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ABSTRACT

Development of β-selective glucuronolysis reaction using phenyl 2,4-di-O-acyl-1-thio-β-glucopyranosiduronolactone was described. Glycosylations of this glycosyl donor with hexosamine derivatives proceeded with excellent yield and β-stereoselectivity to afford glycosaminoglycan-type disaccharides.

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Uronic acids are an important class of monosaccharides and are defined as aldohexoses in which primary alcohol is oxidized to a carboxylic acid. Polysaccharides containing uronic acid entities are widespread in nature and display an array of physical properties and biological functions. For example, glycosaminoglycans (GAGs) are ubiquitous components of the extracellular matrix and play essential roles in biological systems. Because of irregular modifications of their skeleton, structurally defined GAG fragments are not easily prepared from natural resources. Thus, novel and efficient methods of the preparation of GAG fragments are crucial to advance our understanding of this important class of biomolecules. Extensive efforts have been made to improve the synthesis of oligosaccharides containing uronic acids. However, the low reactivity of uronic acid derivatives as glycosyl donors in chemical glycosylation steps has impeded progress. This low reactivity results from the electron withdrawing property of the carboxylate group.

Currently, the following two strategies have been adopted to overcome this problem: i) “post-glycosylation oxidation” and ii) “arming” the reactivity by the arrangement of protecting groups. Conformational arming effect is also an attractive concept for the activation of uronate donors. Van der Mareel et al. demonstrated glycosylation reactions of a glycuronate-type donor whose conformation was locked by 6,3-lactone bridge to give 1,2-cis glycoside by using the reduced reactivity by the torsional effect of the 6,3-lactone and high reactivity as glycosyl acceptor. They also prepared the 6,3-lactone type donor of gluc- and mannurionate. However, they did not demonstrate any conformational arming effect of this class of donor and used common C4 type uronate donors in the following studies of glycosylations.

Hence, we designed glucuronate derivative 1 as a key synthon for synthesis of GAGs fragments (Fig. 1), since GAGs contain the β-GlcA moiety in repeating disaccharide units. The conformation of 1 is fixed by a 6,3-lactone bridge in C4, with all substituents in an axial orientation. In more axial rich conformation, the reactivity increase is expected due to the different mode of σ-σ* or dipole interactions. Also, to enhance 1,2-trans stereoselectivity, to suppress side reactions in coupling steps, and to enhance the activation of the thioglycoside, we selected a well-used 2-O-acetyl protection to employ neighboring group participation. In addition, the rigid 3,6-lactone structure might also activate the orthoester-type intermediate formed by the participation of the 2-O-acetyl group on donor 1.
To investigate the efficiency of our strategy, we prepared 1, O-benzyl protected glucuronolactone derivative 2, and a corresponding set of standard methyl glucuronic derivatives 3 and 4 as disarmed and armed counterparts with C1 conformation, respectively. As acceptors, hexosamine derivatives 5, 6 and 7 were prepared to afford chondroitin sulfate (CS), hyaluronic acid (HA), and heparan sulfate (HS) type disaccharide units in GAGs, respectively. (Fig. 1)

The simultaneous lactonization with C6-oxidation\(^8\) of 8 using 2,2,6,6-tetramethylpiperidinylhydroxy free radical (TEMPO) and [bis(acetoxy)iodo]benzene (BAIB) reagent\(^1\) afforded 6,3-lactone donor 1 only in 22% but uncyclized derivative 9 in 73% yield. EDC/HOBt system, however, was found to give the lactone 1 in 93% from 9. (Scheme 1)

**Scheme 1.** Synthesis of lactone donor 1.

**Table 1.** Glycosylations with GalN\(_3\) derivative 5 under NIS/\(\text{TiO}_2\) system

<table>
<thead>
<tr>
<th># donor</th>
<th>Conditions(^a)</th>
<th>product/α:β ratio(^b)</th>
<th>Yield (%).(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>quant.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>trace(^d)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>11</td>
<td>trace(^d)</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>13 (α:β = 2:3)</td>
<td>93</td>
</tr>
</tbody>
</table>

\(^a\) A donor (1.5 eq.), acceptor (1.0 eq.), NIS (1.5 eq.), \(\text{TiO}_2\) (0.2 eq.), DCM (0.07 M), -40 °C 1 h, then -10 °C 1 h; B donor (1.2 eq.), acceptor (1.0 eq.), NIS (1.2 eq.), \(\text{TiO}_2\) (0.2 eq.), DCM (0.1 M), -40 °C 2 h.

\(^b\) Isolated ratio.

\(^c\) Isolated yields based on the acceptor.

\(^d\) Detected only by MALDI-TOF MS.

**Tetrahedron Letters**

Table 1 shows the result of glycosylation reactions of the glycosyl donors 1-4 with \(\alpha\)-galactosamine 5 as an acceptor substrate. The diacetyl-protected donor 1 under the promotion with NIS/\(\text{TiO}_2\) at -40 °C and stirring at -10 °C for 1 h afforded CS-type disaccharide 10 in a quantitative yield. (# 1) Meanwhile, the glycosylation of dibenzyl-protected donor 2\(^\alpha\) provided a trace amount of disaccharide 11. (# 2) Interestingly, this donor 2 was found extremely fragile to be used as a donor substrate even at -40 °C. (# 6) Analyses of the major product from donor 2 suggested that de-benzylation at 0 °C seemed to be proceeded, although the exact structure could not be identified. When \(\text{C}_1\) type donor 3 was employed, the corresponding \(\beta\)-linked disaccharide 12 was obtained in 83% yield. (# 3) Unexpectedly, an armed counterpart 4 afforded disaccharide 13 in 67% yield with a slight \(\alpha\)-selectivity. (# 4) When the reaction of donor 1 was performed under the conventional condition B (quenched at -40 °C), orthoester 14 was identified as the main product and 24% of acceptor 5 was recovered. (# 5) In the case of donor 4 without elevating temperature, the coupling product 13 was isolated in 93% yield as a slight \(\beta\)-rich mixture. (# 7) The result of # 1 and 5 indicates that a glycosidic bond was formed via orthoester, and product 10 was stable to an acid catalyst at -10 °C. In contrast, from the result of # 4 and 7, disaccharide especially \(\beta\)-glycoside may be unstable under the condition A.

To confirm the versatility of 6,3-lactone and 2-O-acetyl substituents, we carried out reactions of glucosamine derivative 6 as an acceptor substrate to afford HA type disaccharide. (Table 2, # 1-4) The coupling of donor 1 and acceptor 6 under an optimized condition afforded \(\beta\)-linked disaccharide 15 quantitatively. (# 1) Meanwhile, when \(\text{C}_1\) donor 3 was employed, disaccharide 16 was obtained only in 22% yield. (# 2) The glycoside 17 which was obtained from the donor 4 was also unstable at -10 °C. (# 3) By keeping the reaction at -40 °C, however, disaccharide 17 was isolated quantitatively with a slight increase of \(\beta\)-selectivity. (# 4) Thus, the remarkable high yield, \(\beta\)-selectivity, and stability under acidic conditions of glycoside 15 clarified the advantage of donor 1.

In the last place, synthesis of HS type disaccharide was evaluated by using a 2,3-carbamate protected glucosamine derivative 7 as an acceptor which can apply to a donor for \(\alpha\)-selective glycosylation\(^8\) at further glycosylation steps. (# 5-8) Glycosylation reaction to 4-OH of \(N\)-acyethylglucosamine derivatives is a well-known challenge because of the poor nucleophilicity.\(^20\) Indeed, the coupling of donor 1 and acceptor 7 at -10 °C gave HS-type disaccharide 18 only in 22% yield. (# 5) Analysis of this reaction revealed the existence of orthoester at -10 °C. This fact suggested that the conversion to the glycoside was incomplete. Tracing the reaction at 0 °C identified that the orthoester disappeared in 3 hours and the \(\beta\)-glycoside 18 was isolated in 63% yield. (# 6) The yield was still moderate but enough for the fragment synthesis as in previous excellent studies.\(^22\)\(^\alpha\)\(^21\) As expected, a donor 3 with acetyl group at O-2 afforded \(\beta\)-linked disaccharide 19 only in 18% yield. (# 7) Interestingly, disaccharide 20 was stable even at 0 °C and the yield was 93% yield as a \(\alpha\)-rich mixture. (# 8)

In summary, we designed a novel glucuronate donor with 6,3-lactone and 2-O-acetyl protection for the construction of glucuronic acid-containing entities. The 6,3-lactone donor 1 demonstrated an excellent versatility via a series of GAGs disaccharide syntheses. In particular, ideal \(\beta\)-selectivities, high yields, and stable glycosides are noteworthy features. Therefore, we believe that donor 1 provides a practical approach to prepare various glycosides and glycoconjugates containing unonate moieties. Currently, further application of this glucuronate donor for longer GAG oligosaccharide synthesis is in progress.

**Acknowledgments**
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Table 2. Glycosylation with glucosamine derivatives 6 and 7 under NIS/TfOH system

<table>
<thead>
<tr>
<th>#</th>
<th>donor</th>
<th>acceptor</th>
<th>Conditions</th>
<th>product/α:β ratio</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>6</td>
<td>A</td>
<td>15 (β only)</td>
<td>quant.</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6</td>
<td>A</td>
<td>16 (β only)</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
<td>A</td>
<td>17 (α:β = 5:2)</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>6</td>
<td>B</td>
<td>17 (α:β = 5:3)</td>
<td>quant.</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>7</td>
<td>A</td>
<td>18 (β only)</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>7</td>
<td>C</td>
<td>19 (β only)</td>
<td>83</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>7</td>
<td>C</td>
<td>20 (α:β = 7:3)</td>
<td>93</td>
</tr>
</tbody>
</table>

(A) donor (1.5 eq.), acceptor (1.0 eq.), NIS (1.5 eq.), TfOH (0.2 eq.), DCM (0.07 M), -40 °C, 1 h, then -10 °C, 1 h; (B) donor (1.2 eq.), acceptor (1.0 eq.), NIS (1.2 eq.), TfOH (0.2 eq.), DCM (0.1 M), -40 °C, 2 h; (C) donor (1.5 eq.), acceptor (1.0 eq.), NIS (1.5 eq.), TfOH (0.2 eq.), DCM (0.07 M), -40 °C, 2 h, then 0 °C, 3 h.

1 Isolated ratio.
2 Isolated yields based on the acceptor.
3 Determined by 1H-NMR analysis.

References and notes


17. See supporting information, Scheme 2.


