Synthesis of the α-Linked Digitoxose Trisaccharide Fragment of Kijanimicin: An Unexpected Application of Glycosyl Sulfonates

J. Colin Mizia,† Mohammed U. Syed,† and Clay S. Bennett*  

ABSTRACT: Previously, we demonstrated that glycosyl tosylates are effective for the synthesis of β-glycosides of gluco-configured 2-deoxy sugars. Here, we show the same sulfonate system can be used for the selective synthesis of α-glycosides containing the allo-configured 2-deoxy sugar digitoxose. As with previous work, optimal selectivity is obtained through matching the donor with the appropriate arylsulfonyl chloride promoter. The utility of this method is demonstrated through the synthesis of the α-linked digitoxose trisaccharide fragment of kijanimicin.

Bacterial secondary metabolites represent a diverse class of compounds, many of which have potential as leads for new antibacterial and antineoplastic agents.1 Approximately one-third of all bacterial secondary metabolites are glycosylated, often consisting of sugar residues exotic to the mammalian glycome.2,3 Altering the composition of the oligosaccharide fragments within glycosylated compounds has been shown to have an effect on their pharmacodynamic properties.4,5 This approach, termed glycorandomization, opens a path for further development of these secondary metabolites into new therapeutics.

A major hurdle in applying this approach to drug development lies in the construction of these glycans, which often contain 2,6-dideoxy sugars. Stereoselective glycosylation reactions with these molecules are uniquely challenging due to the absence of a directing group adjacent to the anomeric center and the increased reactivity of deoxy sugars,6,7 which can lead to reactions which span the SN1/SN2 continuum.8−10 Our lab and others have made significant strides in recent years toward the stereoselective synthesis of 2-deoxy glycosides,7,11−30 but an effective, generally applicable method remains elusive. In particular, while good progress has been made with gluco- and galacto-configured donors, other classes of deoxy sugars remain underexplored.

Digitoxose is one such sugar, naturally occurring in cardiac glycosides as the D-enantiomer and in bacterial secondary metabolite antibiotics as both D- and L-enantiomers (Figure 1).5,31 Methods for the direct synthesis of β-digitoxose have been well established, often relying upon the use of remote participation or steric buttressing from protecting groups at the C3 position.32−34 In contrast, α-digitoxose, often found in bacterial natural products such as the jadomycins,35,36 saccharomycins,37 and tetrocarcins,38,39 is generally considered to be more challenging to synthesize. In particular, the axial C3-hydroxyl group can hinder approach by the nucleophilic glycosyl acceptor from the α-face of the molecule. This has led
to methods of selective α-glycosylation with digitoxose being more diverse, including N-iodosuccinimide-mediated activation of glycal,\textsuperscript{46} mild activation of digitoxose thioglycosides,\textsuperscript{41,42} and chelation-controlled O-alkylation of digitoxose hemiacetals.\textsuperscript{43} However, a general, mild method using readily accessible and stable starting materials has yet to be reported.

Given our past work on the reagent controlled synthesis of β-linked 2-deoxy sugar glycosides,\textsuperscript{14-16} we sought to extend this methodology to the construction of β-linked digitoxose residues, such as those found in digitoxin, without the use of participating groups at the C3 position. Our initial test of this approach involved glycosylating p-methoxyphenol (PMP-OH) with a selectively protected digitoxose hemiacetal donor 3 under our previously published conditions. To our surprise these conditions afforded the product predominantly as the α-glycoside 4 (Scheme 1). While this was not our anticipated outcome, we realized that this system could possibly provide an efficient route to α-digitoxosides. To the best of our knowledge, this also would represent the first direct, dehydrative approach to the synthesis of α-digitoxose.

To further explore this unexpected result, we studied the glycosylation of glucose acceptor 5 as a model system (Table 1). Applying the same conditions that had been used for the initial PMP-OH glycosylation afforded disaccharide 6 in both moderate yield and selectivity, again predominantly as the α-anomer (Table 1, entry 1). Extending the activation time for these conditions afforded the product predominantly as the α-glycoside 4 (Scheme 1). While this was not our anticipated outcome, we realized that this system could possibly provide an efficient route to α-digitoxosides. To the best of our knowledge, this also would represent the first direct, dehydrative approach to the synthesis of α-digitoxose.

Table 1. Optimization of α-Glycosylation with Digitoxose Donor

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor/Acceptor</th>
<th>Ar</th>
<th>Activation Time</th>
<th>Yield</th>
<th>α:β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1</td>
<td>p-Me(C₆H₄)</td>
<td>5 min</td>
<td>63%</td>
<td>3:1:1</td>
</tr>
<tr>
<td>2</td>
<td>1:1</td>
<td>p-Me(C₆H₄)</td>
<td>15 min</td>
<td>65%</td>
<td>3:1:1</td>
</tr>
<tr>
<td>3</td>
<td>1:1</td>
<td>p-Me(C₆H₄)</td>
<td>30 min</td>
<td>57%</td>
<td>1:1</td>
</tr>
<tr>
<td>4</td>
<td>1:1</td>
<td>p-NO₂(C₆H₄)</td>
<td>5 min</td>
<td>36%</td>
<td>1:1:3</td>
</tr>
<tr>
<td>5</td>
<td>1:1</td>
<td>p-OPr(C₆H₄)</td>
<td>5 min</td>
<td>46%</td>
<td>6:6:1</td>
</tr>
<tr>
<td>6</td>
<td>1.5:1</td>
<td>p-OPr(C₆H₄)</td>
<td>10 min</td>
<td>82%</td>
<td>4.5:1</td>
</tr>
</tbody>
</table>

\textsuperscript{a}All reactions run with 100 mg of acceptor. \textsuperscript{b}Time between ii and iii. \textsuperscript{c}Isolated yield. \textsuperscript{d}Anomeric ratio determined by NMR (500 MHz).

Having established an approach to α-linked digitoxose linkages with good selectivity, we sought out an appropriate target to examine the utility of this chemistry. We identified the oligosaccharide fragment of kijanimicin as a model system (Figure 1). First isolated from Actinomadura kijaniata by Waitz et al. in 1981,\textsuperscript{47} kijanimicin possesses antibiotic activity against Gram-positive bacteria, as well as demonstrated in vivo activity toward rodent malaria on par with Daraprim. Additionally, it was shown to have moderate antitumor activity against lymphatic leukemia in mice.\textsuperscript{48} Importantly for this study, the oligosaccharide fragment of kijanimicin contains a branched tetrasaccharide consisting of a chain of three α-linked digitoxose residues, with an additional β-linked digitoxose at the nonreducing end. To date, there have been two previous syntheses of this target from the Thiem lab. In the first approach, α-digitoxose was synthesized through NIS activation of digitoxal, followed by reductive dehalogenation, reaching the desired trisaccharide in 0.2% yield from commercial sources. In the second synthesis, the stereochemistry was set through α-selective glycosylation with olivose donors followed by post-glycosylation modification, synthesizing the complete tetrasaccharide in 1.5% yield from commercial starting materials.\textsuperscript{49} While this represented a significant improvement on the previous synthesis, the approach lacks the general applicability we hoped our new method would provide. We envisioned that the α-linked trisaccharide would serve as an excellent target to demonstrate the utility of this methodology. Retrosynthetically, we envisioned the trisaccharide structure 8 would arise through three subsequent glycosylations with the same selectively protected digitoxose hemiacetal donor 3 (Scheme 2).

The digitoxose hemiacetal donor 3 was prepared on gram scale from commercially available di-O-acetyl-l-rhamnol (see Supporting Information).\textsuperscript{12,50} Initial studies began with
returning to the glycosylation of p-methoxyphenol as an aglycone analogue. Using p-O-isopropylbenzensulfonyl chloride as a promoter, we were able to obtain PMP-glycoside 4 in 55% yield as a 10:1 (α:β) mixture of anomers (Scheme 3).

Subsequent selective removal of the 2-naphthylmethyl protecting group at C3 via oxidative cleavage with DDQ 51 led to the two anomers of 10 being separated chromatographically, yielding the desired acceptor.

With acceptor 10 in hand, we turned our attention to the synthesis of disaccharide 11. Using the same optimized conditions from our model glycosylation, we were able to synthesize 11 in 81% yield as a 4.4:1 (α:β) mixture of anomers (Scheme 4), in good agreement with our observations of the reaction with model acceptor 5. To confirm that the p-O-isopropylbenzenesulfonyl chloride promoter truly represented a general improvement to glycosylation with digitoxose, we repeated the same coupling using tosyl chloride as a promoter.

Using 3 equiv of KHMDS in 0.5 M toluene solution, we were able to obtain 8 in 3% overall yield from commercially available material.

Finally, we turned our attention to global deprotection of the trisaccharide. To our knowledge, the deprotection of a hindered deoxy sugar oligosaccharide such as 8 had not previously been reported. Hence, this compound would serve as a testing ground for existing methodologies for benzyl deprotection on congested systems. We initially examined hydrogenation with Raney nickel, which we and others had used successfully in previous syntheses.53,54 Unfortunately, these conditions led to only partial removal of the benzyl ethers (Table 3, entry 1). More traditional hydrogenation over palladium on carbon and the reportedly more reactive mixture of palladium on carbon and palladium hydroxide55 were both ineffective (entries 2−3). Since reduction with Raney nickel was the most promising result, we sought a method to drive THF to toluene did not lead to an increase in yield, and using NaHMDMS as the base completely failed to activate the reaction (entries 4−6). Interestingly, in this instance we found using tosyl chloride as a promoter did not result in any loss of selectivity (entry 7), in contrast with our previous observations. It has been noted in previous studies of the synthesis of kijanimicin oligosaccharides that reactions at this particular hydroxyl group of this digitoxose disaccharide tend to be difficult, due to the steric bulk around the hydroxyl group.52 Still, even with the moderate yield of the final glycosylation, we were able to obtain 8 in 3% overall yield from commercially available material.

Having established a route to the α-linked disaccharide 9, we sought to complete the synthesis of trisaccharide 8. Once again using p-O-isopropylbenzenesulfonyl chloride as a promoter, we were able to couple 9 and 3 to afford 8 exclusively as the α-anomer, albeit in a moderate 24% yield (Table 2, entry 1). Increasing the ratio of donor to acceptor failed to improve this yield (entry 2). Subsequent attempts to drive the reaction using excess acceptor only provided a small increase in yield (entry 3) Switching the solvent of our KHMDS solution from

### Table 2. Synthesis and Optimization of Digitoxose Trisaccharide

<table>
<thead>
<tr>
<th>Entry</th>
<th>Yield</th>
<th>Recovered 9</th>
<th>α:β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24%</td>
<td>52%</td>
<td>α-only</td>
</tr>
<tr>
<td>2</td>
<td>23%</td>
<td>42%</td>
<td>α-only</td>
</tr>
<tr>
<td>3</td>
<td>33%</td>
<td>43%</td>
<td>α-only</td>
</tr>
<tr>
<td>4</td>
<td>25%</td>
<td>46%</td>
<td>α-only</td>
</tr>
<tr>
<td>5</td>
<td>31%</td>
<td>48%</td>
<td>α-only</td>
</tr>
<tr>
<td>6</td>
<td>0%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>33%</td>
<td>42%</td>
<td>α-only</td>
</tr>
</tbody>
</table>

PoIsCl = p-O-isopropylbenzenesulfonyl chloride. Based on recovered acceptor. Ratio determined by NMR. Using TsCl as promoter.

### Table 3. Global Deprotection of Trisaccharide 8

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reductant</th>
<th>Solvent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Raney Ni</td>
<td>EtOH/THF (4:5:1)</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>H2, Pd/C</td>
<td>MeOH</td>
<td>N.R.</td>
</tr>
<tr>
<td>3</td>
<td>H2, Pd/C (10%), Pd(OH)2/C (20%)</td>
<td>MeOH</td>
<td>N.R.</td>
</tr>
<tr>
<td>4</td>
<td>H2, Raney Ni</td>
<td>EtOH/THF (4:5:1)</td>
<td>43%</td>
</tr>
</tbody>
</table>
the reduction to completion. To this end, we repeated the reduction running it under an atmosphere of H2. Pleasingly, these conditions afforded the desired trisaccharide tetraol in 43% yield (entry 4).

In conclusion, we have demonstrated that our sulfonyl chloride promoted dehydrative glycosylation chemistry can be used for the direct construction of α-linked digalactose residues. As proof of principle, we successfully applied this chemistry to the synthesis of the oligosaccharide fragment of kijananicin. The selectivity with these substrates stands in contrast to our previous studies on glycosyl arylsulfonates which provided β-linked products through the direct displacement of a reactive α-sulfonate intermediate. The results from this work suggest that the glycosylation with digalactose is proceeding through a different mechanism than our previously developed chemistry.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c04190.

Experimental details and compound characterization (PDF)

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Notes

The authors declare no competing financial interest.

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