Rapid communication

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

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Received 1 October 2001; accepted 31 October 2001

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 1H–1H and 13C–1H 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. © 2002 Elsevier Science Ltd. All rights reserved.

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 6R- or 6S-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 chitin10 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-methoxybenzyldiene derivative of 1 to the corresponding free O-6 hydroxyl group16 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-butylidemethylsilyl ether. Aldehyde 3 was obtained by Swern oxidation17 and was readily reduced by NaBD₄ to yield 6-C-monodeuterated glucosamine derivative 4 as a 3:1 mixture of

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oxidation of the THP-protected 6-

diastereomers, providing reference compounds for the NMR conformational studies. Assignment of 6R- and 6S-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 6S:6R stereoselectivity using AlMe3 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 AlMe3. Replacing the tetrahydropyranyl (THP) group with a benzyl ether lowered the stereoselectivity, whereas a 2-methoxyethylmethyl (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 oxidation17 of the THP-protected 6-C-methyl adduct to the corresponding ketone, followed by reduction with 1-Bu2AlH in the presence of ZnCl2 (6:1 6R:6S 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 6R- and 6S-protons can thus be analyzed simultaneously.

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 acetal under acidic conditions. Glycosylation of O-4 with 2-deoxy-2-phthalimido-3,4,6-triacyethylglucopyranose activated as a Schmidt trichloro-
Table 1

<table>
<thead>
<tr>
<th>β-GlcNAc derivative</th>
<th>3^J_{5,6}</th>
<th>2^J_{C-6,H-5}</th>
<th>3^J_{C-7,H-5}</th>
<th>C-5–C-6 conformational preferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>(6S/6R)-C-d Monosaccharide (7)</td>
<td>6.2/2.6</td>
<td>–</td>
<td>–</td>
<td>gt ≥ gg &gt; tg</td>
</tr>
<tr>
<td>(6S/6R)-C-d Disaccharide (16)</td>
<td>4.9/2.0</td>
<td>–</td>
<td>–</td>
<td>gt ≥ gg &gt; tg</td>
</tr>
<tr>
<td>(6S)-C-Methyl monosaccharide (8)</td>
<td>1.8</td>
<td>2.9</td>
<td>1.6</td>
<td>gg &gt; tg, gt</td>
</tr>
<tr>
<td>(6S)-C-Methyl disaccharide (17)</td>
<td>1.5</td>
<td>2.3</td>
<td>1.2</td>
<td>gg &gt; tg, gt</td>
</tr>
<tr>
<td>(6R)-C-Methyl monosaccharide (9)</td>
<td>3.9</td>
<td>4.2</td>
<td>3.4</td>
<td>gt &gt; tg &gt; gg</td>
</tr>
<tr>
<td>(6R)-C-Methyl disaccharide (18)</td>
<td>2.4</td>
<td>4.5</td>
<td>3.8</td>
<td>gt &gt; tg &gt; gg</td>
</tr>
</tbody>
</table>

^a^ 1H 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 Bruker spectrometer in methanol-d_4 at 298 K.

^b^ In Hz (±0.25 Hz for 3^J_{5,6}, ±0.3 Hz for 2^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_{5,6,e}/3^J_{5,6,g} 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.

^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 1H–1H coupling constants (3^J_{5,6}) from nuclear magnetic resonance (NMR) spectroscopy, supported by ^13^C–^1^H coupling constants (Table 1).^3^ 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. Two- and three-bond ^13^C–^1^H coupling constants (3^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. The 3^J_{C,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.

(6S)-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-7,H-5} constant of 1.6 Hz. In comparison, (6R)-C-methyl β-GlcNAc 9 has a relatively large 3^J_{C-6,H-5} value of 4.2 Hz, which correlates with a depletion of the gg conformer. 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 (6S)-C-methyl substituted 17, the preference for the gg conformation is increased (3^J_{5,6} 1.5 Hz); in the case of (6R)-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-
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.  

Acknowledgements

This work was supported by the American Chemical Society Petroleum Research Foundation (33341-G4, 36069-AC1), the American Heart Association Midwest Affiliates (30399Z), the American Cancer Society (IRG-58-006-41), and the Purdue Research Foundation in the form of a fellowship to J.A. The authors gratefully acknowledge Dr Klaas Hallenga and Dr Edwin Rivera for NMR assistance, and Fabien Boulineau for contributing toward the chemoselective methylation study.

References