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Evaluation of steric effects on the exocyclic conformations of 6-*C*-methyl-substituted 2-acetamido-2-deoxy-β-D-glucopyranosides

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Abstract

Introduction of a stereodefined methyl group at the C-6 position of *N*-acetylglucosamine mono- and disaccharides creates a strong and predictable orientational bias on the geminal C-6 hydroxyl in solution, as determined by $^{1}H-^{1}H$ and $^{13}C-^{1}H$ NMR coupling constants. The conformational directing effect is more pronounced in the disaccharides because of the greater steric demand imposed by the neighboring glycosidic unit.

Keywords: Chitin; Glucosamine; Hydroxymethyl; Conformational analysis; NMR spectroscopy

The hydroxymethyl group plays a key role in the structural and chemical biology of pyranoside carbohydrates whose interactions with receptor proteins and other carbohydrate species are critical for cell–cell recognition and other biological functions.[1,2] The hydroxymethyl C-5–C-6 bond is conformationally mobile and does not exhibit a strong preference for a single staggered conformation.[3] However, one or more rotamers can be destabilized by introducing a small but sterically demanding unit such as a methyl group at the 6*R*- or 6*S*-position. It is known that 1,3-diaxiallike Me···OH interactions increase torsional strain energy by over 2 kcal/mol;[4] therefore, the exocyclic C-5–C-6 bond is expected to demonstrate a preference for staggered conformers that avoid such interactions (Fig. 1). Sterically driven conformational bias has been demonstrated on 6-*C*-substituted gluco- and galactopyranosides,[5,6] and has been used to probe the effect of conformation on the recognition or enzymatic hydrolysis of 1,6-linked disaccharides.[7–9]

Herein we report the stereoselective synthesis and conformational analysis of 6-*C*-methyl β-*N*-acetylglucosaminopyranosides (β-GlcNAc) and their corresponding 1,4-linked disaccharides. We demonstrate that the stereodefined methyl group at C-6 introduces a strong and predictable conformational bias on the C-5–C-6 bond, with a subsequent directing effect on the C-6 hydroxyl groups. 6-*C*-Substituted glucosamines are expected to be useful for investigating conformational effects in the protein–carbohydrate and carbohydrate–carbohydrate interactions of chitin[10] and the glycosaminoglycans.[11,12]

6-*C*-Substituted β-GlcNAc monosaccharides were synthesized according to Scheme 1. All new compounds were fully characterized by NMR spectroscopy and elemental analysis or mass spectrometry. Protected monosaccharide derivative **1** was prepared in multigram quantities from glucosamine hydrochloride using literature procedures.[13–15] Conditions for the reductive cleavage of the 4,6-*O*-*p*-methoxybenzylidene derivative of **1** to the corresponding free O-6 hydroxyl group[16] were found to be incompatible with the anomeric allyl protecting group, but compound **2** could be obtained in good yield by regioselectively protecting the primary alcohol as an intermediate *tert*-butyldimethylsilyl ether. Aldehyde **3** was obtained by Swern oxidation[17] and was readily reduced by $NaBD_4$ to yield 6-*C*-monodeuterated glucosamine derivative **4** as a 3:1 mixture of

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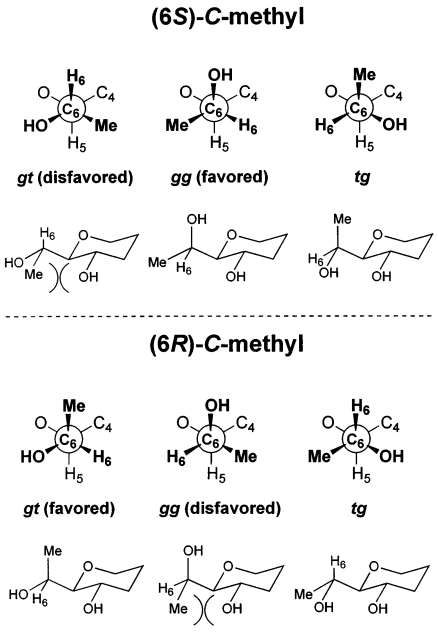
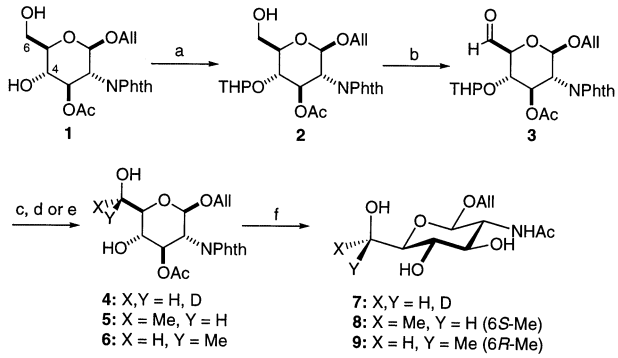


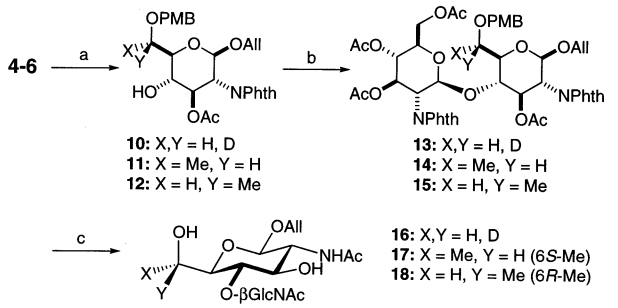
Fig. 1. Staggered conformations of the exocyclic C-5–C-6 bond in 6-*C*-methyl-substituted pyranosides.

diastereomers, providing reference compounds for the NMR conformational studies.† Assignment of 6*R*- and 6*S*-stereochemistry was achieved via vicinal coupling constant analysis of the corresponding 4,6-*O*-isopropylidene or *p*-methoxybenzylidene ketal. Aldehyde **3** was also subjected to chemoselective methylation conditions but was found to be a surprisingly unreactive electrophile. After extensive experimentation, it was determined that methylation of **3** could be achieved in good yield with 6:1 6*S*:6*R* stereoselectivity using $AlMe_3$ with CuCN as an additive. It is noteworthy that the protecting group at O-4 had a significant influence on the efficiency and stereochemical outcome of the methylation by $AlMe_3$. Replacing the tetrahydropyranyl (THP) group with a benzyl ether lowered the stereoselectivity, whereas a 2-methoxyethoxymethyl (MEM) ether reduced reactivity. Removal of the THP group enabled separation of the diastereomers, affording 6-*C*-methyl-substituted **5** and **6** in 58% combined yield after two steps. Additional **6** could be obtained by Swern oxidation[17] of the THP-protected 6-*C*-methyl adduct to the corresponding ketone, followed by reduction with *i*-Bu_2AlH in the presence of $ZnCl_2$ (6:1 6*R*:6*S* ratio). Conversion of the phthalimide group at C-2 into an acetamide followed by global deprotection, yielded the desired C-6-substituted β-GlcNAc monosaccharides **7**–**9**.

† Diastereomeric monodeuteration greatly simplifies conformational analysis of the C-5–C-6 bond by reducing the H-5–H-6 coupling to a two-spin system. The diastereotopic 6*R*- and 6*S*-protons can thus be analyzed simultaneously.



Scheme 1. Reagents and conditions: (a) (i) TBSCl, Et_3N, imidazole, CH_2Cl_2–THF (96%); (ii) dihydropyran, PPTS; (iii) $(n\text{-}Bu)_4NF$, THF (76% over two steps); (b) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; Et_3N, 0 °C (64%); (c) (i) $NaBD_4$, CH_2Cl_2–MeOH, 0 °C; (ii) *p*-TsOH, MeOH (70% isolated yield of **4** over two steps); (d) (i) $AlMe_3$ (5 equiv), CuCN (1 equiv), THF, −45 °C to rt; (ii) *p*-TsOH, MeOH (50% isolated yield of **5** over two steps); (e) (i) $AlMe_3$ (5 equiv), CuCN (1 equiv), THF, −45 °C to rt; (ii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; Et_3N, 0 °C; (iii) *i*-Bu_2AlH, $ZnCl_2$, THF, −78 °C; (iv) *p*-TsOH, MeOH (37% isolated yield of **6** over four steps); (f) (i) ethylenediamine, *n*-BuOH, 100 °C; (ii) Ac_2O, C_5H_5N, 0 °C to rt; (iii) NaOMe, MeOH–CH_2Cl_2, 0 °C to rt (87% over three steps). All = allyl, Phth = phthalimido, TBS = *tert*-butyldimethylsilyl, THP = tetrahydropyranyl.



Scheme 2. Reagents and conditions: (a) (i) *p*-$MeO(C_6H_4)CH(OMe)_2$, camphorsulfonic acid, 4 Å mol sieves, toluene, 90 °C; (ii) $NaBH_3CN$, HCl, 4 Å mol sieves, THF–Et_2O, −30 °C (35–76% over two steps); (b) 2-deoxy-2-phthalimido-3,4,6-*O*-triacetylglucosyl trichloroacetimidate (2 equiv), TMSOTf (0.2 equiv), 4 Å mol sieves, CH_2Cl_2, −30 °C (78–98%); (c) (i) DDQ, *t*-BuOH, pH 7 buffer, THF, 0 °C; (ii) ethylenediamine, *n*-BuOH, 100 °C; (iii) Ac_2O, C_5H_5N, 0 °C to rt; (iv) NaOMe, MeOH–CH_2Cl_2, 0 °C to rt (64–76% over four steps). All = allyl, Phth = phthalimido, PMB = *p*-methoxybenzyl.

Disaccharides containing C-6-substituted β-GlcNAc were also prepared to determine the relative influence of a neighboring glycosidic unit on exocyclic conformation (Scheme 2). Monosaccharides **4**–**6** were protected as 6-*O*-*p*-methoxybenzyl (PMB) ethers **10**–**12** via reductive cleavage of the corresponding 4,6-*O*-*p*-methoxybenzylidene acetals under acidic conditions. Glycosylation of O-4 with 2-deoxy-2-phthalimido-3,4,6-*O*-triacetylglucopyranose activated as a Schmidt trichloro-

Table 1
^{1}H–^{1}H and ^{13}C–^{1}H coupling constants of 6-C-substituted glucopyranosides [a]

β-GlcNAc derivative	$^{3}J_{5,6}$ [b]	$^{2}J_{C\text{-}6,H\text{-}5}$ [b]	$^{3}J_{C\text{-}7,H\text{-}5}$ [b]	C-5–C-6 conformational preferences [c]
(6*S*/6*R*)-*C*-*d* Monosaccharide (**7**)	6.2/2.6	–	–	$gt \simeq gg > tg$ [d]
(6*S*/6*R*)-*C*-*d* Disaccharide (**16**)	4.9/2.0	–	–	$gt \simeq gg > tg$ [d]
(6*S*)-*C*-Methyl monosaccharide (**8**)	1.8	2.9	1.6	$gg > tg,\ gt$ [e]
(6*S*)-*C*-Methyl disaccharide (**17**)	1.5	2.3	1.2	$gg > tg,\ gt$ [e]
(6*R*)-*C*-Methyl monosaccharide (**9**)	3.9	4.2	3.4	$gt > tg > gg$ [f]
(6*R*)-*C*-Methyl disaccharide (**18**)	2.4	4.5	3.8	$gt \gg tg > gg$ [f]

[a] ^{1}H NMR spectra were obtained using a 600 MHz Varian spectrometer in MeOH-d_4 at 298 K. Coupled ^{13}C NMR spectra were obtained using a 500 MHz Brüker spectrometer in methanol-d_4 at 298 K.

[b] In Hz (± 0.25 Hz for $^{3}J_{5,6}$, ± 0.3 Hz for $^{2,3}J_{C,H}$).

[c] Order of conformational preferences was established by $^{3}J_{5,6}$ coupling constant analysis and correlated with $^{2}J_{C,H}$ and $^{3}J_{C,H}$ values.

[d] Theoretical $^{3}J_{5,6R}/^{3}J_{5,6S}$ values for the staggered conformers of 1,2-dialkoxypropane are: 10.7/3.1 for *gt*; 5.0/10.7 for *tg*; 0.9/2.8 for *gg*.[3,22,23]

[e] Theoretical $^{3}J_{5,6}$ values for the staggered conformers of 2,3-(*S*,*S*)-dialkoxybutane (calculated using empirical parameters in Ref. 19) are: 9.2 for *gt*; 3.9 for *tg*; 0.7 for *gg*.

[f] Theoretical $^{3}J_{5,6}$ values for the staggered conformers of 2,3-(*S*,*R*)-dialkoxybutane are: 2.3 for *gt*; 9.2 for *tg*; 2.3 for *gg*.

acetimidate[18] produced 1,4-β-linked disaccharides **13**–**15**, and was followed by global deprotection to give the desired 6-*C*-substituted disaccharides **16**–**18** in high overall yields.

The conformational preferences of the C-5–C-6 bonds of **7**–**9** were evaluated as a function of C-6 substitution using vicinal ^{1}H–^{1}H coupling constants ($^{3}J_{5,6}$) from nuclear magnetic resonance (NMR) spectroscopy, supported by ^{13}C–^{1}H coupling constants (Table 1).‡ Changes in conformational preference as a function of C-6 substitution were evaluated to the first degree of approximation by correlating $^{3}J_{5,6}$ coupling constants with values derived from Karplus equations parameterized for 1,2-dialkoxypropanes or 2,3-dialkoxybutanes.[19] Two- and three-bond ^{13}C–^{1}H coupling constants ($^{2}J_{C,H}$, $^{3}J_{C,H}$) were obtained from coupled ^{13}C spectra and correlated with empirical values reported by Serianni[20] and Murata.[21] The $^{3}J_{H,H}$ coupling constants in the pyranose rings of **8** and **9** describe stable chair conformations which are essentially unaffected by C-6 methyl substitution.

$^{3}J_{5,6}$ coupling constant analysis of 6-*C*-monodeuterated β-GlcNAc **7** indicates an approximately equal mixture of *gt* and *gg* conformations at 298 K in MeOH-d_4, in general agreement with earlier reports on hydroxymethyl conformation (Table 1).[3,22,23] Introducing a stereodefined 6-*C*-methyl group dramatically changes the conformational preference of the C-5–C-6 bond. (6*S*)-*C*-Methyl β-GlcNAc **8** at 298 K has a small $^{3}J_{5,6}$ value of 1.8 Hz, indicating a strong preference for the *gg* conformation ($^{3}J_{5,6}$(theor.) 0.7 Hz) relative to the *tg* or *gt* conformers ($^{3}J_{5,6}$(theor.) 3.9 and 9.2 Hz, respectively). This conformational assignment is supported by a small $^{3}J_{C\text{-}7,H\text{-}5}$ constant of 1.6 Hz. In comparison, (6*R*)-*C*-methyl β-GlcNAc **9** has a relatively large $^{2}J_{C\text{-}6,H\text{-}5}$ value of 4.2 Hz, which correlates with a depletion of the *gg* conformer.[20,21] Evaluation of the remaining two staggered conformations using the $^{3}J_{5,6}$ constant (3.9 Hz) and the appropriately parameterized Karplus equation indicates that the *gt* conformer is strongly favored over the *tg* conformer ($^{3}J_{5,6}$(theor.) 2.3 and 9.2 Hz, respectively).

Conformational analysis of **16**–**18** at 298 K in MeOH-d_4 indicates that the C-4 glycoside reinforces the conformational preference of the exocyclic C-5–C-6 bond (Table 1). In the case of (6*S*)-*C*-methyl substituted **17**, the preference for the *gg* conformation is increased ($^{3}J_{5,6}$ 1.5 Hz); in the case of (6*R*)-*C*-methyl substituted **18**, *gt* is more strongly favored ($^{3}J_{5,6}$ 2.4 Hz). These observations suggest that the neighboring glycosidic unit enhances the directing effect of the 6-*C*-methyl group by increasing steric demand. Intramolecular hydrogen bonding, if any, does not appear to have any significant influence on the exocyclic conformation of **16**–**18** under these conditions. This is in accord with previous solution conformation studies on *C*-glycosides, whose secondary structures are determined essentially by local steric effects on torsional strain.[24]

In conclusion, we have demonstrated that stereoselective methylation at C-6 can be used to direct the conformational preference of the exocyclic O-6 hydrox-

‡ It is important to note that NMR coupling constant analyses in solution are derived from time-averaged conformational distributions and cannot precisely measure the percentage of individual conformers in a general sense. However, such analyses are useful for probing relative changes in conformational preference.

yls in β-GlcNAc derivatives. These conformationally modified carbohydrates are particularly relevant for investigating polysaccharides such as chitin and the glycosaminoglycans, whose physical and biochemical properties are strongly dependent on the interactions between O-6 hydroxyl groups.[10–12,25]

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