On the Reactivity of Gulose and Guluronic Acid Building Blocks in the Context of Alginate Assembly


Abstract: A novel set of L-gulose and L-guluronic acid building blocks, comprising both monosaccharide and disaccharide synthons, was assembled to investigate how structural changes in these synthons influence the outcome of glycosylation reactions in which they are involved. Our studies corroborate previous reports that identified the gulosyl C4-OH group as a relatively poor nucleophile. We did not find a major influence of the neighboring C5 functionality on the reactivity of the alcohols, and the gulose acceptors did not provide more productive glycosylation reactions than their guluronic acid counterparts. The conformational behavior of the synthons was found to be of prime importance to the outcome of the reactions.

Introduction

Gulose (Figure 1) is a rare monosaccharide that can be found in bacteria, archaea, and algae. It can be considered to be the C3 epimer of galactose or the C5 epimer of mannose. L-Guluronic acid and D-mannuronic acid are the two constituting monomers of alginate (see Figure 1), an important cell-wall polysaccharide of brown algae that is used in the pharmaceutical industry and food industry because of its gelating properties.[1]

Alginate also represents the exopolysaccharide of Pseudomonas aeruginosa, an opportunistic pathogen that is responsible for, amongst others, urinary tract, kidney, lung, and burn wound infections.[13] P. aeruginosa uses alginate to create a protective biofilm, which makes it difficult to combat the bacterium by the host immune system and antibiotic therapies. Short alginate fragments can interact with the innate part of our immune system through interactions with Toll-like receptors (TLRs),[3] and polymannuronic acid alginates have been used as a carbohydrate antigen in protein conjugate vaccine modalities to generate a potential Pseudomonas aeruginosa vaccine.[4]

Well-defined synthetic fragments of the alginate polysaccharide are very valuable tools to unravel the mode of action of alginate at the molecular level.[5] Therefore, we and others[6] have developed synthetic strategies to assemble different stretches of the alginate polymer. We devised efficient routes towards the assembly of oligomannuronates,[7] short oligoguluronates,[8] and alternating mannuronic acid–guluronic acid oligomers.[9] During these studies, it became apparent that gulosyl donor building blocks have the tendency to provide 1,2-cis-glycosidic linkages with unusual selectivity.[10] We rationalized this behavior by taking into account the reactivity of the intermediate oxocarbenium ion (\(\cdot\)) intermediates. An L-gulose oxocarbenium ion can adopt a 4H3 half-chair conformation, in which all substituents occupy an orientation considered favorable for the stability of the cation.[11] Attack on this ion by the incoming nucleophile occurs from the diastereotopic face that leads, via a chairlike transition state, to the 1,2-cis-product. Thus, the stereoselective introduction of the \(\alpha\)-gulosyl linkage can be effected with relative ease. During our synthetic efforts it became apparent, however, that the gulosyl C4-OH group is a relatively poor nucleophile, which makes the assembly of guluronic acid containing alginate fragments a challenging undertaking. Hung and co-workers reported on the poor nucleophilicity of gulosyl acceptors as well, and they successfully employed 1,6-anhydrogulose synthons,[6] which changed the steric and electronic surroundings of the alcohol. Functional groups on a carbohydrate not only influence the reactivity of a carbohydrate donor building block but also the nucleophilicity of the carbohydrate acceptors, and it is often surmised that uronic

Figure 1. Structures of L-gulose, L-guluronic acid, and mixed sequence alginate.

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acid acceptors are relatively poor nucleophiles because of the electron-withdrawing effect of the C5 carboxylate group.\textsuperscript{12} To find an effective gulose/guluronic acid acceptor building block for the assembly of mixed alginate sequences and to shed light on the influence of the neighboring C5 functionality on the reactivity of the gulose acceptors, we studied a panel of gulose acceptors in a variety of glycosylation reactions. The results of these studies are reported herein.

Results and Discussion

We investigated a set of glycosylation reactions by using gulosyl acceptors, varying in the nature of their C5 functionality, and by using coupling partners of varying size. Both monomeric and dimeric donors and acceptors were combined and both guluronic acid acceptors and gulose acceptors were examined. We also varied the nature of the C6 O-protecting group in the gulose acceptors to see whether this had any influence on the efficiency of the condensation reactions.

The set of donors and acceptors and the synthesis of the new gulosyl acceptors (see compounds 3–6, 8, 9, 11, and 12) are shown in Scheme 1. Starting from silyliden-protected \(\alpha\)-azidopropyl L-guloside 1, the synthesis of which we reported previously,\textsuperscript{[9]} monomeric acceptors 3–6 were obtained. Thus, the silyliden functionality was removed to provide diol 2, of which the primary alcohol was protected with an acetyl group (in 3), as an allyl ether (in 4), or masked with a cyanoethoxymethyl (CEM) group (in 5). The latter group has not been employed in oligosaccharide synthesis before but has found applications in RNA assembly and serves as a minimally intrusive base-labile alcohol protecting group.\textsuperscript{[13]} All these regioselective protections were achieved by using Taylor’s 2-aminoethyl diphenylborinate catalyst in conjunction with the appropriate electrophiles (i.e., acetyl chloride, allyl bromide, cyanoethoxymethyl chloride).\textsuperscript{[14]} Guluronic ester acceptor 6 was obtained from 2 by regioselective oxidation by using a combination of 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and bisacetoxy iodobenzene (BAIB) and ensuing ester formation as previously described.\textsuperscript{[9]} The assembly of the disaccharide acceptors is also depicted in Scheme 1. We generated a set of four disaccharide acceptors (see compounds 8, 9,\textsuperscript{[9]} 11, and 12\textsuperscript{[9]}), having either a guluronic ester or a gulose acceptor at the nonreducing end and with either an anemic \(\alpha\)-thiocresol (STol) or an \(\beta\)-azidopropyl group attached to the mannuronic acid side. The disaccharide acceptors were obtained from fully protected gulose–mannuronic acid disaccharides 7 and 10.\textsuperscript{[9]} Unmasking of the silyliden as described above and ensuing regioselective acetylation of the C6-OH group, again by using Taylor’s borinic acid catalyst and acetyl chloride, gave gulose–mannuronic acid coupling partners 8 and 11. Oxidation of the liberated primary alcohol functionalities and methyl ester formation gave guluronic acid–mannuronic acid acceptors 9 and 12.

With the set of donors (i.e., 13\textsuperscript{[7d]} and 14\textsuperscript{[4]}),\textsuperscript{[4]} and acceptors in hand, we performed the series of glycosylation reactions summarized in Table 1. First, we combined mannuronic acid monosaccharide donor 13 with the three differentially protected monomeric gulose acceptors 3–5 (Table 1, entries 1–3). The three condensation reactions proceeded under trimethylsilyl trifluoromethanesulfonate (TMSOTf) catalysis and gave disaccharides 15–17 with excellent stereoselectivities but in relatively poor yields. Where it could be reasoned that a more electron-rich protecting group at C6 would lead to a more nucleophilic C4-OH group, this was not apparent from the obtained results: the C6-OAc gulose acceptor outperformed the acceptors protected with the allyl ether or cyanoethoxymethyl protecting groups (Table 1, entries 1–3). In the next set of optimizations, we found that the efficiency of the condensation of mannuronic acid donor 13 and C6-OAc gulose acceptor 3 could be improved by the use of tert-butyl(dimethyl)silyl trifluoromethanesulfonate (TBSOTf), but not trifluoromethanesulfonic acid (TfOH), instead of TMSOTf under otherwise unchanged conditions (Table 1, entries 4 and 5). Upon using TBSOTf as a promoter in the condensation of guluronic acid acceptor 6 and donor 13, disaccharide 18 was obtained in 55 % yield. Notably, the stereoselectivity of this coupling reaction was significantly worse than that obtained in the other glycosylations of mannuronic acid donor 13, for which we currently do not have an adequate explanation.

We then moved to the glycosylations of disaccharide donor 14. In the first instance, 14 was treated with either C6-OAc gulose acceptor 3 or guluronic ester acceptor 6 under the agency of a catalytic amount of TBSOTf (Table 1, entries 7 and...
Table 1. Glycosylation reactions by using different gulosyl acceptors with mannuronic acid donors.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>Acceptor</th>
<th>Conditions[a]</th>
<th>Product</th>
<th>Yield [%] (ratio α/β)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>3</td>
<td>TMSOTf</td>
<td>15</td>
<td>49 (0:1)</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>4</td>
<td>TMSOTf</td>
<td>16</td>
<td>23 (0:1)</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>5</td>
<td>TMSOTf</td>
<td>17</td>
<td>35 (0:1)</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>3</td>
<td>TROH</td>
<td>15</td>
<td>30 (0:1)</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>3</td>
<td>TBSOTf</td>
<td>15</td>
<td>65 (0:1)</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>6</td>
<td>TBSOTf</td>
<td>18</td>
<td>55 (1:3)</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>3</td>
<td>TBSOTf</td>
<td>19</td>
<td>69 (0:1)</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>6</td>
<td>TBSOTf</td>
<td>20</td>
<td>84 (0:1)</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>11</td>
<td>TBSOTf</td>
<td>21</td>
<td>33 (0:1)</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>12</td>
<td>TBSOTf</td>
<td>22</td>
<td>26 (0:1)</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>8</td>
<td>TBSOTf</td>
<td>23</td>
<td>80 (0:1)</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
<td>9</td>
<td>TBSOTf</td>
<td>24</td>
<td>91 (0:1)</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>8</td>
<td>TBSOTf</td>
<td>25</td>
<td>77 (0:1)</td>
</tr>
<tr>
<td>14</td>
<td>13</td>
<td>9</td>
<td>TBSOTf</td>
<td>26</td>
<td>100 (0:1)</td>
</tr>
</tbody>
</table>

[a] In CH2Cl2, –78 to –20 °C over several hours. [b] Determined by analysis of the isolated products by NMR spectroscopy.

8). Here, the guluronic acid acceptor gave the most productive glycosylation reaction (84 vs. 69 % for the C6-OAc acceptor), and trisaccharide 20 was obtained as a single anomer, in contrast to the condensation of 6 with monosaccharide donor 13 (Table 1, entry 6). We then investigated disaccharide acceptors 8, 9, 11, and 12. Upon condensing azidopropyl-functionalized dimers 11 and 12 with dimer donor 14, tetrasaccharides 21 and 22 were obtained in low yields (33 and 26 %, respectively; Table 1, entries 9 and 10). Apparently, the larger size of the disaccharide nucleophiles has a large impact on the reactivity of the gulosyl and guluronic acid C4-OH group.

Switching to the acceptor disaccharides with the anomeric α-thiocresol moiety gave a significant increase in yield both for C6-OAc gulose acceptor 8 and guluronic acid acceptor 9. Tetrasaccharides 23 and 24 were obtained in yields of 80 and 91 %, respectively (Table 1, entries 11 and 12). We rationalize the striking differences observed for the glycosylation of the α-STol and β-azidopropyl acceptors (i.e., 8 and 9 vs. 11 and 12) to the different conformational behavior of these saccharides. Whereas β-configured mannuronic acids 11 and 12 adopt a fixed 4C₄ chair conformation, corresponding α-configured mannuronic acids 8 and 9 display considerable conformational flexibility. The conformational flexibility of 8 and 9 is reflected in their ¹H NMR and ¹³C NMR spectra; the signals of the mannuronic acid ring appear as broad and poorly resolved resonances at room temperature. Figure 2 displays the NMR spectra of acceptor 9 recorded at different temperatures. At low temperature (~60 °C), two resonance sets are apparent that coalesce with increasing temperature. The two resonance sets belong to the disaccharides with the mannuronic acid in a “normal” 4C₁ chair conformation or taking up a 1C₄ chair conformation. It becomes clear from the spectra that the 1C₄ chair conformer is the most prevalent acceptor species present in the mixture. Upon ring flipping of the reducing end mannuronic acid to a 1C₄ chair, the overall structure of the disaccharide becomes more open, which makes the C₄ hydroxy group more accessible and, therefore, more reactive. This effect manifests itself in both the guluronic acid (i.e., 9) and gulose (i.e., 8) dimer acceptors. Upon condensing flexible acceptors 8 and 9 with monosaccharide donor 13 (Table 1, entries 13 and 14), trisaccharides 25 and 26 were obtained in good yields with excellent stereoselectivities. In light of the poor coupling of the same donor with monomeric acceptor 6, the latter outcome is striking. It may be that the ring flip of the mannuronic acid in 8 and 9 not only allows for a more open structure (a shape that also monosaccharide 6 could attain) but that it forces the guluronic acid ring to adopt a particular geometry leading to more effective exposure of the C4-OH group.
Conclusions

In conclusion, we described a set of glycosylation reactions to produce fully protected mixed-sequence alginate oligomers up to the tetrasaccharide level. It was found that the gulosyl C4 hydroxy group was a relatively poor nucleophile that was hard to glycosylate. From the results presented in Table 1, it can be concluded that the functional group close to the acceptor alcohol group had little influence on its reactivity, and at least in the set of glycosylations studied herein, we did not find an important disarming effect of the C5 carboxylate group on the reactivity of the C4-OH group. In fact, the C5 carboxylic acid ester acceptors outperformed their nonoxidized counterparts (see Table 1, entry 7 vs. 8, 11 vs. 12, and 13 vs. 14). An all-important factor, influencing the effectiveness of the glycosylations, turned out to be the conformational flexibility of the acceptors at hand. For cases in which the presence of a rigid β-mannuronic acid O-glycoside reducing end in the disaccharide acceptors led to poor glycosylation reactions, the flexible α-tolyl mannuronic acid reducing ends endowed the acceptors with excellent nucleophilicity. Further studies are required to provide detailed insight into how the conformational behavior of mannuronic acid reducing ends influences the steric and electronic surroundings of the gulose C4′ alcohol moiety. Conformational flexibility may prove to be important in many other glycosylations, as glycosylation reactions involving secondary alcohol acceptors generally proceed through a very crowded transition state.

Experimental Section

General Procedure for the Glycosylation Reactions: The imidate donor (1.5–3.0 equiv.) and acceptor (1.0 equiv.) were coevaporated with toluene (3×). The residue was dissolved in dry CH2Cl2 (0.1 M acceptor in CH2Cl2). The solution was cooled to –78 °C, which was followed by the addition of TBSOTf or TMSOTf (0.2–0.6 equiv.), and the mixture was stirred for 12–48 h at –78 to –20 °C. The reaction was quenched by the addition of Et3N, and the mixture was diluted with EtOAc and washed with satd. aq. NaCl. The organic phase was dried with Na2SO4 and concentrated in vacuo. Purification by column chromatography yielded the product.

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