Studies toward the Synthesis of Neu5Acα-(2-6)-Galβ-(1-4)-GlcNAc

Trisaccharide

Master's Thesis

Presented to

The Faculty of the Graduate School of Arts and Sciences
Brandeis University
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Professor Isaac Krauss, Advisor

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of the Requirements for

Master's Degree

by
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Abstract

Studies toward the Synthesis of Neu5Acα-(2-6)-Galβ-(1-4)-GlcNAc Trisaccharide

A thesis presented to the Chemistry Department

Graduate School of Arts and Sciences
Brandeis University
Waltham, Massachusetts

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Neu5Acα-(2-6)-Galβ-(1-4)-GlcNAc trisaccharide has been shown to be a known sialic acid glycan linkage. The synthesis towards Neu5Acα-(2-6)-Galβ-(1-4)-GlcNAc trisaccharide involved using acetylation, deacetylation, benzylidenation, debenzylidenation, Koenigs-Knorr glycosylation, Schmidt glycosylation, and sialylation reactions. The Galβ-(1-4)-GlcNAc disaccharide was successfully synthesized and will be employed in the studies towards the synthesis of Neu5Acα-(2-6)-Galβ-(1-4)-GlcNAc trisaccharide.
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**Introduction**

The sialic acid-binding immunoglobulin-like lectins (Siglecs) are members of the Immunoglobulin (Ig) super-family that bind to sialic-acid. Siglecs are expressed on mainly on cells of the immune system. Siglecs contain an N-terminal V-set Ig domain that binds to the sialic acid-containing ligand, followed by C2-set Ig-like domains of varying numbers. Siglecs are classified into two subsets based on their similarities. The first is Sialoadhesin (Siglec-1), CD22 (Siglec-2), CD33 (Siglec-3) and myelin-associated glycoprotein, or MAG (Siglec-4) and the second is CD33-related Siglecs. All siglecs have a preference for a certain sialic acid containing glycans that they will bind to, such as Neu5Ac α(2-6) or α(2-3) to Galactose.

One specific sialoside linkage that is of particular interest is the Neu5Ac(α2-6)-Galβ(1-4)-GlcNAc which has a strong binding to CD22 (Siglec-2). CD22 (Siglec-2) which is expressed on B-cells specifically. CD22 controls B-cell receptor signaling, and has a high selective binding for α2,6 sialic acid linkage with a sulfate group on the 6-postion of the GlcNAc moiety. CD22 is of particular interest because it was identified as marker of B-cell lymphomas. There are anti-CD22 antibodies in clinical trials for B-cell non-Hodgkins lymphoma. Since the expression of siglecs is specific for only a few cell types, siglecs are prime targets for cell-directed immunotherapy.
The Sialic acids

Sialic acids are a unique family of natural α-keto acids. This very diverse family of sialic acids was originally discovered by Klenk and Blix back in the late 1930s. There are more than 50 structurally different molecules in nature that are involved in numerous biological processes. Sialic acids are typically found at the non-reducing end of many glycans. The most abundant and well known sialic acid is N-acetylneuraminic acid (Neu5Ac). The ring of the nine-carbon backbone of sialic acid is structurally unique compared to other pyranose ring systems. The sialic acid ring system has a \(^2\)C\(_5\) conformation. The anomeric center of sialic acids have a very sterically hindered tertiary center with an electron-withdrawing carboxylic acid group present. The naturally occurring \(\alpha\)-configuration has an equatorial glycoside and the \(\beta\)-configuration has an unnatural axial glycoside. In the sialic acid ring system the C-3 lacks a hydroxyl group, the C-6 has an equatorial bulky side chain and the C-5 has an equatorial acetamido group. These unique structural characteristics for sialic acids make glycosylation reactions difficult.

\[
\text{\(\alpha\text{Neu5Ac}\)} \quad \text{\(\beta\text{Neu5Ac}\)}
\]

Figure 1.

A typical glycosylation to form a 1, 2-trans glycoside uses neighboring group participation to help control the stereochemical outcome. Sialic acids lack a hydroxyl
group at the C-3 position and therefore cannot use neighboring group participation to control stereochemistry. The use of sialic acid as a glycosyl donor is further complicated by the sterically hindered tertiary anomeric center. Also, the lack of the hydroxyl group at C-3 and the electron-withdrawing carboxylate group at the anomeric center makes these compounds prone to 2, 3-elimination. These complications with sialic acids are the reasons for low yielding unselective glycosylation reactions.

**Previous Work**

Bovin and co-workers have previously described a successful synthesis of α2, 6-sialooligosaccharides under classical Koenigs-Knorr conditions with sialyl chloride donor 3 and 4, 6-diols 35. The sialoside 36 was obtained in a 95% yield of the α-anomer with trace amounts of the β-anomer. (Scheme 1)

\[\text{Scheme 1. Synthesis of trisaccharide 36.}\]
Bovin and coworkers have also successfully synthesized O-sulfonated sialoside 38 from the free hydroxyl at C-6 position of the GluNAc moeity 37 with Pyr'SO₃ in pyridine.¹¹,¹²,¹³ (Scheme 2)

![Scheme 2. Synthesis of the O-sulfated trisaccharide 38.](image)

**Goal of the Project and Synthetic Consideration**

Bovin’s successful sialylation reaction of trisaccharide 36 gave inspiration for the synthetic plan of our trisaccharide 1 as detailed in Scheme 3. The structure of trisaccharide 1 will be different from Bovin’s trisaccharide 36. The trisaccharide will contain a cyclohexyl linker instead of a straight chain aminopropyl linker.

The glucosamine cyclohexylamine 9 is synthesized from glucosamine hydrochloride using an extension of the Koenigs – Knorr glycosylation, known as the phthalimido glycosylation method.¹⁴ The glucosamine hydrochloride can be converted to the β-chloride 10¹⁵,¹⁶ and employed in a AgOTf coupling reaction to give the
Importantly, the phthalimido glycosylation gives the $\beta$-glycoside when the phthalimido halide glycosyl donor is used. The reason for this is because the halide is rapidly abstracted to give a bicyclic cation intermediate which the acceptor alcohol can attack from the top face to give the $\beta$-glycoside. The glucosamine cyclohexyl can be deprotected, benzylidenated, acetylated, selectively debenzylidenated and then deacetylated to give diol 6. The coupling of diol 6 and the $\alpha$-trichloroacetimidate galactose donor 7 in a Schmidt glycosylation gives disaccharide 5. Importantly, the use of the diol of the glucosamine cyclohexyl increases the reactivity of the C-4 hydroxyl group for the coupling reaction. The necessity for using the diol is because when there is a protecting group is at the C-3 position it is known that the 4-OH group is a very weak nucleophile when compared to other hydroxyl acceptors. This can be contributed to the steric hinderance that surrounds the 4-OH group so the diol is used to decrease the bulk in the C-3 position. The disaccharide can be deprotected, benzylidenated, and debenzylidenated to give diol 2. The coupling of diol 2 and 3 used a sialylation reaction to give the trisaccharide. Importantly, the sialylation reaction must give the $\alpha$-glycoside, which can be difficult. One reason that this can be difficult is the presence of the carboxyl group at anomeric center which is electron-withdrawing and there is no participating group at the C-2 position of the sialic acid to help ensure stereochemistry at the anomeric center. Also there is a competing undesirable 2, 3-elimination reaction. All these factors result in low stereoselectivity in sialylation reactions. The trisaccharide can then be debenzylated, sulfonated, and deprotected to give 1.
Scheme 3. Retrosynthesis of 1 from Glucosamine Hydrochloride.
The stereochemistry of each glycosylation reaction mentioned is important to determine the synthesis of 1. The stereochemistry of each anomeric substituent will be determined to be either α or β by $^1$H and $^{13}$C NMR. Typically, the axial anomeric proton of a β-glycoside has a larger $^3J_{1,2}$ coupling constant than the equatorial anomeric proton of a α-glycoside, ($^3J_{1,2} = 8$-$10$ Hz vs. $^3J_{1,2} = 2$-$3$ Hz). Also, the equatorial anomeric proton has a larger coupling constant with the anomeric carbon than the axial anomeric proton does with the anomeric carbon, ($J_{\text{CH}} = 170$ Hz vs. $J_{\text{CH}} = 160$ Hz).$^{25}$ On the other hand, the stereochemistry for a sialylation reaction cannot be determined the same way since there is no anomeric proton at C-2. There are few ways the anomeric configuration of the sialoside can be determined. One is based on the chemical shifts of H'-3 equatorial proton, the α-anomer is at a lower field (δ = 2.67 to 2.72ppm) than the β-anomer (δ = 2.25 to 2.40ppm)$^{26}$ and another is based on the chemical shifts of H'-4, the α-anomer is at a higher field (δ = 4.89 to 4.93ppm) than the β-anomer (δ = 5.68 to 5.81ppm).$^{27}$ The $J_{H-7,H-8}$ coupling constant is larger for the α-anomer (6.2-8.2Hz) than the β-anomer (2.4-2.6Hz), which suggests different conformations of the side chain.$^{27}$ Another way to determine configuration is based on the value of $\Delta \delta \{H'^{9a}-H'^{9b}\}$, the α-anomer value is smaller than 0.5ppm and the β-anomer is around 1.0ppm.$^{28}$ Also, the $J_{C-1,H-3ax}$ coupling constants of the α-anomer is larger than that of the β-anomer.$^{29}$
Results and Discussion

Synthetic Plan

The synthetic strategy for compound 1 was inspired from previous compounds synthesized by Bovin, with some modifications.\textsuperscript{11,12} The synthesis of 9 is detailed in Scheme 4. First, the amino group of the glucosamine hydrochloride 11 was protected with a phthalimido group by treatment with sodium methoxide and then phthalic anhydride, in methanol; to give the phthalimido protected 12.\textsuperscript{15} Compound 12 was converted to the tetra-O-acetyl-protected 13, in a 88% yield, with acetic anhydride and 5 mol\% DMAP in pyridine.\textsuperscript{15} The tetra-O-acetyl-protected 13 was treated with α,α-dichloromethyl methyl ether and boron trifluoride etherate in chloroform to give β-chloride 10, in a 61% yield.\textsuperscript{16} β-Chloride 10 was employed as the donor for an extension of the Koenigs-Knorr glycosylation with trans(4-hydroxycyclohexyl) benzenesulfonamide as the acceptor using AgOTf to give 9, in 91% yield.\textsuperscript{17} (Scheme 4)

Next, the diol 6 was prepared as detailed in Scheme 5. The reaction of 9 with sodium methoxide in methanol gave deprotected 14, in a 99% yield. Compound 14 was treated with benzaldehyde dimethylacetal and NaHSO₄·SiO₂ in acetonitrile to give the benzylidene protected 8, in a 80% yield. The benzylidene protected 8 was then acetylated with acetic anhydride and 5 mol% DMAP in pyridine, in a 74% yield. Then was selectively deprotected with NaBH₃CN and HCl in diethyl ether, in a 84% yield, and followed by deprotection with sodium methoxide in methanol, giving the diol 6 with a free hydroxyl at C-4, in a 99% yield. (Scheme 5)
The galactose Schmidt donor 7 was prepared as detailed in Scheme 6. First, galactose 16 was converted to the tetra-O-acetyl-protected bromide 17, in an 88% yield, with acetic anhydride and HBr in HOAc. Bromide 17 was then converted to the tetra-O-acetyl-protected hydroxyl 18, in a 72% yield, with Ag$_2$CO$_3$ in water and acetone. The reaction of 18 with trichloroacetonitrile and cesium carbonate in dichloromethane gave the trichloroacetimidate donor 7, in a 75% yield.\textsuperscript{21} (Scheme 6)
**Scheme 6. Synthesis of α-trichloroacetimidate 7.**

The trichloroacetimidate donor 7 was then used for a Schmidt glycosylation with diol 6 as the acceptor in the presence of TMSOTf in dichloromethane to give disaccharide 15, in a 57% yield. ²⁰ (Scheme 7)

**Scheme 7. Synthesis of disaccharide 15.**
Challenges

There were many challenges encountered while proceeding through the synthesis described above, in particular, the two glycosylations that were performed. The problems involved either low yields of the desired product, undesired side products, or no reaction at all.

Following Bovin’s precedent, our first synthetic strategy involved the use of the oxazoline method to couple tetra-O-acetyl-oxazoline\textsuperscript{19} and \textit{trans-}(4-hydroxycyclohexyl) trifluoro-acetamide\textsuperscript{20,31}(Scheme 8).

\begin{center}
\includegraphics[width=\textwidth]{synthesis_attempts.png}
\end{center}

\textit{Scheme 8. Synthesis attempt of monosaccharide 21.}

Bovin’s procedure\textsuperscript{31} for the coupling reaction called for 5 mol\% of TsOH in nitromethane/1,2-dichloroethane; however, this resulted in no conversion to product 21. Various attempts of this coupling reaction were made and detailed in Table 1. All attempts began with multiple drying treatments with toluene and 4Å molecular sieves.
Table 1. Attempts at the glycosylation reaction with the oxazoline 19 and trans-(4-hydroxycyclohexyl) trifluoro-acetamide 20.

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Solvent</th>
<th>Temp</th>
<th>Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mol% TsOH</td>
<td>Nitromethane/Dichloroethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>5 mol% TsOH</td>
<td>Nitromethane/Dichloroethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>5 mol% TsOH</td>
<td>Dichloroethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>TMSOTf / Tetramethyl urea</td>
<td>Dichloroethane</td>
<td>0°C → Reflux</td>
<td>Overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>30 mol % Yb(OTf)₃</td>
<td>Dichloromethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>30 mol % Yb(OTf)₃</td>
<td>Dichloromethane</td>
<td>120°C (sealed tube)</td>
<td>Overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>15 mol % Yb(OTf)₃</td>
<td>Dichloromethane</td>
<td>80°C (microwave)</td>
<td>1 hour</td>
<td>No reaction</td>
</tr>
<tr>
<td>30 mol % Yb(OTf)₃</td>
<td>Dichloromethane</td>
<td>100°C (microwave)</td>
<td>30 minutes</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

It was thought that maybe the trifluoro-acetamide protected amine of the trans (4-hydroxycyclohexyl) trifluoro-acetamide was having deactivating effect on the free hydroxyl group. The protecting group was changed to a benzenesulfonamide protecting group and various attempts of the coupling reaction were made and are detailed in Table 2. (Scheme 9)

![Scheme 9. Synthesis attempt of monosaccharide 23.](image-url)
Table 2. Attempts at the glycosylation reaction with the oxazolidine 19 and trans-(4-hydroxycyclohexyl) benzenesulfonamide.

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Solvent</th>
<th>Temp</th>
<th>Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mol% Yb(OTf)$_3$</td>
<td>Dichloromethane</td>
<td>80°C</td>
<td>1 hour</td>
<td>25% (1/3 SM)</td>
</tr>
<tr>
<td>5 mol% TsOH</td>
<td>Dichloroethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No Reaction</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Dichloroethane</td>
<td>0°C $\rightarrow$ Reflux</td>
<td>Overnight</td>
<td>No Reaction</td>
</tr>
<tr>
<td>5 mol% TsOH</td>
<td>Dichloroethane/ Nitromethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No Reaction</td>
</tr>
<tr>
<td>TsOH</td>
<td>Dichloroethane/ Nitromethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No Reaction</td>
</tr>
<tr>
<td>6 mol% TsOH</td>
<td>Dichloroethane</td>
<td>Reflux</td>
<td>Overnight</td>
<td>No Reaction</td>
</tr>
</tbody>
</table>

Since no glycosylation attempts using the oxazoline donor 19 were successful, an alternative synthetic route was constructed. A search of the literature suggested that only very reactive alcohol nucleophiles are able to open the oxazoline ring and achieve the glycosylated product. As a result it was thought that the phthalimido glycosylation method would be better suited to achieve the desired product. The β-chloride donor with a phthalimido group protecting the nitrogen of the glucosamine was synthesized. The β-chloride donor was then employed as the glycosyl donor with trans-(4-hydroxycyclohexyl) benzenesulfonamide as the acceptor and the use of AgOTf as the promoter and gave the glycosylated product in a 91% yield. The β configuration of the glycosylic linkage was determined using an HSQC experiment. The $J_{C-1,H-1}$ coupling constant was found to be 167Hz, which corresponds to the literature values of a β-glycosylic linkage.

Another glycosylation reaction that proved to be complicated was the Schmidt glycosylation of the galactose trichloroacetimidate donor 7 and glucosamine acceptor 24. We initially followed a procedure which employed 1 equivalent of the galactose...
trichloroacetimidate donor 7 with 1 equivalent of the C-4 free hydroxyl of the glucosamine acceptor 24 using 5 mol% TMSOTf. This resulted in undesired side reactions such as the rearrangement of the Schmidt donor and the hydrolysis of the Schmidt donor and gave a yield lower than 5% for the desired product 25. (Scheme 10)

Scheme 10. Synthesis attempt of disaccharide 25.

One potential problem with the glycosylation is that the trichloroacetimidate Schmidt donor 7 may be hydrolyzed 39 or rearrange to 40 if it is not quickly intercepted by the acceptor alcohol. (Figure 2)

Figure 2. The trichloroacetamide and hydrolyzed galactose, respectively.

In an attempt to address this problem, we increased the amount of donor to 1.5 equivalents with 1 equivalent of the acceptor with using 10 mol% TMSOTf, but this resulted in another low yielding reaction of 5%. Since TMSOTf as the catalyst did not
seem to be catalyzing the desired reaction, BF₃Et₂O was attempted for the glycosylation reaction. Again, only a 5% yield was obtained with undesired products. Various attempts of this coupling reaction were made and detailed in Table 3. All attempts began with multiple drying treatments with toluene and all reactions included 4Å molecular sieves.

**Table 3. Attempts at the Schmidt glycosylation reaction with trichloroacetimidate donor 7 and glucosamine acceptor 24.**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>Promoter</th>
<th>Solvent</th>
<th>Temp</th>
<th>Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 equiv</td>
<td>1 equiv</td>
<td>5 mol% TMSOTf</td>
<td>DCM</td>
<td>r.t.</td>
<td>5 h</td>
<td>Less than 5%</td>
</tr>
<tr>
<td>1.5 equiv</td>
<td>1 equiv</td>
<td>10 mol% TMSOTf</td>
<td>DCM</td>
<td>r.t.</td>
<td>5 h</td>
<td>Less than 5%</td>
</tr>
<tr>
<td>1.2 equiv</td>
<td>1 equiv</td>
<td>1 equiv BF₃Et₂O</td>
<td>Et₂O</td>
<td>-78°C → 0°C → r.t.</td>
<td>3 h</td>
<td>Less than 5%</td>
</tr>
<tr>
<td>1.2 equiv</td>
<td>1 equiv</td>
<td>20 mol% TMSOTf</td>
<td>Et₂O</td>
<td>-78°C → 0°C → r.t.</td>
<td>3 h</td>
<td>Less than 5%</td>
</tr>
<tr>
<td>1.2 equiv</td>
<td>1 equiv</td>
<td>1 equiv BF₃Et₂O</td>
<td>Et₂O</td>
<td>-78°C → 0°C → r.t.</td>
<td>Overnight</td>
<td>Less than 5%</td>
</tr>
<tr>
<td>2 equiv</td>
<td>1 equiv</td>
<td>20 mol% TMSOTf</td>
<td>DCM</td>
<td>-10°C</td>
<td>1.5 h</td>
<td>20% (impure)</td>
</tr>
<tr>
<td>5 equiv</td>
<td>1 equiv</td>
<td>2 equiv BF₃Et₂O</td>
<td>DCM</td>
<td>r.t</td>
<td>4 h</td>
<td>30% (impure)</td>
</tr>
<tr>
<td>5 equiv</td>
<td>1 equiv</td>
<td>2 equiv BF₃Et₂O</td>
<td>DCM</td>
<td>r.t → 40°C</td>
<td>3.5 h</td>
<td>Less than 10%</td>
</tr>
</tbody>
</table>

Our next approach to the Schmidt glycosylation was to use N-phenyl-trifluoroacetimidate³³ as the Schmidt donor. This donor was employed to avoid the undesired rearrangement of the trichloroacetimidate. The procedure called for the use of 1.25 equivalent of N-phenyl-trifluoroacetimidate 26 and 1 equivalent of acceptor 24 using 5 mol% of TMSOTf in dichloromethane from -78°C → -40°C. The reaction also resulted in a low yield of the glycosylated product 25.³³ (Scheme 11)
Since all different attempts were carried out by varying the equivalents of the Schmidt donor 7 and promoter, it was determined that the problem was the acceptor 24. The OH on C-4 of the glucosamine acceptor must be a very unreactive nucleophile because of steric hinderance that surrounds the 4-OH or the electron-withdrawing effects of the neighboring C3-acetate. Therefore, the acetate on the C-3 of the glucosamine acceptor was deprotected to give the diol glucosamine acceptor 6.

The Schmidt glycosylation was carried out with 2 equivalents of the trichloroacetimidate donor 7 and 1 equivalent of the diol acceptor 6 using 20 mol% TMSOTf in dichloromethane at -10°C. In order to reprotect the C-3 hydroxyl group, the crude material from the glycosylation reaction was then carried through acetylation reaction and resulted in a 51% yield. (Scheme 12) The acetylation reaction was performed to confirm that the isolated product was the same as the previous products obtained from the low yielding reactions. Given that the product was the same, the

Scheme 11. Unsuccessful and revised approaches to disaccharide 25.
glycosylation reaction was carried out successfully to give the desired disaccharide. The β configuration of the glycosylic linkage will be determined using an HSQC experiment.


Synthetic Plan Continued

The deprotection of the phthalimido group of disaccharide 15 gave the free amine 27 by ethylene diamine in absolute ethanol, in a 99% yield. The free amine 27 was converted to the acetylated 5 in the presence of acetic anhydride with 5 mol% DMAP in pyridine, in a 55% yield. The reaction of disaccharide 5 with sodium methoxide in methanol gave deprotected 28, in a 98% yield. The deprotected galactose unit of the disaccharide 28 was treated with benzaldehyde dimethylacetal and NaHSO₄-SiO₂ in
acetonitrile and gave the benzylidene protected galactose of the disaccharide 29, in an unoptimized 20% yield. The remaining free hydroxyl groups of the disaccharide were converted to the acetylated 4 by treatment with acetic anhydride and 5 mol% DMAP in pyridine, in a unoptimized 50% yield. The benzylidene protecting group can be removed to give a diol 2 with NaHSO₄·SiO₂ in dichloromethane and methanol. (Scheme 13)

**Scheme 13. Synthetic plan for diol 2.**
Future Synthetic Considerations

In order to complete the synthesis of compound 1 the following synthetic considerations can be employed. The diol (2) can then be used in a sialylation reaction with a sialyl chloride (3) in the presence of a silver salt to give the trisaccharide (30).\(^\text{10}\) There are many methods that can be employed for the sialylation reaction. Many donors can be used instead of the sialyl chloride such as using sialyl phosphates, thioglycosides, participating auxiliaries at C-3 or 5-N,4-O-oxazolidinone group.\(^\text{8,7}\) Once the sialylation reaction has been completed the free hydroxyl at the C-4 position of the galactose unit can be acetylated (31). Next the benzyl group on the C-6 of the glucoamine unit can be removed by hydrogenation to give the free hydroxyl at the C-6 position and the benzenesulfonamide can be removed to give the free amine (31). After that a sulfation reaction can then be carried out to give the O-sulfated trisaccharide by employing Bovin’s conditions (32).\(^\text{11}\) Once the sulfate is installed on the trisaccharide, all O-acetyl protected hydroxyls can be completely deprotected to give compound 1. (Scheme 14)
Scheme 14. Synthetic plan for 1.
Conclusions

In summary, we have synthesized disaccharide 15 and there were many challenging glycosylation reactions that slowed down the progress towards the synthesis of trisaccharide 1. The syntheses described above were preformed from modified literature procedures and provided good yields. Future work will include the complete synthesis of trisaccharide 1.
Experimental

General Methods: All reagents were purchased from Sigma-Aldrich, Acros Organics, Alfa Aesar, or Strem, and used without further purification. All reactions involving air or moisture sensitive reagents were carried out under nitrogen atmosphere. Dry solvents used in all reactions were obtained from Pure Process Technology’s solvent purification system or purchased in their dry form (Acetonitrile and Methonal from Sigma-Aldrich and Chloroform from Acros Organics). Reactions were monitored by TLC on Silicycle Glass Backed TLC Extra Hard Layer 60Å F254 plates. Column chromatography was performed on Silicycle SiliaFlash P60 40-63μm particle size silica gel. NMR spectra were obtained on a Varian Inova 400 MHz instrument. Deuterated chloroform and methanol were used as solvents and internally referenced (TMS at 0ppm and methanol at 3.31ppm) LC/MS data was obtained on a Waters Acquity UPLC chromatograph and a Waters Micromass Z/Q mass detector. Infrared spectra were obtained on a Varian 640-IR controlled by Resolution Pro Software. All known compounds were synthesized according to literature procedures, and are not discussed here.
To a 250mL flask was added 13g (28.72mmol) of starting material 10\textsuperscript{16}. Toluene was added and was removed under vacuum, this was repeated two more times and dried overnight under vacuum. A mixture of 5g (19.58mmol) of \textit{trans}-(4-hydroxycyclohexyl)-benzenesulfonamide and 7.38g (28.72mmol) of silver trifluoromethanesulfonate were dried overnight under vacuum over P\textsubscript{2}O\textsubscript{5} in the dark. Then, 3.8mL (28.72mmol) of 2,4,6-trimethylpyridine, 2g of flame-dried powdered 4Å molecular sieves, and 185mL of dry dichloromethane were added to the 500mL flask and the reaction mixture was stirred at -78°C for 30 minutes. A solution of 13g of starting material in 81mL of dry dichloromethane was then added to the reaction mixture. The reaction mixture was then stirred at -30°C for 1 hour. After this time, the reaction mixture was warmed to room temperature and stirred for 24 hours. The reaction mixture was diluted with 200mL of dichloromethane and filtered through a Celite plug. The filtrate was washed with 200mL of water, 200mL of 1M HCl, 200mL of saturated aqueous NaHCO\textsubscript{3} solution and saturated aqueous NaCl solution. The combined organic layers were dried with MgSO\textsubscript{4}, filtered and concentrated. Purification by flash column chromatography with 4:3:3 dichloromethane/ethyl acetate/hexanes. To afford 12g (17.84mmol, 91%) of 9. \textsuperscript{1}H-NMR (400MHz, CDCl\textsubscript{3}) \textsuperscript{1}H-NMR (400MHz, CDCl\textsubscript{3}) \(\delta\) 7.85-7.79 (m, 4H), 7.76-7.74 (m, 2H), 7.55 (t, 1H, \(J = 7.3\)Hz), 7.47 (t, 2H, \(J = 7.5\)Hz), 5.74 (dd, 1H, \(J = 10.6\)Hz, 9.1Hz), 5.40 (d, 1H, \(J = 8.5\)Hz), 5.15 (dd, 1H, \(J = 9.3\)Hz, 10.0Hz), 4.68 (d, 1H, \(J = 7.3\)Hz), 4.32-4.23 (m, 2H), 4.15 (bs, 1H), 4.12-4.04 (m, 1H), 3.82 (dq, 1H, \(J = 10\)Hz, 2.4Hz), 3.56-3.47 (m, 1H), 3.05-2.92 (m, 1H), 2.09 (s, 3H), 2.02 (s, 3H), 1.85 (s, 3H), 1.79-1.59 (m, 3H), 1.35-1.23 (m, 1H), 1.21-0.89 (m, 3H) \textsuperscript{13}C-NMR (400MHz, CDCl\textsubscript{3}, selected signals) \(\delta\) 20.4, 20.6, 20.7, 29.3, 30.7, 51.3, 54.7, 62.0, 68.9, 70.7, 71.7, 76.1, 96.5, 123.6, 126.7, 129.0, 131.2, 134.4, 140.82, 140.83,
To a flask containing 12g (17.84mmol) of 9 was added 35.4mL of methanol, 59mL of THF and 5mL of 0.5M NaOMe solution in methanol. This was allowed to stir for 4 hours, then the reaction mixture was diluted with methanol and Amberlite IR-120 H⁺ ion exchange resin was added until the solution was at a pH 7. The mixture was then filtered and concentrated. To afford 9.7g (17.75mmol, 99%) of 14. ¹H-NMR (400MHz, CDCl₃) δ 7.89-7.73 (m, 6H), 7.60-7.44 (m, 3H), 5.21 (d, 1H, J = 8.5Hz), 4.19 (dd, 1H, J = 10.6Hz, 8.4Hz), 3.91 (d, 1H, J = 7.9Hz), 3.87 (d, 1H, J = 5.3Hz), 3.69 (dd, 1H, J = 11.7Hz, 5.4Hz), 3.62-3.51 (m, 1H), 3.41-3.31 (m, 2H), 3.29 (bs, 1H), 2.90-2.80 (m, 1H), 1.84 (bs, 1H), 1.65 (bs, 1H), 1.69-1.54 (m, 1H), 1.48 (d, 1H, J = 12.1Hz), 1.17 (p, 2H, J = 9.1Hz), 1.08 (q, 1H, J = 11.6Hz), 0.85 (q, 1H, J = 11.6Hz) ¹³C-NMR (400MHz, CDCl₃, selected signals) δ 30.7, 31.5, 31.7, 32.2, 52.6, 58.7, 62.7, 72.5, 72.7, 76.8, 78.3, 97.9, 124.0, 124.3, 127.7, 130.1, 132.9, 133.4, 135.6, 143.1, 169.5(br), 169.9(br). IR (cm⁻¹): 3407(br), 2946, 2882, 1773, 1709, 1447, 1390, 1157, 1072, 1020, 721
To a flask containing 10g (18.295mmol) of 14 was added 500mL of acetonitrile and 17.12mL (114.016mmol) of benzaldehyde dimethyl acetal. To the stirring solution was added 15g (93.798mmol) of activated NaHSO₄·SiO₂ (dried at 105°C for 10 hours before use) at room temperature. This was allowed to stir for 3 hours and 5mL of triethylamine was added to quench the reaction. The reaction mixture was filtered through Celite and concentrated. Purification by flash column chromatography with 1:1:1 ethyl acetate/hexanes/dichloromethane. To afford 9.3g (14.65mmol, 80%) of 8. ¹H-NMR (400MHz, CDCl₃) δ 7.90-7.71 (m, 6H) 7.55 (t, 1H, J = 7.5Hz), 7.49 (d, 4H, J = 6.8Hz), 7.38 (d, 3H, J = 4.5Hz), 5.55 (s, 1H), 5.33 (d, 1H, J = 8.4Hz), 4.59 (t, 1H, J = 9.2Hz), 4.36 (dd, 1H, J = 14.0Hz, 10.0Hz), 4.29 (d, 1H, J = 7.3Hz), 4.20 (t, 1H, J = 9.5Hz), 3.82 (t, 1H, J = 9.8Hz), 3.61 (d, 2H, J = 7.9Hz), 3.57-3.48 (m, 1H), 3.06-2.94 (m, 1H), 2.44 (bs, 1H), 1.85 (d, 1H, J = 12.5Hz), 1.75 (d, 1H, J = 12.5Hz), 1.67 (bs, 1H), 1.31-1.23 (m, 1H) 1.21-0.89 (m, 4H) ¹³C-NMR (400MHz, CDCl₃, selected signals) δ 21.1, 29.4, 30.8, 30.9, 51.4, 56.6, 66.1, 68.7, 75.9, 82.2, 97.3, 101.9, 123.6, 126.3, 126.8, 128.4, 129.1, 129.4, 131.5, 132.6, 134.3, 136.9, 140.8, 168.0(br), 168.4(br). IR (cm⁻¹): 3503, 3272, 2940, 2865, 1774, 1708, 1448, 1387, 1158, 1075, 1043, 993, 722
To flask containing 450mg (.709mmol) of 8 was added 5mL of pyridine. This was cooled to 0°C and 167μL of acetic anhydride and 4.5mg of DMAP was added. The reaction mixture was warmed to room temperature and allowed to stir. After 2 hours, the reaction mixture was concentrated. To a flask containing the crude material 349.9mg (.5171mmol) was added 5mL of THF, .5g of flame-dried powdered 4Å molecular sieves, and 185g (2.942mmol) of NaBH₃CN. This was allowed to stir for 50 minutes at room temperature. The reaction mixture was cooled to 0°C and ice cold HCl in diethyl ether was added slowly to acidify the mixture to pH 2-3. The reaction mixture was then stirred for 1 hour and then diluted with dichloromethane and filtered through Celite. The filtrate was washed with 100mL of saturated aqueous NaCO₃ solution, 100mL of water and 100mL of saturated aqueous NaCl solution. The combined organic layers were dried with MgSO₄, filtered and concentrated. To a flask containing the crude material 252mg (.3709mmol) was added 375μL of methanol, 2.5mL of THF and 3.2μL of 0.5M NaOMe solution in methanol. This was allowed to stir for 6 hours, then the reaction mixture was diluted with methanol and Amberlite IR-120 H⁺ ion exchange resin was added until the solution was at a pH 7. The mixture was then filtered and concentrated. To afford 236mg (.3706mmol, 99%) of 6. **¹H-NMR (400MHz, CDCl₃)** 7.87-7.78 (m, 4H) 7.77-7.71 (m, 2H), 7.55 (t, 1H, J = 8Hz), 7.47 (t, 2H, J = 8Hz), 7.39-7.29 (m, 5H), 5.27 (d, 1H, J = 8Hz), 4.60 (q, 2H, J = 12Hz), 4.28 (bs, 1H), 4.22 (d, 1H, J = 4Hz), 4.09 (dd, 1H, J = 9Hz, 10Hz) 3.77 (q, 2H, J = 8Hz), 3.61 (s, 3H) 3.49 (bs, 2H), 3.05 (s, 1H), 3.00 (bs, 1H), 2.36
Further characterization is in progress.

To a flask was added 236mg (.3706 mmol) of 6 and 365.2g (.7413 mmol) of 7. Toluene was added and was removed under vacuum; this was repeated two more times and dried overnight under vacuum. Then, 10.5mL of dry dichloromethane and 25mg of flamed dried 4Å molecular sieves were added and the reaction mixture was cooled to -10°C. Then 13μL of TMSOTf was and the reaction was stirred for 45 minutes at -10°C. The reaction mixture was diluted with dichloromethane and filtered through Celite. The filtrate was then washed with saturated aqueous NaCO₃ solution and water. The combined organic layers were dried with Na₂SO₄, filtered and concentrated. Purification by flash column chromatography with 1.5:1 ethyl acetate/hexanes. To afford 203.9mg of 15 (.2109mmol, 57%) ¹H-NMR (400MHz, CDCl₃) 7.86-7.78 (m, 4H), 7.77-7.69 (m, 2H), 7.55 (t, 1H, J = 8Hz), 7.48 (t, 2H, J = 8Hz), 7.40-7.29 (m, 5H), 6.31 (d, 1H, J = 4Hz), 5.47 (d, 1H, J = 4Hz), 5.33 (d, 1H, J = 4Hz), 5.25 (d, 1H, J = 8Hz), 5.21-5.15 (m, 1H), 4.93 (dd, 1H, J = 4Hz, 12Hz) 4.71 (d, 1H, J = 12Hz), 4.62 (s, 1H), 4.49 (t, 1H, J = 8Hz),
4.39-4.24 (m, 2H), 4.20-4.02 (m, 1H), 3.97 (s, 1H), 3.95-3.83 (m, 1H), 3.72-3.63 (m, 1H), 3.60 (d, 1H J = 8Hz), 3.56-3.46 (m, 1H), 3.07-2.95 (m, 1H), 2.19 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 1.98 (s, 3H), 1.90 (s, 3H), 1.81-1.63 (m, 3H), 1.46-1.28 (m, 1H), 1.20-0.97 (m, 3H) Further characterization is in progress.
References


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