact that Le^{a} derivatives in D_2O adopt conformations in which the fucosyl H-5″ is close to O-3 glucosamine as well as O-5′ Gal leading to its deshielding by 0.8 ppm relative to H-5 of methyl α-L-fucopyranoside. From these observations, we propose that the protected analogues **1**–**3** adopted conformations in CD_3CN and DMSO-d_6 that were similar to the global minimum energy conformation adopted by Le^{a} derivatives in D_2O. In contrast, their conformation in $CDCl_3$, and presumably CD_2Cl_2, was quite different and included distortion of the glucosamine ring as well as rotation around the rhamno- or fucosidic bond, which brought these residues in close proximity to the *N*-acetamido group. It is important to mention that such glucosamine ring distortions have neither been observed in the glucosamine monosaccharide building block nor in the disaccharide building block [(Ac)$_4$-β-D-Gal-(1→3)-β-D-GlcNAc-OMe] that were used to prepare trisaccharides **1**–**3**.[5,6] Furthermore, and as mentioned above, such distortions were not observed for the methylimidate trisaccharide **4** nor for the di-N-acetylated trisaccharide **5**. Given the experimental data presented here, we propose that the unusual conformations observed for compounds **1**–**3** in $CDCl_3$ and CD_2Cl_2 resulted from the formation of an intramolecular hydrogen bond between the acetamido NH group and a hydrogen bond-accepting group on the fucose or rhamnose rings, which could not be identified with certainty.

When working with *N*-acetylglucosamine-containing oligosaccharides, the unusual behavior of the glucosamine ring in $CDCl_3$ reported here must be taken into account when analyzing NMR data of synthetic intermediates because it can lead to misinterpretation. In our study, a $^{1}C_{4}$ conformation adopted by the glucosamine ring is not supported by the distance that was measured by NMR spectroscopy between the acetamido NH and H-5. We have strong evidence that these conformational distortions resulted from the formation of an intramolecular hydrogen bond involving the *N*-acetamido group. When such unexpected coupling constants are observed, the recording of NMR data in a hydrogen bond-accepting solvent such as CD_3CN or DMSO-d_6 is recommended.

1. Experimental

1.1. NMR experiments

^{1}H NMR (400.13 and 600.13 MHz) and ^{13}C NMR (100.6 and 150.9 MHz) spectra were recorded for 10 mM solutions of compounds **1**–**3** in $CDCl_3$ (internal standard, for ^{1}H residual $CHCl_3$ δ 7.24; for ^{13}C: $CDCl_3$ δ 77.0), CD_2Cl_2 (internal standard, for ^{1}H residual CH_2Cl_2 δ 5.32; for ^{13}C: CD_2Cl_2 δ 53.1), CD_3CN (internal standard, for ^{1}H residual CH_3CN δ 1.93; for ^{13}C: CD_3CN δ 1.3), and Me_2SO-d_6 (internal standard, for ^{1}H residual Me_2SO δ 2.49; for ^{13}C: Me_2SO-d_6 δ 39.5). Chemical shifts and coupling constants were obtained from a first-order analysis of one-dimensional spectra. The digital resolution was 0.16 and 0.27 Hz, respectively, at 400 and 600 MHz. Assignments of proton and carbon resonances were based on two-dimensional ^{1}H–^{1}H and ^{13}C–^{1}H correlation experiments.

All 2D NOESY spectra were recorded at 600 MHz on a Bruker Avance spectrometer and using the noesygpph

pulse sequence. These experiments were acquired using states-TPPI and a total of 256 (t_1) × 2 K (t_2) data points were recorded. Data acquisition and processing were performed using XWINNMR software (Bruker). The data were zero-filled to 1 K (t_1) and the processed data was then carefully phase corrected followed by automatic baseline correction in both dimensions. Integrations of NOE cross-peaks were defined using XWINNMR at the longest mixing time and then measured sequentially at all mixing times. Six experiments at different mixing times 300–800 ms were recorded for each of **1**–**3** in $CDCl_3$, CD_2Cl_2. Only the NOEs building up linearly and having a zero intercept mixing time were considered representative of a short distance between two hydrogens. From cross-peak intensities (I), the apparent internuclear distances r_A between two hydrogens was calculated using the isolated spin pair approximation: $r_A = (I_B/I_A)^{1/6} r_B$ where I_B and r_B are, respectively, the cross-peak intensity and internuclear distances of a reference pair of nuclei. The reference pairs of nuclei were selected on the basis of a well-isolated cross-peak in the 2D-NOESY spectra. The distances used as references were H-4Ga–H-3Ga (2.44 Å) for compounds **1** and **2** in $CDCl_3$; H-4Ga–H-5Ga (2.47 Å) for compound **3** in $CDCl_3$ as well as **1** in CD_2Cl_2; H-1R–H-2R (2.51 Å) for compound **2** in CD_2Cl_2; H-1F–H-2F (2.42 Å) for compound **3** in CD_2Cl_2. The calculated distances were plotted against the mixing time and linear regression extrapolated to zero mixing time. The accuracy of the measurements were estimated to be less than ±10%.[18]

1.2. Conformational search

A systematic search of the possible conformations that methyl 2-acetamido-3,4,6-trimethyl-2-deoxy-β-D-glucopyranoside can adopt was performed using the Molecular Operating Environment Software suite MOE2004.03. In this search, all ring bonds were rotated by 15° increments while other bonds were rotated by 120° increments. It is important to point out that when carrying out a systematic search applied to small ring systems (up to eight-membered rings) using MOE, all angle increments that would produce eclipsed conformations of heavy atoms are removed. This means that in the interest of speed, boat conformations were not produced. The 938 generated conformations were minimized with Amber94 in vacuo and arbitrarily only those 339 conformations within 10 kcal/mol of the global minimum were examined.

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