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Stereoselective Construction of β-Mannopyranosides via Anomeric O-Alkylation: Synthesis of the Trisaccharide Core of N-linked Glycans

Hai Nguyen, Danyang Zhu, Xiaohua Li,† and Jiangong Zhu*†

Abstract: A new and efficient approach for direct and stereoselective synthesis of β-mannopyranosides via anomic O-alkylation has been developed. This anomic O-alkylation of manno- pyranose-derived lactols was believed to occur under synergistic control of kinetic anomeric effect and metal chelation. It was found that the presence of a conformationally flexible C6-oxygen in the sugar-derived lactol donors is required for this anomic O-alkylation to be efficient, probably due to its chelation with cesium ion. In contrast, the presence of C2-oxygen atom was found to play minor role. This glycosylation method has been successfully utilized for the synthesis of the trisaccharide core of complex N-linked glycans.

Protein glycosylation is known as one of the major types of post-translational modification. In general, there are two types of glycans attached to proteins: 1) N-linked glycans attached to asparagine and 2) O-linked glycans attached to serine or threonine. Recent biological studies have demonstrated that the glycans of glycoconjugates play essential roles in numerous biological processes. Cell-surface glycans serve as receptor ligands for proteins, e.g., enzymes, antibodies, and lectins. In addition, it was also found that the degree of cell-surface carbohydrate antigen expression is closely associated with tumor progression, and diagnostic results may guide the use of corresponding approach for cancer treatment. Furthermore, carbohydrate moieties are known to stabilize protein folding and modify physical, chemical, and biological properties of their carrier molecules.

Due to the heterogeneous glycoforms of glycoproteins, it is difficult to understand the exact function of these complex glycans. To address the challenge, researchers seek to obtain single glycoform of biologics by either total chemical synthesis or chemo-enzyme synthesis. Although great progress has been achieved, the synthesis of complex N-linked glycans remains a daunting task. In particular, stereoselective construction of the β-mannopyranoside, one of the key glycosidic linkages existing in structurally complex N-linked glycans, is a long-standing challenge by suitable hydrogen atom donors. Synthesis of β-mannopyranosides involving intramolecular aglycone delivery[17] by suitable hydrogen atom donors has been found to play minor role. This glycosylation method has been successfully utilized for the synthesis of the trisaccharide core of complex N-linked glycans.

Our laboratory has recently developed a method for stereoselective synthesis of 2-deoxy-β-glycosides via anomic O-alkylation controlled by kinetic anomeric effect. In addition, we have reported stereoselective synthesis of 2-deoxy-α-glycosides via chelation-controlled anomeric O-alkylation. Based on aforementioned success, we wondered if kinetic anomeric effect in conjunction with chelation control can be applied to the stereoselective synthesis of β-mannopyranosides. As shown in Scheme 1, after deprotonation of D-mannose with a suitable base, a mixture of dianion and 4 may be produced and interconvert into each other via open intermediate 3. Due to the chelation effect (colored in blue), equatorial anomeric alkoxy 4 would be preferentially formed over the axial counterpart 2. In addition, equatorial anomeric alkoxy 4 should be more nucleophilic than the axial counterpart 2 due to the double-electron donation. Furthermore, the axial anomeric alkoxy 4 would be more nucleophilic than the equatorial counterpart 3 due to the double-electron donation. Eventually, alkoxy 4 would be produced.

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success was also achieved by others\(^{[3]}\) when 1a or other partially protected D-mannopyranoses were converted to their corresponding 1,2-O-dibutylstannylene complexes followed by O-alkylation with various electrophiles. However, organostannanes are highly toxic and the use of stoichiometric amounts of organostannanes is certainly not desirable.

**Table 1.** Anomeric O-alkylation of 3,4,6-tri-O-benzyl-D-mannopyranose 1a with D-galactose-derived C4-triflate 6a.\(^{[4]}\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction condition</th>
<th>Yield,(^{[1]}) α/β ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cs(_2)CO(_3) (1.5 eq.), triflate 6a (1.5 eq.)</td>
<td>25%, β only</td>
</tr>
<tr>
<td>2</td>
<td>Cs(_2)CO(_3) (1.5 eq.), triflate 6a (1.5 eq.)</td>
<td>48%, β only</td>
</tr>
<tr>
<td>3</td>
<td>Cs(_2)CO(_3) (2.5 eq.), triflate 6a (2.0 eq.)</td>
<td>67%, β only</td>
</tr>
<tr>
<td>4</td>
<td>CsPO(_3) (2.5 eq.), triflate 6a (2.0 eq.)</td>
<td>59%, β only</td>
</tr>
<tr>
<td>5</td>
<td>Cs(_2)CO(_3) (2.5 eq.), triflate 6a (2.0 eq.)</td>
<td>64%, β only</td>
</tr>
<tr>
<td>6</td>
<td>Cs(_2)CO(_3) (3.0 eq.), triflate 6a (2.5 eq.)</td>
<td>75%, β only</td>
</tr>
</tbody>
</table>

[a] Unless otherwise noted, all reactions were performed using 0.1 mmol of 3,4,6-tri-O-benzyl-D-mannopyranose 1a in 1 mL CH\(_3\)CN at 40 °C for 24 hours. \([b]\) Isolated yield (calculated based on the lactol donor 1a). \([c]\) CH\(_3\)CN was used as solvent. \([d]\) This reaction was carried out at 50 °C.

In consideration of the natural β-(1→4)-linked mannopyranosidic linkage in complex N-linked glycans, we chose to study the anomeric O-alkylation reaction between 3,4,6-tri-O-benzyl-D-mannopyranose 1a and D-galactose-derived C4 secondary triflate 6a for selective production of the corresponding β-mannopyranoside 7 (Table 1). Initially, we applied the optimal conditions that we had discovered previously\(^{[2,3,5]}\) for the synthesis of 2-deoxy glycosides to this type of β-mannopyranosylation; however, only trace amount of product was obtained. Changing the solvent to dichloromethane\(^{[2,4,6]}\) gave similar results. Base-mediated 1,2-elimination of triflate 6a was found to be the major problem. During the search for bases which could react with 1a to form more nucleophilic anomeric alkoxides, we were excited to discover that warming a mixture of D-mannopyranose 1a (1 eq.), triflate 6a (1.5 eq.), and Cs\(_2\)CO\(_3\) (1.5 eq.) in acetonitrile at 40 °C afforded desired β-mannopyranoside 7 in 25% isolated yield (β only)\(^{[1]}\) (entry 1, Table 1). After screening a range of polar and non-polar solvents as well as co-solvents,\(^{[12]}\) we found that 1,2-dichloroethane was the optimal solvent for this reaction which provided desired β-mannopyranoside 7 in 48% yield (β only) (entry 2). Increasing the amounts of triflate 6a to 2.0 equivalents and Cs\(_2\)CO\(_3\) to 2.5 equivalents increased the isolated yield of β-mannopyranoside 7 to 67% (β only) (entry 3). Changing the base to CsPO\(_3\) or elevating the reaction temperature afforded inferior isolated yields (entries 4 and 5, respectively). Finally, use of triflate 6a (2.5 eq.) and Cs\(_2\)CO\(_3\) (3.0 eq.) furnished desired β-mannopyranoside 7 in 75% yield (β only) (entry 6). Attempting to let the reaction proceed longer (40 hours) gave comparable results.\(^{[13]}\) No over alkylation at O2 was observed in all these experiments. It should be noted that the use of 2-aminoethyl diphenyborinate (0.1 eq.), a catalyst well-demonstrated by Taylor\(^{[15]}\) for regioselective alkylation of cis-diols, in this type of β-mannopyranosylation was not effective.\(^{[12]}\) These results suggested that the anomeric cesium alkoxide was the key active intermediate for anomeric O-alkylation with sugar-derived triflates. The reason why cesium bases turned out to be efficient for this type of β-mannosylation is not entirely clear, probably due to well-known “cesium effect”.\(^{[14]}\) In addition, in contrast to traditional glycosylations which are usually performed under anhydrous conditions, this anomeric O-alkylation with cesium carbonate is not very moisture-sensitive.

With the optimal condition established, we next performed studies on the reaction scope using 3,4,6-tri-O-benzyl-D-mannopyranose 1a and 4-O-benzyl-3,6-di-O-(4-methoxybenzyl)D-mannopyranose 1b with various sugar-derived triflates 6b-g. As shown in Table 2, under optimal conditions, β-mannopyranosides 8, 9, and 10 were produced from 1a/b and relatively unreactive triflates 6a-c in synthetically useful to good yields and excellent anomeric selectivity, respectively.\(^{[13]}\) In addition, β-mannopyranosides 11, 12, and 13 were obtained in good to excellent yields and excellent anomeric selectivity\(^{[13]}\) from more reactive triflates 6d-f,\(^{[22]}\) even less amount of the triflates (2.0 eq.) were used. Furthermore, if most reactive primary triflate 6g was employed, only 1.5 eq. of the triflate was needed for the reactions and β-mannopyranosides 14 and 15 were gained in excellent yields and anomeric selectivity.\(^{[13]}\)

To demonstrate the utilization of this method in complex oligosaccharide synthesis, we next performed the synthesis of the trisaccharide core of the N-linked glycans 21 (Scheme 2). The synthesis commenced with the traditional glycosylation between known glycosyl donor 16\(^{[35]}\) and acceptor 17\(^{[36]}\) under previously reported condition\(^{[13]}\) which afforded desired β-linked disaccharide 18 in 97% yield. Deacetylation of 18 afforded desired alcohol 19 which was subsequently subjected to tritylation to produce triflate 20. Finally, cesium carbonate-mediated anomeric O-alkylation of 3,4,6-
tri-O-benzyl-D-mannopyranose 1a with triflate acceptor 20 (2.5 eq.) gave the desired trisaccharide core of the N-linked glycans 21 in 72% yield (β only).

Scheme 2. Synthesis of the trisaccharide core of the N-linked glycans via anomeric O-alkylation. Reagents and conditions: a) NIS, TIOH, 4Å molecular sieves, CH₂Cl₂, -20 °C, 97%; b) NaOMe, MeOH/THF (1:1), RT, 72% yield; c) Tf₂O, pyridine, CH₂Cl₂, 0 °C, 90% yield; d) 1a (1.0 eq.), 20 (2.5 eq.), Cs₂CO₃ (3.0 eq.), CICH₂CH₂Cl, 40 °C, 24h, 72% (yield calculated based on the lactol donors 1a). NIS = N-Iodosuccinimide; TIOH = Trifluoromethanesulfonic acid; Tf₂O = Trifluoromethanesulfonic anhydride.

In order to gain insight into this type of anomeric O-alkylation, we studied various D-mannopyranose type donors, such as 3,4-di-O-benzyl-6-O-tert-butylidiphenylsilyl-D-mannopyranose 22, 3-O-benzyl-4,6-O-benzylidene-D-mannopyranose 24, 3,4-di-O-benzyl-D-rhamnose 26, 3,4,6-tri-O-benzyl-2-deoxy-D-glucose 28, and 3,4-di-O-benzyl-D-olivose 30 for this type of anomeric O-alkylation with triflate 6a (2.0 eq.). As shown in Table 3, while β-mannopyranosides 23(b) was obtained in 40% yield from D-mannopyranose 22 bearing 6-O-TBDDS ether (entry 1), surprisingly, we did not observe the production of β-mannopyranosides 25 from 4,6-benzylidene protected D-mannopyranose 24 (entry 2). In addition, use of D-rhamnose donor 26 lacking C6-oxygen atom in the reaction afforded only 30% yield of the β-linked disaccharide 27(c) (entry 3). Furthermore, use of 2-deoxy-D-glucose donor 28 (also can be viewed as 2-deoxy-D-mannose) gave the desired β-linked disaccharide 29 in 64% yield (entry 4), and this result was comparable to the reaction outcome using 3,4,6-tri-O-benzyl-D-mannopyranose 1a (entry 3, Table 1). However, use of D-olivose 30 (2,6-dideoxy-D-glucose/mannose) only afforded 15% yield of the corresponding 2,6-dideoxy glycoside 31, albeit excellent anomeric selectivity was achieved. These results suggested that the presence of a conformationally flexible C6-oxygen in the sugar-derived lactol donors is required for this anomeric O-alkylation to be efficient, probably due to its chelation with cesium ion. In contrast, the presence of C2-oxygen atom was found to play minor role in this type of reaction.

In conclusion, an efficient approach for stereoselective synthesis of challenging β-mannopyranosides has been developed via anomeric O-alkylation of mannopyranoside-derived lactols. It was believed that this type of β-mannosylation occurs under synergistic control of kinetic anomeric effect and chelation effect. It was found that the presence of a conformationally flexible C6-oxygen in the sugar-derived lactol donors is indispensable for this anomeric O-alkylation to be efficient, while the presence of C2-oxygen atom was found to play minor role. This approach has been successfully utilized for the synthesis of the trisaccharide core of the N-linked glycans. Further mechanistic studies of this cesium carbonate-mediated β-mannosylation and application of this methodology to the synthesis of complex N-linked glycans are currently underway.

Table 3. Anomeric O-alkylation of various D-mannopyranose type donors.\[a\][b]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>Product</th>
<th>Yield, α/β ratio</th>
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<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
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<td>5</td>
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</table>

[a] General conditions: lactol donors (1.0 eq.), triflate 6a (2.0 eq.), Cs₂CO₃ (2.5 eq.), CICH₂CH₂Cl, 40 °C, 24 h. P = Protecting group. [b] Isolated yield (calculated based on the lactol donors).

Acknowledgments

We are grateful to National Science Foundation (CHE-1464787), The University of Toledo, and University of Michigan-Dearborn for support. This research was also supported in part by a grant from National Science Foundation (CHE-1213352). We thank Mr. James K. Dunaway and Mr. Rodney Park from University of Toledo, Mr. Justin Woodward and Mr. Ali Hourani from University of Michigan-Dearborn for experimental assistance.

Keywords: anomeric O-alkylation · β-mannosylation · glycosylation · oligosaccharides · N-linked glycans

The β-configuration of all these mannoside linkages (7-15, 21, 23, and 27) was unambiguously assigned by measuring the J_{C2,6} for the anomeric carbon. As a result, all the J_{C2,6} were measured to be in the range of 157 to 160 Hz, which proved the β-configuration. For using J_{C2,6} for determination of β-configuration of mannosidic linkages, see: K. Bock, C. Pedersen, J. Chem. Soc., Perkin Trans 2 1974, 293-297.

[32] See Supporting Information for detailed results on screening a range of polar and non-polar solvents as well as co-solvents for this anomeric O-alkylation.


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