Synthesis And Á-Stereoselective Sialylation Of Kdn And Late Stage Modification Of Neuraminic Acid Sialosides

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SYNTHESIS AND $\alpha$-STEREOSELECTIVE SIALYLATION OF KDN AND LATE STAGE MODIFICATION OF NEURAMINIC ACID SIALOSIDES

by

CHANDRA SEKHAR NAVULURI

DISSERTATION

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of Wayne State University,

Detroit, Michigan

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DOCTOR OF PHILOSOPHY

2013

MAJOR: CHEMISTRY (Organic)

Approved by:

_______________________________
Advisor

_______________________________
Date
DEDICATION

Dedicated to my parents, sister and brother-in-law
ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude to my supervisor Prof. David Crich, for his unfailing support, mentorship, inspiration and encouragement throughout the length of my graduate studies, without which this work would not have been possible. My sincere thanks to my thesis committee members Prof. Cha, Prof. Verani, Prof. Montogomery and Prof. Groysman for spending their valuable time to be a part of my thesis committee. I am most thankful to Prof. Groysman for agreeing to be a part of my thesis committee at such a short notice.

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>Ada</td>
<td>Adamantyl</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>Ar</td>
<td>Aryl</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AWMS</td>
<td>Acid washed molecular sieves</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butoxycarbonyl</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
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<tr>
<td>BSP</td>
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</tr>
<tr>
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<td>m-CPBA</td>
<td>m-Chloroperbenzoic acid</td>
</tr>
<tr>
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<td>Camphorsulfonic acid</td>
</tr>
<tr>
<td>DAST</td>
<td>diethylaminosulfur trifluoride</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
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<td>DDQ</td>
<td>2,3-Dichloro-5,6-dicyano-1,4-benzoquinone</td>
</tr>
<tr>
<td>DIPEA</td>
<td>Diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(Dimethylamino)-pyridine</td>
</tr>
<tr>
<td>DMP</td>
<td>Dimethoxy propane</td>
</tr>
<tr>
<td>DMTST</td>
<td>Dimethyl(methylthio)sulfonium triflate</td>
</tr>
<tr>
<td>DPSO</td>
<td>Diphenyl sulfoxide</td>
</tr>
<tr>
<td>Abbr.</td>
<td>Full Form</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>ESIHRMS</td>
<td>Electrospray ionization high resolution mass spectroscopy</td>
</tr>
<tr>
<td>equiv</td>
<td>Equivalent</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
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<td>Fmoc</td>
<td>9-Fluorenlymethoxycarbonyl</td>
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<td>Glucosamine</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>KDN</td>
<td>Keto deoxy nonulosonic acid</td>
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<td>KDO</td>
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</tr>
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<td>Melting point</td>
</tr>
<tr>
<td>mmol</td>
<td>Milli mole</td>
</tr>
<tr>
<td>MTBE</td>
<td>Methyl tertiary butyl ether</td>
</tr>
<tr>
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<tr>
<td>Neu5Ac</td>
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</tr>
<tr>
<td>Neu5Gc</td>
<td>5-Glycolylneuraminic acid</td>
</tr>
<tr>
<td>Np</td>
<td>Naphthyl</td>
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</table>
NBz  $p$-Nitrobenzoyl
NBS  $N$-Bromosuccinimide
NIS  $N$-Iodosuccinimide
NMO  $N$-Methyl morpholine $N$-oxide
NMR  Nuclear magnetic resonance
nOe  Nuclear Overhauser effect spectroscopy
PCC  Pyridinium chlorochromate
PEP  Phosphoenol pyruvate
PG  Protecting group
Ph  Phenyl
Phth  Phthaloyl
Piv  Pivaloyl
PMB  $p$-Methoxybenzyl
ppm  Parts per million
PPTS  Pyridinium-$p$-toluenesulfonate
pTSA  4-Toluene sulfonic acid
Py  Pyridine
rt  Room temperature
SE  (2-Trimethylsilyl)ethyl
Sia  Sialic acid
Siglec  Sialic acid-binding immunoglobulin-like lectin
SFORD  Single frequency off resonance decoupling
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>sTn</td>
<td>Sialyl Tn antigen (sia $\alpha(2\rightarrow6)$galNAc)</td>
</tr>
<tr>
<td>TACA’s</td>
<td>Tumor associated cancer antigens</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBS</td>
<td>$\text{tert-}$Butyldimethylsilyl</td>
</tr>
<tr>
<td>TBSOTf</td>
<td>$\text{tert-}$butyldimethylsilyl triflate</td>
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<td>$\text{tert-}$Butyldiphenylsilyl</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
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<td>TfOH</td>
<td>Trifluoromethanesulfonic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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<tr>
<td>Troc</td>
<td>2,2,2-Trichloroethoxycarbonyl</td>
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<tr>
<td>TTBP</td>
<td>2,4,6-tri-$\text{tert-}$butylpyrimidine</td>
</tr>
<tr>
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<td>Toluencyl</td>
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CHAPTER 1
INTRODUCTION

1.1 Sialic acids

Sialic acids are eight and nine carbon ulosonic acids featuring an anomeric carboxylic acid and a deoxygenated C-3 methylene group. They are found in most vertebrates occupying the outermost positions on the glycans coating the cell surface and are vital elements in various physiological and pathological processes. The pre-eminent member of this monosaccharide family of sugars, N-acetyl neuraminic acid 1 is a nine carbon deoxy sugar with an acetamido substituent at the C-5 position (Figure 1). The other important ulosonic acids of this class include keto deoxy nonulosonic acid 2, N-glycolyl neuraminic acid 3 and keto deoxy octulosonic acid 4. Post translational modifications on the sialic acid backbone, such as acetylation, methylation, sulfation, and phosphorylation impart diversity to this class of molecules and about fifty such sialic acids have been found to date. Legionaminic acid 5 and pseudaminic acid 6 are the 9-deoxy 7-amino variants of the sialic acid skeleton and are found in non-terminal positions in bacterial glycans.3

![Figure 1: Naturally occurring sialic acids](image-url)
1.2 Linkage diversity in sialic acids

The naturally occurring sialoside linkages are equatorial glycosidic linkages and are said to have the $\alpha$-configuration. The linkage diversity of sialic acids is well defined. Sialic acids are primarily linked to either a galactose or a galactosamine through either the 6-OH or 3-OH of the latter (Figure 2). The other important linkage of sialic acid is that of a homopolymer, linked $\alpha(2\rightarrow8)$ or $\alpha(2\rightarrow9)$. The anomeric carboxylic acid, usually negatively charged at physiological pH, is also found lactonized with hydroxyl groups on the adjacent glycans (Figure 2).

![Lactosyl neuraminic acid](image1)
![sTn antigen](image2)
![poly KDN](image3)

![sia $\alpha(2\rightarrow3)$ gal](image4)
![sia $\alpha(2\rightarrow6)$ gal/galNAc](image5)

Figure 2: Naturally occurring sialoside linkages

1.3 Biological roles of sialic acids

Sialic acids are structurally the most diverse among all sugars. This structural diversity translates into the multitude of biological functions they perform. $N$-Acetyl neuraminic acid and KDN are the two sialic acids that have been found in humans. Due to their size, charge and hydrophilic character, these sialic acids have functions ranging from simple physiological effects on the cellular environment to specific phenomena relating to molecular and cellular recognition. The specificity of their interactions arises from either the specific sialic acid...
derivative present at the terminal position or the specific linkage of the terminal sialic acid with
the preceding carbohydrate epitope on the glycan. Sialic acids act primarily in two ways, either
by acting as a biological receptor themselves or by masking a biological receptor.\textsuperscript{7} Well explored
cases of sialic acids acting as receptors for pathological agents include their binding of viral
influenza causing lectins, and the high specificity of α-sialoside in the GM3 saccharide for the
cholera toxin. The remarkable structural specificity of sialoside recognition is such that the
addition of a single acetate ester at \( \text{O-9} \) of the terminal sialic acid prevents their recognition and
cleavage by influenza causing neuraminidases.\textsuperscript{6} In rainbow trout eggs, the presence of the C-5
deaminated sialic acid keto deoxy nonulosonic acid (KDN) as a capping residue to the sialic acid
polymers is thought to be the reason for the non recognition of these cell surface glycans by viral
neuraminidases, thus affording a critical shield necessary to fend off pathogenic attacks during
early embryonic development of the organism.\textsuperscript{8} Initially believed to be limited to lower
vertebrates, KDN has been shown to be widely occurring in humans and has intriguing roles in
early development.\textsuperscript{9} Studies conducted with paired samples of blood obtained from mothers and
newborns of healthy human individuals showed that most of the KDN was found in red blood
cells as free sugar and that the amount of free KDN in fetal newborn red blood cells was 2.4 fold
higher than in red blood cells from the mother or from healthy non pregnant women.\textsuperscript{10} This
elevated expression of KDN was also found in human ovarian cancer cells\textsuperscript{11,12} and the level of
expression was found to have a correlation with the progression towards malignancy. As with
neuraminic acid, which has been widely recognized as an cancer marker by assessing the over
expression of sialyl Lewis X, the elevated expression of KDN containing glycans in ovarian
cancer cells and human lung cancer cells is paving the way for KDN to be recognized as an
important bio-marker.\textsuperscript{10,13} KDN containing glycans and polymers have been extensively found in
various human tissues albeit in lower concentrations compared to neuraminic acid. Research
towards the development of cancer vaccines and immunotherapies utilizing the antigenic
properties of neuraminic acid glycosides to trigger immune responses is increasingly showing
promise. The increasing understanding of the relevance of KDN overexpression in human
disease in such context holds promise for the use of KDN containing glycans as TACA’s.14

1.3.1 Recent progress in the synthesis of sialic acid inhibitors
Sialic acids have been implicated in various pathological processes like cholera,15 influenza,15
and Salla disease.15 Structural knowledge of the viral and bacterial neuraminidases have led to
rational design, synthesis16 and commercialization of popular drugs like Zanamivir, Relenza and
Tamiflu which have been successful in reducing the mortality caused by these diseases.17 Even
as the resistant strains of these viruses and bacteria gain effect,18-20 research towards the
discovery of newer inhibitor drugs is an active research goal in the scientific community,
showcasing the importance of exosialidase inhibition.17 The following two examples show the
growing understanding of sialic acid based diseases found in the recent years.
Adeno virus type 37 (Ad 37), known to cause severe epidemic ocular infections is characterized
by pain, inflammation, lacrimation and decreased vision that lasts for months or even years.21
This viral epidemic conjunctivitis did not have any treatments until recently when it was found
that Ad 37 virus contains sialic acid binding receptors and a glycan array analysis revealed a
tight binding to GD1α glycan (Figure 3) revealing the mode of action of the virus.21
The virus was further found to contain three sialic acid binding domains which led to a clever inhibitor design of trivalent α-sialosides. Screening experiments revealed trivalent sialic acid inhibitor ME0322 14 (Figure 4) to be highly potent in inhibiting Ad 37 infection with an IC₅₀ of 0.38 mM.²² This finding has led to the further development of sialic acid containing anti viral inhibitors for topical treatment of epidemic kerato conjunctivitis.

![Figure 3: The GD1a glycan](image)

The other important discovery pertains to Aspergillosis,²³ an infection known to cause severe respiratory complications in immunocompromised individuals. A sialidase isolated from the causative *Aspergillus fumigates* soil fungus was recently shown to be a KDNase.²⁴,²⁵ The well known neuraminidase inhibitor Neu5Ac2en 15 (Figure 5) did not have inhibitory effects on this sialidase while the KDN analog KDN2en 16 (Figure 5) successfully inhibited the *A. fumigatus* sialidase. Further studies showed that the enzyme active site does not possess the acetamide binding pocket typical to neuraminidases and a crystal structure with fluorinated KDN showed
two molecules of KDN bound at adjacent sites in the crystal structure leading to the possibility of poly KDN molecules being substrates to *A. fumigatus*. This study showed the isolation and structural determination of the first KDNase and opens opportunities for inhibitor design against aspergillosis.\textsuperscript{24}

![Image of chemical structures]

*Figure 5: 2.3 anhydro sialoside inhibitors*

### 1.4 Chemical synthesis of sialic acid glycoconjugates

The growing understanding of the sialome, defined as the complete sialic acid profile of an organism at a given time,\textsuperscript{26} has shed light on the plethora of roles sialic acids play in vertebrates and puts in context the development of methods to access well defined sialic acid containing glycoconjugates. The micro heterogeneous nature and the miniscule concentrations of these molecules makes their isolation from Nature cumbersome, and highlights the significance of efficient chemical or enzymatic methodology development for their synthesis. Although enzymes, or combinations of them, of high substrate promiscuity\textsuperscript{27} are finding use in synthesis of sialyl glycoconjugates, the development of chemical tools to synthesize these molecules efficiently remains an important frontier to facilitate access to meaningful quantities and expand access to non natural linkages.\textsuperscript{3} The naturally occurring sialosides are $\alpha$-linked making their chemical synthesis difficult and challenging. The complex sialic acid architecture and an electron withdrawing carboxylic acid anomeric functionality imposes a certain degree of difficulty in their activation and synthetic manipulations. Also, the electron-deficient anomeric center makes
the 2,3-elimination reaction more facile upon oxocarbenium ion formation (Scheme 1), and leads to the formation of the glycal 21, the main by-product in most sialylations. In addition, the formation of the equatorial α-glycosidic bond is contra-thermodynamic requiring the over-riding of anomeric effect. These factors contribute to the low yields and poor selectivity often encountered in the glycosylation reactions of sialic acids.

Scheme 1: Mechanism of a typical sialylation reaction

Due to these impediments, the development of α-selective sialylation methodology has been a formidable frontier in synthetic carbohydrate development. A multitude of varied approaches have been applied to this problem that can be broadly classified into three main types, i) auxiliary mediated sialylation, ii) installation of electron withdrawing groups at C-5, and iii) use of cyclic protecting groups.

1.4.1 Auxiliary mediated sialylation

Neighboring group participation is a very popular and widely used handle in controlling the stereoselectivity of a glycosylation reaction.\textsuperscript{28} The absence of a participating group close to the anomeric center in sialic acids has led scientists to take to the recourse of installing an additional
participating auxiliary group into the sialic acid skeleton. These initial attempts at α-selective sialylation using auxiliaries centered around the incorporation of a participating auxiliary on either the C-1 or C-3 carbons, or a non-participating auxiliary on O-4. The use of the C-1 auxiliary has been showcased by Gin\textsuperscript{29} and Takahashi\textsuperscript{30} groups who used 2-thioethyl ester (Scheme 2) and \(N, N\) glycolamide auxiliaries (Scheme 3). These strategies rely on stabilizing the putative oxocarbenium ion intermediate by participation of the auxiliary on the sterically less demanding β-face providing overall the desired α-sialoside.

Scheme 2: Takahashi’s 2-thioethyl ether C-1 auxiliary

Unfortunately, the Takahashi protocol incorporating 2-thioethyl ether auxiliary\textsuperscript{30} (Scheme 2) proved to be only modestly α-selective, which was rationalized by computing the stabilities of the α and β sulfonium ion intermediates, revealing them to be of similar energy.

Scheme 3: Gin’s \(N, N\)-glycolylamide auxiliary
Gin’s sialylation using a $N,N$ glycolamide auxiliary was carried out using a thiomethyl leaving (27) group as well in dehydrative fashion using a hemiketal donor 28. The glycolamide auxiliary was somewhat more $\alpha$-selective than the ethyl sulfonium auxiliary using primary carbohydrate alcohol acceptors but was only modestly $\alpha$-selective with hindered secondary alcohol acceptors. 29

The alternative installation of participating auxiliaries at C-3, 31 including halides, acetyl esters, thioethers, and phenyl selenides (33), is typically successful in affording entry to the $\alpha$-sialosides with a variety of leaving groups as displayed in Scheme 4.

**Scheme 4: Achieving $\alpha$-stereoselectivity with the aid of a participating group on C-3**

Wu and coworkers studied sialylation with a nonparticipating morpholine auxiliary at C-4 using sulfoxide activation methodology in a dehydrative sialylation reaction (Scheme 5). 32 The rationale in the auxiliary installation at C-4 involved the destabilization of the $\alpha$-sulfoxonium ion intermediate due to non-bonding interactions between the C-4 auxiliary and the $\alpha$-sulfoxonium ion 39. The alcohol would then attack the congested anomeric center form the less hindered $\alpha$-face on the $\beta$-sulfoxonium ion intermediate 38 leading to the desired $\alpha$-glycoside 40. The methodology was successful using unhindered primary alcohol acceptors, while the selectivity fell considerably when secondary alcohol acceptors were employed. No coupling products were isolated using hindered tertiary alcohols using this methodology.
Scheme 5: C-4 Auxiliary mediated sialylation

Though the methodologies using an auxiliary were successful in achieving good to modest α-selectivity, the extra steps necessary for their installation, with the correct stereochemistry for the C-3 and C-4 variations, and the extra steps needed to remove them make these strategies less attractive ways to conduct α-selective sialylation.

1.4.2 Installation of electron withdrawing groups at C-5

The use of an electron withdrawing group at the C-5 position (Figure 6) on sialic acid donors has been found to have significant influence on glycosylation selectivities. The initial observation by Boons that the N-diacetyl protected sialic acid donors were more reactive in comparison to peracetyl donor was attributed to the loss of hydrogen bonding effects between the donor and the acceptor. These reactions, although they conferred a modest increase in α-selectivity, were accompanied by glycal formation. The use of an NAc₂₃,₃₄ azide,₃₅-₃₇ trifluoroamide₃₈-₄₁ and N-Troc₄₂,₄₃ groups at the C-5 position allowed a significant increase in α-selectivity using primary alcohol acceptors, but was accompanied by complications in activation arising out of the disarming nature of these protecting groups. The increase in selectivity was also limited to the use of unhindered primary alcohol acceptors.
1.4.3 Use of cyclic protecting groups to achieve α-selectivity

The most notable improvement in the α-selective sialylation arena came about through the introduction of cyclic protecting groups such as the N-acetyl 5N, 4O oxazolidinone protected donors 46, 47 introduced by Crich and Li$^{44,45}$ (Figure 7). These donors did not suffer from unnatural modifications of the donor and did not necessitate the use of an auxiliary to control the stereoselectivity. Reactions using these donors could be run using premixing conditions employing NIS/TfOH activation conditions and were found to confer high α-selectivities. The Crich method$^{44,45}$ uses an extra acetyl group on the oxazolidinone moiety, in contrast to
Takahashi’s\textsuperscript{46} and De Meo’s oxazolidinone donors\textsuperscript{47 48, 49} which was found to improve the reactivity due to beneficial effects of double protection but also greatly facilitated the regeneration of the native C-5 acetamide under mild Zemplen deacetylation conditions.

\begin{center}
\begin{tikzpicture}
\node at (0,0) [draw, align=center, text width=0.75cm,minimum height=0.75cm] {\(46\) \(R = \text{Ph}\)}; \\

\node at (1.5,0) [draw, align=center, text width=0.75cm,minimum height=0.75cm] {\(47\) \text{Adamantanyl}}; \\

\node at (3,0) [draw, align=center, text width=0.75cm,minimum height=0.75cm] {\(50\) \(R = \text{Adamantyl, excellent a selectivity for primary secondary and tertiary alcohol acceptors}\)}; \\

\draw[->, thick] (0,0) -- (1.5,0); \\

\node at (4.5,0) [draw, align=center, text width=0.75cm,minimum height=0.75cm] {\(51\)}; \\

\end{tikzpicture}
\end{center}

\textbf{Scheme 6: Crich’s \(\alpha\)-selective acetyl oxazolidinone donors}

The selectivities in Crich’s methodology using the initial thiophenyl glycosyl donor\textsuperscript{45} (Scheme 6) were excellent for primary alcohol acceptors and fell slightly for hindered secondary alcohol acceptors. A significant improvement was brought about by changing the leaving group from thiophenyl to thioadamantanyl,\textsuperscript{44} which lowered the activation temperature from -40 °C to -78 °C and afforded excellent \(\alpha\)-selectivity for hindered secondary and tertiary alcohol acceptors.

The excellent \(\alpha\)-selectivity observed using the \(N\)-acetyl oxazolidinone donor \(47\) extended to the analogous \(N\)-glycolyl oxazolidinone donor\textsuperscript{48 52}, and was used to develop a one-pot glycosylation protocol to build oligosaccharides containing \(N\)-glycolyl neuraminic acid at the terminal position (Scheme 7).
Scheme 7: One-pot glycosylation to synthesize glycolyl sialic acid oligosaccharides

Wong and co-workers extended the high α-selectivity displayed by the N-acetyl oxazolidinone thioglycosides to the corresponding sialyl phosphate\(^{49}\) 56 (Scheme 8) and found that activation using milder TMSOTf conditions were successful for the construction of α-sialosides with hindered and unhindered acceptors.

Scheme 8: Wong’s α-selective sialyl phosphates

Sialoside mimics, the C- and S α-glycosides of sialic acid were efficiently synthesized by Crich and co-workers in a highly stereoselective fashion using a large array of nucleophiles, both C- and S-based. These first examples of electrophilic C-sialylation\(^{50,51}\) and S-sialylation\(^{51}\) showcase the beneficial effect of the N-acetyl oxazolidinone cyclic protecting group system in enabling synthesis of unnatural sialosides with excellent α-selectivity.
Scheme 9: Crich’s α-sialoside mimics

Sialyl sulfoxides are excellent glycosyl donors with mild activation conditions and have been extensively studied, but not applied in the sialic acid series until recently when the sialyl sulfoxide donor carrying an N-acetyl oxazolidinone protecting group 62 was synthesized by Xing and co-workers. This donor was successfully activated using triflic anhydride and was found to be highly α-selective under preactivation conditions (Scheme 10).

Scheme 10: α-Selective sialylation employing sialyl sulfoxides
In this sialylation reaction, the use of a stoichiometric DPSO under pre-activation conditions led to lower glycosylation yields and a significant yield of the elimination glycal byproduct $66$. Interestingly the use of higher equivalents of DPSO led to a decrease in the glycal formation and a significant increase in the glycosylation yield, indicating the stabilization of the intermediate oxocarbenium ion $62$ or of sialyl triflates by DPSO.$^{52}$

The use of a 5,7-oxazionone protecting group on the sialic acid scaffold has been studied by Crich and Wu$^{53}$ (Scheme 11) in an effort to mirror the beneficial effects of benzylidene-like six membered cyclic protecting groups, known to be highly stereodirecting in the mannose and glucose series.$^{54}$ However, the donor $69$ was found to be unselective in glycosylation reactions, probably due to the pseudoaxial side chain blocking the $\alpha$-face in the glycosylation event. Interestingly, however, a double $N$-protected donor $71$ revealed by Hanashima$^{55}$ employing bis-silyl groups and analogous to $69$ developed by Crich, was found to be $\alpha$-stereodirecting depending on the nucleophiles used in glycosylations.

**Scheme 11: Oxazinone protected sialosides donors developed by Crich and Hanashima**
1.5 Synthesis of α-KDN sialosides

The classical chemical methods for the synthesis of α-KDN sialosides mainly employ Koenigs-Knorr and Helfrich protocols in which the anomeric chlorides and bromides of KDN are employed as glycosyl donors. The 2-chloro derivative of KDN\textsuperscript{56} 78 (Scheme 13), synthesized by treating the corresponding O-acetate with dry hydrogen chloride gas, was found to be more stable than the anomeric bromide 73 and is stable enough to be purified by silica gel chromatography. The methyl-α-glycoside of KDN 74 was synthesized in 75% yield using the anomeric bromide 73 as the donor,\textsuperscript{56} and silver carbonate as the promoter. However, when cholesterol 74 was used as the acceptor and silver triflate as promoter the corresponding glycoside 76 was synthesized only in 12% yield as a 2:1 α/β anomeric mixture with the main side product, the glycal 77, isolated in 37% yield (Scheme 12).

![Scheme 12: Koenigs-Knorr glycosylation of KDN C-2 bromide](image)

The aryl α-glycosides of KDN have been synthesized using the glycosyl chloride as the donor (Scheme 13). The displacement of the anomeric chloride 78 was carried out in N,N-
methylformamide using the corresponding sodium salts of the aryl alcohols in excellent yields.\textsuperscript{56}

Scheme 13: Synthesis of α-aryl glycosides of KDN

In the Koenigs-Knorr glycosylation of the donor 73 with silver triflate as the promoter, and uridine 80 as acceptor, the coupled products of furanose ring structure 82 were found in addition to the expected pyranose structures 80 (Scheme 14). A mechanism involving participation of O-5 and a bridged intermediate 84 was proposed to account for the observed ring contraction.\textsuperscript{57}

Scheme 14: Ring contraction in glycosylation of C-2 bromides of KDN
An improved glycosylation of KDN was reported by Hasegawa and co-workers in the synthesis of the KDN-gangliosides GM3 and GM4 (Scheme 15) employing the peracetylated phenyl thioglycoside of KDN as the donor.\textsuperscript{58} The glycosylation of this donor with the galactose 3-OH acceptor in acetonitrile at -40 °C in presence of NIS and TMSOTf, gave exclusively the α-glycoside 88 in 49% yield. Also, a glycosylation with the lactose derived disaccharide 89 gave the α-linked trisaccharide 90 in 46% yield.

Scheme 15: Glycosylation of KDN thioglycoside

An elegant nucleophilic C-glycosylation of KDN phenyl sulfone 91 mediated by samarium iodide was reported by Linhardt and co-workers (Scheme 16).\textsuperscript{59} The phenylsulfone donor was coupled with a number of ketones and aldehydes forming α-C-glycosides in excellent yields. Importantly the galactose aldehyde coupling partners, when subjected to this methodology yielded α-C-glycosides in excellent yields.
Scheme 16: Linhardt’s samarium iodide mediated C-glycosylations

1.6 Synthesis of KDN

In higher animals the biosynthesis of KDN occurs by the condensation of mannose 6-phosphate with phosphoenol pyruvate mediated by KDN 9-phosphate synthetase, with the subsequent 9-dephosphorylation achieved by phosphatases (Figure 8). \(^{60}\)

\[
\text{Mannose} + \text{ATP} \longrightarrow \text{Man-6-P} + \text{ADP}
\]

\[
\text{Man-6-P} + \text{PEP} \longrightarrow \text{KDN-9-P} + \text{Pi}
\]

\[
\text{KDN-9-P} \longrightarrow \text{KDN} + \text{Pi}
\]

Figure 8: Biosynthesis of KDN

The intricate architecture of KDN, coupled with the range of its biological properties, have attracted considerable attention to the provision of a suitable synthetic route to this otherwise
sparse substance. The efforts towards the synthesis of KDN have centered around two main approaches.

1. Biomimetic chiral pool strategies hinging on the fusion of a mannose equivalent with a pyruvate synthon and

2. Total synthesis approaches from non-carbohydrate starting materials.

1.6.1 Enzymatic approaches to KDN

Several syntheses of KDN have been achieved using a biomimetic strategy by enzymatic, chemoenzymatic and chemical routes. Wong and co-workers initially showed the synthesis of KDN starting from D-mannose mediated by sialic acid aldolase (Scheme 17).\textsuperscript{61,62} The enzymatic system employed by them was compatible with various unprotected aldoses allowing synthesis of different sialic acids. More recently, Seeberger reported the discovery of a highly substrate promiscuous aldolase\textsuperscript{27} useful in the syn selective coupling of fully protected aldose precursors with oxaloacetate. Coupling of the mannose derived aldehyde 98 with oxaloacetate afforded the syn diastereomer 100 in 39% yield, which was deprotected using 10% TFA to give KDN in 45% yield (Scheme 17).

![Scheme 17: Enzymatic and Chemo-enzymatic syntheses of KDN](image-url)
1.6.2 Ogura’s synthesis of KDN

The biomimetic coupling of D-mannose with oxaloacetate as the pyruvate synthon was studied by Ogura using aqueous basic conditions (Scheme 18). The coupling reaction proceeded with 4.3:1 syn/anti selectivity and the decarboxylation step was optimized to improve the reproducibility in the presence of catalytic quantities of nickel. This approach was used to synthesize KDN itself in up to a 100 gm scale in 73% yield over 2 steps.

![Scheme 18: Biomimetic coupling of mannose with oxaloacetic acid to synthesize KDN](image)

1.6.3 Chan’s indium mediated synthesis of KDN

The indium-mediated Barbier alkylation reaction functions well under aqueous conditions. Chan and co-workers coupled D-mannose with the Vasella pyruvate synthon 103 under aqueous conditions using indium as mediator. The reaction afforded a 4:1 syn/anti ratio (Scheme 19) of the coupled products, which were separated by crystallization to afford the pure syn-diastereomer 104 in 64% yield. Ozonization under aqueous conditions then afforded KDN in 95% yield.
Scheme 19: Indium-mediated syn-selective synthesis by Chan

1.6.4 Banwell’s synthesis of KDN from quinic acid

Banwell reported an elegant synthesis of KDN starting from a disopropyl acetal\textsuperscript{66} protected methyl shikimate derivative \textbf{108} accessed from quinic acid derivative \textbf{106}.\textsuperscript{67} The synthesis of the core structure of KDN was arrived at using a clever ozonolysis sequence of \textbf{109} to form the ketoester aldehyde \textbf{110} in 63% yield which exists in the hemi-ketal form (Scheme 20). A Wittig homologation with (triphenylphosphorinylidene)acetaldehyde afforded the enal \textbf{111} in 78% yield, which was isomerized photolytically to afford enal \textbf{112} in 42% yield. Luche reduction\textsuperscript{68} and Upjohn oxidation\textsuperscript{69} furnished the protected derivative \textbf{113} of KDN in excellent yield, and acidic hydrolysis afforded KDN in 42% yield (Scheme 20).\textsuperscript{67}
Scheme 20: Banwell’s total synthesis of KDN

In an effort to synthesize unnatural KDN stereoisomers, the enal 111 was reduced using Luche conditions and subjected to Upjohn oxidation to afford a 1:1 mixture of the sialic acid triols 115 and 116 in 68% combined yield. The diastereomers were separated and were subjected to a similar deprotection regime as for 113 to afford 7 epi-KDN and 8 epi-KDN (Scheme 21). 67

Scheme 21: Synthesis of 7 and 8 epi KDN
1.6.5 Burke’s ring closing metathesis strategy for synthesis of KDN

Burke and Voight undertook a total synthesis approach using a desymmetrization by RCM strategy. The synthesis began with acid catalyzed ketalization of enantiomerically pure diol 118 and the γ-haloketone 117 to afford the ketal 119 in 90% yield (Scheme 22). Displacement of the bromo group by O-nitrophenylselenocyanate followed by an oxidation reduction sequence furnished a triene 120 in 96% yield. RCM using the Grubbs 1st generation catalyst afforded entry to the dioxabicyclo[3.2.1] octane core 121, which was subjected to a double Sharpless epoxidation to give 94% of the tetraol 122 that had the correct stereochemistry for the C-8 alcohol. In order to invert the C-4 alcohol stereocenter a tin acetal mediated tosylation was carried out to afford a fully protected derivative 123, which was subjected to double tosyl displacement using cesium acetate 18-crown-6 conditions to set the C-4 stereocenter.

Scheme 22: Burke’s desymmetrization strategy for the synthesis of KDN
Acid catalyzed methanolysis of ketal 123, followed by acetylation afforded the \( \beta \)-KDN C-glycoside 124, which was oxidized to the corresponding carboxylic acid using \textit{in-situ} generated RuO\(_4\) in 90% yield. The crude acid was converted to the methyl ester 125 by diazomethane treatment to complete the formal synthesis of KDN (Scheme 22).\(^70\)

\section*{1.6.7 Banwell’s first synthesis of KDN from a non-carbohydrate source}

Banwell published the first total synthesis of KDN from a non-carbohydrate source.\(^72\) The synthesis is inspired by the findings of Hudlicky and co-workers on the value of \textit{cis}-1,2-dihydroxy catechols as excellent starting materials for pentoses and hexoses.\(^73\) The synthesis began from the \textit{cis}-1,2-dihydroxy catechol 126 accessed using microbial oxidation from chloro benzene using Hudlicky’s protocol. An osmium tetroxide oxidation followed by a double silyl protection afforded 128 in 83% overall yield (Scheme 23). An ozonolysis/reduction sequence, afforded the methyl ester 129, which was protected as a TBS silyl ether and was subjected to a reduction/oxidation sequence to afford the six carbon aldehyde fragment 130, set for the coupling with the Vasella pyruvate equivalent. The Reformatsky reaction afforded a 2:3 \textit{syn/anti} selective separable mixture of homoallylic alcohols 131 as a separable. The \textit{syn}-diastereomer was ozonized to form the ketoester 132, which was subjected to global deprotection under acidic conditions using aqueous TFA to afford KDN in 68% yield (Scheme 23).
Scheme 23: First total synthesis of KDN from a non-carbohydrate source

1.6.8 Dondoni’s thiazole auxiliary mediated synthesis of KDN

Dondoni et al. utilized a mannose aldehyde (133) and coupled it with the thiazole derived phosphorane 134 to afford the thiazole enone 135 in excellent yield (Scheme 24). A Michael addition of benzyl alcohol at 0 °C proceeded with syn preference to afford the masked KDN 136 in 70% yield. Deprotection and methylation afforded the methyl glycoside 138, at which stage the aldehyde synthon thiazole was cleaved off and the aldehyde was oxidized to the carboxylic acid functionality. Global deprotection finally afforded native KDN in 95% yield (Scheme 24).
1.6.9 Li’s synthesis of 4-epi KDN

A homopropargylic alcohol group was shown by Crich and Li to serve as an excellent pyruvate synthon in the synthesis of ulosonic acids. The osmium-mediated oxidative pyruvate formation was modified by Li and co-workers using aqueous potassium permanganate to afford a higher yielding protocol with which to synthesize methyl pyruvates. The Li group sought to use this protocol to synthesize KDN and conducted a Barbier type propargylation of a protected mannose aldehyde 140 to form the anti isomer 141 preferentially in 77% yield (Scheme 25). Several attempts to invert the sterically encumbered homopropargylic alcohol 141 were unsuccessful. However, the protected anti diastereomer was brominated and oxidized using potassium permanganate to form the methyl pyruvate 143 in 79% yield. A deprotection under acidic conditions and acetylation afforded a fully protected 4-epi KDN 144, which was found to exist in the furanose form (Scheme 25).
Scheme 25: Li’s Synthesis of 4-epi KDN
CHAPTER TWO

STEREOSELECTIVE SYNTHESIS OF α-KETO-DEOXY-ᴅ-GLYCERO-ᴅ-GALACTO-NONULOSONIC ACID GLYCOSIDES BY MEANS OF 4,5-O-CARBONATE GROUP

2.1 Background

One of the major reasons for the retarded discovery of KDN, by about 50 years after the isolation of neuraminic acid, was its scarce abundance in nature and the absence of powerful glycoanalysis tools. Thanks to improved analytical tools KDN was eventually found to be widespread in living systems and to mirror neuraminic acid in terms of glycosyl linkage diversity. The discovery of KDN in humans, and the recognition of KDN containing glycoconjugates as markers for disease states, coupled with their scarce natural abundance, microheterogeneity, and complex isolation processes has highlighted the need for efficient access to well-defined α-KDN linked glycoconjugates. The chemical synthesis of α-KDN glycossides, thus, a prominent synthetic challenge, suffers from the same problems associated with sialylation by neuraminic acid, viz., poor selectivity and glycal formation. The 4,5 acetyloxazolidinone and oxazolidinone protected sialic acid donors developed by Crich and others have shown a high α-stereodirecting effect towards various alcohol nucleophiles. Since KDN possesses a very similar pyranose skeleton to neuraminic acid, it was reasoned that the use of a 4,5-O-carbonate protected KDN donor would be highly α-stereodirecting, also taking into considering the beneficial effects of this cyclic protecting groups in stereoselective glycosylations. Accordingly, the synthesis of such a 4,5-O-carbonate protected KDN donor and the study of its glycosylation reactions is the focus of the work described in this chapter.
2.2 Results and discussion

2.2.1 Synthesis of 4,5-O-carbonate protected donors

Among the various thiols available, the electron rich adamantane thiol was selected for the construction of the thioglycoside-based KDN donor methyl adamantylthio-4,5-O-carbonate-7,8,9-tri-O-acetyl-D-glycero-D-galacto-nonulosonate 146 because of the anticipated ease of activation at – 78 °C.44

![Figure 9: 4,5-O-carbonate protected thioadamantyl donor](image)

2.2.2 Synthesis of KDN

Due to low abundance from natural sources, KDN is a very expensive material to purchase. In order to avoid the long syntheses needed to access the gram quantities of KDN required for the synthesis of donors, KDN was sourced by a modified Zbiral deamination79 of peracetyl neuraminic acid. The original Zbiral deamination sequence used nitrosyl acetate, prepared by reacting gaseous dinitrogen tetroxide generated by decomposition of lead nitrate with sodium acetate, to prepare the nitrosyl sialic acid 148 (Scheme 26). The nitrosyl sialic acid 148 was converted to the 5-diazo sialic acid 140 by treatment with a trifluoroethanol/sodium isopropoxide couple, used to prevent uncontrolled Zemplen deacetylation. The diazo sialic acid was immediately quenched with tetrabutylammonium acetate/acetic acid to afford peracetyl KDN 150 in 53% yield.79
Scheme 26: Original Zbiral synthesis of KDN

In the present more environmentally conscious times alternatives to the use of lead nitrate were imperative. Therefore, nitrosation was carried out by treating peracetyl β-neuraminic acid 147 with nitrosonium tetrafluoroborate and pyridine, which furnished the nitrosyl sialic acid in quantitative yield (Scheme 27). The nitroso sialic acid was deaminated using Zbiral’s trifluoroethanol/sodium isopropoxide couple and acetic acid to synthesize peracetyl KDN. The crude deamination reaction mixture was ozonized in methanol at −78 °C in order to destroy the elimination by-products with a view to facilitating chromatographic purification and scale up. Multiple gram quantities of peracetyl KDN 150 could be accessed using this procedure.

Scheme 27: Modified Zbiral deamination sequence

The desired adamantyl thioglycosides 151α, 151β of peracetyl KDN were synthesized in excellent yield by treating the peracetyl KDN 150 with adamantane thiol in the presence of BF₃ etherate (Scheme 28). The purified anomers were then subjected to Zemplen deacetylation
conditions to afford the unprotected thioglycosides in quantitative yield, which were then converted to the acetonide-protected derivatives 152 under standard conditions in excellent yield.

Scheme 28: Preparation of 8,9-O-isopropylidene protected KDN triols

2.2.3 4,5-O-Carbonate formation and donor synthesis

The initial attempts to form the 4,5-O-carbonate group using phosgene, or triphosgene at -78 °C, or -40 °C were unsuccessful due to the poor reactivity of the secondary alcohols under these conditions. At 0 °C the formation of a mono chloroformate of the compound 152β was deduced using mass spectrometry (observation of the corresponding methyl carbonate adduct). Prolonged reaction times under these conditions resulted in complex reaction mixtures. Attempted phosgenations by gradual warming to room temperature also gave complex reaction mixtures. Careful mass spectrometric analysis revealed formation of multiple chloroformate adducts, observed as their corresponding methyl carbonates. Furuhata and co-workers have shown that the reactivity of the 5-OH in the 8,9-O-acetonide-protected methyl glycoside of KDN is dependent on the anomeric stereochemistry. In the case of the α-methyl glycoside of KDN the order of hydroxyl group reactivity is 4-OH > 5-OH > 7-OH, whilst in the β-methyl glycoside the order is 4-OH > 7-OH > 5-OH. They further showed that treatment of the α-methyl KDN glycoside 153a with excess dimethoxypropane under pTSA catalysis, furnished the 6,7;8,9-di-O-acetonide derivative of KDN 154 in good yield upon acetylation (Scheme 29). The group further
showed that under similar reaction conditions the corresponding β-anomer 153β provided the 5,8;7,9-di-O-acetonide protected derivative 156 as the major product in 75% yield.  

Scheme 29: Lessons from Furuhata’s 5,7;8,9-di-O-acetonide protection

Taking a cue from the Furuhata study, bis acetonide protection for the unprotected KDN 158 was envisaged to further protect the closely reactive alcohol groups. The reaction proceeded smoothly in presence of excess dimethoxypropane to afford the bisacetonide derivative 159 in 63% yield for the α anomer and 52% for the β anomer (Scheme 30). The bisacetonide protected derivatives 159α and 159β then were reacted with 4-nitrophenyl chloroformate using Hunig’s base to afford the corresponding nitrophenyl carbonates 160β/α in excellent yields. The NMR spectra of these compounds clearly indicate a downfield shift of H-4 confirming the regiochemistry of the acetonide and subsequent carbonate installations.
Scheme 30: Bisacetonide protection and nitrophenyl carbonate formation

With the nitrophenyl carbonate in place at the desired 4-OH position, the derivatives 160α and 160β were subjected to acidic conditions (1/1/8, HCl/H2O/MeOH) to cleave the acetonide protecting groups (Scheme 31). The tetraol compounds 161α/β were then subjected to mild basic conditions using cesium bicarbonate in wet acetonitrile to form the trans-fused carbonate derivative, acetylation of which gave the desired donors 146α/β in excellent overall yield. Noteworthy in this synthetic transformation is the stability of the 4-nitrophenyl carbonate group to acidic conditions in an overnight reaction.

Scheme 31: Carbonate formation and donor synthesis
Although this route to the donor was efficient on an exploratory scale, its scale up was unsuccessful due to the instability of 4-nitrophenvlcarbonate protected tetraol compound 161 to silica gel chromatography in gram scales.

In order to improve the scalability of the donor synthesis, after several unsuccessful reactions with the acetonide protected KDN triol 152β, the 4,5-\textit{O-trans}-carbonate group eventually was installed using excess bis 4-nitrophenvl carbonate and Hunig’s base (Scheme 32). The use of triethylamine as base is detrimental in this reaction as it results in the cleavage of the formed carbonate 163 by the nucleophilic amine. The carbonate-protected KDN compounds 163α/β were subjected to mild acidic conditions to cleave the 8,9-acetonide group and the glycerol side chain was acetylated to form the donors 146α/β of both the series in excellent yield.

\textbf{Scheme 32: Improved carbonate formation and donor synthesis}

\textbf{2.2.4 Glycosylation study with 4,5-\textit{O-carbonate protected donors}}

With the 4,5-\textit{O-carbonate protected adamantyl thioglycoside donors 146α/β in hand, a glycosylation reaction with primary alcohol acceptor, methyl 2,3,4 tri-\textit{O-benzyl-α-D-glucopyranoside 165 was surveyed (Table 1). The reaction was carried out by premixing the donor 146β and acceptor in a mixture of CH₂Cl₂ and CH₃CN (2:1) using the NIS and catalytic TfOH promoter system. The donor was cleanly activated at \textdegree 78 \textdegree C and delivered a single α-}
isomer of the disaccharide 168 in 85% yield. The stereochemistry at the anomeric center was assigned based on the $^3J_{C-H}$ coupling constant$^{82}$ between the axial hydrogen at C3 and carboxy carbon of the KDN moiety, which measured 5.5 Hz, characteristic of $\alpha$-sialosides. Further glycosylations were then carried out using the primary alcohol acceptors methyl 2,3,4 tri-$O$-benzyl-$\alpha$-D-galactopyranoside 166, and 1,2;3,4-di-$O$-isopropylidene-$\alpha$-D-galactopyranoside 167. In both of these cases excellent $\alpha$-selectivity and yields were obtained, thus demonstrating efficient construction of the important KDN $\alpha$-(2$\rightarrow$6) gal linkage.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acceptor</th>
<th>Product</th>
<th>Yield ($\alpha/\beta$)</th>
<th>$^3J_{C-H}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Acceptor 1" /></td>
<td><img src="image" alt="Product 1" /></td>
<td>85%, 5.5 Hz</td>
<td>Only $\alpha$</td>
</tr>
<tr>
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<td><img src="image" alt="Acceptor 2" /></td>
<td><img src="image" alt="Product 2" /></td>
<td>82%, 5.5 Hz</td>
<td>Only $\alpha$</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Acceptor 3" /></td>
<td><img src="image" alt="Product 3" /></td>
<td>84%, 5.5 Hz</td>
<td>Only $\alpha$</td>
</tr>
</tbody>
</table>

**Table 1: Glycosylation of donor 146$\beta$ with primary carbohydrate alcohol acceptors**

Encouraged by the high $\alpha$-selectivity observed, the donor 146$\beta$ was reacted with various sterically hindered alcohol acceptors (Table 2). In the reaction of 146$\beta$ with adamantanol in
CH$_2$Cl$_2$/CH$_3$CN mixture using NIS/TfOH at -78 °C, the α-anomer product 174 was isolated as a single anomer in an excellent 86% yield.

\[ \text{AcO}_2\text{O} \quad \text{OAc} \quad \text{CO}_2\text{Me} \]

\[ + \quad \text{ROH} \quad \frac{\text{NIS} / \text{TfOH}}{\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN} (2:1)} \quad \text{-78 °C, 3 Å AWMS} \]

\[ \xrightarrow{\text{174}} \]

\[ \text{AcO}_2\text{O} \quad \text{OAc} \quad \text{CO}_2\text{Me} \]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acceptor</th>
<th>Product</th>
<th>Yield (α/β)</th>
<th>$^{3}$J$_{\text{C-H}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HO 171</td>
<td>174</td>
<td>86%, only α</td>
<td>5.5 Hz</td>
</tr>
<tr>
<td>2</td>
<td>HO, OBn 172</td>
<td>175</td>
<td>82%, only α</td>
<td>5.5 Hz</td>
</tr>
<tr>
<td>3</td>
<td>OBn, OMe 173</td>
<td>176</td>
<td>84%, only α</td>
<td>5.5 Hz</td>
</tr>
</tbody>
</table>

Table 2: Glycosylation of Donor 146β with hindered alcohol acceptors

Next, the regioselective sialylation of 3-OH of methyl 2,6-di-O-benzyl-β-D-galactopyranoside 172 was carried out. The reaction proceeded with excellent α-selectivity to furnish the disaccharide 175 in 81% isolated yield. The glycosylation of the galactose 3-OH acceptor methyl 2,4,6-tri-O-benzyl-β-D-galactopyranoside 173 also provided a single isolated α-anomer of compound 176 in 80% yield (Table 2), thus efficiently putting together the KDN α(2→3) gal linkage, which is the most common linkage for KDN found in nature. It is noteworthy that all sialylations were complete in 1 hour and that the sialosides could easily be isolated as single α-anomers using standard silica gel chromatographic techniques.
2.2.5 Glycosylation in absence of acetonitrile

To probe the role of acetonitrile in the α-selective sialylation using the 4,5-O-carbonate protected donor 146β of KDN, a reaction with the acceptor methyl 2,3,4 tri-O-benzyl-α-D-galactopyranoside 165 was carried out using otherwise identical conditions in neat dichloromethane at -78 °C (Scheme 33). The reaction afforded a slightly lower α-selectivity of 13:1 in comparison to the single α-anomer isolated using 2:1 CH2Cl2/CH3CN conditions, revealing the role of the nitrile effect38-86 in improving α-selectivity.

![Scheme 33: Glycosylation of donor 146β in dichloromethane](image)

2.2.6 Glycosylation with α-donor 146α

In order to probe the role of the anomeric configuration of the thioglycoside donor on the stereochemical outcome of the glycosylation, a reaction was carried out using α-KDN carbonate donor 146α, with methyl 2,3,4 tri-O-benzyl-α-D-galactopyranoside 166 using identical conditions to those used for β-KDN carbonate donor 146β (Scheme 34). The reaction proceeded smoothly at -78 °C, and afforded a single isolated α-anomer of the disaccharide 169 in 86% yield, showing that the anomeric configuration of the donor is irrelevant in achieving high α-selectivity.
2.2.7 Synthesis of the glycal elimination product

The 4,5-O-cyclic carbonate group not only confers high α-selectivity but also thwarts the unwanted elimination reaction leading to the glycal formation, which is reflected in the high yields observed in the glycosylation reactions. In order to carefully verify the absence of the glycal formation in the glycosylation reactions a synthesis of an authentic sample of the glycal was undertaken. The reaction of the donor 146β with NIS and TfOH in CH₂Cl₂ in the absence of an acceptor led to a complex mixture of products. In a second approach the thioglycoside 146β was oxidized with m-CPBA in CH₂Cl₂ at -20 °C, which led to the conversion of the thioglycoside to a mixture of the corresponding sulfoxides and sulfone (Scheme 35). Mass spectral analysis revealed the concomitant formation of the desired elimination glycal product in the oxidation reaction. To aid the formation of the glycal the reaction temperature was elevated to 40 °C. The sulfoxide/sulfone mixture was thus converted to the elimination product 177 which was isolated in 21% yield.
Careful comparison of the NMR spectra of the crude glycosylation reactions with that of the glycal 177, confirmed that the cyclic carbonate-mediated glycosylations proceed essentially (<5%) with the absence of the glycal formation.

2.2.8 Glycosylation of donor 1β with sulfoxide activation systems

The compatibility of the cyclic carbonate protected donor to sulfoxide-based activation systems was checked using preactivation conditions with 2,3,4 tri-O-benzyl-α-D-galactopyranoside 166 as acceptor and the diphenyl sulfoxide/triflic anhydride promoter combination (Scheme 36). The reaction delivered a 78% yield of the disaccharide 169 favoring the α-anomer in a 9:1 ratio. The reaction between this donor acceptor pair was then conducted at -78 °C using benzenesulphenyl piperidine and triflic anhydride, with tri-tert butylpyrimidine as a hindered base. The reaction gave a good yield of 73%, although the selectivity was a modest 7:1. These experiments highlight the compatibility of the α-selective 4,5-O-carbonate protected KDN donor 146β to the popular sulfoxide activation systems.

Scheme 36: Activation with DPSO and BSP
2.2.9 Glycosylation study with peracetyl KDN donor 151

To verify the importance of the 4,5-\(O\)-cyclic carbonate group in imparting \(\alpha\)-selectivity and thwarting the glycal formation in the sialylation reactions, a series of glycosylations were conducted using the peracetyl KDN donors 151\(\beta\) and 151\(\alpha\) (Table 3). These glycosylations proceeded with moderate \(\alpha\)-selectivity of 12:1 and 13:1 with the primary alcohol acceptors methyl 2,3,4 tri-\(O\)-benzyl-\(\alpha\)-\(\beta\)-glucopyranoside 165 and methyl 2,3,4 tri-\(O\)-benzyl-\(\alpha\)-\(\beta\)-galactopyranoside 166. However the yields of the disaccharides were reduced to 60 and 62%, respectively, owing to the formation of the glycal byproduct 77 in 28% and 26%, respectively. In the case of the hindered 3-\(OH\) acceptor methyl 2,6-di-\(O\)-benzyl-\(\beta\)-\(\alpha\)-galactopyranoside 172 the selectivity fell considerably to 1.6 :1 favoring the \(\alpha\)-anomer, while the yield was a modest 52% due to the formation of the glycal 77 in 34% yield. A reaction with the peracetyl \(\alpha\)-donor 155\(\alpha\) furnished the corresponding disaccharide 169 in 60% yield and a 12:1 \(\alpha\)/\(\beta\) ratio which is comparable to the result using the corresponding \(\beta\) donor 151\(\beta\), reinforcing the observation that the anomic configuration of the donor does not have an effect on the stereoselectivity of glycosylation in these reactions. Overall these experiments show that the cyclic carbonate protecting group is highly beneficial in imparting \(\alpha\)-selectivity as well in inhibiting the formation of the glycal by-product.
The coupled sialosides were subjected to Zemplen deacetylation conditions to remove the cyclic carbonate and the acetate groups (Table 4). The reactions proceeded to completion in one hour in quantitative yield for all the coupled glycosides.
<table>
<thead>
<tr>
<th>Sialoside</th>
<th>Product</th>
</tr>
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<tbody>
<tr>
<td>[structure image]</td>
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<td>[structure image]</td>
<td>[structure image]</td>
</tr>
</tbody>
</table>

Table 4: Zemplen deacetylation of coupled sialosides
2.2.11 Assignment of configuration for coupled sialosides

The routine methods for configurational assignment for aldopyranoglycosides such as measurement of $^3J_{\text{H1-H2}}$ and $^1J_{\text{C1-H1}}$ NMR coupling constants fail in the case of the sialic acid glycosides owing to the lack of the anomeric proton handle. In view of this, several alternative methods to establish the anomeric configuration of sialosides have been described in the literature based on the chemical shift of the $H$-3eq$^{87}$, and/or $H$-4$^{88,89}$ resonances, the magnitude of the $^3J_{C-1, H3ax}$ heteronuclear coupling constant$^{82,90,91}$ previously mentioned, on the $\Delta\delta$ value of the resonances for $H9a-H9b$92 and on the $\delta$ values of $H$-7 and $H$-8.92 While most of these methods are empirical, the $^3J_{C-1, H3ax}$ coupling constant provides an unambiguous approach to this problem, in which $\alpha$-isomers have a coupling constant of 5-7 Hz, while $\beta$-anomer have a coupling constant of 0-2 Hz provided that, as is the case for all systems studied here, that the $^2C_5$ chair conformation is retained.90 This difference in the coupling constants for anomers can be rationalized using a Karplus relationship, wherein an $\alpha$-glycoside has a C1-C2-C3-H3ax dihedral angle of 180° and a $\beta$-glycoside has one of 60° (Figure 10), leading to the expected higher coupling constants for the $\alpha$-glycosides.90

![diagram](image)

**Figure 10: Dihedral angles of $\alpha$ and $\beta$ sialosides**
Most of the aforementioned sialoside configurational determination methods are not broadly applicable ones, and hence the configurations of the various sialosides described in this work were ascertained using the more rigorous $^3J_{C-1, \ H_3ax}$ coupling constant measurements. To measure $^3J_{C-1, \ H_3ax}$ coupling constants accurately three different NMR experiments were carried out. In the first experiment the standard broadband proton decoupled $^{13}$C NMR spectrum was acquired, which shows the four carbonyl carbon signals for the C-1 ester and the three side chain acetate groups (Figure 11). In a second experiment a $^{13}$C NMR spectrum was acquired with the broadband decoupler turned off providing the complete proton coupling profile for the four carbonyl carbons. A third $^{13}$C NMR spectrum was acquired with selective proton irradiation of the methyl group of the C-1 ester, reducing the C-1 resonance to a doublet and revealing the value of the $^3J_{C-1, \ H_3ax}$ coupling constant.

Figure 11: Example of $^3J_{C-H}$ determination using the SFORD experiment
2.3 Conclusion

In conclusion, a highly stereoselective methodology for the synthesis of $\alpha$-KDN glycosides has been developed. The use of the trans-fused 4,5-$O$-cyclic carbonate group imparts high $\alpha$-selectivity while inhibiting the glycal formation. The high stereoselectivity is consistent across the board for a range of primary, secondary and tertiary acceptors. The stereochemical outcome of the glycosylation was found to be independent of the anomeric configuration of the donor in both the carbonate protected and peracetyl KDN series. The methodology is compatible with the popular sulfoxide activation systems and the ability to conduct efficient glycosylation at -78 °C opens up possibilities in the design of one-pot glycosylation protocols$^{93}$ for KDN containing oligosaccharides.
CHAPTER 3

DISSECTING THE INFLUENCE OF OXAZOLIDINONES AND CARBONATES IN α-SIALYLATION

3.1 Background

α-Sialic acid linkages are ubiquitous in living systems and are key to various recognition and signaling events.\textsuperscript{94} The chemical synthesis of these α-sialic acid linkages has long been of considerable synthetic interest which is reflected in the myriad strategies that have been developed towards this goal.\textsuperscript{3,95,96} Among these strategies, the use of $N$-acetyl-4$O,5N$-oxazolidinone developed by Crich\textsuperscript{44,45,48} and 4$O,5N$-oxazolidinone protecting strategies developed by Takahashi\textsuperscript{46} and De Meo\textsuperscript{47} groups present the best available solutions to the challenging synthesis of α-sialosides (Scheme 37), with the doubly protected $N$-acetyl oxazolidinone being advantageous in delivering the native acetamide under mild deprotection conditions. The $N$-acetyl-4$O,5N$-oxazolidinone-based system has also been shown to be highly α-stereodirecting towards $C$ and $S$ nucleophiles.\textsuperscript{51,97}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {$\text{AcO}\quad \text{AcO}\quad \text{OAc}\quad \text{LG}\quad \text{CO}_2\text{Me}$};
\node at (2,0) {X = NH, NHAc, NHCH$_2$OAc, O};
\node at (2,-1) {LG = SPh, SAda, OPO(Bu)$_3$};
\node at (4,0) {$\text{Nucleophile}$;}
\node at (6,0) {$\text{Promotor}$;}
\node at (8,0) {$\text{AcO}\quad \text{OAc}\quad \text{CO}_2\text{Me}$};
\node at (10,0) {$\text{YR}$};
\node at (8,-1) {$\text{Y = O, C, S}$};
\end{tikzpicture}
\end{center}

Scheme 37: α-Selective 4,5 trans fused sialyl donors

The analogous $N$-glycolyl donor showed excellent α-directing effect towards $O$-nucleophiles and was employed in a one-pot strategy to synthesize biologically relevant sialic acid constructs.\textsuperscript{48} In the related KDN series the 4,5-$O$-carbonate protected thiosialoside system was found to be
highly $\alpha$-stereodirecting with $O$-nucleophiles.$^{98}$ The high $\alpha$-stereodirecting effect of the $N$-acetyl oxazolidinone system was also showcased in the case of sialyl phosphates by Wong and co-workers.$^{49}$ This chapter delineates mass spectrometric experiments conducted in an attempt to understand the origin of the high $\alpha$-stereodirecting effect in sialylations with donors carrying an $N$-acetyl oxazolidinone, oxazolidinone, or carbonate protecting group $\textit{trans}$ fused at the 4,5 position of the sialic acid.

3.2 Results and Discussion

3.2.1 Conformational analysis of the putative oxocarbenium ion intermediate

In order to explain the high $\alpha$-selectivity in sialylations using a 4,5 cyclic protecting group in sialic acids, an understanding of the mechanistic pathway is critical. The mechanism of a glycosylation reaction can either be unimolecular ($S_N 1$), involving an oxocarbenium ion, or bimolecular ($S_N 2$), or somewhere between these two extremes, i.e., involving a more or less tightly associated ion pair. Due to the presence of a $\textit{trans}$-fused cyclic protecting group the likely conformation of any intermediate oxocarbenium ion would be a $^4H_5$ half chair $^{190}$. An attack from the $\alpha$-face on this intermediate would give a $\alpha$-glycoside $^{191}$ in a $^4S_2$ twist boat conformation, while an approach from the $\beta$-face would directly give a $^2C_3$ chair $\beta$-sialoside $^{193}$. Attack by the incoming nucleophile on either face is considered to be facile as there are no pseudo-axial substituents on either face of the $^4H_5$ half chair intermediate $^{190}$ (Scheme 38). The apparent absence of any compelling reasons for preferential nucleophilic attack on the $\alpha$-face of the oxocarbenium ion suggest its intermediacy in the highly $\alpha$-selective sialylations is unlikely. This would imply a mechanism closer to the $S_N 2$ end of the spectrum for the $\alpha$-selective sialylations.
Scheme 38: Conformational analysis of nucleophilic attack on the putative oxocarbenium ion intermediate

To probe this hypothesis a study of the energetics of the intractable oxocarbenium ions was undertaken using a mass spectrometric method. The use of threshold fragmentation energies has been previously identified by Denekamp and Sandlers as a useful means of assessing the influence of protecting groups on the stability of glycosyl oxocarbenium ions, albeit not in the sialic acid series.\textsuperscript{99,100}

3.2.2 In-source fragmentation studies of sialyl donors

Mass spectrometry of appropriately ionized analytes and mass spectral fragmentation studies, routinely used in oligosaccharide and peptide sequencing, can furnish a rich source of structural information. In-source fragmentation, also known as cone-voltage induced fragmentation, is a convenient technique that can be performed on a ESI mass spectrometer without the necessity for a collision chamber. The ESI is a soft ionization technique that occurs at atmospheric pressure and typically precludes fragmentation of the formed ions. The fragmentation though can be induced if necessary by increasing the voltage at the skimmer cone. The increased voltage
creates a potential difference with respect to the capillary voltage and imparts additional velocity to the formed ion (Figure 12). The molecular ion travelling at a higher velocity may collide with the residual solvent molecules, which leads to an increase in internal energy resulting in the possibility of fragmentation. As oxocarbenium ions are known intermediates in collision-induced mass spectral dissociation of carbohydrates, the in-source fragmentation study is an appropriate technique to study the energetics of sialyl oxocarbenium ions.

![Image](image_url)

**Figure 12: A schematic of in-source fragmentation process**

### 3.2.3 In-source fragmentation of percaetyl KDN adamantyl thioglycoside 151

With this in view, methanolic solutions of peracetylated neuraminic adamantyl thioglycosides and the peracetylated KDN adamantyl thioglycoside 151β were infused into an ESI mass spectrometer under standard conditions, and the acquired spectra showed clean sodiated peaks of the thioglycoside. The experiments were then repeated with incrementing cone voltages with a view to induce fragmentation of the molecular ions in-source. At a cone voltage of 80 V the
onset of fragmentation of the thioglycoside $151\beta$ was observed with the loss of an acetoxy group (60 m/z) (Scheme 39). Upon a further cone voltage increase the desired fragmentation at the anomeric center occurred at 140 V with the loss of the adamantyl thio group to give the eliminated glycal 196, observed as a sodiated adduct.

Scheme 39: In-source fragmentation of peracetyl KDN adamantyl thioglycosides

3.2.4 Synthesis and fragmentation studies of sialyl phosphates

The fact that loss of the anomeric group was not the lowest energy fragmentation in the above system necessitated a redesign of the experiment. Thus, in order to promote primary fragmentation at the anomeric center the more labile neuraminic acid glycosyl phosphate, reported by Wong and co-workers,49 was synthesized from the peracetyl neuraminic acid thioglycoside 197. The reaction was completed in 15 minutes and afforded a good yield of the phosphate 198 (Scheme 40).

Scheme 40: Synthesis of peracetyl neuraminic acid phosphate
The pure β-phosphate 198β was subjected to cone voltage induced fragmentation studies. At a cone voltage of 40 V a clean sodiated molecular ion of the phosphate (m/z: 707) was observed. Upon increasing the cone voltage to 78 V the fragmentation set in and gratifyingly the primary fragmentation occurred at the anomeric center to from three daughter ions (Scheme 41) which correspond to the eliminated glycal 200 (m/z 496.1431), the methanolysis product 201 (m/z 528.1693), and the hydrolyzed product 202 (m/z 514.1537). Upon further increasing the cone voltage the loss of an acetoxy group occurred at 120 V to give the diene daughter ion 203.

Scheme 41: ESI cone voltage induced fragmentation of neuraminic acid phosphate

The mass spectral fragmentation of the phosphate likely occurs by the expulsion of dibutyl phosphate to give the corresponding oxocarbenium ion 204, which then undergoes loss of H-3 to give the observed glycal fragment ion 200. Alternatively, the oxocarbenium ion can combine
with methanol or water from the infusion solvents to give rise to the methanolysis and hydrolysis adducts, which were observed as fragment ions $^{201/202}$ (Scheme 42). Alternatively fragmentation could occur via a concerted McLafferty rearrangement ($^{204}$) to give the same glycal daughter ion. However, as the methanolysis and hydrolysis adducts are observed as fragments the oxocarbenium ion and the path A is the more likely mode of fragmentation.

Scheme 42: Mass spectral fragmentation and adduct formation

In order to study the fragmentation of differentially protected sialyl donors the corresponding sialyl phosphates were synthesized (Scheme 43) using NIS/TfOH conditions. All the reactions were completed within 15 minutes and afforded the phosphates in good yields as β anomers except in the case of peracetyl KDN in which case the mixture was highly enriched with the β anomer.
Scheme 43: Synthesis of sialyl phosphates

The various sialyl phosphates 206-209 were subjected to cone voltage induced fragmentation to find the effect of the different protecting groups.

3.2.5 In-source fragmentation of 5N,4O-oxazolidinone protected neuraminic acid phosphate 206

In the case of the oxazolidinone protected sialyl phosphate 206 a clean sodiated molecular ion is observed at the standard 40V cone voltage. Upon increase of the cone voltage to 93 V the onset of fragmentation is observed, with formation of the glycal and methanolysis daughter ions (Figure 13).
3.2.6 In-source fragmentation of N-acetyl-5N, 4O-oxazolidinone protected neuraminic acid phosphate 207

The acetyl oxazolidinone protected sialyl phosphate 207 was then studied for its mass spectral fragmentation. As before, the mass spectra were recorded starting from 40 V where a clean sodiated molecular ion was observed and upon increasing the cone voltage to 83 V the
fragmentation was initiated and gave the glycal and methanolysis daughter ions (Figure 14).

Figure 14: Cone voltage induced fragmentation of acetyl oxazolidinone protected donor
3.2.7 In-source fragmentation of peracetyl KDN phosphate 208

In the case of the peracetyl KDN sialyl phosphate 208 a clean sodiated molecular ion is observed at the standard 40V cone voltage. Upon increase of the cone voltage to 82 V the onset of fragmentation is seen and the glycal and methanolysis daughter ions are observed (Scheme 15).
3.2.8 In-source fragmentation of 4,5-\textit{O}-carbonate protected KDN phosphate 209

In the case of the carbonate protected sialyl phosphate 209 a clean sodiated molecular ion is observed at the standard cone voltage of 40V. Upon increasing the cone voltage to 95 V the fragmentation is induced and gives the glycal and methanolysis daughter ions (Scheme 15).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure16.png}
\caption{Cone voltage induced fragmentation of carbonate protected KDN donor 209}
\end{figure}
3.2.9 Correlation of onset cone voltages with dipole moments of protecting groups

![Chemical structures and voltages]

**Figure 17: Onset fragmentation voltages for sialyl phosphates**

The fragmentation onset voltages shown in the Figure 17 clearly indicate a trend, wherein the use of a cyclic protecting group necessitates higher cone voltage for fragmentation. This clearly suggests that the cyclic protecting group retards the formation of the oxocarbenium ion in comparison to the peracetylated counterparts, perhaps by virtue of a greater electron withdrawing effect. The trend observed for the fragmentation onset voltages can be best correlated with the electron withdrawing ability of the individual protecting groups as manifest in their dipole moments.

![Dipole moments]

**Figure 18: Dipole moments in Debye units of various carbonyl functionalities in non polar solvents**
The dipole moments of the open chain and cyclic carbonyl functionalities measured in non-polar solvents\textsuperscript{101-103} (Figure 18) clearly indicated that the cyclic congeners have a higher overall dipole moment in comparison with the acyclic counterparts.

![Diagram](Image)

**Figure 19: Alignment of protecting group dipoles and their relationship to oxocarbenium ion**

The strong electron withdrawing effect of the oxazolidinone and the cyclic carbonate (212) is a function of their large dipole moment, which is aligned with the carbonyl group in the mean plane of the pyranose ring and which reinforces the inherent electron withdrawing effect of the C4-O4 and C5-N5/O5 bonds (Figure 19). In comparison, the carbonyl dipoles on the O4 ester and the O5/N5 ester/amide systems (211) are roughly orthogonal to the dipoles of C4-O4 and C5-O5/N5 bonds and do not reinforce their electron withdrawing effect to the same extent. In the case of the acetyl oxazolidinone system (213) the orientation of the \textit{N}-acetyl dipole is antiperiplanar to the carbonyl dipole of the oxazolidinone (seen in X-ray crystallographic analyses of 46)\textsuperscript{45} and hence moderates its electron withdrawing ability, which explains the decrease in the cone voltage necessary for the onset of fragmentation.
3.3 Conclusion

The in-source minimum cone voltage fragmentation study of the differentially protected sialoside donors clearly suggests that the trans-fused oxazolidinone and the cyclic carbonate groups are highly electron withdrawing in comparison to their acyclic counterparts by virtue of their higher dipole moments in the mean plane of the pyranose ring. The oxazolidinones and the carbonate group owing to this strong electron withdrawing effect the oxocarbenium ion formation and hence suppress this unselective pathway for sialylation. In effect this favors pathways closer to the \( S_N2 \) end of the \( S_N1 \)- \( S_N2 \) continuum\(^{104,105}\) via exploded transition states or pathways taking place through functionally equivalent contact or tight ion pairs.

Since the anomeric configuration of the donor was found to bear no effect on the stereochemical outcome of sialylation, possible common intermediates, depending on the reaction conditions and the promotion system used, are the \( \beta \)-glycosyl nitriilium ions (214),\(^ {106} \) \( \beta \)-glycosyl triflates (215), \( \beta \)-glycosyl sulfoxonium ions (216),\(^ {29,107} \) and the spirocyclic \( \alpha \)-lactones (217, 218)\(^ {108,109} \) (Figure 20).

![Possible intermediates in sialylation](image-url)

**Figure 20: Possible intermediates in sialylation**
Typically considered strongly disfavored,\textsuperscript{110} the $S_N2$ mechanism at a tertiary carbon center has precedence, stereochemical and kinetic, at tertiary centers adjacent to a carboxylate ester.\textsuperscript{111,112}
CHAPTER 4
SYNTHESIS OF KETO DEOXY NONULOSONIC ACID

4.1 Background
Keto deoxy nonulosonic acid owing to its biological relevance and complex architecture has attracted considerable synthetic attention that resulted in several elegant syntheses of this target as described in Chapter 1. With the exception of the two step approaches by Ogura$^{113}$ and Chan,$^{65}$ most of the reported methods to access KDN suffer from long linear sequences and low yields that deter efficient scale up and so hinder access to multiple gram quantities of this sparsely occurring keto acid. The two step Ogura and Chan biomimetic syntheses involve coupling reactions in highly polar aqueous conditions and tricky separations on resin columns and have been found not to be amenable to scale up. To initiate the studies of KDN chemistry reported in this thesis access to multiple gram quantities of KDN was necessary and stimulated the development of a scalable synthesis. This chapter describes the approaches undertaken towards the development of such a practical and scalable synthesis of KDN.

4.2 Retrosynthetic analysis for KDN
The biomimetic coupling or so called Cornforth $6+3$ approach using a chiral pool strategy is an attractive approach by which to make a rapid entry into the KDN skeleton. The initial strategy centered around this biomimetic approach wherein the target keto acid $1$ is disconnected into a protected six carbon mannose-configured aldehyde derivative $3$ and methyl bromomethylacrylate $4$, a three carbon pyruvate surrogate (Scheme 44).
Scheme 44: Retrosynthetic analysis for the 6+3 coupling

4.3 Synthesis of 4-epi KDN

The synthesis began with the preparation of gram quantities of the mannitol 222, the precursor to the desired six carbon aldehyde fragment 220, in five steps starting from D-mannose according to Schmidt’s reported procedure. The mannitol 222 was oxidized using the Swern conditions and the aldehyde 220 was subjected to the key Barbier reaction mediated by zinc metal (Scheme 45). The reaction proceeded cleanly to afford a separable mixture of diastereomers 223 in a 5:1 ratio in overall 86% yield.

Scheme 45: Barbier reaction with 2-bromomethyl acrylate 221

The major diastereomer of 223 was subjected to ozonolysis to form the keto acid 224, which was subjected to acidic conditions to remove the acetal protecting groups, and then acetylation using...
acetic anhydride in pyridine to afford the peracetylated KDN derivative 225 in 31% over 3 steps (Scheme 46). Examination of the $^1$H NMR spectrum of 225 revealed a chemical shift of δ 2.50 and 2.73 (Figure 21) for the deoxy protons which compares well with the deoxy proton chemical shift of 4-epi KDN$^{76}$, confirming a furanose structure for 225. This clearly indicates the stereochemistry of the major isomer 223 in the Barbier reaction (Scheme 45) as the anti isomer.

Scheme 46: Conversion of homoallylic alcohol 223 to 4-epi KDN 225

Figure 21: H-3$_{ax}$ and H-3$_{eq}$ protons in 4-epi-KDN 225
4.4 Inversion of homoallylic alcohol by oxidation-reduction sequence and completion of KDN synthesis

In an attempt to invert the anti stereochemistry of the homoallylic alcohol 223-anti, Mitsunobu and mesylate (226) inversion strategies were attempted (Scheme 47). The reactions unfortunately did not give any of the desired syn diastereomer probably due to the steric congestion at the homoallylic alcohol center due to the adjacent acetonide group.

![Scheme 47: Attempted inversion of the homoallylic alcohol 223-anti](image)

At this point an oxidation/reduction protocol was attempted. The initial oxidation of the homoallylic alcohol 223 under Swern oxidation conditions led to double bond migration to give the conjugated enone 227 in 89% yield (Scheme 5). However, oxidation to the desired ketone was successful using PCC oxidation when 228 was obtained in 62% yield. The reduction of the ketone 228 was gratifyingly successful with sodium borohydride in methanol at -15 °C and furnished a single diastereomer of the syn homoallylic alcohol 223 in excellent yield. The oxidation reaction was optimized to give an improved 88% yield of the ketone 228 when conducted using the Dess-Martin periodinane (Scheme 48).
Scheme 48: Oxidation and syn selective reduction

The excellent syn selectivity observed in the reduction of the homoallylic ketone 228 can be explained using a polar Felkin Ahn model as shown in Scheme 49.

Scheme 49: Felkin Ahn model for hydride reduction of homoallylic ketone 228

With the syn diastereomer syn-223 in hand, the homoallylic alcohol was protected as a benzoate ester to avoid lactonization. Ozonolysis was conducted to form an unstable ketoester, which was exposed to acidic conditions to remove the isopropylidene protection followed by acetylation using acetic anhydride in pyridine to furnish the fully protected KDN 229 in 38% yield over three steps (Scheme 7), thus completing the synthesis in 12 steps.
Scheme 50: Completion of initial KDN synthesis

4.5 Retrosynthetic analysis of KDN using a lactol intermediate

In this synthesis sequence the desired syn diastereomer syn-223 was obtained by the reduction of the homoallylic ketone intermediate 228. In an effort to reduce the steps in this linear sequence and make it more amenable to scale up, it was reasoned that the 7-debenzylated variant of the key ketone intermediate 228 could be accessed from the corresponding γ-lactol derivative 230 (Scheme 51), which could be synthesized in one step from the commercially available diacetone mannolactone 231, thereby considerably reducing the nine step sequence for the synthesis of the syn diastereomer syn-223.

Scheme 51: Retrosynthesis using a lactol intermediate

Indeed the syn-selective reduction of sugar lactols has been shown by Hannesian in his synthesis of pseudouridines114 (Scheme 52), wherein the D-ribonolactol 232, was reduced under chelation control using L-selectride and zinc chloride at -78 °C. In a study conducted to rationalize this syn selectivity Robertson and co-workers showed using substrates lacking the γ-alcohol that
reductions of 1,3-dioxolan-4-yl ketones are \textit{anti} selective irrespective of the presence or absence of zinc chloride (Scheme 52).\textsuperscript{115} Using related experiments they proposed the importance of the \(\gamma\)-alcohol and an internal delivery of the hydride from the \(\gamma\)-alkoxy borohydride as a likely model (237) to account for the observed \textit{syn} selectivity.

![Chemical structures](image)

\begin{center}
\textbf{Scheme 52:} Hanessian and Robertson’s stereoselective ketone reductions
\end{center}

4.6 \textit{syn}-Selective 6 + 3 coupling by propargyl lactol reduction

In the forward synthesis starting from the commercially available mannolactone 231, propargyl bromide was used as the pyruvate synthon due to its commercial availability and to avoid the lactonization problems encountered during the reduction of 230 (Scheme 51). The use of propargyl bromide as a pyruvate synthon was previously demonstrated by Crich and Chen\textsuperscript{75} who converted their initial homopropargyl alcohol adducts to 1-halo homopropargyl alcohol acetates.
241 (Scheme 53), by acetylation and halogenation, and then revealed the α-ketoester moiety by osmylation reactions conducted in methanol to afford the methyl pyruvates 242 from 47-86% yields.

![Scheme 53: Crich and Chen’s propargyl pyruvate synthon](image)

The addition reaction of freshly prepared propargyl magnesium bromide to mannolactone 231 proceeded smoothly to deliver a clean lactol adduct 243 in 94% yield (Scheme 11). An nOe experiment conducted on this lactol revealed a positive correlation between the propargylic protons and an acetonide methyl group thereby confirming the endo stereochemistry of the formed lactol. The preferential endo adduct formation, previously observed in the literature for the addition of Grignard nucleophiles to furanolactones,116 is likely due to the co-ordination between the multiple ether groups on the top face of the manno-lactone with the propargyl magnesium reagent.

![Scheme 54: Formation and stereochemical confirmation of homopropargyl lactol 243.](image)
The lactol 243 was then subjected to the key reduction reaction to give the diol products with various reducing agents as tabulated below (Table 5). Reduction with sodium cyanoborohydride under acidic conditions gave a complex reaction mixture. The use of the reactive lithium borohydride afforded 61% of the diol with \textit{syn:anti} preference of 68:32. The best condition as with the \textit{syn} selective reduction of ketone 228 (Scheme 48), was with sodium borohydride. The reaction using 10 eq of sodium borohydride at -15 °C, gave a 91\% yield of the diol 244 with a 100:3 \textit{syn:anti} selectivity. Under Hanessian’s chelation controlled conditions\textsuperscript{114} using L-selectride and zinc chloride, the reaction proceeded to give a good yield of the diol products 244 but was unselective. The use of LAH gave a modest yield of the diol 244, but the selectivity was reversed to a 1:9 \textit{anti} preference.

![Reduction reaction diagram](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reducing agent</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Ratio</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaBH₃CN</td>
<td>MeOH</td>
<td>0 °C</td>
<td>Complex mixture</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LiBH₄</td>
<td>Et₂O</td>
<td>0 °C</td>
<td>68:33</td>
<td>61%</td>
</tr>
<tr>
<td>3</td>
<td>NaBH₄</td>
<td>MeOH</td>
<td>-15 °C</td>
<td>100:3</td>
<td>91%</td>
</tr>
<tr>
<td>4</td>
<td>L-Selectride/ZnCl₂</td>
<td>CH₂Cl₂</td>
<td>-78 °C</td>
<td>1:1</td>
<td>83%</td>
</tr>
<tr>
<td>5</td>
<td>LiAlH₄</td>
<td>THF</td>
<td>0 °C</td>
<td>1:9</td>
<td>68%</td>
</tr>
</tbody>
</table>

\textbf{Table 5: Optimization of the propargylic lactol reduction}
The propargylation/reduction sequence using 1.05 equivalents of propargyl magnesium bromide for the lactol formation could be scaled up to five grams without loss in selectivity using crude lactol 243, but when conducted on a 25 g scale the reaction selectivity fell slightly to a 100:6 syn:anti Nevertheless enabling isolation of the syn diastereomer 244 in 79% yield over two steps (Scheme 12) as a free flowing white powder by recrystallization from diethyl ether/pentanes. The yield observed in this reaction is a function of the quality of the sodium borohydride used, with moisture free non-basic samples giving the observed yield, whilst moist samples gave an allenic by product 245 in up to 10% yield (Scheme 55).

Scheme 55: Stereoselective lactol reduction using sodium borohydride

The high stereoselectivity observed in the reduction of the lactol 243 can be explained using a model 246 shown in Scheme 56 with the delivery of the hydride along a Felkin-Ahn trajectory.
Scheme 56: Model for stereoselective lactol reduction of 243

In a related sequence diacetone manno lactol 247 on reaction with propargyl magnesium bromide delivered the coupling product 244 in 94% yield, but in a syn:anti 1:2 selectivity (Scheme 57).

Scheme 57: Homopropargylation of manno lactol 247

In a related study, the propargylation/reduction strategy for the synthesis of ulosonic acid precursors was tested using erythrono lactone 248 and arabino lactone 250. Both the reactions afforded endo propargyl lactol adducts, as confirmed by nOe experiments, which underwent syn selective reduction with sodium borohydride (Scheme 58). The assignments of configuration of the diastereomers were made by comparison of the propargylic methyl proton chemical shifts with the syn and anti adducts of the KDN series.
Scheme 58: Homopropargylation/reduction sequence to achieve syn selective coupling

4.7 Oxidative unmasking of the propargyl pyruvate synthon

With a rapid entry to this syn coupled intermediate 244 in hand the stage was set for the oxidation of the alkyne moiety to the corresponding pyruvate. To achieve this goal the alkyne syn-244 was converted to the corresponding alkynyl bromide by treatment with NBS and silver nitrate, affording the desired compound 252 in 84% yield (Scheme 59). The oxidation of the alkynyl bromide 252 was then attempted using Crich and Chen’s osmium tetroxide protocol.  

Scheme 59: Osmoylation of alkynyl bromide 252
The reaction proceeded to deliver a complex reaction mixture along with the formation of a decarbonylated ester product 253 in 34% yield and with 38% of the recovered starting material (Scheme 59). Modification of the reaction conditions using potassium osmate and by the addition of amines to improve the reaction turnover were fruitless.

After several other unsuccessful attempts at revealing the pyruvate moiety, the alkynyl bromide 252 was subjected to treatment with acetic acid/water to furnish the unprotected polyhydroxy alkynyl bromide 254 in 73% yield (Scheme 60). This highly crystalline alkynyl bromide was then subjected to ozonolysis in a 1:1 acetic acid/water mixture when it gave KDN in 43% yield. This reaction sequence was conducted on a 3 g scale to afford 1.3 g of KDN, whose $^1$H and $^{13}$C NMR spectra were identical to the reported data for KDN.$^{63}$ Thus the synthesis of KDN was completed in 5 steps in 22% overall yield.

![Scheme 60: Ozonolysis of unprotected alkynyl bromide to furnish KDN](image)

Due to the highly crystalline nature of the alkynyl bromide 254, this reaction could not be further scaled up beyond the 3 g scale owing to the large volumes of solvent needed to dissolve it. Attempts to oxidize the more soluble alkynyl bromide 252 by ozonolysis were unsuccessful and gave exclusively the decarbonylated product 253 (Scheme 61), which resulted from the over oxidation of the mol-ozonide intermediate.
Scheme 61: Ozonolysis of alkynyl bromide 252

To facilitate further scale up of the KDN synthesis, the diol 252 was protected as the corresponding diacetate 255. The fully protected alkynyl bromide 255 was then subjected to acidic conditions to give a tetraol intermediate which was subjected to ozonolysis and then acetylated to give the peracetyl KDN 150 in 29% overall yield (Scheme 62).

Scheme 62: Ozonolysis of protected alkynyl bromide 255

In order to improve the overall yield of the final oxidative unmasking of the pyruvate and facilitate scale up, the syn diol 244 was protected as a bis silyl ether 256 under rigorous conditions followed by conversion to the corresponding alkynyl bromide 257 using NBS and silver nitrate in 72% yield over two steps (Scheme 63). The fully protected alkynyl bromide 257 was then subjected to oxidation with potassium permanganate in methanol water mixture at -10 °C, to afford methyl keto ester intermediate 258. The unstable ketoester was then exposed to HCl/methanol, and then was acetylated to give the per acetylated KDN glycoside 150 in 61% yield over the three steps with a single chromatographic purification. This three step sequence
could be efficiently run on a 12 g scale of the alkynyl bromide 257 to furnish 6 grams of the peracetylated KDN 150, thus completing the synthesis of peracetylated KDN in 6 steps in 35% overall yield starting from the commercially available mannolactone 231.

Scheme 63: Large scale preparation of peracetylated KDN

In an effort to synthesize selectively protected KDN derivatives, the unprotected alkynyl bromide 254 was protected at the terminal alcohol position as TBDPS ether 259, chosen to increase solubility at lower temperatures, with DMAP catalysis using pyridine as the solvent in 87% yield (Scheme 64). This TBDPS protected alkynyl bromide was completely soluble in methanol at -78 °C and the ozonolysis of this alkynyl bromide proceeded to afford a crude methyl ketoester, which was acetylated to afford the 9-TBDPS protected KDN 260 in 42% yield.
Scheme 64: Preparation of 9-TBDPS protected KDN by ozonolysis at -78 °C

The homopropargylic alcohol in compound syn-244 could be selectively protected as a TBDPS ether, using the reactive silyl nitrate generated in-situ by the addition of stoichiometric silver nitrate to TBDPS-Cl in DMF, to afford 261 in 77% yield (Scheme 20). The silyl protected derivative 261 was converted to the corresponding alkynyl bromide and the actonide protecting groups were deprotected using aqueous acetic acid to afford the 4- TBDPS protected alkynyl bromide 262 in 63% yield over two steps. The alkynyl bromide 262 was completely soluble in methanol up to -60 °C and when subjected to ozonolysis furnished the 4-TBDPS protected methyl ester of KDN 263 in an excellent 73% yield as a white powder (Scheme 65).
Scheme 65: Synthesis of 4-TBDPS protected KDN by ozonolysis at -60 °C

When the fully protected alkynyl bromide was subjected to ozonolysis, keto ester 258 was isolated in 42% yield along with 33% of the decarbonylated by product 264. The successful conversion of the unprotected alkynyl bromides 259, 262 to the keto esters 260, 263 and the decomposition of the acetonide protected ketoester formed upon ozonolysis of the protected alkynyl bromide 252 (Scheme 66), and the formation of 264 during the ozonolysis of the protected alkynyl bromide 257 clearly indicate that the presence of free hydroxyl groups more remote than the homopropargylic position stabilizes the keto ester by cyclization, thus preventing further attack on the electrophilic ketone center which may be the cause of the decomposition to form the decarbonylated by product 253 and other by-products.
Scheme 66: Comparison of ozonolysis reactions of protected and unprotected propargylic bromides in methanol

4.8 Conclusion

In summary, an efficient and short synthesis of KDN has been achieved that permits access to multiple grams of KDN. The highly stereoselective lactol reduction allows rapid entry to the KDN core structure, thus reducing the steps from the initial synthesis of KDN. The successful ozonolytic oxidative unmasking of pyruvate on variedly protected polyol’s furnishes differentially protected KDN derivatives.
CHAPTER 5
LATE STAGE C-5 MODIFICATION OF NEURAMINIC ACID SIALOSIDES

5.1 Background
The understanding and appreciation of carbohydrates has improved immensely from the initial belief that carbohydrates are structural and energy storage materials at the beginning of 20th century. Carbohydrates are now recognized for their wider biological roles in signaling, recognition events, as diversity imparting post translational modification moieties, and are critical to various developmental processes of an organism. This comprehension of the biological roles of carbohydrates has paved the way for the development of drugs targeting their carbohydrate function or malfunction, and has led to entirely carbohydrate based drugs.\textsuperscript{117-119} Sialic acids are higher carbon carbohydrates and by virtue of their position at the terminal ends of cell surface glycan chains are primed towards various critical signaling events including their involvement in various pathological and bacterial recognition events. Glycan array analysis using libraries of sialic acid containing oligosaccharides\textsuperscript{21,120-122} has been successfully used to identify receptors and potential ligands to various glycan binding viral sialosides, which in turn has led to the development of inhibitors for infections such as keratoconjunctivitis.\textsuperscript{21,22} The various modifications of sialic acids, the most diverse class of carbohydrates known, are vital elements of the highly specific interactions mediated by them. The C-5 substituent on the sialic acid skeleton, the key diversity imparting point in this class,\textsuperscript{2} is one of the key recognition elements in sialidase/siglec-glycan interactions. In this context, the synthesis of well-defined sialoside libraries with varying substituents at the C-5 position has been an important medicinal chemistry objective which previously has been mainly addressed using enzymatic tools.\textsuperscript{3,123-131} The
development of chemical methods for efficient synthesis of C-5 modified sialic acid containing oligosaccharides is the objective of the work described in this chapter.

5.2 Enzymatic synthesis of sialoside libraries

5.2.1 Synthesis of GM3 sialoside library

Chen and co-workers found that multifunctional enzymes derived from *Pasteurella multipoda* are excellent α(2→3) sialyl transferases at pH 8.5 for galactose acceptors. Utilizing these enzymes in combination with the well-studied sialic acid aldolases employed in sialic acid synthesis, the Chen group developed a one pot three enzyme combination consisting of a sialic acid aldolase, a CMP-sialic acid synthetase and the sialyl transferase for the synthesis of the GM3 oligosaccharides with varying substituents at the C-5 position, including the Neu5Ac, KDN, and Neu5Gc saccharides (Scheme 67). In this enzymatic transformation the substituent on the mannose 2-position becomes the 5-position substituent on the sialic acid. The enzyme combination was promiscuous enough to tolerate an alkyne on the mannosamine thereby enabling the synthesis of a Neu5Gc GM3 saccharide with an alkyne handle at C-5.
Scheme 67: Chen’s one pot, three enzyme synthesis of GM3 saccharides

5.2.2 Synthesis of a GD3 library

The same group then used a α(2→8) sialyl transferase in a one-pot three enzyme combination utilizing the GM3 oligosaccharides as acceptors to form the corresponding GD3 saccharides 272 (Scheme 68) in excellent yields (51-76%).\textsuperscript{128} Though the system was well tolerant to the various substitutions on the GM3 acceptors, C-5 OMe and glycolyl azide bearing acceptors were incompatible and delivered no coupled products. In a related library synthesis the use of an α(2→6) sialyl transferase allowed the synthesis of trisaccharide libraries 273 in excellent yields.\textsuperscript{133}
Scheme 68: Enzymatic synthesis of GD3 tetrasaccharide and $\alpha(2\rightarrow6)$ linked sialoside libraries

5.2.3 Synthesis of C-5 glycosylated sialoside libraries

The high promiscuity of sialic acid aldolases is further reflected in their conversion of disaccharides with 2-$O$-glycosylated mannoses 274 at the reducing end to the corresponding sialosides in excellent yields, an example of which is shown in Scheme 69.\textsuperscript{134}

Scheme 69: C-5 glycosylated sialosides

Although the enzymatic protocols described allow synthesis of modified sialic acid-based libraries using unprotected sugars, the building blocks for the preparation of unnatural C-5
modified sialosides are not readily accessible and need to be prepared before they can be submitted to the enzymatic recipe. One example of such a preparation, that required for the synthesis of 5-fluoro sialic containing disaccharides, illustrates the six step sequence necessary to prepare the 2-deoxy-2-fluoro sugar 278 substrate for the multienzymic step (Scheme 70).\textsuperscript{129}

![Scheme 70: Enzymatic synthesis of a 5-fluorosialyli disaccharide illustrating the chemical steps required for substrate preparation](image)

5.2.4 Sialidase substrate specificity analysis with C-5 modified disialosides

Similarly, a number of other 2-substituted mannose derivatives 281 had to be prepared from D-mannose in order to access a C-5 modified sialoside library for the study of the substrate specificity of six different sialidases as a function of the C-5 substituent (Scheme 71).\textsuperscript{129}
5.3 Results and discussion

The chemical synthesis of C-5 modified sialosides by glycosylation of previously modified donors presents difficulties in addition to those usually encountered in α-sialoside formation as the substituents at C-5 position are known to have a significant impact on the stereochemical outcome of sialylations. In this context, the chemical synthesis of C-5 modified sialosides poses an important synthetic challenge. The use of the trans-fused cyclic protecting groups across the 4,5-positions of sialic acid donors presents the best available solution to efficient α-sialoside synthesis, as delineated in this thesis. The combination of this silylation methodology with a late stage modification of the C-5 substituent after α-sialoside formation was considered to be a potentially efficient method for the preparation of C-5 modified sialosides (Scheme 72) and a viable alternative to the enzymatic processes described above. The Zbiral deaminative synthesis of KDN from N-acetyl neuraminic acid deployed earlier in this thesis is an excellent example of the introduction of nucleophiles at the C-5 position of the sialic acid skeleton and was considered an ideal method for the late stage modification despite the fact that previously it had only been employed at the monosaccharide level.
Scheme 72: Strategy for synthesis of C-5 modified sialosides

5.3.1 Nitrosation of sialic acid thioglycosides

In order to test the compatibility of the methodology with the presence of thioglycosides, which would allow further extension of the modified sialosides, nitrosation was initially investigated on thioglycoside-based substrates. Gratifyingly, the methodology employed earlier for the nitrosation of peracetyl sialic acid amide using nitrosyl tetrafluoroborate and 10 equivalents of pyridine at -10 °C \(^{135}\) was successful in nitrosating the adamantyl thioglycoside \(164\) in quantitative yield in 2 h (Scheme 73).

Scheme 73: Nitrosation of a sialic acid thioglycoside

This reaction is particularly noteworthy is in view of the known use nitrosyl tetrafluoroborate as a promoter for the activation of disarmed thioglycosides \(289\) in glycosylation reactions (Scheme 74). \(^{136}\) The compatibility with thioglycosides illustrated in Scheme 73 is likely due to
the in situ formation of the lesser reactive nitrosylpyridinium tetrafluoroborate \( \text{288} \),\(^{137} \) which is indicated by the characteristic light green colored reaction mixture upon addition of the reagent.

\[ \text{AcO} \begin{array} {c} \text{AcO} \\ \text{NPth} \end{array} \longrightarrow \text{O} \begin{array} {c} \text{O} \\ \text{SMe} \end{array} + \text{HO} \begin{array} {c} \text{O} \\ \text{OMe} \end{array} \xrightarrow{\text{NOBF}_4, \text{CH}_2\text{Cl}_2, 90\%} \text{AcO} \begin{array} {c} \text{AcO} \\ \text{NPth} \end{array} \longrightarrow \text{O} \begin{array} {c} \text{O} \\ \text{OMe} \end{array} \]

Scheme 74: Literature use of Nitrosyl tetrafluoroborate to activate disarmed thioglycosides

5.3.2 Exploratory deaminations with nitrosated sialic acid thioglycoside \( \text{292} \)

With the nitrosated thioglycoside \( \text{292} \) in hand, several deamination reactions were attempted. The initial deamination conditions using Zbiral’s trifluoroethanol/sodium isopropoxide couple\(^{79} \) and neat acetic acid as nucleophile provided 58% of the peracetylated KDN thioglycoside \( \text{151} \) along with an inseparable mixture of elimination byproducts (Scheme 75). Deamination was then conducted under the same conditions except for the use of the relatively nucleophilic thioacetic acid. Clean conversion to the C-5 thioacetate-modified sialic acid thioglycoside derivative \( \text{292} \) was observed in 63% yield. Next the deamination was attempted using hydrogen fluoride in pyridine at -10 °C as nucleophile. The reaction afforded a 53% yield of the 5-fluoro sialic acid \( \text{293} \) and an impurity of similar polarity on silica gel chromatography that was characterized as the C-5 isopropanol adduct \( \text{294} \) (Scheme 75).
Scheme 75: Exploratory C-5 modifications on sialic acid

In an effort to avoid formation of the unwanted isopropyl adduct (294) the use of alternatives to the use of sodium isopropoxide in isopropanol in the deamination process was indicated. Sodium trifluoroethoxide was prepared and was found to be insoluble in dichloromethane, but the use of stoichiometric 18-crown-6 solubilized the salt completely in the same solvent at room temperature and at -10 °C. Deamination of the nitrosyl sialoside 287 was then attempted using the modified deamination conditions. The reaction was completed in less than 5 minutes and afforded 63% of the 5-fluoro sialic acid 293 (Scheme 76).

Scheme 76: Modified deamination conditions
Encouraged by the excellent yields for the deamination of sialic acid using thioacetic acid and fluoride nucleophiles, a series of other nucleophiles were tested (Scheme 77). Unfortunately none of these reactions afforded nucleophile incorporation and gave complex reaction mixtures of the elimination products.

![Scheme 77: Unsuccessful attempts at incorporation of various nucleophiles](image)

Intriguingly, in view the unwanted isopropanol adduct 294 (Scheme 75) formation in the fluorination reaction, the use of propargyl alcohol as nucleophile in the deamination reaction, did not deliver the desired product.

This observation clearly suggested an additional role of HF in promoting the isopropanol adduct formation as an acid, apart from that of fluoride nucleophile source. This line of thought clearly suggested the sequence of events in the deamination reaction, wherein in the diazo intermediate 294 is first protonated by the acid(acetic/thioacetic acid, or the HF) leading to a diazonium ion, from which nitrogen is expelled by the neighboring acetate ester providing an acetoxonium ion. The conjugate base of the acid then attack at the C-5 of the so-formed intermediate 295 (Scheme 78) resulting in the nucleophile incorporation with retention at C-5.
5.3.3 Incorporation of non-acidic nucleophiles

Keeping in view the necessity of a proton source in the deamination reactions, a reaction with the non acidic nucleophile propargyl alcohol was conducted using fluoroboric acid as a proton source, along with propargyl alcohol as nucleoside. Thus, upon formation of the diazo intermediate, a mixture of twenty equivalents of propargyl alcohol and two equivalents of fluoroboric acid were added to the reaction mixture. Gratifyingly, when conducted in this manner, deamination took place successfully with the propargyl alcohol incorporation in 66% yield (Scheme 79). This reaction was also successful using sulphuric acid in place of fluoroboric acid as the additive.

Scheme 78: Role of proton source in deamination

Scheme 79: Successful C-5 incorporation of propargyl alcohol
5.4 Deamination of disaccharides

5.4.1 Synthesis of α-sialosides using N-acetyl-5N,4O-oxazolidinone technology

With conditions in hand for the deamination reaction, C-5 modifications were attempted on disaccharides, each of which were synthesized using the acetyl oxazolidinone methodology using adamantyl thioglycosides\textsuperscript{44} or the glycosyl phosphates\textsuperscript{49} as leaving groups as described in Table 6.
### Table 6: Synthesis of α-sialosides using N-acetyl-5N,4O-oxazolidinone technology

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>Yield A/Ratio(α:β)</th>
<th>Yield B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>166</td>
<td>300, 81% only α</td>
<td>301, 77% over 2 steps</td>
</tr>
<tr>
<td>A</td>
<td>165</td>
<td>302, 84% only α</td>
<td>303, 88% over 2 steps</td>
</tr>
<tr>
<td>A</td>
<td>177</td>
<td>304, 83% 10:1</td>
<td>305, 76% over 2 steps</td>
</tr>
<tr>
<td>B</td>
<td>306</td>
<td>307, 86%, only α</td>
<td>308, 91% over 2 steps</td>
</tr>
<tr>
<td>B</td>
<td>309, 310</td>
<td>311, 74% over 3 steps, only α</td>
<td></td>
</tr>
</tbody>
</table>
The acceptors 306 and 309 were synthesized using standard protocols shown below in Scheme 80. The known peracetyl galactosamine 312 was converted to the corresponding phenyl thioglycoside 313 using BF₃ etherate in 77% yield. The phthalalide protected galactosamine 313 was subjected to Zemplen deacetylation to afford a quantitative yield of the deacetylated product, which was protected as a 6-O-TBS ether and then converted to 314 in the same pot using sodium hydride as a base and benzyl bromide as alkylation agent in 61% yield over two steps. The silyl ether was deprotected with pTSA in methanol to afford the galactosamine acceptor 306 in 87% yield. The acceptor 309 was prepared by acetylation of known 8,9-O-acetonide protected neuraminic acid 315 and subsequent acetic acid mediated cleavage of the acetonide to afford 309 in 72% yield.

![Scheme 80: Synthesis of acceptors 306 and 309](image)

5.4.2 Deamination of disaccharides

The naturally occurring Neu5Ac α(2→6)gal based disaccharide 301 was subjected to nitrosation with NOBF₄ using 10 equivalents of pyridine (Scheme 81). The nitrosation proceeded without
disturbing the glycosidic bond to furnish the nitrosated disaccharide 317 in quantitative yield. The disaccharide was then subjected to deamination reaction using acetic acid as nucleophile when it afforded the KDN disaccharide 179 in 51% yield.

Scheme 81: Deamination on a Neu5Ac α(2→6)gal disaccharide

The deamination reaction was then carried out on another disaccharide consisting of a neu5Ac α(2→6)glu disaccharide 303 (Scheme 82) when it afforded the C-5 modification to give the KDN glycoside 180 in 48% yield.

Scheme 82: Deamination on a Neu5Ac α(2→6)glu unit
With the successful conversion of the neuraminic acid glycosides to KDN glycosides the
important Neu5Ac $\alpha(2\rightarrow3)$gal linkage $305$ was tested for compatibility with the deamination
conditions. Nitrosation using the standardized conditions afforded a quantitative yield of the
disaccharide $319$. Deamination with acetic acid as the nucleophile was successful in affording
54% yield of the KDN $\alpha(2\rightarrow3)$gal linkage $320$ (Scheme 83), and the use of thioacetic acid as the
nucleophile gave the C-5 thioester $321$ in 67% yield. Employing the modified deamination
conditions and the triethylamine HF complex as the fluoride source, fluorination was carried out
successfully in 63% yield (Scheme 83).

![Scheme 83: Unnatural C-5 modifications on the Neu5Ac $\alpha(2\rightarrow3)$gal disaccharide](image)

Deamination on the Neu5Ac $\alpha(2\rightarrow6)$galNAc disaccharide $308$, a protected version of the sTn
antigen, using acetic acid as nucleophile furnished the KDN sTn $324$ in 49% yield (Scheme 84).
This result is important in so far as sTn antigens with modifications at the C-5 amide have been shown to display improved immunogenicity. The reaction also showcases the compatibility of the deamination sequence to the presence of another nitrogen atom in the saccharide, protected as a pthalimide to avoid competing nitrosation. This reaction also reiterates the compatibility of the methodology with the presence of a thioglycoside.

**Scheme 84: Deamination of the sTn antigen**

KDN α(2→9)-linked disialosides have been proposed as the receptor targets in the *Aspergillus fumigatus* propagated aspergillosis disease in humans. In an effort to synthesize a disialoside of KDN, the deamination reaction was attempted on an α(2→9) disialoside of neuraminic acid (311). Using tetrabutylammonium acetate in acetic acid as the nucleophile (Scheme 85) afforded cleanly the corresponding double deamination product 326 in 42% yield with retention of the stereochemistry at both the C-5 positions.
Scheme 85: Double deamination

5.5 Deamination of a GM3 trisaccharide

The fully protected GM3 saccharide prepared form the known trisaccharide 327 by acetylation was subjected to the deamination methodology. Nitrosation using the standardized conditions provided the nitrosated GM3 329 in quantitative yield. Subsequent deamination using acetic acid as the nucleophile provided the KDN GM3 330 in 55% yield (Scheme 86).

Scheme 86: Oxidative deamination of a GM3 saccharide
5.6 Deamination of a GM2-like tetrasaccharide

In an effort to test the scope of the methodology on larger saccharides a branched GM2-like tetrasaccharide saccharide 332 was prepared from the peracetyl glucosyl thioglycoside donor 331 and the trisaccharide acceptor 327 in 73% yield using the standard NIS/TfOH preactivation glycosylation protocol to afford the tetrasaccharide (Scheme 87).

Scheme 87: Synthesis of a GM2-like tetrasaccharide

The GM2-like tetrasaccharide 332 was exposed to the nitrosation protocol affording a clean conversion in quantitative yield to the nitrosated tetrasaccharide 333. This oligosaccharide, when exposed to the deamination reaction with acetic acid as the nucleophile, cleanly afforded the KDN GM2-like tetrasaccharide 334 in 58% yield (Scheme 88) after purification by HPLC. This application to a sterically demanding branched tetrasaccharide attests to the broad applicability of the late stage deaminative nucleophile incorporation technology as an efficient means for the modification at the C-5 position of neuraminic acid containing oligosaccharides.
Scheme 88: Synthesis of a KDN GM2-like tetrasaccharide by deamination of 331

5.7 Assignment of regio- and stereochemistry of deamination products

The regiochemistry and the stereochemistry of the various deamination products were assigned based mainly on the $^1$H NMR spectra of the products and the analysis of coupling constant data. The H-5 resonance of a sialic acid with the usual D-glycero-D-galacto stereochemistry is typically a 9-11 Hz triplet due to the two trans diaxial couplings with H-4 and H-6. For a system with the opposite configuration at C-5, i.e., and axial C5 substituent and an equatorial H5, a triple with two small couplings with H-4 and H-6 would be expected as shown in Figure 22. Any substitution at C-4 would necessarily lead to an axial substituent at that position resulting in a smaller $^3J_{HH5}$ coupling constant resulting in the presentation of the H-5 resonance as a doublet of doublets with one large coupling with H-6 and a small one with H-4. With this in mind, H-H Cosy spectra were used to locate the H-5 resonance on the sialic acid for all of the deamination products. Subsequent inspection of this resonance revealed it in every case to be a triplet with an
~10 Hz coupling constant, thereby confirming the region- and stereochemistry of the substitution reactions.

![Chemical structures](image)

**Figure 22**: Anticipated coupling constants for substitution at C-4 and C-5 (A, at C5 with retention; B, at C5 with inversion, and C, at C4 with inversion)

### 5.8 Conclusion

Overall an excellent methodology for a late stage modification of the C-5 substituent on the sialic acid is disclosed in this chapter. This methodology for accessing C-5 modified sialoside libraries obviates the necessity of carrying out multistep syntheses of oligosaccharides with C-5 modified sialic acid donors, which are hard to access themselves and which could potentially lead to unselective transformations. The excellent regio and stereocontrol for the nucleophile incorporation is consistent across a range of important sialoside linkages including the conversion of a neuraminic acid containing branched tetrasaccharide to a KDN containing tetrasaccharide in two simple steps. Late stage modifications in the form of C-H oxidations have been reported by White$^{139,140}$ and others$^{141-144}$ as means to add diversity to natural products accessed through multi step synthesis, but no such modifications at a late stage have been reported for oligosaccharides. The incorporation of the fluoride, thiocarboxylate and alcohol nucleophiles at a late stage offers a chance to access unnatural modifications at the C-5 position.
of sialic acid oligosaccharides. The methodology opens up the possibility for incorporation of
other nucleophiles at C-5 of sialic acids and also for potential modifications of other acetamide
containing glycosides.
CHAPTER 6
CONCLUSION

A novel trans-4,5-O-carbonate group installed on a KDN adamantyl thioglycoside allowed highly stereoselective α-sialoside formation at -78 °C. The donor was found to give uniformly high yields due to the lack of elimination byproduct formation and was found to be compatible with sulphoxide activation systems. The carbonate group can be cleanly removed using Zemplen deacetylation conditions in quantitative yield.

Cone voltage induced fragmentations in a ESI mass spectrometer have been used to assess the relative energetics of sialyl oxocarbenium ions throwing light on the mechanism of α-selective sialylations employing a N-acetyl 5N,4O-oxazolidinone, 5N,4O-oxazolidinone or 4,5-O-carbonate systems. The higher onset cone voltages for fragmentation observed in the presence of a 4,5 cyclic protecting group suggest a strong electron withdrawing effect for such groups, by virtue of which the oxocarbenium ion formation is retarded and associative pathways for glycosylation are promoted.

A highly syn stereoselective manno lactol reduction strategy has been demonstrated to reliably deliver multiple gram quantities of the masked KDN syn 244. The ozonolytic unmasking of the pyruvate completed the five step synthesis of KDN on a 1 g scale. The oxidation of fully protected alkynyl bromide 254 furnished peracetylated KDN on a 6 g scale, thereby demonstrating the scalability of this synthesis.
A nitrosoative deamination strategy using various nucleophiles has allowed late stage modifications on neuraminic acid containing sialosides, opening up possibilities for synthesis of C-5 modified libraries of sialosides. The deamination reaction has been demonstrated on all of the important α-sialyl linkages and the important sTn, GM3, and GM2-like oligosaccharides, making it a viable synthetic alternative to enzymatic sialic acid C-5 modification.
CHAPTER 7

EXPERIMENTAL SECTION

General

All solvents were dried using standard protocols. All reactions were performed in an atmosphere of nitrogen or argon unless otherwise stated. All organic extracts were dried over sodium sulfate and concentrated under aspirator vacuum. Optical rotations were recorded in CHCl₃ solution unless otherwise specified, on an Autopol® III automatic polarimeter. HRMS were carried out on LCT premiere Waters Mass Spectrometer. Stereochemical assignments of coupled sialosides are based on ¹H-¹H-DEPT values. HPLC was performed on a Varian Prostar 300 system.

Methyl (2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-5-[anti/syn-N-nitrosoacetimido]-α-glycero-β-D-galacto-2-onulopyranosid)onate (148): A mixture of methyl (2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-α-glycero-β-D-galacto-2-onulopyranosid)onate (147), (6.2 g, 11.6 mmol) and anhydrous pyridine (9.3 mL, 116 mmol) in anhydrous CH₂Cl₂ (116 mL) was stirred for 5 min and cooled to -10 °C. After stirring for 10 min, nitrosyl tetrafluoroborate (2.72 g, 23.2 mmol) was added in one portion to the mixture. The mixture was stirred at -10 °C for 45 min and then was warmed to 5 °C and was maintained for 4 h with monitoring by TLC (MeOH/ CH₂Cl₂ 1/20). The reaction mixture was diluted with CH₂Cl₂ and washed with cold 1N HCl (150 mL), then with cold saturated aqueous NaHCO₃ (100 mL). The organic layer was washed with cold brine (150 mL) and dried over Na₂SO₄ and then concentrated, below 10 °C to obtain 1 (6.47 g, 11.6 mmol, 99%) as a yellow foam with spectral data consistent with those reported in the literature.¹⁴⁵
Methyl (2,4,5,7,8,9-hexa-O-acetyl-3-deoxy-\(\beta\)-\(\alpha\)-glycero- \(\beta\)-\(\alpha\)-galacto-2-nonulopyranosid)onate (150): A solution of 148 (6.47 g, 11.5 mmol) in CH\(_2\)Cl\(_2\) (115 mL) at -10 °C was mixed with trifluoroethanol (1.24 mL, 17.2 mmol) and freshly prepared sodium isopropoxide in isopropanol (69 mL, 0.2N, 13.8 mmol). The resulting mixture was stirred at -10 °C for exactly 1 min before a solution of glacial acetic acid (13.8 g, 230 mmol) in CH\(_2\)Cl\(_2\) (50 mL) was added. After stirring for 3 min the reaction mixture was warmed to 0 °C and was carefully quenched with saturated aqueous NaHCO\(_3\) solution. The extracted organic layer was washed with brine (150 mL), dried over Na\(_2\)SO\(_4\) and concentrated. The crude reaction mixture was taken up in methanol (120 mL) was cooled to -78 °C and was sparged with ozone with mild stirring for 30 min. Dimethyl sulphide (24 mL, 325 mmol) was then added and the reaction mixture slowly warmed to room temperature and stirred overnight. The reaction mixture was concentrated and the residue was purified by flash chromatography eluting with ethyl acetate/hexane (1:1) to obtain 150 (3.14 g, 51%) with spectral data consistent with those reported in the literature.\(^{145}\)

Methyl (1-adamantanyl 4,5,7,8,9-penta-O-acetyl-3-deoxy-2-thio-\(\alpha\)/\(\beta\)-glycero-\(\alpha\)-galacto-2-nonulopyranosid)onate (151\(\alpha/\beta\)): A mixture of 150 (2.87 g, 5.4 mmol) and 1-adamantanethiol (1.36 g, 8.1 mmol) in anhydrous CH\(_2\)Cl\(_2\) (55 mL) was treated with BF\(_3\).OEt\(_2\) (1.7 mL, 13 mmol) and stirred for 12 h. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) (100 mL) and washed with saturated aqueous NaHCO\(_3\), brine and dried over Na\(_2\)SO\(_4\) and concentrated. The crude residue, diluted with ethyl acetate (100 mL), was treated with 3 g of activated charcoal and stirred for 3 h, then filtered through a pad of silica gel and Celite\(^\circledR\). The decolorized reaction mixture was purified by flash chromatography eluting with ethyl acetate/hexanes (1/4 to 1/1) to obtain 151\(\beta\) (2.89 g, 84%), 151\(\alpha\) (0.382 g, 11%). 151\(\beta\): [\(\alpha\)]\(_D\)\(^{24}\) = -30.8 (c = 1, CHCl\(_3\)), \(^1\)H NMR (500 MHz,
CDCl3) δ: 5.44 (t, J = 2.5 Hz, 1H), 5.34 (ddd, J = 11.8, 9.5, 4.5 Hz, 1H), 5.22 (td, J = 8.5, 2 Hz, 1H), 4.96 (dd, J = 12.5, 2Hz, 1H), 4.83 (t, J = 10 Hz, 1H), 4.71 (dd, J = 10, 5.5 Hz, 1H), 4.23 (dd, J = 12.5, 8.5 Hz, 1H), 3.81 (s, 3H) 2.60 (dd, J = 14, 5 Hz, 1H), 2.09 (s, 3H), 2.05 (s, 3H), 1.96-2.0 (m, 15H), 1.85-1.89 (m, 3H), 1.65 (broad s, 7H). 13C NMR (125 MHz, CDCl3) δ: 170.9, 170.6, 170.3, 170.1, 170.0 (C-1, 3JC-1-H3ax = 0 Hz), 86.1, 77.5, 77.2, 77.0, 73.3, 71.3, 69.2, 68.5, 63.4, 53.0, 50.8, 43.6, 39.7, 36.1, 30.0, 21.2, 21.0, 20.9, 20.8, 20.8. ESIHRMS Calcd. for C30H42O13Na [M + Na]+, 665.2244; found: 665.2233. 151α: [α]24D = +25.06 (c = 1, CHCl3), 

1H NMR (500 MHz, CDCl3) δ: 5.34 (dd, J = 9.5, 1.5 Hz, 1H), 5.32-5.30 (m, 1H), 4.82-4.80 (m, 2H), 4.32 (dd, J = 12.5, 2.5 Hz, 1H), 4.18 (dd, J = 12.5, 3.5 Hz, 1H), 4.13-4.12 (m, 1H), 3.81 (s, 3H), 2.74 (dd, J = 13.5, 4.5 Hz, 1H), 2.2 (s, 3H), 2.1 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.0 (s, 3H), 1.97-1.96 (m, 8H), 1.90-1.87 (broad d, 4H), 1.68 (broad s, 7H). 13C NMR (125 MHz, CDCl3) δ: 170.9, 170.3, 170.1, 170.0, 168.8 (C-1, 3JC-1-H3ax = 7.5 Hz), 84.6, 73.0, 69.9, 68.8, 68.1, 66.1, 66.6, 61.8, 53.0, 51.4, 43.7, 39.7, 36.2, 30.1, 30.0, 21.3, 21.6, 20.94, 20.90. ESIHRMS Calcd. for C30H42O13Na [M + Na]+, 665.2244; found: 665.2256.

Methyl (1-adamantyl 3-deoxy-8,9-O-isopropylidene-2-thio-β-d-glycero-β-d-galacto-2-nonulopyranosid)onate (152β): Compound 151β (2.8 g, 4.4 mmol) in anhydrous MeOH (44 mL) was treated with a few drops of sodium methoxide solution (25 wt. %) in methanol and was stirred for 5 h. The reaction mixture was neutralized by the addition of Amberlyst 15 (H+) resin, filtered and concentrated. The crude reaction mixture in acetone (45 mL) was treated with 2,2-dimethoxypropane (0.67 mL, 5.5 mmol) and 10 mg of pTSA. The mixture was stirred for 1.5 h and was treated with Dowex 1 anionic resin (1 g), and stirring was continued for 10 min. The reaction mixture was then filtered and concentrated. The residue was purified by flash
chromatography (1:20 MeOH/CH₂Cl₂) to obtain \[\text{152\(\beta\)}\] (1.87 g, 91%) as a white foam.

\[\alpha_{D}^{24} = -132.5 \ (c = 1, \text{MeOD}), \text{H NMR} (500 \text{MHz, MeOD}) \delta: 4.19 (m, 2H), 4.15 (dd, \ J = 9,6 Hz, 1H), 3.99 (dd, \ J = 8, 5.5 Hz, 1H), 3.94 (m, 1H), 3.83 (s, 3H), 3.79 (d, \ J = 9 Hz, 1H), 3.41 (t, \ J = 10 Hz, 1H), 2.4 (dd, \ J = 3.5, 5 Hz, 1H), 2.0 (broad s, 10H), 1.7 (broad s, 6H), 1.4 (s, 3H), 1.38 (s, 3H). \text{¹³C NMR} (125 MHz, MeOD) \delta: 172.9, 109.3, 86.2, 74.2, 73.1, 70.78, 70.71, 68.7, 67.8, 52.2, 49.4, 48.3, 48.1, 48.0, 47.8, 47.6, 47.5, 47.33, 47.30, 42.5, 36.0, 30.1, 25.9, 24.6.

ESIHRMS Calcd. for C₂₃H₃₆O₈SNa [M + Na]⁺: 495.2029; found: 495.2032.

**Methyl (1-adamantanyl 3-deoxy-8,9-O-isopropylidene-2-thio-α-D-glycero-β-D-galacto-2-nonulopyranosid)onate (152\(\alpha\)):** 152\(\alpha\) (0.455 g, 86%) was prepared in two steps from 3\(\alpha\) (0.72 g, 1.1 mmol) following the same procedure used to make 4\(\beta\). \[\alpha_{D}^{24} = +8.0 \ (c = 0.6, \text{MeOD}), \text{H NMR} (500 \text{MHz, MeOD}) \delta: 4.32-4.27 (m, 1H), 4.12 (dd, \ J = 8.5, 5.5 Hz, 1H), 3.88 (dd, \ J = 8.5, 6.5 Hz, 1H), 3.80 (s, 3H), 3.7 (d, \ J = 9Hz, 1H), 3.68 (dd, \ J = 10, 1.5 Hz, 1H), 3.54 (t, 9.5 Hz, 1H, 3.39-3.36 (m, 1H), 2.60 (dd, \ J = 12.5, 4.5 Hz, 1H), 2.1-1.9 (m, 9H), 1.71 (broad s, 6H), 1.65 (t, \ J = 12 Hz, 1H), 1.39(s, 3H), 1.34 (s, 3H). \text{¹³C NMR} (125 MHz, MeOD) \delta: 171.0, 109.1, 85.0, 76.9, 75.2, 70.1, 70.0, 69.9, 67.9, 51.5, 50.0, 43.7, 42.1, 36.0, 30.2, 30.1, 25.9, 24.5.

ESIHRMS Calcd. for C₂₃H₃₆O₈SNa [M + Na]⁺, 495.2029; found: 495.2034.

**Methyl (1-adamantanyl 3-deoxy-5,7,8,9-di-O-isopropylidene-2-thio-β-D-glycero-β-D-galacto-2-nonulopyranosid)onate (159\(\beta\)):** Compound 151\(\beta\) (0.6 g, 0.93 mmol) in anhydrous MeOH (10 mL) was treated with a few drops of sodium methoxide solution (25 wt. %) in methanol and was stirred for 5 h. The reaction mixture was neutralized by the addition of Amberlyst 15 (H⁺) resin, filtered and concentrated. The crude reaction mixture in acetone (10 mL) was treated with 2,2-
dimethoxypropane (2.28 mL, 18.6 mmol) and 40 mg of pTSA. The mixture was stirred overnight and was treated with 0.2 mL of triethylamine, and stirring was continued for 10 min. The reaction mixture was then concentrated. The residue was purified by flash chromatography (3:1 PhMe/EtOAc) to obtain 159β (0.34 g, 72%) as a white foam. [α]$_D^{24}$ = +17.0 (c = 0.8, CDCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 4.08-4.03 (m, 2H), 3.83-3.78 (m, 4H), 3.76-3.67 (m, 3H), 3.37 (t, J = 10 Hz, 1H), 2.47 (dd, J = 13, 5 Hz, 1H), 2.02-1.87 (m, 9H), 1.78 (t, J = 14 Hz, 1H), 1.62 (s, 6H), 1.40 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 1.31(s, 3H), $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 171.4, 101.8, 98.2, 85.6, 75.4, 73.8, 72.9, 67.1, 62.7, 62.3, 52.6, 50.2, 43.3, 41.5. ESIHRMS Calcd. for C$_{26}$H$_{40}$O$_6$SNa [M + Na]$^+$, 535.2342; found: 535.2353.

**Methyl (1-adamantanyl 3-deoxy-5,7;8,9-di-O-isopropylidene-2-thio-α-D-glycero-D-galacto-2-nonulopyranosid)onate (159α):** 152α (0.20 g, 63%) was prepared from 151α (0.410 g, 0.06 mmol) following the same procedure used to make 159β. [α]$_D^{24}$ = +7.3 (c = 0.8, CDCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 3.79-3.75 (m, 4H), 3.71-3.62 (m, 4H), 3.46-3.43 (m, 2H), 2.71 (dd, J = 16, 4 Hz, 1H), 2.10 (d, J = 14.5 Hz, 3H), 1.97-1.94 (m, 6H), 1.87-1.81 (m, 2H), 1.66 (s, 3H), 1.44 (s, 3H), 1.40 (s, 3H), 1.34 (s, 3H), 1.33 (s, 3H), $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 171.1, 102.0, 98.3, 84.0, 77.2, 75.8, 72.2, 68.0, 62.8, 62.4, 52.5, 51.2, 43.6, 41.2, 36.2, 30.0, 29.1, 24.9, 24.5, 18.7. ESIHRMS Calcd. for C$_{26}$H$_{40}$O$_6$SNa [M + Na]$^+$, 535.2342; found: 535.2351.

**Methyl (1-adamantanyl 3-deoxy-5,7;8,9-di-O-isopropylidene-4-nitrophenyl-2-thio-β-D-glycero-D-galacto-2-nonulopyranosid)onate (160β):** Compound 159β (0.3 g, 0.58 mmol) in anhydrous CH$_2$Cl$_2$ (4 mL) and Hunig’s base (1.08 mL, 5.8 mmol) was treated with 4-nitrophenyl chloroformate (621 mg, 2.92 mmol) and was stirred overnight. The reaction mixture was
concentrated to dryness under reduced pressure and the residue was purified by silica gel chromatography (Hexanes/EtOAc 3:1) to obtain 160β (0.34 g, 72%) as a yellow foam. [α]$_D^{24}$ = -11.3 (c = 2, CHCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 8.27 (d, J = 10 Hz, 2H), 7.36 (d, J = 9.5 Hz, 2H), 5.26 (m, 1H), 4.24 (t, J = 10Hz, 1H), 3.86-3.83 (m, 2H), 3.81 (s, 3H), 3.80-3.72 (m, 2H), 3.72-3.66 (m, 2H), 2.73 (dd, J = 13.5, 5.5 Hz, 1H), 2.09-1.90 (m, 10H), 1.67-1.62 (m, 7H), 1.81 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H), 1.33 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.83, 155.41, 151.48, 145.34, 125.31, 121.56, 101.93, 98.35, 85.15, 77.23, 75.34, 74.76, 74.03, 69.43, 62.70, 62.51, 58.83, 50.76, 43.27, 39.51, 36.11, 30.93, 29.96, 24.57, 24.50. ESIHRMS Calcd. for C$_{33}$H$_{43}$NO$_1$2SNa [M + Na]$^+$, 700.2404; found: 700.2429.

**Methyl (1-adamantany1 4,5-O-carbonyl-3-deoxy-2-thio-β-D-glycero-D-galacto-2-nonulopyranosid)onate, 162β:** Compound 160β (0.39 g, 0.58 mmol) was dissolved in a mixture of MeOH/Water/HCl (8:1:1, 10 mL) and was stirred overnight. The reaction mixture was diluted with MTBE (20mL) and then water (10 mL). The separated aqueous layer was washed with MTBE (2x5 mL) and the combined organic layer was washed with brine (2x10 mL), dried and concentrated to afford 161β as yellow foam. The crude 161β was dissolved in acetonitrile/water (5 mL 98:2) and was treated with cesium bicarbonate (111mg, 0.58 mmol). After 15 minutes the reaction was completed and the yellow crude reaction mixture was diluted with EtOAc and was washed with water, dried and concentrated. The crude reaction mixture was purified by silica gel chromatography (EtOAc/CH$_2$Cl$_2$, 1:1) to afford 162β (216 mg, 82% over steps) as a white foam. [α]$_D^{24}$ = +14.8 (c = 0.5, CHCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 4.87 (dt, J = 12.5, 4 Hz, 1H), 4.78 (dd, 10, 1.5 Hz, 1H), 4.23 (t, J = 11Hz, 1H), 3.90 (s, 2H), 3.86 (s, 3H), 3.85-3.79 (m, 1H), 3.62 (d, J = 8.5 Hz, 1H), 3.11-3.68 (m, 2H), 2.85 (dd, J = 13, 4 Hz, 1H), 2.28 (t, J = 12.5 Hz,
1H), 2.05-1.91 (m, 9H), 1.67 (s, 6H). 13C NMR (125 MHz, CDCl3) δ: 170.6, 153.6, 85.8, 78.1, 77.7, 71.5, 70.7, 69.8, 63.7, 53.3, 51.3, 43.2, 39.8, 35.9, 29.8. ESIHRMS Calcd. for C21H30O5SNa [M + Na]+, 481.1508; found: 481.1519.

Methyl (1-adamantanyl 7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-2-thio-β-D-glycero-β-D-galacto-2-nonulopyranosid)onate (146β): Compound 162β (200 mg, 0.43 mmol) was dissolved in pyridine (4 mL) and was treated with acetic anhydride (4 mL). The mixture was stirred at room temperature for 4 h, and was concentrated to dryness under reduced pressure. Purification of the crude reaction mixture by silicagel chromatography (EtOAc/Hexanes 4:6) afforded 146β as a colorless foam (0.24 g, 97%). [α]24 D = -38.1 (c = 1.9, CHCl3), 1H NMR (500 MHz, CDCl3) δ: 5.4 (t, J = 5 Hz, 1H), 5.3 (m, 1H), 4.8 (dt, J = 12.5, 4 Hz, 1H), 4.7 (q, 5 Hz, 1H), 4.5 (dd, J = 12.5, 3 Hz, 1H), 4.3 (dd, J = 12.5, 7.5 Hz, 1H) 4.0 (t, J = 10 Hz, 1H), 3.8 (s, 3H), 2.8 (dd, 13, 4.5 Hz, 1H), 2.2 (t, J = 12.5 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.0-1.98 (m, 7H), 1.8 (broad d, 3H), 1.6 (m, 5H). 13C NMR (125 MHz, CDCl3) δ: 170.7, 170.4, 169.5 (C-1, 3J C1-4H3ax = 0 Hz), 169.2, 153.3, 86.4, 79.1, 77.9, 77.5, 77.2, 77.1, 77.0, 70.7, 70.3, 62.4, 53.3, 51.7, 43.5, 39.8, 36.1, 30.7, 21.1, 20.9, 20.8. ESIHRMS Calcd. for C27H36O12SNa [M + Na]+, 607.1825; found: 607.1823.

Bis (4-nitrophenyl) carbonate: To a mixture of 4-nitrophenol (4 g, 28.7 mmol) and Hunig’s base (17.6 mL, 100 mmol) in THF (150 mL), cooled to 0 °C, was added drop wise a solution of triphosgene (5.97 g, 20.1 mmol) in CH2Cl2 (20 mL) over 5 min. The reaction mixture was stirred for 0.5 h (TLC, 100% CH2Cl2), then was poured into 1N HCl (150 mL), and was extracted with CH2Cl2 (3 x 30mL). The combined organic extracts were washed with brine, dried over Na2SO4,
and concentrated to give a white solid, which was purified by flash chromatography (eluent: CH₂Cl₂), to give bis-4-nitrophenyl carbonate (3.98 g, 91%). Mp 137-139 °C, Lit Mp 140-141 °C.

**Methyl (1-adamanantyl 4,5-O-carbonyl-3-deoxy-8,9-O-isopropylidene-2-thio-β-D-glycero-D-galacto-2-nonulopyranosid)onate, 163β:** A mixture of 152β (1.61 g, 3.3 mmol) and bis(4-nitrophenyl) carbonate (5.15 g, 16.9 mmol) in THF (33 mL) at room temperature, was treated with Hunig’s base (5.95 mL, 33 mmol). The reaction mixture was stirred for 6 h then was diluted with ethyl acetate and was washed with 0.5 N HCl (75 mL), brine, dried over Na₂SO₄ and concentrated. The mixture was purified by flash chromatography (ethyl acetate/hexane: 1/10 to 1/1) to obtain compound 163β (1.02 g, 61%). [α]²⁴D = -8.0 (c = 1, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ: 4.8 (dt, J = 12.5, 3.2 Hz, 1H), 4.7 (d, J = 5 Hz, 1H), 4.2 (m, 2H), 4.1 (m, 1H), 4.0 (dd, J = 8.5, 5.5 Hz, 1H), 3.85 (s, 3H), 3.65 (d, J = 8.5 Hz, 1H), 2.86 (dd, J = 12.5, 9.5 Hz, 1H), 2.24 (t, J = 12.5 Hz, 1H), 1.9-2.0 (m, 10H), 1.67 (s, 6H), 1.42 (s, 3H), 1.30 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ: 170.2, 153.7, 109.9, 86.1, 77.8, 77.7, 77.5, 77.2, 77.0, 73.7, 71.6, 71.3, 67.9, 53.4, 51.5, 43.3, 40.2, 36.1, 30.1, 29.9, 25.3. ESIHRMS Calcd. for C₂₄H₄₉O₉SnA [M + Na]⁺, 521.1821; found: 521.1827.

**Methyl (1-adamanantyl 4,5-O-carbonyl-3-deoxy-8,9-O-isopropylidene-2-thio-α-D-glycero-D-galacto-2-nonulopyranosid)onate (163α):** 163α (0.28 g, 60%) was prepared from 152α (0.450 g, 0.95 mmol) following the same procedure used to make 163β. [α]²⁴D = +6.08 (c = 1, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ: 4.4 (d, J = 10 Hz, 1H), 4.25(t, J = 10.5 Hz, 1H), 4.16-4.13 (m, 2H), 4.08 (dd, J = 12.5, 4 Hz, 1H), 4.04-4.02 (m, 1H), 3.87 (s, 3H), 3.67 (t, 8 Hz, 1H), 3.15 (dd, J
= 12, 3.5 Hz, 1H), 2.22-2.17 (m, 2H), 2.0 (b, 6H), 1.89 (broad s, 3H), 1.68 (broad s, 6H), 1.43 (s, 3H), 1.38 (s, 3H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\): 169.9, 153.4, 109.8, 86.1, 77.7, 76.9, 75.5, 74.8, 70.9, 67.4, 53.4, 43.9, 39.7, 36.1, 38.1, 27.0, 25.5. ESIHRMS Calcd. for \(\text{C}_{24}\text{H}_{34}\text{O}_{9}\text{SNa}[\text{M} + \text{Na}]^+\), 521.1821; found: 521.1826.

In order to fully assign the spectra and verify the location of the carbonate in 163\(a\), the substance was converted to its acetate as follows:

**Methyl (1-adamantanyl 7-O-acetyl-4,5-O-carbonyl-3-deoxy-8,9-O-isopropylidene-2-thio-\(\alpha\)-d-glycero-\(\alpha\)-galacto-2-nonulopyranosid)onate (163\(a\)-acetate):** Compound 163\(a\) (60 mg, 0.12 mmol) was dissolved in a mixture of pyridine (3 mL) and acetic anhydride (3 mL) and stirred for 3 h. The volatiles were removed under reduced pressure and the crude mixture was diluted with ethyl acetate, and was washed with 1N HCL, followed by aqueous NaHCO\(_3\), dried over Na\(_2\)SO\(_4\), and concentrated. Purification on silica gel with ethyl acetate/hexanes (1/2.5) gave afforded 163\(a\)-acetate (62 mg, 96\%). \([\alpha]\)^{24}_D = +20.9 (c = 0.5, CHCl\(_3\)), \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\): 5.28 (dd, \(J = 6, 3.5\) Hz, 1H), 4.37 (q, \(J = 6\) Hz, 1H), 4.34 (dd, \(J = 9, 3.5\) Hz, 1H), 4.05-3.95 (m, 3H), 3.88-3.85 (m, 4H), 3.14 (dd, \(J = 12, 3.5\) Hz, 1H), 2.23 (t, \(J = 12.5\) Hz, 1H), 2.15 (s, 3H), 2.62 (broad d, 6H), 1.93 (broad d, 3H), 1.68 (broad s, 6H), 1.42 (s, 3H), 1.38 (s, 3H). NMR (125 MHz, CDCl\(_3\)) \(\delta\): 169.9, 153.4, 109.8, 86.1, 77.7, 77.0, 75.5, 74.8, 70.9, 67.4, 53.4, 51.8, 43.9, 39.7, 30.1, 27.0, 25.5. ESIHRMS Calcd. for \(\text{C}_{26}\text{H}_{36}\text{O}_{10}\text{SNa}[\text{M} + \text{Na}]^+\), 563.1927, found: 563.1932.

**Methyl (1-adamantanyl 7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-2-thio-\(\beta\)-d-glycero-\(\beta\)-galacto-2-nonulopyranosid)onate (146\(\beta\)):** Compound 163\(\beta\) (1.02 g, 2.04 mmol) was dissolved
in 20 mL of THF/water/2 N HCl (4.5/4.5/1) and stirred for 3 h [TLC (1/20 MeOH/CH₂Cl₂)]. The mixture then was diluted with water (40 mL) and was extracted with ethyl acetate (2 x 20 mL) dried over Na₂SO₄ and concentrated. The crude reaction mixture was dissolved in a mixture of pyridine (10 mL) and acetic anhydride (10 mL) and stirred for 8 h, before it was concentrated, diluted in ethyl acetate, and washed with 1N HCl, and aqueous NaHCO₃, and brine. The organic layer was dried over Na₂SO₄, and was concentrated and purified by flash chromatography (1/20-1/1 ethyl acetate/hexanes) to afford the title compound 146β (1.05 g, 88%). [α]D²⁴ = -38.1 (c = 1.9, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ: 5.4 (t, J = 5 Hz, 1H), 5.3 (m, 1H), 4.8 (dt, J = 12.5, 4 Hz, 1H), 4.7 (q, 5 Hz, 1H), 4.5 (dd, J = 12.5, 3 Hz, 1H), 4.3 (dd, J = 12.5, 7.5 Hz, 1H) 4.0 (t, J = 10 Hz, 1H), 3.8 (s, 3H), 2.8 (dd, 13, 4.5 Hz, 1H), 2.2 (t, J = 12.5 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.0-1.98 (m, 7H), 1.8 (broad d, 3H), 1.6 (m, 5H). ¹³C NMR (125 MHz, CDCl₃) δ: 170.7, 170.4, 169.5 (C-1, ³Jc₁-h3ax = 0 Hz), 169.2, 153.3, 86.4, 79.1, 77.9, 77.5, 77.2, 77.1, 77.0, 70.7, 70.3, 62.4, 53.3, 51.7, 43.5, 39.8, 36.1, 30.7, 21.1, 20.9, 20.8. ESIHRMS Calcd. for C₂₇H₃₆O₁₂SNa [M + Na]⁺, 607.1825; found: 607.1823.

Methyl (1-adamantanyl 7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-2-thio-α-D-glycero-α-D-galacto-2-nonulopyranosid)onate (146α): 146α (144 mg, 82%) was prepared from 163α (0.15 g, 0.3mmol) following the same procedure used to make 146β. [α]D²⁴ = +34.1 (c = 1, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ: 5.46 (dd, J = 8.5, 3 Hz, 1H), 5.32-5.29 (m, 1H), 4.41 (dd, J = 9.5, 3 Hz, 1H), 4.32 (dd, J = 13, 2.5 Hz, 1H), 4.22 (dd, J = 12.5, 4 Hz, 1H), 3.99-3.91 (m, 2H), 3.83(s, 3H), 3.08 (dd, J = 12.5, 3 Hz, 1H), 2.2 (t, J = 12 Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.06 (s, 3H), 2.03-1.97 (m, 3H), 1.97-1.85 (broad d, 6H), 1.67 (broad s, 6H). ¹³C NMR (125 MHz, CDCl₃) δ: 170.9, 169.9, 169.8 (C-1, ³Jc₁-h3ax = 7.3 Hz), 169.4, 153.0, 85.6, 77.9, 77.3, 74.1, 68.6, 68.0,

**General coupling Protocol with Donors 146α or 146β:** A mixture of donor 146α or 146β (0.1 mmol), acceptor (0.11 mmol), and powdered AW-300 molecular sieves (2 g/mmol of donor) in CH_{2}Cl_{2}/CH_{3}CN (2:1, 2 mL) was stirred for 2 h at room temperature then was cooled to -78 °C and was treated with NIS (0.12 mmol) and one drop of TfOH (2-3 μL, 0.02 mmol). The resulting mixture was stirred for 1 h, at -78 °C, then was quenched by adding diisopropylethylamine (18 μL, 0.1 mmol), and warmed to room temperature. The molecular sieves were filtered off and the organic layer was washed with 20% aqueous Na_{2}S_{2}O_{3}, brine, dried over Na_{2}SO_{4} and concentrated. The crude mixture was purified by flash chromatography using ethyl acetate/hexanes as eluent.

For Scheme 33 the standard protocol was followed with the modification that the solvent was CH_{2}Cl_{2} (2 mL).

**Coupling Protocol of donor 146β using Ph_{2}SO/TTBP/Tf_{2}O:** A mixture of 146β (0.60 mg, 0.1 mmol), diphenyl sulfoxide (62 mg, 0.3 mmol), TTBP (51 mg, 0.2 mmol), and activated 4Å powdered molecular sieves were stirred in a mixture of CH_{2}Cl_{2}/CH_{3}CN (2:1, 1 mL) for 2 h at room temperature. The mixture was then stirred for 1 h at -78 °C, before triflic anhydride (19 μL, 0.11 mmol) was added. After stirring for an additional 10 min the acceptor (166) (95 mg, 0.2 mmol) in CH_{2}Cl_{2}/CH_{3}CN (2:1, 1 mL) was added to the reaction mixture. The mixture stirred at -78 °C for 2.5 h, warmed to room temperature, filtered through Celite, washed immediately with...
aqueous NaHCO₃, dried and concentrated. Purification by flash chromatography (0/1-1/1 ethyl acetate/hexanes) then afforded 169 (71 mg, 78%).

**Coupling Protocol of donor 146β using BSP/TTBP/Tf₂O:** A mixture of 146β (0.60 mg, 0.1 mmol), 1-benzenesulfinyl piperidine (64 mg, 0.3 mmol), TTBP (51 mg, 0.2 mmol), and activated 4Å powdered molecular sieves were stirred in a mixture of CH₂Cl₂/CH₃CN (2:1, 1 mL) for 2 h at room temperature. The mixture was then stirred for 1 h at -78 °C, and then was treated with triflic anhydride (19 µL, 0.11 mmol). After stirring for an additional 10 min the acceptor (10) (95 mg, 0.2 mmol) in CH₂Cl₂/CH₃CN (2:1, 1 mL) was added to the reaction mixture. The mixture stirred at -78 °C for 2.5 h, warmed to room temperature, filtered through Celite, washed immediately with aqueous NaHCO₃, dried and concentrated. Purification by flash chromatography (0/1-1/1 ethyl acetate/hexanes) then afforded 169 (66 mg, 73%).

**Methyl [methyl (7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-α-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-2(→6) 2,3,4-tri-O-benzyl-β-D-glucopyranoside (168):**

[α]²⁴º = -6.8 (c = 1, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ: 7.37-7.21 (m, 15H), 5.43 (dd, J = 10, 2.5 Hz, 1H), 5.32 (ddd, J = 9.5, 4, 2.5 Hz, 1H), 4.94 (d, J = 11 Hz, 1H), 4.82-4.75 (m, 4H), 4.67 (d, J = 12 Hz, 1H), 4.61 (d, J = 4 Hz, 1H), 4.51 (dd, J = 10, 2 Hz, 1H), 4.17 (dd, J = 10, 4.5 Hz, 1H), 4.09-4.04 (m, 2H), 3.96 (t, J = 9.5 Hz, 1H), 3.84 (t, J = 10 Hz, 1H), 3.80-3.76 (m, 2H), 3.74 (s, 3H), 3.55 (t, J = 9.5 Hz, 1H), 3.51 (dd, J = 9.5, 3.5 Hz, 1H), 3.45 (dd, J = 11, 2 Hz, 1H), 3.37 (s, 3H), 3.03 (dd, J = 12, 4 Hz, 1H), 2.17 (t, J = 12.5 Hz, 1H), 2.14 (s, 3H), 2.03 (s, 3H), 1.87 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ: 170.8, 169.9, 169.0, 168.1 (C-1, ³J₁-C₁-H₃ax = 5.5 Hz), 153.3, 138.9, 138.6, 138.3, 128.7, 128.66, 128.61, 128.3, 128.2, 128.0, 127.9, 127.0, 100.2, 98.4,
82.1, 79.7, 76.8, 76.0, 75.0, 73.6, 72.5, 69.6, 67.1, 66.9, 64.7, 61.5, 55.4, 53.4, 37.8, 21.2, 20.9, 20.5. ESIHRMS Calcd. for C_{45}H_{52}O_{18}Na [M + Na]^+; found: 903.3058.

**Methyl (1-adamantanyl 7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-α-D-galacto-non-2-ulopyranoside)onate (174):** [α]_{D}^{24} = +3.3 (c = 1, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ: 5.48 (dd, J = 8.5, 2.5 Hz, 1H), 5.35 (m, 1H), 4.70 (dd, J = 10, 2.5 Hz, 1H), 4.31 (dd, J = 12.5, 2.5 Hz, 1H), 4.23 (dd, J = 13, 4.5 Hz, 1H), 4.03 (m, 1H), 3.91 (t, J = 10 Hz, 1H), 3.0 (s, 3H), 2.92 (dd, J = 12.5, 4 Hz, 1H), 2.18-2.16 (m, 7H), 2.12 (broad s, 3H), 2.08 (s, 3H), 1.82 (broad s, 6H), 1.60 (broad s, 6H). ¹³C NMR (125 MHz, CDCl₃) δ: 171.3 (C-1, ²J_{C1-H3ax} = 5.5 Hz), 170.9, 170.0, 169.4, 153.5, 99.1, 80.1, 77.3, 72.9, 68.4, 68.0, 61.6, 52.9, 43.3, 40.6, 36.2, 31.2, 21.0, 20.9, 20.8. ESIHRMS Calcd. for C_{27}H_{36}O_{13}Na [M + Na]^+, 591.2054; found: 591.2064.

**Methyl (7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-α-D-galacto-non-2-ulopyranosyl)onate-(2→6)-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (170):** [α]_{D}^{24} = +18.0 (c = 1, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ: 5.52 (d, J = 5, 1 Hz, 1H), 5.48 (dd, J = 7.5, 3 Hz, 1H), 5.41-5.39 (m, 1H), 4.60 (dd, J = 8, 2.5 Hz, 1H), 4.5 (dd, J = 10, 2.5 Hz, 1H), 4.3 (q, J = 2.5 Hz, 1H), 4.28 (d, J = 3 Hz, 1H), 4.24 (d, J = 4.5 Hz, 1H), 4.23 (dd, J = 7.5, 1.5 Hz, 1H), 4.16-4.11 (m, 1H), 3.95 (t, J = 10 Hz, 1H), 3.90 (dd, J = 7.5, 1.5 Hz, 1H), 3.84 (dd, J = 9.5, 6 Hz, 1H), 3.81 (s, 3H), 3.56 (dd, J = 9.5, 7 Hz, 1H), 2.99 (dd, J = 12.5, 4 Hz, 1H), 2.2 (t, J = 12.5 Hz, 1H), 2.16 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 1.55 (s, 3H), 1.43 (s, 3H), 1.34 (s, 6H). ¹³C NMR (125 MHz, CDCl₃) δ: 170.8, 169.8, 169.3, 168.2 (C-1, ²J_{C1-H3ax} = 5.6 Hz), 153.4, 109.5, 108.8, 100.0, 96.5, 77.6, 77.1, 72.8, 70.9, 70.83, 70.80, 68.1, 67.8, 66.6, 64.2, 61.7, 53.2, 37.5,

**Methyl [methyl (7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-α-D-glycero-α-L-galacto-non-2- ulopyranosyl)onate]-(2→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (169):** \([\alpha]^{24}_D = -8.4 \) (c = 1, CHCl₃), \(^1\)H NMR (500 MHz, CDCl₃) \(\delta: 7.38-7.25 \) (m, 15H), 5.46 (dd, \(J = 8\), 2.5 Hz, 1H), 5.47-5.33 (m, 1H), 5.0 (d, \(J = 11.5\) Hz, 1H), 4.89 (d, \(J = 11\) Hz, 1H), 4.80-4.72 (m, 3H), 4.66 (d, \(J = 11.5\) Hz, 1H), 4.46 (dd, \(J = 10\), 3 Hz, 1H), 4.31 (d, \(J = 8\) Hz, 1H), 4.28 (dd \(J = 12.5\), 2.5 Hz, 1H), 4.19 (dd, \(J = 13\), 5 Hz, 1H), 4.15-4.08 (m, 2H), 3.94 (t, \(J = 10.5\) Hz, 1H), 3.86-3.76 (m, 3H), 3.65 (s, 3H), 3.57 (s, 3H), 3.56-3.53 (m, 2H), 2.94 (dd, \(J = 12\), 3.5 Hz, 1H), 2.18-2.14 (m, 4H), 2.12 (s, 3H), 2.05 (s, 3H). \(^13\)C NMR (125 MHz, CDCl₃) \(\delta: 170.8, 169.8, 169.3, 168.0\) (C-1, \(3J_{C1,H3} = 5.6\) Hz), 153.3, 139.0, 138.9, 138.7, 128.6, 128.59, 128.52, 128.37, 128.35, 127.9, 127.7, 127.6, 127.5, 105.1, 100.1, 82.6, 79.7, 75.3, 74.4, 73.4, 73.3, 72.9, 72.7, 68.1, 68.0, 63.9, 61.8, 57.3, 53.4, 35.7, 21.1, 20.9, 20.8. \(3J_{C1,H3} = 5.6\) Hz, ESIHRMS Calcd. for C_{45}H_{52}O_{18}Na [M + Na]^+; 903.3051; found: 903.3055.

**Methyl [methyl (7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-α-D-glycero-α-L-galacto-non-2- ulopyranosyl)onate]-(2→3)-2,6-di-O-benzyl-β-D-galactopyranoside(175):**

\([\alpha]^{24}_D = -3.6 \) (c = 0.5, CHCl₃), \(^1\)H NMR (500 MHz, CDCl₃) \(\delta: 7.36-7.28 \) (m, 10H), 5.45 (dd, \(J = 8\), 2.5 Hz, 1H), 5.41-5.37 (m, 1H), 4.83 (d, \(J = 11.5\) Hz, 1H), 4.66 (d, \(J = 12\) Hz, 1H), 4.58 (s, 2H), 4.40 (dd, \(J = 10\), 2.5 Hz, 1H), 4.34 (d, \(J = 8\) Hz, 1H), 4.28 (dd, \(J = 12.5\), 2.5 Hz, 1H), 4.16-4.10 (m, 1H), 4.16-4.10 (m, 1H), 4.06-4.00 (m, 2H), 3.87 (t, \(J = 10\) Hz, 1H), 3.82 (m, 2H), 3.81 (s, 3H), 3.74 (dd, \(J = 10\), 5.5 Hz, 1H), 3.61 (t, \(J = 6\) Hz, 1H), 3.58 (s, 3H), 3.56 (d, \(J = 2.5\) Hz, 1H),
2.88 (dd, $J = 12.5$, 4 Hz, 1H), 2.25 (t, $J = 12.5$ Hz, 1H), 2.11 (s, 3H), 2.05 (s, 3H), 1.95 (s, 3H). 

$^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.8, 170.1, 169.2, 168.3 (C-1, $^3$$J_{C1-H3ax}$ = 5.6 Hz), 153.2, 139.0, 138.2, 128.6, 128.4, 128.07, 128.01, 127.9, 127.8, 105.0, 99.5, 76.8, 76.6, 75.1, 73.8, 72.88, 72.82, 69.3, 68.6, 68.1, 67.7, 61.9, 67.2, 53.6, 36.3, 21.2, 20.9, 20.5 ESIHRMS Calcd. for C$_{38}$H$_{46}$O$_{18}$Na [M + Na]$^+$, 813.2582; found: 813.2579.

Methyl [methyl (7,8,9-tri-O-acetyl-4,5-O-carbonyl-3-deoxy-α-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]- (2→3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (176): [$\alpha$]$^D_{24}$ = -10.0 (c = 1, CHCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.36-7.28 (m, 15H), 5.42 (dd, $J = 8.5$, 2 Hz, 1H), 5.42-5.40 (m, 1H), 4.86 (d, $J = 12$ Hz, 1H), 4.84 (d, $J = 11$ Hz, 1H), 4.67 (d, $J = 11.5$ Hz, 1H), 4.54 (d, $J = 12$ Hz, 1H), 4.48 (d, $J = 11.5$ Hz, 1H), 4.42 (d, $J = 11.5$ Hz, 1H), 4.31 (d, $J = 7.5$ Hz, 1H), 4.29 (t, $J = 7.5$ Hz, 1H), 4.27 (d, $J = 2$ Hz, 1H), 4.23-4.17 (m, 1H), 3.98 (dd, $J = 12.5$, 4.5 Hz, 1H), 3.90 (dd, $J = 10$, 5 Hz, 1H), 3.85 (t, $J = 10$ Hz, 1H), 3.78 (d, $J = 2.5$Hz, 1H), 3.73 (s, 3H), 3.71-3.66 (m, 2H), 3.64-3.61 (m, 1H), 3.55 (broad s, 4H), 2.76 (dd, $J = 12.5$, 4 Hz, 1H), 2.37 (t, $J = 13$ Hz, 1H), 2.12 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.7, 196.7, 169.1, 167.9 (C-1, $^3$$J_{C1-H3ax}$ = 5.6 Hz), 153.4, 139.2, 138.8, 138.1, 128.6, 128.4, 128.37, 128.30, 128.27, 128.1, 128.0, 127.8, 127.8, 127.4, 105.3, 100.6, 77.8, 77.5, 77.1, 76.7, 75.1, 73.8, 73.3, 72.4, 68.6, 68.1, 67.6, 61.6, 57.3, 53.4, 35.3, 21.1, 20.9, 20.5. ESIHRMS Calcd. for C$_{48}$H$_{52}$O$_{18}$Na [M + Na]$^+$, 903.3051; found: 903.3065.

Methyl 7,8,9-tri-O-acetyl-4,5-O-carbonyl-2,6-anhydro-3-deoxy-α-D-glycero-α-D-galacto-non-2-enoate (177): To a stirred solution of 146β (20 mg, 0.03 mmol) in CH$_2$Cl$_2$ (150 µL), at −20°C was added mCPBA (11 mg, 0.05 mmol) in CH$_2$Cl$_2$ (150 µL). Stirring was continued for 1 h
before the reaction mixture was quenched by addition of Hunig’s base (120 μL, 0.68 mmol), and the reaction mixture was warmed to 45 °C, and stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (ethyl acetate / hexanes : 1/45 to 1/1) to afford 177 (3 mg, 21%). \([\alpha]^{24}_D = +6.0 \) (c = 0.15, CHCl₃), \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\): 6.48 (d, \(J = 2 \) Hz, 1H), 5.52 (dd, \(J = 6, 3.5 \) Hz, 1H), 5.39 (sextet, \(J = 3 \) Hz, 1H), 5.08 (dd, \(J = 10, 2.5 \) Hz, 1H), 4.80 (dd, \(J = 11, 3.5 \) Hz, 1H), 4.50 (dd, \(J = 12.5, 3 \) Hz, 1H), 4.28 (t, \(J = 10.5 \) Hz, 1H), 4.23 (dd, \(J = 13, 6 \) Hz, 1H), 3.83 (s, 3H), 2.17 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H). \(^1\)C NMR (125 MHz, CDCl₃) \(\delta\): 170.7, 170.0, 169.3, 160.7, 153.5, 144.5, 105.8, 76.2, 75.9, 75.6, 69.7, 68.8, 61.7, 53.1, 29.9, 21.0, 20.9, 20.7. ESIHRMS Calcd. for C₁₇H₂₀O₁₂Na [M + Na]⁺, 439.0852; found: 439.0861.

**General coupling Protocol with Donors 151α and 151β:** A mixture of donor (0.11 mmol), acceptor (0.17 mmol), and AW-300 molecular sieves (2 g/mmL of donor) was stirred in 2.1 mL of CH₂Cl₂/CH₃CN (2:1) for 2 h, then was cooled to -78 °C and was treated with NIS (31 mg, 0.14 mmol) and one drop of TfOH (2-3 μL, 0.02 mmol). The reaction mixture was stirred for 1 h, at -78 °C, and was quenched by adding Hunig’s base (20 μL, 0.11 mmol) and warmed to room temperature. The molecular sieves were filtered off and the organic layer was washed with 20% aqueous Na₂S₂O₅, brine, dried over Na₂SO₄ and concentrated. The crude mixture was purified by flash chromatography ethyl acetate/hexanes.

**Methyl 4,5,7,8,9-penta-O-acetyl-2,6-anhydro-3-deoxy-α-glycero-α-galacto-non-2-enoate** (77): \([\alpha]^{24}_D = +28.4 \) (c = 0.6, CHCl₃), \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\): 5.9 (d, \(J = 3 \) Hz, 1H), 5.57 (dd, \(J = 7, 3 \) Hz, 1H), 5.51 (q, \(J = 3 \) Hz, 1H), 5.3 (dt, \(J = 6, 3 \) Hz, 1H), 5.2 (dd, \(J = 9.5, 7 \) Hz,
1H), 4.5 (dd, J = 12.5, 3Hz, 1H), 4.3 (dd, J = 9.5, 3 Hz, 1H), 4.1 (dd, J = 12.5, 6 Hz, 1H), 3.8 (s, 3H), 2.1 (s, 3H), 2.08 (s, 3H), 2.069 (s, 3H), 2.063 (s, 3H), 2.05 (s, 3H) $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.8, 170.6, 170.1, 169.94, 169.91, 161.6, 145.5, 107.9, 75.8, 70.1, 69.0, 67.0, 65.8, 62.0, 52.8, 21.0, 20.9, 20.7. ESIHRMS Calcd. for C$_{20}$H$_{28}$O$_{13}$Na [M + Na]$^+$, 497.1271; found: 497.1289.

Methyl [methyl (4,5,7,8,9-penta-O-acetyl-3-deoxy-β-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-2,6,3,4-tri-O-benzyl-β-D-galactopyranoside (179): [α]$^{24}_D = +1.8$ (c = 0.5, CHCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.28-7.23 (m, 15H), 5.40-5.38 (m, 1H), 5.3 (dd, J = 9.2 Hz, 1H), 4.96(d, J = 11.5 Hz, 1H) 4.94-4.86 (m, 3H), 4.78-4.71 (m, 3H), 4.6 (d, J = 11 Hz, 1H), 4.3 (d, J = 7.5 Hz, 1H), 4.2 (d, J = 2.5 Hz, 1H), 4.15-4.12 (m, 2H), 3.92-3.89 (m, 2H), 3.80 (dd, J = 10, 8 Hz, 1H), 3.64 (s, 3H), 3.58 (s, 3H), 3.56-3.52 (m, 3H), 2.67 (dd, J = 13.5, 5Hz, 1H), 2.1 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 1.93 (t, 12.5Hz, 1H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.8, 170.3, 170.1, 170.0, 169.9, 167.2 (C-1, $^3$J$_{C1-H3ax} = 5.5$ Hz), 139.1, 139.0, 138.7, 128.5, 128.4, 128.36, 128.30, 127.8, 127.7, 127.6, 127.4, 105.1, 98.8, 82.2, 79.7, 75.3, 74.5, 73.5, 73.1, 72.8, 71.5, 69.5, 68.3, 68.0, 66.8, 63.1, 62.2, 57.3, 53.0, 37.6, 21.2, 21.0, 20.96, 20.93, 20.86. ESIHRMS Calcd. for C$_{48}$H$_{58}$O$_{19}$Na [M + Na]$^+$, 961.3470; found: 961.3469.

Methyl [methyl (4,5,7,8,9-penta-O-acetyl-3-deoxy-β-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-2,6,3,4-tri-O-benzyl-α-D-glucopyranoside (180): [α]$^{24}_D = +1.7$ (c = 0.6, CHCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.29-7.37 (m, 15H), 5.29-5.36 (m, 1H), 5.28 (dd, J = 10, 2.5 Hz, 1H) 4.93-4.86 (m, 3H), 4.84-4.75 (m, 4H), 4.66 (d, J = 12.5 Hz, 1H), 4.61(d, J = 4 Hz, 1H), 4.22 (dd, J = 11, 4 Hz, 1H), 4.15 (dd, J = 10.5, 2.5 Hz, 1H), 4.02 (dd, J = 12.5, 2Hz,
1H), 3.95 (t, 9.5 Hz, 1H), 3.79-3.77 (m, 2H), 3.76 (s, 3H), 3.66 (t, $J = 10$ Hz, 1H), 3.52 (dd, $J = 10$, 4 Hz, 1H), 3.44 (dd, $J = 10.5$, 1.5 Hz, 1H), 3.37 (s, 3H), 2.75 (dd, $J = 13$, 5.5 Hz, 1H), 2.15 (s, 3H), 2.01 (s, 3H), 2.0 (s, 3H), 1.98 (s, 3H) 1.94 (t, 12.5 Hz, 1H), 1.78 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.8, 170.3, 170.1, 170.0, 169.8, 167.7 (C-1, $^3J_{C1-H3ax} = 6.5$ Hz), 139.0, 138.8, 138.4, 128.6, 128.5, 128.3, 128.18, 128.12, 127.98, 127.93, 127.7, 98.7, 98.4, 82.2, 79.7, 76.0, 75.0, 73.6, 71.3, 69.6, 69.5, 68.0, 67.4, 66.3, 63.7, 61.9, 55.4, 53.0, 37.9, 21.3, 21.0, 20.9, 20.9, 20.5. ESIHRMS Calcd. for C$_{48}$H$_{58}$O$_{19}$Na $[M + Na]^+$, 961.3470; found: 961.3461.

**Methyl [methyl (4,5,7,8,9-penta-O-acetyl-3-deoxy-$\beta$-glycero-$\alpha$-$\beta$-galacto-non-2-ulopyranosyl)onate]-(2→3)-2,6-di-O-benzyl-$\beta$-$\beta$-galactopyranoside (181a):** $[\alpha]_{D}^{24} = -7.8$ (c = 0.5, CHCl$_3$), $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.44-7.39 (d, 2H), 7.36-7.32 (m, 6H), 7.31-7.25 (m, 2H), 5.47-5.43 (m, 1H), 5.34 (dd, $J = 9$, 2.5 Hz, 1H), 4.94-4.87 (m, 2H), 4.82 (dd, $J = 11.5$ Hz, 1H), 4.70 (d, $J = 12$ Hz, 1H), 4.6 (s, 2H), 4.37 (d, $J = 8$ Hz, 1H), 4.2 (dd, $J = 13$, 2.5 Hz, 1H), 4.1 (dd, $J = 10$, 3.5 Hz, 1H), 4.0 (dd, $J = 12.5$, 5 Hz, 1H), 3.83 (m, 2H), 3.80 (s, 3H), 3.75 (m, 2H), 3.65 (t, $J = 5.5$ Hz, 1H), 3.57 (s, 3H), 3.52 (dd, $J = 9.5$, 7.5 Hz, 1H), 2.64 (dd, $J = 13$, 4.5 Hz, 1H), 2.59 (broad s, 1H), 2.1 (s, 3H), 2.03 (s, 4H), 2.0 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.8, 170.2, 170.1, 170.0, 168.5 (C-1, $^3J_{C1-H3ax} = 6.5$ Hz), 139.5, 139.3, 138.4, 128.6, 128.3, 127.96, 127.90, 127.83, 127.5, 104.8, 98.0, 77.8, 76.0, 75.0, 73.7, 72.8, 71.6, 69.56, 69.50, 68.4, 68.2, 67.8, 66.7, 66.3, 57.2, 53.3, 36.7, 21.3, 21.0, 20.9, 20.8, 20.6. ESIHRMS Calcd. for C$_{44}$H$_{52}$O$_{19}$Na $[M + Na]^+$, 871.3000; found: 871.3008.

**Methyl [methyl (4,5,7,8,9-penta-O-acetyl-3-deoxy-$\beta$-glycero-$\beta$-$\beta$-galacto-non-2-ulopyranosyl)onate]-(2→3)-4-O-acetyl-2,6-di-O-benzyl-$\beta$-$\beta$-galactopyranoside (181b):** $[\alpha]_{D}^{24}$
= -2.0 (c = 0.5, CHCl₃), νH NMR (500 MHz, CDCl₃) δ: 7.37-7.26 (m, 10H), 5.42 (t, J = 2.5 Hz, 1H), 5.38 (td, J = 9, 2.5 Hz, 1H), 5.32 (d, J = 3 Hz, 1H), 5.21-5.14 (m, 1H), 5.11 (dd, J = 12, 2.5 Hz, 1H), 4.85 (t, J = 10 Hz, 1H), 4.7 (d, J = 11 Hz, 1H), 4.66 (dd, J = 10, 2 Hz, 1H), 4.64 (m, 2H), 4.57-4.52 (m, 2H), 4.39 (d, J = 7.5 Hz, 1H), 4.0 (t, J = 6.5 Hz, 1H), 3.91 (dd, J = 12.5, 9.5 Hz, 1H), 3.57 (s, 3H), 3.57-3.53 (m, 3H), 3.31 (s, 3H), 2.70 (dd, J = 13.5, 5 Hz, 1H), 2.16 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.86 (dd, J = 13.5, 12 Hz, 1H). ¹³C NMR (125 MHz, CDCl₃) δ: 171.2, 170.9, 170.4, 170.3, 170.2, 170.1, 166.7, 138.7, 138.4, 128.8, 128.5, 128.2, 127.9, 127.7, 127.1, 105.1, 99.3, 78.3, 75.1, 73.6, 72.4, 72.2, 71.7, 71.6, 70.9, 69.3, 69.1, 68.8, 67.8, 62.9, 57.4, 52.7, 37.7, 21.6, 21.0, 20.9, 20.8. ³J_C-H₃ax = 0 Hz ESIHRMS Calcd. for C₄₃H₅₄O₂₀Na [M + Na]⁺, 913.3106; found: 913.3115.

**General Procedure for Saponification of Carbonate-Protected Saccharides:** The sialoside (0.05 mmol) in methanol (1 mL), was treated with sodium methoxide solution (25 wt. %) in methanol (~ 2-4 μL). The reaction mixture was stirred for 45 min, followed by the addition of Amberlyst 15 (H⁺) resin to neutralize the mixture. The mixture was filtered through a pad of silica gel and Celite® and concentrated to afford the saponified sialosides in quantitative yield.

**Methyl (methyl (3-deoxy-α-glycero-α-galacto-non-2-ulopyranosyl)onate-(2→6) -2,3,4-tri-O-benzyl-β-D-glucopyranoside (182): [α]^{24}_D = +24 (c = 0.5, MeOH), νH NMR (500 MHz, MeOD) δ: 7.38-7.25 (m, 15H), 4.81-4.72 (m, 3H), 4.70-4.65 (m, 3H), 3.90 (dd, J = 6.5 Hz,1H), 3.86-3.77 (m, 4H), 3.75 (s, 3H), 3.70 (dd, J = 11, 2 Hz, 1H), 3.65-3.62 (m, 2H), 3.53-3.38 (m, 6H), 3.35 (s, 3H), 2.63 (dd, J = 12.5, 4 Hz, 1H), 1.70 (t, J = 12Hz, 1H). ¹³C NMR (125 MHz, MeOD) δ: 169.6, 138.9, 138.49, 138.42, 128.28, 128.26, 128.1, 128.0, 127.8, 127.7, 127.5,
127.4, 99.2, 97.8, 81.8, 80.2, 77.7, 75.3, 74.8, 74.7, 72.8, 71.9, 70.1, 70.1, 69.9, 68.7, 63.7, 63.2, 63.1, 54.3, 52.1, 39.6. ESIHRMS Calcd. for C_{38}H_{48}O_{14}Na [M + Na]^+, 751.2942; found: 751.2930.

**Methyl (1-adamantanyl 3-deoxy-β-glycero-α-d-galacto-non-2-ulpopyranosyl)onate (185):**

$[\alpha]_D^{24} = +18.0$ (c = 0.5, MeOH), $^1$H NMR (500 MHz, MeOD): 3.89-3.86 (m, 1H), 3.84-3.83 (m, 1H), 3.82 (s, 3H), 3.75 (dd, $J = 9.5$, 1.5 Hz, 1H), 3.64 (q, $J = 5.5$ Hz, 1H), 3.60-3.51 (m, 1H), 3.4 (t, $J = 9.5$ Hz, 1H), 3.37-3.33 (m, 1H), 2.56 (dd, $J = 12.5$, 4.5 Hz, 1H), 2.08 (broad s, 3H), 1.91 (broad s, 6H), 1.71 (t, $J = 12.5$ Hz, 1H), 1.63 (broad s, 6H). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 172.5, 98.4, 78.0, 75.1, 72.4, 70.2, 69.9, 68.6, 63.8, 63.2, 52.0, 48.6, 43.4, 43.1, 36.0, 31.3. ESIHRMS Calcd. for C$_{20}$H$_{32}$O$_{9}$Na [M + Na]$^+$, 439.1944; found: 439.1953.

**Methyl [methyl (3-deoxy-β-glycero-α-d-galacto-non-2-ulpopyranosyl)onate]-2→6)-1,2;3,4-di-O-isopropylidene-α-d-galactopyranose (184):**

$[\alpha]_D^{24} = +21$ (c = 1, MeOH), $^1$H NMR (500 MHz, MeOD) $\delta$: 5.46(d, $J = 5$ Hz, 1H), 4.62 (dd, $J = 8$, 2.5 Hz, 1H), 4.35 (q, $J = 2.5$ Hz, 1H), 4.23(dd, $J = 8$, 2 Hz, 1H), 3.92-3.80 (m, 2H), 3.83 (s, 3H), 3.82-3.80 (m, 2H), 3.68-3.52 (m, 3H), 3.52-3.46 (m, 3H), 2.61 (dd, $J = 13$, 4 Hz, 1H), 1.71 (t, $J = 12$ Hz, 1H), 1.50 (s, 3H), 1.39 (s, 3H), 1.33 (s, 6H). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 169.6, 109.2, 108.7, 99.0, 96.7, 74.7, 71.9, 71.0, 70.8, 70.7, 70.1, 70.0, 68.8, 67.0, 63.8, 62.9, 52.1, 39.6, 25.17, 25.13, 23.9, 23.5. ESIHRMS Calcd. for C$_{22}$H$_{36}$O$_{14}$Na [M + Na]$^+$, 547.2003; found: 547.2002.

**Methyl [methyl (3-deoxy-β-glycero-α-d-galacto-non-2-ulpopyranosyl)onate]-2→6)-2,3,4-tri-O-benzyl-β-d-galactopyranoside (183):**

$[\alpha]_D^{24} = +16.25$ (c = 0.6, MeOH), $^1$H NMR (500 MHz,
MeOD) δ: 7.36-7.25 (m, 15H), 4.75-4.64 (m, 3H), 4.61-4.5 (m, 2H), 4.30 (d, J = 7.5 Hz, 1H), 4.0-3.96 (m, 2H), 3.88-3.82 (m, 3H), 3.71 (s, 3H), 3.70-3.57 (m, 6H), 3.52 (s, 3H), 3.51-3.45 (m, 3H), 2.61 (dd, J = 12.5, 3.5 Hz, 1H), 1.69 (t, J = 12.5 Hz, 1H). 

13C NMR (125 MHz, MeOD) δ: 169.2, 138.9, 138.8, 138.7, 128.8, 128.1, 128.0, 127.9, 127.8, 127.6, 127.4, 127.3, 105.0, 98.7, 82.0, 79.4, 74.78, 74.71, 74.5, 73.7, 73.1, 72.7, 71.8, 70.2, 70.0, 68.6, 63.7, 63.2, 62.3, 56.2, 39.8. 


**Methyl [methyl 3-deoxy-α-glycero-α-β-galacto-non-2-ulopyranosyl]onate-(2→3)-2,6-di-O-benzyl-β-D-galactopyranoside (186):** Compound (175) (40 mg, 0.05 mmol) in anhydrous methanol (1 mL) was treated with 1-2 μL of sodium methoxide solution (25 wt.% ) in methanol. The mixture was stirred for 20 minutes followed by the addition of Amberlyst 15 (H+) resin to neutralize the mixture. The mixture was filtered through a pad of silica gel and Celite® and concentrated to afford the saponified sialoside (186) in quantitative yield. [α]D24 = -5.4 (c = 0.5, MeOH), 1H NMR (500 MHz, MeOD) δ: 7.41-7.24 (m, 10H), 4.73(m, 2H), 4.57 (s, 2H), 4.30 (d, J = 7.5 Hz, 1H), 4.13 (dd, J = 10, 3.5 Hz, 1H), 3.84-3.80 (m, 2H), 3.79 (s, 3H), 3.78-3.74 (m,1H), 3.68 (d, J = 4 Hz, 1H), 3.67-3.63 (m, 4H), 3.53 (s, 3H), 3.50-3.47 (m, 4H), 2.62 (dd, J = 13, 4 Hz, 1H), 1.87 (t, J = 12.5 Hz, 1H). 

13C NMR (125 MHz, MeOD) δ: 170.0, 138.7, 138.4, 128.2, 128.0, 127.6, 127.5, 127.4, 104.9, 99.3, 77.6, 75.1, 75.0, 74.9, 73.2, 71.9, 70.2, 70.1, 69.3, 68.7, 68.6, 63.7, 56.1, 52.2, 38.6. ESIHRMS Calcd. for C31H42O14Na [M + Na]+, 661.2472; found: 661.2445.

**Methyl [methyl 3-deoxy-α-glycero-α-β-galacto-non-2-ulopyranosyl]onate-(2→3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (187):** [α]D24 = -9.0 (c = 0.5, MeOH), 1H NMR (500
MHz, MeOD) δ: 7.41-7.24 (m, 15H), 4.89 (d, J = 11 Hz, 1H), 4.74 (d, J = 6.5 Hz, 1H), 4.53-4.51 (m, 3H), 4.31 (d, J = 7.5 Hz, 1H), 4.20 (dd, J = 10, 3 Hz, 1H), 3.88-3.79 (m, 4H), 3.76 (s, 3H), 3.74 (t, J = 6Hz, 1H), 3.69-3.64 (m, 2H), 3.60-3.55 (m, 4H), 3.51-3.50 (m, 5H), 2.58 (dd, J = 13, 4 Hz, 1H), 1.95 (t, J = 12.5, 1H). 13C NMR (125 MHz, MeOD) δ: 169.9, 139.1, 138.6, 128.3, 128.2, 128.0, 127.99, 127.94, 127.7, 127.5, 127.4, 127.3, 105.0, 100.2, 77.6, 77.34, 75.4, 75.07, 75.04, 74.8, 73.1, 73.0, 72.6, 72.0, 70.6, 68.7, 68.5, 63.7, 63.2, 56.1, 52.3, 37.7, 29.5.

ESIHRMS Calcd. for C_{38}H_{48}O_{14}Na [M + Na]^+; 751.2942; found: 751.2934.

**General protocol for sialylphosphate synthesis:**

The thiosialoside, dibutyl phosphate (3 eq), pulverized acid washed-300 molecular sieves in dry CH_{2}Cl_{2} were stirred under argon overnight. The mixture was cooled to 0 °C, and NIS (1.05 eq), and triflic acid (0.09 eq) in Et_{2}O were added. The mixture was stirred for 15 min and was quenched by addition of Hunig’s base. The molecular sieves were filtered off and reaction mixture was washed with sat. sodium thiosulfate and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to give crude reaction mixtures which were purified by chromatography over silica gel using the eluents indicated.

**Methyl (7,8,9-tri-O-acetyl-5-N, 4-O-carbonyl-2-(dibutylphosphoryl)-3,5-dideoxy-ß-n-galacto-non-2-ulopyranoside)onate (207):** This compound was synthesized using the general procedure from thiosialoside 206\(^3\) (100 mg, 0.171 mmol), dibutyl phosphate (101 uL, 0.514 mmol) AW-MS300 (300 mg), NIS (46 mg, 0.205 mmol) and TfOH (1.3 uL, 0.015 mmol, 0.09 eq in 50 uL of Et_{2}O) in CH_{2}Cl_{2} (2 mL). The reaction was complete in 15 min and was quenched with Hunig’s base (100 uL). Purification by silica gel chromatography using toluene/
EtOAc (3:1) gave the phosphate \( 207 \) (86 mg, 8\%). \( [\alpha]^2^3 \D = +14 \) (c = 1, CHCl\(_3\)). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 5.36 (brs, 1H), 5.29 (m, 1H), 5.17 (dd, \( J = 8, 2 \) Hz, 1H), 4.55 (ddd, \( J = 12, 4 \) Hz, 1H), 4.48 (t, \( J = 2 \) Hz, 1H), 4.45 (d, \( J = 2 \) Hz, 1H), 4.35 (dd, \( J = 12.8, 6.4 \) Hz, 1H), 4.11-4.02 (m, 4H), 3.83 (s, 3H), 3.19 (t, \( J = 11.2 \) Hz, 1H), 2.82 (dd, \( J = 12.8, 4 \) Hz, 1H), 2.21 (dt, \( J = 12.8, 3.2 \) Hz, 1H), 2.18 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 1.63 (m, 4H), 1.38 (m, 4H), 0.94 (t, \( J = 6.8 \) Hz, 6H). \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 171.3, 170.5, 170.0, 165.7 (C-1, \( ^3\)J\(_{C-H} = 0 \) Hz), 158.9, 99.7, 99.6, 75.6, 74.2, 69.6, 69.0, 68.4, 68.4, 68.4, 68.3, 61.6, 57.7, 53.3, 37.2, 37.1, 32.1, 32.1, 32.0, 20.9, 20.7, 20.6, 18.6, 18.5, 13.5 ESIHRMS: m/z calcd. for \( \text{C}_{25}\text{H}_{40}\text{NO}_{13}\text{PNa} (\text{M} + \text{Na})^+ \) 690.2033, found 648.2042.

**Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-2-(dibutylphosphoryl)-3,5-dideoxy-\( \alpha \)-\( \alpha \)-galacto-non-2-ulopyranoside)onate (198):** This compound was synthesized using the general procedure from thiosialoside \( 197^3 \) (100 mg, 0.156 mmol), dibutyl phosphate (92 uL, 0.463 mmol), AW-MS300 (300 mg), NIS (36 mg, 0.163 mmol) and TfOH (1.2 uL, 0.014 mmol, in 50 uL of Et\(_2\)O) in DCM (3 mL). The reaction was complete in 15 min and was quenched with Hunig’s base (100 uL). Purification on silica gel using acetone/toluene (1:5) gave phosphate \( 198 \) (78 mg, 73\%). \( [\alpha]^2^3 \D = -31 \) (c = 1, CHCl\(_3\)). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 5.44-5.42 (m, 1H), 5.37 (d, \( J = 10 \) Hz, 1H), 5.32-5.24 (m, 2H), 4.57 (dd, \( J = 12, 2.8 \) Hz, 1H), 4.38 (dd, \( J = 10.4, 2.8 \) Hz, 1H), 4.24 (d, \( J = 8 \) Hz, 1H), 4.20 (t, \( J = 10 \) Hz, 1H), 4.15-4.01 (m, 5H), 3.83 (s, 3H), 2.63 (dd, \( J = 13.2, 4.8 \) Hz, 1H), 2.13 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.88 (s, 3H), 1.69-1.60 (m, 4H), 1.45-1.35 (m, 4H), 0.94 (m, 6H). \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 170.8, 170.5, 170.4, 170.2, 170.1, 165.9 (C-1, \( ^3\)J\(_{C-H} = 0 \) Hz), 99.5, 99.4, 73.4, 71.7, 68.4, 68.3, 68.3, 68.3, 68.3, 68.1, 68.0,
62.4, 53.2, 48.5, 37.3, 37.2, 32.1, 32.1, 32.0, 23.1, 20.9, 20.8, 20.8, 20.7, 18.6, 18.6, 13.5.

ESIHRMS: $m/z$ calcd. for $C_{28}H_{46}NO_{16}PNa (M + Na)^+$ 706.2452, found 706.2454

**Methyl (5-acetamido-7,8,9-tri-$O$-acetyl-5-N, 4-$O$-carbonyl-2-(dibutylphosphoryl)-3,5-dideoxy-$
\alpha$-glycero-$\beta$-$\alpha$-galacto-non-2-ulopyranoside)onate (208):** This compound was synthesized using the general procedure from thiosialoside 47 (60 mg, 0.096 mmol), dibutyl phosphate (56 uL, 0.28 mmol) AW-MS300 (180 mg), NIS (23 mg, 0.105 mmol) and TfOH (0.7 uL, 0.008 mmol, 0.09 eq in 50 uL of Et$_2$O) in DCM (2 mL). The reaction was complete in 15 min and was quenched with Hunig’s base (50 uL). Purification by silica gel chromatography using EtOAc/Hexanes (1:1) gave the phosphate 208 (55 mg, 86%). $[\alpha]^{23}_D = +43$ (c = 1, CHCl$_3$).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.52 (q, $J = 2$ Hz, 1H), 5.25 (m, 1H), 4.72 (dd, $J = 9.6$, 2 Hz, 1H), 4.58 (m, 2H), 4.15-4.01 (m, 5H), 3.85 (s, 3H), 3.76 (dd, $J = 11.2$, 9.2 Hz, 1H), 2.89 (dd, $J = 12.8$, 3.2 Hz, 1H), 2.50 (s, 3H), 2.30 (dt, $J = 12.8$, 3.2 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H), 1.65 (sext, $J = 7.2$ Hz, 4H), 1.44-1.35 (sext, $J = 7.2$ Hz, 4H), 0.94 (dt, $J = 7.2$, 2.8 Hz, 6H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.1, 170.6, 170.5, 169.7, 165.5 (C-1, $^3J_{C-H} = 0$ Hz) 153.4, 98.8, 98.7, 76.6, 74.0, 72.5, 71.7, 68.5, 68.4, 68.3, 68.3, 62.8, 58.8, 53.4, 36.0, 36.0, 32.1, 32.0, 32.0, 24.6, 20.9, 20.7, 20.7, 18.5, 18.5, 13.5. ESIHRMS: $m/z$ calcd. for $C_{27}H_{42}NO_{16}PNa (M + Na)^+$ 690.2139, found 690.2104.

**Methyl (4,5,7,8,9-penta-$O$-acetyl-2-(dibutylphosphoryl)-3-ddeoxy-$\alpha$-glycero-$\beta$-$\alpha$-galacto-non-2-ulopyranoside)onate (209):** This compound was synthesized using the general procedure from thiosialoside 151 (100 mg, 0.155 mmol), dibutyl phosphate (92 uL, 0.467 mmol) AW-MS300 (300 mg), NIS (36 mg, 0.163 mmol) and TfOH (1.2 uL, 0.014 mmol in 50 uL Et$_2$O) in DCM (3
mL). The reaction was complete in 15 min and was quenched with Hunig’s base (100 uL). Purification on silicagel using toluene/EtOAc (1:1) gave phosphate 209 (77mg, 72%). $\alpha^{23}_D = +63$ (c = 1, CHCl$_3$). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.43 (dd, $J = 5.2$, 2 Hz, 1H), 5.34 (dt, $J = 11.2$, 4.8 Hz, 1H), 5.29-5.26 (m, 1H), 4.95 (t, $J = 10$ Hz, 1H), 4.51 (dd, $J = 13.2$, 2 Hz, 1H), 4.45 (dd, $J = 11.2$, 2 Hz, 1H), 4.26-4.05 (m, 4H), 3.83 (s, 3H), 2.74 (dd, $J = 13.2$, 6.2 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.02 (s, 4H), 2.0 (s, 3H), 1.71-1.63 (m, 4H), 1.44-1.37 (m, 4H), 0.97 (dt, $J = 7.2$, 3.2 Hz, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.5, 170.0, 170.0, 169.8, 169.8, 165.8 (C-1, $^3J_{C-H} = 0$ Hz), 99.2, 99.1, 77.2, 71.6, 70.5, 68.5, 68.4, 68.3, 68.3, 68.2, 67.2, 67.2, 62.2, 53.2, 36.8, 36.7, 3.1, 32.0, 20.8, 20.8, 20.7, 20.7, 20.7, 20.6, 20.6, 18.6, 13.5. ESIHRMS: m/z calcd. for $C_{28}H_{45}NO_{17}$PNa (M + Na)$^+$ 707.2292, found 707.2278.

Methyl (7,8,9-tri-O-acetyl-4,5-O-carbonyl-2-(dibutylphosphoryl)-3-deoxy-$\beta$-$\alpha$-galacto-non-2-ulopyranoside)onate (210): This compound was synthesized using the general procedure from thiosialoside 146$^\beta$ (58 mg, 0.09 mmol), dibutyl phosphate (59 uL, 0.29 mmol) AW-MS300 (180 mg), NIS (23 mg, 0.1 mmol) and TfOH (0.7 uL, 0.009 mmol in 50 uL Et$_2$O) in DCM (2 mL). The reaction was complete in 15 min and was quenched with Hunig’s base (50 uL). Purification on silica gel using EtOAc/Hexanes (1:1) gave phosphate 210 (48 mg, 78%). $[\alpha]^{23}_D = -13$ (c = 0.5, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.47 (dd, $J = 4.4$, 2.8 Hz, 1H), 5.29 (dt, $J = 4.4$, 2.0 Hz, 1H), 4.68 (m, 1H), 4.40 (dd, $J = 10$, 2.5 Hz, 1H), 4.28 (dd, $J = 10$, 4.8 Hz, 1H), 4.12-4.05 (m, 5H), 3.85 (s, 3H), 2.91 (dd, $J = 10$, 3.2 Hz, 1H), 2.30 (dt, $J = 10$, 2.4 Hz, 1H), 2.14 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.70-1.61 (m, 4H), 1.44-1.35 (m, 4H), 0.94 (t, $J = 6$ Hz, 1H) $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 171.8, 170.6, 169.5, 169.2, 167.2 (C-1, $^3J_{C-H} = 0$ Hz), 152.9, 98.4, 98.4, 76.0, 73.9, 68.1, 68.0, 68.0, 67.9, 67.3, 61.2, 53.6, 53.5, 37.1, 37.1, 32.09, 32.06, 32.04, 32.0, 20.7,
20.7, 20.5, 18.6, 18.5, 13.5 ESIHRMS: m/z calcd. for C_{25}H_{39}NO_{16}PNa (M + Na)^+ 649.1873, found 649.1859. NMR contains succinimide as impurity.

**In-source fragmentation study of sialophosphates:**

The mass spectrometry study was carried out using a Waters LCT Premier Xe mass spectrometer. The spectra were recorded in positive ion mode with a source temperature of 120 °C using a desolvation gas flow of 800 L/h. The samples were injected as methanolic solutions (10 nM).

**Ethyl-1,2,3-trideoxy-5,6:8,9-di-O-isopropyldene-D-glycero-D-galacto-2-methylene-nonanoate (223- syn), Ethyl-1,2,3-trideoxy-5,6:8,9-di-O-isopropyldene-D-glycero-D-talo-2-methylene-nonanoate (223-anti):** The aldehyde 220 was prepared according to literature procedure and was submitted to the Barbier reaction immediately. To a stirred solution of the aldehyde 220 (1.26 g, 3.6 mmol) and acrylate 221 (2.76 g, 14.4 mmol) in a mixture of THF/NH$_4$Cl (1:1, 24 mL) at room temperature under vigorous stirring was added zinc powder (0.98 g, 14.4 mmol). The mixture was stirred for 2 h and was filtered through a pad of Celite. The filtrate was extracted with EtOAc (20 mL), and the organic layer was washed with brine (2 x 20 mL), dried and concentrated to get a yellow oil which was purified by flash chromatography over silica gel (Hexanes/EtOAc 9/1 to 1/1) to afford 223 (1.43 g, 86%) as a 5:1 mixture of diastereomers. **223-anti: [α]$^{[23]}_{D}$ = +52 (c = 1, CHCl$_3$). H NMR (400 MHz, CDCl$_3$) δ 7.37-7.23 (m, 5H), 6.21 (d, J = 1.6 Hz, 1H), 5.61 (d, J = 1.6 Hz, 1H), 4.98 (d, J = 12, 4 Hz, 1H), 4.68 (d, J = 12, 4 Hz, 1H), 4.32-4.27 (m, 1H), 4.23-4.15 (m, H), 4.07-4.00 (m, 2H), 3.97-3.92 (m, 2H), 3.46 (brs, 1H), 2.84 (d, J = 14.4, 4 Hz, 1H), 2.40 (dd, J = 13.6, 3.2 Hz, 1H), 1.46 (s, 3H), 1.41 (s,
3H), 1.34 (s, 3H), 1.32 (s, 3H), 1.27 (t, $J = 4$ Hz, 1H). 169.2, 139.2, 137.2, 128.7, 128.3, 127.3, 127.3, 109.2, 108.2, 79.4, 78.6, 78.6, 77.2, 73.9, 69.6, 65.9, 61.6, 37.8, 26.2, 26.6, 26.0, 25.4, 14.3. ESIHRMS: m/z calcd. for C$_{25}$H$_{36}$O$_{8}$Na (M + Na)$^+$ 487.2303, found 487.2312.

**Ethyl 2,4,6,8,9-hexa-O-acetyl-7-O-benzyl-2,5-anhydro-3-deoxy-D-erythro-D-mannofuranos-2-ulosonate (225):** A solution of the major diastereomer 223 (0.29 g, 0.625 mmol) in anhydrous methanol (5 mL) was cooled to -78 °C and was sparged with ozone for 5 min followed by sparging with oxygen for 5 min. The mixture was stirred for 5 min and was treated with dimethyl sulfide (460 uL, 6.25 mmol). The mixture was allowed to warm to room temperature over 2 h, and was stirred for a further 4 h at room temperature. The volatiles were removed under reduced pressure to afford a yellow oil which was dissolved in pyridine (5 mL) and under an atmosphere of argon at 0 °C was treated with acetic anhydride (5 mL) and was stirred overnight. The volatiles were removed under reduced pressure to afford a yellow oil that was purified by flash chromatography over silica gel (Hexanes/EtOAc 9/1 to 1/2) to afford 225 (106 mg, 31%) as a white foam. [α]$^{23}D = -24$ (c = 1, CHCl$_3$). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.34-7.26 (m, 5H), 5.31-5.16 (m 2H), 5.05-4.97 (m, 2H), 4.80 (t, $J = 6.8$ Hz, 1H), 4.61-4.41 (m, 5H), 4.28-4.18 (m, 2H), 4.02-3.79 (m 2H), 2.89-2.17 (m, 1H), 2.12 (s, 3H), 2.07 (s, 6H), 2.04 (s, 3H). 171.2, 170.7, 170.1, 170.0, 169.9, 169.6, 169.5, 137.4, 130.1, 128.8, 128.6, 128.6, 128.5, 128.2, 128.2, 128.1, 85.2, 84.4, 80.1, 74.9, 74.8, 74.6, 74.0, 73.7, 70.5, 70.6, 69.3, 69.3, 62.6, 62.6, 62.5, 61.2, 42.6, 40.9, 21.1, 21.0, 20.9, 20.9, 20.8, 20.7, 14.1. ESIHRMS: m/z calcd. for C$_{23}$H$_{32}$O$_{15}$Na (M + Na)$^+$ 571.1639, found 571.1652.
Ethyl-1,2,3-trideoxy-7-O-benzyl-5,6:8,9-di-O-isopropylidene-4-O-methanesulfonyl-D-glycero-D-talo-2-methylene-nonanoate (226): To a stirred solution of 223-anti (0.15 g, 0.323 mmol) in pyridine (5 mL) was added mesyl chloride (50 μL, 0.646 mmol) under an argon atmosphere at 0 °C and the mixture was stirred at room temperature for 6 h. The volatiles were removed under reduced pressure and the residue was dissolved in EtOAc (10 mL), and was washed with sat. NaHCO₃ (10 mL), brine (10 mL), dried and concentrated to afford a yellow oil that was purified by flash chromatography over silica gel (Hexanes/EtOAc 9/1 to 3/1) to afford the mesylate 226 (169 mg, 97%) as a yellow oil. [α]°D = +52 (c = 1, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.36–7.25 (m, 5H), 6.26 (s, 1H), 5.71 (s, 1H), 5.32–5.29 (m, 1H), 4.89 (d, J = 11.2 Hz, 1H) 4.77 (d, J = 11.2 Hz, 1H), 4.50–4.48 (m, 1H), 4.39 (t, J = 7.2 Hz, 1H), 4.33 (t, J = 7.2 Hz, 1H), 4.20–4.14 (m, 3H), 4.10–4.05 (m, 1H), 3.95–3.90 (m 1H), 2.96 (s, 3H), 2.87 (d, J = 14.4 Hz, 1H), 2.63 (dd, J = 14.8, 5 Hz, 1H), 1.55 (s, 3H), 1.41 (s, 3H), 1.38 (s, 3H), 1.34 (s, 3H), 1.27 (t, J = 7.2 Hz, 1H). 166.8, 138.7, 135.7, 128.8, 128.4, 128.0, 127.8, 110.1, 109.1, 80.2, 80.0, 78.4, 76.9, 74.3, 67.9, 61.1, 38.9, 34.5, 26.2, 26.1, 25.9, 25.3, 14.4. ESIHRMS: m/z calcd. for C₂₆H₃₈O₁₀SnA (M + Na)+ 565.2083, found 565.2074.

Ethyl-1,2,3-trideoxy-7-O-benzyl-5,6:8,9-di-O-isopropylidene-4-keto-D-glycero-D-talo-2-methylene-nonanoate (228): To a stirred solution of 223-anti (60 mg, 0.12 mmol) and 4 Å° molecular sieves (100 mg) in anhydrous CH₂Cl₂ under an argon atmosphere were treated with PCC (83 mg, 0.38 mmol) and NaOAc (3 mg, 0.037 mmol). The mixture was stirred for 2 h at room temperature and was concentrated to afford a brown solid which was diluted with MTBE and was filtered through a small plug of Celite/silica, and concentrated to afford a yellow oil which was purified by chromatography over silica gel (Hexanes/EtOAc 9/1 to 4/1) to afford 228.
as a yellow oil (36 mg, 62%). [α]_{D}^{23} = +14 (c = 1, CHCl₃). \(^1\)H NMR (400 MHz, CDCl₃) δ 7.33-7.24 (m, 5H), 6.01 (s, 1H0, 5.00 (d, J = 12.5 Hz, 1H), 4.69 (s, 1H), 4.60 (d, J = 8 Hz, 1H), 4.53 (dd, J = 8, 2 Hz, 1H), 4.34 -4.30 (m, 1H), 4.20-4.17 (m, 3H), 4.13-4.10 (m, 2H), 4.00 (brs, 1H), 3.85 (d, J = 17.5 Hz, 1H), 3.09 (d, J = 17.5 Hz, 1H), 1.60 (s, 3H), 1.36 (s, 3H), 1.32 (s, 3H), 1.27 (t, J = 7.2 Hz, 1H), 1.29 (s, 3H). 207.5, 167.0, 139.1, 134.0, 128.7, 128.7, 128.5, 128.0, 127.2, 126.1, 110.0, 108.0, 80.4, 79.9, 78.2, 75.1, 72.2, 65.8, 43.1, 26.5, 26.5, 25.1, 24.9, 14.9. ESIHRMS: m/z calcd. for C\textsubscript{25}H\textsubscript{34}O\textsubscript{8}Na (M + Na)+ 485.2151, found 485.2148.

**Ethyl-1,2,3-trideoxy-7-O-benzyl-5,6:8,9-di-O-isopropylidene-4-keto-D-glycero-D-talo-2-methylene-nonanoate (228):** To a stirred solution of the diastereomeric mixture 223 (0.18g, 0.387 mmol) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (5 mL) under an argon atmosphere was added Dess-Martin periodinane (197 mg, 0.465 mmol). The mixture was stirred for 4 h, diluted with CH\textsubscript{2}Cl\textsubscript{2} (5 mL), and was washed with sat. NaHCO\textsubscript{3} (2 x 10 mL). The organic layer was washed with brine (10 mL), dried and concentrated to afford a colorless oil, that was purified by flash chromatography over silica gel (Hexanes/EtOAc 9/1 to 4/1) to afford the desired ketone 228 (157 mg, 88%) as a colorless oil.

**Ethyl-1,2,3-trideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-galacto-2-methylene-nonanoate (223- syn):** A stirred solution of the ketone 228 (92 mg, 0.2 mmol) in anhydrous methanol (1 mL) under an argon atmosphere was added at 0 °C sodium borohydride (11 mg, 0.3 mmol) in one lot. The mixture was stirred for 90 min at 0 °C and was quenched by addition of solid NH\textsubscript{4}Cl. The solvent was removed under reduced pressure to afford a yellow residue that was dissolved in EtOAc (5 mL) and was washed with brine (5 mL), dried and concentrated to
afford a colorless oil that was purified by flash chromatography over silica gel (Hexanes/EtOAc 9/1 to 1/1) to afford syn-223 (83 mg, 90%) as a colorless oil. \([\alpha]^{23}_D = +21 (c = 1, \text{CHCl}_3)\). 

\(^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta 7.35-7.25 (m, 5H), 6.24 (d, \(J = 1.6 \text{ Hz, 1H}\)), 5.67 (s, 1H), 4.86 (d, \(J = 11.2 \text{ Hz, 1H}\)), 4.73 (d, \(J = 11.2 \text{ Hz, 1H}\)), 4.24-4.17 (m, 5H), 4.08-3.39 (m, 6H), 2.71 (d, d, \(J = 4.8 \text{ Hz, 1H}\)), 2.53 (m, 2H), 1.51 (s, 3H), 1.40 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H), 1.29 (t, \(J = 7.2 \text{ Hz, 1H}\)). 167.4, 138.1, 137.3, 128.5, 128.2, 127.9, 127.7, 108.9, 108.3, 79.7, 78.5, 78.6, 77.5, 76.7, 74.5, 68.4, 66.7, 60.9, 37.1, 26.5, 26.4, 25.5, 25.1, 14.4. ESIHRMS: m/z calcd. for C\(_{25}\)H\(_{36}\)O\(_8\)Na (M + Na\(^+\)) 487.2303, found 487.2310.

**Ethyl (2,5,7,9-tetra-O-acetyl-4-O-benzoyl-8-O-benzyl-3-deoxy-D-glycer-D-galacto-2-nonulopyranose)onate 225:** A stirred solution of syn 223 (0.3 g, 0.646 mmol) in anhydrous CH\(_2\)Cl\(_2\) (3.5 mL) and pyridine (3.5 mL) under an argon atmosphere was treated with benzoyl chloride (82 uL, 0.711 mmol) at 0 °C. The mixture was warmed to room temperature and stirred overnight. The volatiles were removed under reduced pressure to afford a yellow oil that was dissolved in EtOAc (10 mL) and was washed with 1N HCl (10 mL) and sat. NaHCO\(_3\) (10 mL), brine (5 mL), dried and concentrated to afford the benzoylated syn-diastereomer as a colorless oil, which was dissolved in anhydrous methanol (5 mL) and was cooled to -78 °C. The mixture was sparged with ozone for 5 min followed by sparging with oxygen for 5 min. The mixture was stirred for further 5 min and was treated with dimethyl sulfide (473 uL, 6.46 mmol). The mixture was allowed to warm to room temperature over 2 h, and was stirred for a further 4 h at room temperature. The volatiles were removed under reduced pressure to afford a yellow oil which was dissolved in pyridine (10 ml) and under an atmosphere of argon at 0 °C was treated with acetic anhydride (10 ml). The mixture was stirred overnight at room temperature. The volatiles
were removed under reduced pressure to afford a yellow oil that was purified by flash chromatography over silica gel (Hexanes/EtOAc 9/1 to 1/2) to afford 225 (161 mg, 38%), a mixture of anomers as a white foam.

**β-anomer**: $^1$H NMR (400 MHz, CDCl$_3$) 7.99-7.97 (m, 2H), 7.58-7.54 (t, $J = 6.4$ Hz, 1H), 7.45-7.25 (m, 7H), 5.52-5.45 (m, 1H), 4.69-4.64 (m, 2H), 4.55-4.52 (m, 1H), 4.38-4.33 (m, 1H), 4.22-4.13 (m, 4H), 3.81 (brs, 1H), 2.77 (dd, 13.2, 4.4 Hz, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H).

1,2,3-Trideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-galacto-1-yno-nonitol (syn-244) and 1,2,3-Trideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-talo-1-yno-nonitol (anti-244) by Grignard reaction with diacetone manno lactone: Magnesium turnings (9.41 g, 0.38 mol) and mercuric chloride (125 mg, 0.46 mmol) were placed under an argon atmosphere in an oven dried three neck flask fitted with a condenser, a rubber septum and a dropping funnel. Anhydrous ether (30 mL) was added to the flask and under vigorous stirring 80% propargyl bromide in toluene (0.65 mL, 5.8 mmol, 0.06 eq) was added through the septum. The flask was warmed with a heat gun to induce reflux, upon which it was immediately immersed in an ice bath, followed by dropwise addition of propargyl bromide (11.21 mL, 100.6 mmol) in ether (80 mL) over 45 min under vigorous stirring. The reaction mixture was vigorously stirred for 1 h at 0 °C, and was transferred to a solution of lactone 231 (25 g, 0.096 mol) in THF (400 mL) maintained at -40 °C under an argon atmosphere over 15 minutes. The mixture was maintained at -40 °C for 1 h before it was slowly warmed to 0 °C over 30 min and was quenched by addition of sat. NH$_4$Cl (400 mL). The mixture was extracted with EtOAc (200 mL) and the separated aqueous layer was washed with EtOAc (2 x 100 mL). The combined organic layer was washed with brine (300 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo to furnish a yellow oil. The oil was dried
under high vacuum for 1 h, to afford crude lactol 243 (27.4 g), which was carried forward to the
next step. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 4.75\) (dd, \(J = 6.0, 3.5\) Hz, 1H), \(4.45\) (d, \(J = 5.5\) Hz, 1H),
4.28 (dd, \(J = 12.0, 7.5\) Hz, 1H), 4.01–3.97 (m, 2H), 3.98–3.91 (q, \(J = 4.5\) Hz, 1H), 3.55 (s, 1H),
2.62 (ddd, \(J = 9.5, 16.5, 3.0\) Hz, 2H), 2.06 (t, \(J = 3.0\) Hz, 1H), 1.38 (s, 3H), 1.36 (s, 3H), 1.28 (s,
3H), 1.24 (s, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta 113.1, 109.3, 104.4, 85.0, 80.4, 79.5, 79.0,
73.3, 71.8, 66.8, 27.0, 26.6, 26.0, 25.4, 24.6. ESIHRMS: \(m/z\) calcld. for C\(_{13}\)H\(_{22}\)O\(_6\)Na (M + Na)
\(^+\) 321.1306, found 321.1314. The relative stereochemistry of 247 was assigned based on the
observation of an NOE interaction between the propargylic hydrogens and an acetonide methyl
groups, and on the absence of NOE interactions between the propargylic hydrogens and H’s 6
and 7.

To a vigorously stirred solution of the crude lactol 243 (27.4 g, 0.091 mol) in anhydrous MeOH
(920 mL) under an argon atmosphere in a 2 L 2 neck flask fitted with a thermometer was added
free flowing dry sodium borohydride powder (34.7 g, 0.918 mol) in one lot at -15 °C. The
mixture was stirred vigorously maintaining the internal temperature at -15 °C for 2 h by which
time the reaction was complete as indicated by TLC (1:1 EtOAc/ hexanes). The mixture was
diluted with EtOAc (400 mL) and half the volume was transferred to a separatory funnel and sat.
NH\(_4\)Cl (500 mL) was added portion wise carefully. The separated aqueous layer was acidified to
\(pH 6\) using 0.5 N HCl and was extracted with EtOAc (200 mL). The combined organic layer was
washed with 0.5 N HCl (200 mL), sat. NaHCO\(_3\) (250 mL), brine (250 mL) and dried over
Na\(_2\)SO\(_4\). The same procedure was repeated for the other half of the reaction mixture. The dried
organic layer was then concentrated \(in\ vacuo\) and was purified by chromatography over silica gel
eluting with EtOAc /hexanes (1/5 to 1/1) to afford the diol as a white solid 27.9 g (100:6
\(syn/anti\)), which was recrystallized from diethyl ether and pentanes to afford the pure diol \(syn-\)
244 (22.96 g, 79% over 2 steps) as a white powder. Mp 88-89 °C; [α]23D = +43 (c = 1, CHCl3).

1H NMR (500 MHz, CDCl3) δ 4.44 (d, J = 8.0 Hz, 1H), 4.37 (dd, J = 7.5, 1.5 Hz, 1H), 4.15-4.09 (m, 2H), 4.06-3.98 (m, 2H), 3.69 (dd, J = 8.0, 5.5 Hz, 1H), 3.43 (d, J = 5.0 Hz, 1H), 3.34 (d, J = 6.0 Hz, 1H), 2.61 (ddd, J = 17.0, 6.5, 3.0 Hz, 1H), 2.52 (ddd, J = 16.5, 7.5, 2.5 Hz, 1H), 2.07 (t, J = 3.0 Hz, 1H), 1.55 (s, 3H), 1.43 (s, 3H), 1.42 (s, 3H), 1.37 (s, 3H); 13C NMR (125 MHz, CDCl3) δ 109.4, 108.6, 80.3, 76.0, 75.7, 70.7, 70.2, 68.1, 67.2, 26.8, 26.2, 25.2, 24.9, 26.6. ESIHRMS: m/z calcd. for C15H24O6Na (M + Na)+ 323.1471, found 323.1469.

The known146 minor anti-isomer anti-244 was identified in the crude reaction mixture by the following signals: 1H NMR (500 MHz, CDCl3) δ 4.42 (d, J = 6.0 Hz, 1H), 4.14-4.08 (m, 3H), 4.07-4.04 (m, 2H), 3.98 (d, J = 12.5 Hz, 1H), 2.70-2.66 (td, J = 17.0, 3.0 Hz, 1H), 2.50-2.45 (ddd, J = 17.0, 7.0 Hz, 1H), 2.08 (t, J = 2.5 Hz, 1H), 1.46 (s, 3H), 1.41 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H); 13C NMR (125 MHz, CDCl3) δ 109.4, 108.3, 80.1, 78.0, 76.3, 75.5, 71.2, 69.2, 67.6, 67.0, 26.9, 26.8, 25.3, 24.8, 24.7.

1,2,3-Trideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-galacto-1-yno-nonitol (syn-244) and 1,2,3-Trideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-talo-1-yno-nonitol by Grignard reaction with diacetone mannofuranose (anti-244): Magnesium turnings (150 mg, 6.19 mmol) and mercuric chloride (1 mg, 0.003 mmol) were placed under an argon atmosphere in an oven dried 2 neck flask fitted with a condenser, and a rubber septum. Anhydrous ether (0.5 mL) was added to the flask and under vigorous stirring 80% propargyl bromide in toluene (18 uL, 0.15 mmol) was added. The flask was warmed with a heat gun to induce reflux, upon which it was immediately immersed in an ice bath, followed by dropwise addition of propargyl bromide (155 uL) in ether (1 mL) over 5 min under vigorous stirring. The reaction mixture was vigorously stirred for 1 h at 0 °C, and was transferred to a solution of lactol 247 (200 mg, 0.76 mmol) in
THF (7 mL) maintained at -60 °C under an argon atmosphere over 15 minutes. The reaction mixture was warmed to 0 °C over 1 h and stirred further for 2 h. The reaction mixture was quenched by adding sat. NH₄Cl (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), dried over Na₂SO₄ and concentrated in vacuo to give a white powder which was purified by chromatography over silica gel to afford 244 (215 mg, 94%, anti/syn: 2:1) as a white powder, the NMR spectra of which are identical to compounds syn-244 and anti-244 described above. ESIHRMS: m/z calcd. for C₁₅H₂₄O₆Na (M + Na)⁺ 323.1471, found 323.1465.

1,2,3-Trideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-galacto-1,2-dieno-nonitol (245):

Syrup. [α]²³ D = + 23 (c = 1, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ 5.37 (q, J = 6.5 Hz, 1H), 4.93 (dd, J = 7.0, 2.0 Hz, 2H), 4.35 (m, 1H), 4.43 (d, J = 7.5 Hz, 1H), 4.27 (dd, J = 7.5, 4.5 Hz, 1H), 4.12 (dd, J = 8.0, 5.5 Hz, 1H), 4.02-4.09 (m 4H), 3.64 (d, J = 8.0 Hz, 1H), 1.55 (s, 3H), 1.41 (s, 6H), 1.36 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 208.0, 109.3, 108.5, 91.4, 79.3, 77.9, 75.9, 75.5, 70.2, 67.9, 67.3, 26.8, 26.3, 25.2, 24.5. ESIHRMS: m/z calcd. for C₁₅H₂₄O₆Na (M + Na)⁺ 323.1471, found 323.1466.

1,2,3-Trideoxy-5,6-O-isopropylidene-D-lyxo-1-yno-hexitol (syn-249) and 1,2,3-Trideoxy-5,6-O-isopropylidene-D-xylo-1-yno-hexitol (anti-249) by Grignard reaction with erythro lactone 248: Magnesium turnings (47 mg, 1.8 mmol) and mercuric chloride (1 mg, 0.003 mmol) were placed under an argon atmosphere in an oven dried 2 neck flask fitted with a condenser, and a rubber septum. Anhydrous ether (1 mL) was added to the flask and under vigorous stirring 80% propargyl bromide in toluene (9 uL, 0.08 mmol) was added. The flask was warmed with a
heat gun to induce reflux, upon which it was immediately immersed in an ice bath, followed by drop wise addition of propargyl bromide (73 µL) in ether (6.5 mL) over 5 min under vigorous stirring. The reaction mixture was vigorously stirred for 1 h at 0 °C, and was transferred to a solution of lactol 248 (100 mg, 0.63 mmol) in THF (4 mL) maintained at -40 °C under an argon atmosphere over 15 minutes. The mixture was maintained at -40 °C for 1 h before it was slowly warmed to 0 °C over 30 min and was quenched by addition of sat. NH₄Cl (8 mL). The mixture was extracted with EtOAc (10 mL) and the separated aqueous layer was washed with EtOAc (2 x 5 mL). The combined organic layer was washed with brine (10 mL), dried over Na₂SO₄ and concentrated in vacuo to furnish a yellow oil. The oil was dried under high vacuum for 1 h, to afford crude lactol (120 mg), which was carried forward to the next step. To a stirred solution of the lactol (120 mg, 0.606 mmol) in anhydrous methanol (6 mL) at -15 °C under an argon atmosphere was added sodium borohydride (230 mg, 6.06 mmol). The mixture was stirred at this temperature for 2 h and was quenched by addition of sat. NH₄Cl (10 mL), and was extracted with EtOAc (3 x 5 mL). The organic layer was dried and concentrated to afford a 2:1 syn:anti diastereomeric mixture of 249 as a colorless oil, which were purified by flash chromatography over silica gel (Hexanes/ EtOAc 10/1 to 1/1) to afford syn 249 (64 mg, 51%) as a colorless oil, and anti 249 (21 mg, 26%).

\[ \text{syn} \text{ 249} : [\alpha]^{23}_D = +11 \ (c = 0.5, \text{CHCl}_3) \]

\[^1\text{H} \text{NMR} \ (500 \text{ MHz, CDCl}_3) \delta \ 4.32-4.31 \ (m, \ 2H), \ 3.92-3.85 \ (m, \ 3H), \ 3.13 \ (d, \ J = 6.5 \text{Hz}, \ 1H), \ 2.60-2.54 \ (\text{ddd, } J = 16.5, \ 5.5, \ 2.5 \text{ Hz}, \ 1H), \ 2.54-2.48 \ (\text{ddd, } 16.5, \ 5.5, \ 2.5 \text{ Hz}, \ 1H), \ 2.06 \ (t, \ J = 3 \text{ Hz}, \ 1H), \ 1.53 \ (s, \ 3H), \ 1.41 \ (s, \ 3H). \]

\[^{13}\text{C} \text{NMR} \ (125 \text{ MHz, CDCl}_3) \delta \ 108.4, \ 80.4, \ 70.6, \ 67.9, \ 60.9, \ 26.9, \ 24.8, \ 24.8. ]

ESIHRMS: \text{m/z calcd. for C}_{10}\text{H}_{16}\text{O}_4\text{Na (M + Na)}^+ 223.0946, \text{found 223.0939.}
anti 249: \([\alpha]^2_{D} = +49 (c = 0.5, \text{CHCl}_3)\). \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)) \(\delta 4.38-4.34\) (m, 1H), 4.09 (dd, \(J = 10, 5.5\) Hz, 1H), 3.95-3.90 (m, 2H), 3.84-3.81 (m, 1H), 2.98-2.97 (m, 2H), 2.75-2.70 (td, \(J = 16.5, 3\) Hz, 1H), 2.52-2.47 (dd, \(J = 17.5, 7, 3\) Hz, 1H), 2.12 (t, \(J = 3\) Hz, 1H), 1.57 (s, 3H), 1.41 (s, 3H). \(^1\text{C NMR}\) (125 MHz, CDCl\(_3\)) \(\delta 105.8, 78.4, 67.7, 60.8, 27.8, 25.2, 24.5\). ESIHRMS: \(m/z\) calcd. for C\(_{10}\)H\(_{16}\)O\(_4\)Na (M + Na\(^+\)) 223.0946, found 223.0936.

**1,2,3-Trideoxy-6,7-O-isopropylidene-d-allo-1-yno-heptitol (syn-251) by Grignard reaction with erythrono lactone 250:** Magnesium turnings (1 g, 40 mmol) and mercuric chloride (5 mg, 0.015 mmol) were placed under an argon atmosphere in an oven dried 2 neck flask fitted with a condenser, and a rubber septum. Anhydrous ether (4 mL) was added to the flask and under vigorous stirring 80% propargyl bromide in toluene (340 uL) was added. The flask was warmed with a heat gun to induce reflux, upon which it was immediately immersed in an ice bath, followed by drop wise addition of propargyl bromide (1 mL) in ether (8 mL) over 5 min under vigorous stirring. The reaction mixture was vigorously stirred for 1 h at 0 °C, and was transferred to a solution of lactol 250 (940 mg, 5 mmol) in THF (12.5 mL) maintained at -40 °C under an argon atmosphere over 15 minutes. The mixture was maintained at -40 °C for 1 h before it was slowly warmed to 0 °C over 30 min and was quenched by addition of sat. NH\(_4\)Cl (20 mL). The mixture was extracted with EtOAc (10 mL) and the separated aqueous layer was washed with EtOAc (2 x 15 mL). The combined organic layer was washed with brine (10 mL), dried over Na\(_2\)SO\(_4\) and concentrated in vacuo to furnish a yellow oil. The oil was dried under high vacuum for 1 h, to afford crude lactol (1.2 g), which was carried forward to the next step. To a stirred solution of the lactol (1.2 g, 5.2 mmol) in anhydrous methanol (55 mL) at -15 °C under an argon
atmosphere was added sodium borohydride (2 mg, 52 mmol). The mixture was stirred at this
temperature for 2 h and was quenched by addition of sat. NH₄Cl (25 mL), and was extracted with
EtOAc (3 x 15 mL). The organic layer was dried and concentrated to afford a 10:1 syn:anti of
the triol 251 (1.06 g, 88%).

**syn-251**: ¹H NMR (400 MHz, CDCl₃) δ 4.51 (dd, J = 9, 3 Hz, 1H), 4.43-4.28 (m, 2 H), 3.90-
3.76 (m, 5H), 3.67 (t, J = 8.5 Hz, 1H), 3.14 (d, 8.5 Hz, 1H), 2.73-2.67 (ddd, J = 16, 5, 3 Hz, 1H),
2.59-2.47 (m, 3H), 2.07 (t, 4H), 1.51(s, 3H), 1.39 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 108.6,
75.6, 71.6, 70.9, 70.5, 61.3, 61.0, 25.0, 24.4.

**1-Bromo-1,2,3-trideoxy-5,6:8,9-di-O-isopropylidene-Δ-gluceral-Δ-galacto-1-yno-nonitol**

(252): To a stirred solution of **syn 244** (6.8 g, 22.6 mmol) in anhydrous acetone (220 mL) under
an argon atmosphere and was added N-bromosuccinimide (5.04 g, 28.3 mmol) and AgNO₃ (0.96
g, 5.6 mmol) at room temperature. After stirring the mixture in dark for 2 h the reaction mixture
was filtered through Celite, and the Celite was washed with acetone (2 x 10 mL). The filtrate
was concentrated _in vacuo_ and was diluted with EtOAc (100 mL) and washed with water (100
mL) and brine (100 mL). The organic layer was dried over Na₂SO₄ and concentrated _in vacuo_ to
furnish a colorless oil which was purified by chromatography over silica gel (EtOAc/hexanes 1/10
to 1/5) to afford 252 (7.19 g, 84%) as a colorless oil. [α]²νD = + 34 (c = 1, CHCl₃). ¹H NMR (500
MHz, CDCl₃) δ 4.39 (d, J = 8.0 Hz, 1H), 4.28 (d, J = 8.0 Hz, 1H), 4.11-4.03 (m, 2H), 4.01-3.98
(m, 1H), 3.95 (t, J = 7.0 Hz, 1H), 3.65 (d, J = 6.5 Hz, 1H), 2.60-2.49 (ddd, 14.5, 17.5, 6.0 Hz,
2H), 1.50 (s, 3H), 1.39 (s, 3H), 1.38 (s, 3H), 1.33 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 109.4,
108.6, 77.2, 76.3, 76.0, 75.6, 70.0, 67.9, 67.1, 40.6, 26.7, 26.1, 25.9, 25.2, 24.7. ESIHRMS: m/z
calcd. for C₁₅H₂₃O₆BrNa (M + Na)⁺ 401.0576, found 401.0578.
1-Bromo-1,2,3-trideoxy-D-glycero-D-galacto-1-yno-nonitol (254): To the alkynyl bromide 252 (6.23 g, 16.4 mmol) in a 1 L round bottom flask was added an acetic acid/water mixture (500 mL, 6:4). The reaction mixture was stirred at 40 °C for 60 h then concentrated in vacuo at 30 °C to 1/5th of the volume and the solids were filtered off and were washed with diethyl ether (2 x 10 mL) to afford 254 (3.59 g, 73%) as a free flowing white powder. Mp 200-201 °C; [α]²³ D = -12 (c = 1, DMSO). ¹H NMR (500 MHz, DMSO-d₆) δ 4.43 (d, J = 7.6 Hz, 1H), 4.36 (d, J = 6.4 Hz, 1H), 4.32 (t, J = 6.4 Hz, 1H), 4.21 (d, J = 8.0 Hz, 1H), 4.11 (d, J = 7.2 Hz, 1H), 4.06 (d, J = 7.6 Hz, 1H), 3.83 (q, J = 6.8 Hz, 1H), 3.65 (t, J = 8.0 Hz, 1H), 3.61-3.51 (m, 2H), 3.47-3.43 (m, 1H), 3.85-3.34 (m 2H), 2.40 (dd, J = 16.4, 6.8, 1H), 2.32 (dd, J = 16.4, 6.8, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ 79.3, 72.0, 70.6, 70.0, 40.7, 68.9, 68.7, 64.3, 25.1 ESIHRMS: m/z calcd. for C₉H₁₂O₆BrNa (M + Na)⁺ 320.9950, found 320.9958.

3-Deoxy-D-glycero-β-D-galacto-2-nonulosonic acid (2): To compound 254 (3 g, 10.0 mmol) in a 2 L round bottom flask was added a mixture of acetic acid/water (800 mL, 1:1). The mixture was warmed with a heat gun until the solids dissolved after which it was allowed to cool to room temperature. The reaction mixture was sparged with ozone for 1 h by which time analysis by mass spectroscopy confirmed the consumption of the starting material. Oxygen was sparged for 10 minutes and dimethyl sulphide (0.81 mL, 11.07 mmol) was added to the mixture which was stirred for 1 h at room temperature. The mixture was concentrated to about 1/4th of the volume in vacuo at room temperature and was lyophilized to afford a frothy solid, which was dissolved in MeOH/water (3 mL, 10:1), and loaded onto a reverse phase silica gel column and eluted with acetonitrile/water (100:0 to 100:10). The fractions were analyzed by reverse phase TLC (9/1 acetonitrile/water) and the fractions corresponding to the less mobile spots were pooled together
and were lyophilized to afford 1.31 g (46%) of a white powder, the NMR of which is identical with that of 2 as reported in the literature.¹⁴⁷ [α]₂³ D = - 43 (c = 1.4, H₂O). Lit [α]₂³ D = - 40.7 (c = 2.4, H₂O). ESIHRMS: m/z calcd. for C₉H₁₅O₉ (M - H)^+ 267.0716, found 267.0714.

1-Bromo-4,7-di-O-acetyl-1,2,3-trideoxy-5,6:8,9-di-O-isopropyldiene-D-glycero-D-galacto-1-yno-nonitol (255): To a solution of diol 252 (1.42 g, 3.75 mmol) in anhydrous pyridine (20 mL) under an argon atmosphere at 0 °C was added acetic anhydride (20 mL). The mixture was warmed to room temperature over 1 h and was stirred for 6 h. The volatiles were removed in vacuo and the residue was dissolved in EtOAc (20 mL) and was washed with 1N HCl (20 mL), NaHCO₃ (20 mL), and brine (20 mL). The organic layer was dried over Na₂SO₄, concentrated in vacuo and purified by column chromatography over silica gel (hexanes to EtOAc/hexanes 3/10) to afford 255 (1.64 g, 95%) as a colorless syrup. [α]²³ D = + 28 (c = 1, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ 5.26-5.23 (m, 1H), 5.03 (t, J = 7.5 Hz, 1H), 4.47 (dd, J = 6.0, 4.0 Hz, 1H), 4.34 (t, J = 6.5 Hz, 1H), 4.12-4.06 (m, 2H), 3.79-3.75 (m ,1H), 2.62 (dd, J = 16.5, 8.5, 1H), 2.50 (dd, J = 16.5, 8.5, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 1.50 (s, 3H), 1.48 (s, 3H), 1.37 (s, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 170.3, 170.6, 110.2, 109.1, 76.6, 76.1, 75.4, 75.1, 70.3, 69.1, 67.5, 40.7, 26.1, 26.0, 25.7, 25.6, 22.3, 21.1, 20.9. ESIHRMS: m/z calcd. for C₁₉H₂₇O₆BrNa (M + Na)^+ 485.0787, found 485.0771.

Methyl (2,4,5,7,8,9-hexa-O-acetyl-3-deoxy-D-glycero-β-D-galacto-2-nonulopyran-ose)onate (150): A solution of compound 255 (1.9 g, 4.1 mmol) in acetic acid/water (200 mL, 6:4) was heated at 40 °C for 12 h. The mixture was then co-evaporated to dryness with toluene in vacuo and was dried under vacuum to afford a white solid. The crude solid was dissolved in anhydrous
MeOH (20 mL), cooled to -60 °C, and was sparged with ozone for 45 min followed by sparging with oxygen for 5 min. Hunig’s base (0.715 mL, 4.1 mmol), and dimethyl sulphide (3.03 mL, 41.1 mmol) were added sequentially to the reaction mixture which then was warmed to room temperature (over 4 h) and stirred for 1 day. The volatiles were evaporated at 10 °C in vacuo and the residue was dried under vacuum and was treated with pyridine (30 mL) and acetic anhydride (30 mL) at 0 °C under an argon atmosphere. The reaction mixture was warmed to room temperature over 1 h and was stirred overnight before it was concentrated to dryness in vacuo and was dissolved in EtOAc (30 mL) and washed with 1N HCl (20 mL), NaHCO₃ (20 mL), brine (20 mL), dried and concentrated in vacuo to furnish a frothy solid which was purified by chromatography over silica gel (toluene to EtOAc/toluene 3/10) to afford 150 (635 mg, 29 % over 3 steps, β/α: 14/1), the NMR spectra of which are in agreement with those reported in literature.¹⁴⁸ β isomer: [α]²³ D = -21 (c = 1.2, CHCl₃); Lit¹⁴⁸ [α]²⁶ D = -24.3 (c = 0.31, CHCl₃), Mp 101-104 °C; Lit Mp¹⁴⁸ 104-105 °C. ESIHRMS: m/z calcd. for C₂₂H₃₀O₁₅Na (M + Na)+ 557.1482, found 577.1486.

**1-Bromo-9-O-[(t-Butyl)diphenylsilyl]-1,2,3-trideoxy-D-glycero-D-galacto-1-yno-nonitol** (259): To a solution of 254 (3.18 g, 10.63 mmol) in anhydrous pyridine (20 mL) under an argon atmosphere was added TBDPSCI (2.4 g, 15.94 mmol) and DMAP (1.29 g, 10.63 mmol). The mixture was stirred overnight at 40 °C. The volatiles were evaporated in vacuo to afford a yellow solid which was taken up with EtOAc (40 mL) and was washed with brine (2 x30 mL), dried over Na₂SO₄ and concentrated in vacuo. The resulting white powder was purified by chromatography over silica gel (CHCl₃ to 1/10 MeOH/CHCl₃) to afford 259 (4.97 g, 87%) as a white powder. Mp 126-128 °C; [α]²³ D = +71 (c = 1, MeOH). H NMR (500 MHz, CD₃OD) δ
7.72-7.72 (t, J = 7.5 Hz, 4H), 7.45-7.39 (m, 6H), 4.06 (t, J = 7.0 Hz, 1H), 3.95 (d, J = 13.5 Hz, 1H), 3.91 (d, J = 8.0 Hz, 1H), 3.85-3.79 (m, 2H), 3.67 (d, J = 9.5, 1H), 2.57-2.46 (ddd, 16.5, 13.5 Hz, 6.5 Hz, 2H), 1.07 (s, 9H); $^{13}$C NMR (125 MHz, CD$_3$OD) δ 135.3, 135.3, 133.3, 133.2, 129.4, 127.3, 77.1, 72.1, 70.6, 69.5, 69.0, 68.7, 66.1, 38.7, 25.9, 24.1, 18.7. ESIHRMS: m/z calcd. for C$_{25}$H$_{33}$O$_6$BrSiNa (M + Na)$^+$ 559.1127, found 559.1120.

**Methyl (2,4,5,7,8-penta-O-acetyl-9-O-t-butylphenylsilyl-3-deoxy-D-glycero-β-D-galacto-2-nonulopyran-ose)onate (260):** A solution of 259 (1 g, 1.8 mmol) dissolved in anhydrous MeOH (10 mL) was cooled down to -60 °C, and was sparged with ozone until the starting material was consumed (~45 min, as indicated by TLC 1/20 MeOH/CHCl$_3$), after which oxygen was sparged for 5 min before Hunig’s base (0.325 mL, 1.8 mmol) and dimethyl sulfide (1.37 mL, 18.6 mmol) were added sequentially and the mixture was stirred for 1 day at room temperature. The volatiles were evaporated *in vacuo* at 10 °C and the residue was dissolved in EtOAc (15 mL) and was washed with brine (2 x10 mL), dried over Na$_2$SO$_4$ and concentrated *in vacuo* at 10 °C. The residue was then treated with pyridine (15 mL) and acetic anhydride (15 mL) at 0 °C under an argon atmosphere, and was warmed to room temperature over 1 h and stirred overnight. The volatiles were evaporated *in vacuo* and the mixture was diluted with EtOAc (30 mL) and washed with 1N HCl (15 mL), sat. NaHCO$_3$ (15 mL), brine (15 mL), dried over Na$_2$SO$_4$ and concentrated *in vacuo* to afford a yellow foam which was purified by chromatography over silica gel (hexanes to EtOAc/hexanes 3/10) to afford 260 (572 mg, 42%) as a 10/1 α/β mixture. ESIHRMS: m/z calcd. for C$_{36}$H$_{46}$O$_{14}$SiNa (M + Na)$^+$ 753.2555, found 753.2560.
2610 β anomer: $^1$H NMR (500 MHz, CDCl$_3$) δ 7.64-7.61 (m, 4H), 7.40-7.35 (m, 6H), 5.60-5.58 (dd, $J = 7.5$, 2.0 Hz, 1H), 5.28-5.23 (m, 1H), 5.09-5.06 (m, 1H), 4.90 (t, $J = 5.0$ Hz, 1H), 4.24-4.22 (dd, $J = 10.5$, 2.0 Hz, 1H), 3.93-3.90 (dd, $J = 12.0$, 3.0 Hz, 1H), 3.70 (s, 3H), 3.68-3.65 (dd, $J = 12.0$, 5.0 Hz, 1H), 2.63-2.59 (dd, $J = 13.5$, 5.5 Hz, 1H), 2.11 (t, $J = 13.5$ Hz, 1H), 2.10 (s, 3H), 2.04 (s, 3H), 2.01 (s, 6H), 1.98 (s, 3H), 1.03 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.2, 169.7, 169.6, 169.4, 168.1, 166.0, 135.6, 135.3, 133.1, 129.6, 127.6, 97.5, 71.8, 71.4, 68.7, 67.6, 66.5, 61.5, 53.0, 35.2, 26.6, 20.8, 20.8, 20.7, 20.6, 20.5, 19.2.

260 α anomer: $^1$H NMR (500 MHz, CDCl$_3$) δ 5.65-5.62 (dd, $J = 8.5$, 2 Hz), 5.18-5.13 (m, 1H), 4.87 (t, 9.5 Hz), 4.64-4.61 (dd, $J = 11.0$, 2.0 Hz), 3.88-3.85 (dd, $J = 6.5$, 2.5 Hz), 3.77 (d, 9.0 Hz), 3.74 (s), 3.64 (dd, $J = 12.0$, 4.0 Hz) 2.69 (dd, $J = 13.0$, 5.5 Hz).

4-O-[(t-Butyl)diphenylsilyl]-1,2,3-trideoxy-5,6;8,9-di-O-isopropylidene-D-glycero-D-galacto-1-yno-nonitol (261): A stirred solution of the diol syn 244 (1 g, 3.32 mmol), in anhydrous DMF (30 mL) under an argon atmosphere was treated with AgNO$_3$ (1.24 g, 7.3 mmol) and was stirred until the salt dissolved. TBDPSCI (0.602 g, 3.99 mmol) in anhydrous DMF (3 mL) was added to the flask and the resulting turbid mixture was stirred for 1.5 h. The reaction mixture was diluted with EtOAc (30 mL) and was filtered through Celite. The filtrate was washed with brine (2 x 30 mL) and dried over Na$_2$SO$_4$. The organic layer was concentrated in vacuo and was purified by chromatography over silica gel (hexanes to 1/10 EtOAc/hexanes) to afford 261 (1.38 g, 77%) as a colorless oil. [$\alpha$]$^2_{D}^{25} = +61$ (c = 1, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.75-7.71 (m, 4H), 7.43-7.35 (m, 6H), 4.45 (t, $J = 6.5$ Hz, 1H), 4.27 (dd, $J = 6.5$, 3 Hz, 1H), 4.19 (dd, $J = 11$, 6.5 Hz, 1H), 4.05-4.02 (dd, $J = 8.0$, 6.5 Hz, 1H), 3.97-3.93 (dd, $J = 13.5$, 6.0 Hz, 1H), 3.85-3.83 (dd, $J =
8, 5.5 Hz, 1H) 3.52-3.50 (dd, J = 8.0, 2.5 Hz, 1H), 2.52-2.47 (ddd, J = 17.5, 6.5, 2.5 Hz, 1H),
2.33-2.28 (ddd, J = 17.0, 3.5, 3.0 Hz, 1H), 2.08-2.03 (brs, 1H), 1.95 (t, J = 2.5 Hz), 1.42 (s, 3H),
1.32 (s, 3H), 1.28 (s, 3H), 1.25 (s, 3H), 1.06 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 136.2,
134.1, 133.6, 129.9, 129.9, 127.7, 109.4, 108.4, 80.2, 78.8, 76.2, 76.1, 71.1, 70.9, 70.2, 67.4,
27.2, 26.9, 26.5, 25.3, 25.1, 25.0, 19.6. ESIHRMS: m/z calcd. for C31H42O6SiNa (M + Na)+
561.2648, found 561.2641.

1-bromo-4-O-[(t-Butyl)ditolylsilyl]-1,2,3-trideoxy-D-glycero-D-galacto-1-yno-nonitol
(262): To a stirred solution of 261 (1.41 g, 2.6 mmol) in anhydrous acetone (25 mL) under an
argon atmosphere was added N-bromosuccinimide (0.582 g, 3.2 mmol) and AgNO3 (89 mg, 0.52
mmol). The mixture was then stirred in the dark for 1.5 h and the resulting solids were filtered
through Celite and concentrated in vacuo to afford a syrup, which was dissolved in EtOAc (20
mL) and washed with sat. NaHCO3 (15 mL) and brine (15 mL), dried over Na2SO4 and
concentrated in vacuo to furnish a colorless oil (1.46 g). The crude alkynyl bromide was then
dissolved in acetic acid/water (150 mL, 6/4) and was stirred at 40 °C for 24 h. The mixture was
then concentrated in vacuo to dryness and was purified by chromatography over silica gel
(CHCl3 to CHCl3/MeOH 1:10) to afford 262 (0.886 g , 63% over 2 steps) as a colorless syrup.
[α]23D d = +54 (c = 1, MeOH). 1H NMR (500 MHz, CD3OD) δ 7.75 (dd, 15.0, 8.5 Hz, 4H), 7.45-
7.38 (m, 6H), 4.26-4.23 (m, 1H), 4.09 (d, 9.0 Hz, 1H), 3.88 (d, 7.5 Hz, 1H), 3.79 (dd, 11.0, 4.0
Hz, 1H), 3.76 (d, 9.0 Hz, 1H), 3.73-3.69 (m, 1H), 3.64-3.60 (q, 5.5 Hz, 1H), 2.56 (dd, 16.5, 9.5
Hz, 1H), 2.16 (dd, 16.0, 5.0 Hz, 1H), 1.04 (s, 9H); 13C NMR (125 MHz, CD3OD) δ 135.7, 135.7,
133.9, 132.7, 129.6, 129.4, 127.3, 127.1, 76.1, 72.3, 71.4, 70.7, 69.6, 69.1, 63.6, 39.7, 26.1, 23.9,
18.9. ESIHRMS: m/z calcd. for C25H33O6BrSiNa (M + Na)+ 559.1127, found 559.1124.
Methyl (4-O-t-butylidiphenylsilyl-3-deoxy-D-glycero-α-D-galacto-2-nonulopyran-ose)onate (263): A solution of compound 262 (600 mg, 1.1 mmol) in anhydrous MeOH (6 mL) was cooled to -60 °C and was sparged with ozone for 45 min until complete consumption of 262 (indicated by TLC CHCl₃/MeOH 20/1). Hunig's base (194 uL, 1.1 mmol), and dimethyl sulfide (826 uL, 11 mmol) were added sequentially and the reaction mixture was warmed to room temperature over 4 h and stirred for 1 day. The volatiles were removed in vacuo at 10 °C and the crude compound was dissolved in EtOAc (15 mL) and was washed with brine (2 x 10 mL), dried over Na₂SO₄ and concentrated in vacuo at 10 °C to afford a yellow foam which was recrystallized form diethyl ether to afford 263 (425 mg, 73 %, >10/1 α/β) as a white solid. Mp 94-95 °C; [α]²³

13C NMR (125 MHz, CD₃OD) δ 170.8, 135.7, 135.6, 135.6, 134.3, 133.6, 129.3, 129.3, 127.2, 127.1, 95.1, 71.6, 71.5, 70.9, 70.7, 60.5, 63.6, 51.7, 39.5, 26.1, 26.1, 18.7. ESIHRMS: m/z calcd. for C₂₆H₃₆O₇SiNa (M + Na)⁺ 543.2026, found 543.2028.

4,7-Di-O-[(t-butyl)dimethylsilyl]-1,2,3-trIDEOXY-5,6:8,9-DI-isopropyliDene-D-glycero-D-galacto-1-yno-nonitol (256): To a solution of syn-244 (10 g, 0.033 mol) in anhydrous DMF (400 mL) was added (18.13 g, 0.26 mol) of imidazole, TBDMSCl (20 g, 0.13 mol) and DMAP (16.2 g, 0.13 mol). The mixture was stirred at 100 °C for 3 days. The volatiles were then evaporated in vacuo and the mixture was diluted with EtOAc (300 mL) and washed with brine (3 x 300 mL). The organic layer was dried over Na₂SO₄, concentrated in vacuo and purified by chromatography
over silica gel (hexanes to dichloromethane/hexanes, 1/5) to afford 256 (16.02 g, 91%) as a yellow oil. \([\alpha]^2_{D} = +64 (c = 1, \text{CHCl}_3)\). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.34 (dd, \(J = 6.0, 1.5\) Hz, 1H), 4.30 (ddd, 5.0, 4.5, 1.5 Hz, 1H), 4.18 (dd, \(J = 9.0, 6.5\) Hz, 1H), 4.09 (dd, 7.5, 6.0 Hz, 1H), 4.04-3.98 (m, 2H), 3.82 (t, \(J = 7.5\) Hz, 1H), 2.69-2.63 (ddd, \(J = 6.5, 9.5, 2.5\) Hz, 1H), 2.34-2.29 (ddd, \(J = 16.0, 4.5, 3.0\) Hz, 1H), 1.98 (t, \(J = 3.0\) Hz, 1H), 1.50 (s, 3H), 1.44 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 0.92 (s, 9H), 0.87 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H), 0.10 (s, 6H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 109.4, 107.7, 81.1, 80.1, 77.6, 77.2, 71.7, 71.0, 70.1, 67.8, 26.5, 26.4, 26.1, 25.8, 25.5, 25.2, 24.5, 18.2, -3.2, -3.3, -3.6, -3.8. ESI-HRMS: \(m/z\) calcd. for \(\text{C}_{27}\text{H}_{52}\text{O}_6\text{Si}_2\text{Na} (\text{M} + \text{Na})^+\) 551.3200, found 551.3197.

1-Bromo-4,7-di-O-[(\(t\)-butyl)dimethylsilyl]-1,2,3-trideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-galacto-1-yno-nonitol (257): To a solution of 256 (17.3 g, 32.7 mmol) in anhydrous acetone (320 mL) under an argon atmosphere was added N-bromosuccinimide (7.28 g, 40.9 mmol) and AgNO\(_3\) (1.39 g, 8.19 mmol). The mixture was stirred in the dark for 6 h and the resulting solids were filtered off and the Celite layer was washed with acetone (50 mL). The filtrate was concentrated to furnish a yellow oil which was diluted with EtOAc (300 mL) and washed with brine (2 x 150 mL), dried over Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo} to furnish a yellow oil which was purified by chromatography over silica gel (hexanes to 1/5 dichloromethane/hexanes) to afford 257 (15.9 g, 80%) as a yellow oil. \([\alpha]^{23}_{D} = +24 (c = 1, \text{CHCl}_3)\). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.32-4.27 (m, 2H), 4.15-4.11 (m, 2H), 4.06-3.97 (m, 2H), 3.80 (t, \(J = 8.0\) Hz, 1H), 2.67 (dd, \(J = 16.0, 10.0\) Hz, 1H), 2.32 (dd, \(J = 16.5, 4.5, 1.5\) Hz, 1H), 1.49 (s, 3H), 1.44 (s, 3H), 1.34 (s, 6H), 0.91 (s, 9H), 0.86 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H), 0.10 (s, 6H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 109.5, 107.8, 80.2, 77.7, 77.3, 76.2, 71.9, 70.9, 68.2, 39.6, 26.4,

**Methyl (2,4,5,7,8,9-hexa-\textit{O}-acetyl-3-deoxy-\textit{D}-\textit{glycero}-\textit{D}-\textit{galacto}-2-nonulopyran-ose)onate (150):** To a stirred solution of 257 (7.8 g, 12.8 mmol) in MeOH (200 mL) in a 2L flask, was added a mixture of MgSO$_4$ (3.08 g, 25.6 mmol), and NaHCO$_3$ (646 mg, 7.8 mmol) dissolved in water (200 mL). The turbid solution was then treated with MeOH (10 x 100 mL) with 5 min stirring after each addition to give a homogeneous solution. The mixture was cooled to 0 °C and was treated with solid KMnO$_4$ (4.56 g, 28.8 mmol). After 15 min further KMnO$_4$ (1.52 g (9.62 mmol) was added and the mixture was stirred at 0 °C for 3.5 h by which time the reaction was complete as indicated by TLC (1/10 EtOAc/hexanes). One half of the reaction mixture was then transferred to a separating funnel and was diluted with EtOAc (200 mL) and extracted with cold water (700 mL). After separation the aqueous layer was washed with EtOAc (5 x 200 mL). The combined EtOAc layer was washed with brine (3 x 200 mL) and was dried over Na$_2$SO$_4$. The work up process was repeated with the rest of the reaction mixture. The combined organic layer was concentrated \textit{in vacuo} to afford 6.4 g of the crude keto ester 258 as a yellow oil, which was employed directly in the next step. $^1$H NMR (500 MHz, CDCl$_3$) δ 4.66-4.64 (m, 1H), 4.23 (dd, $J$ = 8.5, 5.5 Hz, 1H), 4.10-4.04 (m, 2H), 4.01 (dd, $J$ = 6.0, 2.5 Hz, 1H), 3.96 (dd, $J$ = 9.0, 5.5 Hz, 1H), 3.85-3.82 (m, 4H), 3.29 (dd, $J$ = 17.5, 7.0 Hz, 1H), 3.09 (dd, $J$ = 17.5, 4.0 Hz, 1H), 1.47 (s, 3H), 1.44 (s, 3H), 1.31 (s, 3H), 1.26 (s 3H), 0.90 (s, 9H), 0.86 (s, 9H), 0.14 (s, 3H), 0.11-0.10 (m 9H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 191.7, 161.2, 109.3, 107.9, 80.0, 79.0, 76.7, 71.4, 68.8, 67.1, 52.9, 44.0, 26.4, 26.3, 26.0, 25.8, 25.2, 25.1, 18.3, 18.1, -3.5, -3.5, -3.8, -3.9. ESIHRMS: $m/z$ calcd. for C$_{28}$H$_{54}$O$_9$Si$_2$Na (M + Na)$^+$ 613.3204 found 613.3212.
A solution of the crude keto ester 258 (12.3g, 20.8 mmol) in anhydrous MeOH (420 mL) under an argon atmosphere was treated with conc. HCl (9.5 mL) at 0 °C and was warmed to room temperature over 30 min. The mixture was stirred for 48 h at room temperature and was then cooled to 0 °C and was carefully quenched with solid NaHCO₃. The mixture was then concentrated to dryness under reduced pressure at 10 °C and was taken up with pyridine (200 mL) under an argon atmosphere and at 0 °C before acetic anhydride (200 mL) was added. The mixture was warmed to room temperature over 1 h and was stirred overnight. The volatiles were removed in vacuo, and the residue was diluted with EtOAc (200 mL) and washed with 1N HCl (100 mL), sat. NaHCO₃ (100 mL), and brine (2 x100 mL). The organic layer was dried over Na₂SO₄ and was concentrated in vacuo. The crude product was purified by chromatography over silica gel (hexane to EtOAc /hexane 2/5) to afford 151 (6.79 g, 61%, α/β: 1/15) as a white foam. The NMR spectral values of 151 thus obtained are in agreement with the literature values.¹⁴⁸ β isomer: [α]²³ D = -23 (c = 1, CHCl₃); Lit¹⁴⁸ [α]²⁶ D = -24.3 (c = 0.31, CHCl₃), Mp 103-104 °C; Lit Mp¹⁴⁸ 104-105 °C. ESIHRMS: m/z calcd. for C₂₂H₃₀O₁₃Na (M + Na)⁺ 557.1482, found 577.1486.

**Methyl 3,6-di-O-[(t-butyl)dimethylsilyl]-2-deoxy-4,5:7,8-di-O-isopropylidene-D-glycero-D-galacto-octonoate (264):** A solution of 0.6 g (0.98 mmol) of alkynyl bromide 257 dissolved in methanol (10 mL) was cooled to -60 °C and was sparged with ozone for 45 min by which time the starting material was consumed (TLC: 1/10 EtOAc/hexanes). After sparging the reaction mixture with oxygen for 5 min, Hunig’s base (172 uL, 0.98 mmol) and dimethyl sulfide (729 uL, 9.9 mmol) were added sequentially and reaction mixture was warmed to room temperature over 4 h. After stirring the mixture for 1 day the volatiles were removed in vacuo and residue was
diluted with EtOAc (10 mL) and was washed with brine (10 mL), dried over Na₂SO₄ and concentrated *in vacuo* to afford a colorless oil which was purified by chromatography over silica gel (hexanes to ethyl acetate/hexanes, 1/20) to afford 258 (245 mg, 42%), identical in every way to the sample described above, and 264 (183 mg, 33%) as a colorless oil. [α]₂³_D = +8 (c = 1, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ 4.52-4.49 (m, 1H), 4.20 (dd, J = 8.0, 5.5 Hz, 1H), 4.14 (dd, J = 13.5, 6.0 Hz, 1H), 4.04-4.0 (m, 2H), 3.96 (dd, J = 8.0, 6.5 Hz, 1H), 3.87 (t, J = 8.0 Hz, 1H), 3.65 (s, 3H), 2.88 (dd, J = 16.0, 7.5 Hz, 1H), 2.45 (dd, J = 15.0, 5.5 Hz, 1H), 1.49 (s, 3H), 1.43 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H), 0.89 (s, 9H), 0.86 (s, 9H), 0.12 (s, 3H), 0.10 (s, 6H), 0.09 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 171.6, 109.1, 107.7, 79.8, 78.9, 71.4, 69.3, 66.6, 51.5, 39.5, 26.5, 26.3, 26.0, 25.9, 25.2, 25.2, 18.3, 18.2, -3.4, -3.5, -3.9, -4.2. ESIHRMS: m/z calcd. for C₄₇H₅₄O₈Si₂BrNa (M + Na)⁺ 585.3255, found 585.3248.

**General procedure (1) for nitrosation of sialosides:** A stirred solution of sialoside in anhydrous CH₂Cl₂ (0.1 M) under an argon atmosphere was treated with anhydrous pyridine (10 equiv) and was cooled to -10 °C. After stirring for 10 min, crushed nitrosyl tetrafluoroborate (4 equiv) was added in one portion to the mixture. The mixture was stirred at -10 °C for 3 hours and was diluted with CH₂Cl₂ and washed with cold 1N HCl, followed by a cold sat. NaHCO₃ wash. The organic layer was washed with cold brine, dried over Na₂SO₄ and concentrated below 10 °C to obtain the nitrosated sialoside.

**General procedure (2) for deaminations using acetic acid and thioacetic acid as nucleophiles:** A solution of the nitrosyl sialoside and trifluoroethanol (1.5 equiv) in anhydrous CH₂Cl₂ (0.1 M) at -10 °C was treated with freshly prepared 0.2 N sodium isopropoxide in
isopropanol (1.2 equiv). The resulting mixture was stirred for exactly 2 min before a solution of glacial acetic acid (20 equiv) or thioacetic acid (20 equiv) in CH₂Cl₂ (0.1 M) was added. After stirring for 5 min the reaction mixture was warmed to 0 °C and was carefully quenched with sat. NaHCO₃ solution. The extracted organic layer was washed with brine, dried over Na₂SO₄, concentrated and submitted to flash chromatography over silica gel to afford the deaminated sialosides.

**Methyl (1-adamantanyl 4,7,8,9-penta-O-acetyl-3,5-dideoxy-5-[anti/syn-N-nitrosoacetimido]-\(\alpha\)-glycero-\(\beta\)-galacto-2-onulopyranosid)onate (287):** The nitrosation was performed using the general procedure (1) with the thioglycoside 164 (0.8 g, 1.2 mmol), NOBF₄ (0.58 g, 5.0 mmol) and pyridine (1 mL, 12 mmol) to afford the title compound 287 as a yellow foam (835 gm, qant).

**Methyl (1-adamantanyl 4,5,7,8,9-penta-O-acetyl-3-deoxy-2-thio-\(\beta\)-glycero-\(\beta\)-galacto-2-nonulopyranosid)onate (151β):** The deamination of 287 was carried out using the general procedure (1) with acetic acid as the nucleophile. The nitrosyl sialic acid 287 (0.15 g, 0.22 mmol), was deaminated using the general procedure (2) with 2,2,2 trifluoroethanol (25 uL, 0.33 mmol), 0.2 N sodium isopropoxide in isopropanol (1.34 mL, 0.26 mmol ) and acetic acid (256 uL, 4.4 mmol) to afford 151β after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) as a white foam (84 mg, 58%) with spectral data consistent with those reported in the literature.¹³⁵
Methyl (1-adamantanyl 4,7,8,9-penta-O-acetyl-5-S-acetyl-3-deoxy-2-thio-β-D-glycero-β-D-galacto-2-nonulopyranosid)onate (292): The deamination of 287 was carried out using the general procedure (2) with thiaoctic acid as the nucleophile. The nitrosyl sialic acid 287 (0.143 g, 0.213 mmol), was deaminated using 2,2,2 trifluoroethanol (23 uL, 0.25 mmol), 0.2 N sodium isopropoxide in isopropanol (1.28 mL, 0.25 mmol) and thiaoctic acid (300 uL, 4.26 mmol) to afford 292 after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/3) as a yellow oil (88 mg, 63%). [α]$_D^{23}$ = +23 (c = 0.5, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$) δ 5.68 (t, J = 9.5 Hz, 1H H$_3$), 5.53-5.47 (m, 1H), 5.21 (td, J = 9, 2 Hz, 1H), 4.95 (dd, J = 12, 2 Hz, 1H), 4.88 (dd, J = 9.5, 3 Hz, 1H), 4.21 (dd, J = 12, 9 Hz, 1H), 3.83 (s, 3H), 2.56 (dd, J = 13.5, 4.5 Hz, 1H), 2.05 (s, 4 H), 2.02-2.00 (m, 7H), 1.91-1.86 (m, 7H), 1.66 (brs, 7H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 220.1, 170.8, 170.4, 169.8, 169.7, 169.7, 85.9, 75.0, 73.2, 71.0, 69.1, 68.9, 63.2, 52.8, 50.6, 43.3, 39.4, 35.8, 34.6, 29.7, 21.2, 21.0, 20.9, 20.6. ESIHRMS: m/z calcd. for C$_{30}$H$_{42}$O$_{12}$S$_2$Na (M + Na)$^+$ 681.2015 found 681.2023.

Methyl (1-adamantanyl 4,7,8,9-penta-O-acetyl-5-fluoro-3-deoxy-2-thio-β-D-glycero-β-D-galacto-2-nonulopyranosid)onate (293):

Preparation of 293 by deamination with general procedure (2)

The deamination of 287 was carried out using the general procedure (2) with HF:Pyridine as the nucleophile. The nitrosyl sialic acid 287 (0.15 g, 0.22 mmol), was deaminated using the general procedure with 2,2,2 trifluoroethanol (25 uL, 0.33 mmol), 0.2 N sodium isopropoxide in isopropanol (1.34 mL, 0.26 mmol) and HF:Pyridine (60 uL, 2.23 mmol) to afford 293 after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) as a yellow oil (71 mg, 53%).
$[\alpha]^{23}_{D} = +18 \ (c = 1, \ \text{CHCl}_3)$. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.62-5.59 (m, 1H), 5.53-5.45 (m, 1H), 5.29-5.27 (m, 1H), 4.75 (dd, $J = 12.5, 2$ Hz, 1H), 4.65 (m, 1H), 4.33-4.29 (dd, $J = 12.5, 7.5$ Hz, 1H), 4.30-4.18 (td, $J = 50, 9.5$Hz, 1H H$_5$), 3.81 (s, 3H), 2.68-2.63 (td, $J = 13, 4.5$Hz, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.01-1.99 (m, 6H), 1.86-1.81 (m, 4H), 1.67 (s, 6H). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.5, 170.4, 169.6, 169.6, 169.6, 88.3, 86.8, 85.5, 71.8, 70.3, 70.1, 69.7, 68.6, 68.4, 62.5, 52.8, 50.6, 43.2, 39.0, 36.0, 29.7, 21.0, 20.9, 20.7, 20.6. ESIHRMS: $m/z$ calcd. for C$_{28}$H$_{39}$O$_{11}$SFNa (M + Na)$^+$ 625.2095 found 625.2083.

Preparation of sodium 2,2,2 trifluoroethoxide: 3 g of sodium was added piecewise carefully to an ice cold 2,2,2 trifluoroethanol (300 ml) under an argon atmosphere at 0 °C. The mixture was warmed to room temperature over 1 hour and was stirred till the sodium was completely dissolved. The volatiles were removed under reduced pressure and the resultant solid was dried under high vacuum to afford 2,2,2 trifluoroethoxide (16.5 g, quant) as a free flowing white solid.

Preparation of 293 by deamination using 2,2,2 trifluoroethoxide/18-crown-6:

Methyl (1-adamantanyl 4,7,8,9-penta-O-acetyl-5-fluoro-3-deoxy-2-thio-$\beta$-glycero-D-galacto-2-nonulopyranosid)onate (293) A stirred solution of 18-crown-6 (128 mg, 0.486 mmol) in anhydrous CH$_2$Cl$_2$ (0.5 mL) under an argon atmosphere was treated with 2,2,2 trifluoroethoxide (54 mg, 0.44 mmol). The mixture was stirred for 10 minutes for the solids to completely dissolve and was then cooled to -10 °C. The nitrosyl sialoside 287 (0.15 g, 0.22 mmol) in anhydrous CH$_2$Cl$_2$ (2.2 mL) at -10 °C under an argon atmosphere was treated with the cold solution of 2,2,2 trifluoroethoxide/18-crown-6 solution. The mixture was allowed to stir for exactly 2 minutes for the reddish color to develop at which time HF·Pyridine (60 uL, 2.23 mmol)
was added to the reaction mixture via a plastic syringe. The mixture was stirred for 5 min, diluted with CH$_2$Cl$_2$ (5 mL) and was quenched by addition of sat. NaHCO$_3$ (5 mL). The organic layer was washed with brine (5 mL), dried and concentrated to afford a yellow oil that was purified by flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) to afford 293 (83 mg, 63%) as a yellow oil.

**Methyl (1-adamantanyl 4,7,8,9-penta-O-acetyl-5-prop-2-yn-1-yl-3-deoxy-2-thio-β-D-glycero-D-galacto-2-nonulopyranosid)onate (297):** A stirred solution of 18-crown-6 (173 mg, 0.597 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) under an argon atmosphere was treated with 2,2,2 trifluoroethoxide (73 mg, 0.597 mmol). The mixture was stirred for 10 minutes for the solids to completely dissolve and was then cooled to -10 °C. The nitrosyl sialoside 287 (0.2 g, 0.29 mmol) in anhydrous CH$_2$Cl$_2$ (3 mL) at -10 °C under an argon atmosphere was treated with the cold solution of 2,2,2 trifluoroethoxide/18-crown-6 solution. The mixture was allowed to stir for exactly 2 minutes for the reddish color to develop at which time propargyl alcohol (3.5 mL, 5.97 mmol in 6 mL of CH$_2$Cl$_2$) was added to the reaction mixture via a plastic syringe, followed immediately by addition of HBF$_4$ in Et$_2$O (162 uL, 0.12 mmol, 4 equiv). The mixture was stirred for 5 min, diluted with CH$_2$Cl$_2$ (5 mL) and was quenched by addition of sat. NaHCO$_3$ (5 mL). The organic layer was washed with brine (5 mL), dried and concentrated to afford a yellow oil that was purified by flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) to afford 297 (125 mg, 66%) as a yellow foam. $[\alpha]^{23}_D = +53$ (c = 1, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$) 5.61 (m, 1H), 5.36-5.27 (m, 2H), 4.81 (dd, $J = 12.5$, 2 Hz, 1H), 4.58 (dd, , $J = 9.5$, 3 Hz, 1H), 4.34 – 4.19 (m, 3H), 3.81 (s, 3H), 3.37 (t, , $J = 9.5$ Hz, 1H $\text{H}_3$), 2.61 (dd, , $J = 14$, 4.5 Hz, 1H), 2.44 (t, $J = 2.5$ Hz, 1H), 2.18 (s, 3H), 2.15 (s, 4H), 2.10(s, 6H), 2.08 (s, 3H), 1.86-1.81 (m, 6H),
1.68-1.66 (m 9H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.6, 170.4, 170.1, 169.9, 169.5, 85.4, 75.3, 75.2, 74.6, 72.4, 71.5, 71.4, 70.3, 62.8, 59.3, 52.6, 50.2, 43.1, 38.8, 36.0, 35.9, 21.2, 21.1, 20.9, 20.7.

**Synthesis of disaccharides:** Disaccharides 301, 303 and 305 were synthesized employing the following general procedure (3).

A mixture of the adamantyl thioglycoside donor (47β) and the acceptor (1.1 equiv) and with AW-300 molecular sieves (1 g/mmol) in a mixture of anhydrous CH$_2$Cl$_2$ and CH$_3$CN (2:1, 0.05 M) were stirred overnight at room temperature under an argon atmosphere. The mixture was cooled to -78 °C, and was treated with NIS (1.1 equiv) and triflic acid (0.05 equiv). The mixture was stirred for 1.5 h and was quenched by addition of triethylamine (0.2 equiv). The reaction mixture was filtered through a small plug of Celite and was washed with a 20% solution of sodium thiosulphate, brine, and was concentrated. The reaction mixture was purified by flash chromatography over silica gel to afford the coupled sialosides. To a stirred solution of the coupled sialoside in anhydrous methanol (0.05M) under an argon atmosphere was added a catalytic quantity sodium methoxide and the solution was stirred overnight. The reaction mixture was neutralized using Amberlyst 15 resin and was filtered and concentrated to afford the unprotected sialoside which was dissolved in pyridine and treated with acetic anhydride under an argon atmosphere at 0 °C and was stirred overnight. The volatiles were removed under reduced pressure and the residue was purified by flash chromatography over silica gel to afford the pure peracetylated sialosides.
Methyl (5-acetamido-4,7,8,9-tetra-\(O\)-acetyl-3,5-dideoxy-\(D\)-glycero-\(\alpha\)-\(D\)-galacto-non-2-ulopyranosylonate)-(2→6)-methyl 2,3,4-tri-\(O\)-benzyl-\(\beta\)-\(D\)-galactopyranoside (301): The compound 301 was prepared according to the general procedure (3) using donor 47\(\beta\) (0.13 g, 0.208 mmol) and acceptor 166 (115 mg, 0.249 mmol) in \(\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}\) (4 mL, 2:1) mL, with NIS (56 mg, 0.249 mmol) and TfOH (2 uL, 0.02 mmol). The purification by chromatography over silica gel (Hexanes/EtOAc 4/1 – 1/3) was performed to afford pure 300 (155 mg, 81%) as a white foam and as a single \(\alpha\)-anomer, the spectral data of which matched with the literature report for this compound.44 The sialoside 300 (155 mg, 1.68 mmol) was dissolved in anhydrous methanol (5 mL) and was treated with sodium methoxide (5 mg) and was stirred under an argon atmosphere overnight. The reaction mixture was neutralized with Amberlyst 15 resin and was filtered through a small plug of Celite, concentrated to afford a white solid that was dissolved in pyridine (4 mL). The mixture was cooled to 0 °C and was treated with acetic anhydride (4 mL) and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and was purified by chromatography over silica gel to afford the title compound 301 (121 mg, 77%) as a white foam the NMR of which is identical with that of 301 as reported in the literature.149

Methyl (5-acetamido-4,7,8,9-tetra-\(O\)-acetyl-3,5-dideoxy-\(D\)-glycero-\(\alpha\)-\(D\)-galacto-non-2-ulopyranosylonate)-(2→6)-methyl 2,3,4-tri-\(O\)-benzyl-\(\beta\)-\(D\)-glucopyranoside (303): The compound 302 was prepared according to the general procedure (3) using donor 47\(\beta\) (0.15 g, 0.24 mmol) and acceptor 165 (133 mg, 0.28 mmol) in \(\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}\) (4.8 mL, 2:1) mL, with NIS (64 mg, 0.28 mmol) and TfOH (2 uL, 0.02 mmol). The purification by chromatography over silica gel (Hexanes/EtOAc 4/1 – 1/3) was performed to afford pure 302 (185 mg, 84%) as a
white foam and as a single α-anomer, the spectral data of which matched with the literature report for this compound. The sialoside 302 (185 mg, 0.2 mmol) was dissolved in anhydrous methanol (10 mL) and was treated with sodium methoxide (5 mg) and was stirred under an argon atmosphere overnight. The reaction mixture was neutralized with Amberlyst 15 resin and was filtered through a small plug of Celite, concentrated to afford a white solid that was dissolved in pyridine (5 mL). The mixture was cooled to 0 °C and was treated with acetic anhydride (5 mL) and was stirred overnight. The reaction mixture was concentrated under reduced pressure and was purified by chromatography over silica gel to afford the title compound 303 (165 mg, 88%) as a white foam the NMR of which is identical with that of 303 as reported in the literature.

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl-(2→3)-methyl-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (305). The compound 304 was prepared according to the general procedure (3) using donor 47β (0.5 g, 0.8 mmol) and acceptor 177 (0.36 mg, 0.96 mmol) in CH₂Cl₂/CH₃CN (16 mL, 2:1) mL, with NIS (216 mg, 0.96 mmol) and TfOH (10 uL, 0.08 mmol). The purification by chromatography over silica gel (Hexanes/EtOAc 4/1 – 1/2) was performed to afford pure 304 (551 mg, 83%) as a white foam and as a 10:1 α/β mixture, the spectral data of which matched with the literature report for this compound. The sialoside 304 (520 mg, 0.62 mmol) was dissolved in anhydrous methanol (10 mL) and was treated with sodium methoxide (10 mg) and was stirred under argon overnight. The reaction mixture was neutralized with Amberlyst 15 resin and was filtered through a small plug of Celite, concentrated to afford a white solid that was dissolved in pyridine (15 mL). The anomeric mixture was cooled to 0 °C and was treated with acetic anhydride (15 mL) and was stirred overnight. The reaction mixture was concentrated under reduced pressure
and was purified by chromatography over silica gel to afford the pure α anomer 305 (422 mg, 76%) as a white foam. $[\alpha]_{D}^{23} = -18 \ (c = 0.8, \text{CHCl}_3)$. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.45 (d, $J = 7.5 \text{ Hz, 1H}$), 7.36 (t, $J = 7.5 \text{ Hz, 2H}$), 7.33-7.32 (m, 4H), 7.29-7.25 (m, 2H), 5.55-5.52 (m, 1H), 5.34 (dd, $J = 8, \text{ 3Hz, 1H}$), 5.12 (d, $J = 10.5, \text{ 1H}$), 5.04 (d, $J = 3.5 \text{ Hz, 1H}$), 4.93-4.88 (dt, $J = 12, \text{ 4.5 Hz, 1H}$), 4.82 (s, 2H), 4.55 (d, $J = 12, \text{ 5 Hz, 1H}$), 3.84 (s, 3H), 3.81 (s, 1H), 3.72 (dd, $J = 10.5, \text{ 2.5 Hz, 1H}$), 3.58 (s, 3H), 3.57-3.43 (m, 3H), 2.58 (dd, $J = 12.5, \text{ 5 Hz, 1H}$), 2.14 (s, 4H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.87 (s, 3H), 1.85 (s, 3H) $^{13}$C NMR (125 MHz, CDCl$_3$) 170.9, 170.6, 170.3, 170.2, 170.1, 170.0, 168.0, 139.6, 138.0, 128.0, 127.6, 127.6, 127.0, 104.5, 97.0, 78.3, 74.5, 73.4, 73.0, 72.2, 71.7, 69.4, 68.5, 68.6, 68.5, 67.0, 62.1, 57.4, 53.0, 49.2, 37.5, 23.2, 21.3, 20.8, 20.8, 20.7, 20.5. ESIHRMS: $m/z$ calcd. for C$_{43}$H$_{55}$NO$_{10}$Sn (M + Na)$^+$ 912.3266 found 912.3263.

**Synthesis of acceptor 306:**

**Phenyl-3,4,6-tetra-O-acetyl-1-thio-2-deoxy-2-phthalimido-β-d-galactopyranose (313):** A stirred solution of the compound 312 (1.4 g, 2.9 mmol) in anhydrous CH$_2$Cl$_2$ (25 mL) under a argon atmosphere was treated with thiophenol (1.21 mL, 11.7 mmol) and BF$_3$ etherate (1.45 mL, 11.7 mmol) and was stirred overnight. The reaction mixture was diluted with CH$_2$Cl$_2$ (25 mL), and was carefully quenched with sat. NaHCO$_3$ (50 mL). The organic layer was washed with brine (50 mL), dried and concentrated to give the crude reaction mixture which was purified by flash chromatography over silica gel (EtOAc/Hexanes 1/10 to 2/1) to afford the title compound 313 as a yellow oil (1.19 g, 77%). $[\alpha]_{D}^{23} = +41 \ (c = 1, \text{CHCl}_3)$. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.88-7.84 (m, 2H), 7.77-7.75 (m, 2H), 7.43-7.41(m, 2H), 7.27-7.26 (m, 3H), 5.81 (dd, $J = 11, \text{ 3.5}$
Hz, 1H), 5.70 (d, J = 10.5 Hz, 1H), 5.50 (d, J = 3.5 Hz, 1H), 4.63 (t, J = 10 Hz, 1H), 4.23 (dd, J = 11, 7.5 Hz, 1H), 2.17 (s, 3H), 2.04 (s, 3H), 1.83 (s, 3H) \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 170.4, 170.2, 169.7, 167.9, 167.2, 134.4, 134.3, 132.6, 131.7, 131.5, 131.2, 128.8, 128.1, 123.7, 123.6, 84.0, 77.2, 74.5, 68.8, 66.8, 61.6, 50.0, 20.7, 20.6, 20.5. ESIHRMS: \(m/z\) calc. for C\(_{26}\)H\(_{25}\)NO\(_3\)SNa (M + Na)\(^+\) 550.1148 found 550.1138.

**Phenyl-3,4-di-O-benzyl-6-O-tert-butyldimethylsilyl-1-thio-2-deoxy-2-phthalamido-\(\beta\)-d-galactopyranose (314):** A stirred solution of Compound 313 (1.1 g, 2.08 mmol) in anhydrous methanol (30 mL) was treated with a sodium methoxide (40 mg) and was stirred overnight. The reaction mixture was neutralized with Amberlyst 15 resin and was concentrated under reduced pressure to give a yellow solid that was dissolved in DMF (15 mL) under an argon atmosphere and was treated with triethylamine (0.44 mL, 3.1 mmol) and TBSOTf (530 uL, 2.29 mmol). After 6 h, the reaction mixture was diluted with EtOAc (20 mL) and was washed with brine (3 x 10 mL). The organic layer was dried and concentrated to afford the silylated thioglycoside as a yellow oil. The yellow oil was dissolved in DMF (10 mL), cooled to 0 °C under argon atmosphere and was treated with benzyl bromide (0.75 mL, 6.2 mmol). The mixture was then treated with sodium hydride (184 mg, 4.59 mmol) portion wise over 1 h. After 3 h, the reaction mixture was quenched with crushed ice and was diluted with EtOAc (20 mL) and was washed with brine (2 x 20 mL). The organic layer was dried and concentrated to afford a yellow oil, purification of which by flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/3) afforded 314 (883 mg, 61% over 3 steps) as a yellow oil. \([\alpha]^{23}_{D=+30}\) (c = 1, CHCl\(_3\)). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.81-7.60 (m, 4H), 7.40-7.31 (m, 7H), 7.23-7.18 (m, 3H), 6.98-6.89 (m, 5H), 5.56 (d, J = 11 Hz, 1H), 4.66 (t, J = 10.5, 1H), 4.59 (d, J = 13.5, 2H), 4.53 (d, J = 10.5, 1H),
4.28 (d, J = 2Hz, 1H), 4.19-4.15 (m, 2H), 3.82 (t, J = 10.5, 1H.), 3.75 (t, J = 9 Hz, 1H), 3.70-3.68 (m, 1H), 0.92 (s, 9H), 0.08 (s, 3H), 0.05 (s, 3H) NMR (125 MHz, CDCl3) δ 168.4, 167.2, 137.9, 133.8, 133.6, 132.5, 131.9, 131.7, 128.6, 128.4, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 123.1, 83.0, 78.1, 77.9, 73.4, 72.1, 68.8, 67.5, 50.6, 26.0, 18.5, 4.15, 4.98. ESIHRMS: m/z calcd. for C40H45NO6SSiNa (M + Na)+ 718.2635 found 718.2628.

**Phenyl-3,4-di-O-Benzyl-1-thio-2-deoxy-2-phthalimido-β-D-galactopyranose (306):** A stirred solution of compound 314 (0.41 g, 0.58 mmol) in anhydrous methanol (5 mL) under argon atmosphere was treated with pTSA (25 mg). The mixture was stirred overnight and was neutralized with triethylamine. The reaction mixture was concentrated under reduced pressure and was purified by flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) to afford 306 (297 mg, 87%) as a yellow oil. [α]D23 = +53 (c = 1, CHCl3). 1H NMR (500 MHz, CDCl3) δ 7.86-7.67 (m, 4H), 7.35 (s, 7H), 7.20-7.18 (m, 3H), 7.09-6.99 (m, 5H), 5.56 (dd, J = 10.5, 1Hz, 1H), 5.02 (d, J = 11.5, 1Hz, 1H), 4.84 (t, J = 10.5 Hz, 1H), 4.63 (d, J = 12.5, 2 Hz, 1H), 4.36 (m, 2H), 3.98 (d, J = 1Hz, 1H), 3.86 (dd, J = 11.5, 7 Hz, 1H), 3.64 (t, J = 11.5 Hz, 1H), 3.58 (brs, 1H) NMR (125 MHz, CDCl3) δ 171.3, 171.1, 170.9, 170.5, 170.4, 170.2, 170.1, 169.9, 169.6, 169.4, 168.3, 129.0, 128.2, 125.2, 97.8, 85.7, 74.4, 72.5, 72.0, 69.1, 69.0, 68.8, 67.9, 67.3, 63.6, 62.3, 52.9, 52.4, 50.6, 49.7, 49.4, 43.4, 39.8, 37.8, 35.9, 29.8, 23.2, 23.1, 21.0, 21.0, 20.8, 20.8, 20.7, 20.7, 20.6. ESIHRMS: m/z calcd. for C34H31NO6SSNa (M + Na)+ 604.1770 found 604.1782

**Methyl (5-acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate-α(2→6) Pheny1-3,4-di-O-Benzyl-1-thio-2-deoxy-2-phthalimido-β-D-galactopyranoside (307):** The known sialyl phosphate donor 56 (120 mg,
0.18 mmol) the acceptor 306 (114 mg, 0.197 mmol) and AW300 molecular sieves (400 mg) were stirred in a mixture of a mixture of CH$_2$Cl$_2$/CH$_3$CN (2:1, 4 mL) under argon atmosphere for 2 h. The mixture was cooled to -78 °C and was treated with TMSOTf (32 µL, 0.179 mmol) and after 1 h the reaction mixture was diluted with CH$_2$Cl$_2$ (5 mL) and was quenched with sat. NaHCO$_3$ (10 mL). The mixture was warmed to room temperature, filtered through a plug of Celite, and the organic layer was separated and washed with brine (10 mL), dried and concentrated to afford the crude disaccharide which was purified by flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) to afford 307 (163 mg, 86%), a yellow foam, as a single α-anomer. 

[α]$^{[23]}_D = -18$ (c = 0.6, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.84 (d, $J = 6.5$ Hz, 1H), 7.70-7.65 (m 3H), 7.42-7.40 (m, 2H), 7.37 (d, $J = 7$ Hz, 2H), 7.33 (t, $J = 7.5$ Hz, 2H), 7.27 (m 2H), 7.18-7.14 (m, 3H), 7.05-6.96 (m, 4H), 5.58 (dd, $J = 7.5$, 2 Hz, 1H), 5.54 (d, $J = 11$ Hz, 1H), 5.43 (dt, $J = 7$, 2.8 Hz, 1H), 4.98 (d, $J = 11.5$ Hz, 1H), 4.81 (t, $J = 10.5$ Hz, 1H), 4.68 (d, $J = 11.5$ Hz, 1H), 4.60-4.58 (m, 2H), 4.37 (d, $J = 3$ Hz, 1H), 4.35 (s, 2H), 4.12-3.98 (m, 4H), 3.81 (t, $J = 6.5$ Hz, 1H), 3.72 (dd, $J = 11$, 9.5 Hz, 1H), 3.67 (s, 3H), 3.60 (dd, $J = 9.5$, 6.5 Hz, 1H), 2.86 (dd, $J = 12$, 3.5 Hz, 1H), 2.49 (s, 3H), 2.13 (s, 4H), 2.08 (s, 3H), 2.00 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 172.0, 170.7, 170.1, 170.0, 168.3, 167.3 ($^3$J$_{C-H} = 6.5$ Hz), 150.7, 138.6, 137.4, 133.9, 133.9, 133.0, 131.8, 131.6, 128.6, 120.1, 127.6, 127.5, 127.5, 127.3, 123.5, 123.1, 98.9, 83.8, 77.4, 77.2, 76.9, 75.7, 74.9, 74.1, 71.8, 71.4, 69.3, 63.8, 62.7, 59.0, 53.1, 36.4, 24.7, 21.0, 20.8, 20.7. ESIHRMS: m/z calcd. for C$_{53}$H$_{54}$N$_2$O$_{18}$SNa (M + Na)$^+$ 1061.2290 found 1061.2301.

**Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate-α(2→6) Pheny-3,4-di-O-benzyl-1-thio-2-deoxy-2-phtalimido-β-D-galactopyranoside (308):** A stirred solution of the sialoside 307 (163 mg, 0.182 mmol) in
anhydrous methanol (10 mL) under argon atmosphere was treated with sodium methoxide (10 mg) and was stirred at room temperature overnight. The mixture was neutralized with Amberlyst 15 resin and was filtered through a plug of Celite and concentrated to dryness to afford a white solid. The crude solid was dissolved in pyridine (10 mL) and was treated with acetic anhydride (10 mL) at 0 °C and was stirred overnight under an argon atmosphere. The reaction mixture was concentrated to dryness and was purified by flash chromatography over silica gel to afford the title compound 308 (151 mg, 91%) as a white foam. [α]$_{D}^{23}$ = +12 (c = 0.5, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.84 (d, J = 6.5 Hz, 1H), 7.71-7.60 (m, 3H), 7.44-7.40 (m, 2H), 7.37 (d, J = 7 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.27-7.23 (m, 2H), 7.17 (t, J = 3.5 Hz, 3H), 7.05 - 6.96 (m, 5H), 5.54 (d, J = 10 Hz, 1H), 5.42-5.39 (m, 1H), 5.33 (dd, J = 8, 1.5 Hz, 1H), 5.27 (d, J = 9.5 Hz, 1H), 4.95 (d, J = 11.5 Hz, 1H), 4.95-4.85 (m, 1H), 4.79 (t, J = 10.5, 1H), 4.66 (d, J = 11.5 Hz, 1H), 4.58 (d, J = 12 Hz, 1H), 4.37-4.28 (m, 3H), 4.13 (dd, J = 12.5, 5.5, 1h), 4.10-4.05 (m,3H), 4.00 (dd, J = 9.5, 6.5 Hz, 1H), 3.83 (t, J = 7Hz, 1H), 3.65 (s, 3H), 3.59 (dd, J = 9.5, 7.5 Hz, 1H), 2.60 ( dd, J = 12.5, 5 Hz, 1H), 2.13 (s,3H), 2.11 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.97 (t, J = 12.5 Hz, 1H), 1.88 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 170.9, 170.7, 170.3, 170.1, 169.8, 168.3, 168.0, 167.4, 138.7, 137.5, 133.7, 133.0, 131.9, 131.7, 129.0, 128.6, 128.2, 128.1, 128.1, 128.0, 127.6, 127.5, 127.3, 127.3, 125.2, 123.5, 123.1, 98.6, 83.9, 76.6, 74.2, 72.6, 71.7, 71.3, 69.0, 68.7, 67.3, 62.8, 62.2, 52.8, 51.5, 50.8, 49.4, 37.8, 23.2, 21.0, 20.8, 20.8, 20.7. ESIHRMS: m/z calcd. for C$_{54}$H$_{58}$N$_2$O$_{18}$SNa (M + Na)$^+$ 1017.3303 found 1017.3313.

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate-α(2→9) methyl (1-adamantanyl-5-acetamido-4,7,8-tri-O-acetyl-3,5-dideoxy-2-thio-β-D-glycero-α-D-galacto-non-2-ulopyranosylonate (311): A solution of the
known sialyl phosphate donor 56 (136 mg, 0.203 mmol), the acceptor 309 (147 mg, 0.264 mmol) and AW300 molecular sieves (600 mg) were stirred in a mixture of a mixture of CH₂Cl₂/CH₃CN (2:1, 4.5 mL) under an argon atmosphere for 2 h. The mixture was cooled to -78 °C and was treated with TMSOTf (38 uL, 0.214 mmol) and after 1 h was diluted with CH₂Cl₂ (5 mL) and was quenched with sat. NaHCO₃ (5 mL). The mixture was warmed to room temperature, filtered through a small plug of Celite and the organic layer was separated and washed with brine, dried and concentrated to afford crude 310. The crude reaction mixture was dissolved in anhydrous methanol (5 mL) under an argon atmosphere and was treated with sodium methoxide (5 mg) and was stirred overnight. The mixture was neutralized with Amberlyst 15 resin, filtered and concentrated to dryness to obtain a white foam. The unprotected disialoside was dissolved in pyridine (5 mL) and was treated with acetic anhydride (5 mL) at 0 °C, and was stirred overnight under an argon atmosphere. The volatiles were removed under reduced pressure and the residue was purified by flash chromatography over silica gel to afford the desired disialoside 311 (161 mg, 74%) as a white foam. [α]ᵢ₃D = +28 (c = 1, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ: 5.45-5.42 (m, 3H), 5.29-5.19 (m, 3H), 5.09 (d, J = 8 Hz, 1H), 4.84-4.79 (m, 1H), 4.50 (d, J = 10.5, 1H), 4.25 (d, J = 11, 2H), 4.10-3.97 (m, 5H), 3.90 (s, 3H), 3.85-3.78 (m, 4H), 2.50 (dd, J = 13.5, 5 Hz, 1H), 2.42 (dd, J = 12.5, 4.5, 1H), 2.21 (s, 3H), 2.17 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02-1.98 (m, 13H), 1.87 (m, 8H), 1.65 (s, 6H). ¹³C NMR (125 MHz, CDCl₃) δ: 171.4, 171.1, 170.7, 170.5, 170.4, 170.3, 169.9, 169.6, 169.4, 168.3 (JC,H = 6.5 Hz), 97.8, 85.7, 74.4, 72.5, 72.0, 69.1, 69.0, 68.8, 67.9, 67.3, 63.6, 62.3, 39.8, 37.8, 35.9, 29.7, 23.1, 21.0, 21.0, 20.8, 20.8, 20.7, 20.7, 20.6. ESIHRMS: m/z calcd. for C₄₈H₆₈O₂₃N₂SNa (M + Na)⁺ 1072.3934 found 1072.3940.
Deamination of disaccharides:

Methyl (4,5,7,8,9-penta-O-acetyl-3-deoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]- (2→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (179): The disaccharide 301 (100 mg, 0.106 mmol) was nitrosated using the general procedure (1) using NOBF₄ (50 mg, 0.426 mmol), pyridine (85 uL, 1.06 mmol) to obtain 317 (102 mg, quant) as a yellow foam. The deamination of 317 was carried out using acetic acid as the nucleophile using the general procedure (2) using 2,2,2 trifluoroethanol (12 uL, 0.158 mmol), 0.2 N sodium isopropoxide in isopropanol (0.64 mL, 0.129 mmol) and acetic acid (125 uL, 2.15 mmol) to afford after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) 179 (51 mg, 51%) as a colorless oil the NMR of which is identical with that of 179 as reported in this thesis.

Methyl (4,5,7,8,9-penta-O-acetyl-3-deoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]- (2→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (180): The disaccharide 303 (126 mg, 0.134 mmol) was nitrosated using the general procedure (1) using NOBF₄ (63 mg, 0.537 mmol), pyridine (108 uL, 1.34 mmol) to obtain 318 (129 mg, quant) as a yellow foam. The deamination of 318 was carried out using acetic acid as the nucleophile using the general procedure (2) using 2,2,2 trifluoroethanol (15 uL, 0.203 mmol), 0.2 N sodium isopropoxide in isopropanol (0.8 mL, 0.160 mmol) and acetic acid (152 uL, 2.67 mmol) to afford after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) 180 (60 mg, 48%) as a colorless oil the NMR of which is identical with that of 180 as reported in this thesis.

Methyl (4,5,7,8,9-penta-O-acetyl-3-deoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]- (2→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (320): The
disaccharide 305 (100 mg, 0.112 mmol) was nitrosated using the general procedure (1) using NOBF₄ (52 mg, 0.45 mmol), pyridine (184 uL, 2.24 mmol) to obtain 319 (103 mg, quant) as a yellow foam. The deamination of 319 was carried out using acetic acid as the nucleophile using the general procedure (2) using 2,2,2 trifluoroethanol (12 uL, 0.168 mmol), 0.2 N sodium isopropoxide in isopropanol (0.67 mL, 0.134 mmol) and acetic acid (127 uL, 2.24 mmol) to afford after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) 320 (54 mg, 54%) as a colorless oil, [α]²³ D = + 4 (c = 0.5, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ 7.44 (d, J = 7 Hz, 2H), 7.37-7.32 (m, 6H), 7.30-7.14 (m, 2H), 5.58-5.55 (m, 1H), 5.36 (dd, J = 9, 2.5 Hz, 1H), 5.05 (d, J = 3 Hz, 1H), 4.98-4.95 (m, 1H), 4.93 (t, J = 9.5 Hz, 1H H₃), 4.84-4.78 (m, 2H), 4.55 (d, J = 12 Hz, 1H), 4.50-4.46 (m, 3H), 4.28 (dd, J = 13, 2.5 Hz, 1H), 4.03 (dd, J = 13, 4.5 Hz, 1H), 3.86 (s, 3H), 3.85-3.75 (m, 2H), 3.59 (s, 3H), 3.56-3.52 (m, 1H), 3.48-3.43 (m, 2H), 2.66 (dd, J = 13, 4.5 Hz, 1H), 2.15 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.80 (s, 4H) ¹³C NMR (125 MHz, CDCl₃) δ 178.6, 170.2, 170.1, 170.0, 169.8, 167.8, 139.6, 138.0, 128.3, 128.3, 128.0, 127.6, 127.6, 127.0, 127.0, 104.5, 96.9, 78.3, 74.5, 73.4, 73.2, 71.7, 70.9, 69.7, 68.8, 68.5, 67.9, 67.7, 66.3, 61.9, 57.3, 53.1, 37.0, 21.3, 20.8, 20.8, 20.7, 20.7, 20.3. ESI-HRMS: m/z calcd. for C₄₃H₅₄O₂₀Na (M + Na)⁺ 913.3106 found 913.3101.

Methyl [methyl (4,7,8,9-tetra-O-acetyl-5-S-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-2-→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (321): The deamination of 319 (91 mg, 0.099 mmol) was carried out using thioacetic acid as the nucleophile using the general procedure (2) using 2,2,2 trifluoroethanol (11 uL, 0.148 mmol), 0.2 N sodium isopropoxide in isopropanol (0.59 mL, 0.118 mmol) and acetic acid (0.15 mL, 1.98 mmol) to afford after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/3) to afford 321 (60
mg, 67%) as a colorless oil. $[\alpha]^{23}_{D} = -18$ (c = 0.4, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.47 (d, $J = 7.5$ Hz, 2H), 7.39 (t, $J = 7.5$ Hz, 2H), 7.32 (d, $J = 4.5$, 4H), 7.29-7.25 (m, 2 H), 5.56 (s, 2H), 5.04 (d, $J = 3$ Hz, 1H), 4.94-4.88 (dt, $J = 12$, 4.5 Hz, 1H), 4.84 (s, 2H), 4.54 (d, $J = 11.5$ Hz, 1H), 4.49-4.45 (m, 3H), 4.30 (d, $J = 13$Hz, 1H), 3.97 (d, $J = 13$Hz, 1H), 3.87-3.82 (m, 5H), 3.64 (t, $J = 12$ Hz, 1H $H_3$), 3.59 (s, 3H), 3.55-3.51 (m, 1H), 3.49-3.43 (m, 2H), 2.67 (dd, $J = 12.5$, 4.5 Hz, 1H), 2.30 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.77 (s, 3H), 1.74 (t, $J = 12$ Hz, 1H). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 193.0, 170.7, 170.2, 170.1, 169.7, 169.7, 167.9, 139.7, 138.0, 128.3, 127.6, 127.6, 127.1, 127.0, 104.6, 96.8, 78.3, 77.2, 74.4, 73.4, 72.9, 72.0, 71.7, 69.2, 68.8, 68.5, 68.3, 67.9, 62.0, 57.3, 53.0, 42.7, 38.4, 30.7, 21.3, 20.8, 20.7, 20.7, 20.4.

ESIHRMS: $m/z$ calcd. for C$_{43}$H$_{51}$O$_{19}$SNa (M + Na)$^+$ 929.2878 found 929.2870.

**Methyl [methyl (4,7,8,9-tetra-O-acetyl-5-fluoro-3,5-dideoxy-α-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-(2→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside:** A stirred solution of 18-crown-6 (57 mg, 0.217 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) under an argon atmosphere was treated with 2,2,2 trifluoroethoxide (26 mg, 0.44 mmol). The mixture was stirred for 10 minutes for the solids to completely dissolve and was then cooled to -10 °C. The nitrosyl sialoside 319 (0.1 g, 0.108 mmol) in anhydrous CH$_2$Cl$_2$ (2.2 mL) at -10 °C under an argon atmosphere was treated with the cold solution of 2,2,2 trifluoroethoxide/18-crown-6 solution. The mixture was allowed to stir for exactly 2 minutes for the reddish color to develop at which time HF,NEt$_3$ (52 uL, 0.326 mmol) was added to the reaction mixture via a plastic syringe. The mixture was stirred for 5 min, diluted with CH$_2$Cl$_2$ (3 mL) and was quenched by addition of sat. NaHCO$_3$ (3 mL). The organic layer was washed with brine (4 mL), dried and concentrated to afford a yellow oil that was purified by flash chromatography over silica gel (Acetone/Hexanes
1/20 to 1/4) to afford 322 (57 mg, 63%) as a yellow oil. \([\alpha]_{D}^{23} = +11 (c = 0.5, \text{CHCl}_3)\). \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)) \(\delta\) 7.41 (d, \(J = 7.5\) Hz, 2H), 7.33-7.25 (m, 8H), 5.51 (s, 2H), 5.12-5.00 (m, 2H), 4.82 (d, \(J = 12.5\) Hz, 1H), 4.77 (J = 12.5 Hz, 1H), 4.54 (d, \(J = 11.5\) Hz, 1H), 4.46 (dd, \(J = 12.5, 8\) Hz, 1H), 4.39 (dd, \(J = 10, 3.5\) Hz, 1H), 4.34 (dd, \(J = 12.5, 1.5\) Hz, 1H), 4.29-4.15 (td, \(J = 49.0, 9.5\) Hz, 1H \(H_5\)), 4.10 (dd, \(J = 12.5, 3.5\) Hz, 1H), 3.87 (s, 3H), 3.83-3.74 (m, 2H), 3.59 (s, 3H), 3.55-3.42 (m, 3H), 2.72-2.68 (dd, \(J = 12.5, 4\) Hz, 1H), 2.14 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.84 (s, 3H), 1.71 (t, \(J = 12.5\) Hz, 1H). \(^{13}\text{C NMR}\) (125 MHz, CDCl\(_3\)) \(\delta\) 170.7, 170.1, 170.0, 169.6, 169.3, 167.6, 139.5, 137.9, 128.3, 128.3, 128.0, 127.9, 127.7, 127.6, 127.2, 127.1, 127.1, 104.6, 97.0, 87.2, 85.7, 78.0, 77.2, 74.4, 73.4, 73.3, 71.8, 70.6, 70.4, 69.2, 69.0, 68.8, 68.5, 68.0, 66.9, 61.8, 57.3, 53.2, 36.5, 36.4, 21.2, 20.8, 20.8, 20.7, 20.3. ESIHRMS: \(m/z\) calcd. for \(C_{41}H_{51}FO_{18}Na\) (M + Na)\(^+\) 873.2957 found 873.2963.

**Methyl [Phenyl \(4,5,7,8,9\)-penta-\(O\)-acetyl-3-deoxy-\(\alpha\)-\(\beta\)-galacto-non-2-ulopyranosyl]onate\)-(2→6)-3,4-di-\(O\)-benzyl-1-thio-2-deoxy-2-phthalimido-\(\beta\)-\(\alpha\)-galactopyranose (324):** The disaccharide 308 (160 mg, 0.147 mmol) was nitrosated using the general procedure (1) using NOBF\(_4\) (68 mg, 0.588 mmol), pyridine (120 \(\mu\)L, 1.47 mmol) to obtain 323 (168 mg, quant) as a yellow foam. The deamination of 323 was carried out using acetic acid as the nucleophile using the general procedure (2) using 2,2,2 trifluoroethanol (16 \(\mu\)L, 0.227 mmol), 0.2 N sodium isopropoxide in isopropanol (0.92 mL, 0.82 mmol) and acetic acid (173 \(\mu\)L, 2.15 mmol) to afford after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) 324 (79 mg, 49%) as a colorless oil. \([\alpha]_{D}^{23} = +21 (c = 0.6, \text{CHCl}_3)\). \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)) \(\delta\): 7.84 (d, \(J = 6.5\) Hz, 1H), 7.71-7.60 (m, 3H), 7.44-7.42 (m, 2H), 7.37 (d, \(J = 7\) Hz, 2H), 7.32 (t, \(J = 7.5\) Hz, 2H), 7.27-7.23 (m, 2H), 7.19-7.17 (m, 3H), 7.05 -6.96 (m, 5H), 5.53 (d,
\( J = 11 \text{ Hz, 1H}, 5.48-5.42 \text{ (m, 1H)}, 5.35 \text{ (dd, } J = 9, 2 \text{ Hz, 1H)}, 4.96 \text{ (d, } J = 8 \text{ Hz, 1H)}, 4.94-4.91 \text{ (m, 1H)}, 4.88 \text{ (t, } J = 9.5 \text{ Hz, 1H} H_2), 4.80 \text{ (t, } J = 10.5 \text{ Hz, 1H)}, 4.66 \text{ (d, } J = 11 \text{ Hz, 1H)}, 4.58 \text{ (d, } J = 12.5 \text{, 1H)}, 4.35 \text{ (dd, } J = 11, 3 \text{ Hz, 1H)}, 4.29 \text{ (dd, } J = 13, 2.5 \text{ Hz, 1H)}, 4.18 \text{ (d, } J = 4.5 \text{ Hz, 1H)}, 4.15 \text{ (dd, } J = 9.5, 2.5 \text{ Hz, 1H)}, 4.05 \text{ (d, } J = 2 \text{ Hz, 1H)}, 4.00 \text{ (dd, } J = 9.5, 6 \text{ Hz, 1H)}, 3.81 \text{ (t, } J = 7 \text{ Hz, 1H)}, 3.67 \text{ (s, 3H)}, 3.58 \text{ (dd, } J = 9, 7 \text{ Hz, 1H)}, 2.69 \text{ (dd, } J = 13.5, 4.5 \text{ Hz, 1H)}, 2.16 \text{ (s, 3H)}, 2.07 \text{ (s, 3H)}, 2.03 \text{ (s, 3H)}, 2.02 \text{ (s, 3H)}, 1.94 \text{ (t, } J = 12.5 \text{ Hz, 1H}). ^{13} \text{C NMR (125 MHz, CDCl}_3 \delta 170.7, 170.1, 169.9, 169.8, 169.7, 168.3, 167.4, 138.6, 137.5, 133.9, 133.8, 133.7, 133.0, 131.9, 131.7, 129.0, 128.6, 128.2, 128.1, 128.1, 127.6, 127.5, 127.5, 127.3, 127.3, 125.2, 123.5, 123.1, 98.4, 83.9, 77.3, 76.9, 74.2, 71.7, 71.3, 71.3, 69.3, 67.9, 67.8, 66.4, 62.9, 62.0, 52.9, 51.5, 37.3, 21.4, 21.0, 20.8, 20.7, 20.7, 20.6, 20.6 \text{ (ESIHRMS: } m/z \text{ calcd. for C}_{55}H_{77}NO_{19}SNa, (M + Na)^{+} 1078.3134 \text{ found 1078.3126).}

Methyl (4,5,7,8,9-penta-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-non-2-ulopyranosyl-\( \alpha \rightarrow 9 \) methyl (1-adamantanyl-4,5,7,8,-tetra-O-acetyl-3,5-dideoxy-2-thio-\( \beta \)-D-glycero-a-D-galacto-non-2-ulopyranosyl)ate (326); The disialoside 311 (150 mg, 0.139 mmol) was nitrosated using the general procedure (1) using NOBF\(_4\) (163 mg, 1.39 mmol), pyridine (450 \( \mu \)L, 5.59 mmol) in anhydrous CH\(_2\)Cl\(_2\) (20 mL) to obtain 323 (163 mg, quant) as a yellow foam. The deamination of 311 was carried out using acetic acid as the nucleophile using the general procedure (2) using 2,2,2 trifluoroethanol (25 \( \mu \)L, 0.346 mmol), 0.2 N sodium isopropoxide in isopropanol (1.58 mL, 0.317 mmol) and a mixture of tetrabutylammonium acetate (0.865 gm, 2.78 mmol) acetic acid (330 \( \mu \)L, 5.76 mmol) to afford after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) to afford 324 (79 mg, 49%) as a colorless oil. \([\alpha]^{23}_D = -21 \text{ (c = 1, CHCl}_3\). ^1\text{H NMR (500 MHz, CDCl}_3 \delta 5.44-5.40 \text{ (m, 2H),
5.37-5.31 (m, 2H), 5.16 (dd, J = 5, 2 Hz, 1H), 4.91-4.86 (m, 1H), 4.83 (t, J = 10 Hz, 1H H₃), 4.79 (t, 9.5 Hz, 1H H₅), 4.67 (dd, J = 10, 2.5 Hz, 1H), 4.25 -4.18 (m, 3H), 4.12 (dd, J = 12.5, 4.5 Hz, 1H), 3.90 (s, 3H), 3.83 (dd, J = 7, 4 Hz, 1H), 3.80 (s, 3H), 2.59 (dd, J = 14, 5 Hz, 1H), 2.53 (dd, J = 12.45 Hz, 1H), 2.18 (s, 3H), 2.17 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 6H), 2.00 (s, 9H), 1.98 (s, 3H), 1.92 (t, J = 14 Hz, 1H), 1.86-1.83 (m, 4H), 1.66 (s, 6H) ¹³C NMR (125 MHz, CDCl₃) δ 170.6, 170.5, 170.3, 170.2, 169.9, 169.9, 169.6, 169.5, 169.4, 167.8, 97.8, 85.6, 77.2, 73.4, 71.0, 70.7, 69.2, 69.1, 68.4, 68.4, 67.9, 67.3, 66.6, 63.6, 62.1, 59.2, 52.5, 50.7, 43.3, 39.4, 37.3, 35.9, 29.8, 21.0, 21.0, 20.8, 20.7, 20.7, 20.6, 20.5, 20.5. ESIHRMS: m/z calcd. for C₄₈H₆₆O₂₅SNa (M + Na)⁺ 1097.3512 found 1097.3521.

**Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-D-galacto-non-2-ulpopyranosylate)-(2→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside-(2→4)-benzyl-2,3,6-tri-O-benzyl-β-D-glucopyranoside (328):** A stirred solution of the known GM3 sialoside 327¹⁵⁰ (110 mg, 0.08 mmol) in anhydrous pyridine (4 mL) under an argon atmosphere was treated with acetic anhydride (4 mL) at 0 °C and the mixture was stirred overnight at room temperature. The volatiles were removed under reduced pressure and the residue was purified by flash chromatography over silica gel to afford 328 (113 mg, quant) as a white foam. [α]²³D = +32 (c = 1, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ 7.42-7.17 (m, 30 H), 5.63-5.60 (m, 1H), 5.36-5.33 (m 1H), 5.11 (d, J = 10.5 Hz, 1H), 5.06 (d, J = 3 Hz, 1H), 5.06-4.89 (m, 6H), 4.79-4.63 (m, 6H), 4.55-4.49 (m, 2H), 4.46-4.35 (m, 3H), 4.30 (dd, J = 12.5, 2.5 Hz, 1H), 4.20 (d, J = 11.5 Hz, 1H), 4.13-4.06 (m, 1H), 4.02-3.96 (m, 2H), 3.84 (s, 3H), 3.79-3.62 (m, 5H), 3.57-3.52 (m 1H), 3.48-3.44 (m, 2H), 3.63-3.26 (m 3H), 2.61 (dd, 12.5, 4.5, 1H), 2.10 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.86 (s, 3H), 1.77 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) 170.8, 170.5, 170.3,
Methyl (4,5,7,8,9-penta-O-acetyl-3,5-deoxy-D-glycero-α-D-galacto-non-2-ulopyranosylionate)-(2→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside-(2→4)-benzyl-2,3,6-tri-O-benzyl-β-D-glucopyranoside (330): The GM3 trisaccharide 328 (113 mg, 0.08 mmol) was nitrosated using the general procedure (1) using NOBF₄ (37 mg, 0.32 mmol), pyridine (65 μL, 0.8 mmol) to obtain 329 (115 mg, quant) as a yellow foam. The deamination of 329 was carried out using acetic acid as the nucleophile using the general procedure (2) using 2,2,2 trifluoroethanol (9 μL, 0.121 mmol), 0.2 N sodium isopropoxide in isopropanol (0.49 mL, 0.096 mmol) and acetic acid (92 μL, 1.61 mmol) to afford after flash chromatography over silica gel (EtOAc/Hexanes 1/20 to 1/1) 179 (62 mg, 55%) as a colorless oil. [α]²³ = +20 (c = 1, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ 5.60 (m, 1H), 5.39-5.31 (m 2H), 5.04 (d, J = 2.5 Hz, 1H), 4.99-4.83 (m, 6H), 4.77-4.60 (m, 6H), 4.53-4.46 (m 3H), 4.44-4.32 (m, 3H), 4.22-4.12 (m, 3H), 4.01 (dd, J = 11, 4 Hz, 1H), 3.95 (t, J = 8 Hz, 1H), 3.83 (s, 3H), 3.81-3.72 (m, 2H), 3.69-3.59 (m 3H), 3.54-3.41 (m, 4H), 3.33-3.25 (m, 3H), 2.64 (dd, 10.5, 4 Hz, 1H), 2.08 (s, 3H), 1.99-1.97 (m, 12H), 1.80 (t, J = 10 Hz, 1H), 1.78 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) 170.5, 170.0, 169.9, 169.8, 169.7, 169.5, 139.4, 139.2, 138.6, 138.5, 138.1, 137.5, 129.0, 128.4, 128.3, 128.2 (4), 128.1, 128.0, 127.9 (2), 127.8, 127.7, 127.6 (2), 127.5 (2), 127.4 (2), 127.3, 127.2 (2), 127.1 (2), 127.0, 102.3, 102.0, 97.1, 82.8, 81.8, 79.4, 76.4, 75.0, 74.9, 74.7, 73.9, 73.1, 72.7,
Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-\(\alpha\)-D-galacto-non-2-ulopyranosyl)\(\rightarrow\)4-\(\rightarrow\)1,2,3,4,6-tetra-O-acetyl-\(\beta\)-D-glucopyranosy\(\rightarrow\)1,3-\(\rightarrow\)2,6-di-O-benzyl-\(\beta\)-D-galactopyranoside (2\(\rightarrow\)3)-\(\rightarrow\)1,2,3,6-tri-O-benzyl-\(\beta\)-D-glucopyranoside (332):

A solution of the donor 331 (90 mg, 0.22 mmol), 4 Å molecular sieves (600 mg) in anhydrous CH\(_2\)Cl\(_2\) (2 mL) was stirred for 4 h under an argon atmosphere. The mixture was cooled to -20 °C and was treated with NIS (61 mg, 0.27 mmol), and TfOH (25 uL, 0.27 mmol). The mixture was stirred for 10 min and the GM3 acceptor 327 (156 mg, 0.11 mmol) in anhydrous CH\(_2\)Cl\(_2\) (0.75 mL x 2) was added to the reaction mixture via a syringe over 10 min. The mixture was stirred at -20 °C for 2 h and was quenched by addition of triethylamine (200 uL). The reaction mixture was diluted with CH\(_2\)Cl\(_2\) (5 mL) and was filtered through a pad of Celite, and was washed with 20% sodium thiosulphate (4 mL), and brine (5 mL), dried and concentrated to afford a yellow oil that was purified by flash chromatography over silica gel to afford (1/5 EtOAc/Hexanes – 4/1 EtOAC/Hexanes) to afford 332 (141 mg, 73%) as a colorless oil. \([\alpha]^{23}_{D} = +37\ (c = 0.5, \text{CHCl}_3)\).

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.60 (d, \(J = 7.5\) Hz, 2H), 7.42-7.20 (m, 28 H), 5.28-5.25 (m, 3H), 5.19-5.09 (m, 3H), 5.01 (t, \(J = 9\) Hz, 1H), 4.95-4.90 (m, 3H), 4.85-4.77 (m, 4H), 4.66 (s, 1H), 4.64 (s, 1H), 4.53-4.47 (m, 4H), 4.39-4.29 (m, 3H), 4.16-3.95 (m, 7H), 3.90 (m, 1H), 3.87 (s, 3H), 3.84-3.78 (m, 3H), 3.73 (d, \(J = 2.5\) Hz, 2H), 3.57 (t, \(J = 9\) Hz, 1H), 3.49-3.39 (m, 4H), 2.41 (dd, \(J = 13.5, 5, 1\)H), 2.16 (s, 4H), 2.12 (s, 3H), 2.04 (s, 3H), 2.00 (s, 6H), 1.90 (s, 6H), 1.92 (s, 3H), 1.90 (s, 3H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) 170.7, 170.7m 170.4, 170.3, 170.3, 169.6, 169.5, 169.4, 168.8, 167.8, 139.0, 138.8, 138.6, 138.4, 138.3, 137.6, 129.4, 128.3, 128.2, 128.1,
128.1, 128.1, 120.2, 120.2, 127.9, 127.8, 127.6, 127.5, 127.5, 127.4, 127.3, 127.3, 127.3, 102.5, 102.2, 101.5, 99.6, 82.7, 81.7, 78.7, 77.7, 76.0, 75.2, 75.1, 73.2, 73.2, 73.1, 72.6, 72.2, 71.8, 70.9, 70.7, 69.0, 68.7, 68.2, 68.0, 67.8, 66.7, 61.7, 61.6, 53.2, 49.1, 35.6, 23.2, 21.1. ESIHRMS: m/z calcd. for C_{38}H_{103}NO_{32}Na (M + Na)^+ 1708.6361 found 1708.6348.

Methyl (4,5,7,8,9-penta-\(\text{O}\)-acetyl-3,5-dideoxy-\(\alpha\)-\(\beta\)-galacto-non-2-ulopyranosylate)-(2→3)-[2,3,4,6-tetra-\(\text{O}\)-acetyl-\(\beta\)-\(\text{D}\)-glucopyranosyl-(1→4)]-2,6-di-\(\text{O}\)-benzyl-\(\beta\)-\(\text{D}\)-galactopyranoside-(2→4)-benzyl-2,3,6-tri-\(\text{O}\)-benzyl-\(\beta\)-\(\text{D}\)-glucopyranoside (334):

The tetrasaccharide 332 (140 mg, 0.083 mmol) was nitrosated using the general procedure (1) using NOBF\(_4\) (38 mg, 0.032 mmol) and pyridine (70 uL, 0.83 mmol) to afford the nitrosyl tetrasaccharide 333 (142 mg, quant) as a yellow foam. The tetrasaccharide 333 was deaminated using the general procedure (2) with acetic acid as the nucleophile with 2,2,2 trifluoroethanol (10 uL, 0.12 mmol), 0.2 N sodium isopropoxide in isopropanol (0.5 ml, 0.99 mmol), and acetic acid (95 uL, 1.65 mmol) to afford after purification by normal phase HPLC (Hexanes/EtOAc 80:20 to 30:70) the tetrasaccharide 333 as a colorless oil (79 mg, 58%). [\(\alpha\)]\(_D^{23}\) = + 17 (c = 0.6, CHCl\(_3\)).

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.60 (d, \(J = 7\) Hz, 2H), 7.42-7.20 (m, 6H), 7.34-7.20 (m, 22H), 5.32-5.22 (m, 3H), 5.17 (t, \(J = 10\) Hz, 1H) 5.09 (d, \(J = 10\) Hz, 1H), 5.01 (t, \(J = 10\) Hz, 1H), 4.96-4.90 (m, 3H), 4.85-4.81 (m, 3H), 4.80-4.73 (m, 2H), 4.69-4.64 (m, 2 H), 4.51-4.47 (m, 3H), 4.40-4.37 (m, 3H), 4.16 (dd, \(J = 12.5, 2\) Hz, 1H), 4.12-4.08 (m, 2H), 4.03 (t, \(J = 10\) Hz, 1H), 3.99-3.94 (m, 2H), 3.92-3.89 (m, 3 H), 3.86-3.78 (m, 3H), 3.74 (d, \(J = 1.5\) Hz, 2H), 3.58 (t, \(J = 8.5\) Hz, 1H), 3.50-3.41 (m, 6H), 2.50 (dd, \(J = 13.5, 5\) Hz, 1H), 2.18 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) 170.6, 170.4, 170.3, 169.9, 169.7, 169.5, 169.5, 169.4, 168.8, 167.5, 139.0, 138.7, 138.6, 138.4,
138.1, 137.6, 129.4, 128.3, 128.2, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7,
127.6, 127.5, 127.4, 127.3, 127.3, 102.5, 102.1, 101.5, 99.6, 82.7, 81.7, 78.6, 77.3, 75.2,
75.2, 75.1, 73.2, 73.2, 73.1, 72.6, 71.8, 71.1, 70.9, 70.8, 69.3, 68.7, 68.2, 68.0, 67.4, 67.3, 66.0,
61.6, 61.4, 53.3, 21.1, 20.8, 20.7, 20.6, 20.6, 20.6, 20.4. ESIHRMS: m/z calcd. for C_{88}H_{102}O_{33}Na
(M + Na)\(^+\) 1709.6201 found 1709.6218.
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ABSTRACT

SYNTHESIS AND α-STEROSELECTIVE SIALYLATION OF KDN AND LATE STAGE MODIFICATIONS OF NEURAMINIC ACID SIALOSIDES

by

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Major: Chemistry

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Keto deoxy nonulosonic acid has gained prominence in the recent years after the discovery of its presence in humans and the consequent appreciation of its role in human development and disease, and because of its potential as a marker of disease states. The development of efficient α-KDN sialylation methodology, an investigation of the mechanistic aspects of such sialylations, and the synthesis of this sparsely occurring sialic acid are the focus of this thesis.

In the challenging area of α-sialylation, an efficient methodology was developed for the KDN series by means of a 4,5-O-carbonate group installed on the pyranose ring. The 4,5-O-carbonate moiety proved to be an excellent α-directing group in sialylation for a wide array of acceptors independent of the anomic configuration of the donor, and provided high yields by inhibiting the glycal formation. The donor was further found to be compatible with popular sulphoxide-based activation systems. The lower yields and selectivities of sialylations conducted using the analogous carbonate-free peracetyl KDN donor highlight the beneficial effect of the 4,5-O-carbonate group.
The origins of the excellent α-directing ability of the acetyl oxazolidinone, oxazolidinone and carbonate cyclic protections in neuraminic acid and KDN were investigated by mass spectrometry using in-source fragmentation of the corresponding sialyl phosphates. The trends of the onset cone voltages for fragmentation of the sialyl phosphates clearly indicate a strong electron withdrawing effect of the 4,5 \textit{trans} cyclic protecting group when compared to their peracetyl counterparts. This effect correlates with the dipole moments of the individual protecting groups. The enhanced dipole moment of the cyclic protecting group in the mean plane of the pyranose ring destabilizes any oxocarbenium ion thus necessitating a higher cone voltage to initiate mass spectral fragmentation. By analogy the cyclic protecting group retards oxocarbenium ion formation in the course of glycosylation reactions and so promotes associative pathways with consequent high α-selectivity.

An efficient 5 step synthesis of KDN and a 6 step synthesis of peracetyl KDN were developed, which could be used to access gram quantities of this sparsely occurring sialic acid. A highly \textit{syn}-stereoselective lactol reduction to gain rapid entry to the protected KDN core is central to this efficient strategy. An ozonolytic unmasking of a propargyl group provides a facile entry into the requisite ketoester functionality in this synthesis.

C-5 modified sialic acid containing oligosaccharides, used in glycan arrays and known to be important probes to investigate the binding affinities of various sialosides, were synthesized by late stage modifications of α-sialic acid oligosaccharides prepared using the \textit{N}-acetyl 5\textit{N},4\textit{O}-oxazolidinone technology. This oxidative deamination methodology was successful in late stage conversion of the C-5 acetamido group stereoselectively to the corresponding acetoxy, acetyltthio, fluoro and propargyl alcohol functionalities of potential biologically importance. The
system was applied to the conversion of a neuraminic acid containing branched tetrasaccharide to a KDN containing tetrasaccharide as well as to a number of other systems.
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Publications


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