Matched and Mismatched Glycosylations at O-3 of N-Acetylglucosamine

by

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ABSTRACT

Matched and Mismatched Glycosylations at O-3 of N-Acetylg glucosamine

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University of Guelph, 2014

Dr. France-Isabelle Auzanneau

Glycosylations result in different matched and mismatched pairs of acceptors and donors. In this thesis, glycosylations at O-3 of N-acetylg glucosamine are investigated using β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-β-D-GlcNAc trisaccharide acceptors deprotected at O-3 and O-3″. These acceptors were synthesized with differences in protection at the non-reducing end to determine the effect of benzylidene and trichloroacetamido groups. Glycosylations were completed with D/L, and armed/disarmed donors. Various reaction conditions were investigated to differentiate between kinetic and thermodynamic products. Under acidic, thermodynamic conditions, donors matched to O-3. Removal of the benzylidene gave the same matched pairs, while reduction of the trichloroacetamido group increased reactivity at O-3″. Under kinetic conditions, donors were matched to O-3, while basic conditions resulted in the isolation of an orthoester. We propose that the matching of disarmed donors to the reducing end can be explained by initial substitution or orthoester formation at the non-reducing end, followed by hydrolysis under acidic, high temperature conditions.
Acknowledgements

First and foremost I would like to thank my advisor Dr. France-Isabelle Auzanneau. You have given me the opportunity to learn about carbohydrate synthesis, both educationally and practically in the lab, and you have taught me how to become an accomplished practical chemist. You have provided constant support throughout this research project, and I am most grateful.

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<tr>
<td>aq.</td>
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<td>broad singlet</td>
</tr>
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<tr>
<td>COSY</td>
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<td>δ</td>
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<td>HPLC</td>
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<td>High-Resolution Electrospray Ionization Mass Spectrometry</td>
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<td>Heteronucleur Single Quantum Correlation Spectroscopy</td>
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<td>J</td>
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<td>JMOD</td>
<td>J-Modulated Spin-Echo Nuclear Magnetic Resonance Spectroscopy</td>
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<tr>
<td>qd</td>
<td>quartet of a doublet</td>
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<td>quin</td>
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<tr>
<td>quant.</td>
<td>Quantitative</td>
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<tr>
<td>RDAS</td>
<td>Reciprocal Donor-Acceptor Selectivity</td>
</tr>
<tr>
<td>RP</td>
<td>Reverse-Phase High-Performance Liquid Chromatography</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
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<td>triplet of a doublet</td>
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<tr>
<td>TACA</td>
<td>Tumour-Associated Carbohydrate Antigen</td>
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<td>TLC</td>
<td>Thin-Layer Chromatography</td>
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<td>\mu L</td>
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Chapter 1

Introduction
1.1 Introduction to Carbohydrates

Carbohydrates are the most abundant group of natural products. They have significant biological relevance, being produced by plants through the process of photosynthesis, and acting as the main source of energy for most cells. They are a major structural component in many organisms, which include animal exoskeletons and plant cell walls. They are also expressed as antigens on the outside of cell membranes, allowing them to play a role in cell-cell recognition. Types of carbohydrates include monosaccharides, oligosaccharides, polysaccharides, and their derivatives created by reduction, oxidation, or replacement of their functional groups. Polysaccharides and oligosaccharides are composed of monosaccharide units, and their synthesis is dependent on their monosaccharide components and properties.1-3

1.2 Monosaccharides Structure

1.2.1 Introduction

Monosaccharides are group of polyhydroxylated carbonyl organic compounds with three or more carbons, containing at least two hydroxyl groups, and at least one carbonyl group. Excluding derivates, they are generally subdivided into two categories: aldoses (polyhydroxylated aldehydes) and ketoses (polyhydroxylated ketones). Focusing on the aldose family, the most simple aldose is the triose glyceraldehyde, which has one stereocenter and thus two enantiomers (R and S). Increasing the number of carbon units will increase the number of stereocenters, and thus the number of isomers will increase exponentially.1-3
1.2.2 Aldohexose Structure

We are most interested in the aldohexose series of monosaccharides. Aldohexoses have 4 stereocenters, and thus there are $2^4 = 16$ stereoisomers of aldohexoses. Each of these stereoisomers has one enantiomer, and many diastereoisomers. In order to simplify their categorization, each pair of enantiomers are split into two groups, "D" and "L". When viewed in the Fisher projection, a monosaccharide is designated D if its highest numbered stereocenter has the hydroxyl group on the right (configuration R), while it is designated L if its highest numbered stereocenter has the hydroxyl group on the left (configuration S). This is illustrated below with the aldohexoses D-glucose (the most abundant monosaccharide in nature) and L-glucose (Figure 1.1). D- and L-glucose are indeed enantiomers, as they have opposite configuration at every other stereocenter as well.1-3

![Diagram showing the structures of D-Glucose and L-Glucose](image)

**Figure 1.1.** The structures of D-Glucose and L-Glucose as viewed in the Fisher projection, with emphasis on the position of the hydroxyl group at the highest numbered stereocenter.1-3

While D-glucose has 14 diastereoisomers, of particular interest are its epimers (diastereoisomers which differ in configuration at only one position). Two important epimers of D-glucose are D-galactose, which differs in configuration at C-4, and D-mannose, which differs in configuration at C-2 (Figure 1.2).1-3
The fundamental knowledge of monosaccharides can be traced back to findings by Emil Fischer in the 1880's and 1890's. He was able to synthesize and establish the relative configuration of most sugars known as the time. In particular, he characterized the D-aldohexoses using elongation of aldoses, starting from D-glyceraldehyde, and measuring optical rotations to determine configurations.

1.2.3 Formation of Hexopyranoses

Aldohexoses contain alcohol groups attached to carbons near to their carbonyl group, which allows for cyclization to form an intramolecular hemiacetal (Scheme 1.1). Of the possible cyclizations, the most likely are 5-membered furanose or 6-membered pyranose rings, depending on if the attacking alcohol is located at C-4 or C-5, with the most stable being pyranose rings due to the low energy chair conformation. The monosaccharides used this thesis are all hexopyranoses.
**Scheme 1.1.** The cyclization of D-Glucose into D-Glucopyranose. ¹⁻³

### 1.2.4 Configuration of Hexopyranoses

Following nucleophilic attack of the alcohol, an additional stereocenter is created at C-1, which is known as the anomeric carbon. Since nucleophilic attack can come from above or below the plane of the sp² carbonyl group, both C-1 diastereoisomers will be formed. These are specifically known as anomers. The anomer with the anomeric OH group on the same side of the Fisher projection as the OH group on the highest numbered stereocenter involved in ring formation (C-5 for aldohexoses) is designated α, while the anomer which results in the anomeric OH group being on the opposite side of the Fisher projection is designated β (Scheme 1.2). Since this C-5 OH group is involved in cyclization, a simple rotation of the C-5 carbon in the Fisher projection indicates that, for the α-anomer, the anomeric OH group will be trans to C-6, while for the β anomer the anomeric OH group will be cis to C-6. While in solution, hexopyranoses are in equilibrium with their uncyclized aldohexoses, allowing for constant interconversion of α and β anomers (as well as pyranose and furanose rings and other structures, although 6-membered pyranose rings are more stable). This interconversion is known as mutarotation (specifically anomerization between two anomers). The position of the equilibrium depends on the specific aldohexose.¹⁻³
Scheme 1.2. The configurational structures of anomers α-D-glucopyranose and β-D-glucopyranose, emphasizing the position of the anomeric hydroxyl group. In solution, these anomers are subject to mutarotation.\(^1\)-\(^3\)

### 1.2.5 Conformation of Hexopyranoses

As 6-membered rings, hexopyranoses may adopt two chair conformations through a ring flip. These two conformations are the \(^4\)C\(_1\) chair (C-4 above the plane of the ring, C-1 below) and the \(^1\)C\(_4\) chair (C-1 above the plane of the ring, C-4 below). The more stable conformation will usually have the bulkiest group equatorial, which is the CH\(_2\)OH group at C-5 for hexopyranoses, thus most D-hexopyranoses will adopt the \(^4\)C\(_1\) chair, while most L-hexopyranoses will adopt the \(^1\)C\(_4\) chair (Figure 1.3). If we specifically look at D-hexopyranoses in the \(^4\)C\(_1\) chair and L-hexopyranoses in the \(^1\)C\(_4\) chair, the α anomer will always have the anomeric OH axial (trans to the C-5 substituent), while the β anomer will always have the anomeric OH equatorial (cis to the C-5 substituent). Note that β-D- and β-L-glucose are the stereoisomers of hexopyranoses which place all substituents equatorial.\(^1\)-\(^3\)
Figure 1.3. The conformational structures of β-D-glucopyranose and α-L-glucopyranose, emphasizing the position of the bulky C-5 substituent, and the position of the anomeric hydroxyl group in the more stable conformation.\(^{1-3}\)

1.2.6 Other Hexopyranose Features

When a hexopyranose is functionalized at the anomeric position to become an acetal, it becomes known as a glycoside. Glycosides consist of the glycone (hexopyranose ring and substituents) and the aglycone (functional group at the anomeric position) (Figure 1.4).\(^{1-3}\)

Figure 1.4. Illustration of the glycone and aglycone moieties of β-D-glucopyranose.\(^{1-3}\)

In this thesis, there are five hexopyranoses which are used: N-acetyl-D-glucosamine (2-deoxy-2-acetamido-D-glucopyranose, D-GlcNAc), D-galactose (D-Gal), D-mannose (D-Man),
L-fucose (6-deoxy-L-galactopyranose, L-Fuc), and L-rhamnose (6-deoxy-L-mannopyranose, L-Rha) (Figure 1.5).

Figure 1.5. The structures of N-acetyl-D-glucosamine, D-galactose, D-mannose, L-fucose, and L-rhamnose.

1.2.7 The Anomeric Effect

The anomeric effect was first described by Edward in 1955, and named by Lemieux and Chû in 1958. An electronegative atom attached to C-1 is more stable in the axial position. Normally substituents are less stable in the axial position due to 1,3-diaxial interactions, giving equatorial substituents a lower energy, but this is not the case for hexopyranoses. There are two accepted explanations for this effect.

The first explanation of the anomeric effect is due to the presence of neighbouring dipole moments. For an electronegative atom X (X = O, N, S, F, Cl, Br) in the axial position, the C1-X bond creates a dipole moment. This dipole points away from the combined dipole moment of the endocyclic O-C1 and O-C5 bonds, partially cancelling out the dipoles (Figure 1.6). If X is in the equatorial position, the dipole moment points relatively in the same direction as the combined dipole moment of the O-C1 and O-C5 bonds, causing an additive dipole effect and electrostatic repulsion, destabilizing the molecule. As a result, X is more stable when the C1-X bond is axial, with the relative stability increasing with increasing electronegativity of X. The extent of this relative stability will decrease with increasing solvent polarity.
Figure 1.6. Polar explanation of the anomeric effect, whereby the α-anomer is more stable than
the β-anomer due to the dipole moments.\textsuperscript{1,3}

This partially explains why a C1-X axial bond will be more stable, but it does not explain
one observation: the O-C1 bond for the axial anomer is shorter than the corresponding equatorial
anomer, while the C1-X bond is longer.\textsuperscript{9} To explain this, a molecular orbital theory explanation
is required. With X in the axial position, the C1-X bond is antiperiplanar to one of the endocyclic
oxygen lone pairs, and thus the C1-X antibonding $\sigma^*$ orbital (LUMO) is aligned with the
endocyclic oxygen non-bonding $n_y$ orbital (HOMO) corresponding to one of its lone pairs. This
allows for delocalization of the oxygen non-bonding lone pair electron density from the $n_y$ orbital
to the to the aligned $\sigma^*_{C1-X}$ orbital through hyperconjugation, lowering the energy of the HOMO
and providing overall stabilization (Figure 1.7).\textsuperscript{1,3,10} As a result, the O-C1 bond gains partial
double bond character and becomes shorter, while the C1-X bond lengthens.\textsuperscript{9} This stability
increases with an increasing electronegativity of X.\textsuperscript{11} With the C1-X bond equatorial, the $\sigma^*_{C1-X}$
orbital does not overlap with the oxygen $n_y$ orbital and no such stabilization is possible.
The anomeric effect creates even more stabilization when the C-2 substituent is axial (i.e. mannose), which is known as the Δ2 effect. With the C2-X2 bond axial, its dipole moment points in the general direction of the combined dipole moment of the O-C1 and O-C5 bonds, creating the further need for a C1-X1 dipole to be partially cancelling (Figure 1.8). In fact, mutarotation of mannose in water causes the equilibrium to favour α-mannose, compared to glucose which will favour β-glucose. The corresponding molecular orbital theory for the Δ2 effect is the overlap of the C2-X2 antibonding σ* orbital with the C1-X1 antibonding σ* orbital, lowering the energy.
of the LUMO and increasing the delocalization of the oxygen non-bonding lone pair electron density (Figure 1.8).\textsuperscript{1,3,12}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.8.png}
\caption{Polar and molecular orbital explanation of the $\Delta E_2$ effect, whereby the \( \alpha \)-anomer has increased stability due to the extra C2-X2 dipole moment, and overlap of the antibonding \( \sigma^*_{C1-X1} \) orbital with the antibonding \( \sigma^*_{C2-X2} \) orbital creating a lower energy LUMO (\( \sigma^*_+ \)) thus increasing the energy of stabilization \( (\Delta E) \) by hyperconjugation.\textsuperscript{1,12}}
\end{figure}

Complementary to the anomeric effect is the reverse anomeric effect, which says that if X1 is protonated, it will remove the electrostatic repulsion when in the equatorial position. Thus, the positively charged X1 will be more stable in the equatorial position where it avoids 1,3-diaxial interactions, giving preference for the \( \beta \)-anomer.\textsuperscript{1,3,13}

The anomeric effect plays a clear role in carbohydrate chemistry, but it should be pointed out that it can apply to many other tetrahedral centres attached to two electronegative heteroatoms X (\( X = O, N, S, F, Cl, Br \)) and Y (\( Y = O, N, S \)).\textsuperscript{1,3,14}
1.3 Oligosaccharide Synthesis

1.3.1 Glycosidic Bond Formation

Monosaccharides usually attach to one another using glycosidic bonds in order to make larger oligosaccharides or polysaccharides. $O$-Glycosidic bonds are common, and while others are possible (including $N$-, $S$-, and $C$-glycosidic bonds), only $O$-glycosidic bonds are formed in this thesis. Using synthetic procedures in the absence of enzymes, an $O$-glycosidic bond is formed between two coupling saccharides, each specifically protected in order to achieve good regioselectivity, in a nucleophilic substitution reaction known as glycosylation. The first is the glycosyl acceptor, which is usually protected at all but one of its hydroxyl groups, and that remaining free hydroxyl group acts as the nucleophile (non-saccharide nucleophiles can also act as the acceptor, such as simple alcohols and thiols). The second is the glycosyl donor, which is usually fully protected and also possesses a good leaving group at its anomeric position, making the anomeric carbon the electrophile to be attacked by the acceptor (Scheme 1.3). An activator or promoter is used to assist the departure of the leaving group, leading to an intermediate, resonance stabilized oxocarbenium cation. The $sp^2$ oxocarbenium ion can be attacked by the acceptor from either above or below the ring, both the $\alpha$ or $\beta$ diastereoisomers to form. The resulting glycosidic bond is usually very stable, although it can be hydrolyzed under strong acidic conditions.$^{1,3,15,16}$
Scheme 1.3. Mechanism of glycosidic bond synthesis. When passing through the oxocarbenium ion intermediate, the acceptor can attack from above or below the ring, forming either an α- or β-glycosidic bond. If the acceptor is a glycone (glycosyl acceptor), the product is a disaccharide.$^{1,3,15}$

One of the first known glycosidic bond synthesizes was reported by Fischer in 1893. He developed an acid-catalyzed procedure to form a glycosidic bond between a hemiacetal glycone and a simple alcohol, a method which has become known as Fischer glycosidation.$^{17}$ In this case, protonation of the hemiacetal allowed water to act as the leaving group. However, the glycosidic bond can be hydrolyzed in these acidic conditions, which is augmented by the equivalent of water formed during condensation, creating an equilibrium between the reactants and the product. As a result, there is a synthetic preference to use a better leaving group than the hemiacetal hydroxyl group.$^{16}$

When a linear oligosaccharide is formed, the monosaccharide residue which can be traced back to the initial hemiacetal is known as the reducing end. This term refers to the ability of the linear aldohexose to reduce Cu$^{2+}$ to Cu$^{+}$, simultaneously oxidizing the aldehyde to a
carboxylate. The monosaccharide residue at the opposite end of the oligosaccharide chain is known as the non-reducing end (Figure 1.9).

![Diagram of reducing and non-reducing ends of an oligosaccharide]

**Figure 1.9.** The reducing and non-reducing ends of an oligosaccharide.

Glycosylation reactions are amongst the most difficult in oligosaccharide synthesis. There are many obstacles which are only overcome using very specific procedures. As water is a better nucleophile than an alcohol, these reactions must be performed under anhydrous conditions. Glycosyl donors must have a good leaving group at the anomeric position, but frequently this leaving group is highly unstable, leading to degradation of the donor. As a result, many glycosylations are performed using an excess of donor. In order to control regioselectivity, the acceptor must be selectively unprotected or deprotected at the desired position for the glycosidic bond, which can be achieved through a series of selective protections and deprotections. In addition, controlling stereoselectivity adds another challenge to forming a glycosidic bond.\(^1,15\)

### 1.3.2 Glycosyl Donors

Glycosidic bond formation requires the acceptor to attack the electrophilic anomeric carbon on the donor. A good leaving group is required to achieve this. Three common leaving
groups used in oligosaccharide synthesis are glycosyl halides, trichloroacetimidates, and thioglycosides (Figure 1.10), all of which are used in this thesis.

**Figure 1.10.** Structures of common glycosyl donors in glycosidic bond synthesis.

**1.3.2.1 Glycosyl Halides**

Glycosyl bromides and chlorides are the more commonly used halide donors, although glycosyl fluorides are used too, and sometimes iodides as well despite their instability.\(^1,3,18\) In this thesis only chlorides and bromides are used. Chlorides are more stable than bromides, making bromide donors more reactive. In general, halide donors are unstable and should be prepared in the final step before glycosylation. Glycosyl halides are most commonly prepared from anomeric acetates. A glycosyl bromide can be made from the acetate using HBr in AcOH, while a glycosyl chloride can be made from the hemiacetal using AcCl, which will first acetylate the anomeric position and release HCl simultaneously (Scheme 1.4). Mechanistically, with HX in the reaction, H\(^+\) promotes the anomeric acetate to leave while X\(^-\) attacks the anomeric carbon.\(^1,3,19\) Due to the anomeric effect, \(\alpha\)-bromides and \(\alpha\)-chlorides are more stable and are usually exclusively formed.
Bromide donors were first used by Koenigs and Knörr in 1901. They used stoichiometric Ag(I) salts as activators. If a soluble Ag(I) salt is used (such as AgOTf or AgClO₄), the solubilized Ag⁺ complexes with the halide group, forming an AgX precipitate and the oxocarbenium ion. However, if an insoluble Ag(I) salt is used (such as Ag₂CO₃ or Ag₂O), after complexing with the halide the insoluble complex will remain weakly attached to the anomeric carbon. This allows for S_N2 attack by the acceptor, giving only the β-glycoside (Scheme 1.5).

**Scheme 1.4.** Preparation of glycosyl chlorides and bromides.\(^{1,3,19}\)

**Scheme 1.5.** Koenigs-Knörr activation of a glycosyl halide donor, using either soluble or insoluble Ag(I) salts.\(^{1,3,20,21}\)
A modification of this method was reported by Helferich and co-workers, who used Hg(II) salts (such as Hg(CN)$_2$ or HgBr$_2$) to activate glycosyl halides.$^{22,23}$ While both methods are efficient, they are limited by the required use of toxic heavy metallic salts as activators.$^{1,3}$

1.3.2.2 Trichloroacetimidates

$O$-Glycosyl trichloroacetimidate donors are more reactive compared to halides and thioglycosides, which makes them particularly good for glycosylation reactions and allows for mild activation conditions. However, their instability makes them highly labile in many reaction conditions, so the limitation is that they must be introduced in the final step before glycosylation.$^{1,3,16}$

Trichloroacetimidates were first used by Schmidt and Michel in 1980.$^{24}$ They are made by reaction of the hemiacetal with Cl$_3$CCN in the presence catalytic base. The base first deprotonates the hemiacetal, which then attacks the electrophilic centre on Cl$_3$CCN. The resulting anion is protonated by the now protonated base, regenerating the base catalyst (Scheme 1.6). As the initial hemiacetal is subject to anomerization, the stereochemistry of the trichloroacetimidate can vary. Following deprotonation, the $\beta$-alkoxide is formed first, and is more nucleophilic than the $\alpha$-alkoxide due to electrostatic repulsions with the endocyclic oxygen, thus the kinetic product is the $\beta$-trichloroacetimidate. However, the $\alpha$-trichloroacetimidate is stabilized by the anomeric effect, making it the thermodynamic product. It turns out that the strength of the base used will direct the stereochemical outcome. If a base strong enough to deprotonate the trichloroacetimidate (such as DBU or NaH) is used, the kinetic $\beta$-trichloroacetimidate goes into equilibrium with the alkoxide. This allows the $\beta$-alkoxide to
anomerize to the α-alkoxide, allowing it to attack as well and form the more stable α-trichloroacetimidate. If a weaker base (such as K₂CO₃) is used, the β-trichloroacetimidate will not be deprotonated, making the reaction irreversible (Scheme 1.6). Although β-halides are highly unstable, β-trichloroacetimidates are stable enough to be used as donors.¹³,¹⁶

![Scheme 1.6. Preparation of trichloroacetimidates. If a weak base (K₂CO₃) is used, the kinetic β-trichloroacetimidate is formed, but if the base is strong enough to deprotonate the trichloroacetimidate (DBU), the thermodynamic α-trichloroacetimidate is formed.¹³,¹⁶,²⁴](image.png)

Trichloroacetimidates are commonly activated using a Lewis acid such as BF₃•Et₂O or TMSOTf (Scheme 1.7). As they are so unstable, they tend to fall off quickly in the presence of the activator. However, they can react through either an S_N1 or S_N2 mechanism, which emphasizes the importance of starting with a stereochemically pure trichloroacetimidate.¹³ Schmidt suggested that, in the absence of neighbouring group participation (see section 1.2.6.1), activation using BF₃•Et₂O will facilitate an S_N2 mechanism, while activation using TMSOTf will
facilitate an $S_N1$ mechanism.$^{16}$ Glycosylations with trichloroacetimidate donors are usually performed at low temperature to control the quick activation of the donor,$^1$ although in certain cases higher temperatures can be used if the acceptor is poorly nucleophilic.

**Scheme 1.7.** Activation of a trichloroacetimidate donor, where LA represents a Lewis acid such as BF$_3$•Et$_2$O or TMSOTf.$^{1,3,16}$

### 1.3.2.3 Thioglycosides

Thioglycosides were first introduced by Fischer and Delbrück in 1909.$^{25}$ They have a key advantage as donors as they are fairly stable, and can withstand many other protections and deprotections that the donor might undergo. They can be prepared through glycosylation from anomeric acetates using the corresponding thiol and a Lewis acid activator, forming an $S$-glycosidic bond (Scheme 1.8). As will be discussed in the next section, the stereoselectivity of the thioglycoside can be controlled using the anomeric effect or neighbouring group participation. Thioglycosides can also be prepared from glycosyl halides using the corresponding thiolate in an $S_N2$ glycosylation, giving the $\beta$-thioglycoside.$^{1,3,26-28}$
Scheme 1.8. Preparation of thioglycosides, from anomeric acetates or halides (X).\textsuperscript{1,3,26-28}

Thioglycosides are activated using thiophilic reagents (E\textsuperscript{+}). A common activator is stoichiometric NIS combined with catalytic TfOH or TMSOTf, which provides a source of I\textsuperscript{+}.\textsuperscript{29} Another common activator is MeOTf, which provides a source of CH\textsubscript{3}\textsuperscript{+}.\textsuperscript{30} The nucleophilic sulfur attacks the electrophile with its lone pair, creating a labile sulfonium ion (Scheme 1.9). They can also be easily converted to other donors, including halides and trichloroacetimidates.\textsuperscript{1,3}

Scheme 1.9. Activation of a thioglycoside donor using a thiophilic reagent E\textsuperscript{+}.\textsuperscript{1,3,29,30}

1.3.3 Controlling Stereoselectivity During Glycosidic Bond Formation

Achieving stereoselectivity is very important in oligosaccharide synthesis. Both α or β glycosidic bonds can result from glycosylation, creating two possible relationships between C-1 and C-2: 1,2-cis or 1,2-trans. For hexopyranoses in their stable conformation, whether α (axial)
or β (equatorial) are 1,2-cis or 1,2-trans depends on the substituent at C-2, which is equatorial for glucose (and galactose) but axial for mannose (Figure 1.11).¹,¹⁵

**Figure 1.11.** Types of glycosidic bonds categorized by the relationship between the C-1 and C-2 substituent.¹,¹⁵

There are many factors that affect the anomeric ratio of a glycosidic bond, which include the nature (electronic, steric, and configurational) of the protecting groups and leaving group, and the reaction conditions (activator, solvent, temperature). Despite the number of variables which combine to affect anomeric stereoselectivity, there are methods of controlling stereoselectivity during glycosidic bond formation.¹ In 1982, Paulsen published a review which thoroughly examines the methods to achieve 1,2-cis or 1,2-trans glycosidic bond formation in many common hexopyranoses.¹⁵ Some of the common methods will be discussed below.

**1.3.3.1 Neighbouring Group Participation/Anchimeric Assistance**

Glycosyl donors protected at C-2 with acyl groups (usually esters or amides) will usually achieve 1,2-trans glycosidic bond synthesis. This comes as a result of as participation from this C-2 neighbouring acyl group, also known as anchimeric assistance. After the oxocarbenium ion is formed, the carbonyl oxygen at C-2 will attack the electron deficient anomeric carbon, forming a dioxolane ring known as a dioxocarbenium or acyloxonium ion (resonance stabilized by both cyclic oxygens). The dioxolane ring blocks the cis side of the anomeric carbon, forcing the
acceptor into $S_N2$ attack trans to the dioxolane ring. The attack opens the dioxolane ring onto C-2, recovering the initial acyl protecting group, and forming a 1,2-trans glycosidic bond (Scheme 1.10). With glucose and galactose, participation causes a $\beta$-glycosidic bond to form, while with mannose it causes an $\alpha$-glycosidic bond to form.$^{1,3,15}$

Scheme 1.10. Use of neighbouring (C-2) group participation/anchimeric assistance to form a 1,2-trans glycoside.$^{1,3,15}$

One downside of using ester participation to achieve stereoselectivity is the formation of an orthoester byproduct. The acyloxonium ion is electron deficient on the dioxolane ring carbon, and if the acceptor attacks that position instead of the anomeric carbon, it will form an orthoester instead of a glycosidic bond (Scheme 1.11). In acidic conditions, orthoesters are unstable and will rearrange into the glycosidic bond over time. A mildly acidic activator is frequently used to prevent formation of the orthoester.$^{1,3}$ When the C-2 substituent is axial (i.e. mannose), the resulting acyloxonium ion is stabilized due to the reverse anomeric effect, which increases the likelihood of orthoester formation.$^3$
Scheme 1.11. Undesired formation of an orthoester when the acceptor attacks the electrophilic dioxolane ring instead of the anomeric carbon.\textsuperscript{1,3}

If the participating acyl group is an amide instead of an ester (as is the case with $N$-acetylglucosamine), an issue arises. Following formation of the intermediate, the cyclic nitrogen can lose its proton, forming a stable oxazoline ring which is difficult to open (Scheme 1.12). This can be avoided using acyl groups that will create an unstable oxazoline, such as a phthalimido group, or a trichloroacetamide group as used by Jacquinet and Blatter.\textsuperscript{31} An alternative is to use a large excess of acceptor and high temperature if convenient, which is often the case during Fischer glycosidation of a glycone using a simple alcohol.\textsuperscript{1,15,17}

Scheme 1.12. Undesired formation of a stable oxazoline ring when the C-2 participating group is an amide instead of an ester.\textsuperscript{1,15}
1.3.3.2 The Anomeric Effect

While anchimeric assistance can be used to form a 1,2-trans glycosidic bond, forming a 1,2-cis glycosidic bond can be more difficult. Glycosyl donors without a C-2 acyl group cannot use anchimeric assistance. As a result, they are expected to form an α-glycosidic linkage regardless of whether the C-2 group is equatorial (glucose, galactose) or axial (mannose). This creates a 1,2-cis linkage for glucose and galactose, while a 1,2-trans linkage is formed for mannose. The α-glycosidic linkage is more stable due to the anomeric effect, as described previously in this chapter. Thus, in the absence of other stereocontrolling conditions, the α-glycosidic linkage is the expected product.

1.3.3.3 S_N2 Glycosylation, In Situ Anomerization, and the Ion Pair Mechanism

Most glycosylation methods pass through the oxocarbenium ion, following mainly an S_N1 mechanism which is the cause of a diastereoisomeric mixture. If conditions which would create a strict S_N2 mechanism are created, it would cause inversion of configuration, eliminating the issue of stereoselectivity. This can be achieved with a very good leaving group (such as triflates or thiocyanates) under the right conditions (preferably non-polar solvents). As described previously in this chapter, use of insoluble Ag(I) salt activators leads to an S_N2 mechanism. As well, certain protecting groups, such as esters and acetals, destabilize the oxocarbenium ion, increasing the preference for an S_N2 mechanism. The effect of acetal protecting groups on donor reactivity will be discussed later in this chapter.

Due to the anomeric effect, many good leaving groups are much more stable in the α-configuration, thus an S_N2 mechanism would allow formation of a β-glycosidic bond. In the mannose series, this is one way to make a 1,2-cis glycosidic linkage. The anomeric effect makes
it difficult to isolate a labile β-donor capable of $S_N2$ glycosidation. Thus this method is not practical for direct formation of an α-glycosidic bond, which would be 1,2-cis for glucose and galactose.\textsuperscript{1,3,15,16,32,33}

In one example, Lemieux and co-workers found an alternative procedure to allow for α-glycosidic bond formation through an $S_N2$ mechanism. Using an α-bromide donor, they created \textit{in situ} anomerization of the α- and β-bromides using $\text{Bu}_4\text{NBr}$. The equilibrium favours the α-bromide due to the anomeric effect, however this makes the α-bromide much more stable, while the β-bromide is the much more reactive anomer toward nucleophilic attack. Thus when the β-bromide is formed, it is rapidly attacked by the acceptor to give the α-glycosidic bond (Scheme 1.13).\textsuperscript{1,3,32,33} In order for this to work, the rate of anomerization must be faster than the rate of glycosylation, which allows the glycosidic bond ratio to be determined by the activation energies of the two anomers and not the position of the equilibrium, as dictated by the Curtin-Hammett principle.\textsuperscript{1}

\begin{center}
\textbf{Scheme 1.13.} Use of \textit{in situ} anomerization to form an α-glycosidic bond via an $S_N2$ mechanism.\textsuperscript{1,3,32,33}
\end{center}
Another example has been reported by Crich and co-workers, where thioglycosides and sulfoxides were activated using Tf₂O to create an \textit{in situ} \( \alpha \)-triflate. This covalent triflate would normally yield the \( \beta \)-linkage through \( S_N2 \) glycosylation, however studies have shown that it is in rapid equilibrium with an \( \alpha \)-contact ion pair, in which the triflate ion blocks the axial face of the anomeric carbon and promotes \( \beta \)-glycosylation through an "\( S_N2 \) like" mechanism, and a solvent-separated ion pair, which promotes \( \alpha \)-glycosylation due to the anomeric effect (Scheme 1.14). The position of the equilibrium is dependent on the configurational nature of the donor (i.e. glucose vs mannose) and on the C-2, C-3, C-4, and C-6 protecting groups.\(^{34-38}\) Of particular interest are 4,6-benzylidene acetal groups and their effect on the equilibrium, and thus the resulting stereoselectivity. This specific case which will be discussed in section 1.3.4.2.

\[ \text{Covalent } \alpha \text{-triflate} \iff \text{\( S_N2 \) ROH} \iff \text{\( S_N1 \) ROH} \]

\[ \text{\( \alpha \)-Contact Ion Pair} \iff \text{"\( S_N2 \) like" ROH} \iff \text{\( \alpha \)-Glycoside} \]

\[ \text{Solvent-Separated Ion Pair} \iff \text{\( \beta \)-Glycoside} \]

\textbf{Scheme 1.14}. Equilibrium between the covalent \( \alpha \)-triflate, the \( \alpha \)-contact ion pair, and the solvent-separated ion pair, and resulting glycosylations and stereoselectivities.\(^{34-38}\)

The specific examples reported by Crich and co-workers can be generalized into a complete ion pair mechanism. The concept of ion pairs in substitution was first suggested by
Winstein and co-workers in 1948 for solvolysis reactions, which has since been applied to glycosylation reactions, and extended by Lemieux and co-workers during their studies on \textit{in situ} anomerization. The mechanism illustrates how S$_N$1 and S$_N$2 mechanisms of glycosylation are the two extremes, and how ion pairs create an interrelationship of the anomers and intermediates (Scheme 1.15).

**Scheme 1.15.** The ion pair mechanism of glycosylation, demonstrating how the anomers and intermediates are interrelated through ion pairs.

**1.3.3.4 Solvent Participation**

Use of particular solvents can assist stereoselective control during glycosidic bond formation. Polar solvents will help 1,2-trans glycosidic bond formation by stabilizing the
acyloxonium ion during participation. In the absence of a C-2 participating group, non-polar solvents increase the possibility of an $S_{N}2$ mechanism. Combined with *in situ* anomerization, an $\alpha$-glycosidic bond would be favoured.\(^1\)

It is possible for a solvent to participate by forming a stabilizing complex with the oxocarbenium ion at either the axial or equatorial position, blocking nucleophilic attack at that site in a similar fashion to neighbouring group participation. Et$_2$O will stabilize the oxocarbenium ion by forming an oxonium ion from the equatorial side (due to the reverse anomeric effect), thus forcing the acceptor to attack axial giving an $\alpha$-glycosidic bond (Scheme 1.16).\(^1\)

![Scheme 1.16. Use of solvent assistance from Et$_2$O to form an $\alpha$-glycosidic bond.\(^1\)](image)

In contrast, CH$_3$CN will stabilize the oxocarbenium ion from the axial side (despite the reverse anomeric effect) by forming a nitrilium ion, forcing the acceptor to attack equatorial giving a $\beta$-glycosidic bond (Scheme 1.17).\(^1,43,44\)

![Scheme 1.17. Use of solvent assistance from CH$_3$CN to form a $\beta$-glycosidic bond.\(^1,43,44\)](image)
1.3.4 Protecting Groups

Protecting groups are critical to achieve regioselective and stereoselective glycosidic bond synthesis. As previously discussed, in order to achieve regioselectivity, an acceptor is usually protected at every position except for the nucleophilic OH, while a donor is usually fully protected with a good leaving group at the anomeric position. These protecting groups also affect stereoselectivity, particularly with the possibility of anchimeric assistance. The size, flexibility, and electronic nature of protecting groups can play a role as well.\textsuperscript{1,3} The role of protecting groups will be discussed in upcoming sections on the armed/disarmed and matched/mismatched concepts.

In order to prepare acceptors and donors with these specific features, a clever synthetic sequence of protection and deprotection must be devised, which may potentially require many steps to achieve starting from fully unprotected monosaccharides. These protecting groups must be carefully selected with a clear vision of all reactions required to reach the target. Ideally, a good protecting group will be easy to introduce selectively and easy to remove selectively when desired, but will also be stable under most other reaction conditions. The stability of protecting groups will vary under acidic, basic, oxidizing, and reducing conditions. The result is a time consuming, expensive sequence resulting in a significant loss of yield in order to synthesize ideal acceptors and donors for glycosylation.\textsuperscript{1,3}

The protecting groups used in this thesis are outlined below in Table 1.1, along with selected methods of introduction and removal for each group.
Table 1.1. List of protecting groups used in this thesis.

<table>
<thead>
<tr>
<th>Protecting Group</th>
<th>Method of Introduction</th>
<th>Method of Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O$-Acetate (OAc)</td>
<td>Ac$_2$O/pyridine or AcCl</td>
<td>NaOMe/MeOH</td>
</tr>
<tr>
<td>$O$-Chloroacetate (OAcCl)</td>
<td>ClAcCl/pyridine</td>
<td>Thiourea or BnNH$_2$</td>
</tr>
<tr>
<td>$O$-Benzoate (OBz)</td>
<td>BzCl/collidine</td>
<td>Na/NH$_3$(l)</td>
</tr>
<tr>
<td>$O$-Pivaloate (OPiv)</td>
<td>PivCl/collidine</td>
<td>Na/NH$_3$(l)</td>
</tr>
<tr>
<td>$N$-Acetate (NHAc)</td>
<td>Ac$_2$O/MeOH</td>
<td>N/A</td>
</tr>
<tr>
<td>$N$-Trichloroacetate (NHTCA)</td>
<td>Cl$_3$AcCl/MeOH</td>
<td>Zn/AcOH (reduces to NHAc)</td>
</tr>
<tr>
<td>$O$-Benzyl (OBn)</td>
<td>BnBr/NaH</td>
<td>Na/NH$_3$(l)</td>
</tr>
<tr>
<td>$O$-Pentyl</td>
<td>Pentanol/Hg(CN)$_2$ (from Cl)</td>
<td>N/A</td>
</tr>
<tr>
<td>di-$O$-Benzyldiene (O&gt;CHPh)</td>
<td>PhCHO/ZnCl$_2$</td>
<td>AcOH/H$_2$O</td>
</tr>
<tr>
<td>di-$O$-Triethylorthoester</td>
<td>CH$_3$C(OEt)$_3$/CSA</td>
<td>AcOH/H$_2$O (opens into OAc)</td>
</tr>
<tr>
<td>O-Trichloroacetimidate</td>
<td>Cl$_3$CCN/DBU</td>
<td>BF$_3$•Et$_2$O/ROH or TMSOTf/ROH</td>
</tr>
<tr>
<td>$S$-Ethyl (SEt)</td>
<td>NaSEt/EtSH (from Br)</td>
<td>NIS/TMSOTf/ROH or Bu$_4$NBr/CuBr$_2$ (through Br)</td>
</tr>
<tr>
<td>$S$-Tolyl (STol)</td>
<td>HSTol/BF$_3$•Et$_2$O (from OAc)</td>
<td>NIS/TfOH/H$_2$O</td>
</tr>
<tr>
<td>Chloride (Cl)</td>
<td>AcCl (through OAc)</td>
<td>Hg(CN)$_2$/ROH</td>
</tr>
<tr>
<td>Bromide (Br)</td>
<td>HBr/AcOH (from OAc)</td>
<td>Hg(CN)$_2$/ROH</td>
</tr>
</tbody>
</table>

1.3.4.1 The Armed/Disarmed Concept

In Paulsen's 1982 review, he noted that glycosyl bromides protected with electron donating benzyl groups were more reactive than those protected with electron withdrawing acetyl or benzoyl groups.$^{15}$ Similarly, in 1988 Fraser-Reid and Mootoo reported the hydrolysis of various protected n-pentenyl donors using NBS and an aqueous solvent, where acetyl groups slowed the hydrolysis significantly compared to various ether protecting groups.$^{45}$ The observation of an ester-protected donor being hydrolyzed slower than an ether-protected donor
led to Fraser-Reid and Mootoo proposing that the n-pentenyl group can be "armed" or "disarmed" by the electronic nature of the C-2 protecting group.\textsuperscript{46-48}

The basis of the armed-disarmed concept is, following electrophilic activation of a leaving group, electron donating substituents on the donor will donate electron density toward the anomeric position, thus stabilizing this intermediate and increasing the rate of activation, and also stabilizing the oxocarbenium ion. These protecting groups are considered "arming", and a donor with these protecting groups is considered to be "armed". On the contrary, electron withdrawing substituents on the donor will pull electron density away from the anomeric position, thus destabilizing the activated intermediate and slowing down activation, while also destabilizing the oxocarbenium ion. These protecting groups are considered "disarming", and a donor with these protecting groups is considered to be "disarmed".\textsuperscript{46-48}

Fraser-Reid and Mootoo used this concept to gain a synthetic advantage. Using a glucosyl acceptor and donor, both with anomeric n-pentenyl groups, he afforded selective glycosylation between the two sugars over potential self-glycosylation of the acceptor by introducing acetyl protecting groups on the acceptor and benzyl protecting groups on the donor. These protections armed the donor leaving group while disarming the acceptor leaving group, causing the donor anomeric centre to be the preferred site of regioselective nucleophilic attack. Once the desired disaccharide was made, he converted the acetyl groups to benzyl groups, turning the reducing end into an armed donor, allowing for further glycosidation.\textsuperscript{46} After Fraser-Reid and Mootoo reported the success of glycosylation using the armed-disarmed concept, van Boom and co-workers used the concept to complete oligosaccharide synthesis using thioglycosides.\textsuperscript{29,49} Fraser-Reid and co-workers published their own set of armed-disarmed glycosylation results using thioglycosides as well.\textsuperscript{50}
Although the armed-disarmed effect is evident with simple electron donating/withdrawing substituents such as ethers and esters, other protecting groups can contribute to the arming or disarming of a donor. A particular case relevant to this thesis are acetals, which are investigated in the next section. In addition, the electronic nature of the protecting groups on the acceptor should not be overlooked as they may greatly influence the reactivity (nucleophilicity) of the acceptor.

1.3.4.2 Acetals: Torsional and Electronic Disarming, and Effect on Stereoselectivity

During studies on arming and disarming of n-pentenyl donors, Fraser-Reid and co-workers reported that acetal protecting groups are disarming, and that the activation energy for oxidative hydrolysis is greater with acetal protection. When the oxocarbenium ion forms, the ring undergoes a conformational change from the chair. Acetals create a fused bicyclic system with the carbohydrate ring, rendering it conformationally rigid, and creating additional torsional strain upon formation of the oxocarbenium ion. This led Fraser-Reid to suggest that acetal disarming is not due the same electronic effects as was the case with ester and ether groups, but rather through torsional effects.\(^{48,51-53}\)

Despite this suggestion from Fraser-Reid, the disarming nature of acetals may not be a purely torsional effect.\(^{37}\) In 2004, Bols and co-workers reported results that suggest the disarming nature of acetals is due to electronic effects as well.\(^{54}\) They determined the rates of hydrolysis of dinitrophenyl donors with and without a 4,6-methylene acetal, as well as donors with a 4,6-fused cyclohexane ring leaving O-6 exocyclic (Figure 1.12). The donors with the fused cyclohexane ring and O-6 exocyclic were hydrolyzed faster than the acetal protected donor. This prompted an electronic explanation for the disarming effect, where the C6-O6 bond
of the acetal-protected donor is locked in the *trans-gauche* conformation, causing a 180° torsional angle between O5-C5 and C6-O6 (O-5 being the endocyclic oxygen). This means the dipole created by O-6 points away from the electron deficient oxocarbenium ion, destabilizing it and making it more difficult to hydrolyze the leaving group. However, the cyclohexane-fused donors were hydrolyzed slower than the freely rotating donor without the acetal or cyclohexane ring, confirming that fused bicyclic systems are disarming even without the C6-O6 bond locked in the *trans-gauche* conformation, albeit to a lesser extent.\(^{37,54}\) As a result, the disarming effect of acetals is likely due to a combination of torsional and electronic effects.

![Figure 1.12](image_url) The electronic disarming effect of fused bicyclic protecting groups on donors.\(^{54}\)

In addition to their disarming role, Crich and co-workers have extensively studied the effect of 4,6-benzylidene protection on the stereoselectivity of thioglycoside donors, using *in situ* conversion to an anomeric triflate as activation.\(^{35-37}\) Since the benzylidene destabilizes the oxocarbenium ion as proposed by Bols,\(^{54}\) the benzylidene group alters the equilibrium between the covalent triflate, the ion contact pair, and the solvent-separated ion pair, shifting it toward the least electropositive covalent triflate (Scheme 1.15). As a result, 4,6-benzylidene, 2,3-benzylated mannosyl donors are more β-stereoselective compared to freely rotating 4,6-benzylated-protected donors.\(^{35-37}\)

However, in contrast to that explanation, Crich and co-workers also found that 4,6-benzylidene, 2,3-benzylated glucosyl donors are α-stereoselective, which is not explained by the
electronic disarming effect of the benzylidene. To explain the opposite stereoselectivities of the mannosyl and glucosyl donors, Crich proposed that the key factor is the C2-C3 group interaction. The loss of the covalent triflate into solution gives way to the oxocarbenium ion, altering the conformation of the ring. For mannose, this causes increased torsional strain between C2-C3 and their protecting groups, favouring the covalent triflate and its initial chair conformation. While for glucose, the conformational change decreases conformational strain between C2-C3 and their protecting groups, shifting the equilibrium toward the solvent-separated ion pair. This explanation was supported by experiments using 2-deoxy and 3-deoxy donors, which resulted in a loss of β-stereoselectivity for mannose, and a loss of α-stereoselectivity for glucose.35-37

A further complication in stereoselectivity of benzylidene donors is the nature of the O-3 protecting group. The above results were established using benzyl groups, and they hold for most ether protecting groups. However, when Crich and co-workers replaced the O-3 substituent with an ester protecting group, it caused the mannosyl donors to become α-stereoselective. While the exact reasoning is not known, Crich proposed that the alignment of the C-3 acyl group C=O dipole with the anomeric α-triflate C-O dipole causes destabilization (as was the case with the polar explanation of the anomeric effect), causing the equilibrium to favour the solvent-separated ion pair.37,38 Another factor that can play a role is the bulkiness of the C-3 substituent. A bulky C-3 substituent (i.e. TBDMS) on a mannose donor will create steric hindrance with the axial C-2 substituent, limiting the C-2 substituent's flexibility and causing it to block the β-face of the anomeric carbon, thus reducing the β-selectivity of the glycosidic bond.37,55 Using similar arguments, Crich and co-workers have shown that these factors will affect the anomeric equilibrium of a solvated mannosyl hemiacetal following hydrolysis of the leaving group.56
Benzylidene acetals also play a conformational role on acceptors. Glycosylation of acceptors will often lead to a small conformational distortion in the ring, which can be seen in the \(^1\)H-NMR coupling constants (\(J_{H-1,H-2}\) gets smaller) and the \(^{13}\)C-NMR chemical shifts (\(\delta_C\) C-4 shifts upfield). However, acceptors protected with acetals are stuck in the locked bicyclic conformation, and might not undergo this conformational ring distortion upon glycosylation.\(^{57-60}\) Whether or not this locked conformation limits the reactivity of the acceptor will be investigated during this thesis.

### 1.3.5 The Matched/Mismatched Concept

The matched/mismatched concept in carbohydrate chemistry dates back to a 1982 review by Paulsen on selective synthesis of oligosaccharides.\(^{15}\) In discussing the methods for selective glycosidic bond synthesis, he suggested that some acceptor/donor pairs were well tuned for glycosylation, while other pairs were not. He would later postulate that acceptors and donors must be "matched" in order to achieve successful results. Based on this concept, acceptor/donor pairs that couple in high regioselectivity and stereoselectivity while also achieving a desirable yield are called a "matched" pair, while pairs that achieve poor regioselectivity or stereoselectivity, or do not react in good yield, are called a "mismatched" pair. Frequently, examples in the literature are given in pairs to show how one particular difference can lead to a matched acceptor/donor coupling and a mismatched coupling, giving way to the term "matched/mismatched pair" for a pair of reactions, one of which is matched and the other mismatched.

The matched/mismatched concept was later elaborated by Fraser-Reid and co-workers, who related it to their armed/disarmed concept by showing that armed and disarmed donors often
exhibit opposite regioselectivities to acceptors, which they called "reciprocal donor acceptor selectivity" (RDAS). Thus a donor competing for multiple acceptors can be matched to one acceptor and mismatched to the other, based on the regioselectivity. A corresponding concept for different acceptor/donor pairs that are matched to one stereochemical outcome and mismatched to the other (i.e. α vs β glycosidic bonds) is double diastereodifferentiation, which was described in 1985 by Masamune and co-workers as double asymmetric synthesis, and later illustrated using carbohydrate coupling by van Boeckel and Spijker, while more recently being reviewed by Crich and Bohé.

While the matched and mismatched terms classify the results of glycosylations, the reasons why certain pairs might be matched or mismatched are not completely understood. Past results do not necessarily provide a good guideline how to determine good matching pairs. Mismatched pairs often come from failed reactions which are frequently not reported due to their unsuccessful nature and lack of progress toward specific synthetic goals, making it more difficult to determine why some pairs are matched and others are mismatched. As well, while some pairs are mismatched, certain reaction conditions can sometimes be used to force the reaction to work as desired, so a reaction reported as successful might have actually been a reaction of a mismatched pair modified using forcing conditions to make the reaction work and advance the targeted synthetic pathway.

There are many factors that will play a role in determining if an acceptor/donor pair are matched or mismatched. Acceptor and donor reactivity and steric hindrance are two of the biggest factors. Both are dependent on the nature of the protecting groups. Whether the acceptor has electron donating or electron withdrawing substituents will affect its nucleophilicity. While the electronic nature of the protecting groups on the donor will affect the donor reactivity based
on the armed-disarmed concept. In addition, C-2 protecting group determine if participation is possible or not. Meanwhile, the size and conformational flexibility of the protecting groups will play a role when considering the potential for steric hindrance. The reaction conditions are also important. Temperature, solvent, choice of leaving group, choice of activator, and length of reaction can all contribute to determining if an acceptor/donor pair is matched or mismatched.\textsuperscript{15}

Because so many factors contribute to matched glycosylations, predicting which pairs will be matched or mismatched is quite difficult. Whether or not acceptor/donor pairs will match becomes a case-by-case basis, and patterns that might apply to a series of reactions are difficult to find. This difficulty was best described by Paulsen in 1982, who said that “...each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic research and a good deal of know-how. There are no universal reaction conditions for oligosaccharide syntheses.”,\textsuperscript{15} and despite the advances in the field since then, Crich and Bohé re-iterated the difficulty in 2010, warning that “glycosidic bond formation, though a highly developed art form, is still, a somewhat immature science and many pitfalls await the unwary.”\textsuperscript{65}

1.3.5.1 Reciprocal Donor Acceptor Selectivity (RDAS)

In 2002, Fraser-Reid and co-workers reported a series of glycosylations where armed donors and disarmed donors exhibited different matched pairs with acceptors.\textsuperscript{62} In an attempted glycosylation of a myo-inositol diol acceptor using both armed and disarmed D-n-pentenyl mannosyl donors, they found that the armed donor reacted preferentially at the more accessible equatorial hydroxyl group in a 3:1 ratio with the axial hydroxyl group, while the disarmed donor reacted exclusively at the less accessible axial hydroxyl group (Scheme 1.18). They explained
this result by introducing the concept of reciprocal donor acceptor selectivity (RDAS). This concept suggests that a donor can be matched to one acceptor and mismatched to another, while a different donor can exhibit the opposite preferences.  

Further experiments by Fraser-Reid and co-workers showed a pattern of armed donors being matched to more accessible hydroxyl groups more often, while disarmed donors are more often matched to less accessible hydroxyl groups. The mechanistic explanation for the opposite regioselectivities is uncertain. One would expect both donors to react at the more accessible position. The disarmed donor will react slower and as a result is possible that it is more likely to reach the less accessible position.

More studies led Fraser-Reid to suggest that RDAS is not necessarily based on steric hindrance. Fraser-Reid and co-workers also completed reactions with thioglycoside and trichloroacetimidate donors to determine if the donor leaving group had an effect on matched and mismatched pairs. In all cases the matched pairs were the same as with n-pentenyl donors, suggesting that the type of leaving group on the glycosyl donor does not affect necessarily the
matched pairings. Whether or not steric hindrance and the leaving group play a role in the matched and mismatched pairs is likely a case-by-case basis.

While the RDAS concept is easy to see using an acceptor diol, results from van Boom and co-workers show that a diol is not required for the reciprocal relationship to occur. Their glycosylation of a disarmed α-D-thiomannoside donor with an axial acceptor gave only a 10% yield, while the reaction of the same donor with an equatorial acceptor gave a favourable 84% yield (Scheme 1.19). These results show that the donor is matched to the equatorial acceptor and mismatched to the axial acceptor, thus showing reciprocal donor-acceptor selectivity.

![Scheme 1.19](image)

**Scheme 1.19.** Matched and mismatched glycosylations of a disarmed donor with axial and equatorial acceptors, giving a near identical trisaccharide in different yields. 49,61,62,67,68

One would expect that an equatorial acceptor would be more reactive than an axial acceptor because of 1,3-diaxial interactions hindering the axial acceptor. However Fraser-Reid and co-workers used a similar disarmed donor and found that it preferred the axial acceptor site in his reactions. 61,62 The contrast in their results show that matched glycosylations will differ on a
case-by-case basis, and a pattern found in one set of reactions will not necessarily repeat itself in a different set of reactions.

1.3.5.2 Double Diastereodifferentiation

In 1985, Masamune and co-workers suggested that a double asymmetric synthesis involving two chiral, enantiopure compounds is capable of creating a new, diastereoselective chiral centre in a predictable and controlled manner.\textsuperscript{63} Using the same terminology as Paulsen, they called two compounds reacting in high stereoselectivity a matched pair, and two compounds reacting in poor stereoselectivity a mismatched pair. More recently this concept has been applied to glycosylation reactions, which couple an enantiopure acceptor and donor, whereby different diastereoisomeric ratios can result. The configurational nature of an acceptor/donor pair can create a preference for an \(\alpha\)- or \(\beta\)-glycosidic linkage, while changing the configuration of the acceptor and/or donor can cause the pair to prefer to the other configurational linkage, or yield poor stereoselectivity overall. The most evident case is with a change in absolute configuration (D vs L) which alters the conformation of the ring.

van Boeckel and Spijker reported a good example of this concept in 1991.\textsuperscript{64} The glycosylation of a 2-phthalimido-4,6-isopropylidene N-acetylglucosamine acceptor was completed with both D and L perbenzoylated fucosyl bromide donors (Scheme 1.20). The D donor preferred the \(\alpha\)-glycosidic linkage in a 2:1 ratio with the \(\beta\)-linkage, even though only the \(\beta\)-linkage was expected due to anchimeric assistance. Meanwhile, the L donor gave the expected \(\beta\)-glycosidic linkage in an 8.4:1 ratio to the \(\alpha\)-linkage. Their explanation was that the transition state in the reaction with the D donor leading to the \(\beta\)-linked disaccharide was sterically hindered due to interactions between the bulky 2-N-phthalimido group on the acceptor and the 2-O-
benzoyl group on the donor, so the α-linked disaccharide was the major product (Figure 1.13). However no such steric hindrance arises in the transition state when the L donor is used, so the expected β-linked disaccharide was the major product. This led them to suggest that D and L donors can provide different stereoselectivity in glycosylation reactions based on steric hindrance.\(^6^4\)

**Scheme 1.20.** The matched glycosylations of an acceptor with identical D and L donors, giving α-linked and β-linked disaccharides in different ratios.\(^6^4\)
Figure 1.13. Steric hindrance during formation of the β-glycosidic bond between the 2-NPhth group on the acceptor and 2-OBz group on the D-donor. No such steric hindrance arises during formation of the α-glycosidic bond, nor during formation of the β-glycosidic bond using the same L-donor.

Although van Boeckel and Spijker demonstrated this concept for identical donors with opposite absolute configuration, the general role of configuration in stereoselectivity should not be overlooked. Given their explanation for the result was based on steric hindrance, there is potential for other pairs of diastereoisomers to exhibit different stereoselectivities during glycosylation. In this case, given the steric hindrance was caused by the C-2 positions of both the acceptor and donor, changing the C-2 configuration of either substrate might greatly affect the resulting stereoselectivity.

Fraser-Reid warned that the matching and mismatching of acceptor/donor pairs should not be confused with double diastereodifferentiation created by the coupling of enantiopure acceptors and donors. However, in a 2010 review by Crich and Bohé on double diastereodifferentiation and other factors affecting stereoselectivity during glycosylation, they described the coupling of an acceptor/donor pair in high stereoselectivity as matched, and a pair coupling in poor stereoselectivity as mismatched. In this review, Crich and Bohé presented many other examples of double diastereodifferentiation found in the literature, putting an emphasis on sets of two reactions in which there was a matched/mismatched pair, while also
pointing out the role of the acceptor in stereoselectivity. They also highlighted some unexpected matched pairings to reiterate the difficulty of predicting matched pairs.65

Crich and Bohé also suggested that the regioselectivity of donors in glycosylations with diol and polyol acceptors can be influenced by, amongst other factors, diastereochemical matching and mismatching through the configurations of the acceptor and donor, thus connecting the RDAS and double diastereodifferentiation concepts. While the regioselective matching of a donor to one site of a diol acceptor can be difficult to predict, there is an important synthetic advantage of a successful regioselective matching as it would allow a second glycosylation at the mismatched acceptor site with a different donor under new conditions tuned for successful glycosylation. This provides a potential method for double regioselective glycosylation without selective protection, and even the potential for one-pot matched double glycosylation.65

To conclude this introduction to the matched/mismatched concept, an all-encompassing result was reported by Martín-Lopez and co-workers in 2006, who combined several variables into one complex example.69 They glycosidated glucosamine and galactosamine trichloroacetimidate D donors possessing non-participating 2-azido groups with both perbenzylated and perbenzoylated D and L chiro-inositol diol acceptors (Table 1.2). In addition to the regiodifferentiation of the donors with each acceptor, the non-participating nature of the donor created a higher probability of stereodifferentiation at each reaction site, putting four possible disaccharides per acceptor/donor pair into play, in addition to the possibility of trisaccharide products.69
Table 1.2. Glycosidic linkages formed upon the glycosylation of D/L and Bn/Bz diol acceptors with D-glucosamine or D-galactosamine donors.\(^{69}\)

<table>
<thead>
<tr>
<th></th>
<th>D-OBz</th>
<th>L-OBz</th>
<th>D-OBn</th>
<th>L-OBn</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlcN</td>
<td>α-2 (27%)</td>
<td>α-2 (58%)</td>
<td>α-2 (23%)</td>
<td>α-2 (21%)</td>
</tr>
<tr>
<td></td>
<td>α-3 (40%)</td>
<td>α-3 (9%)</td>
<td>β-2 (16%)</td>
<td>β-2 (13%)</td>
</tr>
<tr>
<td>GalN</td>
<td>α-2 (28%)</td>
<td>α-2 (42%)</td>
<td>α-2 (12%)</td>
<td>α-2 (5%)</td>
</tr>
<tr>
<td></td>
<td>α-3 (35%)</td>
<td>α-3 (6%)</td>
<td>β-2 (14%)</td>
<td>β-2 (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>α-3 (6%)</td>
<td>α-3 (10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>β-3 (22%)</td>
<td>α-3 (10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>trisaccharides (17%)</td>
<td>trisaccharides (4%)</td>
</tr>
</tbody>
</table>

The results led Martín-Lopez to suggest that absolute configuration (D or L) of the perbenzoylated acceptors afforded opposite regioselectivities from the donors (although poorer regioselectivity from the D acceptors), a phenomenon they called simultaneous regio- and enantiodifferentiation in acceptor/donor coupling. The perbenzoylated acceptors also afforded high stereoselectivity, preferring the α-glycosidic linkage in almost every case. However, the perbenzylated acceptors provided poor stereoselectivity, and the D and L acceptors did not afford opposite regioselectivities. Thus, they emphasized the roles of both stereoelectronic factors and protecting groups on the matching of acceptor/donor pairs. Further analysis led them to make some interesting conclusions. They found that an acyl group neighbouring a free hydroxyl group on an acceptor assists glycosylation by providing stabilization through hydrogen bonding. In addition, they highlight the role of hydrogen bonding interactions between the acceptor and
donor leading to the resulting regio- and stereoselectivity. More recently, Demchenko and Yasomanee have demonstrated the use of hydrogen bonding protecting groups on the donor to achieve highly stereoselective nucleophilic attack by the acceptor.

While it is much easier to analyze one variable at a time, this example from Martín-Lopez and co-workers illustrates that many variables will affect matched and mismatched pairings, making them very difficult to predict.

1.4 Matched Glycosylations at O-3 of N-Acetylglucosamine

1.4.1 D-Galactosylation of a Trisaccharide Diol Acceptor

Recently a β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-β-D-GlcNAc trisaccharide with deprotected hydroxyl groups at O-3 and O-3" (the reducing and non-reducing end N-acetylglucosamine residues) was synthesized in the Auzanneau lab by Guillemineau. An attempt at a selective galactosylation with this trisaccharide diol acceptor provided an unexpected result. The galactosylation using 5 equivalents of a disarmed α-D-galactosyl bromide donor was regioselective to O-3 (the reducing end) giving a β-linked tetrasaccharide, which was unexpected due to the steric hindrance created by the β-D-galactosyl residue (Scheme 1.21). Thus O-3 of the acceptor and the disarmed α-D-galactosyl donor are matched, while the donor and O-3" of the acceptor (the non-reducing end) are mismatched.
Scheme 1.21. The matched galactosylation of O-3 on a trisaccharide diol acceptor with a disarmed α-D-galactosyl bromide donor, giving a β-linked tetrasaccharide.\textsuperscript{57}

1.4.2 L-Fucosylation of a Trisaccharide Diol Acceptor

Guillemineau attempted fucosylation of the same trisaccharide diol acceptor using 3 equivalents of an armed β-L-thiofucoside donor, which provided the expected result. The reaction was selective to O-3'' (at the more accessible non-reducing end), giving an α-linked tetrasaccharide in 57% yield, with 20% recovered acceptor (Scheme 1.22). This means that the armed β-L-thiofucosyl donor and O-3'' are matched while the donor and O-3 are mismatched, the opposite result of the disarmed α-D-galactosyl donor. Increase to 4 equivalents of donor gave a yield of 63%, but also caused the formation of the difucosylated pentasaccharide in 17% yield.\textsuperscript{57}
Scheme 1.22. The matched fucosylation of O-3'' on a trisaccharide diol acceptor with an armed β-L-thiofucoside donor, giving an α-linked tetrasaccharide.\(^\text{57}\)

1.4.3 Analysis of Matched Pairs

These results from Guillemineau agree with Fraser-Reid’s RDAS results, where disarmed donors were matched to the less accessible acceptor, while armed donors were matched to the more accessible acceptor.\(^\text{48,61,62,66}\) While this result can be characterized by the RDAS concept, the mechanistic explanation for the opposite regioselectivities is still uncertain.

These results can also be analyzed using the concept of double diastereodifferentiation. If we focus on the non-reducing end N-acetylglucosamine residue, it is structurally similar to the acceptor used by van Boeckel and Spijker, both protected with 4,6-benzylidene groups and bulky C-2 groups (Schemes 1.21, 1.22). As well, the D-donor used in that case was similar to the D-donor in Guillemineau’s case, both being disarmed bromide donors. van Boeckel and Spijker argued that steric hindrance between C-2 groups blocked formation of the β-glycosidic bond.
A similar argument can be made for Guillemineau's result, where the bulky C-2'' trichloroacetamide group might be blocking formation of the desired β-glycosidic bond with the participating D-galactosyl donor, causing the donor to react at the reducing end instead. Thus it is possible that a change in C-2 configuration (D-mannosyl donor) or absolute configuration (L-fucosyl donor) will change the regioselectivity of the disarmed bromide donor. In addition to potentially playing a steric role, the trichloroacetamide group is a highly electron withdrawing substituent, possibly lowering the nucleophilicity of the O-3'' hydroxyl group.

In addition to the potential steric role of the trichloroacetamide, it is interesting to note the general role of steric hindrance created by the acceptor and donor protecting groups, and the resulting kinetics and thermodynamics. It was initially assumed that O-3'' would be more reactive as it is more accessible than O-3, due to steric hindrance created by the galactosyl residue. The galactosyl donor which reacted at O-3 was protected by smaller acetyl groups, while the fucosyl donor which reacted at O-3'' was protected by bulkier benzyl groups. Therefore it is possible that the bulky fucosyl donor had a difficult time accessing O-3'', while the smaller galactosyl donor was able to access it quicker. However, Guillemineau also attempted selective chloroacetylation of the diol acceptor, which resulted in chloroacetylation at O-3'' in quantitative yield, discouraging that theory. It is also interesting to note that increasing the number of equivalents of benzylated fucosyl donor eventually led to glycosylation at O-3 as well. Guillemineau also slowed down the reaction using in situ anomerization with Bu4NBr, and was able to isolate both tetrasaccharides as well as the pentasaccharide. These results give way to a possible kinetic/thermodynamic product explanation, whereby O-3'' is more accessible and glycosylation at that position is kinetic, however a glycosidic bond at O-3 might be more stable, thus being the thermodynamic product. This explanation is supported by results reported in our
lab by Hendel, where attempted difucosylation at O-3 and O-3'' N-acetylglucosamine residues of a similar linear tetrasaccharide using NIS/TMSOTf activation resulted in fucosylation at O-3'' first, followed by O-3, but eventually the less stable fucosyl residue at O-3'' started to fall off, giving a mixture of both pentasaccharides and the hexasaccharide.\textsuperscript{71}

As discussed in section 1.3.4.2, acceptors undergo a conformational ring distortion upon glycosylation, however acceptors protected with acetals are stuck in a locked bicyclic conformation, and may not undergo this conformational ring distortion upon glycosylation.\textsuperscript{57-60} NMR analysis of Guillemineau's tetrasaccharide products showed that, for galactosylation at O-3, \(J_{H-1,H-2}\) changed from 8.0 Hz to 4.6 Hz, and \(\delta_C\) C-4 shifted upfield from 80.4 ppm to 71.1 ppm, confirming that galactosylation at O-3 (the reducing end with no benzylidene) caused distortion of the \(4C_1\) chair conformation. However fucosylation at O-3'' (the non-reducing end with a benzylidene) did not lead to distortion of the chair conformation (\(J_{H-1'',H-2''}\) = 8.0 Hz, \(\delta_C\) C-4 at 80.0 ppm).\textsuperscript{57} It is possible that this locked conformation is limiting the reactivity at O-3''.

1.5 Scope of Thesis

This thesis investigates the factors leading to matched and mismatched glycosylations at O-3 of \(N\)-acetylglucosamine. It describes synthesis of a \(\beta\)-D-GlcNAc-(1→3)-\(\beta\)-D-Gal-(1→4)-D-\(\beta\)-GlcNAc trisaccharide diol acceptor similar to the trisaccharide used by Guillemineau,\textsuperscript{57} and the subsequent change in protection of that trisaccharide to remove the 4'',6'' benzylidene acetal and C-2'' trichloroacetamido groups (Figure 1.14). By removing these protecting groups, glycosylations can be completed on these diol acceptors to determine if the benzylidene and trichloroacetamido affect the resulting matched pairs.
Figure 1.14. Structure of β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-β-GlcNAc trisaccharide diol acceptors being investigated.

The thesis also looks at the effect of the configuration of the donor on the resulting regioselectivity. Participating D-galactosyl, D-mannosyl, L-rhamnosyl, and L-fucosyl donors are investigated, providing all possible glycosidic bond formations (β-D, β-L, α-L, and α-D) to determine if each one is matched to O-3 or O-3\textquoteright.
Chapter 2

Synthesis of Trisaccharide Acceptors to be Used in Matched Glycosylations
2.1 Introduction

In order to investigate matched glycosylations at O-3 of N-acetylglucosamine, we proposed the synthesis of a series of β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-β-GlcNAc trisaccharide acceptors with deprotected hydroxyl groups at both O-3 and O-3". The structures of these trisaccharides are shown below in Figure 2.1. These trisaccharides are structurally similar to each other, with differences in protection at the non-reducing end. Trisaccharide 1 is similar in structure to the trisaccharide synthesized by Guillemineau, with the exceptions of a benzoyl group at O-6 and a pentyl group at O-1, as opposed to a benzyl group at O-6 and a chlorohexyl group at O-1. It is protected with a benzylidene acetal at O-4" and O-6", while trisaccharide 2 has the benzylidene replaced by acetyl groups. Trisaccharide 3 has the C-2" trichloroacetamino group converted into an acetamido group. These protecting group differences will be the backbone for the research on matched glycosylations.

![Figure 2.1](image.png)

**Figure 2.1.** Structures of the primary synthetic targets, trisaccharides 1-3.

2.2 Synthetic Strategy

The primary synthetic targets were trisaccharide diol acceptors 1-3, which come from a common intermediate, protected trisaccharide 4. We proposed a retrosynthesis of trisaccharide 4
through a linear synthesis from glycosyl acceptor 7, galactosyl donor 6, and N-acetylglucosamine donor 5 (Scheme 2.1).\textsuperscript{31,72} The use of a linear synthesis to make a $\beta$-D-GlcNAc-(1→3)-$\beta$-D-Gal-(1→4)-D-$\beta$-GlcNAc trisaccharide has been reported many times in our lab, with trichloroacetimidate donors giving favourable results.\textsuperscript{71,73,74}

![Scheme 2.1](image.png)

**Scheme 2.1.** The structure of protected trisaccharide 4 and trisaccharide diol acceptors 1-3, and their proposed retrosynthesis from donors 5 and 6, and acceptor 7.\textsuperscript{31,72}

### 2.2.1 Structural Properties of Monosaccharides 5-7

Monosaccharides 5-7 were proposed with certain protecting groups in order to allow for regioselective and stereoselective synthesis of protected trisaccharide 4 and eventual deprotection to give trisaccharide acceptors 1-3.

Both glycosyl acceptor 7 and N-acetylglucosamine donor 5 have chloroacetyl groups at O-3. These groups can be selectively deprotected at the trisaccharide stage, giving way to
trisaccharide acceptors 1-3. Galactosyl donor 6 also has a chloroacetyl group at O-3, which can be selectively deprotected at the disaccharide stage to give a disaccharide acceptor.

Galactosyl donor 6 has an acetyl group at O-2. This group will allow for anchimeric assistance during glycosylation with glycosyl acceptor 7, giving selective 1,2-trans glycosidic bond formation. N-Acetylglucosamine donor 5 also has an ester protecting group at C-2 to allow for selective formation of a β-glycosidic bond. In this case a trichloroacetamido group was selected as opposed to a simpler acetamido group. The acetamido group would cause oxazoline formation during glycosylation, while the trichloroacetamido group used by Jacquinet is less stable and will afford successful 1,2-trans glycosylation.31

N-Acetylglucosamine donor 5 also has a benzylidene acetal at O-4 and O-6, which will be one of the protecting groups investigated during matched the glycosylations of the trisaccharide targets.

### 2.2.2 Structural Properties of Trisaccharides 1-4

Protected trisaccharide 4 has chloroacetyl groups at O-3 and O-3", which can be selectively deprotected in one step to give the O-3/O-3" diol on trisaccharide acceptors 1-3. Acceptor 1 is similar in structure to the trisaccharide synthesized by Guillemin,57 and it has an O-4",O-6" benzylidene acetal. This will be one of the protecting groups investigated to determine its impact on the matched glycosylation. Acceptor 2 has the benzylidene deprotected and replaced by acetyl groups at O-4"and O-6", which will allow for matched glycosylations without the benzylidene group. Acceptor 3 has the C-2" trichloroacetamido group converted into an acetamido group, which will allow for investigation of the impact of the trichloroacetamido group on the matched glycosylations.
2.3 Synthesis of Monosaccharide Components 5-7

2.3.1 Synthesis of Glycosyl Acceptor 7

Glycosyl acceptor 7 was synthesized in 7% yield over 9 steps, starting from D-glucosamine hydrochloride (8) (Scheme 2.2). D-Glucosamine HCl was first converted to the free amine using stoichiometric NaOMe (1 equiv) in MeOH at 0 °C, and then acetylated in situ at C-2 using Ac₂O giving N-acetylglucosamine (9) in 70% yield over two steps, as described by Inouye and co-workers.⁷⁵ N-Acetylglucosamine was then acetylated using AcCl, and HCl formed in situ attacked the anomeric position giving Horton's chloride (10) in 48% yield, contained in a crude mixture with peracetylated glucosamine in 15% yield, as described by Horton.⁷⁶ The α-chloride was selectively formed due to the anomeric effect.⁵⁻⁷ Horton's chloride was then glycosidated by excess pentanol (10 equiv) under Helferich conditions²²,²³ using Hg(CN)₂ (1.5 equiv) at 55 °C, giving known pentyl glycoside 11 in 73% yield, as described by Hendel.⁷⁷ The β-glycoside was selectively formed due to anchimeric assistance from the C-2 N-acetyl group, with oxazoline formation avoided using mild acidic conditions and a large excess of pentanol. NMR analysis of 11 showed that $J_{H-1,H-2} = 8.3$ Hz, confirming the formation of a β-glycosidic bond. The pentyl glycoside was then de-acetylated under Zemplén conditions⁷⁸ using catalytic NaOMe in MeOH, followed by ion-exchange resin giving known⁷⁷ deprotected triol 12 in 95% yield. The triol was selectively protected at O-4 and O-6 through introduction of a benzylidene acetal using PhCHO and ZnCl₂, giving known⁷⁷ benzylidene 13 in 84% yield. This left only O-3 deprotected, a key step in introducing a protecting group at O-3 which can be selectively deprotected at the trisaccharide stage. The benzylidene was chloroacetylated at O-3 using ClAcCl and pyridine giving known⁷⁷ chloroacetate 14 in 98% yield. The O-4, O-6 benzylidene acetal was then hydrolyzed using 90% AcOH in H₂O at 50 °C giving diol 15 in 76% yield.
Finally, the diol was selectively benzoylated at O-6 using stoichiometric BzCl (1.1 equiv) and collidine, giving glycosyl acceptor 7 in 51% yield. The primary hydroxyl group at O-6 was selectively protected as it is more reactive than the secondary hydroxyl group at O-4. This left only O-4 deprotected, as desired for glycosylation.

Scheme 2.2. The synthesis of glycosyl acceptor 7, starting from D-glucosamine HCl (8).\textsuperscript{75-77}

2.3.2 Synthesis of Galactosyl Donor 6

Galactosyl donor 6 was synthesized in 14% yield over 10 steps, starting from D-galactose (16) (Scheme 2.3). D-Galactose was acetylated using Ac\textsubscript{2}O and pyridine at 50 °C giving peracetylated galactose 17 in 95% yield. Peracetylated galactose was then glycosidated by p-thiocresol (HSTol) (3 equiv) using BF\textsubscript{3}·Et\textsubscript{2}O (5 equiv) giving known\textsuperscript{79} thiogalactoside 18 in 50% yield, as described by Meldal and co-workers.\textsuperscript{80} The β-thioglycoside was selectively formed due to anchimeric assistance from the O-2 acetyl group. The thiogalactoside was then de-acetylated
under Zemplén conditions\textsuperscript{78} using catalytic NaOMe in MeOH, followed by ion-exchange resin giving known\textsuperscript{79,80} tetraol 19 in quantitative yield. The deprotected thiogalactoside was then selectively pivaloated at O-6 using stoichiometric PivCl (1.5 equiv) and collidine, giving known pivaloate 20 in 69% yield, as described by Chen and co-workers.\textsuperscript{81} The primary hydroxyl group at O-6 was selectively protected, as it is more reactive than the secondary hydroxyl groups at O-2, O-3, and O-4. This triol was then selectively acetylated at O-2 and O-4 using a 3 step synthesis. First, an orthoester was formed at O-3 and O-4 using CH\textsubscript{3}C(OEt)\textsubscript{3} and catalytic CSA. This left only O-2 deprotected, so O-2 was then acetylated in situ using Ac\textsubscript{2}O and pyridine. Finally, the orthoester was selectively opened using 80\% AcOH in H\textsubscript{2}O to make an O-4 acetate, giving known diacetate 21 in 63\% yield, as described by Guilleminneau.\textsuperscript{72} This left only O-3 deprotected, a key step in introducing a protecting group at O-3 which can be selectively deprotected at the disaccharide level. The diacetate was chloroacetylated at O-3 using ClAcCl and pyridine giving known\textsuperscript{72} chloroacetate 22 in quantitative yield.

The thiotolyl group at the anomeric position was then removed using NIS (1.1 equiv), catalytic TfOH, and H\textsubscript{2}O (in wet CH\textsubscript{3}CN). Under these conditions, \textit{in situ} migration of the O-2 acetate to the anomeric position occurred, as reported by Hendel\textsuperscript{71} and described by Guilleminneau,\textsuperscript{72} whereby the acyloxonium ion is attacked by water to form an unstable hemiorthoacetate, which rearranges into the anomeric acetate and C-2 alcohol. The α-acetate was selectively formed due to the cis-migration of the acetate. This reaction gave known\textsuperscript{72} alcohol 23 as the major product in a mixture with hemiacetal 24, for a combined 74\% yield. Finally, the mixture of alcohols were converted to a single trichloroacetimidate donor using Cl\textsubscript{3}CCN and catalytic DBU giving known\textsuperscript{72} donor 6 in 94\% yield. During this reaction, the anomeric acetate migrates back to O-2, which occurs under basic conditions as described by Hendel\textsuperscript{71} and
Guillemineau.\textsuperscript{72} The α-trichloroacetimidate was selectively formed due to the use of DBU, as described by Schmidt,\textsuperscript{16} allowing for the more stable trichloroacetimidate to form, which is the α-trichloroacetimidate due to the anomeric effect. NMR analysis of 6 showed that \( J_{H-1,H-2} = 3.6 \) Hz, confirming the formation of an α-trichloroacetimidate.

**Scheme 2.3.** The synthesis of galactosyl donor 6, starting from D-galactose (16).\textsuperscript{26,72,79,81}

### 2.3.3 Synthesis of N-Acetylglucosamine Donor 5

\( N \)-Acetylglucosamine donor 7 was synthesized in 12% yield over 5 steps, starting from D-glucosamine HCl (8) (Scheme 2.4). D-Glucosamine HCl was converted to the free amine using NEt\(_3\), and trichloroacetylated in situ at C-2 using Cl\(_3\)COCl in MeOH. A trichloroacetamido group was introduced as an alternative to an acetamido group, which would form the oxazoline during future glycosylation.\textsuperscript{31} The trichloroacetamide was then selectively protected at O-4 and
O-6 in situ through introduction of a benzylidene acetal using PhCHO and ZnCl₂, giving known benzylidene 25 in 57% yield, as described by Jacquinet and Blatter. The benzylidene was then chloroacetylated at both O-1 and O-3 using ClAcCl and pyridine giving known dichloroacetate 26 in quantitative yield. The dichloroacetate was selectively dechloroacetylated at the anomeric position using stoichiometric BnNH₂ (1 equiv), giving known hemiacetal 27 in 31% yield, contained in a crude mixture with diol 25 in 26% yield, and the N-chloroacetylbenzylamine by-product. Finally, the crude mixture of hemiacetal 27 and diol 25 was converted to a trichloroacetimidate donor using excess Cl₃CCN and catalytic DBU giving known donor 5 in 66% yield from 27, and known 1,3-ditrichloroacetimidate 28 in 67% yield from 25. The anomeric α-trichloroacetimidates were selectively formed due to the use of DBU ($J_{H-1,H-2} = 3.8$ Hz for 5).
2.4 Synthesis of Disaccharide Acceptor 30

Glycosyl acceptor 7 was glycosylated with galactosyl donor 6 (4 equiv), using BF$_3$·Et$_2$O (2 equiv) at 40 °C, giving β-linked disaccharide 29 in 65% yield (Scheme 2.5). The β-glycosidic bond was selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor. NMR analysis of 29 showed that $J_{H-1',H-2'} = 7.9$ Hz, confirming the formation of a β-glycosidic bond. The protected disaccharide was then selectively dechloroacetylated at O-$3'$ using stoichiometric thiourea (1.2 equiv) in 2:1 pyridine/EtOH at 55 °C giving disaccharide acceptor 30 in 52% yield. Selective dechloroacetylation occurred at O-$3'$ as it was more
accessible (less sterically hindered) compared to O-3. This left only O-3’ deprotected, as desired for glycosylation. NMR analysis of 30 showed an upfield shift of H-3″ from $\delta_H$ 4.93 ppm to 3.68 ppm and the disappearance of the chloroacetyl group at 3.91 ppm, while H-3 and the other chloroacetyl group retained their chemical shifts, confirming the selective dechloroacetylation at O-3’.

**Scheme 2.5.** The synthesis of disaccharide acceptor 30, starting from glycosyl acceptor 6 and galactosyl donor 5.

### 2.4.1 Effect of O-6 Group on Galactosylation at O-4 of N-Acetylglucosamine

Previous studies on how the aglycone and the O-3 substituent affect glycosylation at O-4 of N-acetylglucosamine have been reported by Hendel.$^{82}$ The results of the glycosylation of glycosyl acceptor 7 and galactosyl donor 6 suggest that the O-6 protecting group might play a role as well. Glycosyl acceptor 7 is protected with a benzoyl group at O-6, while in previous research completed in our lab, O-6 is protected with a benzyl group.

The yield of the glycosylation of acceptor 7 with galactosyl donor 6 was 65%. Similar glycosylations using the same reaction conditions (4 equiv donor, 2 equiv BF$_3$·Et$_2$O, 40 °C) have been reported in our lab by Hendel$^{82}$ and Guillemineau.$^{72}$ Hendel reported the glycosylation of
the same acceptor except protected with an O-6 benzyl group with a peracetylated galactosyl trichloroacetimidate donor in 83% yield.\textsuperscript{82} Meanwhile, Guillemineau reported the glycosylation of galactosyl donor 6 with the O-6 benzyl protected N-acetylglucosamine acceptor also containing an anomeric chlorohexyl group in 79% yield (Table 2.1).\textsuperscript{72} Comparing these previous results to the 65% yield for glycosyl acceptor 7 with galactosyl donor 6, there is a strong indication that the electron-withdrawing benzoyl group at O-6 is deactivating O-4 toward glycosylation in comparison to the electron-donating benzyl group.

\textbf{Table 2.1.} Yields of glycosylation reactions between glycosyl acceptors (N-acetylglucosamine deprotected at O-4) and galactosyl trichloroacetimidate donors, performed under the same reaction conditions (4 equiv donor, 2 equiv BF\textsubscript{3}·Et\textsubscript{2}O, 40 °C), which demonstrates the potential effect of the O-6 substituent on the acceptor.\textsuperscript{72,82}

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<th>Donor</th>
<th>Acceptor</th>
<th>Yield</th>
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<tr>
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<td><img src="image2" alt="Chemical Structure 2" /></td>
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<td><img src="image5" alt="Chemical Structure 5" /></td>
<td><img src="image6" alt="Chemical Structure 6" /></td>
<td>79%</td>
</tr>
</tbody>
</table>
2.4.2 Attempts to Reduce the Number of Equivalents of Galactosyl Donor 6 During Glycosylation

In an attempt to economically optimize the glycosylation of glycosyl acceptor 7 with galactosyl donor 6, the reaction was completed using fewer equivalents of donor. When the reaction was completed using only 2 equivalents of 6, a low yield of 48% was obtained for disaccharide 29. Next we scaled up to 3 equivalents of 6, and a similar yield of 47% was obtained for 29 (Table 2.2). This indicates that the reaction requires around 4 equivalents of galactosyl donor 6 in order to obtain a more desirable yield. In contrast, Kuir reported the glycosylation of a similar N-acetylglucosamine acceptor having a benzyl group at O-6, trichloroethoxycarbonyl group at O-3, and chlorohexyl group at O-1 with only 2 equivalents of galactosyl donor 6 under the same reaction conditions in 88% yield. This once again shows the possible effect of the O-6 benzoyl group on O-4 glycosylation efficiency.

Table 2.2. Yields of glycosylation reactions between glycosyl acceptor 7 and galactosyl donor 6 using varying equivalents of the donor.

<table>
<thead>
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<th>Equivalents of Galactosyl Donor 6</th>
<th>Yield</th>
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<tr>
<td>2</td>
<td>48%</td>
</tr>
<tr>
<td>3</td>
<td>47%</td>
</tr>
<tr>
<td>4</td>
<td>65%</td>
</tr>
</tbody>
</table>

2.5 Synthesis of Protected Trisaccharide 4

Disaccharide acceptor 30 was glycosylated with N-acetylglucosamine donor 7 (3 equiv), using TMSOTf (2 equiv) at 0 °C, giving β-linked trisaccharide 4 in 75% yield (Scheme 2.6). The β-glycosidic bond was selectively formed due to anchimeric assistance from the C-2
trichloroacetyl group on the donor. NMR analysis of 4 showed that $J_{H-1',H-2'} = 8.0$ Hz, confirming the formation of a β-glycosidic bond.

![Scheme 2.6](image)

**Scheme 2.6.** The synthesis of protected trisaccharide 4 from disaccharide acceptor 30 and N-acetylglucosamine donor 5.

### 2.5.1 Unexpected Side Product During the Synthesis of Trisaccharide 4

The reaction of disaccharide acceptor 30 with donor 5, using TMSOTf (2 equiv) at 0 °C gave trisaccharide 4 in 75% yield. However, a side product was also isolated. NMR analysis showed that this product has a trimethylsilyl (TMS) group at $\delta_H 0.01$ ppm, while the O-H peak at 2.80 ppm disappeared. This side product was identified as TMS-disaccharide 31, obtained in 11% yield, where the deprotected O-3' on disaccharide acceptor 30 had attacked the electrophilic TMS group from TMSOTf instead of N-acetylglucosamine donor 5 (Scheme 2.7). Mass spectrometry data (MW = 874.3471, [M+H]$^+$) confirmed that this product was formed. Since TMS groups are acid labile and easily cleaved, the disaccharide acceptor could be easily recovered in this case. TMS-disaccharide 31 was de-silylated using H+ ion-exchange resin in MeOH, giving disaccharide acceptor 30 in quantitative yield.
Scheme 2.7. The unexpected formation TMS disaccharide 31 as a side product during the glycosylation of disaccharide acceptor 30, and the recovery of 30 using acidic hydrolysis.\textsuperscript{84}

2.6 Synthesis of Trisaccharide Acceptors 1-3

2.6.1 Synthesis of Benzylidene Trisaccharide Acceptor 1

Protected trisaccharide 4 was dechloroacetylated at both O-3 and O-3'' using excess thiourea (10 equiv) in 1:1 pyridine/EtOH at 70 °C, giving trisaccharide diol acceptor 1 in 98% yield (Scheme 2.8). This left both O-3 and O-3'' deprotected, as desired for future matched glycosylations. NMR analysis of 1 showed an upfield shift of H-3 from $\delta_H$ 5.16 ppm to 4.03 ppm, and H-3'' from 5.45 ppm to 4.34-4.23 ppm, and the disappearance of both chloroacetyl CH$_2$Cl peaks at 4.15-4.06 and 4.04-3.97 ppm, confirming the dechloroacetylation at O-3 and O-3''.

\[ \text{AcO} \] \[ \text{OPiv} \] \[ \text{O} \] \[ \text{ClAcO} \] \[ \text{O} \] \[ \text{OAc} \] \[ \text{OBz} \] \[ \text{HNAc} \] \[ \text{30 (quant.)} \] \[ \text{TMSOTf} \] \[ \text{0 °C} \] \[ \text{MeOH} \] \[ \text{H+ Resin} \] \[ \text{AcO} \] \[ \text{OPiv} \] \[ \text{O} \] \[ \text{ClAcO} \] \[ \text{O} \] \[ \text{OAc} \] \[ \text{OBz} \] \[ \text{HNAc} \] \[ \text{31 (11%)} \]
**Scheme 2.8.** The synthesis of benzylidene trisaccharide acceptor 1 from protected trisaccharide 4.

### 2.6.2 Synthesis of Diacetyl Trisaccharide Acceptor 2

Benzylidene trisaccharide 4 was hydrolyzed in 90% AcOH in H₂O at 80 °C, removing the O-4", O-6" benzylidene acetal and giving trisaccharide diol 32 in quantitative yield (Scheme 2.9). The diol was acetylated using Ac₂O and pyridine giving diacetyl trisaccharide 33 in 99% yield. The diacetyl trisaccharide was then dechloroacetylated at both O-3 and O-3" using excess thiourea (10 equiv) in 1:1 pyridine/EtOH at 70 °C, giving trisaccharide diol acceptor 2 in 89% yield. NMR analysis of 2 showed a downfield shift of H-4" from $\delta_H$ 3.73-3.66 ppm to 4.83 ppm, and the appearance of two additional acetate CH₃ singlet peaks at 2.07 ppm and 2.06 ppm, combined with the disappearance of the aromatic benzylidene peaks at 7.48-7.41 ppm and 7.35 ppm and the hemiacetal singlet at 5.51 ppm, confirming the conversion of the benzylidene to the diacetate. Dechloroacetylation at O-3 and O-3" was confirmed by chemical shifts of H-3 ($\delta_H$ 4.04-3.93 ppm) and H-3" (4.30-4.08 ppm) and the disappearance of both chloroacetyl CH₂Cl peaks.
Scheme 2.9. The synthesis of diacetyl trisaccharide acceptor 2, starting from protected trisaccharide 4.

2.6.3 Synthesis of Acetamido Trisaccharide Acceptor 3

Trichloroacetamido trisaccharide diol 2 was reduced using Zn in AcOH under sonication at 50 °C, converting the C-2'' trichloroacetamide into an acetamido group, giving trisaccharide diol acceptor 3 in 89% yield (Scheme 2.10). NMR analysis showed an upfield shift of the N-H'' from δH 7.19 ppm to 5.96 ppm, and the appearance of an additional acetate CH₃ singlet peak at
1.99 ppm, combined with the disappearance of the CCl₃ quaternary peak at δC 92.1 ppm, confirming the reduction of the trichloroacetamide into the acetamide.

**Scheme 2.10.** The synthesis of acetamido trisaccharide acceptor 3 from trichloroacetamido trisaccharide 2.

### 2.6.3.1 Isolation of Chloroacetamido Trisaccharide Intermediate 34

The reduction of the trichloroacetamido group to the acetamido group to make trisaccharide 3 is a slow reaction which goes through an intermediate chloroacetamido group. When the reaction is halted prior to completion, or does not reach completion due to non-forcing reaction conditions, the chloroacetamido trisaccharide can be isolated, as previously reported in our lab by Guillemineau" and Kuir. A reaction where fewer equivalents of Zn were used gave both acetamido trisaccharide 3 in 47% yield and chloroacetamido trisaccharide 34 in 35% yield (Scheme 2.11). NMR analysis of 34 showed an N-H" shift of δH 6.65 ppm, upfield from 2 but downfield from 3, and the appearance of an additional chloroacetate CH₂Cl peak at 4.06-4.00 ppm with no additional acetate CH₃ peak appearing, combined with the disappearance of the CCl₃ quaternary peak at δC 92.1 ppm, confirming the reduction of the trichloroacetamide into the...
chloroacetamide. Mass spectrometry data (MW = 1045.3769, [M-H]) confirmed that this product was formed.

Scheme 2.11. The synthesis of chloroacetamido trisaccharide 34 from trichloroacetamido trisaccharide 2.

2.7 Conclusion

Protected trisaccharide 4 was successfully synthesized from glycosyl acceptor 7, galactosyl donor 6, and N-acetylglucosamine donor 5. Glycosylations of glycosyl acceptor 7 with galactosyl donor 6, and disaccharide acceptor 30 with N-acetylglucosamine donor 5 were completed on a large scale with reasonable yields obtained. The target molecules, trisaccharide diol acceptors 1-3, were successfully synthesized from protected trisaccharide 4 through simple, high yielding deprotections. 

$^1$H- and $^{13}$C-NMR analysis and mass spectrometry data confirms the structures of products 1-3. Trisaccharide diol acceptors 1-3 can now be glycosylated by various donors to gather information on matched glycosylations at O-3 of N-acetylglucosamine.
Chapter 3

Matched Glycosylations at O-3 of N-Acetylglucosamine
3.1 Introduction

With trisaccharide acceptors 1-3 synthesized, the next step is to investigate the factors leading to matched and mismatched glycosylations at O-3 of N-acetylglucosamine.

The first factor being investigated is the effect of the benzylidene and trichloroacetamido protecting groups on the resulting matched pairs. Glycosylations with trisaccharide diols 1-3 will determine whether the two protecting groups are limiting the reactivity at O-3”.

The other main factor investigated is the structure of the donor. The donors selected to be used in the matched glycosylation reactions selected are peracetylated bromide donors, to remain consistent with the results reported by Guillemineau.57 Four peracetylated bromide donors were chosen, each to give a different expected glycosidic linkage upon glycosidation: D-galactose 35 (β-D), D-mannose 36 (α-D), L-rhamnose 37 (α-L), and L-fucose 38 (β-L) (Figure 3.1) This will help determine which glycosidic linkages are preferred at O-3 vs O-3” on the acceptors. In addition, perbenzylated thioethyl L-fucosyl donor 39 was selected as an armed donor to confirm the results reported by Guillemineau,57 and to differentiate between both armed and disarmed donors, and bulky protecting groups (Bn) vs smaller protecting groups (Ac).

Figure 3.1. The structures of peracetylated bromide donors 35-38 and perbenzylated thioethyl donor 39, and their expected glycosidic linkages when used in matched glycosylations.
The reaction/activation conditions were selected in order to remain consistent with the results reported by Guillemineau.\textsuperscript{57} Bromide donors 35-38 (5 equiv) were chosen to be activated using Hg(CN)\textsubscript{2} (6 equiv) at 50 °C for 5 h, in a solvent mixture of 1:1 anhydrous PhMe/CH\textsubscript{3}NO\textsubscript{2} with activated 4 Å powdered molecular sieves. In addition to aiding solubility of the acceptor, the use of CH\textsubscript{3}NO\textsubscript{2}, a polar aprotic hydrogen bonding solvent, prevents any potential intramolecular hydrogen bonding between O-H and N-H groups which might affect the reactivity of O-3 or O-3''. Thioethyl donor 39 (4 equiv) was activated using NIS (5 equiv) and catalytic TMSOTf at 0 °C for 4-7 h in CH\textsubscript{2}Cl\textsubscript{2}. In situ anomerization conditions were also investigated for donor 39, using Bu\textsubscript{4}NBr (5 equiv) and CuBr\textsubscript{2} at RT for 40 h in a solvent mixture of 1:1 DMF/CH\textsubscript{2}Cl\textsubscript{2}. The use of DMF, a polar aprotic hydrogen bonding solvent, also prevents any potential intramolecular hydrogen bonding (like CH\textsubscript{3}NO\textsubscript{2}).

The goal of these glycosylations was to find whether the donor was matched to O-3 or O-3'', and to obtain as many results and as much information as possible. Isolation and characterization of the products was chosen as a priority over obtaining optimized yields. As glycosylations introduce a 4th (and sometimes 5th) monosaccharide residue, from this point on the numbering system will use specific letters to designate each monosaccharide. The reducing end N-acetylglucosamine residue is labelled A, the middle galactosyl residue is labelled B, and the non-reducing end N-acetylglucosamine residue is labelled A'. Any glycosyl residue which reacts at O-3A is labelled C, and any glycosyl residue which reacts at O-3A' is labelled C' (Figure 3.2).
Figure 3.2. Numbering system with respect to trisaccharide acceptors 1-3, including substitutions at O-3A and/or O-3A'.

3.2 Synthesis of Donors to be Used for Matched Glycosylations

Peracetylated D-galactosyl bromide donor 2 was synthesized in our lab by Guillemineau, using a known synthesis starting from D-galactose (16) as described by Kartha and Jennings (Scheme 3.1).

D-Mannose (40) was acetylated using Ac₂O, and then HBr attacked the anomeric position in situ giving peracetylated D-mannosyl bromide donor 36 in 65% yield, as described by Kartha and Jennings (Scheme 3.2). The α-bromide was selectively formed due to the anomeric effect. The same procedure was applied to L-rhamnose monohydrate (41), giving peracetylated L-rhamnosyl bromide donor 37 in 87% yield (Scheme 3.3), and L-fucose (42), giving peracetylated L-fucosyl bromide donor 38 in 71% yield (Scheme 3.4).
Scheme 3.2. The synthesis of peracetylated D-mannosyl bromide donor 36 from D-mannose (40).\textsuperscript{19}

Scheme 3.3. The synthesis of peracetylated L-rhamnosyl bromide donor 37 from L-rhamnose monohydrate (41).\textsuperscript{19}

Scheme 3.4. The synthesis of peracetylated L-fucosyl bromide donor 38 from L-fucose (42).\textsuperscript{19}

Perbenzylated thioethyl L-fucosyl donor 39 was synthesized in our lab by Moore, using a known 4 step synthesis starting from peracetylated L-fucosyl bromide (38), as described by Lönn (Scheme 3.5).\textsuperscript{85}
Scheme 3.5. The synthesis of perbenzylated thioethyl L-fucosyl donor 39 from peracetylated L-fucosyl bromide (38).85

3.3 Matched Glycosylations Using Benzylidene Trisaccharide Acceptor 1

3.3.1 With D-Galactosyl Donor 35

Trisaccharide acceptor 1 was glycosylated with D-galactosyl donor 35 (5 equiv), using Hg(CN)₂ (6 equiv) at 50 °C in 1:1 PhMe/CH₃NO₂, giving O-3A β-linked tetrasaccharide 43 in 35% yield, while also recovering acceptor 1 in 37% yield (Scheme 3.6). The β-glycosidic bond was selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 43 showed that $J_{H-1A,H-2A}$ changed from 8.1 Hz to 3.8 Hz, demonstrating distortion of the chair caused by glycosylation at O-3A, while also showing a downfield shift of N-HA from $\delta_H$ 5.59 ppm to 6.46 ppm, and an upfield shift of C-4A from $\delta_C$ 82.1 ppm to 71.4 ppm. Integration of ring $^1$H and acetate CH₃ peaks confirms the presence of a new galactosyl residue B'. In addition, $J_{H-1C,H-2C} = 8.0$ Hz, confirming the formation of a β-glycosidic bond.
Scheme 3.6. Matched glycosylation of trisaccharide acceptor 1 and D-galactosyl donor 35, giving O-3A β-linked tetrasaccharide 43.

3.3.2 With D-Mannosyl Donor 36

Trisaccharide acceptor 1 was glycosylated with D-mannosyl donor 36 (5 equiv), using Hg(CN)₂ (6 equiv) at 50 °C in 1:1 PhMe/CH₃NO₂, giving O-3A α-linked tetrasaccharide 44 in 22% yield, O-3A' orthoester tetrasaccharide 45 in 11% yield, and O-3A' orthoester pentasaccharide 46 in 12% yield, while also recovering acceptor 1 in 20% yield (Scheme 3.7). The α-glycosidic bond and orthoester were formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 44 ($J_{H-1A,H-2A} = 4.0$ Hz, $\delta_N$ N-HA 6.42 ppm, $\delta_C$ C-4A 73.8 ppm) and integration of ring $^1$H and acetate CH₃ peaks confirms mannosylation at O-3A. In addition, $J_{C-1C,H-1C} = 177$ Hz, confirming the formation of an α-glycosidic bond.⁸⁶
NMR analysis of 45 showed no significant difference in $J_{H-1A,H-2A}$, $\delta_H$ N-HA, and $\delta_C$ C-4A from 1, demonstrating no chair distortion which would have been created by glycosylation at O-3A, while also showing a small an upfield shift of C-4A' from $\delta_C$ 81.2 ppm to 79.9 ppm, as is consistent with glycosylation at O-3A'. Evidence of orthoester formation is found with an upfield $^1H$ shift of one of the acetate CH$_3$ peaks to $\delta_H$ 1.67 ppm, and of H-2C from 5.42 ppm (on the donor) to 4.58 ppm, along with a downfield $^{13}C$ shift of one of the acetate CH$_3$ peaks to $\delta_C$ 25.7 ppm, and the appearance of a quaternary peak at 124.1 ppm combined with the disappearance of a carbonyl quaternary peak.$^{87}$ Integration of ring $^1H$ and acetate CH$_3$ peaks confirms the presence of a new mannosyl residue C'.

NMR analysis of 46 ($J_{H-1A,H-2A} = 2.5$ Hz, $J_{C-1C,H-1C} = 177$ Hz, $\delta_H$ N-HA 6.45 ppm, H-2C 4.58 ppm, CH$_3$ 1.67 ppm, $\delta_C$ C-4A 79.9 ppm, C-4A' 73.6 ppm, CH$_3$ 25.7 ppm, $^4$C 124.1 ppm) and integration of ring $^1H$ and acetate CH$_3$ peaks confirms $\alpha$-mannosylation at O-3A and an orthoester at O-3A'.$^{86}$
Scheme 3.7. Matched glycosylation of trisaccharide acceptor 1 and D-mannosyl donor 36, giving O-3A α-linked tetrasaccharide 44, O-3A' orthoester tetrasaccharide 45, and O-3A' orthoester pentasaccharide 46.
3.3.3 With L-Rhamnosyl Donor 37

Trisaccharide acceptor 1 was glycosylated with L-rhamnosyl donor 37 (5 equiv), using Hg(CN)₂ (6 equiv) at 50 °C in 1:1 PhMe/CH₃NO₂, giving O-3A α-linked tetrasaccharide 47 in 27% yield, and O-3A/O-3A' α-linked pentasaccharide 48 in 29% yield (Scheme 3.8). The α-glycosidic bonds were selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 47 \( (J_{H-1A,H-2A} = 3.8 \text{ Hz}, \ J_{C-1C,H-1C} = 173 \text{ Hz}, \ \delta_H \text{ N-HA} 6.45 \text{ ppm}, \ \delta_C \text{ C-4A} 71.5 \text{ ppm}) \) and integration of ring \(^1H\) and acetate CH₃ peaks confirms α-rhamnosylation at O-3A.\(^8\)

NMR analysis of 48 \( (J_{H-1A,H-2A} = \text{bs}, \ J_{C-1C,H-1C} = 175 \text{ Hz}, \ \delta_H \text{ N-HA} 6.49 \text{ ppm}, \ \delta_C \text{ C-4A} 71.5 \text{ ppm}) \) confirms α-rhamnosylation at O-3A. Meanwhile, there was also a small upfield shift of C-4A' from \( \delta_C 81.2 \text{ ppm to 79.8 ppm} \), as is consistent with glycosylation at O-3A'. In addition, \( J_{C-1C',H-1C'} = 171 \text{ Hz} \), confirming the formation of an α-glycosidic bond.\(^8\) Integration of ring \(^1H\) and acetate CH₃ peaks confirms the presence of two new rhamnosyl residues, C and C'. The chemical shift of H-2C' at \( \delta_H 5.28-5.23 \text{ ppm} \) and the acetate CH₃ peaks in the normal region confirm that this new C' residue is not an orthoester.
Scheme 3.8. Matched glycosylation of trisaccharide acceptor 1 and L-rhamnosyl donor 37, giving O-3A α-linked tetrasaccharide 47, and O-3A/O-3A' α-linked pentasaccharide 48.

3.3.4 With L-Fucosyl Donor 38

Trisaccharide acceptor 1 was glycosylated with L-fucosyl donor 38 (5 equiv), using Hg(CN)$_2$ (6 equiv) at 50 °C in 1:1 PhMe/CH$_3$NO$_2$, giving O-3A β-linked tetrasaccharide 49 in 50% yield (Scheme 3.9). The β-glycosidic bond was selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor.
NMR analysis of 49 ($J_{H-1A,H-2A} = 3.8$ Hz, $J_{H-1C,H-2C} = 7.9$ Hz, $\delta_H$ N-HA 6.26 ppm, $\delta_C$ C-4A 74.8 ppm) and integration of ring $^1H$ and acetate CH$_3$ peaks confirms $\beta$-fucosylation at O-3A.

Scheme 3.9. Matched glycosylation of trisaccharide acceptor 1 and L-fucosyl donor 38, giving O-3A $\beta$-linked tetrasaccharide 49.

3.3.5 With L-Fucosyl Donor 39

Trisaccharide acceptor 1 was glycosylated with L-fucosyl donor 39 (4 equiv), using NIS (5 equiv) and catalytic TMSOTf at 0 °C in CH$_2$Cl$_2$, giving O-3A' $\alpha$-linked tetrasaccharide 50 in 55% yield, while also recovering acceptor 1 in 23% yield (Scheme 3.10). The $\alpha$-glycosidic bond was selectively formed due to the anomeric effect.

NMR analysis of 50 ($J_{H-1A,H-2A} = 3.6$ Hz, $\delta_C$ C-4A' 80.0 ppm, and no significant change in $J_{H-1A,H-2A}$, $\delta_H$ N-HA, and $\delta_C$ C-4A from 1), and integration of ring $^1H$ and benzyl CH$_2$ peaks confirms $\alpha$-fucosylation at O-3A'.
Scheme 3.10. Matched glycosylation of trisaccharide acceptor 1 and L-fucosyl donor 39, giving O-3A' α-linked tetrasaccharide 50.

The glycosylation of trisaccharide acceptor 1 with L-fucosyl donor 39 was also completed under in situ anomerization conditions, using Bu₄NBr (5 equiv) and CuBr₂ at RT in 1:1 DMF/CH₂Cl₂, giving O-3A/O-3A' α-linked pentasaccharide 51 in 47% yield, and α-linked tetrasaccharide 50 in 12% yield (Scheme 3.11). The α-glycosidic bonds were selectively formed due to the anomic effect.

NMR analysis of 51 ($J_{H-1A,H-2A} = 3.9$ Hz, $J_{H-1C,H-2C} = 3.8$ Hz, $J_{H-1C',H-2C'} = 3.7$ Hz, $\delta_H$ N-HA 6.18 ppm, $\delta_C$ C-4A 72.0 ppm, C-4A' 80.0 ppm) and integration of ring $^1$H and benzyl CH₂ peaks confirms α-fucosylation at both O-3A and O-3A'.
Scheme 3.11. Matched glycosylation of trisaccharide acceptor 1 and L-fucosyl donor 39 under in situ anomerization conditions, giving O-3A/O-3A' α-linked pentasaccharide 51, and α-linked tetrasaccharide 50 in 12% yield.

3.3.6 Discussion of Results

The results reported by Guillemineau\textsuperscript{57} were confirmed for trisaccharide acceptor 1. The reaction of 1 and D-galactosyl donor 35 resulted in a matched glycosylation at O-3A (Scheme 3.7), while the reaction of 1 with L-fucosyl donor 39 using NIS/TMSOTf activation resulted in a matched glycosylation at O-3A' (Scheme 3.10). In addition, the reaction of 1 with 39 using in situ anomerization gave the pentasaccharide as the major product (Scheme 3.11), confirming the
increased reactivity at O-3A when the reaction is slowed down. Results from the glycosylations with trisaccharide acceptor 1 are summarized in Table 3.1.

Table 3.1. Selected NMR data for products of glycosylations with trisaccharide acceptor 1.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Product</th>
<th>$J_{H-1A,H-2A} (\text{Hz})$</th>
<th>$\delta_H N$-$\text{HA} (\text{ppm})$</th>
<th>$\delta_H H$-$2C' (\text{ppm})$</th>
<th>$\delta_C C$-$4A (\text{ppm})$</th>
<th>$\delta_C C$-$4A' (\text{ppm})$</th>
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<tr>
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<td>3.8</td>
<td>6.46</td>
<td>-</td>
<td>71.4</td>
<td>81.3</td>
</tr>
<tr>
<td>36</td>
<td>44</td>
<td>4.0</td>
<td>6.42</td>
<td>-</td>
<td>73.8</td>
<td>81.3</td>
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<td>45</td>
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<td>5.56</td>
<td>4.58</td>
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<td>79.9</td>
</tr>
<tr>
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<td>6.45</td>
<td>4.58</td>
<td>73.6</td>
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</tr>
<tr>
<td>37</td>
<td>47</td>
<td>3.8</td>
<td>6.45</td>
<td>-</td>
<td>71.5</td>
<td>81.2</td>
</tr>
<tr>
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<td>48</td>
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<td>5.28-5.23</td>
<td>71.5</td>
<td>79.8</td>
</tr>
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<td>80.0</td>
</tr>
<tr>
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<td>51</td>
<td>3.9</td>
<td>6.18</td>
<td>-</td>
<td>72.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Based on the reactions of benzylidene acceptor 1 with peracetylated bromide donors 35-38, it appears that the peracetylated bromide donors are all matched to O-3A of the acceptor. The comparison of D vs L donors does not yield much information in this case as both are matched to O-3A, however when comparing $\alpha$ (D-Man, L-Rha) vs $\beta$ (D-Gal, L-Fuc) glycosidic bonds, it appears that the donors forming $\alpha$-glycosidic bonds showed additional reactivity at O-3A'. D-mannosyl donor 36 gave two orthoester products at O-3A' (23% total, Scheme 3.7), while L-rhamnosyl donor 37 gave a pentasaccharide in slightly higher yield (29%) to the O-3A tetrasaccharide (27%, Scheme 3.8). This indicates that there is indeed some reactivity at O-3A' despite the pattern for glycosylation at O-3A. Using $\beta$-glycosidic bond forming donors 35 and 38, the only new products formed were O-3A tetrasaccharides. While all four donors are
matched to O-3A, it appears that O-3A' is more willing to form an α-glycosidic bond than a β-glycosidic bond. However, considering the kinetic/thermodynamic product theory, there is a possibility that orthoesters or glycosidic bonds are first formed at the more accessible O-3A', but under these reaction conditions (5 equiv donor, 50 °C, weak acidic conditions) they might be easily hydrolyzed, while glycosidic bonds eventually formed at O-3A might be much more stable. The comparison of D vs L donors does not yield much information in this case as both are matched to O-3A.

As all four peracetylated bromide donors were matched to O-3A, it is still possible that the benzylidene is affecting the reactivity at O-3A'. Glycosylations with diacetyl trisaccharide acceptor 2 will determine if that is indeed the case.

3.4 Matched Glycosylations Using Diacetyl Trisaccharide Acceptor 2

3.4.1 With D-Galactosyl Donor 35

Trisaccharide acceptor 2 was glycosylated with D-galactosyl donor 35 (5 equiv), using Hg(CN)₂ (6 equiv) at 50 °C in 1:1 PhMe/CH₃NO₂, giving O-3A β-linked tetrasaccharide 52 in 35% yield, and O-3A’ orthoester pentasaccharide 53 in 6% yield, while also recovering acceptor 1 in 33% yield (Scheme 3.12). The β-glycosidic bond and orthoester were formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 52 (Jₜₜ₁A,H₂A changed from 8.2 Hz to 3.7 Hz, Jₜₜ₁C,H₂C = 7.9 Hz, δₜₜ₁ N-HA shifted downfield from 5.61 ppm to 6.46 ppm, and δₜₜ₂ C-4A shifted upfield from 82.1 ppm to 71.9 ppm) and integration of ring ¹H and acetate CH₃ peaks confirms β-galactosylation at O-3A.

NMR analysis of 53 (Jₜₜ₁A,H₂A = 4.1 Hz, Jₜₜ₁C,H₂C = 7.7 Hz, δₜₜ₁ N-HA 6.50 ppm, H-2C shifted downfield from 5.03 ppm on the donor to 4.32 ppm, an acetate CH₃ shifted downfield to
1.60 ppm, δ_C C-4A 71.7 ppm, C-4A' shifted upfield from 71.3 ppm to 66.1 ppm, an acetate CH₃ shifted upfield to 26.7 ppm, and a 4° C 123.4 ppm) and integration of ring 'H and acetate CH₃ peaks confirms β-galactosylation at O-3A and an orthoester at O-3A'.

3.4.2 With D-Mannosyl Donor 36

Trisaccharide acceptor 2 was glycosylated with D-mannosyl donor 36 (5 equiv), using Hg(CN)₂ (6 equiv) at 50 °C in 1:1 PhMe/CH₃NO₂, giving O-3A α-linked tetrasaccharide 54 in 50% yield (Scheme 3.13). The α-glycosidic bond was selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 54 ($J_{H-1A,H-2A} = 3.3$ Hz, $J_{C-1C,H-1C} = 176$ Hz, $\delta_H$ N-NA 6.42 ppm, $\delta_C$ C-4A 73.8 ppm) and integration of ring $^1H$ and acetate CH₃ peaks confirms α-mannosylation at O-3A.

![Scheme 3.13](image)

**Scheme 3.13.** Matched glycosylation of trisaccharide acceptor 2 and D-mannosyl donor 36, giving O-3A α-linked tetrasaccharide 54.

3.4.3 With L-Rhamnosyl Donor 37

Trisaccharide acceptor 2 was glycosylated with L-rhamnosyl donor 37 (5 equiv), using Hg(CN)₂ (6 equiv) at 50 °C in 1:1 PhMe/CH₃NO₂, giving O-3A α-linked tetrasaccharide 55 in
41% yield, and O-3A/O-3A' α-linked pentasaccharide 56 in 18% yield (Scheme 3.14). The α-glycosidic bonds were selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 55 ($J_{H-1A,H-2A} = \text{bs}$, $J_{C-1C,H-1C} = 175 \text{ Hz}$, $\delta_H \text{ N-HA } 6.44 \text{ ppm}$, $\delta_C \text{ C-4A } 73.4 \text{ ppm}$) and integration of ring $^1\text{H}$ and acetate CH$_3$ peaks confirms α-rhamnosylation at O-3A.$^{86}$

NMR analysis of 56 ($J_{H-1A,H-2A} = \text{bs}$, $J_{C-1C,H-1C} = 176 \text{ Hz}$, $J_{C-1C',H-1C'} = 173 \text{ Hz}$, $\delta_H \text{ N-HA } 6.47 \text{ ppm}$, N-HA' shifted downfield from 7.19 ppm to 7.50-7.41 ppm, H-2C' 5.16 ppm, COCH$_3$ peaks in the regular region, $\delta_C \text{ C-4A } 73.2 \text{ ppm}$, and C-4A' 70.3 ppm) and integration of ring $^1\text{H}$ and acetate CH$_3$ peaks confirms α-rhamnosylation at both O-3A and O-3A', with no orthoester at O-3A.$^{86}$
Scheme 3.14. Matched glycosylation of trisaccharide acceptor 2 and L-rhamnosyl donor 37, giving O-3A α-linked tetrasaccharide 55, and O-3A/O-3A' α-linked pentasaccharide 56.

3.4.4 With L-Fucosyl Donor 38

Trisaccharide acceptor 2 was glycosylated with L-fucosyl donor 38 (5 equiv), using Hg(CN)₂ (6 equiv) at 50 °C in 1:1 PhMe/CH₃NO₂, giving O-3A β-linked tetrasaccharide 57 in 54% yield (Scheme 3.15). The β-glycosidic bond was selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor.
NMR analysis of 57 ($J_{\text{H-1A,H-2A}} = \text{bs}, \delta_{\text{H}} \text{ N-HA} 6.29 \text{ ppm}, \delta_{\text{C}} \text{ C-4A} 72.7 \text{ ppm}$) and integration of ring $^1\text{H}$ and acetate CH$_3$ peaks confirms $\beta$-fucosylation at O-3A.

Scheme 3.15. Matched glycosylation of trisaccharide acceptor 2 and L-fucosyl donor 38, giving O-3A $\beta$-linked tetrasaccharide 57.

3.4.5 With L-Fucosyl Donor 39

Trisaccharide acceptor 2 was glycosylated with L-fucosyl donor 39 (4 equiv), using NIS (5 equiv) and catalytic TMSOTf at 0 °C in CH$_2$Cl$_2$, giving O-3A' $\alpha$-linked tetrasaccharide 58 in 37% yield (Scheme 3.16). The $\alpha$-glycosidic bond was selectively formed due to the anomeric effect.

NMR analysis of 58 ($J_{\text{H-1C',H-2C'}} = 8.2 \text{ Hz}, \delta_{\text{C}} \text{ C-4A'} 69.9 \text{ ppm}$, and no significant change in $J_{\text{H-1A,H-2A}}, \delta_{\text{H}} \text{ N-HA},$ and $\delta_{\text{C}} \text{ C-4A from 2}$), and integration of ring $^1\text{H}$ and benzyl CH$_2$ peaks confirms $\alpha$-fucosylation at O-3A'.

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Scheme 3.16. Matched glycosylation of trisaccharide acceptor 1 and L-fucosyl donor 39, giving O-3A' α-linked tetrasaccharide 58.

3.4.6 Discussion of Results

Based on the reactions of diacetyl acceptor 2 with peracetylated bromide donors 35-38, it once again appears that the peracetylated bromide donors are all matched to O-3A of the acceptor. In fact, it seems that even higher regioselectivity was achieved for O-3A on 2 in comparison to O-3A on 1, as is evident by D-mannosyl donor 36 resulting in only a single O-3A tetrasaccharide in 50% yield (Scheme 3.13), and L-rhamnosyl donor 37 showing a higher ratio of O-3A tetrasaccharide to pentasaccharide (41:18, Scheme 3.14). This indicates that the benzylidene is not playing a role in the reactivity at O-3A', and that the matching of the peracetylated bromide donors to O-3A is being driven by another factor. In addition, the high regioselectivity of donors 35-38 at O-3A suggests that the absolute configuration (D vs L) and glycosidic linkage (α vs β) are not defining factors in the matched pairings. Results from the glycosylations with trisaccharide acceptor 2 are summarized in Table 3.2.
### Table 3.2. Selected NMR data for products of glycosylations with trisaccharide acceptor 2.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Product</th>
<th>$J_{H-1A,H-2A}$ (Hz)</th>
<th>$\delta_H$ N-HA (ppm)</th>
<th>$\delta_H$ H-2C' (ppm)</th>
<th>$\delta_C$ C-4A (ppm)</th>
<th>$\delta_C$ C-4A' (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>2</td>
<td>8.1</td>
<td>5.61</td>
<td>-</td>
<td>82.1</td>
<td>71.3</td>
</tr>
<tr>
<td>35</td>
<td>52</td>
<td>3.7</td>
<td>6.46</td>
<td>-</td>
<td>71.9</td>
<td>71.6</td>
</tr>
<tr>
<td>35</td>
<td>53</td>
<td>4.1</td>
<td>6.50</td>
<td>4.32</td>
<td>71.7</td>
<td>66.1</td>
</tr>
<tr>
<td>36</td>
<td>54</td>
<td>3.3</td>
<td>6.42</td>
<td>-</td>
<td>73.8</td>
<td>71.3</td>
</tr>
<tr>
<td>37</td>
<td>55</td>
<td>bs</td>
<td>6.44</td>
<td>-</td>
<td>73.4</td>
<td>71.4</td>
</tr>
<tr>
<td>37</td>
<td>56</td>
<td>bs</td>
<td>6.47</td>
<td>5.16</td>
<td>73.2</td>
<td>70.3</td>
</tr>
<tr>
<td>38</td>
<td>57</td>
<td>bs</td>
<td>6.29</td>
<td>-</td>
<td>72.7</td>
<td>71.4</td>
</tr>
<tr>
<td>39</td>
<td>58</td>
<td>8.2</td>
<td>5.56</td>
<td>-</td>
<td>82.0</td>
<td>69.9</td>
</tr>
</tbody>
</table>

It is interesting to note that the reaction of 2 with D-galactosyl donor 35 gave an O-3A' orthoester product in 6% yield (Scheme 3.12). While this is a very minor product, it confirms that a donor expected to make a β-glycosidic bond is reacting, in some capacity, at O-3A'. This observation gives additional merit to the kinetic/thermodynamic product theory, where orthoesters might be forming at the more accessible O-3A', but under the reaction conditions they easily fall off. The reaction of 2 with L-fucosyl donor 39 using NIS/TMSOTf activation at 0 °C gave the same O-3A' matched pair as with acceptor 1 (Scheme 3.16). Under these kinetic driven conditions with no orthoester possible (O-2 benzyl group), it is clear that the donor is matched to O-3A'. The potential formation (and resulting instability under acidic conditions) of orthoesters when using O-2 acetylated donors complicates the formation of a kinetic product at O-3A'. In order to investigate what might be happening at O-3A' with the peracetylated donors, we decided to test kinetic conditions (AgOTf activation, 2 equiv donor, 0 °C → RT) under basic conditions (TMU) to stabilize any orthoesters being formed (see section 3.6).
As all peracetylated bromide donors were matched to O-3A for reactions with acceptors 1 and 2, it is still possible that the trichloroacetamido group is affecting the reactivity at O-3A'. Using acetamido trisaccharide acceptor 3 will determine if that is indeed the case.

3.5 Glycosylation Using Acetamido Trisaccharide Acceptor 3

Trisaccharide acceptor 3 was glycosylated with D-galactosyl donor 35 (5 equiv), using Hg(CN)_2 (6 equiv) at 50 °C in 1:1 PhMe/CH_3NO_2, giving O-3A/O-3A' β-linked pentasaccharide 59 in 41% yield (Scheme 3.18). The β-glycosidic bonds were selectively formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 59 (J_{H-1A,H-2A} changed from 8.3 Hz to 3.6 Hz, J_{H-1C,H-2C} = 7.7 Hz, J_{H-1C',H-2C'} = 7.9 Hz, δ H N-HA shifted downfield from 5.59 ppm to 6.32 ppm, N-HA' shifted upfield from 5.96 ppm to 5.56 ppm, H-2C' 5.07-4.97 ppm, COCH_3 peaks in the normal region, δ C C-4A shifted upfield from 82.4 ppm to 71.6 ppm, and C-4A' from 71.0 ppm to 68.7 ppm) and integration of ring ^1H and acetate CH_3 peaks confirms β-galactosylation at both O-3A and O-3A', with no orthoester at O-3A'.

The formation of pentasaccharide 59 as the major product indicates that the conversion of the trichloroacetamido group into the acetamide increased the reactivity at O-3A' significantly. As a result, under these reaction conditions, donor 35 no longer exhibits regioselectivity to O-3A or O-3A' of 3, and reacts at both positions. In addition, no orthoester is isolated, possibly indicating the increased stability of the glycosidic bond at O-3A'.

3.6 Matched Glycosylation Under Basic/Kinetic Conditions

The use of Hg(CN)₂ as an activator creates mild acidic reactions conditions. Under these conditions, orthoesters are unstable and might be hydrolyzed, thus making it seem as if no reaction took place at that reaction site. This is particularly interesting at the O-3A' reaction site.
The isolation of orthesters 45, 46, and 53 gives evidence of orthoester formation at this position. For any particular reaction with a participating donor, if an orthoester is not isolated, that does not mean an orthoester was not formed at that position. It is not surprising that mannosyl orthoesters were isolated in significant yields, but other orthoesters at O-3A' might be quick to hydrolyze and fall off under acidic conditions. As these reactions were completed at 50 °C using 5 equivalents of donor, conditions were ideal for glycosylation at both O-3A' and O-3A. The more accessible O-3A' might be reacting first, but primarily forming an unstable orthoester which falls off, followed by glycosylation at O-3A. This is a possible explanation for the observed regioselectivity of donors 35-38 at O-3A.

To determine if an orthoester is indeed forming at the more accessible O-3A', we turned to basic reaction conditions to stabilize any orthoester formed. At the same time, the conditions were altered to promote formation of a single kinetic product, using only 2 equivalents of donor at lower temperature.

Trisaccharide acceptor 1 was glycosylated with D-galactosyl donor 35 (2 equiv), using AgOTf (2 equiv) and TMU (2.1 equiv) in CH₂Cl₂, starting at 0 °C with natural warming to RT, giving O-3A' β-linked tetrasaccharide 60 in 13% yield, and O-3A' orthoester tetrasaccharide 61 in 11% yield, while also recovering acceptor 1 in 30% yield (Scheme 3.17). The β-glycosidic bond and orthoester were formed due to anchimeric assistance from the O-2 acetyl group on the donor.

NMR analysis of 60 (J₃H₂C₃H₂C = 8.1 Hz, δ₃H H-2C' 5.15-5.08 ppm, COCH₃ peaks in the regular region, δC C-4A' shifted upfield from 81.2 ppm to 78.0 ppm, and no significant change in J₃H₂A₂C₂A, δ₃H N-HA, and δC C-4A from 1), and integration of ring ¹H and acetate CH₃ peaks confirms β-galactosylation at O-3A' (not an orthoester).
NMR analysis of 61 ($J_{\text{H-1C',H-2C'}} = 4.5$ Hz, H-2C 4.20 ppm, CH$_3$ 1.63 ppm, $\delta_{\text{C}}$ C-4A' 79.8 ppm, CH$_3$ 25.6 ppm, $4^\circ$ C 121.7 ppm, and no significant change in $J_{\text{H-1A',H-2A'}}$, $\delta_{\text{H}}$ N-HA, and $\delta_{\text{C}}$ C-4A from 1) and integration of ring $^1$H and acetate CH$_3$ peaks confirms an orthoester at O-3A'.

Scheme 3.18. Matched glycosylation of trisaccharide acceptor 1 and D-galactosyl donor 35 under basic/kinetic conditions, giving O-3A' $\beta$-linked tetrasaccharide 60, and O-3A' orthoester tetrasaccharide 61.

This result, along with the regioselectivity at O-3A' using perbenzylated donor 39, provides evidence that the more accessible O-3A' is the initial reaction site. The instability of orthoesters and labile glycosidic bonds under acidic conditions and increased temperature
provides an explanation as to why few products were isolated with donors 35-38 substituted at O-3A'. Under these reactions conditions, it is no surprise that the donors were able to react at the less accessible O-3A as well, clearly forming a more stable product. It is unknown why orthoesters are forming at O-3A' but not O-3A, although the steric hindrance at O-3A and the trichloroacetamido group might be factors in orthoester/glycosidic bond formation and stability.

3.7 Conclusions and Future Considerations

When considering all of the above results, the most plausible explanation for the observed matched glycosylations and the results reported by Guillemineau⁵⁷ is that, between O-3A and O-3A' trisaccharide acceptors 1-3, O-3A' is the first to react, and substitution at O-3A' is a kinetic product, however use of more forcing conditions (increased temperature/donor or in situ anomerization) eventually leads to substitution at O-3A as well. In addition, the instability of orthoesters under increased temperature and acidic conditions provides an explanation for the lack of substitution at O-3A' when using acceptors 1 and 2 with peracetylated donors 35-38. The trichloroacetamido group appears to limit the reactivity at O-3A' as well, although it is unknown if this is due to a steric, electronic, or another effect.

Given these findings, the arming/disarming nature of the donor protecting groups do not appear to play a significant role in these particular set of matched glycosylations. As well, it is no surprise that the smaller peracetylated donors reacted more frequently at O-3A than the larger perbenzylated donors. One possibility is to replace the bromide donor acetate groups with benzoate groups, and the thioethyl donor benzyl groups with methoxy groups. This would make the disarmed donor much larger than the armed donor, which could provide more insight into the role of protecting group arming/disarming and steric hindrance on matched pairings. Another
possibility is to compete two disjoint monosaccharide acceptors over a single donor, which would eliminate any steric hindrance at O-3A created by the galactosyl residue on trisaccharides 1-3. Ongoing studies in our research group are looking at competing monosaccharide acceptors similar to the reducing and non-reducing end N-acetylglucosamine residues on trisaccharide 1.

3.8 Application: The Synthesis of Dimeric Lewis X Fragments

An application of matched glycosylations at O-3 of N-acetylglucosamine is using them toward the synthesis of tumour-associated carbohydrate antigens (TACAs). In particular, trisaccharides 1-3 are protected linear trisaccharide fragments of the TACA Dimeric Lewis X (DimLe\(^x\)), an antigen first isolated from the surface of colorectal adenocarcinomas by Hakomori and co-workers in 1984.\(^{88}\) DimLe\(^x\) is a hexasaccharide consisting of two Le\(^x\) trisaccharides, each with a D-N-acetylglucosamine residue α-1→3-linked to an L-fucosyl residue, and β-1→4-linked to a D-galactosyl residue. The two trisaccharides are connected by a β-1→3-linkage between the D-galactosyl residue of the reducing end trisaccharide and the D-N-acetylglucosamine residue of the non-reducing end trisaccharide (Figure 3.3).

![Figure 3.3. The structure of the hexasaccharide TACA DimLe\(^x\).\(^{88}\)](image)
This hexasaccharide was first synthetically prepared in the Auzanneau lab by Hendel for use in monoclonal antibody studies toward the synthesis of an anti-cancer vaccine. The synthesis of selected fragments of this hexasaccharide are also of interest. Given that 1-3 are DimLe$^x$ trisaccharides fragments deprotected at O-3 of both N-acetylg glucosamine residues, α-fucosylation at one (or both) of the O-3 positions would give a protected tetra- or pentasaccharide fragment of DimLe$^x$. Tetrasaccharides 50 and 58, and pentasaccharide 51 are indeed protected fragments of DimLe$^x$. While a synthetic strategy to synthesize these fragments might normally involve selective protection/deprotection of O-3A and O-3A' separately to achieve high regioselectivity and high yield, the above matched glycosylations demonstrate the ability to selectively α-fucosylate O-3A', or α-fucosylate both O-3A and O-3A', to achieve either the protected tetra- or pentasaccharide fragment in a single step from the diol, without selective protection/deprotection required. A one-step deprotection of these fragments using Na/NH$_3$(l) would give the deprotected tetra- and pentasaccharide fragments of DimLe$^x$ (Figure 3.4).

![Figure 3.4. Tetra- and pentasaccharide fragments of the TACA DimLe$^x$.](image)

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Chapter 4

Experimental Section
4.1 General Methods

$^1$H NMR (300, 400, or 600 MHz) and $^{13}$C NMR (100 or 150 MHz) spectra were recorded in CDCl$_3$ (calibrated at $\delta_C$ 77.0 ppm, and using residual CHCl$_3$ at $\delta_H$ 7.24 ppm), CD$_3$OD (calibrated at $\delta_C$ 49.0 ppm, and using residual CH$_3$OH at $\delta_H$ 3.30 ppm), and DMSO-d$_6$ (calibrated at $\delta_C$ 39.0 ppm, and using residual DMSO at $\delta_H$ 2.50 ppm). All chemical shifts are reported in parts per million (ppm). All coupling constants ($J$) are reported in hertz (Hz) and were obtained from analysis of $^1$H NMR spectra. Assignments of proton and carbon peaks were made using assistance from 2D COSY and HSQC experiments. Multiplicities are abbreviated as singlet (s), broad singlet (bs), doublet (d), doublet of a doublet (dd), doublet of a doublet of a doublet (ddd), triplet (t), doublet of a triplet (dt), triplet of a doublet (td), quartet (q), doublet of a quartet (dq), quartet of a doublet (qd), quintet (quin), and multiplet (m). Anhydrous solvents were freshly distilled. Organic solutions were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. All products were dried over high vacuum. Molecular sieves were activated by heating at 300 °C under reduced pressure. Zinc powder was activated by successive washings with 2M HCl, H$_2$O, MeOH, and Et$_2$O. Sonication was carried out in a Branson 3510 thermostated ultrasonic bath at 40 kHz. Thin layer chromatography (TLC) was performed on aluminum plates coated with silica gel and charred with 20% H$_2$SO$_4$ in EtOH. Compounds were purified by column chromatography using silica gel unless otherwise stated. Reverse-phase HPLC purifications were carried out on a Prep Nova Pak HR C18, 6 µm 60 Å column using HPLC grade acetonitrile and milli-Q water. Optical rotations were measured at 22 °C on a Rudolph Research Autopol III polarimeter and reported as follows: $[\alpha]_D$ (c in g per 100 mL, solvent). High resolution electrospray ionization mass spectra (HRESIMS) were recorded by the analytical services of the

4.2 Experimental Section for Chapter 2

4.2.1 Synthetic Procedures for the Preparation of Glycosyl Acceptor 7

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride (10)

Sodium (5.33 g, 232 mmol, 1 equiv) was dissolved in MeOH (250 mL), which was then cooled to 0 °C. D-Glucosamine HCl (8) (50.0 g, 232 mmol) was added to the solution. The mixture was stirred at 0 °C for 1.5 min, and then filtered. The precipitate was washed with cold MeOH (2 × 50 mL). Ac₂O was added to the filtrate, and solution was warmed to RT and stirred for 3 h, then stored at -20 °C for 18 h. The mixture was filtered and washed with cold MeOH (2 × 20 mL), giving N-acetylglucosamine (9) as a white powder. The mother liquor was concentrated, and acetone (100 mL) was added to the residue to promote crystallization. The precipitate was filtered to recover additional 9 (total yield 35.68 g, 70%). 9 (35.68 g, 161 mmol) was dissolved in AcCl (70 mL, 984 mmol, 6.1 equiv). The solution was stirred at RT for 18 h, then diluted with CH₂Cl₂ (240 mL) and washed with water (60 mL in 240 g ice) then sat. aq. NaHCO₃ (240 mL in 60 g ice). The organic layer was dried and concentrated. Et₂O (250 mL) was added to the residue to promote crystallization. The mixture was stored in at -20 °C for 18 h, then filtered, which gave a crude mixture of Horton's chloride (10) (28.58 g, 48%) and peracetylated glucosamine (9.13 g, 15%) as purple crystals. ¹H NMR for 10 (CDCl₃, 400 MHz): δH 6.17 (d, J = 3.7 Hz, 1H, H-1),
5.74 (d, $J = 8.7$ Hz, 1H, NH), 5.30 (t, $J = 9.5$ Hz, 1H, H-2), 5.20 (t, $J = 9.8$ Hz, 1H, H-4), 4.52 (ddd, $J = 3.7$, 8.8, 12.5 Hz, 1H, H-2), 4.30-4.20 (m, 2H, H-5, H-6a), 4.12 (m, 1H, H-6b), 2.09, 2.04, 2.04, 1.97 (4 s, 12H, COCH$_3$). The NMR data are in agreement with those reported in the literature.

**Pentyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (11)**

![Chemical Structure](image)

C$_{19}$H$_{31}$O$_5$N
417.45 g/mol

The crude mixture (37.71 g) containing Horton's chloride (10) (28.58 g, 78.1 mmol) was dissolved in anhydrous PhMe (425 mL). Activated 4 Å MS (28.6 g), Hg(CN)$_2$ (29.61 g, 117 mmol, 1.5 equiv), and pentanol (85 mL, 781 mmol, 10 equiv) were added and the mixture was stirred at 55 °C for 6 h. The solution was filtered over Celite and the filtrate washed with sat. aq. NaHCO$_3$ (600 mL), and the aqueous layers re-extracted with CH$_2$Cl$_2$ (2 × 150 mL). The organic layers were dried and concentrated. Column chromatography (EtOAc/hexanes, 6:4 → 9:1) gave pentyl glycoside 11 (23.85 g, 73%) as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz): δH 5.59 (d, $J = 8.0$ Hz, 1H, NH), 5.28 (t, $J = 9.4$ Hz, 1H, H-3), 5.03 (t, $J = 9.6$ Hz, 1H, H-4), 4.65 (d, $J = 8.3$ Hz, 1H, H-1), 4.23 (dd, $J = 4.8$, 12.2 Hz, 1H, H-6a), 4.10 (dd, $J = 2.3$, 12.2 Hz, 1H, H-6b), 3.86-3.74 (m, 2H, H-2, OCHHCH$_2$), 3.67 (qd, $J = 2.4$, 4.7, 9.9 Hz, 1H, H-5), 3.43 (td, $J = 6.8$, 9.6 Hz, 1H, OCHHCH$_2$), 2.05, 2.00, 1.99, 1.92 (4 s, 12H, COCH$_3$), 1.54 (h, 2H, OCH$_2$CH$_2$), 1.31-1.23 (m, 4H, CH$_2$CH$_2$CH$_3$), 0.86 (t, $J = 6.8$ Hz, 3H, CH$_2$H$_3$). The NMR data are in agreement with those reported in the literature.
Pentyl 2-acetamido-4,6-benzylidene-2-deoxy-β-D-glucopyranoside (13)

Pentyl glycoside 11 (23.85 g, 57.1 mmol) was dissolved in MeOH (382 mL). Sodium (1.71 g, 74.3 mmol, 1.3 equiv) was added to the solution, which was stirred at RT for 3.5 h, then deionized with H⁺ resin and filtered. The solids were washed with MeOH (4 × 100 mL). The filtrate was concentrated giving triol 12 (15.82 g, 95%) as an orange solid. 12 (15.82 g, 54.3 mmol) and ZnCl₂ (15.54 g, 114 mmol, 2.1 equiv) were dissolved in PhCHO (137 mL, 1.36 mol, 25 equiv). The solution was stirred at RT for 7.5 h, then poured into 1:1 hexane/cold water (600 mL) and stirred at 0 °C for 1 h to promote crystallization. The precipitate was filtered and washed with water (300 mL), then hexanes (300 mL), then Et₂O (100 mL) giving benzylidene 13 (17.31 g, 84%) as a white solid. ¹H NMR for 13 (CDCl₃, 400 MHz): δH 7.46 (m, 2H, Ph), 7.32 (m, 3H, Ph), 6.35 (s, 1H, NH), 5.43 (s, 1H, CHPh), 4.58 (d, J = 8.3 Hz, 1H, H-1), 4.28 (dd, J = 4.9, 10.4 Hz, 1H, H-6a), 4.07 (t, J = 9.5 Hz, 1H, H-3), 3.83 (td, J = 6.6, 9.6 Hz, 1H, OCH₂HCH₂), 3.73 (t, J = 10.3 Hz, 1H, H-5), 3.59-3.48 (m, 2H, H-2, H-4), 3.48-3.39 (m, 2H, H-6b, OCH₂HCH₂), 2.09 (s, 3H, COCH₃), 1.57 (m, 2H, OCH₂CH₂), 1.34-1.23 (m, 4H, CH₂CH₂CH₃), 0.88 (t, J = 6.8 Hz, 3H, CH₂CH₃). The NMR data are in agreement with those reported in the literature.⁷⁷
Pentyl 2-acetamido-4,6-benzylidene-3-O-chloroacetyl-2-deoxy-β-D-glucopyranoside (14)

![Chemical Structure](image)

Benzylidene 13 (7.46 g, 19.7 mmol) was dissolved in CH₂Cl₂ (200 mL). Pyridine (6.4 mL, 78.6 mmol, 4 equiv) was added, followed by ClAcCl (3.1 mL, 39.3 mmol, 2 equiv). The solution was stirred at RT for 5 min, then diluted with CH₂Cl₂ (100 mL) and washed with 2 M HCl (2 × 200 mL) followed by sat. aq. NaHCO₃ (2 × 200 mL). The aqueous layers were re-extracted with CH₂Cl₂ (2 × 100 mL each), and the organic layers were dried and concentrated giving chloroacetate 14 (8.74 g, 98%) as a yellow solid.¹H NMR (CDCl₃, 300 MHz): δH 7.40 (m, 2H, Ph), 7.30 (m, 3H, Ph), 5.86 (d, J = 9.3 Hz, 1H, NH), 5.48 (s, 1H, CHPh), 5.38 (t, J = 10.1 Hz, 1H, H-3), 4.51 (d, J = 8.3 Hz, 1H, H-1), 4.30 (dd, J = 5.0, 10.5 Hz, 1H, H-6a), 4.10-3.97 (m, 3H, COCH₂Cl, OCHHCH₂), 3.83-3.67 (m, 3H, H-2, H-4, H-5), 3.52 (dd, J = 4.9, 9.8 Hz, 1H, H-6b), 3.37 (td, J = 6.8, 9.6 Hz, 1H, OCHHCH₂), 1.95 (s, 3H, COCH₃), 1.52 (m, 2H, OCH₂CH₂), 1.34-1.21 (m, 4H, CH₂CH₂CH₃), 0.90-0.83 (t, J = 6.8 Hz, 3H, CH₃CH₃). The NMR data are in agreement with those reported in the literature.⁷⁷

Pentyl 2-acetamido-3-O-chloroacetyl-2-deoxy-β-D-glucopyranoside (15)

![Chemical Structure](image)

Chloroacetate 14 (13.00 g, 28.5 mmol) was dissolved in 90% AcOH (233 mL, 4 mol, 143 equiv) in water (26 mL). The solution was stirred at 50 °C for 80 min, then co-concentrated with PhMe (2 × 200 mL). Et₂O (200 mL) was added to the residue to promote crystallization. The precipitate was filtered and washed with Et₂O (2 × 100 mL), giving diol 15 (8.00 g, 76%) as a
white powder. \([\alpha]_D -45.8 \text{ (c 1.0, MeOH)}\). \(^1\)H NMR (DMSO-\(d_6\), 600 MHz): \(\delta_H 7.87 \text{ (d, } J = 9.2 \text{ Hz, 1H, NH)}\), 5.37 \(\text{ (d, } J = 5.7 \text{ Hz, 1H, OH-4)}\), 4.88 \(\text{ (dd, } J = 9.2, 10.4 \text{ Hz, 1H, H-3)}\), 4.63 \(\text{ (t, } J = 5.7 \text{ Hz, 1H, OH-6)}\), 4.44 \(\text{ (d, } J = 8.4 \text{ Hz, 1H, H-1)}\), 4.33 \(\text{ (d, } J = 15.2 \text{ Hz, 1H, COCHHCl)}\), 4.21 \(\text{ (d, } J = 15.2 \text{ Hz, 1H, COCHHCl)}\), 3.72 \(\text{ (td, } J = 6.4, 9.8 \text{ Hz, 1H, OCHHCH}_2\), 3.68 \(\text{ (dd, } J = 3.6, 11.8 \text{ Hz, 1H, H-6a)}\), 3.60 \(\text{ (dd, } J = 9.0, 19.1 \text{ Hz, 1H, H-2)}\), 3.50 \(\text{ (dd, } J = 5.9, 12.0 \text{ Hz, 1H, H-6b)}\), 3.41-3.32 \(\text{ (m, 2H, OCHHCH}_2\), 3.21 \(\text{ (ddd, } J = 1.8, 5.3, 9.6 \text{ Hz, 1H, H-5)}\), 1.73 \(\text{ (s, 3H, COCH}_3\), 1.46 \(\text{ (quin, } J = 6.8 \text{ Hz, 2H, OCH}_2CH_2\), 1.30-1.20 \(\text{ (m, 4H, CH}_2CH_2CH_3\), 0.85 \(\text{ (t, } J = 6.9 \text{ Hz, 3H, CH}_2CH_3\). \(^{13}\)C NMR (DMSO-\(d_6\), 150 MHz) \(\delta_C 169.0, 167.0 \text{ (2 C=O)}\), 100.3 \(\text{ (C-1)}\), 78.3 \(\text{ (C-3)}\), 76.4 \(\text{ (C-5)}\), 68.5 \(\text{ (OCH}_2CH_2\), 67.7 \(\text{ (C-4)}\), 60.4 \(\text{ (C-6)}\), 53.1 \(\text{ (C-2)}\), 41.2 \(\text{ (COCH}_2\text{Cl)}\), 28.6, 27.6, 21.8 \(\text{ (CH}_2CH_2CH_2CH_3\), 22.3 \(\text{ (COCH}_3\), 14.0 \(\text{ (CH}_2CH_3\). HRESIMS calculated for C\(_{15}\)H\(_{26}\)O\(_7\)NClNa [M+Na]^+ 390.1295, found 390.1309. 

4.2.2 Synthetic Procedures for the Preparation of Galactosyl Donor 6

**Pentyl 2-acetamido-6-O-benzoyl-3-O-chloroacetyl-2-deoxy-\(\beta\)-D-glucopyranoside (7)**

Diol **15** (8.00 g, 21.7 mmol) was dissolved in anhydrous CH\(_2\)Cl\(_2\) (350 mL). Collidine (8.6 mL, 65.2 mmol, 3 equiv) was added, and the solution was stirred at RT under N\(_2\) for 1 h. Benzoyl chloride (2.8 mL, 23.9 mmol, 1.1 equiv) was added, and the stirring continued at RT under N\(_2\) for 19 h. The solution was diluted with CH\(_2\)Cl\(_2\) (500 mL), then washed with 2 M HCl (500 mL), followed by sat. aq. NaHCO\(_3\) (500 mL). The aqueous layers were re-extracted with CH\(_2\)Cl\(_2\) (2 \(\times\) 100 mL each), and the organic layers were dried and concentrated giving acceptor **7** (5.28 g,
51% as a light brown powder. $[\alpha]_D^{22.6} -22.6$ (c 0.8136, MeOH). $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta_H$ 7.98 (d, $J = 7.0$ Hz, 2H, Bz), 7.93 (d, $J = 9.2$ Hz, 1H, NH), 7.67 (t, $J = 7.4$ Hz, 1H, Bz), 7.54 (t, $J = 7.9$ Hz, 2H, Bz), 5.94 (dd, $J = 9.0, 10.4$ Hz, 1H, H-3), 4.52 (d, $J = 8.5$ Hz, 2H, H-1, H-6a), 4.40 (dd, $J = 5.4, 12.0$ Hz, 1H, H-6b), 4.36, 4.23 (d, $J = 15.2$ Hz, 2H, CH$_2$), 3.72-3.58 (m, 3H, H-2, H-5, OCH/HCH$_2$), 3.54 (t, $J = 13.4$ Hz, 1H, H-4), 3.40 (td, $J = 6.6, 9.9$ Hz, 1H, OCH/HCH$_2$), 1.73 (s, 3H, COCH$_3$), 1.41 (quin, $J = 6.9$ Hz, 2H, OCH$_2$CH$_2$), 1.23-1.12 (m, 4H, CH$_2$CH$_2$CH$_3$), 0.78 (t, $J = 6.8$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta_C$ 169.1, 167.0, 165.6 (3 C=O), 133.5 (Bz), 129.6 (4° Bz), 129.2 (2 Bz), 128.9 (2 Bz), 100.4 (C-1), 77.8 (C-3), 73.1 (C-5), 68.8 (OCH$_2$CH$_2$), 68.0 (C-4), 63.6 (C-6), 53.0 (C-2), 41.2 (COCH$_2$Cl), 28.7, 27.5, 21.8 (CH$_2$CH$_2$CH$_2$CH$_3$), 22.7 (COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{22}$H$_{30}$O$_8$NClH $[M+H]^+$ 472.1738, found 472.1726.

**p-Tolyl 2,3,4,6-tetra-O-acetyl-β-1-thio-D-galactopyranoside (18)**

D-Galactose (16) (40.0 g, 222 mmol) was dissolved in a mixture of pyridine (200 mL, 2.12 mol, 9.5 equiv) and Ac$_2$O (200 mL, 2.47 mol, 11.1 equiv). The solution was stirred at 50 °C for 4 h, then co-concentrated with PhMe (6 × 100 mL). The residue was dissolved in CH$_2$Cl$_2$ (500 mL), then washed with 2 M HCl (2 × 500 mL), followed by sat. aq. NaHCO$_3$ (2 × 500 mL). The aqueous layers were re-extracted with CH$_2$Cl$_2$ (100 mL each), and the organic layers were dried and concentrated giving peracetylated galactose (17) (82.07 g, 95%) as a clear gel. 17 (82.07, 210 mmol) and 4-thiocresol (52.23 g, 420 mmol, 3 equiv) were dissolved in anhydrous CH$_2$Cl$_2$ (450 mL). BF$_3$•Et$_2$O (133 mL, 1.05 mol, 5 equiv) was added, and the solution was stirred at RT
under N₂ for 18 h. The solution was diluted with CH₂Cl₂ (200 mL), then washed with sat. aq. NaHCO₃ (2 × 500 mL). The organic layers were dried and concentrated. The residue was dissolved in 1:1 boiling EtOAc/hexanes (120 mL) and then stored at -20 °C to promote recrystallization. The precipitate was filtered, then washed with 1:1 cold EtOAc/hexanes (2 × 50 mL), giving thiogalactoside 18 as a white solid. The mother liquor was concentrated, and the recrystallization process was repeated to recover additional 18 (total yield 47.82 g, 50%). ¹H NMR for 18 (CDCl₃, 400 MHz): δ H 7.39 (d, J = 8.1 Hz, 2H, Ar), 7.10 (d, J = 7.9 Hz, 2H, Ar), 5.39 (dd, J = 0.8, 3.3 Hz, 1H, H-4), 5.20 (t, J = 10.0 Hz, 1H, H-2), 5.01 (DD, J = 3.3, 9.9 Hz, 1H, H-3), 4.62 (d, J = 10.0 Hz, 1H, H-1), 4.15 (dd, J = 6.9, 11.3 Hz, 1H, H-6a), 4.08 (dd, J = 6.3, 11.3 Hz, 1H, H-6b), 3.89 (t, J = 7.2 Hz, 1H, H-5), 2.33 (s, 3H, COCH₃). The NMR data are in agreement with those reported in the literature.¹⁰

**p-Tolyl β-1-thio-D-galactopyranoside (19)**

Sodium (3.14 g, 137 mmol, 1.3 equiv) was dissolved in MeOH (100 mL). This solution was added to a mixture of thiogalactoside 18 (47.82 g, 105 mmol) in MeOH (500 ml). The solution was stirred at RT for 2 h, then deionized with H⁺ resin and filtered. The solids were washed with MeOH (5 × 75 mL). The filtrate was concentrated giving deprotected thiogalactoside 19 (30.48 g, quant.) as yellow powder. ¹H NMR (CD₃OD, 400 MHz): δ H 7.44 (d, J = 8.1 Hz, 2H, Ar), 7.10 (d, J = 7.8 Hz, 2H, Ar), 4.49 (d, J = 9.6 Hz, 1H, H-1), 3.88 (d, J = 3.2 Hz, 1H, H-4), 3.74 (dd, J = 6.8, 11.4 Hz, 1H, H-6a), 3.69 (dd, J = 5.3, 11.4 Hz, 1H, H-6b), 3.60-3.44 (m, 3H, H-2, H-3, H-5), 2.30 (s, 3H, ArCH₃). The NMR data are in agreement with those reported in the literature.¹⁰
Deprotected thiogalactoside 19 (30.48 g, 106 mmol) was dissolved in anhydrous CH₂Cl₂ (500 mL). Collidine (56 mL, 426 mmol, 4 equiv) was added, and the solution was stirred at RT under N₂ for 30 min. Pivaloyl chloride (13.8 mL, 112 mmol, 1.05 equiv) was added, and the stirring continued at RT under N₂ for 18 h. More pivaloyl chloride (2.6 mL, 21.3 mmol, 0.2 equiv) was added, and the stirring continued at RT under N₂ for 6 h. More pivaloyl chloride (2.0 mL, 16.0 mmol, 0.15 equiv) was added, and the stirring continued at RT under N₂ for 18 h. More pivaloyl chloride (1.3 mL, 10.6 mmol, 0.1 equiv) was added, and the stirring continued at RT under N₂ for 4 h. The solution was washed with 2 M HCl (2 × 400 mL). The aqueous layers were re-extracted with CH₂Cl₂ (4 × 100 mL), and the organic layers were dried and concentrated, giving a crude precipitate, which was re-crystallized in 95:5 Et₂O/EtOH (200 mL), filtered, and washed with in 95:5 Et₂O/EtOH (50 mL), then 9:1 Et₂O/H₂O (50 mL), then Et₂O (50 mL), giving pivaloate 20 as a white powder. The mother liquor was purified by column chromatography (CH₂Cl₂/MeOH, 20:1) to recover additional 20 (total yield 27.21 g, 69%). ¹H NMR (CDCl₃, 400 MHz): δH 7.42 (d, J = 8.1 Hz, 2H, Ar), 7.09 (d, J = 7.9 Hz, 2H, Ar), 4.43 (d, J = 9.5 Hz, 1H, H-1), 4.48 (dd, J = 6.3, 11.5 Hz, 1H, H-6a), 4.23 (dd, J = 6.6, 11.5 Hz, 1H, H-6b), 3.86 (dd, J = 1.2, 2.7 Hz, 1H, H-4), 3.68 (dt, J = 1.0, 6.5 Hz, 1H, H-5), 3.63-3.58 (m, 2H, H-2, H-3), 2.31 (s, 3H, ArCH₃), 1.18 (s, 9H, C(CH₃)₃). The NMR data are in agreement with those reported in the literature.³¹
p-Tolyl 2,4-di-O-acetyl-6-O-pivaloyl-β-1-thio-D-galactopyranoside (21)

![Chemical Structure]

Pivaloate 20 (27.21 g, 73.4 mmol) and CSA (1.36 g, 5.9 mmol, 0.08 equiv) were dissolved in anhydrous CH$_3$CN (500 mL). CH$_3$C(OEt)$_3$ (54 mL, 294 mmol, 4 equiv) was added, and the solution was stirred at RT under N$_2$ for 90 min. A mixture of pyridine (237 mL, 2.94 mol, 40 equiv) and Ac$_2$O (139 mL, 1.47 mol, 20 equiv) was added, and the stirring continued at RT under N$_2$ for 16 h. The solution was filtered, and the filtrate was co-concentrated with PhMe (5 × 100 mL). The residue was diluted with CH$_2$Cl$_2$ (200 mL), then washed with 2 M HCl (200 mL). The aqueous layer was re-extracted with CH$_2$Cl$_2$ (50 mL), and the organic layers were dried and concentrated. The residue was dissolved in 80% AcOH (200 mL, 3.49 mol, 48 equiv) in water (50 mL). The solution was stirred at RT for 1 h, then diluted with CH$_2$Cl$_2$ (300 mL), washed with sat. aq. NaHCO$_3$ (2 × 500 mL), then with 2 M HCl (2 × 500 mL). The aqueous layers were re-extracted with CH$_2$Cl$_2$ (2 × 100 mL each), and the organic layers were dried and concentrated. Et$_2$O (100 mL) was added to the residue to promote crystallization. The precipitate was filtered, giving diacetate 21 as a white powder. The mother liquor was purified by column chromatography (EtOAc/hexanes, 3:7 → 4:6) to recover additional 21 (total yield 20.91 g, 63%).

$^1$H NMR (CDCl$_3$, 400 MHz): δ$_H$ 7.39 (d, $J = 8.1$ Hz, 2H, Ar), 7.09 (d, $J = 7.9$ Hz, 2H, Ar), 5.31 (dd, $J = 0.8$, 3.5 Hz, 1H, H-4), 4.97 (t, $J = 9.8$ Hz, 1H, H-2), 4.61 (d, $J = 10.0$ Hz, 1H, H-1), 4.20 (d, $J = 6.7$ Hz, 2H, H-6a, H-6b), 3.88-3.81 (m, 2H, H-3, H-5), 2.31 (s, 3H, ArCH$_3$), 2.16, 2.13 (2 s, 6H, COCH$_3$), 1.16 (s, 9H, C(CH$_3$)$_3$). The NMR data are in agreement with those reported in the literature.$^{72}$
**p-Tolyl 2,4-di-O-acetyl-3-O-chloroacetyl-6-O-pivaloyl-β-1-thio-D-galactopyranoside (22)**

Diacetate 21 (10.82 g, 23.8 mmol) was dissolved in CH₂Cl₂ (230 mL). Pyridine (7.7 mL, 95.2 mmol, 4 equiv) was added, followed by ClAcCl (3.8 mL, 47.6 mmol, 2 equiv). The solution was stirred at RT for 20 min, then diluted with CH₂Cl₂ (75 mL) and washed with 2 M HCl (2 × 150 mL) followed by sat. aq. NaHCO₃ (2 × 150 mL). The aqueous layers were re-extracted with CH₂Cl₂ (2 × 30 mL each), and the organic layers were dried and concentrated giving chloroacetate 22 (13.20 g, quant.) as a yellow solid.¹ H NMR (CDCl₃, 400 MHz): δH 7.39 (d, J = 8.1 Hz, 2H, Ar), 7.10 (d, J = 7.9 Hz, 2H, Ar), 5.37 (dd, J = 0.9, 3.3 Hz, 1H, H-4), 5.23 (t, J = 10.0 Hz, 1H, H-2), 5.09 (dd, J = 3.3, 9.9 Hz, 1H, H-3), 4.67 (d, J = 9.9 Hz, 1H, H-1), 4.16 (dd, J = 6.9, 11.3 Hz, 1H, H-6a), 4.10 (dd, J = 6.6, 11.3 Hz, 1H, H-6b), 3.95-3.89 (m, 3H, COCH₂Cl), 2.32 (s, 3H, ArCH₃), 2.10, 2.09 (2 s, 6H, COCH₃), 1.15 (s, 9H, C(CH₃)₃). The NMR data are in agreement with those reported in the literature.⁷²

**1,4-Di-O-acetyl-3-O-chloroacetyl-6-O-pivaloyl-α-D-galactopyranose (23)**

Chloroacetate 22 (13.20 g, 24.9 mmol) was dissolved in a mixture of CH₃CN (200 mL) and H₂O (3.25 mL). NIS (6.34 g, 27.3 mmol, 1.1 equiv) was added, followed by TfOH (220 µL, 2.5 mmol, 0.1 equiv). The solution was stirred at RT for 20 min, then quenched with NEt₃, and concentrated. The residue was diluted with CH₂Cl₂ (150 mL), then washed with 20% Na₂S₂O₃ (200 mL). The aqueous layer was re-extracted with CH₂Cl₂ (2 × 50 mL), and the organic layers
were dried and concentrated. Column chromatography (EtOAc/hexanes, 3:7 → 4:6) gave a mixture of alcohol 23 and hemiacetal 24 (total yield 7.77 g, 74%) as a yellow solid. $^1$H NMR for 23 (CDCl₃, 400 MHz): δH 6.30 (d, J = 3.8 Hz, 1H, H-1), 5.42 (dd, J = 1.4, 3.4 Hz, 1H, H-4), 5.09 (dd, J = 3.3, 10.5 Hz, 1H, H-3), 4.27 (t, J = 7.5 Hz, 1H, H-5), 4.20 (ddd, J = 3.9, 7.9, 11.6 Hz, 1H, H-2), 4.13-4.03 (m, 4H, H-6a, H-6b, COCH₂Cl), 2.17, 2.12 (2 s, 6H, COCH₃), 1.90 (d, J = 7.9 Hz, 1H, OH-2), 1.14 (s, 9H, C(CH₃)₃). The NMR data are in agreement with those reported in the literature.²²

2.4-Di-O-acetyl-3-O-chloroacetyl-6-O-pivaloyl-1-O-trichloracetimidate-α-D-galactopyranose (6)

Alcohol 23 and hemiacetal 24 (7.00 g, 16.5 mmol) were dissolved in anhydrous CH₂Cl₂ (185 mL). Cl₃CCN (5.0 mL, 49.4 mmol, 3 equiv) was added, followed by DBU (0.62 mL, 4.1 mmol, 0.25 equiv). The solution was stirred at RT under N₂ for 4 h, and then was concentrated. Column chromatography (EtOAc/hexanes, 6:4, using 0.1% NEt₃) gave galactosyl donor 6 (8.80 g, 94%) as a yellow/light brown solid. $^1$H NMR (CDCl₃, 400 MHz): δH 8.65 (s, 1H, C=NH), 6.56 (d, J = 3.6 Hz, 1H, H-1), 5.53 (dd, J = 1.2, 3.2 Hz, 1H, H-4), 5.48 (dd, J = 3.2, 10.7 Hz, 1H, H-3), 5.38 (dd, J = 3.6, 10.6 Hz, 1H, H-2), 4.43 (t, J = 6.1 Hz, 1H, H-5), 4.11 (d, J = 6.7 Hz, 2H, H-6a, H-6b), 3.97 (s, 2H, COCH₂Cl), 2.15, 2.01 (2 s, 6H, COCH₃), 1.13 (s, 9H, C(CH₃)₃). The NMR data are in agreement with those reported in the literature.²²
4.2.3 Synthetic Procedures for the Preparation of \( N \)-Acetylglucosamine Donor 5

4,6-Benzylidene-2-deoxy-2-trichloroacetamido-D-glucopyranose (25)

D-Glucosamine HCl (8) (7.20 g, 33 mmol) was dissolved in anhydrous MeOH (360 mL). NEt\(_3\) (28 mL, 200 mmol, 6 equiv) was added, followed by Cl\(_3\)COCl (7.5 mL, 66.8 mmol, 2 equiv). The solution was stirred at RT under N\(_2\) for 48 h. The solution was co-concentrated with PhMe. The precipitate and ZnCl\(_2\) (10.92 g, 80.1 mmol, 2.4 equiv) were dissolved in PhCHO (84 mL, 835 mmol, 25 equiv), and the solution was stirred at RT for 18 h. A mixture of cold 2:1 hexanes/Et\(_2\)O (450 mL) was added and the solution was stirred at 0 °C for 2 h to promote crystallization. The mixture was filtered and washed with cold H\(_2\)O (2 × 100 mL), then cold hexanes (2 × 200 mL), then Et\(_2\)O (2 × 200 mL). The crude product was re-crystallized in the minimal amount of hot EtOH and stored at -20 °C for 18 h. The mixture was filtered and washed with cold EtOH (60 mL), giving benzylidene 25 as a white powder. The mother liquor was concentrated and the re-crystallization process repeated to recover additional 25 (total yield 7.96 g, 57%, \( \alpha/\beta \) ratio 5:1). \(^1\)H NMR (DMSO-\( d_6\), 400 MHz): \( \delta \)H 8.75 (d, \( J = 9.6 \) Hz, 1H, NH\( \beta \)), 8.54 (d, \( J = 7.9 \) Hz, 1H, NH\( \alpha \)), 7.50-7.43 (m, 2H, Ph), 7.42-7.34 (m, 3H, Ph), 7.02 (d, \( J = 4.5 \) Hz, 1H, OH-1\( \alpha \)), 7.01 (d, \( J = 6.8 \) Hz, 1H, OH-1\( \beta \)), 5.62 (s, 2H, \( CH\)Ph\( \alpha \), \( CH\)Ph\( \beta \)), 5.39 (d, \( J = 5.5 \) Hz, 1H, OH-3\( \beta \)), 5.23 (d, \( J = 6.2 \) Hz, 1H, OH-3\( \alpha \)), 5.11 (t, \( J = 3.9 \) Hz, 1H, H-1\( \alpha \)), 4.79 (t, \( J = 7.2 \) Hz, 1H, H-1\( \beta \)), 4.20 (dd, \( J = 4.4, 9.7 \) Hz, 1H, H-6a\( \beta \)), 4.13 (dd, \( J = 4.6, 9.7 \) Hz, 1H, H-6a\( \alpha \)), 4.00 (td, \( J = 6.2, 9.6 \) Hz, 1H, H-3\( \alpha \)), 3.91-3.82 (m, 2H, H-5\( \alpha \), H-3\( \beta \)), 3.80-3.68 (m, 3H, H-2\( \alpha \), H-6b\( \alpha \), H-6b\( \beta \)), 3.57-3.49 (m, 3H, H-2\( \beta \), H-6a\( \beta \), H-6a\( \alpha \)), 3.28 (s, 3H, OCH\( 3 \)), 2.11-2.01 (m, 2H, H-5\( \beta \), H-6\( \beta \)), 1.98 (s, 3H, OCH\( 3 \)).
3.60-3.42 (m, 2H, H-2β, H-4β), 3.50 (t, $J = 9.3$ Hz, 1H, H-4α), 3.30 (m, 1H, H-5β). The NMR data are in agreement with those reported in the literature.\textsuperscript{31}

\textbf{4,6-Benzyldene-2-deoxy-1,3-di-O-chloroacetyl-2-trichloracetamido-D-glucopyranose (26)}

Benzylidene 25 (7.96 g, 19.3 mmol) was dissolved in CH$_2$Cl$_2$ (120 mL). Pyridine (9.4 mL, 116 mmol, 6 equiv) was added, followed by ClAcCl (4.6 mL, 57.9 mmol, 3 equiv). The solution was stirred at RT for 15 min, then diluted with CH$_2$Cl$_2$ (25 mL) and washed with 2 M HCl (2 × 100 mL) followed by sat. aq. NaHCO$_3$ (2 × 100 mL). The aqueous layers were re-extracted with CH$_2$Cl$_2$ (2 × 30 mL each), and the organic layers were dried and concentrated giving dichloroacetate 26 (11.79 g, quant., α/β ratio 4:1) as a brown solid. $^1$H NMR (CDCl$_3$, 600 MHz):

$\delta$H 7.45-7.40 (m, 2H, Ph), 7.38-7.34 (m, 3H, Ph), 6.93 (d, $J = 9.3$ Hz, 1H, NHβ), 6.89 (d, $J = 8.6$ Hz, 1H, NHα), 6.32 (d, $J = 3.8$ Hz, 1H, H-1α), 5.92 (d, $J = 8.7$ Hz, 1H, H-1β), 5.55 (s, 1H, CHPHα), 5.52 (s, 1H, CHPHβ), 5.51 (t, $J = 10.2$ Hz, 1H, H-3α), 5.45 (t, $J = 10.3$ Hz, 1H, H-3β), 4.45 (ddd, $J = 4.9$, 6.8, 8.6 Hz, 1H, H-2α), 4.33 (dd, $J = 4.9$, 10.6 Hz, 2H, H-2β, H-6aα), 4.16 (d, $J = 14.5$ Hz, 1H, COCHHCl), 4.11 (d, $J = 14.5$ Hz, 1H, COCHHCl), 4.10 (d, $J = 15.3$ Hz, 1H, COCHHCl), 4.05 (d, $J = 15.3$ Hz, 1H, COCHHCl), 3.99 (dt, $J = 4.9$, 9.8 Hz, 2H, H-5α, H-6aβ), 3.89 (t, $J = 9.7$ Hz, 1H, H-4α), 3.85-3.78 (m, 3H, H-6bα, H-4β, H-6bβ), 3.68 (dt, $J = 4.9$, 9.7 Hz, 1H, H-5β). The NMR data are in agreement with those reported in the literature.\textsuperscript{31}
4,6-Benzylidene-2-deoxy-3-O-chloroacetyl-2-trichloroacetamido-1-O-trichloroacetimidate-\(\alpha\)-D-glucopyranose (5) & 4,6-benzylidene-2-deoxy-2-trichloroacetamido-1,3-di-O-trichloroacetimidate-\(\alpha\)-D-glucopyranose (28)

\[
\begin{align*}
\text{C}_{19}H_{17}O_2N_2Cl_7 & \quad 633.52 \text{ g/mol} \\
\text{C}_{19}H_{16}O_6N_3Cl_9 & \quad 701.42 \text{ g/mol}
\end{align*}
\]

Dichloroacetate 26 (9.40 g, 16.6 mmol) was dissolved in anhydrous THF (300 mL). BnNH\(_2\) (1.8 mL, 16.6 mmol, 1 equiv) was added, and the solution was stirred at RT under N\(_2\) for 19 h. The solution was poured into H\(_2\)O (200 mL) then extracted with CH\(_2\)Cl\(_2\) (4 \(\times\) 100 mL). Column chromatography (PhMe/EtOAc/, 5:1) gave a mixture of hemiacetal 27 (2.54 g, 31%), 1,3-diol 25 (0.90 g, 26%), and the N-chloroacetyl benzylamine by-product (2.50 g, 83%) as a brown gel (total yield 5.94 g). The mixture containing 27 (2.54 g, 5.2 mmol, 1 equiv) and 25 (0.90 g, 2.2 mmol, 0.42 equiv) was dissolved in anhydrous CH\(_2\)Cl\(_2\) (150 mL). Cl\(_3\)CCN (7.0 mL, 69.9 mmol, 13.4 equiv) was added, followed by DBU (0.52 mL, 3.5 mmol, 0.67 equiv). The solution was stirred at 0 °C under N\(_2\) for 4 h, and then was warmed to RT and stirred under N\(_2\) for an additional 2.5 h. The solution was concentrated. Column chromatography (EtOAc/hexanes, 2:8 \(\rightarrow\) 4:6, using 0.1% NEt\(_3\)) gave N-acetylglucosamine donor 5 (2.18 g, 66% from 27) as a yellow solid, and ditrichloroacetimidate 28 (1.02 g, 67% from 25) as a yellow solid.

\(^{1}\)H NMR for 5 (CDCl\(_3\), 600 MHz): \(\delta_H\) 8.82 (s, 1H, C=NH), 7.43 (m, 2H, Ph), 7.36 (m, 3H, Ph), 6.98 (d, \(J = 9.0\) Hz, 1H, NH), 6.45 (d, \(J = 3.8\) Hz, 1H, H-1), 5.58 (t, \(J = 10.1\) Hz, 1H, H-3), 5.57 (s, 1H, CHPh), 4.52 (ddd, \(J = 3.8, 8.9, 12.7\) Hz, 1H, H-2), 4.37 (dd, \(J = 4.9, 10.6\) Hz, 1H, H-6a),
4.13-4.04 (m, 3H, H-5, COCH₂Cl), 3.93 (t, J = 9.7 Hz, 1H, H-4), 3.83 (dd, J = 4.9, 10.6 Hz, 1H, H-6b). The NMR data are in agreement with those reported in the literature.¹³¹
¹H NMR for 28 (CDCl₃, 600 MHz): δ_H 8.75, 8.58 (2 s, 2H, C=NH), 7.84 (d, J = 7.3 Hz, 1H, NH), 7.44 (m, 2H, Ph), 7.32 (m, 3H, Ph), 6.58 (d, J = 3.6 Hz, 1H, H-1), 5.79 (t, J = 10.5 Hz, 1H, H-3), 5.67 (s, 1H, CHPh), 4.53 (ddd, J = 3.5, 7.3, 14.0 Hz, 1H, H-2), 4.41 (dd, J = 4.4, 10.4 Hz, 1H, H-6a), 4.14-4.08 (m, 2H, H-4, H-5), 3.97 (t, J = 10.3 Hz, 1H, H-6b). The NMR data are in agreement with those reported in the literature.¹³¹

4.2.4 Synthetic Procedures for the Preparation of Disaccharide Acceptor 30

2,4-Di-O-acetyl-3-O-chloroacetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl acetamido-3-O-chloroacetyl-2-deoxy-β-D-glucopyranoside (29)

Acceptor 7 (1.00 g, 2.1 mmol) and galactosyl donor 6 (4.82 g, 8.5 mmol, 4 equiv) were dissolved in anhydrous CH₂Cl₂ (100 mL). The solution was warmed to 40 °C. BF₃•Et₂O (0.54 mL, 4.2 mmol, 2 equiv) was added, and the solution was stirred at 40 °C for 2 h, then quenched with NEt₃. The solution was dilute with CH₂Cl₂ (50 mL), then washed with sat. aq. NaHCO₃ (150 mL). The aqueous layer was re-extracted with CH₂Cl₂ (3 × 50 mL), and the organic layers were dried and concentrated. Column chromatography (EtOAc/hexanes, 3:7 → 6:4) gave disaccharide 29 (1.20 g, 65%) as a light brown solid. [α]D +9.4 (c 1.0, MeOH). ¹H NMR (CDCl₃, 600 MHz): δ_H 8.02 (d, J = 7.1 Hz, 2H, Bz), 7.60 (t, J = 7.4 Hz, 1H, Bz), 7.48 (t, J = 8.0 Hz, 2H, Bz), 5.67 (d, J = 9.2 Hz, 1H, NH), 5.26 (d, J = 3.3 Hz, 1H, H-4'), 5.23 (dd, J = 8.5, 9.8 Hz, 1H, H-3), 5.09 (dd,
J = 7.9, 10.3 Hz, 1H, H-2'), 4.93 (dd, J = 3.5, 10.4 Hz, 1H, H-3'), 4.70 (dd, J = 2.5, 11.9 Hz, 1H, H-6a), 4.55 (d, J = 7.7 Hz, 1H, H-1), 4.51 (d, J = 7.9 Hz, 1H, H-1'), 4.36 (dd, J = 4.9, 11.9 Hz, 1H, H-6b), 4.14-4.03 (m, 4H, H-6a', H-6b', COCH₂Cl), 3.97 (dd, J = 3.5, 10.4 Hz, 1H, H-3), 4.93 (dd, J = 3.5, 10.4 Hz, 1H, H-3').

13C NMR (CDCl₃, 150 MHz) δC 177.8, 170.3, 170.3, 169.3, 167.2, 166.5, 165.9 (7 C=O), 133.5 (Bz), 129.6 (2 Bz), 129.5 (4° Bz), 128.7 (2 Bz), 100.8 (C-1), 100.7 (C-1'), 75.5 (C-4), 74.1 (C-3), 72.5 (C-3', C-5), 70.7 (C-5'), 69.9 (OCH₂CH₂), 68.8 (C-2'), 66.3 (C-4'), 62.6 (C-6), 60.5 (C-6'), 53.5 (C-2), 40.7, 40.3 (2 COCH₂Cl), 38.7 (C(CH₃)₃), 29.0, 28.0, 22.3 (CH₂CH₂CH₂CH₃), 27.0 (C(CH₃)₃), 23.3, 20.6, 20.6 (3 COCH₃), 14.0 (CH₂CH₃). HRESIMS calculated for C₃₉H₅₉O₁₇NCl₂H [M+H]+ 878.2769, found 878.2750.

**2,4-Di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-3-O-chloroacetyl-2-deoxy-β-D-glucopyranoside (30)**

Method A: Disaccharide 29 (500 mg, 0.57 mmol) and thiourea (52 mg, 0.68 mmol, 1.2 equiv) were dissolved in a mixture of 2:1 pyridine/EtOH (15 mL). The solution was stirred at 55 °C for 3.5 h, then cooled to RT and diluted with CHCl₃ (150 mL), then washed with 2 M HCl (150 mL). The aqueous layer was re-extracted with CHCl₃ (5 × 25 mL), and the organic layers were dried and concentrated. Column chromatography (CH₂Cl₂/MeOH, 50:1 → 20:1) gave disaccharide acceptor 30 (235 mg, 52%) as a yellow solid.
**Method B:** Trimethylsilyl disaccharide 31 (354 mg, 0.41 mmol) was dissolved in MeOH (35 mL). H⁺ ion-exchange resin (2.36 g) was added, and the mixture was stirred at RT for 90 min, then filtered and washed with MeOH (5 × 30 mL). The filtrate was concentrated, giving disaccharide acceptor 30 (321 mg, quant.) as a yellow solid.

[α]D +4.5 (c 1.0, MeOH). ¹H NMR (CDCl₃, 600 MHz): δH 8.00 (d, J = 7.3 Hz, 2H, Bz), 7.58 (t, J = 7.4 Hz, 1H, Bz), 7.46 (t, J = 7.7 Hz, 2H, Bz), 5.82 (d, J = 9.2 Hz, 1H, NH), 5.22 (dd, J = 8.8, 10.0 Hz, 1H, H-3), 5.19 (d, J = 3.4 Hz, 1H, H-4'), 4.82 (dd, J = 8.0, 9.8 Hz, 1H, H-2'), 4.70 (dd, J = 2.0, 11.9 Hz, 1H, H-6a), 4.55 (d, J = 8.0 Hz, 1H, H-1), 4.42 (d, J = 7.9 Hz, 1H, H-1'), 4.39 (dd, J = 4.9, 12.0 Hz, 1H, H-6b), 4.16-4.02 (m, 4H, H-6a', H-6b', COCH₂Cl), 3.95 (dd, J = 9.2, 17.9 Hz, 1H, H-2), 3.84-3.77 (m, 2H, H-4, OCH₂HCH₂), 3.74 (m, 1H, H-5), 3.68 (t, J = 6.5 Hz, 2H, H-3', H-5'), 3.42 (td, J = 6.8, 9.5 Hz, 1H, OCH₂HCH₂), 2.13, 2.10, 1.93 (s, 3H, COCH₃), 1.52 (h, 2H, OCH₂CH₂), 1.27-1.21 (m, 4H, CH₂CH₂CH₂CH₃), 1.18 (s, 9H, C(CH₃)₃), 0.82 (t, J = 6.8 Hz, 3H, CH₂CH₃). ¹³C NMR (CDCl₃, 150 MHz) δC 177.9, 171.1, 170.7, 170.2, 167.4, 165.9 (6 C=O), 133.5 (Bz), 129.5 (2 Bz), 128.6 (2 Bz), 100.9 (C-1), 100.7 (C-1'), 75.7 (C-4), 74.2 (C-3), 72.8 (C-2'), 72.5 (C-5), 71.2 (C-3'), 71.1 (C-5'), 69.9 (OCH₂CH₂), 69.2 (C-4'), 62.6 (C-6), 61.3 (C-6'), 53.6 (C-2), 40.8 (COCH₂Cl), 38.7 (C(CH₃)₃), 29.0, 28.0, 22.3 (CH₂CH₂CH₂CH₃), 27.1 (C(CH₃)₃), 23.2, 20.9, 20.7 (3 COCH₃), 14.0 (CH₂CH₃). HRESIMS calculated for C₃₇H₅₂O₁₆NClH [M+H]⁺ 802.3053, found 802.3027.
4.2.5 Synthetic Procedures for the Preparation of Trisaccharide Acceptors 1-3

4,6-Benzylidene-2-deoxy-3-O-chloroacetyl-2-trichloroacetamido-β-D-glucopyranosyl-
(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-3-O-
chloroacetyl-2-deoxy-β-D-glucopyranoside (4) & 2,4-di-O-acetyl-6-O-pivaloyl-3-O-
trimethylsilyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-3-O-chloroacetyl-2-deoxy-
β-D-glucopyranoside (31)

Disaccharide acceptor 30 (500 mg, 0.62 mmol) and N-acetylgalactosamine donor 5 (1.18 g, 1.87 mmol, 3 equiv) were dissolved in anhydrous CH₂Cl₂ (50 mL) containing activated 4Å MS (1.0 g). The mixture was cooled to 0 °C under N₂. TMSOTf (226 µL, 1.25 mmol, 2 equiv) was added, and the mixture was stirred at 0 °C under N₂ for 75 min, then quenched with NEt₃. The mixture was warmed to RT and diluted with CH₂Cl₂ (150 mL), then filtered over celite and washed with CH₂Cl₂ (150 mL). The filtrate was washed with sat. aq. NaHCO₃ (200 mL). The aqueous layer was re-extracted with CH₂Cl₂ (3 × 50 mL), and the organic layers were dried and concentrated. Column chromatography (EtOAc/hexanes, 4:6 → 6:4) gave trisaccharide 4 (594 mg, 75%) as a yellow solid, and trimethylsilyl disaccharide 31 (58 mg, 11%) as a yellow solid.

Analytical Data for 4: [α]D -8.2 (c 1.0, MeOH). ¹H NMR (CDCl₃, 600 MHz): δH 7.99 (d, J = 7.1 Hz, 2H, Bz), 7.59 (t, J = 7.4 Hz, 1H, Bz), 7.47 (t, J = 7.9 Hz, 2H, Bz), 7.38 (d, J = 6.5 Hz, 2H, Ph), 7.32 (dd, J = 6.4, 14.0 Hz, 3H, Ph), 6.94 (d, J = 8.8 Hz, 1H, NH'), 5.77 (d, J = 9.3 Hz, 1H,
NH), 5.46 (s, 1H, CHPh), 5.45 (t, J = 9.8 Hz, 1H, H-3"), 5.26 (d, J = 3.5 Hz, 1H, H-4'), 5.16 (dd, J = 8.2, 9.3 Hz, 1H, H-3), 5.01 (dd, J = 8.0, 9.9 Hz, 1H, H-2'), 4.77 (d, J = 8.0 Hz, 1H, H-1"), 4.64 (dd, J = 2.8, 12.0 Hz, 1H, H-6a), 4.50 (d, J = 7.3 Hz, 1H, H-1), 4.37 (dd, J = 4.9, 11.9 Hz, 1H, H-6b), 4.35 (d, J = 7.3 Hz, 1H, H-1'), 4.30 (dd, J = 5.0, 10.5 Hz, 1H, H-6a"), 4.15-4.06 (m, 3H, H-6a', COCH₂Cl), 4.04-3.97 (m, 4H, H-2, H-6b', COCH₂Cl), 3.85-3.77 (m, 3H, H-4, H-2', H-6b''), 3.75 (dd, J = 3.6, 9.9 Hz, 1H, H-3'), 3.74-3.66 (m, 4H, H-5, H-5', H-4'', H-6b''), 3.48 (dt, J = 4.8, 9.6 Hz, 1H, H-5''), 3.39 (td, J = 6.8, 9.5 Hz, 1H, OCHHCH₂), 1.52 (m, 2H, OCH₂C₃H₃), 0.83 (t, J = 6.9 Hz, 3H, CH₂C₃H₃). ¹³C NMR (CDCl₃, 150 MHz): δC 177.9, 170.1, 170.0, 169.1, 167.4, 167, 166.0, 162.1 (8 C=O), 136.5 (4° Ph), 133.6 (Bz), 129.6 (2 Bz), 129.5 (4° Bz), 129.3 (Ph), 128.7 (2 Bz), 128.3 (2 Ph), 126.0 (2 Ph), 101.4 (CHPh), 100.8 (C-1, C-1'), 100.1 (C-1''), 78.0 (C-4''), 75.6 (C-3'), 74.9 (C-4), 73.7 (C-3), 72.5 (C-5), 72.1 (C-3''), 71.3 (C-5'), 70.8 (C-2'), 69.9 (OCH₂CH₂), 68.5 (C-4''), 68.2 (C-6'), 65.2 (C-6), 61.5 (C-6'), 56.7 (C-2''), 52.9 (C-2), 40.7, 40.4 (2 COCH₂Cl), 38.8 (C(CH₃)₃), 29.0, 28.0, 22.3 (CH₂CH₂CH₂CH₃), 27.1 (C(CH₃)₃), 23.2, 21.1, 20.7 (3 COCH₃), 14.0 (CH₂CH₃). HRESIMS calculated for C₅₄H₆₇O₂₂N₂Cl₅H [M+H]⁺ 1271.2706, found 1271.2725.

Analytical Data for 31: [α]D -3.6 (c 1.0, MeOH). ¹H NMR (CDCl₃, 400 MHz): δH 8.01 (d, J = 7.1 Hz, 2H, Bz), 7.60 (t, J = 7.4 Hz, 1H, Bz), 7.49 (t, J = 7.9 Hz, 2H, Bz), 5.78 (d, J = 9.3 Hz, 1H, NH), 5.18 (dd, J = 8.1, 9.6 Hz, 1H, H-3), 5.10 (dd, J = 0.6, 3.7 Hz, 1H, H-4'), 4.90 (dd, J = 8.1, 9.6 Hz, 1H, H-2'), 4.70 (dd, J = 2.7, 12.0 Hz, 1H, H-6a), 4.51 (d, J = 7.4 Hz, 1H, H-1), 4.37 (dd, J = 4.9, 10.0 Hz, 1H, H-6b), 4.35 (d, J = 8.0 Hz, 1H, H-1'), 4.20-4.05 (m, 4H, H-6a', H-6b', COCH₂Cl), 4.02 (m, 1H, H-2), 3.85-3.78 (m, 2H, H-4, OCHHCH₂), 3.74 (m, 1H, H-5), 3.67 (t, J = 7.4 Hz, 1H, H-5''), 3.61 (dd, J = 3.7, 9.6 Hz, 1H, H-3'), 3.41 (td, J = 6.8, 9.6 Hz, 1H,
OCHHCH₂), 2.10, 2.04, 1.94 (s, 3H, COCH₃), 1.52 (m, 2H, OCH₂CH₂), 1.28-1.21 (m, 4H, CH₂CH₂CH₂), 1.18 (s, 9H, C(CH₃)₃), 0.82 (t, J = 6.8 Hz, 3H, CH₂C(CH₃)₃), 0.01 (s, 9H, Si(CH₃)₃).

¹³C NMR (CDCl₃, 100 MHz) δC 177.9, 170.2, 170.1, 169.3, 167.3, 165.9 (6 C=O), 133.5 (Bz), 129.5 (2 Bz), 128.6 (2 Bz), 100.9 (C-1, C-1'), 7517 (C-4), 73.9 (C-3), 72.5 (C-5), 72.1 (C-2'), 70.8 (C-3', C-5'), 69.9 (OCH₂CH₂), 68.9 (C-4'), 62.8 (C-6), 61.2 (C-6'), 52.9 (C-2), 40.8 (COCH₂Cl), 38.7 (C(CH₃)₃), 29.0, 28.0, 22.3 (CH₂CH₂CH₂CH₃), 27.0 (C(CH₃)₃), 23.3, 20.9, 20.7 (3 COCH₃), 14.0 (CH₂CH₃), -0.4 (Si(CH₃)₃). HRESIMS calculated for C₄₀H₆₀O₁₆NSiClH [M+H]+ 874.3448, found 874.3471.

**4,6-Benzyldiene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (1)**

Trisaccharide 4 (325 mg, 0.26 mmol) and thiourea (194 mg, 2.55 mmol, 10 equiv) were dissolved in a mixture of 1:1 pyridine/EtOH (32 mL). The solution was stirred at 70 °C for 18 h, then cooled to RT and diluted with CHCl₃ (100 mL), then washed with 2 M HCl (100 mL). The aqueous layer was re-extracted with CHCl₃ (5 × 25 mL), and the organic layers were dried and concentrated. The crude product was re-dissolved in CHCl₃ (50 mL) and washed with 2 M HCl (50 mL) followed by sat. aq. NaHCO₃ (50 mL). The aqueous layers were re-extracted with CH₂Cl₂ (5 × 10 mL each), and the organic layers were dried and concentrated giving gave trisaccharide diol acceptor 1 (281 mg, 98%) as a yellow solid. [α]D -4.5 (c 1.0, MeOH). ¹H NMR (CDCl₃, 400 MHz): δH 8.00 (d, J = 7.2 Hz, 2H, Bz), 7.58 (t, J = 7.3 Hz, 1H, Bz), 7.48-7.41 (m,
4H, 2 Bz, 2 Ph), 7.35 (t, $J = 3.9$ Hz, 3H, Ph), 6.96 (d, $J = 7.1$ Hz, 1H, NH"), 5.59 (d, $J = 7.8$ Hz, 1H, NH), 5.51 (s, 1H, CHPh), 5.32 (d, $J = 3.0$ Hz, 1H, H-4'), 5.16 (dd, $J = 8.1, 9.9$ Hz, 1H, H-2'), 5.09 (d, $J = 8.0$ Hz, 1H, H-1"), 4.76 (d, $J = 8.1$ Hz, 1H, H-1), 4.51 (d, $J = 9.8$ Hz, 1H, H-6a), 4.45 (d, $J = 8.0$ Hz, 1H, H-1'), 4.34-4.23 (m, 3H, H-3", H-6b, H-6a"), 4.20 (d, $J = 3.7$ Hz, 1H, OH-3), 4.17 (dd, $J = 4.3, 11.6$ Hz, 1H, H-6a'), 4.01 (t, $J = 9.1$ Hz, 1H, H-3), 3.95 (dd, $J = 7.9, 11.6$ Hz, 1H, H-6b'), 3.89-3.82 (m, 2H, H-3', H-5'), 3.78 (td, $J = 6.7, 9.6$ Hz, 1H, OCHHCH$_2$), 3.74-3.66 (m, 2H, H-5, H-6b"), 3.54-3.66 (m, 5H, H-2, H-4, H-4", H-5", OCHHCH$_2$), 3.27 (td, $J = 7.8, 10.0$ Hz, 1H, H-2"), 2.89 (s, 1H, OH-3'), 2.12, 2.10, 1.97 (3 s, 9H, COCH$_3$), 1.52 (m, 2H, OCH$_2$CH$_2$), 1.28-1.21 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.17 (s, 9H, C(CH$_3$)$_3$), 0.83 (t, $J = 6.8$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$C 178.0, 170.4, 169.9, 169.6, 166.1, 162.2 (6 C=O), 136.7 (4° Ph), 133.3 (Bz), 129.6 (2 Bz, 4° Bz), 129.4 (Ph), 128.5 (2 Bz), 128.4 (2 Ph), 126.2 (2 Ph), 101.8 (CHPh), 101.6 (C-1"), 100.1 (C-1), 99.1 (C-1'), 92.2 (CCl$_3$), 82.1 (C-4), 81.2 (C-4"), 75.8 (C-3'), 71.9 (C-5), 71.8 (C-5'), 71.5 (C-3), 70.3 (C-2'), 69.9 (OCH$_2$CH$_2$), 68.8 (C-4'), 68.6 (C-3"), 68.3 (C-6"), 66.0 (C-5"), 63.2 (C-6), 62.2 (C-6'), 59.7 (C-2"), 56.7 (C-2), 38.7 (C(CH$_3$)$_3$), 29.1, 28.0, 22.3 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.5, 21.8, 20.8 (3 COCH$_3$), 14.0 (CH$_2$CH$_3$).

HRESIMS calculated for C$_{50}$H$_{65}$O$_{20}$N$_2$Cl$_3$H [M+H]$^+$ 1119.3274, found 1119.3292.
2-Deoxy-3-O-chloroacetyl-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-penty1 2-acetamido-3-O-chloroacetyl-2-deoxy-β-D-glucopyranoside (32)

Trisaccharide 4 (300 mg, 0.24 mmol) was dissolved in 90% AcOH (27 mL, 472 mmol, 2000 equiv) in water (3 mL). The solution was stirred at 80 °C for 2 h, then co-concentrated with PhMe (2 × 100 mL), giving diol 32 (279 mg, quant.) as a white/light brown powder. [α]D -2.0 (c 1.0, MeOH). 1H NMR (CD3OD, 400 MHz): δH 8.23 (d, J = 9.3 Hz, 1H, NH), 8.03 (d, J = 7.1 Hz, 2H, Bz), 7.65 (t, J = 7.4 Hz, 1H, Bz), 7.52 (t, J = 8.0 Hz, 2H, Bz), 5.43 (d, J = 3.3 Hz, 1H, H-4'), 5.25 (dd, J = 9.0, 10.7 Hz, 1H, H-3''), 5.18 (dd, J = 8.5, 10.6 Hz, 1H, H-3), 4.97 (dd, J = 8.0, 10.1 Hz, 1H, H-2'), 4.90 (s, 1H, N-H''), 4.84 (d, J = 8.2 Hz, 1H, H-1''), 4.63 (dd, J = 1.8, 12.2 Hz, 1H, H-6a), 4.55 (d, J = 8.4 Hz, 1H, H-1), 4.48 (d, J = 8.0 Hz, 1H, H-1''), 4.40 (dd, J = 4.5, 12.2 Hz, 1H, H-6b), 4.30 (dd, J = Hz, 1H, H-6a''), 4.25-4.06 (m, 6H, H-6a', H-6b', 2 COCH2Cl), 4.02 (dd, J = 3.5, 10.2 Hz, 1H, H-3'), 3.90-3.83 (m, 3H, H-2, H-5', H-6a''), 3.82-3.76 (m, 3H, H-4, H-5, OCHHCH2), 3.67-3.60 (m, 2H, H-2", H-6b''), 3.50 (dd, J = 9.8, 18.8 Hz, 1H, H-4''), 3.46 (td, J = 6.7, 9.7 Hz, 1H, OCHHCH2), 3.36 (ddd, J = 2.1, 5.3, 9.8 Hz, 1H, H-5''), 2.14, 2.09, 1.89 (3 s, 9H, COCH3), 1.52 (m, 2H, OCH2CH2), 1.30-1.25 (m, 4H, CH2CH2CH3), 1.23 (s, 9H, C(CH3)3), 0.85 (t, J = 6.9 Hz, 3H, CH2CH3). 13C NMR (CD3OD, 100 MHz): δC 179.3, 173.4, 172.5, 171.0, 168.6, 168.5, 167.3, 164.0 (8 C=O), 134.7 (Bz), 131.0 (4° Bz), 130.6 (2 Bz), 129.9 (2 Bz), 102.3 (C-1'), 102.2 (C-1), 100.9 (C-1''), 77.8 (C-5''), 77.5 (C-3'), 77.4 (C-3''), 77.1 (C-4), 75.8 (C-3), 73.8 (C-5), 72.6 (C-5'), 71.8 (C-2'), 71.0 (OCH2CH2), 70.8 (C-4'), 69.3 (C-4''), 63.8 (C-6), 63.1 (C-6'), 62.1 (C-6''), 57.6 (C-2''), 55.1 (C-2), 41.9, 41.7 (2 COCH2Cl), 39.8 (C(CH3)3), 30.3, 29.3,
27.7 (CH₂CH₂CH₂CH₃), 27.7 (C(CH₃)₃), 22.7, 21.4, 20.9 (3 COCH₃), 14.4 (CH₂CH₃). HRESIMS calculated for C₄₇H₆₃O₂₂N₂Cl₅H [M+H]+ 1183.2351, found 1183.2271.

4,6-Di-O-acetyl-2-deoxy-3-O-chloroacetyl-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-3-O-chloroacetyl-2-deoxy-β-D-glucopyranoside (33)

Diol 32 (224 mg, 0.19 mmol) was dissolved in a mixture of pyridine (10 mL, 106 mol, 560 equiv) and Ac₂O (10 mL, 124 mol, 655 equiv). The solution was stirred at RT for 1 h, then co-concentrated with PhMe (2 × 100 mL). The residue was dissolved in CH₂Cl₂ (100 mL), then washed with 2 M HCl (100 mL). The aqueous layer was re-extracted with CH₂Cl₂ (3 × 20 mL), and the organic layers were dried and concentrated giving diacetate 33 (237 mg, 99%) as a yellow solid. [α]D +9.8 (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 400 MHz): δH 7.99 (d, J = 7.1 Hz, 2H, Bz), 7.60 (t, J = 7.4 Hz, 1H, Bz), 7.47 (t, J = 7.9 Hz, 2H, Bz), 6.87 (d, J = 8.0 Hz, 1H, N-H"), 5.71 (d, J = 9.1 Hz, 1H, NH), 5.46 (dd, J = 9.2, 10.7 Hz, 1H, H-3"), 5.30 (d, J = 3.4 Hz, 1H, H-4'), 5.18 (dd, J = 8.1, 9.6 Hz, 1H, H-3), 5.07 (t, J = 12.8 Hz, 1H, H-4"), 5.00 (dd, J = 8.0, 9.9 Hz, 1H, H-2'), 4.94 (d, J = 8.0 Hz, 1H, H-1"), 4.67 (dd, J = 2.6, 12.0 Hz, 1H, H-6a), 4.53 (d, J = 7.4 Hz, 1H, H-1), 4.36 (dd, J = 4.7, 11.9 Hz, 1H, H-6b), 4.33 (d, J = 8.0 Hz, 1H, H-1'), 4.23 (dd, J = 2.4, 12.4 Hz, 1H, H-6a"), 4.16-3.91 (m, 8H, H-2, H-6a', H-6b', H-6b", 2 COCH₂Cl), 4.02 (dd, J = 3.5, 10.2 Hz, 1H, H-3'), 3.84-3.76 (m, 3H, H-4, H-3', OCH₃HCH₂), 3.75-3.57 (m, 4H, H-5, H-5', H-2", H-5"), 3.40 (td, J = 6.9, 9.6 Hz, 1H, OCH₂HCH₂), 2.10, 2.06, 2.05, 2.00, 1.94 (5 s, 15H, COCH₃), 1.52 (m, 2H, OCH₂CH₂), 1.27-1.20 (m, 4H, CH₂CH₂CH₃), 1.17 (s, 9H, C(CH₃)₃), 0.83
(t, J = 6.8 Hz, 3H, CH₂CH₃). ¹³C NMR (CDCl₃, 100 MHz): δC 177.9, 170.7, 170.1, 169.8, 169.4, 169.2, 167.3, 167.0, 166.0, 161.9 (10 C=O), 133.6 (Bz), 129.5 (2 Bz), 129.4 (4° Bz), 128.7 (2 Bz), 100.8 (C-1, C-1’), 99.0 (C-1”), 91.9 (COCl₃), 75.8 (C-3’), 75.0 (C-4), 73.6 (C-3), 72.5 (C-5), 72.3 (C-5”), 71.8 (C-3”), 71.4 (C-5”), 70.7 (C-2”), 70.0 (OCH₂CH₂), 68.6 (C-4’), 68.0 (C-4”), 62.6 (C-6), 61.7 (C-6”), 61.0 (C-6”), 56.7 (C-2”), 53.1 (C-2), 40.7, 40.3 (2 COCH₂Cl), 38.7 (C(CH₃)₃), 29.0, 28.0, 22.3 (CH₂CH₂CH₂CH₃), 27.1 (C(CH₃)₃), 23.3, 21.1, 20.7, 20.7, 20.6 (5 COCH₃), 14.0 (CH₂CH₃). HRESIMS calculated for C₅₁H₆₇O₂₄N₂Cl₅H [M+H]⁺ 1267.2605, found 1267.2572.

4,6- Di-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (2)

Diacetate 33 (236 mg, 0.19 mmol) and thiourea (142 mg, 1.86 mmol, 10 equiv) were dissolved in a mixture of 1:1 pyridine/EtOH (24 mL). The solution was stirred at 70 °C for 18 h, then cooled to RT and diluted with CHCl₃ (100 mL), then washed with 2 M HCl (100 mL), then with sat. aq. NaHCO₃ (100 mL). The aqueous layers were re-extracted with CHCl₃ (3 × 20 mL each), and the organic layers were dried and concentrated. Column chromatography (CH₂Cl₂/MeOH, 20:1) gave diacetate acceptor 2 (248 mg, 89%) as a yellow solid. [α]D +15.6 (c 0.50, CHCl₃). ¹H NMR (CDCl₃, 400 MHz): δH 8.00 (d, J = 7.1 Hz, 2H, Bz), 7.62 (t, J = 7.4 Hz, 1H, Bz), 7.45 (t, J = 7.8 Hz, 2H, Bz), 7.19 (d, J = 7.0 Hz, 1H, N-H”), 5.61 (d, J = 7.9 Hz, 1H, NH), 5.35 (d, J = 3.3 Hz, 1H, H-4”), 5.14 (dd, J = 8.1, 10.1 Hz, 1H, H-2”), 5.03 (d, J = 8.0 Hz, 1H, H-1”), 4.83 (t, J = 125
9.2 Hz, 1H, H-4''), 4.76 (d, J = 8.2 Hz, 1H, H-1), 4.51 (dd, J = 1.9, 11.7 Hz, 1H, H-6a), 4.47 (d, J = 8.0 Hz, 1H, H-1'), 4.30-4.08 (m, 6H, H-6b, H-6a', H-3'', H-6a'', H-6b'', OH-3), 4.04-3.93 (m, 2H, H-3, H-6b'), 3.90-3.82 (m, 2H, H-3', H-5'), 3.79 (td, J = 6.7, 9.6 Hz, 1H, OCHHCH₂), 3.70 (m, 1H, H-5), 3.60 (m, 1H, H-5''), 3.50 (t, J = 9.4 Hz, 1H, H-4), 3.47-3.38 (m, 2H, H-2, OCHHCH₂), 3.27 (td, 1H, H-2''), 3.21 (s, 1H, OH-3''), 2.10, 2.08, 2.07, 2.06, 1.96 (5 s, 15H, COCH₃), 1.52 (m, 2H, OCH₂CH₂), 1.28-1.22 (m, 4H, CH₂CH₂CH₃), 1.16 (s, 9H, C(CH₃)₃), 0.83 (t, J = 6.8 Hz, 3H, CH₂CH₃). \(^{13}\)C NMR (CDCl₃, 100 MHz): \(\delta_C\) 178.1, 170.9, 170.8, 170.4, 169.9, 169.4, 166.2, 162.4 (8 C=O), 133.3 (Bz), 129.6 (2 Bz), 128.5 (2 Bz), 101.5 (C-1'), 100.1 (C-1), 98.7 (C-1''), 92.1 (COCCl₃), 82.1 (C-4), 75.7 (C-3'), 72.0 (C-5), 71.9 (C-5''), 71.8 (C-5''), 71.5 (C-3), 71.3 (C-4''), 70.3 (C-2'), 70.0 (C-3''), 69.9 (OCH₂CH₂), 69.0 (C-4'), 63.2 (C-6), 62.5 (C-6'), 61.4 (C-6''), 59.8 (C-2''), 56.7 (C-2), 38.7 (C(CH₃)₃), 29.1, 28.0, 22.3 (CH₂CH₂CH₂CH₃), 26.9 (C(CH₃)₃), 23.5, 21.1, 20.8, 20.8, 20.7 (5 COCH₃), 14.0 (CH₂CH₃). HRESIMS calculated for C₄₇H₆₅O₂₂N₂Cl₃H [M+H]^+ 1115.3173, found 1115.3164.
4,6- Di-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (3)
& 4,6- di-O-acetyl-2-deoxy-2-chloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (34)

Method A: Trisaccharide diol acceptor 2 (190 mg, 0.17 mmol) was dissolved in AcOH (19 mL, 332 mmol, 1950 equiv). Activated Zn dust (1.11 g, 17.0 mmol, 100 equiv) was added. The mixture was sonicated at 50 °C for 4 h. More Zn (1.11 g, 17.0 mmol, 100 equiv) was added, and the mixture was sonicated at 50 °C for 4 h. More Zn (1.11 g, 17.0 mmol, 100 equiv) was added, and the mixture was sonicated at 50 °C for 4 h. The mixture was cooled to RT, then filtered over celite and washed with CH₂Cl₂ (2 × 100 mL). The filtrate was washed with sat. aq. NaHCO₃ (200 mL). The aqueous layer was re-extracted with CH₂Cl₂ (5 × 50 mL), and the organic layers were dried and concentrated, giving acetamido acceptor 3 (154 mg, 89%) as a white solid.

Method B: Trisaccharide diol acceptor 2 (30 mg, 0.027 mmol) was dissolved in AcOH (3 mL, mmol, 1950 equiv). Activated Zn dust (75.6 mg, 1.16 mmol, 43 equiv) was added. The mixture was sonicated at 50 °C for 4 h. More Zn (75.6 mg, 1.16 mmol, 43 equiv) was added, and the mixture was sonicated at 50 °C for 3 h. More Zn (75.6 mg, 1.16 mmol, 43 equiv) was added, and the mixture was sonicated at 50 °C for 1.5 h. More Zn (75.6 mg, 1.16 mmol, 43 equiv) was added, and the mixture was sonicated at 50 °C for 3 h. More Zn (75.6 mg, 1.16 mmol, 43 equiv) was added, and the mixture was sonicated at 50 °C for 2.5 h. The mixture was cooled to RT, then
filtered over celite and washed with CH$_2$Cl$_2$ (2 × 50 mL). The filtrate was washed with sat. aq. NaHCO$_3$ (100 mL). The aqueous layer was re-extracted with CH$_2$Cl$_2$ (5 × 20 mL), and the organic layers were dried and concentrated. Column chromatography (CH$_2$Cl$_2$/MeOH, 20:1) gave acetamido acceptor 3 (12.7 mg, 47%) as a clear solid, and chloroacetamido acceptor 34 (9.8 mg, 35%) as a clear solid.

**Analytical Data for 3:** \([\alpha]_D^\circ +7.4 (c 0.615, \text{MeOH}).\) $^1$H NMR (CDCl$_3$, 400 MHz): δ $^1$H NMR (CDCl$_3$, 600 MHz): δ $^H$ 8.01 (d, $J = 7.2$ Hz, 2H, Bz), 7.58 (t, $J = 7.4$ Hz, 1H, Bz), 7.45 (t, $J = 7.8$ Hz, 2H, Bz), 5.96 (s, 1H, N-H'), 5.59 (d, $J = 7.4$ Hz, 1H, NH), 5.34 (d, $J = 3.5$ Hz, 1H, H-4'), 5.13 (dd, $J = 8.1, 9.9$ Hz, 1H, H-2'), 4.87 (t, $J = 9.5$ Hz, 1H, H-4''), 4.84 (d, $J = 8.3$ Hz, 1H, H-1), 4.68 (d, $J = 8.1$ Hz, 1H, H-1''), 4.56 (dd, $J = 1.2, 10.3$ Hz, 1H, H-6a), 4.51 (d, $J = 8.0$ Hz, 1H, H-1'), 4.28 (dd, $J = 5.5, 11.7$ Hz, 1H, H-6b), 4.24 (dd, $J = 1.5, 10.4$ Hz, 1H, H-6a''), 4.08 (t, $J = 9.1$ Hz, 2H, H-3), 4.05 (dd, $J = 4.1, 12.4$ Hz, 1H, H-6b''), 4.02-3.95 (m, 2H, H-3', H-3''), 3.86 (dd, $J = 3.7, 8.2$ Hz, 1H, H-5'), 3.81 (td, $J = 6.5, 9.5$ Hz, 1H, OCHHCH$_2$), 3.77 (dd, $J = 3.7, 14.9$ Hz, 1H, H-3'), 3.73 (m, 1H, H-5), 3.55 (qd, $J = 3.8, 9.0$ Hz, 1H, H-5''), 3.51 (dd, $J = 8.3, 9.3$ Hz, 1H, H-4), 3.46 (td, $J = 6.8, 9.5$ Hz, 1H, OCHHCH$_2$), 3.34 (td, $J = 8.1, 9.9$ Hz, 1H, H-2), 3.06 (dd, $J = 1.1, 4.8, 8.4$ Hz, 1H, H-2''), 2.12, 2.10, 2.07, 2.05, 1.99, 1.97 (6 s, 18H, COCH$_3$), 1.53 (m, 2H, OCH$_2$CH$_2$), 1.28-1.22 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.17 (s, 9H, C(CH$_3$)$_3$), 0.83 (t, $J = 6.9$ Hz, 3H, CH$_2$CH$_2$). $^{13}$C NMR (CDCl$_3$, 150 MHz): δ$_C$ 178.1, 172.4, 170.9, 170.5, 170.5, 170.4, 169.5, 166.2 (8 C=O), 133.4 (Bz), 129.6 (2 Bz), 128.5 (2 Bz), 101.3 (C-1'), 99.9 (C-1, C-1''), 82.4 (C-4), 76.2 (C-3'), 72.0 (C-5', C-5''), 71.8 (C-5), 71.4 (C-3), 71.3 (C-3''), 71.0 (C-2', C-4''), 69.1 (OCH$_2$CH$_2$), 69.1 (C-4'), 63.3 (C-6), 62.3 (C-6'), 61.5 (C-6''), 59.4 (C-2''), 57.1 (C-2), 38.7 (C(CH$_3$)$_3$), 29.1, 28.0, 22.3 (CH$_2$CH$_2$CH$_2$CH$_3$).
Analytical Data for 34: $\left[\alpha\right]_D +9.4$ (c 0.435, MeOH). $^1$H NMR (CDCl$_3$, 600 MHz): $\delta$H 8.01 (d, $J = 7.1$ Hz, 2H, Bz), 7.58 (t, $J = 7.3$ Hz, 1H, Bz), 7.45 (t, $J = 7.8$ Hz, 2H, Bz), 6.65 (d, $J = 7.0$ Hz, 1H, N-H"), 5.57 (d, $J = 7.7$ Hz, 1H, NH), 5.34 (d, $J = 3.2$ Hz, 1H, H-4'), 5.14 (dd, $J = 8.1, 10.0$ Hz, 1H, H-2'), 4.86-4.82 (m, 2H, H-1'', H-4''), 4.80 (d, $J = 8.2$ Hz, 1H, H-1), 4.53 (dd, $J = 1.9, 11.8$ Hz, 1H, H-6a), 4.47 (d, $J = 8.0$ Hz, 1H, H-1'), 4.30-4.24 (m, 2H, H-6b, H-6a''), 4.20 (d, $J = 1.2$ Hz, 1H, OH-3A), 4.17 (dd, $J = 4.2, 11.8$ Hz, 1H, H-6a'), 4.07 (dd, $J = 4.3$, 12.3 Hz, 1H, H-6b'), 4.06-4.00 (m, 3H, H-3, COCH$_2$Cl), 3.98 (dd, $J = 8.2, 11.6$ Hz, 1H, H-6b'), 3.86 (q, $J = 4.3$ Hz, 1H, H-5'), 3.80 (td, $J = 6.5, 9.6$ Hz, 1H, OCHHCH$_2$), 3.78 (dd, $J = 3.4, 10.0$ Hz, 1H, H-3'), 3.72 (m, 1H, H-5), 3.57 (qd, $J = 2.7, 3.9, 9.9$ Hz, 1H, H-5''), 3.50 (t, $J = 8.2$ Hz, 1H, H-4), 3.45 (td, $J = 6.8, 9.7$ Hz, 1H, OCHHCH$_2$), 3.38 (dd, $J = 7.9, 17.9$ Hz, 1H, H-2), 3.25 (m, 1H, H-2''), 2.12, 2.10, 2.08, 2.06, 1.97 (5 s, 15H, COCH$_3$), 1.53 (m, 2H, OCH$_2$CH$_2$), 1.28-1.22 (m, 4H, $CH_2CH_2CH_3$), 1.17 (s, 9H, C(CH$_3$)$_3$), 0.83 (t, $J = 6.9$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$C 178.1, 170.8, 170.4, 169.8, 169.6, 167.1, 166.2 (8 C=O), 133.3 (Bz), 129.6 (4° Bz), 129.6 (2 Bz), 128.5 (2 Bz), 101.4 (C-1'), 100.3 (C-1), 99.4 (C-1''), 82.2 (C-4), 75.8 (C-3'), 72.0 (C-5'), 71.9 (C-5), 71.8 (C-5''), 71.5 (C-4''), 71.3 (C-3), 70.9 (C-3''), 70.5 (C-2'), 69.9 (OCH$_2$CH$_2$), 69.0 (C-4'), 63.2 (C-6), 62.3 (C-6'), 61.4 (C-6''), 58.8 (C-2''), 56.9 (C-2), 42.6 (COCH$_2$Cl), 38.7 (C(CH$_3$)$_3$), 29.1, 28.0, 22.3 (CH$_2$CH$_2$CH$_2$CH$_3$), 26.9 (C(CH$_3$)$_3$), 23.6, 21.0, 20.8, 20.8, 20.7 (5 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{47}$H$_{66}$O$_{22}$N$_2$Cl [M-H]$^-$ 1045.3801, found 1045.3769.
4.3 Experimental Section for Chapter 3

4.3.1 Synthetic Procedures for the Preparation of Donors 35-39

2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide (35)

Peracetylated galactosyl bromide donor 35 was previously synthesized in our lab by Guillemineau in one step using known methods, starting from D-galactose (16). \( ^1 \text{H NMR} \) (CDCl\(_3\), 400 MHz): \( \delta \text{H} \) 6.68 (d, \( J = 4.0 \text{ Hz} \), 1H, H-1), 5.50 (dd, \( J = 1.4, 3.3 \text{ Hz} \), 1H, H-4), 5.38 (dd, \( J = 3.2, 10.7 \text{ Hz} \), 1H, H-3), 5.03 (dd, \( J = 3.9, 10.7 \text{ Hz} \), 1H, H-2), 4.46 (t, \( J = 6.3 \text{ Hz} \), 1H, H-5), 4.16 (dd, \( J = 6.3, 11.4 \text{ Hz} \), 1H, H-6a), 4.09 (dd, \( J = 6.8, 11.4 \text{ Hz} \), 1H, H-6b), 2.13, 2.09, 2.04, 1.99 (4 s, 12H, COCH\(_3\)). The NMR data are in agreement with those reported in the literature.\(^{19}\)

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl bromide (36)

D-Mannose (40) (1.00 g, 5.5 mmol) was added to a mixture of Ac\(_2\)O (5.0 mL, 53 mmol, 9.5 equiv) and 33% HBr in AcOH (1.0 mL, 5.8 mmol, 1 equiv). The mixture was stirred at RT for 15 min to allow the mannose to dissolve. More 33% HBr in AcOH (5.0 mL, 29 mmol, 5.2 equiv) was added, and the solution was stirred at RT for 4h. The solution was co-concentrated with PhMe (3 \times 100 mL). Column chromatography (3:7 EtOAc/hexanes, using 0.1% NEt\(_3\)) gave peracetylated mannosyl bromide donor 36 (1.49 g, 65%) as a yellow gel. \( ^1 \text{H NMR} \) (CDCl\(_3\), 600
MHz): $\delta_H$ 6.27 (s, 1H, H-1), 5.69 (dd, $J = 3.4$, 10.1 Hz, 1H, H-3), 5.43 (d, $J = 2.3$ Hz, 1H, H-2), 5.34 (t, $J = 10.1$ Hz, 1H, H-4), 4.30 (dd, $J = 4.9$, 12.5 Hz, 1H, H-6a), 4.20 (ddd, $J = 1.9$, 4.9, 8.4 Hz, 1H, H-5), 4.11 (dd, $J = 1.9$, 12.6 Hz, 1H, H-6b), 2.15, 2.08, 2.05, 1.98 (4 s, 12H, COCH$_3$).

The NMR data are in agreement with those reported in the literature.$^{19}$

2,3,4-Tri-O-acetyl-$\alpha$-L-rhamnopyranosyl bromide (37)

L-Rhamnose monohydrate (41) (1.00 g, 5.5 mmol) was added to a mixture of Ac$_2$O (5.0 mL, 53 mmol, 9.6 equiv) and 33% HBr in AcOH (1.0 mL, 5.8 mmol, 1.1 equiv). The mixture was stirred at RT for 5 min to allow the rhamnose to dissolve. More 33% HBr in AcOH (5.0 mL, 29 mmol, 5.3 equiv) was added, and the solution was stirred at RT for 5h. The solution was co-concentrated with PhMe (2 × 100 mL). Column chromatography (4:6 EtOAc/hexanes, using 0.1% NEt$_3$) gave peracetylated rhamnosyl bromide donor 37 (1.69 mg, 87%) as a light brown solid.$^1$H NMR (CDCl$_3$, 400 MHz): $\delta_H$ 6.23 (d, $J = 0.8$ Hz, 1H, H-1), 5.66 (dd, $J = 3.4$, 10.2 Hz, 1H, H-3), 5.43 (dd, $J = 3.4$, 10.2 Hz, 1H, H-2), 5.34 (t, $J = 10.0$ Hz, 1H, H-4), 4.09 (m, 1H, H-5), 2.14, 2.06, 1.98 (3 s, 9H, COCH$_3$), 1.26 (d, $J = 6.3$ Hz, 3H, H-6). The NMR data are in agreement with those reported in the literature.$^{19}$
2,3,4-Tri-O-acetyl-α-L-fucopyranosyl bromide (38)

![Chemical Structure](image)

L-Fucose (42) (1.00 g, 6.1 mmol) was added to a mixture of Ac₂O (5.0 mL, 53 mmol, 8.7 equiv) and 33% HBr in AcOH (1.0 mL, 5.8 mmol, 0.95 equiv). The mixture was stirred at RT for 10 min to allow the fucose to dissolve. More 33% HBr in AcOH (5.0 mL, 29 mmol, 4.7 equiv) was added, and the solution was stirred at RT for 5 h. The solution was co-concentrated with PhMe (2 $\times$ 100 mL). Column chromatography (3:7 EtOAc/hexanes, using 0.1% NEt₃) gave peracetylated fucosyl bromide donor 38 (1.53 g, 71%) as a light brown jelly. 

$^1$H NMR (CDCl₃, 400 MHz): $\delta$H 6.67 (d, $J = 3.9$ Hz, 1H, H-1), 5.38 (dd, $J = 3.3$, 10.6 Hz, 1H, H-3), 5.33 (dd, $J = 1.2$, 3.3 Hz, 1H, H-4), 5.00 (dd, $J = 3.9$, 10.6 Hz, 1H, H-2), 4.38 (q, $J = 6.5$ Hz, 1H, H-5), 2.15, 2.08, 1.99 (3 s, 9H, COCH₃), 1.20 (d, $J = 6.5$ Hz, 3H, H-6). The NMR data are in agreement with those reported in the literature.¹⁹

Thioethyl 2,3,4-tri-O-benzyl-β-L-fucopyranoside (39)

![Chemical Structure](image)

Perbenzylated thioethyl fucosyl donor 39 was previously synthesized in our lab by Moore in four steps using known methods, starting from peracetylated L-fucosyl bromide 38. $^1$H NMR (CDCl₃, 400 MHz): $\delta$H 7.40-7.25 (m, 15H, Bn), 5.00-4.66 (m, 6H, CHHPh), 4.38 (d, $J = 9.6$ Hz, 1H, H-1), 3.80 (t, $J = 9.4$ Hz, 1H, H-2), 3.60 (dd, $J = 0.8$, 2.8 Hz, 1H, H-4), 3.55 (dd, $J = 2.9$, 9.2 Hz, 1H, H-3), 3.46 (dq, $J = 0.9$, 6.2, 12.6 Hz, 1H, H-5), 2.73 (m, 2H, SCH₂), 1.28 (t, $J = 7.5$ Hz, 3H,
CH$_2$CH$_3$), 1.18 (d, $J = 6.4$ Hz, 3H, H-6). The NMR data are in agreement with those reported in the literature.$^{85}$

4.3.2 General Procedure for Glycosylations with Bromide Donors 35-38 Using Hg(CN)$_2$

Activation

Trisaccharide acceptor 1 or 2 (30 mg, 0.027 mmol) or 3 (30 mg, 0.030 mmol) and donor 35, 36, 37, or 38 (0.134 mmol, 5 equiv) were dissolved in an mixture of 1:1 anhydrous PhMe/CH$_3$NO$_2$ (2 mL) containing activated 4Å MS (200 mg). The mixture was stirred at RT under N$_2$ for 1 h, then warmed to 50 °C for 10 min. Hg(CN)$_2$ (6 equiv, 0.161 mmol for reactions with 1 and 2, 0.148 mmol for reaction with 3) was added, and the mixture was stirred at 50 °C for 5 h. The mixture was cooled to RT, then filtered over celite and washed with CH$_2$Cl$_2$ (3 × 20 mL). The filtrate was washed with sat. aq. NaHCO$_3$ (50 mL). The aqueous layer was re-extracted with CH$_2$Cl$_2$ (3 × 15 mL), and the organic layers were dried and concentrated. Products were purified using reverse-phase HPLC (CH$_3$CN/H$_2$O).
4.3.3 Synthetic Procedures for Glycosylations with Trisaccharide Acceptor 1

4,6-Benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (43)

The general procedure was applied with trisaccharide acceptor 1 and galactosyl donor 35 (55.1 mg), and Hg(CN)$_2$ (40.6 mg). Reverse-phase HPLC (CH$_3$CN/H$_2$O, 40:60 → 70:40) gave a mixture of O-3A tetrasaccharide 43 (13.7 mg, 35%) and recovered acceptor 1 (11.0 mg, 37%) as a white solid. **Analytical Data for 43:** $^1$H NMR (CDCl$_3$, 400 MHz): δ$_H$ 8.04 (d, $J = 7.1$ Hz, 2H, Bz), 7.58 (t, $J = 7.4$ Hz, 1H, Bz), 7.50-7.42 (m, 4H, 2 Bz, 2 Ph), 7.39-7.33 (m, 3H, Ph), 6.81 (d, $J = 7.2$ Hz, 1H, NHA'), 6.46 (d, $J = 9.1$ Hz, 1H, NHA), 5.53 (s, 1H, CPh), 5.39 (d, $J = 2.5$ Hz, 1H, H-4C), 5.35 (d, $J = 3.0$ Hz, 1H, H-4B), 5.21 (d, $J = 8.0$ Hz, 1H, H-1A'), 5.17 (dd, $J = 7.8$, 10.3 Hz, 1H, H-2C), 5.08-5.01 (m, 2H, H-2B, H-3C), 4.93 (d, $J = 8.0$ Hz, 1H, H-1C), 4.67 (dd, $J = 7.4$, 11.5 Hz, 1H, H-6aA), 4.39 (m, 1H, H-6bA), 4.36 (d, $J = 3.8$ Hz, 1H, H-1A), 4.34-4.29 (m, 2H, H-3A', H-6aA'), 4.23-3.98 (m, 8H, H-2A, H-3A, H-5A, H-5B, H-6aB, H-6bB, H-6aC, H-6bC), 3.97-3.91 (m, 2H, H-3B, H-5C), 3.83-3.71 (m, H-6bA', OCHHCH$_2$), 3.65 (t, $J = 6.7$ Hz, 1H, H-4A), 3.53-3.44 (m, 2H, H-4A', H-5A'), 3.35 (td, $J = 7.0$, 9.4 Hz, 1H, OCHHCH$_2$), 3.25 (td, $J = 7.3$, 10.2 Hz, 1H, H-2A'), 2.61 (d, $J = 2.8$ Hz, 1H, OH-3A'), 2.13, 2.11, 2.10, 2.05, 2.03, 2.02, 1.95 (7 s, 21H, COCH$_3$), 1.60-1.50 (m, 2H, OCH$_2$CH$_2$), 1.31-1.22 (m, 4H, CH$_2$CH$_2$CH$_3$),
1.18 (s, 9H, C(CH$_3$)$_3$), 0.86 (t, $J = 6.8$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$C 177.6, 170.9, 170.4, 170.3, 170.2, 169.8, 169.7, 169.6, 166.2, 162.0 (10 C=O), 136.7 (4° Ph), 133.4 (Bz), 129.8 (4° Bz), 129.5 (2 Bz), 129.4 (Ph), 128.6 (2 Bz), 128.4 (2 Ph), 126.2 (2 Ph), 101.8 (CHPh), 100.1 (C-1A), 99.2 (C-1A'), 98.7 (C-1B), 98.1 (C-1C), 92.2 (CCl$_3$), 81.3 (C-4A'), 74.8 (C-3B), 74.3 (C-3A), 73.4 (C-5A), 71.7 (C-5B), 71.4 (C-4A), 71.0 (C-5C), 70.9 (C-2B), 70.7 (C-3C), 69.5 (OCH$_2$CH$_2$), 68.6 (C-4B, C-3A', C-2C), 68.3 (C-6A'), 66.9 (C-4C), 66.1 (C-5A'), 64.8 (C-6A), 60.6 (C-6B), 60.5 (C-6C), 59.7 (C-2A'), 48.2 (C-2A), 38.7 (C(CH$_3$)$_3$), 29.0, 28.1, 22.4 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.0, 21.5, 20.8, 20.8, 20.7, 20.7, 20.6 (7 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{64}$H$_{82}$O$_{29}$N$_2$Cl$_3$ [M-H]$^-$ 1447.4068, found 1447.4045.
4,6-Benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→3)]-penty1 2-acetamido-2-deoxy-β-D-glucopyranoside (44), 3,4,6-Tri-O-acetyl-1,2-O-[4,6-benzylidene-2-deoxy-3-orthoacetyl-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-penty1 2-acetamido-2-deoxy-β-D-glucopyranoside]-α-D-mannopyranose (45), & 3,4,6-Tri-O-acetyl-1,2-O-[4,6-benzylidene-2-deoxy-3-orthoacetyl-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside]-α-D-mannopyranose (46)

The general procedure was applied with trisaccharide acceptor 1 and mannosyl donor 36 (55.1 mg), and Hg(CN)_2 (40.6 mg). Reverse-phase HPLC (CH_3CN/H_2O, 35:65 → 65:35) gave a
mixture of tetrasaccharide 44 (8.7 mg, 22%), orthoester tetrasaccharide 45 (4.4 mg, 11%), orthoester pentasaccharide 46 (5.6 mg, 12%), and recovered acceptor 1 (6.1 mg, 20%) as a white solid.

**Analytical Data for 44:** $^1$H NMR (CDCl$_3$, 400 MHz): δ$_H$ 8.03 (d, $J = 7.1$ Hz, 2H, Bz), 7.58 (t, $J = 7.3$ Hz, 1H, Bz), 7.50-7.42 (m, 4H, 2 Bz, 2 Ph), 7.39-7.33 (m, 3H, Ph), 6.87 (d, $J = 7.0$ Hz, 1H, NHA'), 6.42 (d, $J = 9.0$ Hz, 1H, NHA), 5.52 (s, 1H, CHPh), 5.33 (d, $J = 3.6$ Hz, 1H, H-4B), 5.30-5.24 (m, 3H, H-2C, H-3C, H-4C), 5.14 (d, $J = 8.0$ Hz, 1H, H-1A'), 5.08 (dd, $J = 8.2$, 10.0 Hz, 1H, H-2B), 5.04 (d, $J = 1.3$ Hz, 1H, H-1C), 4.72 (dd, $J = 4.8$, 11.7 Hz, 1H, H-6aA), 4.68 (d, $J = 4.0$ Hz, 1H, H-1A), 4.64 (dd, $J = 5.0$, 11.5 Hz, 1H, H-6bA), 4.37-4.27 (m, 5H, H-1B, H-3A', H-6aA', H-5C, H-6aC), 4.17-4.06 (m, 3H, H-3A, H-6aB, H-6bC), 4.01 (dd, $J = 6.9$, 11.5 Hz, 1H, H-6bB), 3.98-3.84 (m, 5H, H-2A, H-4A, H-5A, H-3B, OCH$_2$CH$_2$), 3.77-3.67 (m, 2H, H-2B, H-6bA'), 3.54-3.44 (m, 2H, H-4A', H-5A'), 3.38 (td, $J = 7.2$, 9.3 Hz, 1H, OCH$_2$CH$_2$), 3.26 (td, $J = 7.7$, 10.0 Hz, 1H, H-2A'), 2.70 (s, 1H, OH-3A'), 2.14, 2.12, 2.11, 2.07, 2.01, 2.00, 1.94 (7 s, 21H, COCH$_3$), 1.64-1.51 (m, 2H, OCH$_2$CH$_2$), 1.31-1.22 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.14 (s, 9H, C(CH$_3$)$_3$), 0.85 (t, $J = 6.8$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ$_C$ 177.6, 170.9, 170.2, 170.1, 169.7, 169.7, 169.6, 166.2, 162.1 (10 C=O), 136.7 (4° Ph), 133.4 (Bz), 129.7 (4° Bz), 129.6 (2 Bz), 129.4 (Ph), 128.6 (2 Bz), 128.3 (2 Ph), 126.2 (2 Ph), 101.8 (CHPh), 100.0 (C-1B), 99.9 (C-1A), 99.2 (C-1A'), 98.9 (C-1C, 177 Hz), 92.3 (CCl$_3$), 81.3 (C-4A'), 75.6 (C-3B), 75.3 (C-3A), 73.8 (C-4A), 72.0 (C-5A), 71.7 (C-5B), 70.6 (C-2B), 69.6 (OCH$_2$CH$_2$), 69.3 (C-3C), 69.0 (C-2C, C-5C), 68.7 (C-4B, C-3A'), 68.3 (C-6A), 66.0 (C-5A', C-4C), 64.1 (C-6A), 62.4 (C-6C), 60.9 (C-6B), 59.8 (C-2A'), 51.0 (C-2A), 38.6 (C(CH$_3$)$_3$), 29.0, 28.1, 22.4 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.1, 21.2, 20.9, 20.8, 20.8, 20.7, 20.6 (7 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{64}$H$_{82}$O$_{29}$N$_2$Cl$_3$ [M-H]$^-$ 1447.4068, found 1447.4091.
Analytical Data for 45: $^1$H NMR (CDCl$_3$, 400 MHz): $\delta_H$ 8.01 (d, $J = 7.2$ Hz, 2H, Bz), 7.58 (t, $J = 7.4$ Hz, 1H, Bz), 7.50-7.42 (m, 4H, 2 Bz, 2 Ph), 7.39-7.33 (m, 3H, Ph), 6.90 (d, $J = 7.3$ Hz, 1H, NHA'), 5.56 (d, $J = 7.8$ Hz, 1H, NHA), 5.48 (s, 1H, CHPh), 5.32 (d, $J = 3.0$ Hz, 1H, H-4B), 5.19-5.12 (m, 3H, H-2B, H-4C'), 5.03 (d, $J = 2.2$ Hz, 1H, H-1C'), 5.00 (d, $J = 8.0$ Hz, 1H, H-1A'), 4.96 (dd, $J = 4.1$, 9.7 Hz, 1H, H-3C'), 4.78 (d, $J = 8.2$ Hz, 1H, H-1A), 4.58 (m, 1H, H-2C'), 4.50 (d, $J = 12.4$ Hz, 1H, H-6aA), 4.44 (d, $J = 8.0$ Hz, 1H, H-1B), 4.35-4.23 (m, 3H, H-3A, H-6bA, H-3A', H-6aA'), 4.20 (s, 1H, OH-3A), 4.15 (dd, $J = 4.4$, 7.8 Hz, 1H, H-6aB), 4.13-4.01 (m, 3H, H-3A, H-6bC', H-6aC'), 3.96 (dd, $J = 8.0$, 11.1 Hz, 1H, H-6bB), 3.90-3.77 (m, 3H, H-3B, H-5B, OCHHCH$_2$), 3.76-3.66 (m, 2H, H-5A, H-6bA'), 3.58-3.37 (m, 6H, H-2A, H-4A, H-4A', H-5A', H-5C', OCHHCH$_2$), 3.28 (td, $J = 7.6$, 9.5 Hz, 1H, H-2A'), 2.12, 2.10, 2.05, 2.02, 2.00, 1.98 (6 s, 18H, COCH$_3$), 1.67 (s, 3H, CH$_3$ orthoester), 1.62-1.50 (m, 2H, OCH$_2$CH$_2$), 1.31 (t, $J = 6.9$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta_C$ 178.0, 170.6, 170.4, 169.9, 169.4, 169.4, 166.1, 161.8 (9 C=O), 136.9 (4° Ph), 133.3 (Bz), 129.6 (2 Bz), 129.1 (Ph), 128.5 (2 Bz), 128.3 (2 Ph), 126.0 (2 Ph), 124.1 (4° orthoester), 101.9 (C-1B), 101.6 (CHPh), 100.0 (C-1A), 99.1 (C-1A'), 97.1 (C-1C'), 92.0 (CCl$_3$), 82.0 (C-4A), 79.9 (C-4A'), 76.1 (C-2C'), 75.1 (C-3B), 71.9 (C-5B), 71.8 (C-5A), 71.6 (C-3A), 71.4 (C-2B), 69.9 (C-3C', C-5A'), 69.8 (OCH$_2$CH$_2$), 68.6 (C-4B, C-3A'), 68.2 (C-6A'), 66.4 (C-5C'), 65.4 (C-4C'), 63.2 (C-6A), 62.2 (C-6B, C-6C'), 58.6 (C-2A'), 56.7 (C-2A), 38.7 (C(CH$_3$)$_3$), 29.1, 28.0, 22.3 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 25.7 (CH$_3$ orthoester), 23.5, 21.1, 20.7, 20.7, 20.6 (6 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{64}$H$_{82}$O$_{29}$N$_2$Cl$_3$ [M-H]$^-$ 1447.4068, found 1447.4093.

Analytical Data for 46: $^1$H NMR (CDCl$_3$, 400 MHz): $\delta_H$ 8.02 (d, $J = 7.2$ Hz, 2H, Bz), 7.58 (t, $J = 7.2$ Hz, 1H, Bz), 7.51-7.43 (m, 4H, 2 Bz, 2 Ph), 7.40-7.30 (m, 3H, Ph), 6.88 (d, $J = 7.6$ Hz, 1H,
NHA'), 6.45 (d, J = 8.9 Hz, 1H, NHA), 5.49 (s, 1H, CHPh), 5.31 (d, J = 3.9 Hz, 1H, H-4B), 5.28-5.21 (m, 3H, H-2C, H-3C, H-4C), 5.15 (t, J = 9.6 Hz, 1H, H-3C'), 5.10-5.00 (m, 3H, H-2B, H-1A', H-1C'), 5.06 (d, J = 2.5 Hz, 1H, H-1C), 4.97 (dd, J = 3.9, 9.8 Hz, 1H, H-3C'), 4.73-4.68 (m, 2H, H-6aA, H-6bA), 4.67 (d, J = 3.0 Hz, 1H, H-1A), 4.58 (m, 1H, H-2C'), 4.40-4.24 (m, 4H, H-3A', H-6aA', H-5C, H-6aC), 4.32 (d, J = 8.0 Hz, 1H, H-1B), 4.16-3.95 (m, 8H, H-2A, H-3A, H-5A, H-6aB, H-6bB, H-6bC, H-6aC', H-6bC'), 3.94-3.84 (m, 3H, H-4A, H-4A', H-3A), 3.76-3.66 (m, 2H, H-5B, H-6bA'), 3.56 (t, J = 7.7, 8.8 Hz, 1H, OCHHCH$_2$), 3.30-3.24 (m, 1H, H-2A'), 2.13, 2.12, 2.10, 2.08, 2.05, 2.02, 2.01, 2.00, 1.94 (10 s, 30H, COCH$_3$), 1.67 (s, 3H, CH$_3$ orthoester), 1.63-1.54 (m, 2H, OCH$_2$CH$_2$), 1.30-1.21 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.14 (s, 9H, C(CH$_3$)$_3$), 0.84 (m, 3H, CH$_2$C(CH$_3$)$_3$). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta_C$ 177.7, 170.9, 170.4, 170.1, 170.0, 169.8, 169.7, 169.7, 169.4, 169.2, 166.2, 161.7 (13 C=O), 137.0 (4° Ph), 133.4 (Bz), 129.6 (4° Bz), 129.6 (2 Bz), 129.1 (Ph), 128.6 (2 Bz), 128.3 (2 Ph), 126.0 (2 Ph), 124.1 (4° orthoester), 101.4 (CHPh), 100.0 (C-1A), 100.0 (C-1B), 99.3 (C-1A'), 98.9 (C-1C, 177 Hz), 97.1 (C-1C'), 92.2 (CCl$_3$), 79.9 (C-4A'), 76.1 (C-2C'), 75.5 (C-5A), 74.7 (C-3B), 73.6 (C-4A), 72.0 (C-5A), 71.7 (C-5B), 71.1 (C-2B), 70.9 (C-5A'), 70.1 (C-3C'), 69.9 (C-3A'), 69.6 (OCH$_2$CH$_2$), 69.4 (C-2C), 69.1 (C-5A'), 69.1 (C-3C), 68.3 (C-6A'), 66.5 (C-5C'), 65.9 (C-4C), 65.4 (C-4C'), 64.2 (C-6A), 62.3 (C-6C), 62.2 (C-6C'), 61.0 (C-6B), 58.5 (C-2A'), 50.5 (C-2A), 38.6 (C(CH$_3$)$_3$), 29.0, 28.1, 22.4 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 25.7 (CH$_3$ orthoester), 23.1, 21.3, 20.9, 20.7, 20.7, 20.7, 20.7, 20.7, 20.6 (10 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{78}$H$_{100}$O$_{38}$N$_2$Cl$_3$ [M-H]$^-$ 1777.5019, found 1777.5081.
4,6-Benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (47) & 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (48)

The general procedure was applied with trisaccharide acceptor 1 and rhamnosyl donor 37 (47.3 mg), and Hg(CN)$_2$ (40.6 mg). Reverse-phase HPLC (CH$_3$CN/H$_2$O, 40:60 → 70:30) gave tetrasaccharide 47 (10.0 mg, 27%) as a white solid, and pentasaccharide 48 (12.8 mg, 29%) slightly impure as a white solid.

**Analytical Data for 47:** $[\alpha]_D$ -16.8 (c 0.6, MeOH). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$H 8.03 (d, $J$ = 7.2 Hz, 2H, Bz), 7.58 (t, $J$ = 7.4 Hz, 1H, Bz), 7.50-7.41 (m, 4H, 2 Bz, 2 Ph), 7.39-7.33 (m, 3H, Ph), 6.93 (d, $J$ = 7.1 Hz, 1H, NHA’), 6.45 (d, $J$ = 9.0 Hz, 1H, NHA), 5.52 (s, 1H, CHPh), 5.37 (d, $J$ = 3.3 Hz, 1H, H-4B), 5.26 (d, $J$ = 1.6 Hz, 1H, H-2C), 5.20-5.13 (m, 2H, H-1A’, H-3C), 5.12-
5.03 (m, 2H, H-2B, H-4C), 5.01 (s, 1H, H-1C), 4.78-4.66 (m, 2H, H-6aA, H-6bA), 4.72 (d, J = 3.8 Hz, 1H, H-1A), 4.38-4.28 (m, 2H, H-3A', H-6aA'), 4.37 (d, J = 8.2 Hz, 1H, H-1B), 4.15-4.08 (m, 2H, H-4A, H-6aB), 4.06-3.90 (m, 5H, H-2A, H-5A, H-3B, H-6bB, H-5A'), 3.89-3.84 (m, 2H, H-3A, OCH2HCH2), 3.77-3.68 (m, 2H, H-5B, H-6bA'), 3.53-3.45 (m, 2H, H-4A', H-5A'), 3.36 (td, J = 7.1, 9.2 Hz, 1H, OCH2HCH2), 3.26 (td, J = 7.7, 9.8 Hz, 1H, H-2A'), 2.83 (s, 1H, OH-3A'), 2.12, 2.11, 2.08, 2.01, 2.01, 1.94 (6 s, 18H, COCH3), 1.55 (quin, J = 7.1 Hz, 2H, OCH2CH2), 1.31-1.23 (m, 4H, CH2CH2CH3), 1.21 (d, J = 6.2 Hz, 3H, H-6C), 1.16 (s, 9H, C(CH3)3), 0.85 (t, J = 6.6 Hz, 3H, CH2CH3). 13C NMR (CDCl3, 100 MHz): δC 177.7, 170.7, 170.3, 169.9, 169.9, 169.7, 166.3, 162.1 (9 C=O), 136.7 (4° Ph), 133.4 (Bz), 129.8 (4° Bz), 129.6 (2 Bz), 129.4 (Ph), 128.5 (2 Bz), 128.4 (2 Ph), 126.2 (2 Ph), 101.8 (CHPh), 99.6 (C-1A'), 99.4 (C-1A), 99.3 (C-1B), 95.6 (C-1C, 173 Hz), 92.2 (CCl3), 81.2 (C-4A'), 75.3 (C-3B), 73.4 (C-3A), 72.2 (C-5A), 71.5 (C-4A), 70.7 (C-5B), 70.5 (C-2B, C-4C), 69.6 (OCH2CH2), 69.3 (C-2C), 69.1 (C-3C), 68.8 (C-4B), 68.6 (C-3A'), 68.3 (C-6A'), 67.1 (C-5C), 66.0 (C-5A'), 64.6 (C-6A), 61.0 (C-6B), 59.6 (C-2A'), 49.0 (C-2A), 38.7 (CH2CH2CH3), 28.9, 28.1, 22.4 (CH2CH2CH2CH3), 27.0 (CH2CH3), 23.1, 21.3, 20.9, 20.8, 20.7 (6 COCH3), 17.4 (C-6C), 13.9 (CH2CH3). HRESIMS calculated for C62H80O27N2Cl3 [M-H] 1389.4054.

Analytical Data for 48: 1H NMR (CDCl3, 400 MHz): δH 8.04 (d, J = 7.1 Hz, 2H, Bz), 7.57 (t, J = 7.7 Hz, 1H, Bz), 7.50-7.41 (m, 4H, 2 Bz, 2 Ph), 7.35-7.29 (m, 3H, Ph), 7.08 (d, J = 7.1 Hz, 1H, NHA'), 6.49 (d, J = 9.0 Hz, 1H, NHA), 5.51 (s, 1H, CHPh), 5.34 (d, J = 3.4 Hz, 1H, H-4B), 5.28-5.23 (m, 3H, H-1A', H-2C, H-2C'), 5.20-5.11 (m, 2H, H-3C, H-3C'), 5.09-5.02 (m, 2H, H-2B, H-4C), 5.01 (s, 1H, H-1C), 4.86 (t, J = 10.1 Hz, 1H, H-4C'), 4.75-4.72 (m, 2H, H-6aA, H-6bA), 4.71 (s, 1H, H-1A), 4.68 (d, J = 2.4 Hz, 1H, H-1C'), 4.43 (t, J = 9.3 Hz, 1H, H-3A'), 4.36 (dd, J = Hz, 1H, H-6aA'), 4.32 (d, J = 8.0 Hz, 1H, H-1B), 4.15-4.07 (m, 2H, H-4A, H-6aB), 4.05-3.92
(m, 6H, H-2A, H-5A, H-3B, H-6bB, H-5C, H-5C'), 3.91-3.94 (m, 2H, H-3A, OCH_2CH_3), 3.78-3.66 (m, 2H, H-5B, H-6bA'), 3.61-3.50 (m, 2H, H-4A', H-5A'), 3.35 (td, J = 7.0, 9.2 Hz, 1H, OCH_2CH_2), 3.19 (td, J = 7.8, 9.7 Hz, 1H, H-2A'), 2.13, 2.12, 2.09, 2.02, 2.01, 2.00, 1.95, 1.93, 1.92 (9 s, 27H, COCH_3), 1.55 (quin, J = 6.9, 13.8 Hz, 2H, OCH_2CH_2), 1.32 (td, J = 7.0, 9.2 Hz, 1H, OCH_2CH_2CH_3), 1.21 (d, J = 6.3 Hz, 3H, H-6C), 1.16 (s, 9H, C(CH_3)_3), 0.85 (t, J = 6.7 Hz, 3H, CH_2CH_3), 0.52 (d, J = 6.2 Hz, 3H, H-6C').

^{13}C NMR (CDCl_3, 100 MHz): \delta_C 177.7, 170.6, 170.3, 170.3, 169.9, 169.9, 169.9, 169.8, 169.6, 166.3, 161.9 (12 C=O), 136.7 (4° Ph), 133.4 (Bz), 129.8 (4° Bz), 129.6 (2 Bz), 129.4 (Ph), 128.5 (2 Bz), 128.2 (2 Ph), 126.4 (2 Ph), 102.2 (CHPh), 99.6 (C-1B), 99.5 (C-1C', 171 Hz), 98.3 (C-1A), 98.1 (C-1A'), 95.5 (C-1C, 175 Hz), 91.8 (CCl_3), 79.8 (C-4A'), 74.4 (C-3B), 73.9 (C-3A'), 73.1 (C-3A), 72.1 (C-5A), 71.5 (C-4A, C-5B), 70.7 (C-2B), 70.6 (C-4C), 70.5 (C-4C'), 69.6 (OCH_2CH_2), 69.3 (C-2C), 69.1 (C-3C, C-2C', C-3C'), 68.8 (C-4B), 68.4 (C-3A'), 67.1 (C-5C), 66.7 (C-5C'), 66.2 (C-5A'), 64.7 (C-6A), 61.0 (C-6B), 60.1 (C-2A'), 48.5 (C-2A), 38.7 (C(CH_3)_3), 28.9, 28.1, 22.4 (CH_2CH_2CH_2CH_3), 27.0 (C(CH_3)_3), 23.0, 21.3, 20.9, 20.9, 20.9, 20.8, 20.7, 20.7, 20.7 (9 COCH_3), 17.4 (C-6C), 16.3 (C-6C'), 13.9 (CH_2CH_3). HRESIMS calculated for C_{74}H_{96}O_{34}N_{2}Cl_3 [M-H]^- 1661.4910, found 1661.4937.
4,6-Benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-acetyl-β-L-fucopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (49)

The general procedure was applied with trisaccharide acceptor 1 and galactosyl donor 35 (47.3), and Hg(CN)₂ (40.6 mg). Reverse-phase HPLC (CH₃CN/H₂O, 40:60 → 70:30) gave tetrasaccharide 49 (18.6 mg, 50%) as a white solid. [α]D -14.6 (c 1.0, MeOH). ¹H NMR (CDCl₃, 600 MHz): δH 8.02 (d, J = 7.6 Hz, 2H, Bz), 7.57 (t, J = 7.3 Hz, 1H, Bz), 7.49-7.40 (m, 4H, 2 Bz, 2 Ph), 7.38-7.32 (m, 3H, Ph), 6.99 (d, J = 7.0 Hz, 1H, NHA'), 6.26 (d, J = 8.6 Hz, 1H, NHA), 5.51 (s, 1H, CPh), 5.37 (d, J = 2.8 Hz, 1H, H-4B), 5.18 (d, J = 2.7 Hz, 1H, H-4C), 5.16 (d, J = 8.0 Hz, 1H, H-1A'), 5.12 (dd, J = 8.4, 10.1 Hz, 1H, H-2C), 5.06 (t, J = 8.6 Hz, 1H, H-2B), 4.96 (dd, J = 3.1, 10.4 Hz, 1H, H-3C), 4.66 (d, J = 7.9 Hz, 1H, H-1C), 4.64 (d, J = 3.8 Hz, 1H, H-1A), 4.62 (dd, J = 6.0, 10.9 Hz, 1H, H-6aA), 4.52 (dd, J = 3.8, 11.2 Hz, 1H, H-6bA), 4.36 (d, J = 7.9 Hz, 1H, H-1B), 4.34-4.27 (m, 2H, H-3A', H-6aA'), 4.10 (s, 1H, H-2), 4.07-4.03 (m, 2H, H-3A, H-6aB), 3.96 (dd, J = 6.2, 11.0 Hz, 1H, H-6bB), 3.91 (dd, J = 3.4, 10.2 Hz, 1H, H-3A), 3.89-3.86 (s, 2H, H-5A, H-3B), 3.84-3.76 (m, 2H, H-5A', OCHHCH₂), 3.72 (t, J = 9.8 Hz, 1H, H-6bA'), 3.67 (t, J = 6.5 Hz, 1H, H-5B), 3.52-3.43 (m, 2H, H-4A', H-5A'), 3.39 (td, J = 7.1, 8.8 Hz, 1H, OCHHCH₂), 3.26 (td, J = 7.9, 9.3 Hz, 1H, H-2A'), 2.93 (s, 1H, OH-3A'), 2.14, 2.13, 2.07, 2.01, 1.97, 1.93 (6 s, 18H, COCH₃), 1.56 (m, 2H, OCH₂CH₂), 1.31-1.25 (m, 4H, CH₂CH₂CH₃), 1.20 (d, J = 6.3 Hz, 3H, H-6C), 1.17 (s, 9H, C(CH₃)₃), 0.85 (m, 3H, CH₂CH₃). ¹³C NMR (CDCl₃, 100
MHz): δC 177.7, 170.7, 170.4, 170.2, 169.6, 169.3, 166.1, 162.1 (9 C=O), 136.7 (4° Ph), 133.4 (Bz), 129.7 (4° Bz), 129.5 (2 Bz), 129.4 (Ph), 128.6 (2 Bz), 128.4 (2 Ph), 126.2 (2 Ph), 101.8 (CHPh), 100.5 (C-1A), 100.2 (C-1C), 99.3 (C-1A'), 99.0 (C-1B), 92.2 (CCl3), 81.2 (C-4A'), 75.1 (C-3A), 74.8 (C-4A), 72.6 (C-3B), 72.4 (C-5A), 71.4 (C-5B), 71.2 (C-3C), 70.7 (C-2B), 70.2 (C-4C), 69.4 (C-5A'), 69.3 (OCH2CH2), 69.1 (C-2C), 68.6 (C-4B), 68.4 (C-3A'), 68.3 (C-6A'), 66.0 (C-5C), 64.7 (C-6A), 60.9 (C-6B), 59.7 (C-2A'), 51.0 (C-2A), 38.7 (C(CH3)3), 29.0, 28.0, 22.4 (CH2CH2CH2CH3), 27.0 (C(CH3)3), 23.2, 21.3, 20.8, 20.8, 20.7, 20.6 (6 COCH3), 15.9 (C-6C), 14.0 (CH2CH3). HRESIMS calculated for C62H80O27N2Cl3 [M-H]- 1389.4014, found 1389.4038.

2,3,4-Tri-O-benzyl-α-L-fucopyranosyl-(1→3)-4,6-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-penty 2-acetamido-2-deoxy-β-D-glucopyranoside (50)

Trisaccharide acceptor 1 (20 mg, 0.018 mmol) and fucosyl donor 39 (34.2 mg, 0.071 mmol, 4 equiv) and NIS (20.7 mg, 0.089 mmol, 5 equiv) were dissolved in anhydrous CH2Cl2 (2 mL). The solution was cooled to 0 °C under N2 for 20 min. A 20 µL solution of TMSOTf (0.646 µL, 0.004 mmol, 0.2 equiv) in anhydrous CH2Cl2 was added, and the mixture was stirred at 0 °C under N2 for 7.5 h, then quenched with NEt3. The mixture was warmed to RT and diluted with CH2Cl2 (10 mL), then washed with 20% aq. Na2S2O3 (15 mL). The aqueous layer was re-extracted with CH2Cl2 (3 × 5 mL), and the organic layers were dried and concentrated. Column
chromatography (CH$_2$Cl$_2$/MeOH, 100:1 → 20:1) gave tetrasaccharide 50 (15.1 mg, 55%) as a yellow solid, while recovering acceptor 1 (4.6 mg, 23%). [α]$_D$ -24.8 (c 0.81, MeOH). $^1$H NMR (CDCl$_3$, 400 MHz): δ$_H$ 8.00 (d, $J = 7.2$ Hz, 2H, Bz), 7.58 (t, $J = 7.4$ Hz, 1H, Bz), 7.45 (t, $J = 7.7$ Hz, 2H, Bz), 7.43-7.38 (m, 2H, Ph), 7.37-7.20 (m, 18H, 3 Ph, 15 Bn), 7.13 (d, $J = 7.1$ Hz, 1H, NHA'), 5.62 (d, $J = 7.9$ Hz, 1H, NHA), 5.45 (s, 1H, CHPh), 5.30 (d, $J = 2.9$ Hz, 1H, H-4B), 5.15 (dd, $J = 8.2$, 9.9 Hz, 1H, H-2B), 5.12 (d, $J = 8.0$ Hz, 1H, H-1A'), 5.08 (d, $J = 3.6$ Hz, 1H, H-1C'), 4.90-4.82 (m, 2H, CHHPh), 4.78 (d, $J = 8.1$ Hz, 1H, H-1A), 4.70-4.51 (m, 4H, 2 CHHPh), 4.49 (m, 1H, H-6aA), 4.42 (d, $J = 8.0$ Hz, 1H, H-1B), 4.34-4.24 (m, 3H, H-6bA, H-3A', H-6aA'), 4.22 (s, 1H, OH-3A), 4.18 (dd, $J = 4.1$, 11.5 Hz, 1H, H-6aB), 4.06-3.76 (m, 8H, H-3A, H-3B, H-5B, H-6bB, H-2C', H-3C', H-5C', OCHHCH$_2$), 3.71 (m, 1H, H-5A), 3.67 (t, $J = 11.3$ Hz, 1H, H-6bA'), 3.58 (t, $J = 9.1$ Hz, 1H, H-4A'), 3.53 (s, 1H, H-4C'), 3.52-3.36 (m, 4H, H-2A, H-5A, H-2A', OCHHCH$_2$), 2.11, 2.01, 1.98 (3 s, 9H, COCH$_3$), 1.52 (m, 2H, OCH$_2$CH$_2$), 1.30-1.21 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.18 (s, 9H, C(CH$_3$)$_3$), 0.82 (m, 3H, CH$_2$CH$_3$), 0.68 (d, $J = 6.4$ Hz, 3H, H-6C').

$^{13}$C NMR (CDCl$_3$, 150 MHz): δ$_C$ 178.0, 170.3, 169.9, 169.4, 166.1, 161.7 (6 C=O), 138.8, 138.4, 138.4 (3 4° Bn), 137.0 (4° Ph), 133.3 (Bz), 129.7 (4° Bz), 129.6 (2 Bz), 129.2 (Ph), 128.5 (2 Bz), 128.4 (2 Ph), 128.4, 128.3, 128.2, 128.2, 127.6, 127.5, 127.3, 127.1 (15 Bn), 126.2 (2 Ph), 101.9 (CHPh), 101.6 (C-1B), 100.1 (C-1A'), 98.9 (C-1A'), 98.2 (C-1C'), 91.8 (CCl$_3$), 82.0 (C-4A), 80.0 (C-4A'), 79.9 (C-3C'), 77.4 (C-4C'), 76.2 (C-2C'), 75.2 (C-3B), 74.9, 73.8, 72.5 (3 CH$_2$Ph), 73.8 (C-3A'), 72.0 (C-5B), 71.8 (C-5A), 71.4 (C-3A), 70.4 (C-2B), 69.8 (OCH$_2$CH$_2$), 68.6 (C-4B), 68.5 (C-6A'), 66.9 (C-3C'), 66.1 (C-5A'), 63.2 (C-6A), 62.3 (C-6B), 60.2 (C-2A'), 56.7 (C-2A), 38.8 (C(CH$_3$)$_3$), 29.1, 28.0, 22.2 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.0, 21.1, 20.8 (3 COCH$_3$), 16.1 (C-6C'), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{77}$H$_{92}$O$_{24}$N$_2$Cl$_3$ [M-H] $^{1533.5105}$, found 1533.5148.
2,3,4-Tri-O-benzyl-α-L-fucopyranosyl-(1→3)-4,6-benzylidene-2-deoxy-2-
trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-
galactopyranosyl-(1→4)-[2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→3)]-pentyl 2-acetamido-
2-deoxy-β-D-glucopyranoside (51)

Trisaccharide acceptor 1 (20 mg, 0.018 mmol) and fucosyl donor 39 (34.2 mg, 0.071 mmol, 4
equiv) were dissolved in an mixture of 1:1 anhydrous CH₂Cl₂/DMF (2 mL) containing activated
4Å MS (200 mg). The mixture was stirred at RT under N₂ for 1 h. CuBr₂ (19.9 mg, 0.089 mmol,
5 equiv) and Bu₄NBr (29.3 mg, 0.091 mmol, 5.1 equiv) were added, and the mixture was stirred
at RT under N₂ for 40 h. The mixture was filtered over celite and washed with CH₂Cl₂ (2 × 15
mL). The filtrate was washed with sat. aq. NaCl (15 mL) then with sat. aq. NaHCO₃ (5 × 15
mL). The aqueous layers were re-extracted with CH₂Cl₂ (3 × 15 mL), and the organic layers
were dried and concentrated. Reverse-phase HPLC (CH₃CN/H₂O, 60:40 → 90:10) gave
pentasaccharide 51 (16.3 mg, 47%) as a white solid, and also gave tetrasaccharide 50 (3.4 mg,
12%) as a white solid. Analytical data for 51: [α]D -52.0 (c 0.74, MeOH). ¹H NMR (CDCl₃, 400
MHz): δH 7.98 (d, J = 7.2 Hz, 2H, Bz), 7.58 (t, J = 7.4 Hz, 1H, Bz), 7.45 (t, J = 7.9 Hz, 2H, Bz),
7.43-7.40 (m, 2H, Ph), 7.38-7.34 (t, J = 7.3 Hz, 4H, 3 Ph, Bn), 7.33-7.20 (m, 29H, Bn), 6.83 (d, J
= 7.4 Hz, 1H, NHA'), 6.18 (d, J = 8.0 Hz, 1H, NHA), 5.48 (s, 1H, CHPh), 5.32 (d, J = 3.4 Hz,
1H, H-4B), 5.12 (d, J = 3.7 Hz, 1H, H-1C'), 5.10 (d, J = 7.7 Hz, 1H, H-1A'), 5.09 (d, J = 3.8 Hz,
1H, H-1C), 4.96-4.79 (m, m, 5H, CHHPh), 4.76 (d, J = 3.9 Hz, 1H, H-1A), 4.75-4.54 (m, 7H,
CHHPh), 4.68 (m, 1H, H-6aA), 4.60 (dd, J = 6.7, 12.0 Hz, 1H, H-6bA), 4.33 (m, 1H, H-6aA'),
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4.32 (d, $J = 7.9$ Hz, 1H, H-1B), 4.27 (t, $J = 9.4$ Hz, 1H, H-3A’), 4.09-4.05 (m, 3H, H-3A, H-2C, H-5C’), 4.04 (dd, $J = 7.9$, 13.0 Hz, 1H, H-6aB), 4.02 (m, 1H, H-5C), 4.00 (dd, $J = 3.7$, 10.1 Hz), 3.94 (dd, $J = 6.7$, 11.2 Hz, 1H, H-6bB), 3.91 (dd, $J = 2.6$, 10.2 Hz, 1H, H-3C’), 3.89-3.85 (m, 4H, H-4A, H-5A, H-3B, H-3C), 3.81 (t, $J = 5.0$ Hz, 1H, H-2A), 3.75-3.66 (m, 3H, H-5B, H-6bA', OCHHCH$_2$), 3.63 (t, $J = 9.5$ Hz, 1H, H-4A’), 3.56 (d, $J = 1.3$ Hz, 1H, H-4C), 3.54 (d, $J = 1.3$ Hz, 1H, H-4C’), 3.53-3.45 (m, 2H, H-5A, H-2A’), 3.37 (td, $J = 7.0$, 9.5 Hz, 1H, OCHHCH$_2$), 2.03, 2.01, 1.86 (3 s, 9H, COCH$_3$), 1.44 (m, 2H, OCH$_2$CH$_2$), 1.22-1.17 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.16 (s, 9H, C(CH$_3$)$_3$), 1.10 (d, $J = 6.5$ Hz, 3H, H-6C), 0.80 (t, $J = 6.7$ Hz, 3H, CH$_2$CH$_3$), 0.74 (d, $J = 6.4$ Hz, 3H, H-6C’).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$C 177.7, 170.1, 169.7, 169.6, 166.0, 161.6 (6 C=O), 138.9, 138.9, 138.8, 138.5, 138.5, 138.4 (6 4° Bn), 136.9 (4° Ph), 133.3 (Bz), 129.8 (4° Bz), 129.5 (2 Bz), 129.2 (Ph), 128.6 (2 Bz), 128.4 (2 Ph), 128.4, 128.3, 128.2, 128.1, 127.6, 127.5, 127.5, 127.3, 127.3, 127.2 (30 Bn), 126.2 (2 Ph), 101.8 (CHPh), 99.8 (C-1B), 99.7 (C-1A), 99.1 (C-1A’), 98.1 (C-1C’), 96.9 (C-1C), 91.9 (CCl$_3$), 80.0 (C-4A’), 79.8 (C-3C’), 79.2 (C-3C), 77.3 (C-4C, C-4C’), 76.3 (C-2C), 76.2 (C-2C’), 74.9, 74.8, 73.7, 73.0, 72.8, 72.5 (6 CH$_2$Ph), 74.8 (C-5A, C-3B), 73.6 (C-3A’), 73.4 (C-2A), 72.3 (C-3A), 72.0 (C-4A), 71.3 (C-5B), 70.6 (C-2B), 69.5 (OCH$_2$CH$_2$), 68.5 (C-4B), 68.4 (C-6A’), 67.0 (C-5C’), 66.8 (C-5C), 66.2 (C-5A’), 64.3 (C-6A), 61.1 (C-6B), 60.0 (C-2A’), 38.7 (C(CH$_3$)$_3$), 29.0, 28.0, 22.4 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.0, 21.2, 20.8 (3 COCH$_3$), 16.6 (C-6C’), 16.1 (C-6C), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{104}$H$_{120}$O$_{28}$N$_2$Cl$_3$ [M-H]$^-$ 1949.7093, found 1949.7154.
4.3.4 Synthetic Procedures for Glycosylations with Trisaccharide Acceptor 2

4,6-Di-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (52) & 3,4,6-Tri-O-acetyl-1,2-O-[4,6-Di-O-acetyl-2-deoxy-3-orthoacetyl-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside]β-D-galactopyranose (53)

The general procedure was applied with trisaccharide acceptor 2 and galactosyl donor 35 (55.2 mg), and Hg(CN)$_2$ (40.7 mg). Reverse-phase HPLC (CH$_3$CN/H$_2$O, 35:65 → 65:35) gave a mixture of tetrasaccharide 52 (13.6 mg, 35%), orthoester pentasaccharide 53 (3.0 mg, 6%), and recovered acceptor 1 (9.8 mg, 33%) as a white solid.
Analytical Data for 52: \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta_H\) 8.04 (d, \(J = 7.1\) Hz, 2H, Bz), 7.58 (t, \(J = 7.2\) Hz, 1H, Bz), 7.46 (t, \(J = 7.8\) Hz, 2H, Bz), 6.91 (d, \(J = 6.9\) Hz, 1H, NHA'), 6.46 (d, \(J = 9.6\) Hz, 1H, NHA), 5.38 (d, \(J = 3.4\) Hz, 1H, H-4C), 5.35 (d, \(J = 3.5\) Hz, 1H, H-4B), 5.17 (dd, \(J = 7.9, 10.4\) Hz, 1H, H-2C), 5.14 (d, \(J = 7.8\) Hz, 1H, H-1A'), 5.08-5.01 (m, 2H, H-2B, H-3C), 4.91 (d, \(J = 7.9\) Hz, 1H, H-1C), 4.84 (t, \(J = 8.8\) Hz, 1H, H-4A'), 4.67 (dd, \(J = 7.2, 11.0\) Hz, 1H, H-6aA), 4.39 (dd, \(J = 6.7, 11.5\) Hz, 1H, H-6bA), 4.35 (d, \(J = 3.7\) Hz, 1H, H-1A), 4.26 (d, \(J = 8.0\) Hz, 1H, H-1B), 4.22-3.94 (m, 12H, H-2A, H-3A, H-5A, H-5B, H-6aB, H-6bB, H-3A', H-6aA', H-6bA', H-5C, H-6aC, H-6bC), 3.90 (dd, \(J = 3.4, 10.2\) Hz, 1H, H-3B), 3.79 (td, \(J = 7.0, 9.4\) Hz, 1H, OCHHCH\(_2\)), 3.68-3.61 (m, 2H, H-4A, H-5A'), 3.34 (td, \(J = 7.2, 9.1\) Hz, 1H, OCHHCH\(_2\)), 3.24 (td, \(J = 6.8, 9.5\) Hz, 1H, H-2A'), 2.92 (d, \(J = 2.9\) Hz, 1H, OH-3A'), 2.13, 2.11, 2.11, 2.10, 2.06, 2.05, 2.03, 2.02, 1.94 (9 s, 27H, COCH\(_3\)), 1.60-1.51 (m, 2H, OCH\(_2\)CH\(_2\)), 1.32-1.22 (m, 4H, CH\(_2\)CH\(_2\)CH\(_3\)), 1.17 (s, 9H, C(CH\(_3\))\(_3\)), 0.85 (t, \(J = 6.7\) Hz, 3H, CH\(_2\)CH\(_3\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta_C\) 177.7, 171.0, 170.9, 170.7, 170.4, 170.3, 170.3, 170.0, 169.7, 169.6, 169.6, 166.1, 166.1, 162.1 (12 C=O), 133.4 (Bz), 129.8 (4° Bz), 129.5 (2 Bz), 128.6 (2 Bz), 100.1 (C-1A), 98.7 (C-1B), 98.6 (C-1A'), 98.1 (C-1C), 92.1 (CCl\(_3\)), 74.9 (C-3B), 74.3 (C-3A), 73.4 (C-5A), 72.1 (C-5A'), 71.9 (C-4A), 71.7 (C-5B), 71.6 (C-4A'), 70.8 (C-3C, C-5C), 70.4 (C-2B), 69.8 (C-3A'), 69.5 (OCH\(_2\)CH\(_2\)), 68.6 (C-4B), 68.3 (C-2C), 67.0 (C-4C), 64.9 (C-6A), 61.7 (C-6A'), 60.7 (C-6B), 60.6 (C-6C), 59.8 (C-2A'), 48.2 (C-2A), 38.7 (C(CH\(_3\))\(_3\)), 29.0, 28.1, 22.4 (CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 27.0 (C(CH\(_3\))\(_3\)), 23.0, 21.5, 20.8, 20.8, 20.8, 20.8, 20.7, 20.7, 20.6 (9 COCH\(_3\)), 14.0 (CH\(_2\)CH\(_3\)).

HRESIMS calculated for C\(_{61}\)H\(_{82}\)O\(_3\)N\(_2\)Cl\(_3\) [M-H]\(^-\) 1443.3967, found 1443.4015.

Analytical Data for 53: \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta_H\) 8.04 (d, \(J = 7.1\) Hz, 2H, Bz), 7.58 (t, \(J = 7.4\) Hz, 1H, Bz), 7.47 (t, \(J = 7.8\) Hz, 2H, Bz), 6.91 (d, \(J = 6.9\) Hz, 1H, NHA'), 6.46 (d, \(J = 9.6\) Hz, 1H, NHA), 5.68 (d, \(J = 4.4\) Hz, 1H, H-1C'), 5.46 (t, \(J = 3.4\) Hz, 1H, H-4A'), 5.39 (d, \(J = 2.8\) Hz, 1H, NHA), 5.21 (d, \(J = 8.8\) Hz, 1H, H-4A), 5.08 (d, \(J = 8.8\) Hz, 1H, H-4A), 3.79 (td, \(J = 3.4, 9.1\) Hz, 1H, OCHHCH\(_2\)), 3.34 (td, \(J = 7.2, 9.1\) Hz, 1H, OCHHCH\(_2\)), 3.24 (td, \(J = 6.8, 9.5\) Hz, 1H, H-2A'), 2.92 (d, \(J = 2.9\) Hz, 1H, OH-3A'), 2.13, 2.11, 2.11, 2.10, 2.06, 2.05, 2.03, 2.02, 1.94 (9 s, 27H, COCH\(_3\)), 1.60-1.51 (m, 2H, OCH\(_2\)CH\(_2\)), 1.32-1.22 (m, 4H, CH\(_2\)CH\(_2\)CH\(_3\)), 1.17 (s, 9H, C(CH\(_3\))\(_3\)), 0.85 (t, \(J = 6.7\) Hz, 3H, CH\(_2\)CH\(_3\)).
Hz, 1H, H-4C), 5.33 (d, J = 3.7 Hz, 1H, H-4B), 5.27 (d, J = 8.2 Hz, 1H, H-1A'), 5.16 (dd, J = 8.0, 10.5 Hz, 1H, H-2C), 5.08-4.97 (m, 3H, H-2B, H-3C, H-4C'), 4.96-4.89 (m, 2H, H-5A', H-3C'), 4.93 (d, J = 7.7 Hz, 1H, H-1C), 4.67 (dd, J = 7.1, 11.3 Hz, 1H, H-6aA), 4.41 (dd, J = 6.9, 11.3 Hz, 1H, H-6bA), 4.34 (d, J = 4.1 Hz, 1H, H-1A), 4.33-3.94 (m, 15H, H-2A, H-3A, H-5A, H-1B, H-5B, H-6aB, H-6bB, H-3A', H-6aA', H-5C, H-6aC, H-6bC, H-2C', H-6aC', H-6bC'), 3.90 (dd, J = 3.6, 10.2 Hz, 1H, H-3B), 3.79 (td, J = 6.8, 9.2 Hz, 1H, OCHHCH2), 3.69-3.58 (m, 3H, H-4A, H-6bA', H-5C'), 3.34 (td, J = 7.2, 9.1 Hz, 1H, OCHHCH2), 3.04 (td, J = 7.8, 9.9 Hz, 1H, H-2A'), 2.12, 2.11, 2.10, 2.10, 2.05, 2.05, 2.02, 2.02, 2.01, 1.94 (12 s, 36H, COCH3), 1.65-1.45 (m, 5H, CH3 orthoester, OCH2CH2), 1.32-1.22 (m, 4H, CH2CH2CH3), 1.16 (s, 9H, C(CH3)3), 0.86 (t, J = 6.7 Hz, 3H, CH2CH3). 13C NMR (CDCl3, 150 MHz): δC 177.6, 170.9, 170.7, 170.4, 170.3, 170.2, 170.1, 169.9, 169.7, 169.6, 169.7, 169.6, 169.4, 169.2, 166.2, 162.5 (15 C=O), 133.4 (Bz), 129.8 (4° Bz), 129.5 (2 Bz), 128.6 (2 Bz), 123.4 (4° orthoester), 100.1 (C-1A), 98.9 (C-1C), 98.1 (C-1A), 97.4 (C-1A'), 96.7 (C-1C'), 92.2 (CCl3), 76.6 (C-2C'), 74.5 (C-3B), 74.4 (C-3A), 73.5 (C-5A), 72.0 (C-3C'), 72.0 (C-5C'), 71.7 (C-4A), 71.5 (C-5B, C-5C'), 70.9 (C-3C, C-4C'), 70.8 (C-5C), 70.7 (C-2B), 69.4 (C-6A', OCH2CH2), 68.6 (C-5A'), 68.3 (C-4B, C-2C), 67.0 (C-4C), 66.1 (C-4A'), 64.9 (C-6A), 60.6 (C-6B), 60.6 (C-6C), 60.5 (C-6C'), 58.9 (C-2A'), 47.5 (C-2A), 38.7 (C(CH3)3), 29.0, 28.1, 22.4 (CH2CH2CH2CH3), 27.0 (C(CH3)3), 26.7 (CH3 orthoester), 23.0, 21.6, 20.8, 20.8, 20.7, 20.7, 20.7, 20.7, 20.7, 20.7, 20.6, 20.6 (12 COCH3), 14.0 (CH2CH3). HRESIMS calculated for C75H100O40N2Cl3 [M-H]- 1773.4917, found 1773.4974.
4,6- Di-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→3)]-pentyl-2-acetamido-2-deoxy-β-D-glucopyranoside (54)

The general procedure was applied with trisaccharide acceptor 2 and mannosyl donor 36 (55.2 mg), and Hg(CN)₂ (40.7 mg). Reverse-phase HPLC (CH₃CN/H₂O, 35:65 → 65:35) gave tetrasaccharide 54 (19.3 mg, 50%) as a white solid. [α]D +28.4 (c 1.0, MeOH). 

1H NMR (CDCl₃, 600 MHz): δH 8.01 (d, J = 7.7 Hz, 2H, Bz), 7.57 (t, J = 7.4 Hz, 1H, Bz), 7.45 (t, J = 7.5 Hz, 2H, Bz), 7.16 (d, J = 6.7 Hz, 1H, NHA’), 6.42 (d, J = 8.8 Hz, 1H, NHA), 5.32 (d, J = 2.3 Hz, 1H, H-4B), 5.31-5.22 (m, 3H, H-2C, H-3C, H-4C), 5.11-5.00 (m, 3H, H-2B, H-1A’, H-1C), 4.83 (t, J = 9.2 Hz, 1H, H-4A’), 4.71 (dd, J = 4.8, 11.6 Hz, 1H, H-6aA), 4.68-4.63 (m, 2H, H-1A, H-6bA), 4.36-4.26 (m, 2H, H-5C, H-6aC), 4.30 (d, J = 7.5 Hz, 1H, H-1B), 4.24-4.09 (m, 4H, H-3A’, H-6aA’, H-6bA’, H-6bC), 4.08-4.04 (m, 2H, H-3A, H-6aB), 4.03-3.92 (m, 3H, H-2A, H-5A, H-6bB), 3.91-3.82 (m, 3H, H-4A, H-3B, OCHHCH₂), 3.69 (t, J = 6.7 Hz, 1H, H-5B), 3.61 (d, J = 9.4 Hz, 1H, H-5A’), 3.38 (td, J = 7.1, 8.6 Hz, 1H, OCHHCH₂), 3.25 (td, J = 7.7, 9.2 Hz, 1H, H-2A’), 2.12, 2.09, 2.08, 2.07, 2.06, 2.03, 2.00, 1.99, 1.93 (9 s, 27H, COCH₃), 1.58 (m, 2H, OCH₂CH₂), 1.30-1.21 (m, 4H, CH₂CH₂CH₃), 1.17 (s, 9H, C(CH₃)₃), 0.84 (m, 3H, CH₂CH₃). 

13C NMR (CDCl₃, 100 MHz): δC 177.7, 171.0, 170.9, 170.7, 170.3, 170.2, 169.8, 169.7, 169.7, 169.6, 166.2, 162.3 (12 C=O), 133.4 (Bz), 129.6 (4° Bz), 129.6 (2 Bz), 128.6 (2 Bz), 100.0 (C-1B), 99.8 (C-1A), 98.9 (C-1C, 176 Hz), 98.6 (C-1A’), 92.1 (CCl₃), 75.5 (C-3B), 75.4 (C-3A), 73.8 (C-4A),
71.9 (C-5A), 71.8 (C-5A'), 71.8 (C-5B), 71.3 (C-4A'), 70.5 (C-2B), 69.9 (C-3A'), 69.6 (OCH₂CH₂), 69.3 (C-3C), 69.0 (C-5C), 69.0 (C-2C), 68.6 (C-4B), 65.9 (C-4C), 64.0 (C-6A), 62.4 (C-6C), 61.7 (C-6A'), 61.1 (C-6B), 59.7 (C-2A'), 51.0 (C(CH₃)₃), 29.0, 28.1, 22.4 (CH₂CH₂CH₂CH₃), 26.9 (C(CH₃)₃), 23.1, 21.2, 20.9, 20.8, 20.7, 20.7, 20.7, 20.6 (9 COCH₃), 14.0 (CH₂CH₃). HRESIMS calculated for C₆₁H₸₂O₃₁N₂Cl₃ [M-H] 1443.3967, found 1443.4006.

4,6- Di-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (55) & 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-di-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (56)

The general procedure was applied with trisaccharide acceptor 2 and rhamnosyl donor 37 (47.5 mg), and Hg(CN)₂ (40.7 mg). Reverse-phase HPLC (CH₃CN/H₂O, 35:65 → 65:35) gave
tetrasaccharide 55 (15.3 mg, 41%) as a white solid, and pentasaccharide 56 (8.2 mg, 18%) as a white solid.

**Analytical Data for 55:** [α]D -8.9 (c 0.78, MeOH). 1H NMR (CDCl₃, 600 MHz): δH 8.03 (d, J = 7.3 Hz, 2H, Bz), 7.57 (t, J = 6.5 Hz, 1H, Bz), 7.46 (t, J = 7.1 Hz, 2H, Bz), 7.05 (d, J = 6.5 Hz, 1H, NHA'), 6.44 (d, J = 8.9 Hz, 1H, NHA), 5.36 (s, 1H, H-4B), 5.25 (s, 1H, H-2C), 5.16 (dd, J = 7.9 Hz, 1H, H-3C), 5.11-5.02 (m, 3H, H-2B, H-1A', H-4C), 5.00 (s, 1H, H-1C), 4.84 (t, J = 9.1 Hz, 1H, H-4A'), 4.77-4.65 (m, 3H, H-1A, H-6aA, H-6bA), 4.34 (d, J = 7.9 Hz, 1H, H-1B), 4.22 (d, J = 7.7 Hz, 1H, H-6aA'), 4.16 (d, J = 13.0 Hz, 1H, H-6bA'), 4.14-4.09 (m, 3H, H-3A, H-4A, H-3B, OCH₂CH₂), 3.71 (t, J = 5.8 Hz, 1H, H-5B), 3.62 (d, J = 8.5 Hz, 1H, H-5A'), 3.36 (td, J = 7.4, 7.9 Hz, 1H, OCH₂CH₂), 3.27 (td, J = 8.1, 8.4 Hz, 1H, H-2A'), 3.13 (s, 1H, OH-3A'), 2.12, 2.09, 2.08, 2.07, 2.05, 2.00, 1.99, 1.93 (8 s, 24H, COCH₃), 1.55 (m, 2H, OCH₂CH₂), 1.30-1.22 (m, 4H, CH₂CH₂CH₃), 1.20 (d, J = 5.9 Hz, 3H, H-6C), 1.14 (s, 9H, C(CH₃)₃), 0.84 (t, J = 6.6 Hz, 3H, CH₂CH₃). 13C NMR (CDCl₃, 100 MHz): δC 177.7, 170.9, 170.8, 170.7, 170.2, 169.9, 169.9, 169.9, 169.6, 166.3, 162.2 (11 C=O), 133.4 (Bz), 129.8 (4° Bz), 129.6 (2 Bz), 128.6 (2 Bz), 99.7 (C-1B), 99.4 (C-1A), 98.8 (C-1A'), 95.7 (C-1C, 175 Hz), 92.1 (CCl₃), 75.4 (C-3B), 73.4 (C-4A), 72.2 (C-5A), 71.9 (C-3A, C-5A'), 71.7 (C-5B), 71.4 (C-4A'), 70.8 (C-2B), 70.4 (C-4C), 70.0 (C-3A'), 69.7 (OCH₂CH₂), 69.4 (C-2C), 69.1 (C-3C), 68.9 (C-4B), 67.1 (C-5C), 64.6 (C-6A), 61.7 (C-6C), 61.3 (C-6B), 59.7 (C-2A'), 38.6 (C(CH₃)₃), 28.9, 28.1, 22.4 (CH₂CH₂CH₂CH₃), 27.0 (C(CH₃)₃), 23.1, 21.2, 20.9, 20.9, 20.8, 20.8, 20.8, 20.7 (8 COCH₃), 17.4 (C-6C), 13.9 (CH₂CH₃). HRESIMS calculated for C₅₉H₈₀O₂₉N₂Cl₃ [M-H]- 1385.3912, found 1385.3957.
Analytical Data for 56: [α]_D 12.7 (c 0.415, MeOH). $^1$H NMR (CDCl$_3$, 600 MHz): δ$_H$ 8.03 (d, $J$ = 7.4 Hz, 2H, Bz), 7.57 (t, $J$ = 7.9 Hz, 1H, Bz), 7.50-7.42 (m, 3H, NHA', 2 Bz), 6.47 (d, $J$ = 8.3 Hz, 1H, NHA), 5.35 (s, 1H, H-4B), 5.28 (d, $J$ = 8.3 Hz, 1H, H-1A'), 5.26 (s, 1H, H-2C), 5.18 (m, 1H, H-3C), 5.16 (d, $J$ = 2.3 Hz, 1H, H-2C'), 5.12 (d, $J$ = 8.2 Hz, 1H, H-3C'), 5.09-5.03 (m, 2H, H-2B, H-4C), 5.01 (s, 1H, H-1C), 5.00-4.95 (m, 2H, H-4A', H-4C'), 4.75 (s, 1H, H-1C'), 4.72 (dd, $J$ = 4.5, 10.5 Hz, 2H, H-6aA, H-6bA), 4.69 (s, 1H, H-1A), 4.37 (t, $J$ = 9.8 Hz, 1H, H-3C'), 4.36 (d, $J$ = 8.0 Hz, 1H, H-1B), 4.20-4.07 (m, 4H, H-3A, H-6aB, H-6aA', H-6bA'), 4.04-3.94 (m, 4H, H-2A, H-5A, H-6bB, H-5C), 3.92 (dd, $J$ = 2.5, 10.4 Hz, 1H, H-3B), 3.70 (t, $J$ = 6.4 Hz, 1H, H-5B), 3.55 (d, $J$ = 8.8 Hz, 1H, H-5A'), 3.36 (td, $J$ = 6.8, 8.2 Hz, 1H, OCHHCH$_2$), 3.17 (td, $J$ = 7.8, 8.3 Hz, 1H, H-2A'), 2.12, 2.12, 2.08, 2.07, 2.06, 2.05, 2.01, 2.00, 2.00, 1.93, 1.91 (11 s, 33H, COCH$_3$), 1.56 (m, 2H, OCH$_2$CH$_2$), 1.31-1.23 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.21 (d, $J$ = 6.1 Hz, 3H, H-6C), 1.15 (s, 9H, C(CH$_3$)$_3$), 1.12 (d, $J$ = 5.9 Hz, 3H, H-6C'), 0.85 (t, $J$ = 6.0 Hz, 3H, CH$_2$CH$_2$). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ$_C$ 177.7, 170.7, 170.7, 170.7, 170.3, 170.2, 169.9, 169.9, 169.9, 169.9, 169.9, 169.7, 169.6, 166.3, 162.3 (14 C=O), 133.2 (Bz), 129.8 (4° Bz), 129.6 (2 Bz), 128.6 (2 Bz), 99.7 (C-1B), 99.7 (C-1C', 173 Hz), 99.5 (C-1A), 97.5 (C-1A'), 95.6 (C-1C, 176 Hz), 91.7 (CCl$_3$), 78.8 (C-3A'), 74.8 (C-3B), 73.2 (C-4A), 72.1 (C-5A), 71.9 (C-5A'), 71.7 (C-3A, C-5B), 70.8 (C-2B), 70.4 (C-4C), 70.3 (C-4A'), 70.2 (C-4C'), 69.7 (C-2C'), 69.6 (OCH$_2$CH$_2$), 69.3 (C-2C), 69.1 (C-3C), 68.9 (C-4B), 68.8 (C-3C'), 67.8 (C-5C'), 67.1 (C-5C), 64.7 (C-6A), 61.6 (C-6C), 61.2 (C-6B), 59.2 (C-2A'), 38.6 (C(CH$_3$)$_3$), 28.9, 28.2, 22.4 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.0, 21.3, 22.2, 20.9, 20.9, 20.8, 20.8, 20.8, 20.7 20.7 (11 COCH$_3$), 17.4 (C-6C), 17.1 (C-6C'), 13.9 (CH$_2$CH$_3$). HRESIMS calculated for C$_{71}$H$_{96}$O$_{36}$N$_2$Cl$_3$ [M-H]$^-$ 1657.4808, found 1657.4851.
4,6- Di-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-acetyl-β-L-fucopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (57)

The general procedure was applied with trisaccharide acceptor \(2\) and fucosyl donor \(35\) (47.5 mg), and \(\text{Hg(CN)}_2\) (40.7 mg). Reverse-phase HPLC (CH\(_3\)CN/H\(_2\)O, 35:65 → 65:35) gave tetrasaccharide \(57\) (20.0 mg, 54%) as a white solid. \([\alpha]_D\) -2.8 (c 1.0, MeOH). \(^1\)H NMR (CDCl\(_3\), 600 MHz): \(\delta_\text{H} 8.01\) (d, \(J = 7.1\) Hz, 2H, Bz), 7.57 (t, \(J = 6.6\) Hz, 1H, Bz), 7.45 (t, \(J = 7.2\) Hz, 2H, Bz), 7.13 (d, \(J = 6.0\) Hz, 1H, NHA'), 6.29 (d, \(J = 8.9\) Hz, 1H, NHA), 5.38 (s, 1H, H-4B), 5.18 (s, 1H, H-4C), 5.14-5.07 (m, 2H, H-1A', H-2C), 5.04 (t, \(J = 8.2\) Hz, 1H, H-2B), 4.96 (d, \(J = 7.9\) Hz, 1H, H-3C), 4.84 (t, \(J = 9.2\) Hz, 1H, H-4A'), 4.70-4.59 (m, 3H, H-1A, H-6aA, H-1C), 4.57 (m, 1H, H-6bA), 4.36 (d, \(J = 7.9\) Hz, 1H, H-1B), 4.23-4.13 (m, 3H, H-3A', H-6aA', H-6bA'), 4.11-4.03 (m, 3H, H-2A, H-3A, H-6aB), 3.96 (m, 1H, H-6bB), 3.91-3.86 (m, 3H, H-4A, H-5A, H-3B), 3.84-3.76 (m, 2H, H-5C, OCH\(_2\)CH\(_2\)), 3.68 (m, 1H, H-5B), 3.62 (d, \(J = 8.1\) Hz, 1H, H-5A'), 3.39 (m, 1H, OCH\(_2\)CH\(_2\)), 3.25 (m, 1H, H-2A'), 2.14, 2.12, 2.08, 2.07, 2.05, 2.01, 1.96, 1.93 (8 s, 24H, COCH\(_3\)), 1.56 (m, 2H, OCH\(_2\)CH\(_2\)), 1.32-1.25 (m, 4H, CH\(_2\)CH\(_2\)CH\(_3\)), 1.20 (d, \(J = 5.8\) Hz, 3H, H-6C), 1.16 (s, 9H, C(CH\(_3\))\(_3\)), 0.86 (m, 3H, CH\(_2\)CH\(_3\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta_\text{C} 177.7, 170.9, 170.7, 170.7, 170.4, 170.3, 170.2, 169.6, 169.3, 166.1, 162.2\) (11 C=O), 133.4 (Bz), 129.7 (4° Bz), 129.5 (2 Bz), 128.6 (2 Bz), 100.5 (C-1A), 100.2 (C-1C), 99.0 (C-1B), 98.7 (C-1A'), 92.2 (CCl\(_3\)), 75.2 (C-3B), 74.8 (C-3A), 72.7 (C-4A), 72.4 (C-5A), 71.9 (C-5A'), 71.6 (C-
2,3,4-Tri-O-benzyl-α-L-fucopyranosyl-(1→3)-4,6-di-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (58)

Trisaccharide acceptor 2 (20 mg, 0.018 mmol) and fucosyl donor 39 (34.2 mg, 0.071 mmol, 4 equiv) and NIS (20.7 mg, 0.089 mmol, 5 equiv) were dissolved in anhydrous CH$_2$Cl$_2$ (2 mL). The solution was cooled to 0 °C under N$_2$ for 20 min. A 20 µL solution of TMSOTf (0.646 µL, 0.004 mmol, 0.2 equiv) in anhydrous CH$_2$Cl$_2$ was added, and the mixture was stirred at 0 °C under N$_2$ for 7.5 h, then quenched with NEt$_3$. The mixture was warmed to RT and diluted with CH$_2$Cl$_2$ (10 mL), then washed with 20% aq. Na$_2$S$_2$O$_3$ (15 mL). The aqueous layer was reextracted with CH$_2$Cl$_2$ (3 × 5 mL), and the organic layers were dried and concentrated. Reverse-phase HPLC (CH$_3$CN/H$_2$O, 60:40 → 90:10) gave tetrasaccharide 58 (10.3 mg, 37%) as a white solid. $[\alpha]_D$ -13.5 (c 0.515, MeOH). $^1$H NMR (CDCl$_3$, 400 MHz): δH 8.01 (d, $J = 7.2$ Hz, 2H, Bz), 7.59 (t, $J = 7.5$ Hz, 1H, Bz), 7.45 (t, $J = 7.7$ Hz, 2H, Bz), 7.35-7.21 (m, 15H, 15 Bn), 7.08 (d, $J = 6.8$ Hz, 1H, NHA'), 5.56 (d, $J = 7.9$ Hz, 1H, NHA), 5.34 (d, $J = 3.2$ Hz, 1H, H-4B), 5.12 (dd, $J =$
8.0, 10.0 Hz, 1H, H-2B), 5.02 (t, J = 9.2 Hz, 1H, H-4A'), 4.99-4.84 (m, 3 H, H-1C', CHHPh),
4.82 (d, J = 7.9 Hz, 1H, H-1A'), 4.76 (d, J = 8.2 Hz, 1H, H-1A), 4.71-4.54 (m, 4H, CHHPh),
4.49 (dd, J = 1.4, 11.6 Hz, 1H, H-6aA), 4.43 (d, J = 8.0 Hz, 1H, H-1B), 4.27 (dd, J = 4.9, 11.8 Hz,
1H, H-6bA), 4.24-4.14 (m, 3H, H-6aA', OH-3A), 4.08-3.92 (m, 4H, H-3A, H-6bB, H-6bA', H-2C'), 3.90-3.76 (m, 6H, H-3B, H-5B, H-3A', H-3C', H-5C', OCHHCH₂), 3.70 (m, 1H, H-5A), 3.64 (s, 1H, H-4C'), 3.57 (td, J = 7.8, 10.4 Hz, 1H, H-2A'), 3.54-3.46 (m, 2H, H-4A, H-5A'), 3.43 (td, J = 8.1, 9.7 Hz, 2H, H-2A, OCHHCH₂), 2.13, 2.07, 2.00, 1.97, 1.96 (5 s, 15H, COCH₃), 1.53 (m, 2H, OCH₂CH₂), 1.30-1.21 (m, 4H, CH₂CH₂CH₃), 1.17 (s, 9H, C(CH₃)₃), 1.03 (d, J = 6.4 Hz, 3H, H-6C'), 0.83 (m, 3H, CH₂CH₃). ¹³C NMR (CDCl₃, 100 MHz): δC 178.1, 170.8, 170.3, 169.9, 169.7, 169.1, 166.2, 162.1 (8 C=O), 138.8, 138.2, 138.2 (3 4° Bn), 133.3 (Bz), 129.7 (4° Bz), 129.6 (2 Bz), 128.6 (2 Bz), 128.5, 128.4, 128.2, 127.8, 127.8, 127.6, 127.4 (15 Bn), 101.7 (C-1B), 100.6 (C-1C'), 100.1 (C-1A), 99.6 (C-1A'), 92.0 (CCl₃), 82.0 (C-4A), 79.9 (C-3A'), 78.4 (C-3C'), 76.8 (C-4C'), 76.5 (C-2C'), 75.0, 74.3, 72.4 (3 CH₂Ph), 74.8 (C-3B), 72.2 (C-5A'), 72.0 (C-5A), 71.8 (C-5B), 71.5 (C-3A), 70.4 (C-2B), 69.9 (C-4A'), 69.9 (OCH₂CH₂), 68.7 (C-4B), 67.8 (C-5C'), 63.2 (C-6A), 62.4 (C-6B), 61.4 (C-6A'), 58.3 (C-2A'), 56.6 (C-2A), 38.7 (C(CH₃)₃), 29.1, 28.0, 22.3 (CH₂CH₂CH₂CH₃), 27.0 (C(CH₃)₃), 23.6, 21.1, 21.0, 20.9, 20.7 (5 COCH₃), 16.4 (C-6C'), 14.0 (CH₂CH₃). HRESIMS calculated for C₇₄H₉₂O₂₆N₂Cl₃ [M-H]⁺ 1529.5003, found 1529.5030.
4.3.5 Synthetic Procedure for Glycosylation with Trisaccharide Acceptor 3

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)]-pentyl 2-acetamido-2-deoxy-β-D-glucoopyranoside (59)

The general procedure was applied with trisaccharide acceptor 3 and galactosyl donor 35 (60.9 mg), and Hg(CN)₂ (44.9 mg). Reverse-phase HPLC (CH₃CN/H₂O, 20:80 → 50:50) gave pentasaccharide 59 (25.3 mg, 51%) as a white solid. [α]D -11.3 (c 0.185, MeOH). ¹H NMR (CDCl₃, 400 MHz): δH 8.04 (d, J = 7.1 Hz, 2H, Bz), 7.58 (t, J = 7.4 Hz, 1H, Bz), 7.46 (t, J = 7.8 Hz, 2H, Bz), 6.12 (d, J = 9.6 Hz, 1H, NHA), 5.65 (d, J = 7.0 Hz, 1H, NHA’), 5.38 (d, J = 3.4 Hz, 1H, H-4C), 5.34 (d, J = 3.8 Hz, 1H, H-4B), 5.31 (d, J = 2.5 Hz, 1H, H-4C’), 5.19 (d, J = 7.7 Hz, 1H, H-1A’), 5.16 (dd, J = 7.9, 10.4 Hz, 1H, H-2C), 5.06-4.97 (m, 3H, H-2B, H-3C, H-2C’), 4.95-4.87 (m, 2H, H-4A’, H-3C’), 4.90 (d, J = 7.7 Hz, 1H, H-1C), 4.66 (dd, J = 7.3, 11.4 Hz, 1H, H-6aA), 4.53 (t, J = 10.2 Hz, 1H, H-3A’), 4.45 (d, J = 7.8 Hz, 1H, H-1C’), 4.43 (m, 1H, H-6bA), 4.40 (d, J = 3.4 Hz, 1H, H-1A), 4.30 (d, J = 8.0 Hz, 1H, H-1B), 4.23-3.92 (m, 13H, H-2A, H-3A, H-5A, H-6aB, H-6bB, H-6aA’, H-6bA’, H-5C, H-6aC, H-6bC, H-5C’, H-6aC’, H-6bC’), 3.85-3.77 (m, 3H, H-3B, H-5B, OCH₂H₂), 3.66 (t, J = 6.9 Hz, 1H, H-4A), 3.60 (ddd, J = 2.8, 4.1, 10.4 Hz, 1H, H-5A’), 3.35 (td, J = 7.0, 9.1 Hz, 1H, OCH₂H₂), 2.84 (td, J = 7.3, 10.2 Hz, 1H, H-2")
1.54 (m, 2H, OCH$_2$CH$_2$), 1.32-1.22 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.16 (s, 9H, C(CH$_3$)$_3$), 0.86 (t, $J = 6.8$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$C 177.6, 171.2, 170.9, 170.7, 170.4, 170.4, 170.2, 170.1, 170.1, 170.0, 169.9, 169.6, 169.3, 169.1, 168.9, 166.1 (16 C=O), 133.3 (Bz), 129.8 (4° Bz), 129.5 (2 Bz), 128.5 (2 Bz), 100.6 (C-1C), 100.0 (C-1A), 98.8 (C-1A'), 98.7 (C-1B), 98.2 (C-1C), 76.0 (C-3A'), 75.5 (C-3B), 73.9 (C-3A), 73.1 (C-5A), 72.0 (C-5C'), 71.7 (C-5A'), 71.6 (C-4A), 70.9 (C-3C'), 70.9 (C-5C), 70.7 (C-2B, C-3C), 70.5 (C-5B), 69.5 (C-2C'), 69.4 (OCH$_2$CH$_2$), 68.7 (C-4B, C-4A'), 68.3 (C-2C), 66.9 (C-4C), 66.7 (C-4C'), 64.8 (C-6A), 61.8 (C-6A'), 60.8 (C-6B), 60.6 (C-6C, C-6C'), 58.6 (C-2A'), 47.9 (C-2A), 38.6 (C(CH$_3$)$_3$), 29.0, 28.1, 22.4 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.5, 23.1, 21.2, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5, 20.5 (12 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{75}$H$_{103}$O$_{40}$N$_2$ [M-H]$^-$ 1671.6087, found 1671.6108.
4.3.6 Synthetic Procedure for Glycosylation Under Basic Conditions

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-4,6-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (60) & 3,4,6-tri-O-acetyl-1,2-O-[4,6-benzylidene-2-deoxy-3-orthoacetyl-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1→4)-pentyl 2-acetamido-2-deoxy-β-D-glucopyranoside]-β-D-galactopyranose (61)

Trisaccharide acceptor 1 (20 mg, 0.018 mmol) and galactosyl donor 35 (14.7 mg, 0.036 mmol, 2 equiv) and AgOTf (9.2 mg, 0.036 mmol, 2 equiv) were cooled to 0 °C under N₂ for 25 min. A solution of TMU (4.5 µL, 0.038 mmol, 2.1 equiv) in anhydrous CH₂Cl₂ (2 mL) was added, and the mixture was stirred under N₂, starting at 0 °C and increasing to RT over 5 h, then for an additional 1 h at RT before being quenched with NEt₃. The mixture was filtered over celite and washed with CH₂Cl₂ (3 × 20 mL). The filtrate was washed with sat. aq. NaHCO₃ (20 mL). The aqueous layer was re-extracted with CH₂Cl₂ (3 × 5 mL), and the organic layers were dried and
concentrated. Reverse-phase HPLC (CH$_3$CN/H$_2$O, 35:65 → 65:35) gave a mixture of tetrasaccharide 60 (3.3 mg, 13%) as a white solid, orthoester tetrasaccharide 61 (2.8 mg, 11%) as a white solid, and recovered acceptor 1 (6.0 mg, 30%).

**Analytical Data for 60:** $^1$H NMR (CDCl$_3$, 600 MHz): $\delta$H 8.00 (d, J = 7.2 Hz, 2H, Bz), 7.58 (t, J = 7.6 Hz, 1H, Bz), 7.49-7.42 (m, 4H, 2 Bz, 2 Ph), 7.38-7.32 (m, 3H, Ph), 7.01 (d, J = 6.6 Hz, 1H, NHA'), 5.56 (d, J = 7.4 Hz, 1H, NHA), 5.50 (s, 1H, CHPh), 5.33 (d, J = 2.8 Hz, 1H, H-4B), 5.28 (d, J = 2.4 Hz, 1H, H-4C'), 5.18 (d, J = 8.0 Hz, 1H, H-1A'), 5.16-5.08 (m, 2H, H-2B, H-2C'), 4.88 (dd, J = 3.4, 10.5 Hz, 1H, H-3C'), 4.80 (d, J = 7.9 Hz, 1H, H-1A), 4.73 (d, J = 8.1 Hz, 1H, H-1C'), 4.54-4.47 (m, 2H, H-6aA, H-3A'), 4.45 (d, J = 7.9 Hz, 1H, H-1B), 4.34-4.26 (m, 2H, H-6bA, H-3A'), 4.19 (dd, J = 4.9, 11.3 Hz, 1H, H-6aB), 4.17 (s, 1H, OCH$_2$), 3.88 (dd, J = 8.3, 8.9 Hz, 1H, H-1A'), 3.85-3.77 (m, 2H, H-5B, OCH$_2$HCH$_2$), 3.75-3.67 m, 4H, H-5A, H-4A', H-6bA', H-5C), 3.51 (t, J = 8.5 Hz, 1H, H-4A), 3.48-3.42 (m, 2H, H-5A', OCH$_2$HCH$_2$), 3.39 (td, J = 8.3, 8.9 Hz, 1H, H-2A), 3.28 (td, J = 7.6, 9.5 Hz, 1H, H-2A'), 2.12, 2.10, 2.08, 1.99, 1.97, 1.92, 1.79 (7 s, 21H, COCH$_3$), 1.61-1.49 (m, 2H, OCH$_2$CH$_2$), 1.29-1.22 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.18 (s, 9H, C(CH$_3$)$_3$), 0.83 (t, J = 6.8 Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$C 177.6, 170.7, 170.2, 170.1, 169.9, 169.7, 169.6, 169.4, 166.2, 162.5 (10 C=O), 133.4 (Bz), 129.8 (4° Bz), 129.5 (2 Bz), 128.6 (2 Bz), 121.7, 101.5 (C-1B), 101.4 (CHPh), 100.1 (C-1A), 98.9 (C-1A', C-1C'), 81.4 (C-4A), 78.0 (C-4A'), 75.5 (C-3B), 75.0 (C-3A'), 71.8 (C-5B), 71.6 (C-5A), 71.0 (C-3A), 70.6 (C-3C'), 70.5 (C-5C'), 70.1 (C-2B), 69.8 (OCH$_2$CH$_2$), 68.5 (C-4B), 68.4 (C-2C'), 68.0 (C-6A'), 66.5 (C-4C'), 65.8 (C-5A'), 62.9 (C-6A), 61.9 (C-6B), 61.1 (C-6C'), 58.9 (C-2A'), 56.7 (C-2A), 39.0 (C(CH$_3$)$_3$), 29.0, 28.1, 22.4 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 23.0, 21.5, 20.8, 20.7, 20.7, 20.7, 20.6 (7 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{64}$H$_{82}$O$_{29}$N$_2$Cl$_3$ [M-H]$^-$ 1447.4068, found 1447.4096.
Analytical Data for 61: $^1\text{H}$ NMR (CDCl$_3$, 400 MHz): $\delta_H$ 8.00 (d, $J = 7.4$ Hz, 2H, Bz), 7.58 (t, $J = 6.5$ Hz, 1H, Bz), 7.49-7.42 (m, 4H, 2 Bz, 2 Ph), 7.38-7.31 (m, 3H, Ph), 6.92 (d, $J = 7.7$ Hz, 1H, NHA'), 5.72 (d, $J = 4.6$ Hz, 1H, H-1C'), 5.56 (d, $J = 7.6$ Hz, 1H, NHA), 5.50 (s, 1H, CHPh), 5.34 (d, $J = 2.9$ Hz, 1H, H-4C'), 5.32 (d, $J = 2.7$ Hz, 1H, H-4B), 5.15 (m, 1H, H-2B), 5.05 (d, $J = 8.6$ Hz, 1H, H-1A'), 4.94 (dd, $J = 3.2$, 6.5 Hz, 1H, H-3C'), 4.80 (d, $J = 8.0$ Hz, 1H, H-1A), 4.50 (m, 1H, H-6aA), 4.45 (d, $J = 8.2$ Hz, 1H, H-1B), 4.34-4.25 (m, 3H, H-6bA, H-3A', H-6aA'), 4.23-4.15 (m, 3H, H-6bA, H-2C', H-5C'), 4.10-4.02 (m, 2H, H-3A, H-6aC'), 4.00 (dd, $J = 5.9$, 11.4 Hz, 1H, H-6bC'), 3.96 (dd, $J = 8.0$, 11.2 Hz, 1H, H-6bB), 3.88 (dd, $J = 3.4$, 9.8 Hz, 1H, H-3B'), 3.83 (m, 1H, H-5B), 3.80 (m, 1H, OCH$_2$CH$_2$), 3.75-3.62 (m, 2H, H-5A, H-6bA'), 3.55-3.48 (m, 2H, H-4A, H-4A'), 3.44 (m, 2H, H-5A', OCH$_2$HCH$_2$), 3.40 (td, $J = 8.7$, 9.0 Hz, 1H, H-2A), 3.28 (td, $J = 7.9$, 9.2 Hz, 1H, H-2A'), 2.12, 2.11, 2.04, 2.01, 1.98, 1.92 (6 s, 18H, COCH$_3$), 1.64 (s, 3H, CH$_3$ orthoester), 1.60-1.48 (m, 2H, OCH$_2$CH$_2$), 1.30-1.22 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.18 (s, 9H, C(CH$_3$)$_3$), 0.84 (t, $J = 6.6$ Hz, 3H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta_C$ 177.6, 170.3, 170.0, 169.9, 169.7, 169.7, 166.2, 162.3 (9 C=O), 136.9 (4° Ph), 133.4 (Bz), 129.7 (4° Bz), 129.6 (2 Bz), 129.5 (Ph), 128.6 (2 Bz), 128.2 (2 Ph), 126.0 (2 Ph), 121.7 (4° orthoester), 101.6 (C-1A), 101.3 (CHPh), 100.1 (C-1B), 98.8 (C-1A'), 97.5 (C-1C'), 92.1 (CCl$_3$), 82.0 (C-4A), 79.8 (C-4A'), 75.5 (C-3B), 74.2 (C-5C'), 72.0 (C-5B), 71.8 (C-5A), 71.3 (C-3A), 71.1 (C-3C'), 70.7 (C-2B), 70.2 (C-3A'), 69.9 (OCH$_2$CH$_2$), 69.2 (C-2C'), 68.5 (C-4B), 68.3 (C-6A'), 66.5 (C-5A'), 65.8 (C-4C'), 63.5 (C-6A), 62.4 (C-6B), 61.1 (C-6C'), 59.0 (C-2A'), 56.8 (C-2A), 38.7 (C(CH$_3$)$_3$), 29.1, 28.0, 22.3 (CH$_2$CH$_2$CH$_2$CH$_3$), 27.0 (C(CH$_3$)$_3$), 25.6 (CH$_3$ orthoester), 22.9, 21.7, 20.8, 20.7, 20.6, 20.6 (6 COCH$_3$), 14.0 (CH$_2$CH$_3$). HRESIMS calculated for C$_{64}$H$_{82}$O$_{29}$N$_2$Cl$_3$ [M-H] $^{+}$ 1447.4068, found 1447.4118.
Chapter 5

References


