An investigation into the synthesis of 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonic acid analogues

Matthew Stephen Zunk

B. Pharm. Sci. (Hons)

Institute for Glycomics
Griffith Sciences
Griffith University

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Statement of Originality

To the best of my knowledge, the content of this thesis has not been previously submitted by me or any other person(s) for a degree or diploma at Griffith University or any other institution. Any information obtained from published or unpublished work of others has been acknowledged in the text. This thesis is less than 100,000 words in length excluding tables, appendices and references.

___________________
Matthew Zunk
May 2014
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Publications, Communications and Awards

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Communications:


Awards:

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<tr>
<td>$^1$H NMR</td>
<td>Proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>Carbon-13 nuclear magnetic resonance</td>
</tr>
<tr>
<td>Aq.</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>BnBr</td>
<td>Benzylbromide (bromotoluene)</td>
</tr>
<tr>
<td>Bu$_2$SnO</td>
<td>Dibutyltin oxide</td>
</tr>
<tr>
<td>Bz</td>
<td>Benzoyl</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>Deuterated Chloroform</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>Chloroform</td>
</tr>
<tr>
<td>CMP</td>
<td>Cytidine 5’ monophosphate</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlated two-dimensional Spectroscopy</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet of doublets</td>
</tr>
<tr>
<td>$N,N$-DMF</td>
<td>$N,N$-dimethylformamide</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>Deuterium oxide</td>
</tr>
<tr>
<td>Eq.</td>
<td>Equivalents</td>
</tr>
<tr>
<td>Et$_2$O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>EtN\textsuperscript{2}\textsubscript{Pr} \textsuperscript{2}</td>
<td>\textit{N},\textit{N}-diisopropylethane</td>
</tr>
<tr>
<td>H\textsubscript{2}</td>
<td>Hydrogen gas</td>
</tr>
<tr>
<td>H\textsubscript{2}SO\textsubscript{4}</td>
<td>Sulphuric Acid</td>
</tr>
<tr>
<td>HCl</td>
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</tr>
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<td>Hex</td>
<td>Hexane</td>
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<td>Hz</td>
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</tr>
<tr>
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<td>kiloDalton</td>
</tr>
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<td>K\textsubscript{i}</td>
<td>Dissociation constant of inhibitor</td>
</tr>
<tr>
<td>K\textsubscript{m}</td>
<td>Michaelis-Menten constant</td>
</tr>
<tr>
<td>LA</td>
<td>Lewis Acid</td>
</tr>
<tr>
<td>Lit</td>
<td>Literature</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>M</td>
<td>Molar(s)</td>
</tr>
<tr>
<td>MeOH and/or Me</td>
<td>Methanol</td>
</tr>
<tr>
<td>mg</td>
<td>milligrams</td>
</tr>
<tr>
<td>\textmu L</td>
<td>Microliters</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>mmol</td>
<td>millimol(s)</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium metal</td>
</tr>
<tr>
<td>Na\textsubscript{2}SO\textsubscript{4}</td>
<td>Sodium sulfate</td>
</tr>
<tr>
<td>N\textsubscript{3}</td>
<td>Azide</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NaHCO\textsubscript{3}</td>
<td>Sodium hydrogen carbonate</td>
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</table>
NaCl  Sodium chloride (Salt)
NaN₃  Sodium Azide
OMs  Mesylate group
OTs  Tosylate group
OTf  Triflate group
Ph  Phenyl
Pd on C  Activated palladium on charcoal
Pd(OH)₂  Palladium hydroxide on Carbon
ppm  Parts per million
psi  Pounds per square inch
Rf  Retention factor
s  Singlet
Sat.  Saturated
TBAF  tetra-butylammonium fluoride
OTBDMS  tert-butyldimethylsilyl ether
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TLC  Thin Layer Chromatography
UV  Ultra violet
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Abstract

This thesis describes the results of a research program aimed at investigating synthetic methodology towards obtaining novel 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonic acid analogues. Syntheses outlined in this thesis comprise varied approaches which include a chemoenzymatic approach and two chemical approaches from differing starting materials.

Pseudaminic acid and legionaminic acid are 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonic acids that play an essential role in the pathogenic capabilities of numerous clinically relevant Gram-negative bacteria. Over the past twenty-five years several structural and functional group derivatives of these carbohydrates have been isolated from many bacterial species, highlighting the fact that these compounds are widespread throughout nature. However, to date their exact biological significance is yet to be clearly determined and the limited syntheses described within the literature are for the parent compounds only, not structural analogues. Therefore, the primary aim of this research program was to fill a void in the literature in regard to creating a versatile and efficient synthesis of pseudaminic acid and legionaminic acid analogues.

Chapter one provides a comprehensive literature overview of the biological role, biosynthesis, chemical synthesis reported to date and key structural differences of 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonic acids, in reference to sialic acids which have been well recognised for near five decades as being important in a wide variety of disease states. Chapter one also highlights the importance of the role 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonic acids have in the life cycle of pathogenic Gram-negative bacteria and describes all naturally occurring derivatives known to date and where they are found within nature.

Chapter two outlines in detail our chemoenzymatic approach toward the synthesis of novel legionaminic acid analogues. The chapter begins with a detailed discussion on the type 1 aldolase, Neu5Ac aldolase enzyme which we used to catalyse all of our enzymatic reactions toward legionaminic acid analogues. Following on from this discussion, chapter two outlines the complete synthesis starting from D-mannose of two novel C-4 nitrogen functionalised Neu5Ac aldolase substrates along with the synthesis of D-rhamnose and D-talose, which were also successfully transformed into their corresponding nonulosonic acid derivatives via a Neu5Ac aldolase catalysed aldol
condensation reaction. The chapter closes with the results of our chemoenzymatic approach toward the synthesis of legionaminic acid analogues, detailing the chemical yields and relative rate of turn over for our two novel Neu5Ac aldolase substrates and that of three known substrates D-mannose, D-rhamnose and D-talose.

Following on from our chemoenzymatic approach, chapter three outlines our attempts at utilising a chemical aldol condensation reaction, with both synthesised and known substrates, in order to obtain pseudaminic acid-based analogues. Chapter three begins with a discussion of reported uses of chemical aldol condensation reactions toward the synthesis nonulosonic acids. This discussion is followed by a detailed account of our attempts to synthesise selectively functionalised 6-deoxy-galactosides and N-acetyl-glucosamine derivatives as substrates to be condensed with oxaloacetic acid in order to create pseudaminic acid analogues. Chapter three finishes with a detailed discussion of the results of our attempted synthetic aldol condensation reactions.

Having investigated the synthesis of both pseudaminic acid and legionaminic acid analogues via two different aldol condensation reaction methods, we were interested in investigating the synthesis of pseudaminic acid analogues from a more advanced precursor as a starting material. Chapter four therefore outlines our successful synthesis of pseudaminic acid analogues from the nine-carbon sialic acid derivative KDN. Chapter four details our novel 10 step synthesis of 8-epi-pseudaminic acid along with structurally important precursors that form branching points within our synthesis for future work that will allow for other structurally distinct naturally occurring pseudaminic acid based analogues to be obtained.

The final chapter, chapter five brings together all experimental details in support of the compounds made and conclusions drawn throughout chapter’s two to four.

NMR spectroscopy was used extensively throughout this research program to unequivocally determine compound structures and aid in assignments of all compounds reported, therefore Appendix 1 contains a selection of ¹H, ¹³C and two-dimensional (¹H-¹H-COSY) spectra used in this regard.
CHAPTER 1

INTRODUCTION TO NONULOSONIC ACIDS

1.1 The natural occurrence and structural diversity of nonulosonic acids

The term nonulosonic acid refers to a structural class of nine carbon carbohydrate monomers that are C-1 oxidised or α-ketose aldonic acids. The most abundant naturally occurring nonulosonic acids are the sialic acids or neuraminic acids and their derivatives. The sialic acid family is widely distributed throughout nature with over fifty naturally occurring derivatives having been identified within nearly all domains of cellular organisms. To date, sialic acids have been isolated from numerous mammalian tissues including embryonic neural and non-neural tissues and also human tumours. Sialic acids have also been isolated from various non-mammalian tissues including fish eggs, insects and are increasingly being discovered within pathogenic bacteria.

The term sialic acid was first put forth by Blix et al. (1957) to describe all acetylated, unsubstituted neuraminic acids like neuraminic acid (Neu5Ac, Figure 1) in order to avoid confusion regarding various names that were being used at the time to describe chemically identical compounds. Over the past fifty years this nomenclature has been extended to account for naturally occurring non-acetylated compounds that clearly contain sialic acid regio- and stereochemical conformations.

![Figure 1](image.png)

Figure 1. Neu5Ac (1) structural features (six-membered heterocyclic ring (red), glycerol side chain (blue) and hemiketal (green)).
Sialic acids are characterised as nine-carbon $\alpha$-keto sugars (Figure 1) and are distinguished by the 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid skeleton$^{3,6}$. Generally all of the unsubstituted naturally occurring sialic acids are made up of a six membered heterocyclic ring (containing oxygen, highlighted in red, Figure 1), which are more commonly found in a $\text{C}_5$ conformation having a glycerol side chain extending from carbon-6 (highlighted in blue, Figure 1), which contains carbons seven through to nine$^3$. All sialic acids contain a carboxylic acid group at carbon-1 which exists in its deprotonated or basic form at physiological pH (Neu5Ac (1) $pK_a = 2.6$) therefore existing as a negative charge$^3$. Carbon-2 of sialic acids forms part of a hemiketal (shown in green, Figure 1) and the numbering of these compounds begins with the carbon of the carboxylic acid and ends at carbon-9 the last carbon of the glycerol side chain$^{2,3}$.

Of all the naturally occurring sialic acids isolated to date there are three major members recognised which all other sialic acids are considered derivatives of. They are 5-acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid which is more commonly referred to as $N$-acetylneuraminic acid or Neu5Ac (1)$^6$, $N$-glycolyneuraminic acid (Neu5Gc, 2) and 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN, 3) (Figure 2)$^{2,3}$. These three carbohydrates contain the regio- and stereochemical detail upon which all other nonulosonic acids are generally based, which is the $D$-glycero-$D$-galacto configuration. Structurally the difference between Neu5Ac (1) and Neu5Gc (2) is at the C-5 position where 1 has a $N$-acetamido group [NHAc] and 2 has a $N$-glycolyl group [NHC(O)CH$_2$OH] present instead. KDN (3) differs from 1 and 2 in that it has a hydroxyl group [OH] at C-5 instead (Figure 2).

![Figure 2](image.png)

Figure 2. Image shows the carbon-5 substituent differences of the sialic acids Neu5Ac (1), Neu5Gc (2) and KDN (3).
*N*-Acetylneuraminic acid (Neu5Ac, 1), is the most commonly found sialic acid within nature and was first isolated in the 1930’s. Neu5Ac (1) has been identified in most mammalian tissues including human tissues, bodily fluids and secretions, where it occurs as a component of cell-surface glycans. Neu5Ac (1) has also been detected in numerous mammalian tumours such as colon, gastric and liver cancers and indeed overexpression of terminal sialylation mechanisms are understood to be associated with cancer malignancies.

Another common naturally occurring sialic acid is *N*-glycolyneuraminic acid (Neu5Gc, 2, Figure 2), which has been isolated from numerous animal tissues and cells, including swine, bovine, sheep, goat, rabbit and rat tissues and has also been found in human tissues, although it is worth noting that in humans Neu5Gc (2) is not an endogenous compound, rather we acquire it from our dietary intake. Neu5Gc (2) has also been identified in mammalian tumours and has been isolated from the poly(oligo)sialyl chains of a variety of salmonoid fish species such as the rainbow trout and is recognised as the major sialic acid found in invertebrates.

In 1986 the defining characteristics of sialic acids had to be broadened with the discovery of a non-*N*-acetamido sialic acid 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN, 3, Figure 2), isolated from glycans found within rainbow trout egg polysialoglycoproteins (PSGPs). Polysialoglycoproteins are a class of glycoproteins which were first discovered in 1978 by Inoue and Iwasaki. PSGPs are characterised by their high sialic acid content (>60% w/w) and by the presence of oligo- or polysialylglycan chains (8-18 residues in length), composed of oligosialyl groups with an average chain length containing six residues. In PSGPs, KDN residues are found to cap the non-reducing termini of the oligo/polysialyl chains. KDN (3) was the only nonulosonic acid component isolated from the vitelline envelope in the trout eggs and has been found to be present in all of the polysialoglycoprotein of salmonoid fish eggs examined. Since the discovery of KDN (3) a number of KDN-glycoconjugates have been discovered in various organisms from fish eggs to bacteria.

KDN (3) has also been isolated as an *O*-linked glycan component of glycoproteins from the egg jelly of two different species of amphibian. KDN-ganglioside GM3 was isolated from rainbow trout sperm in 1991, which could possibly indicate the widespread distribution of this class of ganglioside in nature. Gangliosides are often
bacterial targets, being made up of a membrane bound sphingolipid with a carbohydrate moiety attached\textsuperscript{18}. Gangliosides vary in structure depending on the species and cell type in which they are found\textsuperscript{18}.

The first report of KDN residues within bacteria was published in 1989\textsuperscript{17,18} being isolated from the capsular polysaccharide layer of the Gram-negative bacterium \textit{Klebsiella ozaenae} K4, a bacterial species that occurs in cases of ozena and other chronic diseases of the respiratory tract\textsuperscript{17,18}. Since this report KDN (3) has been isolated from numerous other bacterial sources including \textit{Sinorhizobium fredii}, the cell-wall teichulosonic acids of the actinomycetes \textit{Streptomyces scabei}, \textit{Streptomyces setonii-griseus}, \textit{Streptomyces sp. VKM Ac-2090}, \textit{Streptomyces macrosporus}, \textit{Arthrobacter protophormiae VKM Ac-2101}, \textit{Arthrobacter spp. VKM Ac-2549} and VKM Ac-2550, \textit{Streptomyces sp. VKM Ac-2274}, and \textit{Brevibacterium casei}\textsuperscript{17,19}.

Interestingly, all sialic acids are always found within nature as $\alpha$-glycosidically-linked carbohydrates residues of glycoproteins or glycolipids. They have been isolated as poly-$\alpha$-2,8-linked homopolymers\textsuperscript{11}, however are more commonly keto-linked to either D-galactose via the C-3 or C-6 positions (Figure 3) or N-acetyl-D-galactosamine at the C-6 position\textsuperscript{3}. Sialic acids are rarely found as free acids within mammalian tissues however small amounts have been isolated from trout eggs and in the digestive glands of squid and lobster\textsuperscript{2,12,20}.

![Sialic Acid Structures](image)

\textbf{Figure 3.} An example of a segment of $\alpha$-Neup(2→3)-$\beta$-D-Galp-(1→4)-$\beta$-D-Glc$p$NAc(1→ polysaccharide from \textit{Streptococcus agalactiae}.\textsuperscript{17}
1.2 The natural occurrence and structural diversity of 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonates

Since the discovery of the sialic acids Neu5Ac (1), Neu5Gc (2) and KDN (3) and the emergence of our understanding of their pivotal role in mammalian and bacterial lifecycles, numerous sialic acid analogues have been identified \(^3,17\). Of these newly discovered nonulosonic acid analogues one important sub-class of carbohydrate has been characterised which to date are only found within bacterial species. However, this important new class of nonulosonates are proving to be widespread within numerous clinically important pathogenic bacterium which utilise these carbohydrates in order to become virulent \(^17\).

This newer class of compounds are chemically characterised as 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonate derivatives of which two important parent compounds are known, pseudaminic acid (4) and the legionaminic acid (5) (Figure 4) \(^17\).

![Figure 4. Structures of the bacterial nonulosonic acids pseudaminic acid (4) and legionaminic acid (5).](image)

1.2.1 Pseudaminic acids

In 1984, Knirel and colleagues tentatively characterised a new type of sialic acid-like sugar, which was extracted and purified from the LPS of *Pseudomonas aeruginosa* strains O7 and O9 and *Shigella boydii* Type 7 \(^21\). This new compound was correctly identified at the time as 5,7-diacetamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-non-2-ulosonic acid, which Knirel generically named pseudaminic acid (4) \(^21\).

The discovery of pseudaminic acid (4) was significant, in that it showed not all nonulosonic acid type carbohydrates were direct regio- and stereochemical derivatives
of Neu5Ac (1), which up until this point was thought to be the case\textsuperscript{21}. In nature, the pseudaminic acids can exist as either α or β anomers, whereas the sialic acids are only found as the α-linked anomers\textsuperscript{17,22}. Structurally both pseudaminic acid (4) and neuraminic acid (1) are nine-carbon α-keto acidic sugars (nonulosonic acids), however a number of key stereochemical and functional group differences exist which sets the two classes of compound apart.

Firstly, all pseudaminic acids are 9-deoxy-nonulosonic acids, as C-9 is a methyl group (Figure 5). Secondly, pseudaminic acid (4) has different stereochemistry at C-5, C-7 and C-8 compared with sialic acid, as well as having the extra acetamidofunctionality at C-7 (Figure 5). When comparing the two sugars, it is the stereochemistry at C-8 that defines the nomenclature used to describe the glycerol-fragment, and the stereochemistry of C-7 that determines a D- or L-assignment of the hexose-fragment. Due to the fact the stereochemistry at C-7 and C-8 in 1 compared with 4 is opposite, the overall configuration of pseudaminic acid (4) is designated as an L-glycerol-L-manno configuration compared to Neu5Ac (1) which is designated as D-glycerol-D-galacto (Figure 5).

![Figure 5](image_url)

**Figure 5.** Image showing the stereochemical and substituent differences between the naturally occurring pseudaminic acid (4) and N-acetylneuraminic acid (1)

Since 1984 there have been numerous naturally occurring pseudaminic acid derivatives discovered which further support Knirel’s comment that the pseudaminic acids are indeed a new class of nonulosonic acid\textsuperscript{21}. These derivatives of pseudaminic acid have been identified within and isolated from many Gram-negative bacterial species, as shown in Table 1.
Table 1. Published accounts of isolation and characterisation of naturally occurring pseudaminic acid derivatives (table adapted from Zunk & Kiefel, 2014\(^{23}\))

<table>
<thead>
<tr>
<th>R’</th>
<th>R”</th>
<th>Bacterial Source</th>
<th>Year Published</th>
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<td>NH(O)CH(CHOH)CH₂</td>
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<td><em>Pseudomonas aeruginosa</em> O5, a,b,c,d</td>
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<td>NH(O)H</td>
<td><em>Pseudomonas aeruginosa</em> O5, a,b,c,d</td>
<td>1984(^{14})</td>
</tr>
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<td>NH(O)CH(CHOH)CH₂</td>
<td><em>Pseudomonas aeruginosa</em> O10a</td>
<td>1984(^{14})</td>
</tr>
<tr>
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<td>NH(O)CH(CHOH)CH₂</td>
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<td>1984(^{14}), 1985(^{15}), 1987(^{15})</td>
</tr>
<tr>
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<td>NH(O)H</td>
<td><em>Pseudomonas aeruginosa</em> O7a, 7b, 7d and O7a, 7d (immunotype 6)</td>
<td>1986(^{15}), 1987(^{15})</td>
</tr>
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<td>NH(O)CH(CHOH)CH₂</td>
<td><em>Pseudomonas aeruginosa</em> O9a, 9b</td>
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<td>NH(O)H</td>
<td><em>Pseudomonas aeruginosa</em> O7a, 7b, 7c</td>
<td>1987(^{15})</td>
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<td>NH(O)CH₃</td>
<td>NH(O)CH₃</td>
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<td>NH(O)CH₃</td>
<td>NH(O)CH₃</td>
<td><em>Campylobacter jejuni</em> 11168</td>
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<td>NH(O)CH₃</td>
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<td><em>Campylobacter jejuni</em> 11168</td>
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<td>NH(O)CH₃</td>
<td><em>Piscirickettsia salmonis</em></td>
<td>2013(^{27})</td>
</tr>
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</table>
Within these bacterial species pseudaminic acid (4) and its derivatives are found as components of important cell surface glycans (Figure 6), such as LPS O-antigens\textsuperscript{22}, capsular polysaccharides\textsuperscript{45}, pili\textsuperscript{10} and flagellin\textsuperscript{35,46} all of which are essential for bacterial virulence\textsuperscript{47,48}.

\textbf{Figure 6.} An example of a teichulosonic acid fragment containing C-4 linked and C-7 nitrogen linked pseudaminic acid residue from \textit{Kribbella spp}.$^{17}$
1.2.2 Legionaminic acids

Legionaminic acid (5) (5,7-diacetamido-3,5,7,9-tetradeoxy-D-glycero-D-galacto-non-2-ulosonic acid) was first discovered in 1994 as an α-(2,4)-linked homopolymer component of the *Legionella pneumophila* serotype 1 O-chain LPS. *L. pneumophila*, the causative agent of Legionnaires’ disease, invades and replicates within the alveolar macrophages of the lung leading to a chronic and sometimes fatal pneumonia. Indeed legionaminic acid (5) appears to be important in the virulence of *L. pneumophila* as it is believed to be the LPS of serotype 1 that is the key determinant for the progression of the disease.

The naturally occurring legionaminic acid (Leg5,7Ac, 5) and its derivatives have an overall configuration similar to that of Neu5Ac (1), which is a D-glycero-D-galacto configuration (Figure 7). This overall configuration relates to the stereochemical centres at C-5, C-7 and C-8 being the same in both Neu5Ac (1) and Leg5,7Ac (5). Therefore unlike pseudaminic acid (4), legionaminic acid (5) can be considered a true sialic acid derivative only differing in its substitution pattern not in its overall stereochemical configuration. Like pseudaminic acid (4), legionaminic acid (5) is a 9-deoxy nonulosonic acid.

![Structural similarities and functional group differences between Neu5Ac (1) and Leg5,7Ac (5)](Figure 7)

Thus far legionaminic acid (5) and its naturally occurring derivatives have been isolated from several pathogenic bacterial species, including *Campylobacter*[^42,52], *Pseudomonas*[^27,50,53], *Vibrio*[^50,54-57], *Acinetobacter*[^50,58], *Escherichia*[^59-61] and *Salmonella*[^62] (Table 2).

[^42]: Reference 42
[^52]: Reference 52
[^27]: Reference 27
[^53]: Reference 53
[^50]: Reference 50
[^54]: Reference 54
[^57]: Reference 57
[^58]: Reference 58
[^59]: Reference 59
[^61]: Reference 61
[^62]: Reference 62
Table 2. Published accounts of isolation and characterisation of naturally occurring legionaminic acid derivatives based on a D-glycero-D-galacto configuration (table adapted from Zunk & Kiefel, 2014)

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<th>R''</th>
<th>Bacterial Source</th>
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<td>NH2C(O)CH3</td>
<td>Campylobacter jejuni 11168</td>
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<td>NH2C(O)CH3</td>
<td>Campylobacter jejuni 11168</td>
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<td></td>
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<tr>
<td>NH2C(O)CH3</td>
<td>NH2C(O)CH3</td>
<td>Escherichia coli O161</td>
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<td>NH2C(O)CH3</td>
<td>Escherichia coli O161</td>
<td>2010</td>
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Furthermore, two naturally occurring legionaminic acid structural isomers have been discovered that vary in their overall configuration compared with the parent compound 5. These two naturally occurring structural isomers are 4-epi-legionaminic acid (6) and the 8-epi-legionaminic acid (7), which have configurations of D-glycero-D-talo and L-glycero-D-galacto, respectively (Figure 8).
Figure 8. Naturally occurring legionaminic acid isomers 4-epi-legionaminic acid (6) and 8-epi-legionaminic acid (7).

These legionaminic acid derivatives have been isolated from a range of Gram-negative bacteria, including *Legionella pneumophila*<sup>50,51,64</sup>, *Pseudomonas aeruginosa*, *Escherichia coli* and certain *Salmonella* strains (Tables 3 and 4), having varied substitutions other than that of the parent compound 5.

Table 3. Published accounts of isolation and characterisation of naturally occurring 8-epi-legionaminic acid and its derivatives (table adapted from Zunk & Kiefel, 2014)<sup>23</sup>)

<table>
<thead>
<tr>
<th>N-substitution</th>
<th>R'&lt;br&gt;</th>
<th>R''&lt;br&gt;</th>
<th>Bacterial Source</th>
<th>Year Published</th>
</tr>
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</tr>
<tr>
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<td>NH(O)CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td><em>Salmonella arizonae</em> O61</td>
<td>1992&lt;sup&gt;22&lt;/sup&gt;</td>
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</tr>
<tr>
<td>NH(O)CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;OH</td>
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<td><em>Yersinia ruckeri</em> O1</td>
<td>1994&lt;sup&gt;44&lt;/sup&gt;</td>
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<td>NH(O)CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td><em>Morganella morganii</em> KF 1676 (RK 4222)</td>
<td>2002&lt;sup&gt;26&lt;/sup&gt;</td>
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<td><em>Shewanella putrefaciens</em> A6</td>
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<td><em>Escherichia coli</em> O108</td>
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<td>NH(O)CH(NHAc)CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td><em>Escherichia coli</em> O108</td>
<td>2010&lt;sup&gt;45&lt;/sup&gt;</td>
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</table>
Table 4. Published accounts of isolation and characterisation of naturally occurring 4-epi-legionaminic acid and its derivatives (table adapted from Zunk & Kiefel, 2014).

<table>
<thead>
<tr>
<th>N-substitution</th>
<th>Bacterial Source</th>
<th>Year Published</th>
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<td>NHC(O)CH₃</td>
<td>Legionella pneumophilia serogroup 1</td>
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<td>NH(NH)CH₃</td>
<td>Legionella pneumophilia serogroup 2</td>
<td>2001</td>
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<tr>
<td>NHC(O)CH₃</td>
<td>Shewanella japonica KMM 3601</td>
<td>2011</td>
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</table>

All of these bacteria utilise legionaminic acids as a virulence factor, for example *Campylobacter* strains heavily decorate their flagella via *O*-linked serine and threonine residues with this important carbohydrate moiety also utilising pseudaminic acid pathways in the same manner.

More recently Kandiba et al. identified a 5-N-formyl derivative of legionaminic acid as a terminal residue within the N-glycosylated VP4-derived (structural protein) pentasaccharide glycan of the haloarchaeal pleomorphic virus (HRPV-1). This discovery is the first known account of this typically *O*-linked sugar being isolated from an *N*-linked glycoprotein and also the first time a legionaminic acid derivative has been identified in an archaeal-derived glycan structure. This discovery highlights the ever-increasing knowledge that the legionaminic acids are widespread within nature and have important metabolic and virulent capabilities.

The discovery of pseudaminic and legionaminic acid derivatives within important bacterial structural elements demonstrates that these new higher sugars are more common to bacteria than previously believed. To date, the biological significance of these unique carbohydrate moieties remains to be fully determined. However, the existence of pseudaminic and legionaminic acids and their naturally occurring derivatives in cell surface-associated glycans in numerous clinically important pathogenic Gram-negative bacteria, along with the capability of these bacteria to modify their terminal glycans further to produce strains with particularly virulent and/or invasive properties, has contributed to a growing knowledge that these...
carbohydrate moieties and their derivatives contribute significantly in host-bacterial interactions and therefore virulence\textsuperscript{35}. Moreover, the consensus in the literature at present is that due to the importance of their role in the pathogenic capabilities of many clinically important drug-resistant Gram-negative bacteria, there is an opportunity for exploitation of these novel carbohydrates in the development of potentially new therapeutic agents\textsuperscript{35,72,73}.

1.3 Biological significance of 5,7-diacetamido-3,5,7,9-tetrahydroxynonulosonates

Bacterial infections are a global issue that not only affect people of all socio-economic backgrounds and age, but also place a major burden on the health systems of all countries\textsuperscript{74}. The presence of multi-drug resistant pathogenic bacteria has created a significant health crisis, which is a prominent threat to human health worldwide\textsuperscript{75}. The emergence of multi-drug resistant pathogens can largely be attributed to the over prescribing and misuse of many efficient antibiotics and a notable lack of research over the past 30 years into the development of anti-bacterial drugs that have novel mechanisms of action\textsuperscript{75,76}.

Multi-drug resistance in Gram-negative bacteria is particularly problematic as it is achieved via numerous complex mechanisms, which are largely due to molecular and structural modifications\textsuperscript{77–79}. These modifications may include altered cell wall permeability to anti-bacterials, and efflux mechanisms to remove such compounds from within the cell\textsuperscript{76–79}. Gram-negative bacteria have also developed mechanisms that deactivate antibiotics, alter the target site of antibiotics, and increase genomic mutation rates\textsuperscript{76–79}. Ultimately these mechanisms alter the virulence of a bacterium, in many cases leading to more pathogenic species and infections that are difficult to treat\textsuperscript{77,78}.

Infections caused by Gram-negative bacteria have always been particularly difficult to treat, due to the presence of their outer membrane, which acts as an extra physical barrier to the environment and also to drugs\textsuperscript{77,78}. This outer membrane contains transporters, efflux pumps and selective porins all of which make it difficult for drugs to reach the cytoplasm in order to elicit their pharmacological effect\textsuperscript{77,78}. 
Due to the rapid emergence of multi-drug resistant pathogenic bacteria, it has become increasingly important to investigate possible new drug targets for current medications and new drugs alike\textsuperscript{75,79}. One possible new target that has emerged from the literature in the past 30 years are nonulosonic acid biosynthetic pathways and associated processing enzymes\textsuperscript{72,73}.

Of particular importance with respect to this thesis is the role of the enzymes that create and utilise the nine carbon acidic sugars pseudaminic acid (4) and legionaminic acid (5). The enzymes that process these important carbohydrates are attractive potential drug targets as they are found only within micro-organisms, with pseudaminic acid (4) and legionaminic acid (5) hypothesised to be involved in bacterial-host interactions and biofilm production in many clinically relevant pathogens\textsuperscript{10,17,21,22,35,42,72,73,80–82}. As an example, studies have shown that \textit{Campylobacter jejuni} and \textit{Helicobacter pylori} have their own distinct pseudaminic acid biosynthetic pathways, which are important for the construction of the virulence factors these bacteria use to cause infection\textsuperscript{46,73}. Research into virulence factors utilised by these bacterial strains has shown that, when genetically modified to not express enzymes involved in the biosynthesis of pseudaminic acid, these bacteria are indeed non-pathogenic\textsuperscript{83}. These findings clearly show that interfering with the biosynthesis of pseudaminic acid is a potential opportunity for developing new anti-bacterials that specifically interfere with the pseudaminic acid biosynthetic pathway and subsequent incorporation into glycoconjugates\textsuperscript{72,73,83}.

1.4 The role of 5,7-diacetamido-3,5,7,9-tetra deoxy-nonulosonates in Gram-negative bacterial virulence

Opportunistic Gram-negative bacterial species achieve their pathogenicity via virulence factors which are generally proteins or molecules that are associated with key structural components\textsuperscript{47,84}. Gram-negative bacteria infect a host via numerous mechanisms, including: adhesion, colonisation, invasion, immune response inhibitors (evasion) and toxins. All of these mechanisms have certain virulence factors associated with them\textsuperscript{47,84}. The response to these virulence factors in humans is an innate immune response. However, more specific toxic effects can occur, such as sepsis and anaphylactic shock in susceptible individuals\textsuperscript{47,84}.
Whilst the outer membrane is the major component contributing to the virulence of all pathogenic Gram-negative bacteria, individual bacterial species also contain other components that contribute to their virulence. Structures such as flagella (Figure 9) and pili provide a bacterium motility, which aids in colonisation, invasive and evasive mechanisms. Motility is a key factor in the adaptation and virulence capacity of many bacterial pathogens that colonize mucosal surfaces. For example, the gastroenteritis causing Campylobacter and Helicobacter species demonstrate highly efficient flagella motility under conditions of elevated viscosity such as that found in the gastric lumen. Studies have shown that although flagella are a major structural determinate for bacterial virulence, it is in fact the glycosylated ends of these flagella (Figure 9) that facilitate bacterial-host interactions and therefore largely influence immune responses.

Figure 9. Diagrammatic depiction of O-linked glycosylated flagella from C. jejuni. Image modified from Young et al 2007.

Within Campylobacter and Helicobacter strains the carbohydrate modifications of their flagella glycans are largely based on pseudaminic acid and legionaminic acid. The major modifications identified on flagellum from C. jejuni are O-linked pseudaminic acid, Pse5Ac7Am, Pse5Am7Ac and Pse5,7Pr2. There are also minor amounts of an acetylated form of pseudaminic...
acid Pse5,7Ac28OAc (11)\textsuperscript{34,46} (Figure 10) along with legionaminic acid derivatives Leg5Am7Ac\textsuperscript{42} and Leg5AmNMe7Ac\textsuperscript{42}, which have been isolated and identified.

![Chemical structures of pseudaminic acid derivatives](attachment:structures.png)

**Figure 10.** Example of naturally occurring pseudaminic acid derivatives 8, 9, 10 and 11

Structural investigations of flagella from isogenic mutants of *Campylobacter* and *Helicobacter* strains have provided sufficient proof that pseudaminic acid (4) is an essential glycosylation molecule involved in flagella assembly and function\textsuperscript{34,46,87,88}, and therefore has an important role in the pathogenicity of these bacteria\textsuperscript{34,81}. Indeed mutants of pseudaminic acid biosynthetic genes in several strains of *Campylobacter* are non-motile and accumulate intracellular flagella of reduced molecular mass\textsuperscript{34}. Analysis of the flagellin by mass spectrometry confirmed the lack of any glycosylation, providing specific proof of a functional role for the glycosylation process in flagella assembly\textsuperscript{34}.

Other studies have shown that pseudaminic acid (4) is a potential substrate and signalling molecule for many proteins essential for flagella assembly in both *C. jejuni* and *H. pylori*\textsuperscript{34,35,46,81,87,89}, and can account for up to 10% of the total mass of flagella glycoprotein in certain strains\textsuperscript{90}. Flagellin from *C. jejuni* and *H. pylori* strains have been shown to contain 19 amino acids (Ser/Thr residues) that are \textit{O}-linked to the monosaccharide pseudaminic acid (4) and several of its derivatives, dependent on the strain\textsuperscript{34,35,42,46}. This discovery produced great interest when reported, since it was the
first account of an acidic monosaccharide being directly linked to a protein within a bacterium\(^ {91} \).

Although the exact function of pseudaminic acid (4) and legionaminic acid (5) and their naturally occurring derivatives is not fully understood within those strains of Gram-negative bacteria where they have been found\(^ {32} \), it is known that the presence of these post-translational glycolytic modifications of flagellin subunits is absolutely necessary for the assembly of a functional flagellin\(^ {56, 90} \). Since motility is required for colonisation, the presence of these nonulosonates can be considered a virulence factor in these bacteria\(^ {17, 35, 46, 72, 81} \). Due to this fact it can then be rationalised that any of the enzymes involved in the biosynthetic pathway of O-linked nonulosonates such as pseudaminic acid (4) and legionaminic acid (5) glycosylation, have the potential to be a drug target.

### 1.5 Nonulosonic acids as components of glycans

Bacterial protein glycosylation has become an area of intense interest since it was shown that protein components of surface appendages such as pili and flagella are essential for the virulence of various pathogens\(^ {35, 46} \). The reason for protein glycosylation is not fully understood although it has been shown within many flagellated bacteria that glycosylation appears to be essential for bacterial-host interactions, secretion, and assembly of functional flagellin subunits\(^ {92–94} \).

Glycans themselves have two distinct forms in prokaryotes just as in eukaryotes\(^ {95} \). \(N\)-glycosylation, in which the oligosaccharide is attached to an asparagine residue, and \(O\)-glycosylation, in which the oligosaccharide can be attached to a serine, threonine or tyrosine residue\(^ {95, 96} \). Generally what is known is that the glycans found on prokaryotic glycoproteins are far more varied in terms of carbohydrate arrangement and structure than those found in eukaryotic organisms\(^ {95, 96} \).

At present, bacterial glycoproteins can be divided into two groups: a group in which the carbohydrate modification directly affects protein function, such as subunit interactions and/or assembly of flagellin and pili, and the adherence to host cells. The second group is defined by the way in which the glycosylation influences the interaction with the immune system of the host\(^ {95–97} \).
It has been suggested that bacterial glycoproteins have a diverse role in maintenance of protein conformation, enhancing stability and protection against proteolysis degradation, surface recognition, cell adhesion or immune evasion\textsuperscript{95}. It has been shown that most bacterial glycoproteins are either associated with the surface of the organism as in pili or flagella, or they are secreted into the environment\textsuperscript{95–97}. Either way, these glycoproteins are in an important location, resulting in direct exposure to host cells and defence systems\textsuperscript{95–97}. Therefore, just as sialic acids in eukaryotes encode a unique glycosylation pattern in cell surface glycoconjugates, so too is the case with the pseudaminic acids and legionaminic acids in numerous Gram-negative bacteria, which amounts to a greater ability for bacterial-host interaction and therefore an improved chance for infection\textsuperscript{48,95–97}.

### 1.6 Biosynthesis of 3,5,7,9-tetradeoxy-nonulosonic acids and incorporation into glycans

#### 1.6.1 Pseudaminic acids

Within nature there exists a unique aspect to the biosynthesis of most nine-carbon nonulosonates\textsuperscript{2,3}. The first is that a nucleotide-activated hexose sugar serves as a precursor to nine carbon $\alpha$-keto acids. In the case of sialic acid derivatives, this hexose sugar is a D-mannose derivative, whilst compounds such as pseudaminic acid (4) and legionaminic acid (5) are synthesised from a nucleotide activated N-acetylg glucosamine\textsuperscript{3}. Secondly all known nine-carbon $\alpha$-keto acids are synthesised by an enzyme catalysed aldol condensation reaction, whereby a six carbon unit is condensed with a three carbon unit (typically a pyruvate derivative) to form the desired nine-carbon acidic sugar\textsuperscript{2,3,98}. This being said, in the case of pseudaminic acid (4) biosynthesis, certain chemical transformation must occur to the nucleotide-activated N-acetylg glucosamine in order for the appropriate hexose sugar to be obtained for the aldol condensation step as it is the stereochemical and functional group detail that this compound possesses which directly determines the nine-carbon nonulosonate being produced.
Biosynthetically, pseudaminic acid (4) is generated from UDP-\(N\)-acetylglucosamine (12) in an efficient five step pathway\(^{73,82,99,100}\) (Scheme 1). The first step in the pathway is catalysed by PseB an enzyme that has a dual function as a NAD(P)-dependant dehydratase and C-5 epimerase, converting UDP-\(\alpha\)-D-GlcNAc (12) to UDP-2-acetamido-2,6-dideoxy-\(\beta\)-D-arabino-hexos-4-hexulose (13) (Scheme 1)\(^{73,100}\). Compound 13 is converted by the aminotransferase PseC into UDP-4-amino-4,6-dideoxy-\(\beta\)-L-AltNAc (14) as seen in Scheme 1\(^{73,100,101}\). The intermediate 14 is then shuttled through a further two enzyme catalysed reactions, involving PseH and PseG\(^{73,90,100,102}\), being converted into 2,4-diacetamindo-2,4,6-trideoxy-\(\beta\)-L-altropyranose (15), which is the substrate for PseI, the enzyme that converts it into pseudaminic acid (4) via an aldol condensation with the three-carbon unit phosphoenolpyruvate (PEP) (Scheme 1)\(^{73,100}\).

**Scheme 1.** Biosynthetic pathway of CMP-pseudaminic acid (16) in *C. jejuni* and *H. pylori*\(^{73,100}\)

In order to be incorporated into \(O\)-antigens, or utilised in \(O\)-linked protein glycosylation pathways, pseudaminic acid (4) must be activated as its CMP-linked derivative 16\(^{73,100}\). This reaction is catalysed by PseF, a CMP-pseudaminic acid
synthetase, which utilises CTP, producing CMP-pseudaminic acid (16) and pyrophosphate (Scheme 1)\textsuperscript{73,100}.

The elucidation of the complete biosynthetic pathway for pseudaminic acid (4) was an important step toward understanding the role of this carbohydrate moiety within the bacterial lifecycle. Interestingly it has been noted that further work must be carried out in order to identify the glycosyltransfer mechanisms that the bacteria use to incorporate CMP-activated pseudaminic acid (16) into the glycoconjugates where it is found. These as yet unidentified glycosyltransferase enzymes along with certain biosynthetic enzymes are potential drug targets\textsuperscript{73,100}.

Since the publication of the elucidation of the complete pseudaminic acid biosynthetic pathway there have been a limited number of papers published regarding more in depth studies into enzymes involved in the pathway. One enzyme that has been characterised extensively is PseG, the nucleotide sugar hydrolase responsible for the catalysis of the fourth step in the pseudaminic acid biosynthetic pathway\textsuperscript{73,90,100,102}. PseG was first identified and characterised by Liu and Tanner in 2006\textsuperscript{90} and is responsible for the hydrolysis of UDP-2,4-diacetamido-2,4,6-trideoxy-\(\beta\)-L-altropyranose (not shown in Scheme 1) to generate 2,4-diacetamido-2,4,6-trideoxy-\(\beta\)-L-altropyranose (15) the compound that is condensed with the three carbon unit phosphoenolpyruvate to produce pseudaminic acid (4) (Scheme 1).

In their 2006 paper Liu and Tanner\textsuperscript{90} detailed the functional role of PseG, publishing the catalytic constants for the enzyme and identifying the specific mechanistic catalysis and thereby suggesting the type of enzyme family into which PseG could be classified\textsuperscript{90}. Their study concluded, based on homology and their results, that PseG belongs to the superfamily of inverting glycosyltransferase enzymes known as the metal-independent GT-B family (Family 28)\textsuperscript{90}. However, the actual enzymatic function of PseG was found to be that of a hydrolase enzyme and all of the data obtained indicated that this was the primary function of the enzyme and that UDP-2,4-diacetamido-2,4,6-trideoxy-\(\beta\)-L-altropyranose was the natural substrate\textsuperscript{90}. Therefore Liu and Tanner\textsuperscript{90} ruled out the possibility that PseG could be the missing CMP-pseudaminyltransferase that would be responsible for incorporating CMP-activated pseudaminic acid (16) into glycoconjugates\textsuperscript{90}. The results of this study show that PseG along with NeuC (a UDP-GlcNAc 2-epimerase involved in the biosynthesis of sialic
acids) are unique examples of two sugar nucleotide hydrolases that have evolved from the metal-independent GT-B family of glycosyltransferases\textsuperscript{90}. The findings of Liu and Tanner\textsuperscript{90} were confirmed in 2009 with the publication of the complete crystal structure of PseG in both the apo-form and with a UDP-product bound to the active site at 1.8Å resolution\textsuperscript{102}. This study showed that PseG was indeed a sugar nucleotide hydrolase which belonged to the GT-B glycosyltransferase superfamily\textsuperscript{102}. Another important enzyme involved in the biosynthesis of pseudaminic acid is the first enzyme in the pathway, PseB\textsuperscript{73,100}. PseB is a unique enzyme in that it has a dual function in the biosynthetic pathway, acting as a 4,6-dehydratase and a 5-epimerase converting UDP-\(\alpha\)-D-GlcNAc (12) to UDP-2-acetamido-2,6-dideoxy-\(\beta\)-D-arabinohexos-4-ulose (13)\textsuperscript{73,100}. McNally \textit{et al} (2008)\textsuperscript{73} showed that upon accumulation of 13 the enzyme also catalyses an additional C-5 epimerisation forming UDP-2-acetamido-2,6-dideoxy-\(\alpha\)-D-xylo-hexos-4-ulose (17) (Scheme 2). In \textit{C. jejuni} 17 is used to make 2,4-diacetamido-2,4,6-trideoxy-\(\alpha\)-D-glucopyranoside (\(\alpha\)-D-QuINAc4NAc, 18, Scheme 2) which is an important component of the N-linked glycan that modifies over thirty proteins in this bacterium\textsuperscript{73}. This finding indicates that there exists cross talk between the pseudaminic acid and the \(\alpha\)-D-QuINAc4NAc pathways via PseB\textsuperscript{73}. Considering the dependence of \(O\)-linked flagellin glycosylation on PseB and its apparent contribution to other carbohydrate biosynthetic pathways, PseB could be a viable drug target for inhibition\textsuperscript{73}.

\textbf{Scheme 2.} Pse B catalysed reactions with respect to the CMP-Pse and UDP-\(\alpha\)-D-QuINAc4NAc biosynthetic pathways.
Another important finding from McNally’s 2008 study showed that the CMP-activated pseudaminic acid (16) itself was a potent inhibitor of the enzyme PseB, completely inhibiting the enzyme at low concentrations. The results showed that CMP-pseudaminic acid (16) was a competitive inhibitor with UDP-GlcNAc (12) \((K_i(app) = 18.7\mu M)\). This finding suggests that there exists a negative feedback loop within the biosynthetic pathway of CMP-pseudaminic acid (16) whereby PseB acts as an on/off switch in the pathway, which is turned off by increased levels of CMP-pseudaminic acid (16) and turned on by decreased levels of 16.

1.6.2 Legionaminic acids

Synonymously the legionaminic acid biosynthetic pathway, which in the case of *C. jejuni* is an alternate pathway that operates at the same time as the pseudaminic acid biosynthetic pathway, has also been fully characterised. Legionaminic acid (5) is also generated from a nucleotide activated N-acetylglucosamine derivative in an efficient five step pathway. However, the starting molecule in the legionaminic acid pathway is GDP-N-acetylglucosamine (19). The first step in the biosynthesis of legionaminic acid is catalysed by LegB, an NAD-dependent 4,6-dehydratase which converts GDP-N-acetylglucosamine (19) to GDP-2-acetamido-2,6-dideoxy-\(\alpha-D\)-xylo-hexos-4-ulose (20) (Scheme 3). LegC, a PLP-dependent aminotransferase, then catalysis the next step converting 20 into GDP-4-amino-4,6-dideoxy-\(\alpha-D\)-GlcNAc (21). Compound 21 is processed by the next enzyme in the pathway, a N-acetyltransferase (LegH) which produces GDP-2,4-diacetamido-2,4,6-trideoxy-\(\alpha-D\)-glucopyranose (22), which is then converted into 2,4-diacetamido-2,4,6-trideoxy-D-mannopyranose (23) via the catalysis of an enzyme known as LegG, which acts as a NDP-sugar hydrolase and 2-epimerase.
Compound 23 is a 6-deoxy-mannose derivative or D-rhamnose sugar which is transformed into legionaminic acid (5) by the enzyme LegI, a legionaminic acid synthase\textsuperscript{103} (Scheme 2). As with all nonulosonate biosynthetic pathways the resulting nine carbon $\alpha$-keto sugar must be nucleotide activated before being able to processed and incorporated into the varying glycans where they are found. In the case of legionaminic acid (5) it is CMP-activated by the enzyme LegF, a CMP-legionaminic acid synthetase producing CMP-legionaminic acid (24)\textsuperscript{103}.

Despite a detailed knowledge of the role of the key enzymes in the biosynthesis of the activated CMP-pseudaminic acid (16) and CMP-legionaminic acid (24)\textsuperscript{73,100,103}, the identification and characterisation of possible glycosyltransferase(s) responsible for the incorporation of these CMP-linked nonulosonates into proteins remains elusive\textsuperscript{73,100,103}. However, recently it has been reported that the glycosyltransferase responsible for the transfer of CMP-activated pseudaminic acid (16) onto the flagellin of \textit{Aeromonas caviae} has been found\textsuperscript{104}. Whilst these authors did not show direct evidence that the motility-associated factor \textit{maf} gene provides the enzyme responsible for adding pseudaminic acid onto the flagellin, their studies provide strong evidence that \textit{mafI} is indeed a
pseudaminyltransferase\textsuperscript{104}. This is the first report describing the identification of a probable pseudaminyltransferase, and will perhaps provide important information for further studies aimed at identifying this glycosyltransferase enzyme in other bacteria.

Despite recent studies into pseudaminic acid processing enzymes, a lot of molecular detail is still missing in regard to the overall metabolism or recycling of these nonulosonates within bacteria that utilise these important carbohydrates in order to be pathogenic\textsuperscript{73,100,103}. However, based on what is known it is reasonable to suggest that this crucial missing information could possibly be obtained by using pseudaminic acid or legionaminic acid based compounds as biological probes. These analogues could help identify key glycosyltransfer processes, and also aid in studies regarding possible inhibition of enzymes in the biosynthetic pathway of pseudaminic acid (4) and legionaminic acid (5) and whether or not these bacteria possess mechanisms to overcome such inhibition. To date there has been no single report on disrupting either the pseudaminic acid or legionaminic acid biosynthetic pathways using xenobiotics, or similar inhibition studies where pseudaminic acid or legionaminic acid based analogues are used.

1.7 Pseudaminic and legionaminic acid processing enzymes

Given the importance of pseudaminic acid (4) and legionaminic acid (5) as structural components of glycans within pathogenic bacteria; there is considerable interest in finding the enzymes responsible for their utilisation and processing. In 2009 a sialidase-like enzyme from \textit{Pseudomonas aeruginosa} was identified as a possible pseudaminidase (PA2974)\textsuperscript{105}. This postulated pseudaminidase has key characteristics that make it appear to be a sialidase, but there are some important structural differences within the catalytic site, which indicates that sialic acid compounds are not the natural substrate(s) for this enzyme\textsuperscript{105}. In general sialidase enzymes are a widely distributed and conserved family of enzymes found in both eukaryotes and prokaryotes\textsuperscript{105–107}. All sialidase enzymes have structural similarities such as a six bladed $\beta$-propeller fold and share a number of key amino acid residues in the catalytic site\textsuperscript{105–107}. They generally have highly conserved catalytic residues, which include an arginine triad that interacts with the sialic acid carboxylate group, a nucleophilic tyrosine and an associated
glutamic acid, an aspartic acid that acts as an acid/base, and a hydrophobic pocket that accommodates the acetamido group at C-5 in sialic acids$^{105-107}$ (Figure 11).

**Figure 11.** An example of the highly conserved sialidase catalytic site from the H2N2 influenza viral neuraminidase with bound Neu5Ac (1). Image shows arginine triad (Arg292, 118 and 371), acid/base aspartic acid (Asp 151) and hydrophobic pocket seen above Arg152 (bottom centre). *Image modified from Yen, H.-L. et al 2006*$^{108}$

The new sialidase-like enzyme which Taylor and colleagues identified has been reported to be involved in biofilm production in *Pseudomonas aeruginosa*$^{105}$. The findings reported from studies carried out on PA2794 suggest that the probable substrate for this newly discovered enzyme could indeed be glycans that contain pseudaminic acid (4) itself or its naturally occurring derivatives$^{105}$. This hypothesis was supported by an apo-x-ray crystal structure of the enzyme along with *in silico* docking studies which compared the binding orientation of Neu5Ac (1) Neu5Ac2en (25) and pseudaminic acid (4) to the binding site of the sialidase-like enzyme from *Pseudomonas aeruginosa*.$^{105}$
The active site of the enzyme has several structural similarities that are comparable with other sialidase enzymes, including the presence of two of the usual three arginine’s found in the arginine triad in sialidases, the nucleophilic tyrosine and its associated glutamic acid and a hydrophobic pocket to accommodate the acetamido group found at C-5 in sialic acids (Figure 12)\textsuperscript{105}. However, the first arginine of the arginine triad and the acid/base aspartic acid that are usually present in sialidases are both clearly absent in the apo-structure of the enzyme, whilst the hydrophobic pocket is more suited to a different stereochemistry at C-5 compared with that of sialic acids\textsuperscript{105}. Importantly, Taylor and colleagues showed that due to the altered amino acid residues in the catalytic domain the recombinant enzyme was seen to have no sialidase activity with a standard sialidase fluorescent tagged substrate\textsuperscript{105}.

Structural studies carried out by Taylor et al.\textsuperscript{105}, show further unique elements within the catalytic site of PA2794, which supports the hypothesis that this enzyme could indeed be a pseudaminidase\textsuperscript{105}. Importantly, all sialidases characterised to date have an aspartic acid, located on a loop that is situated above the bound substrate, which has the role of an acid/base in the catalytic site\textsuperscript{105}. The new enzyme also has a similar
loop in the same position however there is no aspartic acid present. Instead where the aspartic acid is normally found there is a His residue (Figure 13)\(^\text{105}\). It has been proposed by Taylor and colleagues\(^\text{105}\) that the His45 residue found in the loop acts as the acid/base, although it is seen to be slightly distant from the substrate in the apo structure\(^\text{105}\). However, the amino acid sequence mentioned could give the loop some flexibility upon substrate binding and in addition, the His45 residue could rotate around its C\(^\alpha\)-C\(^\beta\) bond\(^\text{105}\). This rotation of the His45 residue around its C\(^\alpha\)-C\(^\beta\) bond upon substrate binding appears to bring the His45 residue closer to where the substrate would sit\(^\text{105}\). In this position the His45 could make a hydrogen bond interaction with the ring hydroxyl of Tyr21 that is already in hydrogen bond contact with Glu315 in the crystal structure\(^\text{105}\). Therefore Taylor and colleagues\(^\text{105}\) propose that the charged state of His45 might be controlled through a charge-relay system involving Glu315 and Tyr21, allowing for the His45 residue to act as an acid/base\(^\text{105}\).

![Figure 13. In silico docking of Neu5Ac2en (25) (yellow carbon atoms) and Pse5,7Ac\(_2\) (4) (magenta carbon atoms) into PA2794, His45 has been rotated around its C\(^\alpha\)-C\(^\beta\) bond to highlight possible charge rely interactions (right side)\(^\text{105}\); Structures of Pse5,7Ac\(_2\) (4) and Neu5Ac2en (25) showing stereochemical differences at C-5 and C-7 (left side).](image)

In silico docking studies with Pse5,7Ac\(_2\) (4) show that this compound is expected to bind to PA2794 in a way very similar to that observed for the binding of sialic acids
in sialidases (Figure 13, right)\textsuperscript{105}. The presence of a phenylalanine residue (Phe129) in the hydrophobic region of PA2794 produces a binding orientation quite different to that of the sialic acids in sialidases however; this is presumably due to the difference of the stereochemistry for the acetamido group on C-5 in Pse5,7Ac\textsubscript{2} (4) compared to sialic acids (Figure 13, left)\textsuperscript{105}. Taylor noted in the docking studies that the topology of the active site was quite open and therefore would allow for recognition of the various pseudaminic acid analogues, and also the loop containing the His45 residue contains two adjacent glycine’s, which would decrease the chance of steric hindrance to substitutions at C-5 on the sugar ring, and likewise bulkier substitutions at C-7 could also be accommodated (Figure 13)\textsuperscript{105}.

Furthermore, and of particular importance, Taylor and colleagues showed that the wild type PA2794 showed no enzyme activity against a known fluorescent-tagged sialic acid substrate (MUAN)\textsuperscript{105}. However, molecular modelling suggested that it may be possible to convert PA2794 into a sialidase enzyme by mutation of Phe129 to allow for the difference in stereochemistry at the C-5 position in Pse5,7Ac\textsubscript{2} (4) and Neu5Ac (1)\textsuperscript{105}. Taylor and colleagues were able to create a Phe129Ala mutant of PA2974, which was able to cleave the known fluorescent-tagged sialic acid substrate (MUAN) in a 100-hour reaction releasing the fluorescent agent 4-MU and Neu5Ac (1)\textsuperscript{105}. Moreover, in addition to 4-MU and Neu5Ac, there was also a significant amount of Neu5Ac2en (25) produced in the reaction, the ratio of released Neu5Ac (1)/Neu5Ac2en (25) being $\sim$2:1, which remained constant throughout the reaction from 12 to 120 hours\textsuperscript{105}. In separate experiments, the Phe129Ala mutant was unable to convert Neu5Ac (1) to Neu5Ac2en (25) or Neu5Ac2en (25) to Neu5Ac (1) therefore, as Taylor notes\textsuperscript{105} both Neu5Ac (1) and Neu5Ac2en (25) are direct products released by the mutant from the cleavage reaction on MUAN.

Yet another structural feature that was noted in the study of PA2794 was that in the active site on the side that would be occupied by the aglycon there is a flat topology, which is comparable to that of sialidases that are promiscuous in that they have the ability to cleave $\alpha$2,3-, $\alpha$2,6-, and $\alpha$2,8-linked sialic acids\textsuperscript{105}. All of these structural features suggest that PA2794 could be described as a pseudaminidase, rather than a sialidase, having the ability to cleave pseudaminic acids from a variety of glycoconjugates\textsuperscript{105}. 

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The identification of this new possible pseudaminidase enzyme, although exciting in the area of pseudaminic acid processing enzymes, poses a number of questions regarding the possible role such an enzyme may play. So far pseudaminic acid (4) and its derivatives are believed to be found only within glycoconjugates of bacterial origin, and have been shown to be an essential substrate and signalling molecule for many proteins essential for flagella assembly and biofilm production\textsuperscript{17,22,80,81,83,85}. Pseudaminic acids also appear to play an important role in bacterial-host interactions and appear to be essential for colonisation within mucosal layers\textsuperscript{17,22,30,35,73}. Therefore with these facts in mind we can speculate that a pseudaminidase could form an important part of the regulation of glycosylation mechanisms in order to ensure aggregation of bacterial colonies and therefore colonisation, or possibly play a role in maintenance of flagella glycosylation mechanisms. A pseudaminidase may also have a possible role in defence mechanisms like those seen in \textit{Pseudomonas aeruginosa}, where pseudaminic acid has been shown to be essential for biofilm production in these bacteria\textsuperscript{10,17,26}.

To date there have been no key legionaminic acid processing enzymes identified other than those known to be involved in the biosynthetic pathway.

1.8 Structurally modified pseudaminic and legionaminic acid analogues as biological probes.

It has been well recognised for over a decade now that research into the area of pseudaminic and legionaminic acid glycosylation has been greatly hampered by lack of access to optically pure samples of these two important carbohydrate moieties or their derivatives\textsuperscript{42,73,100}. Little work has been published since 2006 in regard to using pseudaminic acid based compounds as biological probes in order to better understand glycosyl processes used by Gram-negative bacteria in order to become virulent. Early studies set the scene for exciting work into possible small molecule inhibitors of the biosynthetic pathway of pseudaminic acid but since then there has been no follow on publications relating to the subject.

The only paper that has been published in the past five years that is of genuine interest in this area is a study carried out by Liu \textit{et al.} in 2009\textsuperscript{109}. Based on pioneering
work by the Bertozzi group\textsuperscript{110}, which showed that feeding azide modified precursors to eukaryotic cells can result in the display of azide modified sialic acids on their surface, Liu and colleagues\textsuperscript{109} produced an azide modified 6-deoxy-AltdiNAc in the form of 6-deoxy-AltNAc4NAz (\textbf{26}) (Scheme 4) and fed these precursors to mutant strains of \textit{C. jejuni} that were deficient in the pseudaminic acid pathway\textsuperscript{109}. The result from this study was \textit{C. jejuni} mutants having flagella decorated with 7NAz-pseudaminic acid derivatives\textsuperscript{109} (Scheme 4).

\begin{center}
\textbf{Scheme 4.} Pseudaminic acid analogue created from 26 by Liu \textit{et al.} 2009\textsuperscript{109}
\end{center}

This work provides great promise in the area of chemical biology and functional elucidation, especially relating to discovery of the mechanisms involved in pseudaminic acid glycosyltransfer and flagella assembly. The ability to incorporate reactive chemical handles such as azides selectively onto the flagella of pathogenic bacteria could provide a useful tool for further studies. Such tags could be used for labelling of cells to be used in \textit{in vivo} animal models of infection, biophysical studies of bacterial motility, or in studies probing flagella assembly and surface accessibility of glycan modifications on flagellin proteins\textsuperscript{109}. Other more elaborate uses could include using labelled bacteria as drug delivery vehicles or taking advantage of the immunogenicity of the flagellin proteins in the development of novel conjugate vaccines\textsuperscript{109}. Overall this work has provided a great insight into the future possibilities of using pseudaminic acid analogues as biological probes to better understand virulence factors and how bacterium use pseudaminic acid-based compounds in order to be pathogenic.
1.9 Previously published syntheses of pseudaminic and legionaminic acids

1.9.1 Synthesis of pseudaminic acids

To date there has been limited work published regarding the synthesis of the naturally occurring pseudaminic acid (4) and legionaminic acid (5), and only two reported syntheses of novel pseudaminic acid analogues, some of which are in fact isomers of 5\(^{109,111}\). Aside from our own efforts (\textit{vide infra}), currently there are only four papers that discuss the actual synthesis of pseudaminic acids, and two papers regarding the synthesis of legionaminic acids, all of which have been published since 2001\(^{50,72,100,109,111,112}\).

The most comprehensive study into the synthesis of pseudaminic acid derivatives was reported in 2001 by Tsvetkov and colleagues\(^{111}\). This work was carried out in an attempt to identify the precise chemical structure of the naturally occurring pseudaminic acid monomer, as at the time there was still conjecture regarding the exact stereochemistry within the glycerol side chain\(^{111}\). In their paper, Tsvetkov and colleagues\(^{111}\) report the synthesis of all nine possible stereo-isomers of pseudaminic acid, which were obtained by a synthetic aldol condensation of four different 2,4-diacetamido-2,4,6-trideoxyhexose sugars with oxaloacetic acid (27) under basic conditions (Scheme 5). The chemistry involved in producing each of the four hexose sugars to be condensed with oxaloacetic acid (27) involved a long multiple-step process using a different starting material for each process, some of which also had to be synthesised themselves. The overall yields for each synthesis were poor, producing each of the nine pseudaminic acid isomers in less than 5% yield\(^{111}\).

![Scheme 5](image)

\textit{Reagents and Conditions:} (a) oxaloacetic acid, Na\(_2\)B\(_4\)O\(_7\), pH 10.5, rt.

\textbf{Scheme 5.} An example of a pseudaminic acid isomer synthesised by Tsvetkov \textit{et al.} in 2001\(^{111}\).
However, prior to this work by Tsvetkov, the most efficient way to obtain pseudaminic acid was by isolating it out of the cell wall components of those bacteria that contained it. This isolation method is generally fairly inefficient, and involves complex processes and harsh conditions\textsuperscript{72,73}.

More recently Chou and colleagues described a chemoenzymatic synthesis of pseudaminic acid (4)\textsuperscript{72}. Using a synthetically derived form of 2,4-diacetamido-2,4,6-trideoxy-\(\beta\)-L-altropyranose (15), pyruvate (28), and a neuraminic acid synthase (NeuB3) homolog from \textit{C. jejuni}, Chou and colleagues showed it was possible to produce pseudaminic acid (4) itself in an efficient and relatively inexpensive process (Scheme 6)\textsuperscript{72}.

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme6.png}
\caption{Enzymatic conversion from Chou’s chemoenzymatic approach for the synthesis of pseudaminic acid (4)\textsuperscript{72}}
\end{scheme}

Following from the work described by Chou\textsuperscript{72}, and inspired by the elucidation of the biosynthetic pathway of pseudaminic acid in \textit{C. jejuni} and \textit{H. pylori}\textsuperscript{35,73}, Schoenhofen and colleagues synthesised the nucleotide activated CMP-pseudaminic acid (16) from UDP-GlcNAc (12) in what they referred to as a “one pot” method\textsuperscript{100}. By combining the six enzymes involved in the pseudaminic acid biosynthetic pathway, Schoenhofen’s team were able to produce the first complete \textit{in vitro} enzymatic synthesis of the activated form of pseudaminic acid\textsuperscript{100}.

In 2011 Lee \textit{et al.}\textsuperscript{112} published a chemical synthesis of pseudaminic acid (4) from \textit{N}-acetylglucosamine (29) via 6-deoxy-AltdiNAc (15) which is the key intermediate in the biosynthesis of pseudaminic acid (4)\textsuperscript{100,112}. Compound 15 was elongated via an Indium-mediated allylation reaction with a bromomethacrylate ester derivative followed by ozonolysis and hydrolysis to produce the desired pseudaminic acid (4) in moderate overall yield\textsuperscript{112} (Scheme 7).
Regents and Conditions: (a) In, 0.1 N HCl-EtOH (1:6); (b) O₃, MeOH, then 30% H₂O₂, H₂O, HCO₂H; (c) TEA-H₂O (1:3)

**Scheme 7.** Lee’s synthesis of pseudaminic acid (4) from GlcNAc (29) via a 6-deoxy-AltdiNAc (15) derivative.

Lee and colleagues publication was also the first account of a synthetically prepared pseudaminic acid disaccharide, producing the α(2-6)-linked disaccharide (30) in a 35% yield along with 62% yield of the elimination product pseudaminic acid-2-en (Pse-2-en (31)). (Figure 14)₁¹²

**Figure 14.** Two novel compounds produced by Lee and colleagues, a pseudaminic acid disaccharide (30) and Pse-2-en (31)₁¹²
1.9.2 Synthesis of legionaminic acids

In 2000 Tsvetkov and colleagues published the first paper\textsuperscript{113} detailing the synthesis of legionaminic acid based derivatives 8-\textit{epi}-legionaminic acid (7) and 4,8-di-\textit{epi}-legionaminic acid (32) via condensation of 2,4-diacetamido-2,4,6-trideoxy-L-gulose (33) with oxaloacetic acid (27) under basic conditions (Scheme 8). At this time due to the poor overall yields after the aldol condensation reaction, Tsvetkov et al.\textsuperscript{113} were unable to fully characterise the two products 7 and 32. Therefore, their work that followed on from this was primarily aimed at determining the exact configuration of the newly synthesised legionaminic acid derivatives.

\textbf{Scheme 8.} Legionaminic acid derivatives 7 and 32 synthesised by Tsvetkov et al.\textsuperscript{113}

In a further paper published in 2001\textsuperscript{50} Tsvetkov et al. outlined a new synthesis whereby they synthesised legionaminic acid (5) itself and another naturally occurring derivative the 4-\textit{epi}-legionaminic acid (6), which greatly aided the characterisation of all four legionaminic acid compounds (5, 6, 7, 32). However, it should be noted that even though their new synthesis was successful in determining the structural configurations of the legionaminic acid compounds, all four compounds were produced in poor overall yield (<5\%)\textsuperscript{50,113}.

As was the case in the latter work (pseudaminic acid synthesis by Tsvetkov et al. 2001\textsuperscript{111}) these syntheses\textsuperscript{50,113} were aimed at unambiguous identification of legionaminic acids including determination of their absolute configurations in order to identify the
natural monosaccharide’s. These two publications are the only known synthesis of legionaminic acid (5) or any of its naturally occurring derivatives\textsuperscript{50,113}.

The six methods outlined\textsuperscript{50,72,100,111–113} above for the synthesis of pseudaminic and legionaminic acids are to the best of our knowledge and excluding our own work described herein, the only published accounts thus far in regard to generating these important carbohydrates. Each of the six approaches represents a major advance in the area of pseudaminic and legionaminic acid synthesis and carbohydrate chemistry. However, one limitation in all of the reported syntheses of pseudaminic acid is the lack of versatility in the products obtained. In three reports\textsuperscript{72,100,112} the only compound obtained is pseudaminic acid (4) or legionaminic acid (5) itself, whilst the Tsvetkov work\textsuperscript{111} makes different stereoisomers of pseudaminic acid (4), but no functional group analogues. To date no single report has been published with the primary aim of synthesising analogues of pseudaminic acid or legionaminic acid.
1.10 Scope of thesis

The 5,7-diamino-3,5,7,9-tetrahydroxy-nonulosonic acids pseudaminic acid (4) and legionaminic acid (5), are important bacterial carbohydrates that play an essential role in the pathogenic capabilities of numerous clinically relevant Gram-negative bacteria. Since their initial discovery, numerous structural and functional group derivatives have been isolated from many bacterial species, highlighting the fact that these compounds are widespread throughout nature. However, to date their exact biological significance is yet to be fully determined.

To date, a number of publications have noted the major limitation in performing research into the role of these important carbohydrates is access to sufficient quantities of pure compound. Thus far, there have only been a small number of publications reporting the chemical synthesis of the parent compounds, and these syntheses do not lend themselves to creating structural analogues in sufficient quantity, therefore limiting their use.

The work described in this thesis aimed primarily to address the gap in the literature in regard to having an efficient and versatile synthesis method for obtaining structural analogues of pseudaminic acid (4) and legionaminic acid (5). A range of pseudaminic acid and legionaminic acid analogues could serve as valuable compounds to probe enzymes and proteins that bacteria utilise to biosynthesise and/or process these compounds. Limited research has been published in this regard with only work by the Tanner group (see Liu et al. 2009 \(^\text{109}\)), pg. 36 of this thesis for discussion), providing evidence that such analogues could be used as glycoprotein labels. Therefore, further work is needed in developing protocols for the efficient synthesis of pseudaminic acid (4) and legionaminic acid (5) analogues along with potentially important compounds, which could include specific positional modifications (e.g. changes at C-7), 2,3-didehydro derivatives, and fluorescently labelled compounds (e.g. PseMU).

Specifically this research project aimed to investigate three different approaches toward the synthesis of these important 5,7-diamino-3,5,7,9-tetrahydroxy-nonulosonic acids

1. Firstly, we aim to develop a chemoenzymatic approach toward the synthesis of legionaminic acid analogues (described in chapter 2). This chemoenzymatic
approached aims to use the commercially available type 1 aldolase Neu5Ac aldolase to catalyse an aldol condensation reaction between pyruvic acid and some novel 6-deoxy C-4 nitrogen functionalised substrates of D-mannose and D-talose (4-epi-mannose) to produce their corresponding C-7 nitrogen functionalised legionaminic acid analogues. The two target substrates that we propose to use for the enzyme catalysed aldol condensation reaction will be synthesised from commercially available D-mannose. The significance of this approach is that there are only two reported synthesis of legionaminic acid based compounds in the literature. We therefore aimed to improve on reported outcomes for the synthesis of such compounds and potentially establish a more versatile protocol for the synthesis of structural analogues that could aid in mechanistic studies of key legionaminic acid processing enzymes.

2. Secondly, we aim to investigate the use of a chemical aldol condensation reaction in order to create pseudaminic acid analogues (described in chapter 3). This type of approach has been reported by others for the synthesis of 5,7-diamino-3,5,7,9-tetraenolosonic acids however typically in poor overall yields. Therefore the major aim of this aspect of our project is to efficiently create selectively functionalised 6-deoxy-galactopyranosides and N-acetyl-glucosamine derivatives to be condensed with oxaloacetic acid in order to generate a range of pseudaminic acid analogues. In achieving this goal we aimed to establish improved reaction conditions for the efficient synthesis of such compounds.

3. The final aim of this project is to establish an efficient and versatile synthesis of pseudaminic acid analogues from the nine-carbon sialic acid derivative KDN. We will achieve this aim by selectively manipulating KDN at key positions to convert this sialic acid derivative into a 5,7-diamino-3,5,7,9-tetraenolosonic acid, developing potential branching points in our synthesis to allow for naturally occurring pseudaminic acid analogues to be developed if time permits.
CHAPTER 2

A CHEMOENZYMATIC APPROACH TOWARDS THE SYNTHESIS OF 5,7-DIAMINO-3,5,7,9-TETRADEOXY-NONULOSONIC ACID ANALOGUES

2.1 Introduction

The first major aim for this project was to investigate the use of a chemoenzymatic approach towards the synthesis of 5,7-diamino-3,5,7,9-tetra(deoxy-nonulosonic acids. The basic concept was to investigate methods for the synthesis of selectively functionalised hexose substrates, which could then be transformed via an enzyme catalysed reaction in order to create legionaminic acid analogues.

Over the past three decades enzymes have been commonly used as catalysts in carbohydrate chemistry, not only for the synthesis of simple functionalised monomers but also in one-pot synthesis of oligosaccharides\textsuperscript{114–116}. The main attraction of using enzymes for chemical transformations is their ability to produce a regio- and stereoselective product in an efficient manner\textsuperscript{116,117}. Another attractive characteristic of enzymatic reactions is the inherent promiscuous nature of many enzymatic families in accepting selectively functionalised substrates allowing for chemoenzymatic protocols which provide a larger array of possible products that can be obtained from choosing the right enzyme for catalysis\textsuperscript{118,119}.

The term “chemoenzymatic synthesis” covers both chemical modifications of substrates before enzymatic synthesis and/or modification of the products after enzymatic transformations\textsuperscript{120}. A number of enzymes have been used in the synthesis of complex carbohydrates such as nonulosonic acids, producing selectively functionalised monomers; enzymes have also been utilised to create oligosaccharides and other complex linked structures\textsuperscript{114–125}.

With respect to chemoenzymatic synthesis to produce nonulosonic acids, the Type 1 aldolase\textsuperscript{N-acetylneuramininate pyruvate lyase (E.C. 4.1.3.3)} more commonly referred to as Neu5Ac aldolase has been extensively used and studied\textsuperscript{115,116,118,119,122,123,126–128}. To this end, the substrate specificity of this enzyme has been well documented and the catalytic mechanism has been characterised using crystal
structures of bound substrate\textsuperscript{117,120,129}. Numerous publications have reported successful chemical manipulation of modified substrates for Neu5Ac aldolase, consequently producing a number of C-5, C-7, C-8 and C-9 functionalised nonulosonic acids\textsuperscript{13,116,117,119–125,130–132}.

In nature Neu5Ac aldolase catalyses a reversible aldol condensation reaction between the natural substrate \(N\)-acetyl-D-mannosamine (D-ManNAc, 34) and the donor compound pyruvate (28), producing \(N\)-acetylneuraminic acid (Neu5Ac, 1) (Scheme 9)\textsuperscript{119,126}. \textit{In vitro} under controlled conditions, the forward conversion (34 to 1) and the reversible reactions are both possible\textsuperscript{117,119,126,127,133} (Scheme 9).

\begin{center}
\includegraphics[width=\textwidth]{scheme9.png}
\end{center}

\textbf{Scheme 9.} The reversible aldol condensation reaction catalysed by Neu5Ac aldolase

In order to use Neu5Ac aldolase to create legionaminic acid analogues, a number of aspects regarding our intended chemoenzymatic approach had to be considered. Firstly, we had to think about the type of products we wanted or were able to create using Neu5Ac aldolase. This required careful consideration of the types of selectively functionalised hexoses that needed to be created, in order to be efficiently transformed by Neu5Ac aldolase into our desired products. Secondly, we wanted to use a common starting material to create a variety of tetradeoxy-nonulosonic acids, as this would introduce versatility and efficiency into our chemoenzymatic approach. Moreover, if we intended on using Neu5Ac aldolase a number of functional group manipulations and stereochemical detail had be installed within the intended substrates for the enzyme before the enzymatic transformation. Based on what is known regarding the substrate specificity of Neu5Ac aldolase (as outline in Section 2.2) the following stereochemical features of intended substrates must be met.
Substrates must:

- Be a reducing sugar (hydroxyl group at C-1)
- Have a mannose conformation at C-2 (X is axial in Figure 15)
- Have an equatorial group at C-3

![Figure 15. Neu5Ac aldolase substrate structural requirements](image)

Structural and functional deviations at C-4, C-5 and C-6 of D-mannopyranoside-based substrates seemed to be well tolerated by Neu5Ac aldolase\(^\text{117}\). This is indicated by the fact that 6-deoxy-D-mannose (D-rhamnose, (35)) is a good substrate for Neu5Ac aldolase along with D-talose (36) (4-epi-D-mannose) and a host of 2-deoxy compounds\(^\text{117}\). However, importantly, studies have noted that Neu5Ac aldolase would not accept glucose based sugars as substrates, which included N-acetylglucosamine (29), as these sugars have an equatorial C-2 substituent\(^\text{117}\).

With this knowledge in hand we began work on designing a flexible chemoenzymatic synthesis of tetradeoxy-nonulosonic acid analogues using the commercially available D-mannose (37) as our starting material. Our proposed synthesis can be seen in retro-synthetic terms in Scheme 10. Using standard chemistry, commercially available D-mannose (37) will be transformed into a 6-deoxy-mannose derivative depicted in generic terms by compound 38 (Scheme 10). The 6-deoxy-mannose derivative 38 is crucial to the success of this initial aim of this project, since it contains the necessary structural components and stereo-chemical detail to produce legaminic acid configured sugars like 39 after the aldol condensation reaction catalysis by Neu5Ac aldolase\(^\text{117}\).

The most important feature of the mannose derivative 38 is the presence of the 6-deoxy functionality, since this will become C-9 in the legaminic acid analogue (39) produced after the Neu5Ac aldolase catalysed condensation reaction with pyruvic acid (28) (Scheme 10). Similarly, C-2 in the mannose derivative 38 becomes C-5 in our desired nonulosonic acid analogue 39 and C-4 in 38 becomes C-7 in 39 (Scheme 10). Therefore, we can prepare a variety of legaminic acid analogues from the Neu5Ac
The flexibility and versatility of our approach toward legionaminic analogues, and the importance of the structural configuration of our 6-deoxy-mannose acceptor substrate 38 is perhaps best shown in Scheme 11. The key intermediate 40, which is a selectively functionalised form of the mannose derivative 38, is not only a potential substrate for Neu5Ac aldolase (after deprotection) but forms a branching point in our
approach toward other legionaminic acid-based analogues (Scheme 11). Through further protection and deprotection chemistry, the key intermediate 40 has the potential to be transformed into a variety of nonulosonic acids, all of which are legionaminic acid-based analogues. For example, as seen in Scheme 11, C-4 of the key intermediate 40 can be selectively functionalised in order to create compounds like 41 and 42, which could then be deprotected and processed through the aldol condensation reaction to produce C-7 functionalised legionaminic acids, which are exemplified by compounds 43 and 44 in Scheme 11. Additionally, we can also activate the C-2 of our already C-4 functionalised intermediate 41, to produce a C-2 derivative (Scheme 11), which ultimately will allow us, after condensation with Neu5Ac aldolase, to achieve the correct functionality at both C-5 and C-7 of our legionaminic acid analogue 43.

**Scheme 11.** Possible legionaminic and pseudaminic acid analogues that can be created from the key intermediate 40 via a Neu5Ac aldolase catalysed aldol condensation reaction.
Due to the different protecting groups in 40, we can also invert the stereochemistry at C-4 of our key intermediate (40) to produce compound 42, which is a selectively functionalised 6-deoxy-talopyranoside. Once deprotected 42 will be a good acceptor substrate for Neu5Ac aldolase being converted to its corresponding 7-epi-legionaminic acid analogue 44 (Scheme 11). In an analogous manner to 41 (Scheme 11), we could also activate the C-2 of our talopyranoside intermediate 42, to produce a C-2 derivative (Scheme 11), which would then allow us, after condensation with Neu5Ac aldolase, to produce a C-5 and C-7 functionalised compound similar to the legionaminic acid analogue 44. Furthermore, 44 has the potential to be converted into a pseudaminic acid analogue by activation of the C-5 position followed by a displacement reaction which would invert the stereochemistry creating a compound like 45 (Scheme 11).

With a clear picture of our aims for the chemoenzymatic approach toward legionaminic acid analogues now in place, we needed to ensure that all of the selectively functionalised hexose substrates that we intended to prepare would indeed be good substrates for Neu5Ac aldolase before we set out on synthesising each compound. In order to do this we had to turn to the literature and review in detail what was known about the substrate specificity of Neu5Ac aldolase.

2.2 Neu5Ac aldolase substrate specificity

Numerous substrate specificity studies have been conducted using Neu5Ac aldolase. These studies have primarily focused on the use of pentose and hexoses sugars as acceptor substrates,$^{117,119,122,124–126,132–142}$ and pyruvate (28) and its analogues$^{119,143}$ as donors in the synthesis of sialic acids. Furthermore, a large number of studies have looked at the substrate specificity of the forward reaction or cleavage of Neu5Ac derivatives$^{144–154}$. The literature illustrates a clear picture for the mechanism of catalysis for both the forward and reverse reactions catalysed by Neu5Ac aldolase which is shown in Scheme 12.

The mechanism for the transformation of D-ManNAc (34) to Neu5Ac (1), which is the transformation that we are interested in, involves the initial formation of a Schiff
base between the donor (pyruvate (28)) and a lysine residue (Lys 165)\textsuperscript{117,132,155} from the enzyme (Scheme 12). A number of donor molecules have been tested in reactions catalysed by Neu5Ac aldolase, interestingly, only pyruvate (28) is accepted as the nucleophilic component (\(K_m 10 \text{ mM}\))\textsuperscript{119} in order to form the important Schiff base during catalysis. Moreover, it is pyruvate (28) used in excess that is the driving force behind the \textit{in vitro} aldol condensation reactions which has been so readily utilised in chemoenzymatic synthesis, as pyruvate (28) itself competitively inhibits the cleavage of Neu5Ac (1) with a calculated \(K_i\) value of 1.0 mM\textsuperscript{143}. This stringent structural requirement of the donor molecule has been supported by a number of studies\textsuperscript{119,143} that have observed that using C-1, C-2 and C-3 modified derivatives of Neu5Ac (1) typically are not substrates for the enzyme.

Scheme 12. Mechanism for the Neu5Ac aldolase catalysed reversible aldol condensation of D-ManNAc (34) to Neu5Ac (1)
The stringent degree of structural requirements for donor molecules accepted by Neu5Ac aldolase fortunately is not observed for acceptor molecules. The enzyme has been reported to accept a wide variety of hexoses, both D- and L-sugars, as substrates in the synthesis of sialic acids\textsuperscript{117,119,122,124–126,132–143,156–158}. Regarding the aldol condensation reaction of the natural substrate (D-ManNAc (34)) to form Neu5Ac (1) the first three carbons (i.e. C-1, C-2, C-3) of Neu5Ac (1) originate from pyruvate (28), the donor molecule, whereas C-4 to C-9 of Neu5Ac (1) belong to the acceptor molecule D-ManNAc (34) (Scheme 13). With this knowledge, along with the inherent promiscuous nature of the enzyme to acceptor substrates, numerous structurally modified substrates have been synthesised in order to better understand the substrate specificity of Neu5Ac aldolase.

Scheme 13. Neu5Ac aldolase catalysed synthesis and cleavage of Neu5Ac (1)

### 2.2.1 C-1, C-2 and C-3 modified acceptor substrates

To date there has not been a single report regarding the use of C-1 modified acceptor substrates (e.g. C-1 modified derivatives of D-ManNAc (34)) in the synthesis of C-4 modified sialic acids. This is in principal due to the fact that based on results published on the C-4 functionalised derivatives of Neu5Ac (1), it’s clearly apparent that Neu5Ac aldolase has strict structural requirements for substitutions at C-1 of acceptor substrates. Specifically, Neu5Ac aldolase requires good acceptor substrates to be reducing sugars (hydroxyl at C-1), as this is crucial in the mechanism for the formation of the Schiff base intermediate that allows for chain elongation to occur (Scheme 12). Furthermore, numerous studies have shown that indeed C-4 modified derivatives of
Neu5Ac (1), such as 4-deoxy-Neu5Ac are not substrates for the enzyme, but rather inhibitors of Neu5Ac aldolase\textsuperscript{146,147,151}.

Over the past two decades a number of C-2 functionalised hexoses have been tested as substrates for Neu5Ac aldolase\textsuperscript{124–126,132–136,140,157,159}. These studies have shown that replacing the C-2 acetamido (NHAc) group in D-ManNAc (34) with bulky, small, polar or even apolar substitutions has little effect on the enzymes ability to catalyses the aldol condensation reaction. The only limitation that has been reported being that the substituent at C-2 must be axial (i.e. mannose configuration)\textsuperscript{117}. Studies have shown that the enzyme can readily transform D-ManNAc (34)\textsuperscript{117,119}, D-mannose (37)\textsuperscript{117,134}, 2-azido-2-deoxy-D-mannose (46)\textsuperscript{159}, 2-deoxy-2-C-phenyl-D-mannose (47)\textsuperscript{134} and 2-N-thioacetyl-D-mannosamine (48)\textsuperscript{160} into the corresponding C-5 functionalised sialic acids.

Further studies have shown that Neu5Ac aldolase also transforms 2-substituted L-sugars\textsuperscript{117,140} to the analogous L-\textit{glycero} sialic acids but at a rate roughly 100 times lower than the corresponding D-sugars\textsuperscript{140}, which is primarily due to a \textit{re} face attack of the Schiff base in this type of configuration\textsuperscript{117,140,158}.

In contrast to the lack of substituent specificity at C-2, the presence of a free hydroxyl group at C-3 in the hexose or pentose substrate is essential for enzymatic transformation by Neu5Ac aldolase\textsuperscript{117}. Certain studies have shown that the stereochemistry at C-3 of the acceptor substrate directs the orientation of the enamine attack during the enzymatic aldol condensation\textsuperscript{117,158}. Furthermore, results have shown sugars with a C-3(S) configuration are attacked from the \textit{si} face to give C-4(S) configured products\textsuperscript{117}. For example, D-ManNAc (34) and D-mannose (37) following
exposure to Neu5Ac aldolase gave Neu5Ac (1) and KDN (3) in excellent overall yields respectively\textsuperscript{117,140}.

Therefore based on these finding it is essential for good substrates of Neu5Ac aldolase to be reducing sugars (C-1 hydroxyl), have a C-2 axial substituent and have a C-3 hydroxyl group present in an equatorial conformation, if they are to be efficiently processed by the enzyme.

2.2.2 C-4, C-5 and C-6 modified acceptor substrates

Chemical modification of nonulosonic acids at C-7 generally have proven to be quite difficult, primarily due to the steric hindrance around C-7\textsuperscript{161}. For this very reason, Neu5Ac aldolase has proven to be an invaluable tool in catalysing the transformation of C-4 functionalised hexopyranosides to the corresponding C-7 substituted nonulosonic acids. Numerous studies have reported the use of Neu5Ac aldolase in transforming compounds such as 4-deoxy-D-ManNAc (49)\textsuperscript{122}, 4-O-methyl-D-ManNAc (50)\textsuperscript{122}, 4-deoxy-D-mannose (51)\textsuperscript{126,134} and D-talose (36)\textsuperscript{117,140} to the corresponding 7-substituted sialic acids, 7-deoxy-Neu5Ac (52), Neu5Ac7Me (53), 7-deoxy-KDN (54) and 7-epi-KDN (55), respectively. Neu5Ac aldolase has also been reported to transform L-talose (56) to the corresponding 7-epi-L-KDN (57) at a predictable lower rate in contrast to D-talose (36)\textsuperscript{117,140}.

Indeed Neu5Ac aldolase has been shown to tolerate a wide variety of C-4 modified hexopyranosides, however studies carried out to date do not reveal the enzymes ability to accept bulky substituents at C-4 nor do they show the outcome of using substrates with sp\textsuperscript{2} hybridisation (e.g. presence of alkenyl and oxo-functionalities) or the presence of various functional groups (e.g. containing nitrogen or sulphur) at the C-4 position in regard to substrate specificity.
Further studies have shown that Neu5Ac aldolase is also able to catalyse transformations of C-5 functionalised substrates. One particular paper\textsuperscript{117} has shown that exposure of 5-deoxy-D-ManNAc and 5-deoxy-D-mannose to Neu5Ac aldolase and excess pyruvate (28) produces 8-deoxy-Neu5Ac and 8-deoxy-KDN in 75% and 81% yield, respectively. Furthermore, L-gulose is also a substrate for the enzyme being transformed into the corresponding 8-epi-KDN\textsuperscript{117,140}.

Hexopyranosides modified at C-6 are generally well accepted by Neu5Ac aldolase, producing the corresponding nine-substituted nonulosonic acids after catalysis\textsuperscript{117}. Reports have shown esterification of the 6-hydroxyl group in D-ManNAc (34) to a 6-O-acetate derivative 58\textsuperscript{119,142,156} produced a substrate that was freely transformed to the corresponding nine-substituted sialic acid in 84% yield\textsuperscript{119}. Moreover, the 6-azido-6-deoxy-derivative 59, 6-deoxy-6-fluoro-derivative 60, 6-O-(dimethylphosphinyl)-D-ManNAc (61)\textsuperscript{156}, 6-O-methyl-D-ManNAc (62) and D-rhamnose (35)\textsuperscript{117,139} were all transformed by the enzyme.
Despite the many reports into the substrate specificity of Neu5Ac aldolase, there remains scope to further explore the exploitation of this enzyme to aid the synthesis of some of the more interesting and clinically important nonulosonic acids found in bacteria. The work described in this thesis (vide infra) will focus on our efforts toward using Neu5Ac aldolase to prepare key building blocks toward the synthesis of some of the 9-deoxy nonulosonic acids, such as legionaminic acids.

2.3 Synthesis of selectively functionalised D-rhamnopyranoside derivatives as acceptor substrates for Neu5Ac aldolase

With a detailed knowledge of the stereochemical and functional group requirements needed for catalysis by Neu5Ac aldolase, we could begin the synthesis of appropriate hexose substrates that could be used in the synthesis of legionaminic acid analogues.

Based on the data presented above, D-mannose (37) was selected as the starting material as it contained all the stereochemical requirements of a good acceptor substrate for Neu5Ac aldolase. It is a reducing sugar at C-1, has a C-2 axial functional group and also has an equatorial hydroxyl at C-3, which are essential structural requirements of Neu5Ac aldolase substrates. Therefore we intended to start with D-mannose (37) and selectively functionalise this important carbohydrate to produce a compound similar to the key intermediate 63 (Figure 16), which would allow us considerable flexibility in the types of legionaminic acid analogues that we could produce.

As can be seen in Figure 16 the key intermediate 63 is a tris-protected 6-deoxy-mannopyranoside. 6-Deoxy-D-mannopyranoside compounds are commonly referred to as D-rhamnopyranosides. Aside from the differentially protected hydroxyl groups, the
key intermediate 63 contains an important functionality in the form of the 6-deoxy group (in blue, Figure 16). This is important as the C-9 of all known legionaminic acid compounds is a methyl functionality and therefore, by introducing this group into the mannoside structure of 63 early in the synthesis, we ensure that all the legionaminic acid analogues produced by our chemoenzymatic approach will have the correct functionality at C-9.

![Figure 16. Functionality changes to D-mannose (37) to create the key intermediate 63](image)

The following sections describe our approach towards compounds like the protected key intermediate 63, which was prepared from D-mannose (37), and discusses the types of protecting group chemistry that we utilised in order to enable flexibility in the chemistry that would be carried out on this important compound.

### 2.3.1 Synthesis of para-methoxyphenyl rhamnopyranosides

The initial work undertaken towards the synthesis of compounds similar to the key intermediate 63 was largely based on work reported by Fauré and colleagues in 2007[^62], whereby they designed an efficient method to synthesise the p-methoxyphenyl rhamnopyranoside 64 in 5 steps (Scheme 14). Using this work as a template, the initial aim of our synthesis was to put in place the robust glycoside, the p-methoxyphenyl functionality.

The first step towards introduction of the p-methoxyphenyl glycoside (OpMP) involves a relatively straightforward and high yielding acetylation of D-mannose (37), to form the per-O-acetyl-α-D-mannopyranoside 65 (Scheme 14). The reaction was carried out by dissolving D-mannose (37) in pyridine, to which acetic anhydride was added.
The resulting reaction mixture was stirred overnight at room temperature before being worked-up using an aqueous acidic wash (1 N HCl) and purified using column chromatography (Hex/EtOAc 3:1, Rf = 0.5). The $^1$H NMR spectrum of 65 showed five, three proton singlets in the region $\delta$ 2.0-2.2 ppm, consistent with the presence of five acetyl groups.

**Scheme 14.** Published conversion of D-mannose (37) to the p-methoxyphenyl rhamnopyranoside 64 by Fauré $^{162}$.

Compound 65 was then reacted with p-methoxyphenol in the presence of an acid (triflic acid) to form the p-methoxyphenyl glycoside 66 in high yield (98%) (Scheme 15). The formation of the p-methoxyphenyl $\alpha$-D-mannopyranoside 66 was easily confirmed by examination of the $^1$H NMR spectrum, which showed the appearance of a para-substituted benzene ring splitting pattern in the aromatic region ($\delta$ 6.8-7.1 ppm) and a three proton singlet of the methoxy-group at $\delta$ 3.76 ppm, which supported the presence of the newly introduced glycoside. Further inspection of the $^1$H NMR spectrum of 66 also showed that the coupling between H-1 and H-2 was 1.8 Hz, which is consistent with an $\alpha$-configured mannoside $^{163}$ (Scheme 15).
Reagents and conditions: (a) Pyridine/\text{Ac}_2\text{O}, \text{cat. DMAP, rt, 15 hrs, quantitative (α only)}; (b) \text{p-MPOH, TfOH, CH}_2\text{Cl}_2, 0ºC then rt, 15 hrs, 98% (α only); (c) \text{MeOH, 1 N NaOH, rt, 15 hrs, quantitative}.

Scheme 15. Synthesis of the p-methoxyphenyl glycoside 67 from D-mannose (37)

Deprotection of the acetate groups in 66 was the next step, as we required the acetylated groups in 66 to be removed if we were to selectively functionalise C-2 through C-6. To achieve this step, 66 was treated with a basic methanol solution (~12 pH) overnight. The resulting deprotected p-methoxyphenyl α-D-mannopyranoside (67) (Scheme 15) was purified using column chromatography (EtOAc, \( R_f = 0.5 \)) and characterised by NMR. The \(^1\text{H NMR spectrum of 67 showed that along with the disappearance of the five acetate peaks between } \delta \text{ 2.0-2.2 ppm compared with 66, the H-2, H-3, H-4 and the geminal proton (H-6/H-6a) signals had all moved upfield } \sim \delta \text{ 1.0 ppm which would be expected if the acetate groups had been successfully removed}}^{164}. \text{Furthermore a mass spectrum of 67 showed a molecular ion peak of } m/z \text{ 309.1 } [\text{M + Na}]^+, \text{which is consistent with the molecular formula of 67 (C}_{13}\text{H}_{18}\text{O}_7].$

The three initial steps of our synthesis were largely quantitative in yield, requiring essentially only one purification step at the end once compound 67 was obtained. With the p-methoxyphenyl glycoside of D-mannose (67) in hand, our next step was to reduce the C-6 hydroxyl group to give the target methyl group. Whilst there are several possible ways to bring about this transformation, we opted for an approach based on the
idea of introducing a good leaving group at the primary hydroxyl of C-6 (most reactive portion of compound 67), which could then be reduced to the required methyl functionality.

Initially, the leaving group we chose was a tosylate (OTs) functionality which should then be reduced by a good reducing reagent such as lithium aluminium hydride (LAH) (Scheme 16). Our initial reactions showed that the tosylate group could be introduced in 67 in an acceptable yield (~67%) to give compound 68 (Scheme 16). Evidence that we had indeed succeeded in putting the tosylate at C-6 in 67 was seen in the $^1$H NMR spectrum of 68, which showed a second para-substituted aromatic ring signal downfield from that of the $p$-methoxyphenyl signal between ~ $\delta$ 7.2-7.8 ppm and also a sharp three proton singlet at $\delta$ 2.37 ppm (tosyl-methyl group) and a downfield shift of ~$\delta$ 0.4 ppm (compared with 67) of the C-6 methylene protons attached to the newly added sulfonate group.

![Scheme 16](image)

Reagents and conditions: (a) Pyridine, $p$-TsCl, 0ºC then rt, 2 hrs, 67%;
(b) THF, LAH, rt, 2hrs, <20%.

Scheme 16. Proposed synthesis of 64 from 67 via a 6-$O$-tosyl intermediate 68

Once we were sure the tosylate group had been placed at C-6 in 67 to produce 68 (Scheme 16), we could attempt the reduction step in order to obtain our first D-rhamnopyranoside derivative 64. Unfortunately, the attempted reduction of the tosylate at C-6 in 68 using LAH proved to be problematic. The general method used was one that is well documented in the literature$^{165}$, using THF as a solvent to which LAH is added. However, several attempts toward obtaining the desired D-rhamnoside derivative 64, gave poor yields (<20%) of the target compound. The primary reason for this poor yield was probably due to the fact that both 68 and 64 have three secondary hydroxyl groups which coordinate to aluminium salts produced during the reaction, thereby creating a hard to separate complex of sugar and aluminium salts which would
lower the overall yields of product after work-up procedures. After some experimentation including the use of a mesylate group at C-6 instead of a tosylate, it was decided due to low yields, difficult work up and purification steps that the approach via reduction of a tosylate using LAH was an inefficient avenue into D-rhamnosides.

Based on the work originally cited by Fauré\textsuperscript{162} and the lessons learnt over the time spent attempting to reduce the C-6 sulfonate, it was decided that we would attempt to directly introduce an iodo-functionality at C-6 in 67 and reduce this group with a hydride reducing reagent. A routine reaction used in order to introduce iodine to a primary hydroxyl within a hexose compound was first established by Garegg and colleagues in the late 1970’s\textsuperscript{166}. The reaction utilises triphenylphosphine, imidazole and iodine, creating the desired alkyl halide and triphenylphosphine oxide as a by-product.

With this knowledge in hand we proceeded to attempt the reaction of introducing iodine to the C-6 position of our p-methoxyphenyl glycoside compound 67, using the Garegg reaction\textsuperscript{166}. The reaction itself can be considered to proceed as shown in Scheme 17, whereby exposure of the electrophilic triphenylphosphine-iodide complex (\( \text{Ph}_3\text{PI} \)) (formed \textit{in situ}) to the primary alcohol (at C-6) in 67 in the presence of base (in this case imidazole) produces a sugar-phosphine intermediate (seen in square brackets, Scheme 17). The methylene carbon at C-6 in the sugar-phosphine intermediate being electron deficient is then attacked by iodide generating the 6-deoxy-6-iodo product 69 releasing triphenylphosphine oxide in the final step (Scheme 17)\textsuperscript{166}.

This reaction proved to be very efficient, producing 69 in consistently high yields (>90%). The evidence that we were able to introduce the iodo functionality at C-6 in 67 was most notably seen in the \(^{13}\text{C} \) NMR spectrum of 69 with the large upfield shift of the C-6 methylene signal from \( \delta \) 61.2 ppm in 67 to \( \delta \) 7.3 ppm in 69, indicating the expected\textsuperscript{164} shielding effect on the carbon bearing the iodo functionality.

However, the purification of 69 proved to be particularly difficult due to the fact that 69 is again a triol-sugar which lacks the required polarity difference with the triphenylphosphine oxide produced in the iodination reaction, making it impossible to separate the two compounds using column chromatography. Numerous methods were attempted to obtain compound 69 without the presence of triphenylphosphine oxide in the sample, however, with no success.
Despite the presence of triphenylphosphine oxide contaminating our sample of 69, reduction of the 6-iodo group was initially attempted based on work cited by Fauré and colleagues, using tributyltin hydride (Bu₃SnH) in toluene at 110 ºC for 1 hour with 0.1 eq. of AIBN. Although Fauré quoted a yield of 98% for the same transformation, we found after numerous attempts that the best yield we could obtain was always less than 50%. The reasons for this poor yield were hard to ascertain, however it was thought that maybe the triphenylphosphine oxide contaminating the sample was interfering in the reaction and therefore lowering the overall yield. Therefore, we attempted a LAH reduction of the iodo group in 69, which improved the yield of 64 (~60%), however once again the work up for this reaction was difficult. After considerable experimentation we decided to abandon the use of hydride as a reducing agent in our synthesis.

Based on the idea that the p-methoxyphenyl glycoside in 69 should be robust enough to withstand a hydrogenation reaction, the next iodide reduction method attempted was to react 69 in the presence of palladium hydroxide on carbon (Pd(OH)₂/C (20% wt.)) as a catalyst and a base N,N-diisopropylethylamine (EtNPr₂) under
hydrogenation conditions to obtain the desired D-rhamnopyranoside derivative 64\(^{111}\) (Scheme 18). This reaction proved to be very efficient, running overnight at room temperature and 40 psi on a hydrogenator, giving a near quantitative yield (based on molar mass calculations taking into account eq. of PPh\(_3\) used in the reaction) of the desired \(p\)-methoxyphenyl rhamnopyranoside 64. The \(^1\)H NMR spectrum of the rhamnopyranoside 64 provided evidence that we had indeed reduced C-6 of 69 to a methyl group by the presence of a three proton doublet at \(\delta\) 1.25 ppm which showed correlation to H-5 \((J = 6.3\) Hz\) in the COSY spectrum. The \(^{13}\)C NMR spectrum of 64 also showed a methyl group carbon at \(\delta\) 17.5 ppm, consistent with the newly introduced methyl group at C-6.

![Scheme 18. Synthesis of 64 from 67 via a 6-deoxy-6-ido intermediate 69](image)

**Reagents and conditions:** (a) I\(_2\), PPh\(_3\), imidazole, THF reflux, 2hrs, >90%; (b) MeOH, Pd(OH)\(_2\)/C (20% wt.), Et\(_2\)NPr\(_2\), H\(_2\)(g) at 40 psi, 15 hrs, quantitative.

Having successfully created our desired rhamnopyranoside 64 in excellent overall yield from D-mannose (37), we felt it important to explore methods for the removal of the \(p\)-methoxyphenyl glycoside. Literature precedent\(^{167,168}\) states that a \(p\)-methoxyphenyl glycoside is particularly robust to most reaction conditions, and generally is removed by a radical generated reaction using cerium ammonium nitrate (CAN) in a mixture of CH\(_3\)CN and water (Scheme 19). However, initial attempts to remove the \(p\)-methoxyphenyl glycoside in 64 under such conditions proved to be problematic. After considerable optimisation of the reaction conditions and the use of a high quality cerium ammonium nitrate we were indeed able to remove the \(p\)-methoxyphenyl glycoside in 64 to produce D-rhamnose (35) in a 98% yield based on recovered starting material. Unfortunately, however this reaction remained problematic as the yields over numerous reactions were inconsistent, only on occasion producing the
high yields reported in the literature\textsuperscript{162,167,168}. Therefore, it was decided that possibly an alternative glycoside should be used, one which was robust but still could be easily removed under specific reaction conditions. Therefore we decided to pursue an alternate strategy.

Reagents and conditions: (a) CH\textsubscript{3}CN:H\textsubscript{2}O (4:1), CAN, rt, 1 hr.

Scheme 19. Deprotection of 64 using cerium ammonium nitrate to produce D-rhamnose (35)

2.3.2 Synthesis of benzyl α-D-rhamnopyranosides

Despite the problems we had encountered with the \( p \)-methoxyphenyl glycoside work discussed in Section 2.3.1, the essence of our approach converting D-mannose (37) into functionalised D-rhamnopyranoside derivatives was still an attractive option. To avoid some of the problems with removing the \( p \)-methoxyphenyl glycoside, but maintaining a chemically robust functionality, we opted to use a benzyl glycoside in our revised strategy.

To form a benzyl glycoside of D-mannose (37), we reacted benzyl alcohol together with 37 in the presence of an acid catalyst (acetyl chloride) following a method described by Winnik \textit{et al.}\textsuperscript{163}. A mechanism for the formation of the benzyl \( \alpha \)-D-mannopyranoside (70) using this approach is shown in Scheme 20.

Initially there were some problems encountered with the isolation of the benzyl \( \alpha \)-D-mannopyranoside (70) from this reaction, mostly due to the fact that benzyl alcohol has a very high boiling point. Ultimately we found that the optimum method for obtaining the benzyl \( \alpha \)-D-mannopyranoside (70) involved dissolving the reaction mixture in water, diluting it with ethyl acetate (EtOAc), before successively washing the organic phase with water, concentrating the combined aqueous phases under reduced
pressure, and then placing the residue under high vacuum. The crude residue was subsequently recrystallised (hot EtOAc), to give the pure benzyl α-D-mannopyranoside (70) in 92% yield, an improved outcome compared with Winnik et al.163,169.

Scheme 20. Mechanism for the formation of benzyl α-D-mannopyranoside (70), using benzyl alcohol and the acid catalyst acetyl chloride.

Evidence that we had indeed produced the benzyl glycoside of 37 came from examination of the 1H NMR spectrum of 70, which clearly showed the addition of a benzyl glycoside by the presence of a five proton aromatic multiplet at δ 7.24-7.36 ppm, and also a methylene AB spin system centred at δ 4.57 ppm. The coupling constant between H-1 and H-2 was 1.8 Hz, which is indicative of the α-glycoside in mannopyranosides and comparable to that published by Winnik et al.163

Having satisfactorily obtained the benzyl α-D-mannopyranoside (70) in high yield the next step was to reduce the C-6 hydroxyl to the required 6-deoxy functionality. This was achieved in an analogous manner to that described in our previous synthesis (see section 2.3.1), and involved reacting 70 with triphenylphosphine and iodine in the presence of imidazole to yield the benzyl 6-deoxy-6-iodo-α-D-mannopyranoside (71) in 95% yield (Scheme 21). Evidence that we had successfully introduced the iodo-functionality to C-6 could be seen in the 13C NMR spectrum of 71, by the upfield shift of the C-6 methylene group from δ 60.7 ppm in 70 to δ 7.4 ppm in 71, indicating the strong shielding effect that the iodo-functionality has on the C-6 methylene carbon164.
Reagents and conditions: (a) I₂, PPh₃, imidazole, THF reflux, 2hrs, 95%; (b) MeOH, Pd(OH)₂/C (20% wt.), EtNPr₂, H₂(g) at 40 psi, 15 hrs, quantitative.

Scheme 21. Synthesis of benzyl α-D-rhamnopyranoside 72 from 70 via the 6-deoxy-6-iodo intermediate 71

Once we were happy that we had indeed produced 71, the crude reaction mixture was dissolved in methanol to which Pd(OH)₂/C (20% wt.) and EtNPr₂ were added and placed on a hydrogenator overnight at room temperature and 40 psi of hydrogen gas (Scheme 21). It is well documented¹⁰,¹¹,¹¹³ that reduction of the iodo group without loss of the benzyl glycoside can be achieved under these basic conditions. Once the reaction was complete, the resulting mixture was filtered through Celite and concentrated under reduced pressure to produce the benzyl α-D-rhamnopyranoside (72) in a quantitative yield. Compound 72 was characterised by ¹H NMR spectroscopy, with the spectrum showing the presence of a three proton doublet at δ 1.26 ppm which showed correlation with H-5 (J = 6.3 Hz) in the COSY spectrum, indicating that C-6 was now a methyl functionality.

Our synthesis of the benzyl α-D-rhamnopyranoside (72) in 3 steps from D-mannose (37) in an overall yield greater than 90% is, to our knowledge, the most efficient synthesis of a D-rhamnopyranoside reported to date and resulted in a publication of our results in *Tetrahedron Letters*¹⁶⁹. Having successfully prepared the rhamnose derivative 72, we felt it was valuable to investigate methods for the removal of the benzyl glycoside. This would serve two purposes, namely determine appropriate conditions to remove the benzyl glycoside, but also provide us with a four-step synthesis of D-rhamnose (35) from D-mannose (37). Our first attempts at removing the benzyl glycoside from 72 involved the use of a well-documented hydrogenation reaction¹¹¹. Compound 72 was taken up in ethanol to which Pd/C (10% wt.) and a few drops of acetic acid were added. This mixture was placed on a hydrogenator at room
temperature and 40 psi of hydrogen gas for 48 hours and after filtration through Celite to remove the catalyst produced D-rhamnose (35) in a quantitative yield (Scheme 22).

\[
\begin{align*}
(72) & \xrightarrow{(a)} (35) \ \alpha/\beta \text{-D-rhamnose}
\end{align*}
\]

*Reagents and conditions:* (a) EtOH, Pd/C (10% wt.), AcOH, H\textsubscript{2}(g) at 40 psi, 48 hrs, quantitative.

**Scheme 22.** Hydrogenation reaction on 72 to produce D-rhamnose (35).

This reaction proved to be excellent in most instances, however we found that upon scale up (reactions >2.0g) the conversion of 72 to D-rhamnose (35) was inconsistent compared with earlier attempts. After numerous attempts to rectify the issue on a larger scale we decided to abandon the use of the hydrogenation reaction as our preferred method for removing the benzyl glycoside in 72. Benzyl ethers are known to be acid labile and therefore we decided to attempt to remove our benzyl ether protecting group using acidic resin in water as a solvent. To this end, 72 was dissolved in water to which a sufficient amount of Dowex 50W H\textsuperscript{+} resin was added and stirred at 80°C for 24 hours. After filtering off the resin the filtrate was concentrated under reduced pressure to produce the desired D-rhamnose (35) in a quantitative yield. This reaction was performed on scales of up to 10 grams and we found that the overall outcome was always consistent producing 35 in a 100% yield. Evidence that we had indeed created our desired D-rhamnose (35) was best seen in the \(^1\)H and COSY spectra, clearly showing the presence of \(\alpha\) and \(\beta\) anomers. The \(^1\)H NMR spectrum of 35 showed that there were two H-1 proton signals (\(\delta\) 4.75 (\(\beta\)) and \(\delta\) 5.00 (\(\alpha\))) and also two C-6 methyl group signals at \(\delta\) 1.16 ppm (\(\alpha\)) and \(\delta\) 1.18 ppm (\(\beta\)) (Figure 17). Inspection of the HSQC and \(^{13}\)C NMR spectrum of 35 confirmed that we had indeed removed the benzyl glycoside to produce the two anomers \(\alpha\) and \(\beta\) D-rhamnose (35) via an efficient four-step process from D-mannose (37).
2.3.3 Synthesis of the 6-deoxy-hexopyranosides 73 and 75

Having established an efficient synthesis of 72 we looked at using this rhamnopyranoside to create selectively functionalised C-4 derivatives. Such C-4 rhamnosides could provide access to the corresponding C-7 functionalised legionaminic acid analogues using Neu5Ac aldolase. As can be seen in Scheme 23, 73 is a selectively protected benzyl 4-azido-4-deoxy-α-D-rhamnopyranoside which once deprotected can be enzymatically converted into the corresponding novel 7-azido-5-hydroxy-legionaminic acid analogue (74). Similarly, 75 is a benzyl 4-azido-4,6-dideoxy-α-D-talopyranoside, which after deprotection can be enzymatically converted into the corresponding 7-epi-7-azido-5-hydroxy-legionaminic acid analogue (76) (Scheme 23).
Scheme 23. Proposed enzymatic conversion of C-4 functionalised substrates 73 and 75 into legionaminic acid analogues 74 and 76

In order to create our desired 6-deoxy-hexopyranosides 73 and 75 starting from the benzyl α-D-rhamnopyranoside 72 we needed to create a versatile key intermediate which would form a branching point in our synthesis and allow us to produce the two C-4 modified 6-deoxy-hexopyranosides efficiently. In order to achieve this we felt it was necessary to protect both C-2 and C-3 simultaneously in order to leave the C-4 hydroxyl the only unprotected hydroxyl group. This was achieved by reacting 72 in acetone and 2,2-dimethoxypropane (1:1 ratio) in the presence of an acid catalyst (p-toluenesulfonic acid monohydrate)\(^{162}\) producing the 2,3-O-isopropylidene protected 77 (key intermediate) in a 98% yield after chromatography (Scheme 24). With the synthesis of 77 we had indeed achieved our goal of efficiently producing the key intermediate compound that is analogous to 40 as shown in Scheme 11, Section 2.1.

\[72\] \[\text{Reagents and conditions: (a) 2,2-DMP/acetone (1:1), p-TsOH\cdot H_2O, rt, 0.5 hrs, 98%}.\]

Scheme 24. Synthesis of 77 from 72.
Evidence that we had indeed produced our new key intermediate 77 was seen by examination of the NMR spectra, which showed a broad hydroxyl proton peak at δ 2.57 ppm in the $^1$H spectrum, which showed correlation with H-4 ($J = 4.5$ Hz) in the COSY spectrum and was also exchangeable with D$_2$O. The $^1$H NMR spectrum of 77 also showed the coupling between H-2 and H-3 had increased from $J = 3.3$ Hz in 72 to 5.7 Hz in 77, which would be expected if a 2,3-O-isopropylidene group were to be placed at carbons-2 and 3, as this group would make the d-rhamnoside ring more rigid, decreasing the angle between the two protons$^{164}$. Lastly, the $^1$H NMR spectrum of 77 also showed the appearance of two sharp three proton singlets at δ 1.36 and 1.54 ppm, providing further evidence that two additional methyl groups associated with the isopropylidene protecting group had been added to 72.

The selectively protected key intermediate 77 was now ready for C-4 modification in the form of introducing a nitrogen functionality, which would provide access to C-7 functionalised legionaminic acid analogues. Initially it was decided that displacement of a sulfonate group in the form of a triflate (trifluoromethane sulfonate) leaving group at C-4 in the key intermediate 77 with an azide nucleophile would produce the desired 4-epi-product having a d-talopyranoside configuration.

Introduction of the triflate functionality into 77 was achieved in an 80% yield using triflic anhydride in the presence of pyridine (Scheme 25). Evidence that we had prepared the desired product 78 was best seen by examining the $^{13}$C NMR spectrum, where a downfield shift of C-4 from δ 74.5 to δ 89.2 ppm was seen compared with 77, which indicated the presence of a strong electron withdrawing group attached to C-4. Notably, the $^{13}$C NMR spectrum of 78 also showed a distinct quaternary carbon quartet splitting pattern at δ 120.5 ppm with a coupling of 317 Hz, which is consistent with the CF$_3$ carbon being split due to coupling to the three fluorine nuclei$^{164}$. Furthermore a mass spectrum obtained for 78 showed a molecular ion peak of m/z 449.5 [M +Na]$^+$, which is consistent with the molecular formula of 78 (C$_{17}$H$_{21}$F$_3$O$_7$S).
**Reagents and conditions:** (a) CH$_2$Cl$_2$, Pyridine, Tf$_2$O, -78°C 10 min, then 0°C 30 min, 80%.

**Scheme 25.** Synthesis of 78 from the key intermediate 77.

With the synthesis of 78 accomplished, we were now ready to attempt the displacement reaction using sodium azide (NaN$_3$). The general method for this type of SN2 displacement reaction is well documented in the literature$^{170}$, typically employing the use of N,N-DMF as a solvent to which the nucleophilic azide is added. Our initial attempts at displacing the C-4 sulphonate group however proved to be problematic. Several attempts were made, however these attempts failed to produce the desired 4-azido-talopyranoside in any significant amount. Typically these attempts to displace the C-4 triflate in 78 with azide produced a complex mixture of products.

We considered steric hinderance may have been causing problems (*vide infra*), but we found it difficult to understand the complexity of the crude reaction mixture. However, inspection of the literature showed that others had encountered similar complexity when attempting to displace C-4 sulfonates in manno-configured suagrs.$^{171,172}$ These reports$^{171,172}$ describe the formation of furanoside products upon treatment of the C-4 sulfonate pyranoside with a nucleophile, via a ring contraction as summarised in Scheme 26. The complexity of our crude reaction mixture precluded us from determining if we too had formed furanoside products.
Scheme 26. Potential mechanism for the ring contraction and formation of furanoside products when displacing a C-4 sulfonate in manno-configured sugars.\textsuperscript{171,172}

Further literature precedence\textsuperscript{179–182} indicated that displacement of C-4 sulfonates within D-mannose configured sugars has been shown to be problematic in regard to controlling the configuration of products in both D- and L-mannose sugars. Given these circumstances and the outcome of our initial attempts to displace the C-4 sulfonate in 78 it was decided that removing the 2,3-O-isopropylidene group prior to the sodium azide displacement reaction could be advantageous, as this may reduce the rigidity of the compound and may help in possibly overcoming the ring contraction issues.

Towards this end, the crude reaction mixture of 78 was stirred in aqueous 80% acetic acid at 80°C for 2 hour producing the diol-compound 79 directly in 93% yield over the two steps from 77 after purification (Scheme 27).

Reagents and conditions: (a) CH\textsubscript{3}Cl\textsubscript{2}, Pyridine, Tf\textsubscript{2}O, -78°C 10 min, then 0°C 30 min; (b) aqueous 80% acetic acid at 80°C for 2 hour, 93% over two steps.

Scheme 27. Synthesis of 79 from 77.

Evidence that we had indeed produced the 4-O-triflate diol product 79 was best seen upon examination of the NMR spectra, which showed a broad hydroxyl peak at ~δ 2.65 ppm, which integrated to two protons in the \textsuperscript{1}H spectrum, showed correlation with C-2 and C-3 in the COSY spectrum and was also exchangeable with D\textsubscript{2}O. The \textsuperscript{13}C NMR spectrum of 79, as expected was similar to that of 78, showing a downfield
shift of C-4 from δ 74.5 to δ 88.5 ppm which again indicated the presence of a strong electron withdrawing group attached to C-4. As seen in 78, the $^{13}$C NMR spectrum of 79 also showed a distinct quaternary carbon quartet splitting pattern (δ 121.5 ppm) with a coupling of 317 Hz which is consistent with the CF$_3$ carbon being split due to coupling to the three fluorine nuclei$^{164}$. The mass spectrum of 79 supplied further evidence that we had successfully removed the isopropylidene group showing a m/z $[M + Na]^+$ of 409.1 which was consistent with the molecular formula (C$_{14}$H$_{17}$F$_3$O$_7$S).

With 79 now available for azide displacement, an examination of the literature showed numerous papers had been published$^{171-176}$ particularly looking at the displacement reaction outcomes of sulfonates at C-4 in manno-configured sugars. In many of these papers it was noted that when attempting to displace a good leaving group, such as a sulfonate, at C-4 in a manno-configured sugar two common products were formed. Firstly the desired product which has a D-talo-configuration (C-4 axial), however this could be either in a pyranose or furanose configuration and a minor product, which greatly depended on what functionality is present at the neighbouring carbon-3. In work published by Cicero et al.$^{174}$ it was observed that when both the C-2 and C-3 carbons bore a free hydroxyl group the sulfonate displacement reaction produced both the desired C-4 inverted product (D-talo-configuration) and a C-4 retention product, bearing the newly introduced nucleophile in the original D-manno-configuration (Scheme 28). To account for this unexpected retention of configuration product Cicero et al.$^{174}$ suggested that displacement of the C-4 sulfonate initially resulted in two products, one major and one minor. Firstly, the major product was the direct SN2 displacement product the desired D-talo-sugar. The minor product was proposed to be a 3,4-anhydro-sugar, containing a oxirane ring (epoxide) functionality between C-3 and C-4, which under the reaction conditions would then lead to the retention configured D-manno-sugar (Scheme 28).
Scheme 28. The C-4 sulfonate displacement reaction as described by Cicero et al.\textsuperscript{174}

Importantly, what these studies showed was that by removing the isopropylidene-protecting group in 78 to produce 79, we could potentially create both our desired talopyranoside 75 in good yield along with the equally desirable rhamno-configured product 73, after the azide displacement reaction.

Our initial attempts at introducing the desired C-4 azide functionality involved exposure of the triflate 79 to an excess of sodium azide under a variety of standard azide displacement conditions (e.g. \textit{N},\textit{N}-DMF as a solvent; temperatures ranging from room temperature to 80°C; varying reaction times up to 24 hours). In all instances 79 failed to react, with the C-4 triflate being recovered quantitatively.

Interestingly, carrying out the azide displacement reaction on 79 at 0°C (\textit{N},\textit{N}-DMF; 5 eq. NaN\textsubscript{3}), the desired product 75 was obtained along with a second product that was very similar in \( R_f \). Acetylation of the crude reaction mixture resulted in a change in \( R_f \) on T.L.C. (see Figure 18) that allowed the two components to be separated chromatographically. In this way, the second product from the azide displacement was identified as the 2-\textit{O}-acetyl-3,4-anhydro derivative 80 (obtained in a
35% yield), with the major product being the acetylated 4-azido-4,6-dideoxy-taloside 81 (obtained in a 60% yield).

Figure 18. TLC plate comparison showing the two products formed from the azide displacement reaction of 79 before and after acetylation (1:1 Hex/EtOAc).

Confirmation of the structure shown in Figure 18 was obtained from examination of the spectroscopic data for 81, the $^1$H NMR spectrum showed that the coupling constant between H-4 and H-5 had changed from $J = 9.6$ Hz in 79 to $J = 1.2$ Hz in compound 81, indicating a change in the angle between the hydrogens which would be expected if the H-4 substituent was now axial. The $^1$H NMR spectrum of 81 also showed two, three proton acetyl peaks at $\delta$ 2.11 and 2.22 ppm. Furthermore, IR data recorded for 81 showed a sharp peak at 2111 cm$^{-1}$ which is indicative of an azide functionality$^{164}$. Other spectroscopic data that supported the fact we had indeed produced 81 was a mass spectrum that showed a molecular ion peak of $m/z$ 386.1 [M + Na]$^+$, which was consistent with the molecular formula C$_{17}$H$_{21}$N$_3$O$_6$.

Evidence that we had indeed created the 3,4-anhydro sugar 80 came first from the IR spectrum, which showed no evidence of an azide group within the molecule. Secondly, the mass spectrum obtained from 80 showed a molecular ion peak at $m/z$ 301.1 [M + Na]$^+$, which is consistent with the molecular formula C$_{15}$H$_{18}$O$_5$. The $^1$H NMR spectrum of 80 showed only one three proton singlet at $\delta$ 2.16 ppm belonging to the single acetate at C-2, and compared with 79 H-2 had moved downfield from $\delta$ 4.08 ppm to 4.93 ppm, whilst H-3 had moved upfield from $\delta$ 4.12 ppm to 3.60 ppm;
this suggested that C-2 had now been acetylated and C-3 was in a very different chemical environment compared with 79. The $^{13}$C NMR spectrum of 80 showed that the C-4 signal had moved upfield from $\delta$ 88.5 to $\delta$ 53.3 ppm compared with 79 and the C-3 signal had moved from $\delta$ 69.0 to $\delta$ 49.5 ppm, whilst the C-2 signal remained similar. These chemical shifts indicated that C-3 and C-4 were now part of an epoxide ring\textsuperscript{177}, forming a 3,4-anhydro sugar in a talo-configuration (H-H couplings between H-4 and H-5 = $J_{4,5}$ = 0.9 Hz) (Scheme 29). Having viewed all the data we were now confident that the minor product from the azide displacement reaction was indeed the 3,4-anhydro sugar 80 (Scheme 29).

![Scheme 29](image)

*Reagents and conditions: (a) N,N-DMF, NaN$_3$, 0°C, 1 hr.; (b) Py., Ac$_2$O, rt. 15hrs. 81 (60%), 80 (35%).

**Scheme 29.** Synthesis of the talopyranosides 80 and 81 via an azide displacement reaction

The 3,4-anhydro derivative 80 is a potentially very interesting precursor to a range of D-rhamnose or D-talose compounds, and hence we wondered if we could optimise the yield of 80 from 79. After an examination of the literature and some minor refinement of conditions on our part, we found that stirring a mixture of 79 and one molar equivalents of potassium carbonate (K$_2$CO$_3$) in anhydrous methanol produced the 2-hydroxy-3,4-anhydro-sugar 82 in 100% yield after purification.
2.3.4 Investigations into using 3,4-anhydro-talopyranosides as building blocks

Having produced the 3,4-anhydro-talopyranoside 80 it became obvious that this compound could be a valuable building block. The initial impetus behind utilising 80 was in performing a regioselective ring opening of the oxirane functionality. It was hoped that we could regioselectively open the oxirane ring in order to obtain the double inversion product a 4-azido-4-deoxy-rhamnopyranoside like 73. Attempting to achieve a regioselective ring opening of the chiral epoxide functionality in 80 was seen as an attractive pathway to yield the desired C-4 functionalised rhamnopyranosides which themselves once deprotected could be used as substrates for Neu5Ac aldolase in order to create C-7 functionalised legionaminic acid analogues, as discussed previously (See section 2.3).

Initial attempts at opening the oxirane functionality in 80 via methods described in the literature by Sharpless\(^{178}\) proved to be less than satisfactory. These initial reactions used sodium azide as a nucleophile in the presence of ammonium chloride in a solvent mixture of water and methanol. These exact reaction conditions had been used by Sharpless\(^{178}\) for the regioselective ring opening of 3-propyloxiranemethanol yielding regioselective products in high overall yields. However, after considerable effort we found the reaction either produced no product with 100% recovery of 80 or a very poor yield (<2%) of an unknown compound that was never successfully characterised.

Following these preliminary attempts, we looked at other methods of opening the oxirane ring of 80. We wondered if we could exploit a hard-soft acid/base theory approach, whereby we attempted to use the hard base sodium methoxide (NaOMe) and the soft acid sodium acetate (NaOAc) in order to open the oxirane ring. After several experiments using varying temperatures and differing equivalents of reactants no reaction was seen to occur, with 80 being recovered in all cases.

Based on work described by Jacobsen\(^{179}\) on the asymmetric catalysis of epoxide ring opening reactions we turned away from typical methods in order to open the oxirane functionality in 80. In their *Organic Letters* paper published in 2002, Sabitha et al.\(^{180}\) used cerium (III) chloride (CeCl\(_3\)•7H\(_2\)O) in order to selectively open epoxide functionalities in a number of alkyl epoxides and aziridines. The general reaction conditions used involved CeCl\(_3\)•7H\(_2\)O and NaN\(_3\) (as a nucleophile) in acetonitrile and
water mixture (9:1) producing the corresponding 1,2-azidoalcohols and 1,2-azidoamines in regioselective and high yielding manner\textsuperscript{180}. Unfortunately in our hands the reaction conditions failed to convert \textit{80} into the desired azido-product after using varying reaction times, with \textit{80} being recovered quantitatively in all instances. In another attempt made at opening the epoxide functionality in \textit{80} we reacted \textit{80} in the presence of TBDMSOTf and 2,6-lutidene at room temperature for 30 minutes. Interestingly the outcome from this reaction was a complex mixture of products, with the 2-OTBDMS-3,4-anhydro-sugar \textit{83} being the only compound isolated in sufficient amount for characterisation (refer to Section 5.2.2 for full NMR assignment).

The asymmetric ring opening of epoxides has been thoroughly studied and reported in the literature over the past two decades since influential work carried out by Nugent \textit{et al.}\textsuperscript{181} on catalytic desymmetrisation of \textit{meso}-epoxides using azidotrimethylsaline (TMSN\textsubscript{3}) as a nucleophile first appeared. This important work showed that using a chiral Lewis acid greatly enhanced the regioselective outcome of these types of reactions\textsuperscript{181}.

With this knowledge in hand and based on work published by Banaszek \textit{et al.}\textsuperscript{182,183} for the synthesis of 2,4-diazido-2,4,6-trideoxy-L-hexopyranoses, the use of a Lewis acid (as a catalyst) in conjunction with TMSN\textsubscript{3} (as the nucleophile) appeared to be a mild and highly regioselective method for opening epoxy alcohols particularly in sugars. A postulated benefit of using the Lewis acid is that the epoxide oxygen coordinates to the metal of the Lewis acid, which aids in the regioselective ring opening, stabilising the transitory formation of the carbonium ion, which is attacked by the incoming nucleophile as shown in Scheme 30\textsuperscript{182,183}.  


Scheme 30. Proposed Lewis acid mediated epoxide ring opening mechanism\textsuperscript{182,183}

Literature precedence\textsuperscript{177,184} suggests that the type of substituent at C-2 could have an effect on the direction of attack by the incoming nucleophile, thus affecting overall outcome in regard to yield of the ring opened product. To this end, Rao \textit{et al.}\textsuperscript{184} showed that having a bulky protecting group at C-2 in a 3,4-anhydro-sugar greatly enhanced the C-4 directed attack, thereby improving the yield of the desired double inversion product. With this knowledge in hand we firstly reacted our C-2 acetate protected compound 80 with TMSN\textsubscript{3} and BF\textsubscript{3}•OEt\textsubscript{2} at room temperature for 1 hour. Using these conditions we were able to obtain the desired benzyl 2-O-acetyl-4-azido-4,6-dideoxy-\textalpha-D-rhamnopyranoside 84 in a 70% yield after purification (Scheme 31).

\textit{Reagents and conditions: (a) TMSN\textsubscript{3} (as solvent), BF\textsubscript{3}•OEt\textsubscript{2}, 25°C, 1 hr, 70%}.

Scheme 31. Synthesis of the 4-azido-4-deoxy-rhamnopyranoside 84 from 80.
Evidence that we had successfully opened the epoxide ring in 80 came from examination of the NMR spectra of 84. The $^1$H NMR spectrum of 84 showed that the coupling constants between H-3 and H-4 and H-4 and H-5 were above $J = 9.0$ Hz, which would indicate that the substituent at C-4 of the product from the reaction was in an equatorial configuration consistent with being a manno-configured sugar. Furthermore the H-3 also showed a correlation with a D$_2$O exchangeable hydroxyl proton (δ 2.50 ppm), clearly indicating that the newly formed hydroxyl group from epoxide opening was attached to C-3. Secondly the mass spectrum of 84 showed a molecular ion peak at $m/z$ 344.1 [M + Na]$^+$, which was consistent with the desired product (molecular formula C$_{15}$H$_{19}$N$_3$O$_5$) and lastly the IR spectrum of 84 showed that there was a sharp azide peak at 2109 cm$^{-1}$, which indicated that we had successfully introduced the desired azide functionality.

Having successfully opened the epoxide in 80, we also investigated the use of a more bulky benzoyl ester as a protecting group at C-2. To this end we were able to introduce the desired benzoyl protecting group at C-2 by treating the 2-hydroxy-3,4-anhydro derivative 82 with benzoyl chloride in pyridine at 0ºC over two hours, producing 85 in good overall yield (>90%). Treatment of 85 with TMSN$_3$ and BF$_3$•OEt$_2$ at room temperature for 1 hour afforded the 4-azido-2-O-benzoyl-4-deoxy-rhamnopyranoside derivative 86 in a 60% yield after purification (Scheme 32). We also attempted the epoxide opening reaction of the 2-hydroxy-3,4-anhydro compound 82, however we found that having no protecting group indeed lowered the yield for the epoxide opening reaction producing 73 in a 26% yield (Scheme 32).

Reagents and conditions: (a) TMSN$_3$ (as solvent), BF$_3$•OEt$_2$, 25°C, 1 hr.

Scheme 32. Summary of the epoxide opening reaction with variation at C-2.
2.3.5 Synthesis of deprotected C-4 functionalised 6-deoxy-hexose derivatives

The final step needed in obtaining the desired C-4 functionalised 6-deoxy-hexose sugars for the Neu5Ac aldolase reaction, was to deprotect the two C-4 azido-functionalised compounds 84 and 81.

The benzyl 2,3-O-acetyl-4-azido-4,6-dideoxy-α-D-talopyranoside (81) was firstly deacetylated using sodium methoxide in MeOH at room temperature to yield 75 in a quantitative yield (Scheme 33). The $^1$H NMR spectrum of 75 showed that the coupling constant between H-4 and H-5 was $J = 1.2$ Hz, which indicated that the H-4 substituent was axial. Furthermore, IR data recorded for 75 showed a sharp peak at 2100 cm$^{-1}$ which is indicative of an azide functionality. Other spectroscopic data that supported the fact we had indeed formed 75 was a mass spectrum that showed a molecular ion peak of $m/z$ 302.1 [M + Na]$^+$, which was expected for the 2,3-diol compound 75. With 75 in hand work now turned to removing the benzyl glycoside.

Reagents and conditions: (a) Na$_{aq}$, MeOH, 0°C, 15 min, then rt. for 1 hr; (b) Dowex W50 H$^+$ resin, water (as solvent), 80°C, 24 hrs; quantitative yield over two steps.

Scheme 33. Synthesis of 87 from 81.
Following the same approach we had successfully employed for creating D-rhamnose (35) from the benzyl α-D-rhamnoside 72, it was reasoned that an acid catalysed reaction should suffice in removing the benzyl ether in 75. Accordingly, 75 was taken up in water in which it was only partially soluble and to the reaction mixture was added a sufficient amount of H⁺ resin (Dowex 50 H⁺ resin). The resulting reaction mixture was stirred at 80°C for 24 hours, after which time the reaction mixture was filtered to remove the resin and the filtrate was concentrated under reduced pressure and purified using column chromatography (4:1 EtOAc/MeOH, Rf = 0.3) to yield the deprotected taloside 87 quantitatively (Scheme 33).

![Figure 19. ¹H NMR spectrum of 87](image)

Evidence that we had been successful in removing the benzyl glycoside in 75 was seen best in the ¹H NMR spectrum of 87 (Figure 19). Firstly, the spectra of 87 had no aromatic signals around δ 7.20 ppm, which were seen in the spectrum of 75. Secondly, with the removal of the benzyl glycoside two H-1 signals were now seen at δ 5.07 ppm (β) and δ 4.60 ppm (α), belonging to the two anomers of 87 α and β respectively.
(Figure 19). Importantly, there were also two H-6 three proton doublets present at δ 1.28 ppm (β) and δ 1.23 ppm (α), which were the two methyl group peaks from the two anomers now present in the sample (Figure 19).

In an analogous manner the 4-azido-4-deoxy-rhamnoside 84 was exposed to NaOMe in MeOH producing 73, followed by removal of the benzyl glycoside, to give the 4-azido-4-deoxy-rhamnoside 88 in a quantitative yield (Scheme 34). The 1H NMR spectrum of 88 showed similar characteristics analogous to that seen in the spectrum of 87.

![Scheme 34. Synthesis of 88 from 84.](image)

Reagents and conditions: (a) Na, MeOH, 0°C, 15 min, then rt. for 1 hr; (b) Dowex W50 H+ resin, water (as solvent), 80°C, 24 hrs; quantitative yield over two steps.

**2.4 Aldol condensation reactions catalysed by Neu5Ac aldolase**

Having successfully prepared two C-4 modified 6-deoxy hexoses 87 and 88, as well as D-rhamnose (35), our attention turned to using these compounds as substrates in the Neu5Ac aldolase catalysed aldol condensation reaction with pyruvic acid (28). As discussed in Section 2.2, Neu5Ac aldolase has been used extensively in both synthesis and cleavage reactions of Neu5Ac (1) and its analogues. Along with its freely soluble form117,124,137,140,185, Neu5Ac aldolase can be used in an immobilised state126,133–135. However, it has been noted that even though enzyme immobilisation provides an efficient method of recovering the enzyme by simple filtration of the enzyme-polymer complex133–135 and can be used in large scale enzymatic synthesis132,156, the loss of enzyme activity upon immobilisation is a major disadvantage132,134,141. Numerous other methods have been developed over the past three decades regarding *in vitro* enzymatic reactions, especially utilising Neu5Ac aldolase. One such method is membrane-
enclosed enzyme catalysis, which compared with using immobilised enzyme does not lead to loss of activity\textsuperscript{186}. In the case of the membrane enclosed enzyme catalysis protocol generally used for Neu5Ac aldolase, the enzyme can also be recovered by simple removal of the enzyme containing dialysis bag from the reaction mixture. As with immobilised enzyme, when stored correctly at 4\textdegree C, the protein is stable and can be reused for further catalysis\textsuperscript{187}.

In order to achieve the desired enzymatic conversions, we opted to use a membrane enclosed enzyme catalysis\textsuperscript{188} (e.g. dialysis tubing) as our preferred method for catalysis. This method was chosen due to the ease of set up and the overall reproducibility of each reaction. Therefore, in general the methodology used for the desired transformation of our substrates was carried out as follows.

The substrate, excess sodium pyruvate (28) and bovine serum albumin (BSA) were dissolved in water, and the pH of the solution adjusted to \(~7.3\) (the optimal pH for Neu5Ac aldolase). The enzyme from \textit{E. coli} (purchased from Sigma-Aldrich) was then dissolved in a small portion of water and added to the dialysis tubing, and the tubing placed into a solution containing substrate, sodium pyruvate (28) and BSA. After incubation at 37 \textdegree C for 4 days, the dialysis tubing (containing the enzyme) was removed from the solution, leaving behind the product of the reaction, any unreacted substrate, and the excess sodium pyruvate (28) that was used to drive the reaction in the desired direction. The solution containing the various chemical components from the enzyme reaction was then passed through an ion-exchange column (formate type), which works by separating the acidic components (e.g. our desired nonulosonic acid analogues) from non-acidic molecules (e.g. the unreacted substrate and sodium pyruvate (28)). Using this purification method, the desired nonulosonic acid analogue is efficiently and readily isolated from the membrane enclosed enzyme reaction.
2.4.1 Utilising Neu5Ac aldolase to create 5,7-diamino-3,5,7,9-tetraadeoxy-non-ulosonic acids

In a series of initial experiments we felt that it was important to use Neu5Ac aldolase to catalyse the condensation between D-mannose (37) and sodium pyruvate (28). In this way we would ensure that the enzyme we were using was active, we would also be able to determine the best conditions for the reaction and the purification of the product (which would be the known nine-carbon sugar KDN (3)). Furthermore, we would obtain valuable information from the $^1$H NMR analysis of KDN (3) which we could use as a comparison with the NMR spectra of our desired nonulosonic acid analogues. In choosing an appropriate type of dialysis tubing, the enzyme Neu5Ac aldolase has a molecular weight of 98 kDa, whilst the non-ulosonic acid analogues we were intending to generate would have molecular weights under 500 Da. Therefore, we initially tried a membrane enclosed Neu5Ac aldolase catalysed condensation reaction between sodium pyruvate (28) and mannose (37) using dialysis tubing with a molecular weight "cut-off" of 10,000Da. After incubation for four days at 37 °C, and subsequent ion-exchange chromatography, the nine-carbon acidic sugar KDN (3) was obtained in 43% yield.

Evidence of the successful formation of KDN (3) was obtained from examination of the NMR spectrum of the crude reaction mixture. Whilst many of the proton resonances for KDN (3) appear as overlapping multiplets in the range $\delta$ 3.50 ppm to $\delta$ 3.90 ppm, the most diagnostic resonance signals are due to the two H-3 protons, which appear at $\delta$ 1.67 ppm and $\delta$ 2.07 ppm for the H-3 axial and H-3 equatorial protons of the $\beta$-pyranoside configuration, respectively$^{189}$ (Figure 20).

Although pleased with obtaining KDN (3) from our initial reaction attempts, further work was needed in order to improve the overall chemical yield. Additional membrane enclosed Neu5Ac aldolase experiments with mannose (37) as substrate were undertaken in order to improve the efficiency of the reaction, most notably involving varying the concentration of the reactants and changing the molecular weight cut-off of the dialysis tubing. In this way, we could obtain KDN (3) in a 75% yield, using dialysis tubing with a molecular weight cut-off of 2000 Da, and ensuring that careful attention was paid to concentration (1.0 mmol substrate used) and pH (7.3 ± 0.2).
Figure 20. $^1$H NMR spectrum of the crude reaction mixture from the Neu5Ac aldolase catalysed aldol condensation reaction of D-mannose (37) with sodium pyruvate (28).

Having established the appropriate reaction conditions for our enzyme reactions, we began work on investigating the Neu5Ac aldolase catalysed aldol condensation with our 6-deoxy-mannose (D-rhamnose) (35) as a substrate. Following the protocol outlined above, D-rhamnose (35) was condensed with sodium pyruvate (28) in the presence of Neu5Ac aldolase using membrane enclosed enzyme catalysis at pH 7.3 ± 0.2 and 37°C over a period of four days. After this time the solution was concentrated under reduced pressure taken up in a small amount of water and applied to an IRA-400 (formate) ion exchange column for purification. The resulting acidic components from this reaction
were concentrated under reduced pressure and the $^1$H NMR spectrum was analysed. From the spectrum of the crude acidic fraction from the ion-exchange column it was decided that further modifications were needed, as the purification of our acidic portion of the reaction was not sufficient to produce a clean sample. The spectrum did however show that there appeared to be the expected nine-carbon acidic sugar within the sample, as the H-3 axial and H-3 equatorial signals were present at $\delta$ 1.65 ppm (H-3$_{ax}$) and $\delta$ 2.05 ppm (H-3$_{eq}$), coupling to each other ($J = 12.9$ Hz), and both coupling to H-4 ($J_{3eq,4} = 4.8$ Hz and $J_{3ax,4} = 11.4$ Hz). Yet another signal that was of interest was a doublet at $\delta$ 1.06 ppm, which was the C-9 methyl group, indicating to us that the reaction had been successful in converting D-rhamnose (35) into the 9-deoxy-KDN product (89) (Scheme 35).

Scheme 35. Aldol condensation reaction between 35 and pyruvate (28) catalysed by Neu5Ac aldolase to produce the 9-deoxy product 89

To this end the acidic fraction from the initial purification was taken up in anhydrous methanol to which Dowex 50 H$^+$ acidic resin was added and stirred at room temperature for two days. The resulting reaction mixture was filtered to remove the resin and the filtrate was concentrated under reduced pressure to yield the methyl ester 90 as white foam. The $^1$H NMR spectrum of 90 was complex, however it did show the characteristic methyl ester peak at $\delta$ 3.78 ppm along with the H-3$_{ax}$ and H-3$_{eq}$ proton signals $\delta$ 1.80 ppm and $\delta$ 2.10 ppm, which indicated that we had successfully created the desired methyl ester. However, the $^1$H NMR spectrum of 90 also showed that we had a mixture of anomers, as there were three other methyl ester peaks around $\delta$ 3.80 ppm and further H-3 proton signals that complicated the spectra.
Therefore, 90 was reacted further in order to create the methyl ester methyl glycoside compound 91, which could then could be acetylated and purified in order to obtain a clean sample which could be efficiently characterised. Compound 90 was taken up in anhydrous methanol to which Dowex 50 H⁺ acidic resin was added and stirred at 70°C for 15 hours, after which time it was cooled, the resin filtered from the mixture and concentrated under reduced pressure. The resulting white foam (91) was dried without purification then taken up in pyridine to which acetic anhydride and DMAP were added under argon gas and stirred at room temperature overnight yielding the per-O-acetyl-compound 92 as a white amorphous solid in 65% yield from D-rhamnose (35).

![Chemical Structure](image)

**Figure 21.** ¹H NMR spectrum of methyl (methyl-per-O-acetyl-9-deoxy)-KDN1,β2Me₂ (92)

Evidence that we had indeed created the methyl (methyl per-O-acetyl-9-deoxy)-KDN1,β2Me₂ (92) was confirmed by ¹H NMR spectroscopy (Figure 21). Viewing the spectrum of 92 shown in Figure 21, the distinctive C-9 methyl group signal can be seen as a three proton doublet at δ 1.27 ppm, whilst the characteristic H-3ax and H-3eq protons appeared at δ 1.83 ppm and δ 2.49 ppm, respectively, similar to what we had
seen for KDN (3). Other signals in the spectrum of 92 include the methyl ester (δ 3.79 ppm) and β-methyl glycoside (δ 3.18 ppm), with the remaining signals in the ¹H NMR spectrum of 92 consistent with those seen for the analogous protected KDN derivative.

With the successful synthesis of our 9-deoxy compound 92 we were now ready to attempt the conversion of our selectively functionalised C-4 derivatives 87 and 88 in order to create our first legionaminic acid analogues.

### 2.4.2 C-4 modified D-rhamnopyranosides as substrates for Neu5Ac aldolase

Having established optimal conditions for the aldol condensation reaction catalysed by Neu5Ac aldolase in creating KDN (3) from D-mannose (37) and 9-deoxy-KDN 89 from D-rhamnose (35), we began work on investigating the Neu5Ac aldolase catalysed aldol condensation with our 4-azido-4,6-dideoxy-mannose derivative 88. Following the protocol outlined above (Section 2.4.1) for D-rhamnose (35) compound 88 was condensed with sodium pyruvate (28) in the presence of Neu5Ac aldolase using membrane enclosed enzyme catalysis at pH ~7.3 and 37°C over a period of four days. The reaction solution was concentrated under reduced pressure and analysed by ¹H NMR (Figure 22).

From the spectrum (Figure 22) it can be seen that the prominent H-3ax and H-3eq proton signals were present at δ 1.80 ppm and δ 2.10 ppm, along with a 9-methyl doublet at δ 1.25 ppm which indicated that the desired nonulosonic acid was present in the mixture (Figure 22). Confident that we had indeed created the desired 7-azido-9-deoxy-nonulosonic acid (74), the crude reaction mixture was purified using ion-exchange chromatography. As before, the acidic fraction was then treated with Dowex 50 H⁺ acidic resin to create firstly the methyl-ester and then the methyl glycoside followed by global acetylation. This resulted in the isolation of the protected methyl (methyl per-O-acetyl-7-azido)-Leg1,β2Me₂ analogue 93 (Figure 23).
Figure 22. $^1$H NMR spectrum of the crude reaction mixture from the aldol condensation reaction of 88 catalysed by Neu5Ac aldolase

Evidence that we had indeed obtained the legionaminic acid analogue 93 was confirmed by $^1$H NMR spectroscopy. The distinctive C-9 methyl group appeared as a three proton doublet at δ 1.31 ppm, whilst the characteristic H-3$_{ax}$ and H-3$_{eq}$ protons appeared at δ 1.87 ppm and δ 2.49 ppm, respectively, similar to what we had seen for both 3 and 89. Furthermore, IR analysis showed an azide peak at 2109 cm$^{-1}$ and the HRMS spectrum showed an molecular ion peak of $m/z$ 454.1541 [M + Na]$^+$, which is consistent with the molecular formula of 93 (C$_{17}$H$_{25}$N$_3$O$_{10}$Na).
2.4.3 C-4 modified 6-deoxy-D-talopyranosides as substrates for Neu5Ac aldolase

Having created our first legionaminic acid analogue 93 from our selectively functionalised derivative 88 using an aldol condensation reaction catalysed by Neu5Ac aldolase we began work on investigating the Neu5Ac aldolase catalysed aldol condensation with our benzyl 4-azido-4,6-dideoxy-α-D-talopyranoside (87). Initially, we thought that we should investigate how well the enzyme Neu5Ac aldolase would turn over 4-epi-configured D-mannose substrates, which are referred to as D-talopyranosides.

In order to achieve this initial aim for this part of our synthesis, we investigated numerous methods for the synthesis of D-talopyranosides. Surprisingly, what we found was that only a limited number of articles had been published on an efficient synthesis of D-talopyranosides\(^{190-196}\). From these papers, we chose to utilise the convenient conversion outlined by Xiao et al. 2010\(^{196}\) for synthesising D-talosides from D-galactosides (Scheme 36).

The efficient 5 step synthesis as outlined by Xiao et al.\(^{196}\) as shown in Scheme 36, begins with D-Galactose (94). Compound 94 is firstly per-O-acetylated using sodium acetate and acetic anhydride at elevated temperature to produce solely the β-glycoside 95 in high yield. This particular reaction was very efficient producing 95 in 90% yields and above without the need for purification.
**Reagents and conditions:** (a) NaOAc, Ac₂O, 120°C, 92%; (b) 90% Aq. TFA, 60%; (c) (i) Tf₂O, py., CH₂Cl₂; (ii) H₂O/DMF, 70°C, 98% (60%); 99 (35%); (d) MeOH, Na⁺(s), 1 hr, 100%.

**Scheme 36.** Synthesis of D-talose (36) from D-galactose (94) as reported by Xiao et al.¹⁹⁶

Exposure of 95 to a 90% aqueous TFA solution as outlined by Xiao et al.¹⁹⁶ (Scheme 36) and described by Chittenden¹⁹⁷ in the late nineteen eighties, selectively deprotects the acetate group at C-2 in 95, producing the 2-hydroxy-compound 96 in a modest 60% yield (Scheme 36). The ¹H NMR spectrum of 96 was consistent with that reported by Xiao et al.¹⁹⁶. Interestingly, just as Xiao et al.¹⁹⁶ had depicted in their method the acetyl glycoside had converted to the α-anomer (H-1 to H-2 coupling $J = 4.1$ Hz) in 96 compared with that of 95 (H-1 to H-2 coupling of $J = 8.9$ Hz, β-glycoside).

The key inversion of stereochemistry at C-2, to produce the taloside configuration is reported to be achieved by firstly activating the C-2 position with a good leaving group in the form of a trifluoromethanesulfonyl group (triflate), which can then be displaced by an appropriate nucleophile via an SN₂ mechanism, thereby inverting the stereochemistry at C-2 with retention of the hydroxyl functionality. In order to introduce the leaving group at C-2, trifluoromethanesulfonic anhydride was added to a stirred
solution of 96 dissolve in CH₂Cl₂ and pyridine at 0°C, after which time the reaction as allowed to warm to room temperature and stirred for a further 4 hours. After this time the 2-O-triflate-compound 97 was obtained in 75% yield as yellow viscous oil after purification. 97 was not characterised due to poor stability upon storage, therefore being always used promptly in the next reaction of the sequence (Scheme 36). The next step is the S_N2 displacement reaction to give the d-talo-configured sugar, requiring the use of water as a nucleophile in order to displace the C-2 triflate leaving group. In this regard the reaction was run at elevated temperature in N,N-DMF as a solvent to which water was added. The reaction itself was very sluggish taking 4 days to achieve the desired outcome, which was similar to that described by Xiao et al.\textsuperscript{196}.

As indicated by Xiao et al.\textsuperscript{196} the outcome of the S_N2 reaction was a mixture of partially protected products 98 and 99 (Scheme 36), which was due to an acetate shift occurring during the reaction producing the expected 2-hydroxyl-product 98 and the 2-O-acetyl-3-hydroxy-product 99. The $^1$H NMR spectrum of the crude reaction mixture was quite complex due to the two products being very similar in structure, however a few signals gave us the confidence to assume that we had obtained the desired compounds 98 and 99. Firstly the two H-2 and H-3 signals from each compound were roughly one ppm apart due to the acetate shift that had occurred during the reaction. The H-2 peak in 98 (δ 3.75-3.77) was a complex signal coupling to H-1 and H-3 and the 2-OH, whilst the H-2 peak in 99 was one ppm downfield compared with 98 as 99 had the acetate at C-2 (Scheme 36). Similarly, the H-3 proton signals in 98 and 99 were also roughly one ppm apart as C-3 in 98 had an acetate protecting group and C-3 in 99 was a hydroxyl (Scheme 36). The H-1 proton signals in 98 and 99 also had slightly different chemical shifts (98 δ 5.85 ppm and 99 δ 5.95 ppm) due to 98 having a 2-OH group and 99 a 2-OAc substituent, along with the characteristic H-1, H-2 coupling ($J_{1,2} = 1.8$ Hz), for a $\alpha$-glycoside in a mannose configured (C-2 axial) sugar.

Having now produced the two d-taloside products 98 and 99, we wanted to deacetylate these two products to produce our desired fully deprotected d-talose (36) (Scheme 36). In this regard we took the crude reaction mixture of 98 and 99 up in anhydrous methanol and added sodium metal, forming sodium methoxide (CH₃ONa) in situ, thus producing our desired d-talose (36) as a mixture of anomers ($\alpha$ and $\beta$) in a quantitative yield after purification (Scheme 36). The $^1$H NMR spectrum of 36 showed
two H-1 protons at $\delta$ 4.80 ppm ($\beta$) ($J_{1,2} = 3.2$ Hz) and $\delta$ 5.25 ppm ($\alpha$)($J_{1,2} = 1.8$ Hz), in both cases correlating to H-2 protons in the COSY spectra with coupling constants consistent with those expected for a talo-configured sugar. Further evidence that we had indeed created the desired D-talose (36) was obtained from a mass spectrum of 36 giving an exact mass of 203.1 [M + Na]$^+$, which is consistent with the molecular formula of C$_6$H$_{12}$O$_6$.

Now that the C-4 epimer of D-mannose (37), D-talose (36) was in our hands, work began on investigating whether it would be efficiently transformed by Neu5Ac aldolase into the corresponding 7-epi-KDN product (55).

Following the protocol outlined in section 2.4.1 for D-rhamnose (35), 36 was condensed with sodium pyruvate (28) in the presence of Neu5Ac aldolase using membrane enclosed enzyme catalysis at pH ~7.3 and 37°C over a period of four days. In the same manner as outlined in the preceding two sections (2.4.1 and 2.4.2) the crude solution from the reaction of 36 with Neu5Ac aldolase and excess sodium pyruvate (28) was firstly analysed by $^1$H NMR (Figure 24) to ensure the reaction had produced the desired 7-epi-KDN (55) product.

From this $^1$H NMR analysis (Figure 24) it could be seen that obvious H-3$_{ax}$ and H-3$_{eq}$ proton signals were present at $\delta$ 1.75 ppm and $\delta$ 2.21 ppm respectively, which indicated that the desired nonulosonic acid (55) was present in the mixture (Figure 24). Confident that we had indeed created a non-ulosonic acid, the crude reaction mixture was taken up in a small amount of water and applied to an IRA-400 (formate) ion exchange column for purification. After the initial ion-exchange column, the acidic fraction which contained our desired product was further processed without spectroscopic analysis to create the methyl ester, methyl glycoside compound (100) which was per-O-acetylated creating 101 in an analogous manner to what we had done previously (see sections 2.4.1 and 2.4.2). In this way we obtained the methyl (methyl 7-epi-per-O-acetyl)-KDN1,β2Me$_2$ product 101 in a 20% yield from 36 using Neu5Ac aldolase.
Evidence that we had indeed isolated the 7-epi-per-O-acetyl-KDN product 101 was confirmed by $^1$H NMR spectroscopy. The spectrum of 101 showed two three proton singlets at δ 3.33 ppm (methyl glycoside) and δ 3.82 ppm (methyl ester), along with the H-3ax and H-3eq peaks at δ 1.85 and δ 2.51 ppm respectively, which coupled to each other ($J_{3ax,3eq} = 12.0$ Hz) and to H-4 ($J_{3ax,4} = 11.1$ Hz, $J_{3eq,4} = 5.7$ Hz). Importantly, in the $^1$H NMR spectrum of 101 H-7 (δ 4.54 ppm) showed a large coupling with H-6 ($J_{7,6} = 9.0$ Hz), which would be expected if the C-7 enantiomer was in an 7-(S)-configuration that being a 7-epi-KDN configuration compared with 7-(R) seen in KDN (3) (Figure 25).
Although disappointed with the overall chemical yield of the protected 7-epi-product 101, we had successfully used a C-4 epimer in D-talose (36) and two 6-deoxy-substrates (35 and 88) for the Neu5Ac aldolase condensation reaction, we were confident that the final C-4 selectively functionalised substrate 87 would be successfully transformed by the enzyme Neu5Ac aldolase, thus producing the desired legionaminic acid analogue 76.

In the same fashion as outlined in proceeding sections, our 4-azido-4,6-dideoxy talopyranoside 87 was condensed with sodium pyruvate (28) in the presence of Neu5Ac aldolase using membrane enclosed enzyme catalysis at pH ~7.3 and 37°C over a period of four days. The crude reaction solution from the reaction of 87 with Neu5Ac aldolase and excess sodium pyruvate (28) was analysed by $^1$H NMR (Figure 26).

From the $^1$H NMR spectrum (Figure 26) it could be seen that the prominent H-3$_{\text{ax}}$ and H-3$_{\text{eq}}$ proton signals were present at $\delta$ 1.78 ppm and $\delta$ 2.10 ppm, along with a three proton doublet (C-9) at $\delta$ 1.35 ppm which indicated that the desired nonulosonic acid 76 was present in the mixture (Figure 26). Confident that we had indeed created the desired 7-epi-7-azido-9-deoxy-nonulosonic acid 76, the crude reaction mixture was taken up in water and applied to an IRA-400 (formate) ion exchange column for purification. After purification, the acidic fraction from the ion-exchange column was once again further processed to create the desired per-O-acetylated methyl-ester, methyl glycoside compound 102, again to aid purification and characterisation in the same manner as 92, 93 and 101.

**Figure 25.** Comparison of the C-7 configuration seen between 7-epi-KDN (55) and KDN (3)
Figure 26. $^1$H NMR spectrum of the crude reaction mixture from the aldol condensation reaction of 87 catalysed by Neu5Ac aldolase

Analysis of the $^1$H NMR spectrum of 102 showed the distinctive C-9 methyl group, which appeared as a three proton doublet at $\delta$ 1.22 ppm, whilst the characteristic H-3$_{\text{ax}}$ and H-3$_{\text{eq}}$ protons appeared at $\delta$ 1.75 ppm and $\delta$ 2.15 ppm, respectively, similar to what we had seen for 92, 93 and 101. The remaining signals in the $^1$H NMR spectrum of 102 were also consistent with what would be expected for a legionaminic acid derivative similar to those signals seen for 92, 93 and 101 (see Section 5.3.1 for full NMR assignment).
2.5 Conclusion and Future directions

The results of our chemoenzymatic approach toward the synthesis of legionaminic acid analogues clearly shows that we were able to achieve our aim of using standard and well documented synthetic chemistry, in order to create novel 6-deoxy C-4 nitrogen functionalised substrates 87 and 88 that were successfully transformed by the enzyme Neu5Ac aldolase into their corresponding nonulosonic acids 74 and 76, respectively.

The successful transformation of the five substrates (37, 35, 36, 87 and 88) from the aldol condensation reaction catalysed by Neu5Ac aldolase is summarised in Table 5 below.

**Table 5.** Aldol condensation reaction catalysed by Neu5Ac aldolase outcome for the substrates 37, 35, 36, 87 and 88

<table>
<thead>
<tr>
<th>Substrate → Products</th>
<th>X; Y; R substrates and Products</th>
<th>Rel. Rate*</th>
<th>Chemical Yield* (protected products (#))</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 → 3</td>
<td>X = H; Y = OH; R = OH</td>
<td>100</td>
<td>75 % (142)</td>
</tr>
<tr>
<td>35 → 89</td>
<td>X = H; Y = OH; R = H</td>
<td>96</td>
<td>72% (92)</td>
</tr>
<tr>
<td>36 → 55</td>
<td>X = OH; Y = H; R = OH</td>
<td>26</td>
<td>20% (101)</td>
</tr>
<tr>
<td>87 → 76</td>
<td>X = N3; Y = H; R = H</td>
<td>35</td>
<td>26% (102)</td>
</tr>
<tr>
<td>88 → 74</td>
<td>X = H; Y = N3; R = H</td>
<td>42</td>
<td>32% (93)</td>
</tr>
</tbody>
</table>

a: Yields of protected products obtained after enzymatic conversion by Neu5Ac aldolase, subsequent methyl esterification, methyl glycoside and acetylation. Yield calculated based on molar amounts of isolated protected product compared to substrate used.

* Relative rate of turn over calculated using the conversion of D-mannose (37) to KDN (3) as reference standard = 100%

As can be seen in Table 5, the outcome of the overall chemical yield of protected products for the know substrates, 37, 35 were good (>70%), however the yield obtained for the conversion of 36 was considerably lower. This outcome was potentially due to contaminates from the reaction sequence used to produce D-talose (36), as the result was always similar each time this conversion was attempted. The overall yields of the synthetic novel C-4 functionalised substrates (87 and 88) were a little over one third.
relative to 37 and nearly half relative to 35 and better overall compared with 36. A possible reason for these results could be the reaction conditions. Most notably the concentration (1 mmol) of substrate utilised in each reaction was the same for all substrates used. This was of course done on purpose to allow us to make a direct comparison, as shown in Table 5, which is a measure of the relative reaction turnover compared with a known substrate (in this case D-mannose (37)). However, the individual $K_i$ and $K_{cat}$ values for each substrate, most notably 87 and 88 would probably be quite different to those of 37 or 35 and 36, and therefore if the actual enzymatic kinetics for the reaction of 87 and 88 could be measured, we would be able to increase the yields in regard to turnover of the legionaminic acid products 74 and 76.

Despite the modest turnover of the C-4 functionalised substrates 87 and 88, these yields are a marked improvement on those reported to date for the synthesis of legionaminic acids. What is significant about our results is that we were able to achieve our aim, firstly by creating the key intermediate 77 (see section 2.2.3), which proved to be pivotal to the success of this part of our project, as it allowed us the flexibility to produce a number of 6-deoxy-D-hexose derivatives (35, 87 and 88), all of which were transformed into their corresponding nonulosonic acid analogues by Neu5Ac aldolase. This chemoenzymatic approach is novel in regard to the synthesis of legionaminic acid analogues, and we have shown by the work done in this project, that this approach is valid in creating flexibility in the types of analogues which can be produced using this method.

Having successfully fulfilled our intended aim of utilising a chemoenzymatic approach toward the synthesis of C-7 functionalised legionaminic acid-based analogues, a few important points should be discussed regarding the overall procedure that has been described within this chapter. Firstly, due to the stereochemical constraints for the enzyme Neu5Ac aldolase in regard to acceptor substrates, we were limited in the types of compounds that could be used for this type of chemoenzymatic approach. Therefore, future work in this regard would involve investigating further selectively modified 6-deoxy-D-hexose substrates, with a focus on the use of C-2 functionalised compounds which would allow access to C-5 functionalised legionaminic acid compounds. Preliminary work carried out in this regard (not described in this chapter), showed enzymatic turnover of these substrates was extremely sluggish and in most cases
resulted in unidentifiable products (that were not a nonulosonic acids) being produced. Therefore future work is needed in order to better understand the viability of using 2,4,6-trideoxy-hexose substrates in this type of chemoenzymatic approach.

In summary, legionaminic acid is a unique nine-carbon acidic carbohydrate that plays an important role in the pathogenesis of numerous clinically important Gram-negative bacteria. To date there is no efficient method available for creating analogues of this important carbohydrate that can be used as biological probes in order to learn more about the role of legionaminic acid in pathogenesis. This chapter shows that we have successfully designed a chemoenzymatic approach toward the synthesis of novel legionaminic acid analogues, and has demonstrated the validity of using such an approach toward the synthesis of legionaminic acid-based analogues.
CHAPTER 3

A CHEMICAL ALDOL CONDENSATION APPROACH TOWARDS THE SYNTHESIS OF 5,7-DIAMINO-3,5,7,9-TETRADEOXY-NONULOSONIC ACID ANALOGUES

3.1 An alternate strategy toward the synthesis of tetradeoxy-nonulosonic acids

With the successful synthesis of the talopyranoside 87 and rhamnopyranoside 88 and their transformation into the legionaminic acid analogues 74 and 76 using Neu5Ac aldolase, as described in chapter two, we were interested in developing C-2 modified 4,6-dideoxy-hexopyranosides that could be transformed into C-5 modified nonulosonic acids. It was initially thought that by creating such compounds we could potentially use Neu5Ac aldolase to transform these substrates into nonulosonic acids that could be modified into pseudaminic acid analogues after the Neu5Ac aldolase catalysed aldol condensation reaction. Preliminary studies in this regard focused on creating a C-2 activated mannose configured sugar 103 (Scheme 37), which was functionalised with the C-2 mesylate (OMs) leaving group. Having a good leaving group at C-5 in our nonulosonic acid product 104 would allow for its displacement using a nitrogen-based nucleophile, inverting the stereochemistry at this position producing a C-5 axial product 105 that has pseudaminic acid stereochemistry at C-5 (Scheme 37).

Scheme 37. Proposed reaction converting 103 into 104 via an aldol condensation reaction catalysed by Neu5Ac aldolase, followed by potential nucleophilic (Nu) displacement to produce 105

Unfortunately, in our hands the transformation of 103 into the C-5 activated nonulosonic acid analogue 104 (Scheme 37) via an aldol condensation reaction catalysed by Neu5Ac aldolase was never successful. The inability of Neu5Ac aldolase
TRANSFORM

103 could possibly be due to the mesylate at C-2. We reasoned that having a strong electron withdrawing group at C-2 could make this position potentially susceptible to attack by the catalytic Schiff-base responsible for the C-C bond formation and chain elongation in the Neu5Ac aldolase catalysed reaction.

Therefore, due to the inability of Neu5Ac aldolase to convert our C-2 activated compound 103, along with the stereochemical limitations for the use of Neu5Ac aldolase in the synthesis of nonulosonic acid analogues like pseudaminic acids (as described in chapter 2), we became interested in investigating alternate strategies for synthesising tetradeoxy-nonulosonic acids.

One such strategy that was attractive to us was the use of a synthetic aldol condensation reaction using oxaloacetic acid (27). Numerous papers\textsuperscript{50,111,113,198–202} have been published outlining the synthesis of nonulosonic acids from a variety of hexose substrates using this method. Synthetic aldol condensation reactions use a hexose substrate which is condensed with a four-carbon donor (i.e. oxaloacetic acid (27)) in order to produce the corresponding nine-carbon sugar (Scheme 38). Importantly, carbons 1, 2 and 3 in the nonulosonic acid product come from oxaloacetic acid (27) (coloured blue), whilst carbons 4 to 9 come from the hexose substrate (black, Scheme 36).

\textbf{Scheme 38.} Proposed aldol condensation reaction between a hexose substrate and oxaloacetic acid (27) producing the corresponding nine-carbon acidic sugar

The type of approach toward the synthesis of complex carbohydrates shown in Scheme 38 has been successfully used to create a variety of nonulosonic acids in varying yields\textsuperscript{50,111,113,198–202}. Therefore we thought this a viable alternative to overcome the limitations we had encountered in our chemoenzymatic approach especially in regard to the types of substrates that could be used in the aldolase catalysed reaction. However, first we need to understand what type of substrates could be used in this approach and
whether we could use these substrates to obtain our desired tetradeoxy-nonulosonic acids.

### 3.1.1 Synthetic aldol condensation reactions using oxaloacetic acid (27)

The first relevant use of a synthetic aldol condensation reaction in the synthesis of higher order sugars like nonulosonic acids was the Cornforth method reported in 1958\(^ {198}\). This pivotal publication set out to develop a novel approach for the synthesis of \(N\)-acetylneuraminic acid (1), via an aldol condensation reaction between \(N\)-acetyl-D-glucosamine (29) and pyruvic acid (28) under basic conditions (pH 11). However, based on poor overall outcomes encountered with the three carbon donor pyruvic acid (28), it was replaced with the four carbon unit oxaloacetic acid (27), with the expectation that the methylene group in oxaloacetic acid would be more reactive, and the additional carboxyl group could be eliminated in the final step of the condensation\(^ {198}\).

Cornforth’s proposed method\(^ {198}\) for the synthesis of Neu5Ac (1) although producing poor overall yields (<2%) was important in its scope, as it outlined the possible versatility of creating nine-carbon acidic sugars through a six plus four mechanism (Scheme 39).

Scheme 39 shows the six plus four strategy for the aldol condensation reaction between \(N\)-acetyl-D-glucosamine (29) and oxaloacetic acid (27). The initial reaction step occurs between the enolate produced from oxaloacetic acid due to the alkaline conditions, and the aldehyde of the acyclic form of 29. This is the carbon-carbon bond formation step whereby the enolate ion reacts with 29 extending the carbon chain. The final step of the process involves the decarboxylation of the C-3 carboxylate group, producing Neu5Ac (1). As shown in Scheme 39, the initial step whereby the enolate ion attacks the aldehyde carbonyl in 29 can occur from either the \(re\) or \(si\) face, therefore introducing a lack of stereochemical control at C-4 (hydroxyl in red, Scheme 39) in the product Neu5Ac (1). This lack of stereochemical control at this position (C-4) has the potential of creating both Neu5Ac (1) and the 4-epimer, which complicated characterisation and purification processes in the early work\(^ {198,199}\).
Scheme 39. The six plus four strategy for the aldol condensation reaction between \( N \)-acetyl-D-glucosamine (29) and oxaloacetic acid (27)

Since 1958, studies have shown that the use of various additives usually in the form of organic or inorganic metallic salts are required under alkaline conditions to slow or even inhibit multiple side products from being formed\textsuperscript{199,201,202}. Furthermore, it has been shown through the synthesis of sialic acids like KDN (3)\textsuperscript{200} that the product of an aldol condensation reaction between GlcNAc (29) and oxaloacetic acid (27) should in fact be 5-\textit{epi}-Neu5Ac (106) not Neu5Ac (1) itself (Figure 27).

Figure 27. Diagram showing the stereochemical difference at C-5 between Neu5Ac (1) and 5-\textit{epi}-Neu5Ac (106)

An improved synthesis of \( N \)-acetylneuraminic acid (1) using this aldol condensation reaction was published in 1969 by How \textit{et al.}\textsuperscript{199}, which showed the
addition of sodium tetraborate to the aldol condensation reaction gave an improved yield of 21%. In this work, How et al.\textsuperscript{199} reacted 2-acetamido-2-deoxy-D-mannose (ManNAc, 34) and 2-acetamido-2-deoxy-D-glucose (GlcNAc, 29) along with other C-2 functionalised D-manno- and D-gluco- substrates with oxaloacetic acid (27), showing that the addition of the tetraborate ion (in 0.1 M concentration) greatly inhibits the alkaline C-2 epimerisation of 2-acylamino-hexoses, resulting in the increased overall yields and reduction of possible mutarotated side products when compared with those reactions not treated with tetraborate\textsuperscript{199}.

Further work published by McNicholas et al.\textsuperscript{201} in this regard came in an attempt to improve the yield for the synthesis of the eight-carbon acidic sugar KDO. McNicholas et al.\textsuperscript{201} showed that the addition of sodium carbonate (Na\textsubscript{2}CO\textsubscript{3}) to the condensation reaction between oxaloacetic acid (27) and D-arabinose had a positive effect, as it was easier to maintain the pH of the reaction at an optimal level (>pH 10), thus reducing side reactions\textsuperscript{201}. Moreover, and subsequent to this work, Shirai & Ogura\textsuperscript{202} concluded that the major variable in the aldol condensation reaction between oxaloacetic acid (27) and D-arabinose under basic conditions is the decarboxylation step\textsuperscript{202} (final step, Scheme 39). These authors suggested that in some instances the spontaneous decarboxylation of the aldol adduct is very slow, and determined that the addition of Ni\textsuperscript{2+} to the reaction mixture promoted the decarboxylation step, producing better yields with fewer side products\textsuperscript{202}.

3.1.2 Synthesis of 3,5,7,9-tetradeoxy-nonulosonic acids using a synthetic aldol condensation reaction

To date there have been a limited number of papers published that discuss using a synthetic aldol condensation reaction in order to obtain tetradeoxy-nonulosonic acids such as the pseudaminic and legionaminic acids (see chapter 1, section 1.8)\textsuperscript{50,111,113}. A general theme amongst these approaches is that they have been specifically aimed at synthesising the parent structures for the purpose of characterisation, and generally use long and very inefficient routes that are clearly not amenable to generating a range of synthetic analogues.
The first major consideration when attempting a synthetic approach toward tetradeoxy-nonulosonic acids is the configuration of the hexose compound that is intended to be condensed with oxaloacetic acid (27) in order to create the desired nonulosonic acid analogues. The C-2 position of the hexose sugar to be condensed with oxaloacetic acid (27) corresponds to the stereochemistry at C-5 in the resulting nine-carbon sugar. Mannose configured sugars correspond to Neu5Ac (1) type configurations (D-glycero-D-galacto) in the resulting nonulosonic acid after the aldol condensation. Therefore, in order to create pseudaminic acid based analogues (C-5 axial) via this method, a glucose configured (C-2 equatorial) sugar would be required, which would allow direct access to the desired C-5 stereochemistry (L-manno) in the resulting nonulosonic acid product. Therefore, based on the fact that all pseudaminic acid compounds have a acylamino functionality at C-5, the commercially available and inexpensive hexose D-glucosamine (107) or its N-acyl-derivative (N-acetyl-D-glucosamine (29), Scheme 40) would be a more appropriate starting material for direct access to pseudaminic acid-based analogues, whilst manno-configured hexoses would produce legionaminic acid-based analogues.

Scheme 40. Proposed synthesis of a C-5 axial nonulosonic acid analogue from 107 or 29

With this knowledge in hand, we began designing our synthetic aldol condensation approach toward the synthesis of tetradeoxy-nonulosonic acids. Scheme 41 shows in general terms, an overview of the intended synthesis of pseudaminic acid analogues via a synthetic aldol condensation reaction, starting from the commercially available hexoses D-glucosamine (107) or D-mannose (37). An important consideration in the structures seen in Scheme 41 is not only the configuration of the intended starting materials 107 and 37 but also the C-4 functionalised 6-deoxy compound 108. The success of this chemical approach toward pseudaminic acid analogues rests on our ability to create a key intermediate like 108. Important functional group considerations
in 108 are the 6-deoxy group which corresponds to C-9 in the resulting nonulosonic acid analogue 109 after condensation with oxaloacetic acid (27). Starting from either D-glucosamine (107) or D-mannose (37) allows us to introduce variation in substitutions seen at C-2 and C-4 in 108, ultimately both pathways will produce a C-5 axial nonulosonic acid like 109. Furthermore, a number of C-7 functionalised analogues can potentially be prepared from the condensation reaction between 108 and oxaloacetic acid (27), simply by varying the C-4 “Y” group in these substrates. This is the essence of the flexibility and efficiency of our chemical approach toward tetrahydroxy-nonulosonic acid analogues.

Scheme 41. Proposed synthesis of pseudaminic acid and legionaminic acid analogues from 6-deoxy substrates via a synthetic aldol condensation reaction

In order to synthesise pseudaminic acid analogues using this approach, we first had to develop an efficient synthesis of compounds like the important key intermediate 108.
3.2 Synthesis of C-2 modified 4,6-dideoxy-hexopyranosides

3.2.1 Approach from D-mannose (37)

Following on from the previous section, we were interested in using the commercially available D-mannose (37) and selectively manipulating this compound to obtain the C-2 and C-4 functionalised 6-deoxy galactopyranoside compound 110 (Scheme 42).

![Scheme 42. A generalised proposed synthesis of the galactosamine derivative 110 from 37 (OPG = protecting group)](image)

As can be seen in Scheme 42, we intended to use the selectively protected 2,3-isopropylidene key intermediate compound 77 (Scheme 42), which had been produced for our chemoenzymatic approach toward legionaminic acid analogues. We had already developed an efficient synthesis of 77 from 37, which would also serve our aims in this synthesis, making the approach from 37 more efficient. Therefore, with 77 already in hand the next target compound was a 2,4-diol compound like 111 (Scheme 42), which has a protecting group at C-3. The importance of producing the 2,4-diol compound like 111 was to allow direct access to either C-2 modified substrates or 2,4-bis-modified compounds similar to 110, which could be used in an aldol condensation reaction to produce the corresponding 5,7-modified non-ulosonic acid analogues.

Therefore, the first step in our approach towards a compound like 110 was the protection of the C-4 hydroxyl group in the key intermediate 77, using an acetate protecting group, to give compound 112 (Scheme 43). This reaction was achieved by
dissolving 77 in pyridine before the addition of acetic anhydride, followed by stirring the solution at room temperature under argon gas, overnight. The reaction was extremely efficient providing the desired compound 112 in quantitative yield after purification. The $^1$H NMR spectrum of 112 showed the expected change in chemical shift of ~1.5 ppm of the H-4 proton signal from $\delta$ 3.42 ppm in 77 to $\delta$ 4.90 ppm in 112, along with the appearance of a three proton methyl group singlet at $\delta$ 2.12 ppm which clearly indicated that the acetate group had been introduced at C-4. (Scheme 43). With the synthesis of the fully protected compound 112 complete the next step towards a C-2 modified compound was to unmask the C-2 and C-3 hydroxyl groups, which were currently protected by the acetonide functionality in 112. This step was achieved by stirring 112 in an aqueous 80% acetic acid solution at elevated temperature for 1 hour, removing the acetonide protecting group in a quantitative manner producing the 2,3-diol product 113 (Scheme 43).

![Scheme 43. Synthesis of the 2,3-diol compound 113 from the key intermediate 77.](image_url)

Evidence that the acetonide protecting group had been successfully removed was best seen in the $^1$H NMR spectrum of 113, which showed the appearance of a broad two proton singlet at $\delta$ 3.35 ppm belonging to the two hydroxyl protons, which were exchangeable with D$_2$O and showed correlation with both H-2 and H-3 in the COSY spectrum of 113. The mass spectrum of 113 also showed the expected molecular ion peak of $m/z$ 319.1 [M + Na]$^+$ which is consistent with the molecular formula C$_{15}$H$_{20}$O$_6$.

With the successful removal of the acetonide protecting group, a method was required to protect the C-3 hydroxyl selectively, which would allow for the removal of the C-4 acetate producing our intermediate target 2,4-diol compound like 111. Within the literature the use of tert-butyldimethylsilyl (TBDMS) ethers as protecting groups of
secondary hydroxyls is well established having been first described by Corey et al\textsuperscript{203} in the early 1970's.

When looking closely at the C-2 and C-3 hydroxyls in 113, it is worth noting that the C-2 hydroxyl is axial and the C-3 hydroxyl is equatorial. In three dimensional terms, this means that both hydroxyls groups are on the same face of the sugar ring. A method for the selective acylation and alkylation of carbohydrates with axial/equatorial 2,3-diols was first reported by Nashed and Anderson\textsuperscript{204} and since then has been extended and widely used for other vicinal and 4,6-diols\textsuperscript{205}.

Following a method set out by David and Hanessian\textsuperscript{206}, exposure of the 2,3-diol 113 to dibutyltin oxide in methanol at reflux resulted in the formation of the cyclic 2,3-\textit{O}-dibutylstannylene acetal intermediate (Scheme 44). Without isolation, the 2,3-\textit{O}-dibutylstannylene acetal was dissolved in anhydrous \textit{N,N}-DMF and tert-butylidimethylsilyl chloride was added, the resulting mixture was stirred at 110°C for 30 minutes. In this way the selectively protected 3-\textit{O}-TBDMS compound 114 was obtained in 72% yield after purification (Scheme 44).

![Scheme 44](image)

\textit{Reagents and Conditions:} (a) \textit{Bu}_2\textit{SnO}, MeOH, 80°C, 2 hrs; (b) \textit{N,N}-DMF, TBDMSCI, 110°C, 30 min, 72% over two steps\textsuperscript{206}; (c) Aq. 1 N NaOH, rt., 15 hrs, 99%.

\textbf{Scheme 44.} Formation of compound 114 via a cyclic 2,3-\textit{O}-dibutylstannylene acetal intermediate followed by synthesis of the 2,4-diol compound 115.
Evidence that the newly introduced silyl ether was on the C-3 hydroxyl and not the C-2 hydroxyl, could be unequivocally shown by careful examination of the $^1$H NMR spectrum of 114. A broad one proton doublet at δ 2.68 ppm could be assigned to a hydroxyl proton, since this signal disappeared upon D$_2$O exchange. Importantly, in the two-dimensional COSY spectrum of 114, this hydroxyl proton shows a clear correlation to a signal at δ 3.86 ppm, which could be assigned to the proton at C-2 since it shows a correlation to the anomeric proton H-1 ($J_{2,1} = 1.8$ Hz).

With the successful synthesis of the selectively protected 3-O-TBDMS compound 114 complete, the C-4 acetate was removed using Sodium metal dissolved in an anhydrous methanol forming sodium methoxide in situ, producing the desired 2,4-diol 115 (Scheme 44). The $^1$H NMR spectrum of 115 compared with 114, showed that the signals belonging to the C-4 acetate were no longer apparent and the H-4 proton had moved from δ 5.01 ppm in the spectrum of 114 upfield to δ 3.51 ppm in the spectrum of 115, clearly indicating that the acetate protecting group had been removed producing the 2,4-diol 115. Furthermore, the mass spectrum obtained for this compound showed a molecular ion peak at $m/z$ 391.2 [M + Na]$^+$ which is consistent with the molecular formula C$_{19}$H$_{32}$O$_5$Si.

In order to create a compound like 110 (see Scheme 42) from the 2,4-diol compound 115, the C-2 and C-4 hydroxyl groups in 115 needed to be activated to allow for a double inversion step to take place. To this end 115 was stirred in anhydrous dichloromethane and pyridine at -78°C to which trifluoromethanesulfonic anhydride was added. The resulting reaction mixture was allowed to warm to 0°C and stirred for a further 2 hours before being worked up via an aqueous acidic (1 M HCl) wash and concentrated under reduced pressure. The resulting crude yellow oil was purified using column chromatography (5:1 Hex/EtOAc, R$_f$ = 0.4) yielding the 2,4-bis-triflate product 116 in a 87% yield. Examination of the $^{13}$C NMR spectrum of 116, showed a downfield shift of both the C-2 and C-4 peaks from δ 67.8 to δ 85.9 ppm (C-2) and δ 67.9 to δ 85.3 ppm (C-4) had occurred when compared with 115, which indicated the presence of a strong electron withdrawing group attached to these two positions. The $^{13}$C NMR spectrum of 116 also showed two distinct quaternary carbon quartet splitting patterns centred at δ 118.5 ppm, both with a coupling of ~ 317 Hz, which is consistent with the CF$_3$ carbon being split due to coupling to the three fluorine nuclei.
With the characterisation and purification of 116 complete the next reaction to be carried out was the double displacement of the C-2 and C-4 leaving groups to produce the desired benzyl 2,4-diazido-2,4,6-trideoxy-galactopyranoside 117 (Scheme 45).

Reagents and Conditions: (a) Py., Tf₂O, CH₂Cl₂, -78°C, 10 min then 0°C, 2 hrs, 87%; (b) N,N-DMF, NaN₃, rt. 15 hrs.

Scheme 45. Attempted synthesis of the benzyl 2,4-diazido-2,4,6-trideoxy-galactopyranoside 117 from 115.

This reaction was attempted via a standard azide displacement reaction, whereby 116 was dissolved in anhydrous N,N-DMF, to which an excess of sodium azide was added, stirring at room temperature under argon gas, overnight. The resulting reaction mixture was concentrated under reduced pressure and purified directly using column chromatography (3:1 Hex/EtOAc, Rₐ = 0.3), yielding a sole product in 98% yield. Examination of the NMR spectra from the product of this reaction however, clearly showed that in our hands the desired benzyl 2,4-diazido-2,4,6-trideoxy-galactopyranoside 117 was not the product that had been produced. The ¹H NMR spectrum showed clearly that the leaving group at C-4 had been successfully displaced as the coupling constant between H-4 and H-5 was now J = 1.2 Hz, due to the C-4 substituent being axial compared with the coupling in 116 (C-4 substituent being equatorial) between H-4 and H-5 of J = 9.6 Hz. However, further examination of the ¹H and COSY NMR spectra for this product appeared to indicate that there was no H-3 resonance, and that there appeared to be two protons attached to C-2. This latter point was evident from what appeared to be a H-2eq-ax coupling of 14.4 Hz. Examination of the ¹³C NMR spectrum provided the important clue for the characterisation of this unknown compound, since it could be seen in the DEPT spectrum that C-2 was a methylene group (δ 46.1 ppm). Additionally, there was a carbonyl peak seen at δ 199.8 ppm which we attributed to C-3. These important resonance changes compared with 116 hinted toward the structure of the product from this attempted double displacement
reaction to be the 3-oxo derivative 118. The proposed structure of 118 is supported by the mass spectrum (m/z 254.1 [M + Na]+) and IR (azide at 2100 cm\(^{-1}\), carbonyl (C=O) at 1735 cm\(^{-1}\)).

The formation of the 3-oxo product 118 can be considered as shown in Scheme 46. The azide displacement of the C-4 triflate gives the axial 4-azido group as expected. However, the basic conditions of the reaction presumably bring about an elimination of triflic acid across C-3 and C-2, giving the glycal 119, which upon acidic work up lost the silyl ether and tautomerises into the keto form as shown in Scheme 46.

![Scheme 46. E2-elimination mechanism to form 118](image)

With the outcome of our initial attempt at the double displacement reaction, we were interested in having a less labile protecting group at C-3, which we hoped would limit the likelihood of the 3-oxo functionality being obtained. Therefore we opted for the more robust benzyl ether to be introduced at C-3 in 113.

Therefore, the benzyl ether protecting group was introduced into 113 (Scheme 47) following the same method set out by David and Hanessian\(^{206}\), whereby, the 2,3-diol 113 was exposed to dibutyltin oxide in methanol at reflux, which results in the formation of the cyclic 2,3-\(\text{O}\)-dibutylstannylene acetal intermediate. Without isolation, the 2,3-\(\text{O}\)-dibutylstannylene acetal was dissolved in anhydrous \(N,N\)-DMF and benzyl bromide was added, the resulting mixture was stirred at 110°C for 30 minutes. In this
way the energetically favoured selectively protected 3-O-benzyl ether compound 120 was obtained in 80% yield after purification (Scheme 47). The $^1$H NMR spectrum of 120 showed the presence of 5 additional aromatic protons in the aromatic region and a second methylene AB$_4$ spin system that overlapped with the benzyl glycoside signal at $\delta$ 4.63 ppm, which clearly indicated that the benzyl ether at C-3 was present. The acetate group at C-4 in 120 was removed using sodium metal dissolved in methanol producing a sodium methoxide solution creating the 2,4-diol compound 121 in a quantitative yield (Scheme 47). Compound 121 was stirred in anhydrous dichloromethane and pyridine at -78°C to which trifluoromethanesulfonic anhydride was added. The resulting reaction mixture was allowed to warm to 0°C and stirred for a further 2 hours producing the 2,4-*bis*-triflate product 122 in a 90% yield (See Section 5.4.1 for full NMR assignments of all compounds in Scheme 47).

![Scheme 47](image)

**Reagents and Conditions:** (a) Bu$_3$SnO, MeOH, 80°C, 2 hrs; (b) N,N-DMF, BnBr, 110°C, 30 min, 80% over two steps$^{20a}$; (b) Aq. 1 N NaOH, rt., 15 hrs, 99%; (c) Py., Tf$_2$O, CH$_2$Cl$_2$, -78°C, 10 min then 0°C, 2 hrs, 90%; (d) N,N-DMF, NaN$_3$, rt. 15 hrs.

**Scheme 47.** Synthesis of 123 via C-3 benzyl protected intermediate 121.

As can be seen in Scheme 47, when the *bis*-triflate compound 122 was reacted with excess sodium azide, the resulting product was again a 2-deoxy elimination product 123. However, in this case the more robust benzyl ether protecting group was still in place, with the C-2 substituent being eliminated and a C-2-C-3 double bond now in place. The $^1$H NMR spectrum of 123 showed the C-4 displacement had taken place.

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inverting the stereochemistry at this position \((J_{4,5} = 2.4 \text{ Hz})\) and a newly introduced azide functionality could be seen as a sharp peak at \(2101 \text{ cm}^{-1}\) in the IR spectrum of 123. However, the \(^1\text{H NMR}\) spectrum of 123 also showed two doublets at \(\delta \ 5.10 \text{ ppm (H-2, } J_{2,1} = 3.3 \text{ Hz) and } \delta \ 5.33 \text{ ppm (H-1, } J_{1,2} = 3.3 \text{ Hz) and no signal for a H-3 proton. This outcome would be expected if a double bond was now in place between C-2 and C-3, as the C-3 carbon having the benzyl ether protecting group still attached would be in a quaternary carbon environment, whilst the C-2 carbon would no longer be chiral, having a lone proton in an unsaturated environment (expected chemical shift of \(\sim \delta \ 5.0 \text{ ppm}\)), with a calculated coupling being around 4.6 Hz, based on known unsaturated proton couplings in similar environments. Furthermore, evidence that C-3 was indeed in a quaternary unsaturated environment could be seen in the \(^{13}\text{C NMR}\) spectrum of 123, with the signal for C-3 appearing at \(\delta \ 153.7 \text{ ppm}, \) which is consistent for a quaternary carbon in an unsaturated environment.

Interestingly, the outcome of the attempted azide displacement of the \(\text{bis-triflate}\) with the more stable benzyl ether at C-3 supports our thought regarding the formation of the 3-keto-tautomer product 118, which occurred due to the loss of the silyl protecting group from the formed silyl-enol-ether. With the characterisation of 123, the obvious mechanism for the formation of both the 3-keto-tautomer product 118 and the 2,3-unstaturated compound 123 could be easily rationalised. For both 118 and 123 to have formed the mechanism would have had to have followed a probable E2 elimination mechanism as shown in Scheme 46. The basic solution caused by the azide would remove the proton at C-3 causing the E2-elimination mechanism to occur. This type of reaction is set up due to the orientation of the C-3 proton and the leaving group at C-2 being anti-periplanar to one another, causing the \(p\)-orbital overlap required to allow for the elimination of the C-2 leaving group, thus producing the 2-deoxy products. The only difference in the outcome between compounds 118 and 123 is due to the silyl-enol ether formed not being stable under the aqueous acidic work up compared with the benzyl ether, therefore creating the 3-keto-tautomer 118 compared with the 2,3-unstaurated product 123, respectively.

The isolation of both 118 and 123 demonstrates a clear limitation for the double inversion step within \textit{manno}-configured sugars. Given the problems with this double inversion strategy, together with the success of other synthesis protocols that were being
developed at the same time as this method, further C-2 modification attempts of D-rhamnopyranosides in this regard were abandoned in favour of a more direct approach starting with a C-2 nitrogen-based compound as outlined in the following section.

### 3.2.2 Synthesis of \( N \)-acetyl-6-deoxy-glucosamine as a substrate for an aldol condensation reaction

Following on from the difficulties we encountered with the synthesis of C-2 modified rhamnosides described in the previous section, we began investigating a synthesis protocol which would allow access directly to pseudaminic acid analogues via a synthetic aldol condensation reaction with oxaloacetic acid (27), using a C-2 nitrogen based monosaccharide as a starting material. In this regard, \( N \)-acetyl-glucosamine (GlcNAc, 29) was chosen as an appropriate starting compound as it possessed the desired \( N \)-acetyl-group at C-2 in the correct stereochemistry to allow direct transformation to a C-5 axial nonulosonic acid, which is essential if we were to create pseudaminic acid based compounds (Scheme 48).

![Scheme 48](image)

**Scheme 48.** Conversion of GlcNAc (29) to 5-epi-neuraminic acid (106) via an aldol condensation reaction.

In order to obtain pseudaminic acid analogues via a selectively functionalised GlcNAc derivative our first aim was the synthesis of a \( N \)-acetyl-6-deoxy-glucosamine compound. We chose the commercially available monosaccharide glucosamine hydrochloride (107) as our starting material. The most important structural feature of glucosamine (107) is the C-2 amine in the equatorial configuration, which can be easily modified to an acetamide group, which will correspond to the C-5 position in pseudaminic acid analogues after the aldol condensation reaction. The first step toward the synthesis of our desired \( N \)-acetyl-glucosamine derivatives was taking our starting
material glucosamine hydrochloride (107) and converting it into a per-O-acetyl-GlcNAc (124), as seen in Scheme 49. This step was important because not only does the acetylation introduce acetate groups to all the free hydroxyls, but also creates the acetamide group at C-2 by displacing the hydrochloride salt producing the desired C-2 N-acetyl-functionality.

\[
\text{HO} \quad \text{OH} \quad \text{NH}_2 \cdot \text{HCl} \quad \text{AcO} \quad \text{OAc} \quad \text{NHAc}
\]

(107) \quad (124)

Reagents and Conditions: (a) Py, Ac₂O, rt., 15 hrs, 99%.

**Scheme 49.** Synthesis of the per-O-acetyl-α-D-GlcNAc compound 124 from 107.

The acetylation of glucosamine (107) was achieved by adding acetic anhydride to a stirred solution of glucosamine hydrochloride (107) in pyridine following a protocol described by Chaplin et al.\textsuperscript{207}. Evidence that we had indeed created the desired per-O-acetylated GlcNAc derivative 124 was seen in the \textsuperscript{1}H NMR spectrum of 124, which showed the five singlet acetate peaks (δ 1.92 ppm, 2.02 ppm, 2.03 ppm, 2.07 ppm and 2.17 ppm (NHCH₃)), along with couplings of all ring protons consistent with those described in the literature for the α-anomer\textsuperscript{207}.

Having successfully prepared the fully acetylated GlcNAc derivative 124, we next needed to introduce a more stable protecting group at the anomeric position, since anomeric acetate groups are susceptible to cleavage under acidic conditions\textsuperscript{208}. It is well known in carbohydrate chemistry that in an acidic environment the free electrons from the ring oxygen can stabilise the formation of a oxocarbenium ion, thereby allowing the oxygen from the acetamide species to nucleophilically attack the anomeric carbon to form a 1,2-cyclic intermediate known as a oxazoline (Scheme 50)\textsuperscript{209–211}. In our hands, we intended to use this oxazoline intermediate, although quite stable, as such groups are susceptible to attack by alcohols from the equatorial position to form solely β-glycosides with the alcohol now attached at the anomeric position as seen in Scheme 50. Due to this well documented procedure\textsuperscript{209–211}, we thought it possible that we could
quite readily introduce a benzyl glycoside into compound 124, which would allow us a
differentially protected anomeric position.

Scheme 50. Mechanism for the formation of the benzyl β-glycoside 125 from 124 via an
oxazoline intermediate

To produce the benzyl glycoside 125, a one-pot synthesis was used starting with
124. Initially 1.1 eq. of TMSOTf was added to a stirred solution of 124 in
1,2-dichloromethane under argon gas. The resulting reaction mixture was stirred at 50°C
for 24 hours in order to form the oxazoline intermediate (Scheme 50); the formation of
which could be monitored by TLC analysis (EtOAc/Hex 3:1, R_f = 0.6). Without workup
or purification, the reaction was cooled to room temperature and molecular sieves were
added followed by benzyl alcohol, after which the reaction was stirred for a further 24
hours, before workup and purification. Following this procedure we were able to isolate
the desired β-benzyl glycoside 125 in a 52% yield.

The 1H NMR spectrum of 125 showed the appearance of the aromatic benzyl peak
belonging to the newly introduced glycoside as a five proton aromatic multiplet at
δ 7.29-7.38 ppm, along with the accompanying methylene ABq spin system at δ 4.75
ppm. Evidence that we had created the correct glycoside came from the coupling
constant between H-1 and H-2, which was 8.4 Hz, which is consistent with a
β-glycoside in a GlcNAc monosaccharide.

With the anomeric position successfully protected in sufficient yield, the next step
involved removal of the remaining acetates at C-3, C-4 and C-6, which was required to
allow for selective modification at the C-6 position. We were able to deacetylate 125 by
stirring it overnight in an aqueous methanol solution containing aqueous NaOH (1 N) adjusted to pH 12. In this manner we were able to produce 126 quantitatively from 125.

The $^1$H NMR spectrum of 126 showed that with the removal of the acetate groups the chemical shift of H-3, H-4 and H-6 had moved upfield roughly 1.0 ppm, furthermore the acetate methyl group peaks seen in the spectrum of compound 125 at δ 2.01 ppm, 2.02 ppm and 2.10 ppm were not seen in the spectrum of 126. The mass spectrum of 126 showed a molecular ion peak at $m/z$ 334.1 [M + Na]$^+$ which is consistent with the molecular formula C$_{15}$H$_{21}$NO$_6$.

With compound 126 in hand and in excellent yield from 107, the next step toward the synthesis of a selectively functionalised 6-deoxy-GlcNAc derivative was to reduce the C-6 position in order to obtain the desired 6-deoxy derivative as seen in Scheme 51. The formation of the 6-deoxy compound 127 was considered to be crucial for our approach towards the synthesis of pseudaminic acid analogues, since the C-6 position within a hexose compound corresponds to the C-9 position within pseudaminic acid as mentioned previously.

![Scheme 51. Proposed synthesis of the 6-deoxy-GlcNAc derivative 127.](image)

In order to achieve the desired outcome shown in Scheme 51, based on literature precedent$^{166}$ we thought it best to introduce an activating group at C-6, which could then be reduced to the required methyl group. The group we chose to introduce was iodine, based on the success that we had had with this type of group in the synthesis of our selectively functionalised rhamno- and talopyranosides. Once the iodine activating group at C-6 was in place, to give compound 128, the methylene halide moiety could be reduced under hydrogenation conditions to form the 6-deoxy compound 127 (Scheme 51).
Our initial attempts at synthesising the desired compound 128 proved difficult. The major issue encountered was that compound 126 had very poor organic solvent solubility, which made handling this compound in common reaction solvents problematic, we believe this contributed too much of the early failed attempts at introducing our desired iodine group at C-6. The solubility problems we encountered made attempted work-up and isolation of the 6-iodo compound 128 extremely difficult. Numerous attempts were made varying equivalents of reagents, solvents used and temperature. However, none of these attempts were successful in directly transforming 128 from 126.

After numerous unsuccessful attempts at introducing the desired iodine group directly at C-6 of 126, we attempted a series of other approaches where we replaced the primary hydroxyl with an activating group, which included the use of tripsylate or mesylate groups, followed by displacement of the activating group with iodine. We hoped that such a two-step procedure for the introduction of the desired 6-iodo group would overcome some of the solubility problems with 126. Unfortunately these attempts also produced none of the desired compound 128.

Turning back to the literature, we could see that the Garegg reaction was typically carried out at 70°C in toluene, however, the compounds used in the literature166 compared with 126 had greater hydrophobic properties in the form of lipophilic protecting groups which clearly aided their solubility in toluene. While we had been able to produce a small amount (<5%) of the desired 6-deoxy-6-iodo-GlcNAc compound 128 during our attempts at optimising the reaction, the overall poor yields and lack of reproducibility of the reactions forced us to reconsider the introduction of an iodine to the C-6 position using a direct approach.

With the knowledge of the solubility limitations, we felt that if we were able to introduce a protecting group at the C-3 position (shown in red (OPG), Figure 28) this would produce an increase in the lipophilic nature of compound 126, therefore permitting the intended iodination reaction to be carried out in toluene as described in the literature166.
Figure 28. Intended position of the desired C-3 protecting group in 126

To achieve introduction of a protecting group selectively at C-3, we initially thought that introducing a 4,6-O-benzylidene protecting group might be beneficial. Although we were able to introduce 4,6-O-benzylidene group in good overall yield (89%) following a protocol set out by Winnik et al.\textsuperscript{163} (Scheme 52, 129) (For \textsuperscript{1}H NMR assignment see section 5.4.1), subsequent reactions attempting to introduce a protecting group at C-3 on 129 proved to be difficult.

Reagents and Conditions: (a) Benzaldehyde, Formic acid, rt. 15 hrs 89%.

Scheme 52. Synthesis of the benzyl 4,6-O-benzylidene-GlcNAc derivative 129.

Therefore, based on the problems that had been encountered, an alternate protocol was developed that utilised a 4,6-acetonide protecting group which, like the 4,6-O-benzylidene, would allow us to selectively protect C-3 with a large lipophilic group, which was hoped would increase the organic solubility of compound 126.

The 4,6-acetonide protecting group can be added selectively to C-4 and C-6 over the possible formation of a 3,4-acetonide due to the fact that in compound 126, the C-3 and C-4 secondary hydroxyls are trans with respect to one another. Therefore the 3,4-acetonide would be too strained and energetically unfavourable to be able to be formed. In comparison, when adding the acetonide group it would preferentially add to the primary hydroxyl group at C-6 first and then to the closest unhindered secondary hydroxyl (C-4) forming the desired 4,6-acetonide, leaving the C-3 hydroxyl group free for further manipulation (Scheme 53).
Reagents and Conditions: (a) Acetone:2,2-DMP (1:1), p-TsOH, 2 hrs, 93%.

Scheme 53. Synthesis of the 4,6-acetonide compound 130 from 126.

The 4,6-acetonide protecting group was added to compound 126, following a standard protocol, which involved stirring 126 in dry acetone to which an equal volume of 2,2-dimethoxypropane was added along with an acid catalyst (p-toluenesulfonic acid). The resulting reaction mixture was stirred at room temperature for 2 hours before being neutralised with triethylamine and concentrated to dryness. After purification using column chromatography (EtOAc, Rf = 0.8) compound 130 was obtained in a 93% yield. Looking at the 1H NMR spectrum of 130, compared with 126, we could see the appearance of two three proton singlet peaks at δ 1.34 ppm and 1.47 ppm, corresponding to the two methyl groups of the newly introduced 4,6-acetonide. Key signals were also seen in the 13C NMR spectrum of 130 corresponding to the two methyl peaks (δ 23.6 and 29.5 ppm) and quaternary carbon (δ 99.0 ppm) of the newly formed acetonide protecting group. With confirmation that we had indeed been successful in adding the desired 4,6-acetonide, in excellent yield, we now had the C-3 hydroxyl free for further manipulation.

The choice of protecting group at C-3 was important as we needed to introduce a group that could be installed in high yield and be robust enough to withstand future manipulations, whilst being able to be removed selectively if required. Therefore, we chose to utilise a silyl ether protecting group, as this would introduce the required lipophilicity needed for further reactions and would potentially be robust enough in future planned reactions. A bulky silyl protecting group in the form of tert-butyldimethylsilyl ether (TBDMS) was chosen due to the stability of the functional group to withstand the subsequent reactions; also this type of ether is only removed under very specific reaction conditions, which would not affect any of the other protecting groups. Therefore, we treated compound 130 with tert-butyldimethylsilyl
chloride in a solution of anhydrous pyridine over 24 hours at room temperature. The reaction was found to be quite sluggish, with further addition of the silyl reagent being required. After work-up and purification however, we were able to obtain the intended C-3 protected 3-O-TBDMS compound 131 in good yield (80%). The $^1$H NMR spectrum of 131 showed the appearance of the indicative silyl methyl groups at δ -0.02 ppm and -0.04 ppm (2 s, 2 x 3H, Si(CH$_3$)$_2$) and δ 0.81 ppm (s, 9 H, C(CH$_3$)$_3$), respectively, which indicated that we had indeed added the desired protecting group. Furthermore, the $^1$H NMR spectrum of 131 also showed the chemical shift of the H-3 proton had moved downfield from δ 3.84 ppm to 4.07 ppm compared with compound 130, therefore providing further evidence that we had selectively protected the C-3 position with the TBDMS group.

With the addition of the silyl protecting group at the C-3 position we now needed to prepare for the introduction of the 6-iodo group. Accordingly, compound 131 was treated with aqueous 80% acetic acid at room temperature for several hours (Scheme 54), thus removing the 4,6-acetonide protecting group in a quantitative yield based on recovered starting material. The $^1$H and $^{13}$C NMR spectra of 132 showed that peaks associated with the acetonide protecting group seen in the spectrum of 131 were no longer present indicating that we had indeed removed the acetonide group. Furthermore the mass spectrum of 132 had a molecular ion peak of $m/z$ 448.1 [M + Na]$^+$ which is consistent with the molecular formula of 132 (C$_{21}$H$_{35}$NO$_6$Si).

![Scheme 54](image)

Reagents and Conditions: (a) Aq. 80% AcOH, rt., 3 hrs, 99%.

**Scheme 54.** Removal of the 4,6-acetonide in 131 to produce the 4,6-diol 132.

With the synthesis of the selectively protected benzyl 3-O-TBDMS-β-D-GlcNAc compound 132 in good overall yield, we were ready to once again attempt the Garegg iodination reaction in order to produce the 6-deoxy-6-iodo compound 133. Following
our earlier work, exposure of 132 to triphenylphosphine, imidazole and iodine, in anhydrous \textit{N,N}-DMF, resulted in a poor yield (8\%) of the 6-iodo product 133. Therefore, we altered the reaction conditions by changing the solvent to toluene and increasing the temperature. This change in conditions resulted in a dramatic increase in the yield, as we were able to obtain the desired product 133 in 90\% overall yield after purification. The $^{13}$C DEPT NMR spectrum of 133 gave us the necessary evidence that we had indeed introduced the iodine group at C-6 as the C-6 methylene carbon in 133 was seen at $\delta$ 6.3 ppm compared with $\delta$ 62.9 ppm in compound 132 which bore a hydroxyl group at C-6. This dramatic change in upfield chemical shift is common for a methylene carbon, which has been converted from a primary alcohol to an alkyl iodide$^{164}$.

With the desired 6-deoxy-6-iodo compound 133 now in hand, the final reduction step to obtain the C-6 methyl group could be carried out (Scheme 55). In order to achieve this step we intended to use a well-documented basic-hydrogenation procedure$^{50,111,113}$, which would reduce the alkyl halide to a methyl group, without affecting the other protecting groups. To this end the 6-deoxy-6-iodo compound 133 was dissolved in a solution of methanol and diisopropylethylamine to which palladium hydroxide on carbon 20\% wt. (as catalyst) was added. The resulting reaction mixture was stirred under an atmosphere of hydrogen gas (1 atm) for 15 hours. Upon completion (monitored by TLC; Hex/EtOAc 1:1, $R_f$ = 0.5) the reaction mixture was filtered through Celite to remove the palladium on carbon and concentrated under reduced pressure before being purified using column chromatography (Hex/EtOAc 1:1, $R_f$ = 0.5) yielding the desired 6-deoxy compound 134 in a quantitative yield (Scheme 55).

\[
\begin{array}{c}
\text{HO} & \text{O} & \text{OBn} & \text{NHAc} \\
\text{TBDMSO} & \text{HO} & \text{O} & \text{OBn} & \text{NHAc} \\
\text{TBDMSO} & \text{HO} & \text{O} & \text{OBn} & \text{NHAc} \\
132 & 133 & 134
\end{array}
\]

\begin{enumerate}
\item Reagents and Conditions: (a) PPh$_3$,imidazole, I$_2$, toluene, 80°C, 1 hr, 90\%;
\item Pd(OH)$_2$ (20\% wt.), Et$_2$NPr$_2$, MeOH, H$_2$ (atm), rt., 1 atm, 15 hrs, 100\%.
\end{enumerate}

\textit{Scheme 55.} Synthesis of the 6-deoxy compound 134 from the C-3 protected 4,6-diol 132.
The $^1$H NMR spectrum of 134 showed the appearance of the C-6 methyl group doublet peak at $\delta$ 1.30 ppm which coupled to H-5 ($J_{6,5} = 6.3$ Hz), proving we had now reduced the 6-iodo group to the desired methyl group.

With the synthesis and successful purification of 134 complete, work turned to the final deprotection steps in order to remove the C-3 silyl ether and benzyl glycoside protecting groups, which was essential if 134 were to be used in the aldol condensation reaction with oxaloacetic acid (27). The final deprotection sequence was achieved in a single step by stirring 134 in water (as solvent) with acidic resin (Dowex DW50 H$^+$ resin) at 80°C for two days (Scheme 56). The acidic environment hydrolysed both the silyl ether protecting group and the benzyl glycoside, producing the desired fully deprotected compound 135 in a quantitative yield. The $^1$H NMR spectrum of 135 showed the expected H-1 singlets at $\delta$ 5.15 ppm ($\alpha$) and $\delta$ 4.69 ppm ($\beta$), and the two C-6 methyl peak doublets at 1.31 ppm ($\alpha$) and 1.28 ppm ($\beta$), respectively. Furthermore, all peaks associated with the two protecting groups in 134, were not present in the NMR spectrum of 135.

![Chemical structures](image)

Reagents and Conditions: (a) water (as solvent), H$^+$ resin, 80°C, 48 hrs, 100%.

Scheme 56. Final deprotection step to produce compound 135.

With the synthesis of the first 6-deoxy-GlcNAc derivative (135) complete, we were interested in using this compound in a synthetic aldol condensation reaction, thereby producing our first pseudaminic acid analogue.
3.3 Synthetic aldol condensation reactions

Aldol condensation reactions between hexose substrates and oxaloacetic acid (27) have been reported, but most of the published methods either result in very poor yields of the target nonulosonic acids\(^{50,111,113,198}\), or use reaction conditions that appear to very specific for that particular target compound. At the start of this project it appeared that there was no single general method that would efficiently condense oxaloacetic acid (27) with a variety of hexose substrates in order to gain access into a range of nonulosonic acid analogues. We therefore aimed to streamline the process of the aldol condensation by altering the reaction conditions with; heat, base catalyst, the use of sodium tetraborate and other additives, along with varying the concentration of reagents. In order to achieve this aim we firstly wanted to determine an optimised set of reaction conditions, which we would eventually use on the 6-deoxy-GlcNAc derivatives like 135 that had been synthesised.

Therefore, initially work was carried out on a series of aldol condensation reactions utilising monosaccharide’s that had been documented as being good substrates for this type of transformation. These initial reactions were first attempted on commercially available D-mannose (37), which has been proven to be a successful substrate in creating the nine-carbon sialic acid derivative KDN (3)\(^{200}\). The general process (Method 1) for these reactions entailed the hexose substrate 37 being dissolved in water, followed by the addition of base catalyst (e.g. Na\(_2\)CO\(_3\)), then oxaloacetic acid (27) was added portion wise, following this the pH was adjusted to 11 with the use of aqueous NaOH (10 M). After stirring for 2-4 hours at room temperature, the reaction mixture was acidified to pH 2 using acid resin (Amberlite IR 120H\(^{+}\)) heated to 60ºC and stirred for a further 1 hour. The reaction mixture was allowed to cool to room temperature before being neutralised with concentrated ammonia and then concentrated under reduced pressure. The resulting residue was purified using an ion-exchange column (HCO\(_3^−\)) initially eluting with water (~ 200 mL) followed by NH\(_4\)HCO\(_3\) (0.5 M) to elute the acidic fraction containing our desired product. Using these reaction conditions and purification method we were able to obtain the nine-carbon nonulosonic acid KDN (3) in a good overall yield of 60% (Scheme 57).

Examination of the \(^1\)H NMR spectrum of 3 showed the distinctive H-3 equatorial and axial proton signals at \(\delta\) 1.67 ppm (H-3\(_{ax}\)) and \(\delta\) 2.07 ppm (H-3\(_{eq}\)) respectively.
Furthermore, other proton resonances and calculated proton couplings in the $^1$H NMR spectrum of 3 were consistent with those reported in the literature\textsuperscript{189}. Following on from the initial reactions carried out on D-mannose (37), it was seen as important that we attempt similar reactions on a 6-deoxy-hexose substrate. Therefore we also carried out a series of reactions on D-rhamnose (35) using the same reaction conditions and purification techniques. In this manner we were able to obtain 9-deoxy-KDN (89) from D-rhamnose (35) in a 50% yield (Scheme 57). Evidence that we had obtained the desired 9-deoxy-KDN (89) was best seen in the $^1$H NMR spectrum of 89, which showed the diagnostic three proton doublet of the C-9 methyl group at $\delta$ 1.06 ppm, whilst all of the other resonance seen in the spectrum were consistent with those seen in the $^1$H NMR spectrum of the same product obtained from our chemoenzymatic approach (for the full $^1$H NMR assignment refer to Section 5.5.1).

Scheme 57. Aldol condensation reaction between the hexose substrates D-mannose (37) and D-rhamnose (35) with oxaloacetic acid (27) to produce 3 and 89.

Delighted with the outcome of these initial reactions on the manno-configured sugars 37 and 35 we thought it important to investigate the use of $N$-acetyl-glucosamine (29) as a substrate for the aldol condensation reaction. Our initial attempts at transforming 29 into the corresponding 5-epi-Neu5Ac derivative 106, resulted in 95% recovery of starting material, with no reaction seen to take place. This was disappointing based on what we had seen with the manno-configured substrates, so we looked at ways we could potentially drive the reaction to produce the desired nonulosonic acid product. In particular we varied the reaction time, temperature and use of additives, including type of base used, whilst keeping the concentrations of oxaloacetic acid (27) and pH used essentially constant. Table 6 shows examples of the reaction attempts for transforming 29 into 106. As can be seen in Table 6, the reaction attempts using our established reaction conditions failed to produce the desired
nonulosonic acid 106, with recovery of unreacted starting material being the only material identified.

Table 6. Results from the aldol condensation reaction with various hexose sugars

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Temp (°C)</th>
<th>Base</th>
<th>Sodium tetraborate (Y/N)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Mannose (37)</td>
<td>24</td>
<td>NaCO₃</td>
<td>Yes</td>
<td>KDN (60%)</td>
</tr>
<tr>
<td>D-Rhamnose (35)</td>
<td>24</td>
<td>NaCO₃</td>
<td>Yes</td>
<td>9-deoxy-KDN (50%)</td>
</tr>
<tr>
<td>GlcNAc (29)</td>
<td>24</td>
<td>NaCO₃</td>
<td>Yes</td>
<td>Recovered GlcNAc (95%)</td>
</tr>
<tr>
<td>GlcNAc (29)</td>
<td>50</td>
<td>NaCO₃</td>
<td>No</td>
<td>Recovered GlcNAc (87%)</td>
</tr>
<tr>
<td>GlcNAc (29)</td>
<td>24</td>
<td>MgCO₃</td>
<td>Yes</td>
<td>Recovered GlcNAc (98%)</td>
</tr>
<tr>
<td>GlcNAc (29)</td>
<td>50</td>
<td>MgCO₃</td>
<td>Yes</td>
<td>Recovered GlcNAc (97%)</td>
</tr>
</tbody>
</table>

As a result of these disappointing outcomes we looked at changing the reaction conditions. Based on a method published by Dumont et al. we refined the way in which this reaction was carried out. This new protocol (Method 2) consisted of starting with a cooled aqueous solution (0°C) of oxaloacetic acid (27) adjusted to pH 11 with NaOH (10 M), and then adding an ice cold aqueous solution of the hexose substrate. The resulting reaction mixture was then stirred for 24 hours allowing it to warm to room temperature. After this, the pH was tested and adjusted to pH 11 again if needed before being heated to 60°C and stirred for a further 24 hours. The reaction was acidified (pH 6) with acidic resin (Dowex 50 H⁺ resin) before stirring for a further hour. After cooling to room temperature, it was filtered to remove the resin, the pH was adjusted to 7 (if needed) and then the filtrate was concentrated under reduced pressure. The residue was columned on an anion exchange column (HCO₃⁻), initially eluting with water (200 mL) then aqueous NH₄HCO₃ (0.5 M). Using the above method we were able to isolate an acidic component from the ion exchange column, however after some experimentation we were never able to unequivocally produce evidence using NMR spectroscopy that we had obtained the desired 5-epi-compound 106. In all instances the NMR spectrum of this acidic component was extremely complex and lacked important cross correlations within the COSY spectrum to suggest this reaction had been successful.
With the unsuccessful attempts at converting $N$-acetyl-glucosamine (29) into its corresponding nonulosonic acid 106 using either method 1 or 2, we decided to attempt a slightly refined method 2, which would use a trace amount of nickel (II) chloride, to be added once the reaction had been acidified in the last step. We rationalised, following the observation of others$^{202}$, that using the nickel (II) chloride may help reduce possible side products being produced, which could potentially be the reason for the complex NMR spectra we were seeing.

Therefore, in an analogous manner to the reaction conditions described in method 2, $N$-acetyl-glucosamine (29) was reacted with oxaloacetic acid (27) for 24 hours at room temperature, followed by 24 hours at 60ºC, before being acidified (pH 6) and a trace amount of nickel (II) chloride added. After purification however, we saw no improved outcome compared with method 2. Unfortunately, the NMR spectrum of the acidic component from the ion exchange column was far too complex to again unequivocally provide evidence that we had created the desired 5-epi-product 106 (Scheme 58).

Despite the disappointing outcome of our repeated attempts to convert 29 into 106 via a synthetic aldol condensation reaction we decided to attempt the reaction on our 6-deoxy-GlcNAc compound 135 in order to produce the corresponding 7-epi-9-deoxy-pseudaminic acid analogue 136. Using our refined method 2 we reacted 135 with oxaloacetic acid (27) for 48 hours at room temperature followed by a further 24 hours at 60ºC. The reaction was then acidified (pH 6) and a trace amount of nickel (II) chloride added before being stirred for a further 2 hours at 60ºC (Scheme 58). Again we were able to isolate an acidic component from the ion exchange column which was analysed by NMR spectroscopy.

**Scheme 58.** Proposed aldol condensation reaction with oxaloacetic acid (27) between the $N$-acetyl-glucosamine substrates 29 and 135 to produce 106 and 136.
The $^1$H NMR spectrum of the acidic component again was very complex and impossible to unequivocally characterise our desired nonulosonic product 136.

### 3.4 Conclusions and future directions

The aim of this part of our project was to investigate the use of a synthetic aldol condensation approach toward the synthesis of pseudaminic acid analogues. In order to achieve this aim we firstly sought to create two different structural and functional group hexose substrates that had a C-2 nitrogen functionality in an equatorial orientation, that could be condensed with oxaloacetic acid (27) to form the corresponding pseudaminic acid analogues.

As outlined in the beginning of this chapter the synthesis of the desired 2,4-diazido-2,4,6-trideoxy-galactopyranoside 117 proved to be difficult, with two elimination products obtained that were not usable for our intended purpose. Therefore this synthesis was abandoned in favour of the 6-deoxy-GlcNAc synthesis we were performing concurrently.

Not too disheartened by the problem we had encountered with the galactose synthesis; we successfully obtained the 6-deoxy-GlcNAc compound 135 from the commercially available glucosamine hydrochloride (107) in an efficient nine step pathway with an overall yield of 22%.

With a viable substrate in hand, investigations began towards optimising the reaction conditions for the aldol condensation reaction using oxaloacetic acid (27). We aimed to develop a single set of reaction conditions that could be used for all intended substrates of this reaction. However, what we found is that due to the varying chemical characteristics of the different substrates used, a single set of reaction conditions was not a realistic option. This was best exemplified by the fact that we could efficiently obtain KDN derivatives from manno-configured hexoses, but the same reaction conditions returned near 100% recovery of starting material when using gluco-configured substrates like N-acetyl-glucosamine (29).

Table 7 summarises the outcomes of the varied reaction attempts that were used in our attempts of using an aldol condensation reaction to create pseudaminic acid analogues.
Table 7. Reaction outcome for aldol condensation reaction on varied substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Additive</th>
<th>Sodium tetraborate (Y/N)</th>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Mannose (37)</td>
<td>NaCO₃</td>
<td>Y</td>
<td>Method 1</td>
<td>KDN (60%)</td>
</tr>
<tr>
<td>D-Rhamnose (35)</td>
<td>NaCO₃</td>
<td>Y</td>
<td>Method 1</td>
<td>9-deoxy-KDN (50%)</td>
</tr>
<tr>
<td>GlcNAc (29)</td>
<td>NaCO₃</td>
<td>Y</td>
<td>Method 1</td>
<td>Recovered GlcNAc (95%)</td>
</tr>
<tr>
<td>GlcNAc (29)</td>
<td>---</td>
<td>N</td>
<td>Method 2</td>
<td>Unknown acidic Component(s)</td>
</tr>
<tr>
<td>GlcNAc (29)</td>
<td>---</td>
<td>N</td>
<td>Method 3</td>
<td>Unknown acidic Component(s)</td>
</tr>
<tr>
<td>6-deoxy-GlcNAc (135)</td>
<td>---</td>
<td>N</td>
<td>Method 3</td>
<td>Unknown acidic Component(s)</td>
</tr>
</tbody>
</table>

**Method 1**: Dissolve substrate in H₂O; add Na₂CO₃, then oxaloacetic acid; adjust pH to 11; stirred for 2 hrs at RT; acidify (to pH 2); heat 60°C for 1 hr.

**Method 2** Substrate in H₂O at 0°C added to oxaloacetic acid solution at 0°C and pH 11; stirred at 60°C for 48 hrs; acidified (to pH 6); stirred for 1 hr.

**Method 3** Same as Method 2, but nickel (II) chloride added after final acidification.

The results from this work show that we were able to produce the known compounds KDN (3) and 9-deoxy-KDN (89) from D-mannose (37) and D-rhamnose (35), respectively, using method 1 in good yield (Table 7). However, reactions using this method failed to convert N-acetyl-glucosamine (29) with recovery of 95% of unreacted starting material. Following on from this disappointing result, methods 2 and 3 also failed to produce the desired 5-epi-Neu5Ac (106) product from the aldol condensation reaction, instead producing a complex mixture of acidic products which were impossible to characterise. Unfortunately, the attempted conversion of the 6-deoxy-GlcNAc derivative 135 to produce 136 also gave a complex mixture of acidic products that was impossible to characterise.

It is clear that more work needs to be done in an attempt to establish the best reaction conditions for this type of transformation. Following on from my preliminary results there has been some success toward this end with work being carried out within our lab using a modified set of reaction conditions based on Method 3. We believe that the approach toward pseudaminic acid analogues using an aldol condensation reaction whilst having no real success within this PhD project could still produce the types of outcomes we had hoped possible at the beginning of our efforts.
4.1 Introduction

The final approach for this project was to investigate the synthesis of pseudaminic acid analogues via a chemical strategy starting from a more advanced precursor compared with the hexose sugars used in the former two approaches discussed thus far. The rationale behind this approach was to develop a synthesis that would overcome the stereochemical limitations of substrates used in the previous two syntheses, with a view to create pseudaminic acid based compounds directly from a nine-carbon sugar. The main aim of this part of the project was to develop a novel synthesis of pseudaminic acid analogues from the commercially available nine-carbon monosaccharide, 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid KDN (3).

KDN (3) is a nine-carbon acidic sugar which was first reported in 1986\textsuperscript{11} having been isolated in microgram (µg) quantities from the polysialoglycoproteins (PSGPs) of rainbow trout eggs. Structurally KDN (3) is a 5-hydroxy-sialic acid derivative, which has subsequently been isolated from a wide variety of species including, mammals, bacteria, amphibians and fish\textsuperscript{9,11,13,14}, for a full discussion refer to Chapter 1, Section 1.1.

Despite the significant interest in the biological function of KDN (3), surprisingly in contrast to Neu5Ac (1), there have been limited published accounts of the preparation of structurally modified KDN derivatives\textsuperscript{189,200,213–215}. Most research towards the synthesis of KDN derivatives has generally used esterification at C-1 as well as protecting group chemistry, such as acetylation\textsuperscript{189,200,213}. Limited O-glycosylation at the C-2 position of KDN (3) has been reported, with some C-glycosylation also having been reported\textsuperscript{214,215}. However, overall there have been very few reports of chemistry directed at the other carbon centres of KDN (3).

Therefore, based largely on synthetic carbohydrate chemistry carried out on Neu5Ac (1), we wanted to investigate the synthesis of pseudaminic acid analogues via a purely synthetic approach starting with KDN (3). As can be seen in Figure 29,
structurally both KDN (3) and pseudaminic acid (4) are non-ulosonic acids; however KDN (3) has a true sialic acid (D-glycero-D-galacto) configuration, whilst pseudaminic acid (4) has an L-glycero-L-manno-configuration (Figure 29).

**Figure 29:** Structures of KDN (3) and pseudaminic acid (4) highlighting structural and functional group differences at C-5, C-7, C-8 and C-9.

In converting KDN (3) to pseudaminic acid (4), the changes required at C-5 and C-7 are the introduction of a nitrogen-based functionality, in the form of an acetamide, as well as stereochemical interconversion. Additionally, deoxygenation must occur at C-9, as all known pseudaminic acid compounds possess a 9-deoxy functionality, and the stereochemistry at C-8 would also need to be inverted (Figure 29).

The primary impetus behind our approach was that we wanted to expand on the range of chemistry undertaken on KDN (3) by developing a novel general method toward a range of tetradeoxy-nonulosonic acids. We hoped that this new approach would allow more efficient access to structurally modified analogues of pseudaminic acid and would permit access to syntheses of some naturally occurring pseudaminic acid based derivatives.

As discussed in chapter 1 Section 1.2.1, the major variation that sets all of the naturally occurring derivatives of pseudaminic acid (4) apart is the differing N-acyl substitutions found at C-5 and C-7 (see Table 1, Pg. 9, for examples of derivatives isolated from nature). These nitrogen functionalities differ between bacteria from being quite simple acetamides as seen for pseudaminic acid (4) itself, to more complex N-acyl substitutions, for example those seen in some *Pseudomonas aeruginosa* strains.\(^{21,24-27}\) (Figure 30).
Furthermore, there have been no publications to date that aim specifically to generate pseudaminic acid analogues and moreover, previous published approaches toward the synthesis of pseudaminic acid (4) and its naturally occurring derivatives have been rather long and inefficient routes of synthesis, whilst enzymatic approaches have often been arduous and expensive and do not provide access to structural analogues of pseudaminic acid (4).

Our proposed approach for the synthesis of pseudaminic acid analogues from KDN (3) is shown in retrosynthetic terms in Scheme 59. The retro-synthesis illustrates the key intermediate compounds we intended to create toward the synthesis of pseudaminic acid analogues.

**Scheme 59:** Retrosynthetic approach toward the synthesis of pseudaminic acid analogues from KDN (3).
Starting with the 5-hydroxy-sialic acid derivative KDN (3), we initially wanted to selectively protect 3 in order to generate the 5,7-diol 137 (Scheme 59), which is an essential compound for the success of this part of our project. The 5,7-diol-compound 137 should allow direct introduction of the requisite nitrogen functionalities at C-5 and C-7 found in pseudaminic acid (4), with concomitant stereochemical interconversion. Our proposed route was to selectively activate the C-5 and C-7 positions in 137 followed by subsequent displacement with a nitrogen nucleophile (e.g. azide), resulting in inversion of the stereochemistry and introduction of the nitrogen functionalities at C-5 and C-7 in a single step, leading to the bis-azole 138 (Scheme 59). Once the bis-azole 138 is produced, the azides at C-5 and C-7 can be reduced and converted to N-acyl groups, which in the first instance would be acetamide functional groups, which are found in the naturally occurring pseudaminic acid itself.

Following the introduction of the acetamide groups at C-5 and C-7, the 8,9-isopropylidene and C-4 silyl protecting group could then be removed to produce the 4,8,9-triol 139. Deoxygenation of the primary hydroxyl at C-9 in 139 then gives the desired 9-deoxy functionality. If successful, this approach towards pseudaminic acid analogues from KDN (3) would be the simplest method of all avenues we have explored, and would also represent one of the shortest methods reported. Once the C-9 hydroxyl has been reduced, the only structural change needed to obtain pseudaminic acid itself is inversion of the hydroxyl group stereochemistry at C-8. However, since the aim of this project is to prepare pseudaminic acid analogues, we can consider the C-8-epimer to be an analogue of pseudaminic acid itself. The following section outlines the synthesis of the 5,7-diol 137.

4.2 Synthesis of the 5,7-diol 137

The first step towards the synthesis of the selectively protected 5,7-diol 137 involved protecting the anomeric position (C-2) and free carboxylic acid at C-1 in KDN (3). These initial protection steps are important as C-1 and C-2 are extremely reactive and allow for the various conformations of KDN (3) to exist (Figure 31). In solution KDN (3) can exist in various conformers, being the furanose (α or β) and or pyranose
(α or β) conformations (Figure 31), with a small percentage of the acyclic conformation also possible (not shown in Figure 31). These different conformations of KDN (3) make the initial protection of the anomeric hemi-ketal essential in order to lock the compound into a specific conformation, which inhibits further mutarotation or ring size change and allows ease in characterisation and further chemical manipulation.

Figure 31. The α and β anomers of the furanose and pyranose conformers of KDN (3)

Following from the extensive derivatisation of Neu5Ac (1) that has been reported, we sought to protect C-1 and C-2 as the methyl ester methyl glycoside (KDN1,β2Me2, 140). Following a protocol set out by Nakamura et al.189, KDN (3) was dissolved in anhydrous methanol to which an acidic resin (Dowex 50 H+) was added at room temperature under an inert atmosphere of nitrogen gas. The resulting reaction mixture was allowed to stir at room temperature for two days, before the resin was removed by filtration and the filtrate was concentrated under reduced pressure, producing the methyl-ester (KDN1Me) 141 in 98% yield. The 1H NMR spectrum of 141 showed the expected three proton singlet peak at δ 3.82 ppm, which belonged to the newly introduced methyl-ester, whilst all the other peaks seen in the spectra of 141 were consistent with the literature189. With the synthesis of the methyl-ester 141, in excellent overall yield without purification, work began on creating the desired methyl glycoside KDN1,β2Me2 (140).

The introduction of the methyl glycoside into 141 requires a very similar protocol to that used for the introduction of the methyl-ester as set out by Nakamura et al.189. Nakamura et al.189 showed that by altering the reaction conditions for the formation of
the methyl glycoside along with the time of the reaction, different ratios of the various pyranose and furanose conformers could be obtained. In their work Nakamura and colleagues\textsuperscript{189} showed that in order to create primarily pyranose conformers and in particular \( \beta \)-glycosides, elevated temperatures must be used over a very specific time frame\textsuperscript{189}. Therefore based on our need to produce solely methyl \( \beta \)-glycosides, compound 141 was dissolved in anhydrous methanol, to which acidic resin (Dowex 50 H\textsuperscript{+}) was added at room temperature under an inert atmosphere of nitrogen gas. The resulting reaction mixture was heated to 70ºC and left to stir for 15 hours, before being cooled and the resin removed by filtration and the filtrate concentrated under reduced pressure. The resulting mixture of anomers that is produced from the reaction is extremely difficult to separate in order to obtain a specific purified anomer; therefore we took the crude reaction mixture and globally acetylated using acetic anhydride and pyridine to produce 142 (Scheme 60). In this way, the fully acetylated methyl-ester methyl \( \beta \)-glycoside 142 (per-\( O \)-acetyl-KDN1,\( \beta \)2Me\textsubscript{2}) was isolated in a 70% yield after chromatography and recrystallisation from Hexane and EtOAc. This result was comparable with yields reported by Nakamura\textsuperscript{189} as a small fraction of \( \alpha \)-furanoside was obtained along with the \( \alpha \)-pyrano-glycoside, which was produced in less than 20% yield each and every time this reaction process was undertaken.

\textbf{Reagents and Conditions:} (a) Dowex H\textsuperscript{+} resin, MeOH, 48 hrs, 25ºC, 98\% (KDN1Me); (b) Dowex H\textsuperscript{+} resin, MeOH, 15 hrs, 70ºC; (c) Py. Ac\textsubscript{2}O, 15hrs, 25ºC, 70\% per-\( O \)-acetyl- KDN1,\( \beta \)2Me\textsubscript{2} over steps b and c.

\textbf{Scheme 60:} Synthesis of per-\( O \)-acetyl- KDN1,\( \beta \)2Me\textsubscript{2} (142) from KDN (3).

Analysis of 142 by \( ^1 \text{H} \) NMR showed two large peaks each integrating to three protons at \( \delta \) 3.25 ppm and \( \delta \) 3.80 ppm, which are consistent with the methyl groups of the methyl \( \beta \)-glycoside and the methyl-ester, respectively. Additionally to this, five three-proton singlets (\( \delta \) 1.98 ppm, 2.01 ppm, 2.02 ppm, 2.07 ppm, 2.11 ppm) were seen,
which are consistent with the five acetate groups in 142 (Figure 32). Furthermore, examination of the $^1$H NMR spectrum of 142 showed that the H-3$\text{ax}$ peak was seen at $\delta$ 1.83 ppm, whilst the H-3$\text{eq}$ peak was centred at $\delta$ 2.51 ppm, which was consistent with chemical shifts indicated by Nakamura$^{189}$ for the methyl $\beta$-glycoside (Figure 32).

**Figure 32.** $^1$H NMR spectrum of the per-O-acetyl- KDN1,β2Me$_2$ 142

With the successful synthesis of the desired per-O-acetyl-KDN1,β2Me$_2$ 142, work could begin on introducing appropriate protecting groups in order to create the 5,7-diol product 137. However, in order to achieve this from 142, we first had to remove all of the acetate groups that had been introduced into 142 to aid purification and characterisation. To this end, compound 142 was dissolved in anhydrous methanol to which sodium metal was added creating sodium methoxide *in situ*, successfully removing all the acetate groups in a quantitative manner (Scheme 61). The $^1$H NMR spectrum of 140 showed that all of the five acetate peaks seen around $\delta$ 2.00 ppm had
disappeared, and the mass spectrum of 140 was seen to be m/z [M + Na]⁺ = 319.1, which was sufficient evidence that we had successfully removed the five acetate groups.

![Chemical Structures](image)

Reagents and Conditions: (a) Na, MeOH, 0°C, 1 hr, 100%.

Scheme 61. Deprotection of 142 to produce KDN1β2Me₂ 140.

With compound 140 in hand, we were now ready to introduce the first protecting group toward the synthesis of the selectively protected 5,7-diol 137. Compound 140 has five free hydroxyl groups, four of which are secondary hydroxyls (C-4, C-5, C-7 and C-8) and one is a primary hydroxyl (C-9). As C-9 is a primary hydroxyl this group is typically more reactive than the other four secondary hydroxyl groups. Therefore, the first protecting group that we needed to introduce had to protect the C-9 hydroxyl. In order to create the most efficient synthesis possible, we thought the use of a C-9, C-8 protecting group would be an efficient process for protecting the end of the glycerol side chain. A typical functionality used for protecting 1,2-diols like the 8,9-hydroxyls found in KDN (3) or Neu5Ac (1) are cyclic acetals or acetonides, which are easily introduced under acidic conditions in relatively high yields (Scheme 62).

![Chemical Structures](image)

Reagents and Conditions: (a) Acetone, 2,2-DMP, Dowex H⁺ resin, 30 min., 25°C, <50%.

Scheme 62. Synthesis of the 8,9-isopropylidene compound 143.
Following a protocol published by David et al.\textsuperscript{213}, \textbf{140} was taken up in dried acetone in which it was only sparingly soluble, followed by the addition of 2,2-dimethoxypropane and a small amount of acidic resin (Dowex 50 H\textsuperscript{+}, as an acid catalyst), under an inert atmosphere (nitrogen gas) and at room temperature. The resulting mixture was stirred at room temperature for 30 minutes before being filtered to remove the resin and then concentrated under reduced pressure. The initial attempts at this reaction produced the desired 8,9-isopropylidene compound \textbf{143} in modest yields of less than 50%, which was much lower than the reported yield of 69\% by David et al.\textsuperscript{213}.

In an attempt to improve the outcome of this reaction, more stringent controls were implemented in regard to excluding moisture from the reaction, therefore the acetone was dried before use and molecular sieves (4Å) were introduced into the reaction mixture to reduce the amount of free methanol produced during the reaction. Furthermore, instead of using an acidic resin, 0.2 molar equivalents of \textit{p}-toluenesulphonic acid was used as catalyst. This simple adjustment to the published protocol\textsuperscript{213} greatly improved the yields of \textbf{143} up to 90\% (after purification), provided the reaction time was kept below 30 minutes. Interestingly, two products were produced from this reaction. In addition to the desired 8,9-isopropylidene product \textbf{143} (Scheme 63), a second product, the \textit{bis}-isopropylidene \textbf{144} was always obtained. Indeed, the unwanted \textit{bis}-isopropylidene \textbf{144} could be obtained as the major product if too much catalyst was added or the reaction was allowed to proceed for longer than 30 minutes.

A small amount of the unwanted compound \textbf{144} was acetylated for the purpose of characterisation. The \textbf{\textsuperscript{1}}H NMR spectrum of the acetylated product showed four three proton singlets centred at \textbf{\textendash}\textbf{\textsuperscript{\textdelta}} 1.38 ppm which could be attributed to the four methyl groups of the two isopropylidene moieties. Additionally, there was a single acetate peak seen at \textbf{\textendash}\textbf{\textsuperscript{\textdelta}} 2.12 ppm, which was accompanied by a 1.2 ppm downfield shift of the H-7 proton seen at \textbf{\textendash}\textbf{\textsuperscript{\textdelta}} 5.35 ppm in the acetylated product compared with that seen in \textbf{140} (H-7, \textbf{\textendash}\textbf{\textsuperscript{\textdelta}} 4.16 ppm), indicating the newly introduced acetate was at C-7, which indicated that \textbf{144} was a 4,5-8,9-\textit{bis}-isopropylidene derivative (Scheme 63). Therefore, with \textbf{144} being obtained each and every time we attempted the isopropylidene reaction, careful attention to the concentration of acid used and the time of the reaction were crucial in obtaining the desired \textbf{143} in high yields.
Reagents and Conditions: (a) Acetone, 2,2-DMP, TsOH•H₂O (0.2 eq.), 30 min., 25ºC, 90% of 143.

Scheme 63. Outcome of the isopropylidene reaction on KDN1,β2Me₂ 140.

Evidence that we had indeed created the desired 8,9-isopropylidene 143, was best seen in the ¹H NMR spectrum, which showed two three proton singlets at δ 1.31 ppm and δ 1.39 ppm which belonged to the two methyl groups of the acetonide group. The ¹³C NMR spectrum of 143 also showed two methyl group peaks at δ 25.7 ppm and δ 27.2 ppm along with a quaternary carbon peak from the acetonide at δ 110.4 ppm. The mass spectrum of 143 confirmed that a single acetonide had been introduced with the expected mass of m/z 359.5 [M + Na]⁺ found. Furthermore, a sample of 143 was also acetylated to provide evidence that the newly introduced acetonide was indeed at the 8,9-position, with the ¹H NMR spectrum obtained comparable to that reported by David et al.²¹³.

In order to create the 5,7-diol 137, we needed to selectively protect the C-4 position with a protecting group that was both robust and could also be removed selectively if desired. Silyl ethers can be introduced in high yields, are stable under a variety of reaction conditions and can also be cleaved in high yield when required under specific reaction conditions. In our hands, the stability of TBDMS ethers were particularly attractive for the reactions that were going to be undertaken in our approach towards the synthesis of pseudaminic acid analogues. Based on work published by David et al.²¹³ the C-4 hydroxyl could be selectively protected due to its higher
reactivity compared to the C-5 and C-7 hydroxyls, as the latter two hydroxyl groups are more sterically hindered than the C-4 position. With this knowledge in hand the 8,9-isopropylidene compound 143 was reacted with tert-butylidimethylsilyl chloride in the presence of imidazole using N,N-DMF as a solvent (Scheme 64). The reaction was run overnight at room temperature under an inert atmosphere of nitrogen gas, before being concentrated to dryness and purified using column chromatography (EtOAc, Rf = 0.7). In this manner we could isolate the 5,7-diol compound 137 in a 73% yield (99% based on recovered 143).

Scheme 64. Synthesis of the 5,7-diol 137 from 143.

$^1$H NMR analysis of 137 confirmed the successful introduction of the silyl ether, with the spectrum showing two three proton singlets at δ 0.10 ppm and δ 0.12 ppm and a large nine-proton singlet at δ 0.89 ppm, which were consistent with the silyl methyl and tert-butyl methyl groups. Additionally, the hydroxyls at C-5 and C-7 appeared as broad unresolved peaks (δ 3.00 ppm (C-7) and δ 2.65 ppm (C-5)), which exchanged with D$_2$O. A small amount of 137 was taken and acetylated in order to provide further evidence that we had indeed created the desired 5,7-diol 137 (Scheme 65). The $^1$H NMR spectrum of the per-O-acetyl compound 145, showed that the H-4 signal remained at δ 4.09 ppm, whilst the H-5 signal had moved from δ 3.60 ppm in 137 to δ 4.89 ppm and the H-7 from δ 3.89 ppm in 137 to δ 5.25 ppm in the acetylated compound 145. These changes in chemical shifts of H-5 and H-7 indicated that these two positions were now acetylated, therefore indicating that we had indeed been successful in creating the key intermediate 5,7-diol 137.
With the successful synthesis of 137, work could begin on the double inversion step that was required if we were to transform the sialic acid configuration of 137 into pseudaminic acid based analogues. This important step relied on the idea that we would be able to selectively activate the C-5 and C-7 positions in 137 and use a nitrogen based nucleophilic displacement reaction thereby inverting the stereochemistry at both C-5 and C-7. The following section outlines the chemistry that was used in order to achieve this essential transformation toward the synthesis of pseudaminic acid analogues.

4.3 Synthesis of tetraolony-nonulosonic acids from 137

With the successful synthesis of the selectively protected 5,7-diol compound 137, work began on the important double inversion step, which would not only introduce the required nitrogen based functionalities but also introduce the correct stereochemistry at C-5 and C-7 in our target pseudaminic acid analogues. The idea was to take 137 and introduce two activating groups at the C-5 and C-7 positions, and for this purpose we chose a good leaving group in the form of trifluoromethanesulfonate (triflate) functionality, which are well documented to be relatively stable and very susceptible to being displaced via an S$_{N}$2 mechanism$^{216}$.

Bearing this aim in mind, work began for the initial reaction attempts at creating the bis-triflate 146. The 5,7-diol 137 was dissolved in anhydrous dichloromethane to which pyridine and then triflic anhydride were added at -78°C under an inert atmosphere of argon gas. The resulting reaction mixture was allowed to stir at -78°C for ten minutes before being allowed to warm to 0°C and stirred for a further one hour. The

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Reagents and Conditions: (a) Py, Ac$_2$O, DMAP, 15 hrs, 25°C, 99%.

Scheme 65. Acetylation of the key intermediate 137
reaction was monitored by TLC (5:1 Hex/EtOAc; R_f = 0.3) over the course of four more hours at room temperature. Over this time by TLC one could visualise the appearance of firstly a spot 0.1 R_f above the starting material, followed by a second spot roughly 0.2 R_f above the starting material (Figure 33). The first spot to appear at R_f = 0.2 was assumed to be a single triflate product as it was never isolated for characterisation due to poor stability. Whilst the second spot to appear (R_f = 0.3) was the desired bis-triflate compound 146 (Figure 33). This compound was purified using column chromatography (5:1 Hex/EtOAc; R_f = 0.3) giving 146 in a 88% yield.

**Figure 33.** TLC plate showing the reaction progress monitored for the synthesis of the bis-triflate compound 146

The ^13^C NMR spectrum of 146 showed two CF_3 quartets centred at δ 126.0 ppm, both having couplings of about 317 Hz, which is consistent with that expected for carbon-fluorine couplings. With the successful synthesis and purification of 146, the double inversion reaction to displace the two leaving groups now at C-5 and C-7 was attempted. The bis-triflate 146 was reacted with a large excess of sodium azide in N,N-DMF at 0°C for 24 hours. After work-up and purification using column chromatography (5:1 Hex/EtOAc; R_f = 0.25), the bis-azide 138 was obtained in a 93% yield over two steps from the 5,7-diol 137 (Scheme 66).
Reagents and Conditions: (a) Tf₂O, Py., CH₂Cl₂, -78°C to 25°C, 6 hrs, 88%;
(b) NaN₃, N,N-DMF, 24 hrs, 0°C, 93%.

Scheme 66. Synthesis of the 5,7-bis-azide 138

In order to equivocally provide evidence that we had indeed created the desired bis-azide 138, a number of spectroscopic methods were employed. Firstly, analysis of 138 using infrared spectroscopy, showed a sharp peak at 2100 cm⁻¹, which is consistent with the presence of an azide group₁⁶. Mass spectrometry also indicated a correct mass of m/z 500.1 [M + Na]⁺, which is consistent with the molecular formula (C₂₀H₃₆N₆O₇Si) of 138. Additionally, using ¹H and COSY NMR spectra we could see if there had been an inversion of stereochemistry for the C-5 and C-7 substituents, since we would expect to see a difference in the spin-spin couplings between those protons in 138 around C-5 and C-7 compared with the corresponding couplings in the 5,7-diol 137.

Figure 34. Stereochemical differences between 138 and 137 (shown in red)

As you can see in Figure 34, highlighted in red, there should be certain changes in the spin-spin couplings between specific protons in the ¹H NMR spectrum of 138 compared to 137. This is due to the change in stereochemistry where, for example, a once trans-diaxial (for example between H-4 and H-5 in 137, Figure 34) coupling in protons changes to an axial-equatorial coupling in 138. Seeing this change in the H-4 to H-5 coupling therefore would support the notion that there has been a stereochemical
change at the C-5 position. Table 8 summaries the observed spin-spin coupling changes seen in the bis-azide 138 compared with the 5,7-diol 137.

Table 8: Comparison of the $^1$H couplings for specific protons between 138 and 137

<table>
<thead>
<tr>
<th>Coupling Protons</th>
<th>5,7-diol 137 Spin-spin coupling (Hz)</th>
<th>Bis-Azide 138 Spin-spin coupling (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{4,5}$</td>
<td>8.7</td>
<td>2.7</td>
</tr>
<tr>
<td>$J_{5,6}$</td>
<td>9.9</td>
<td>1.5</td>
</tr>
<tr>
<td>$J_{6,7}$</td>
<td>1.5</td>
<td>9.9</td>
</tr>
<tr>
<td>$J_{7,8}$</td>
<td>8.4</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Importantly, as can be seen in Table 8, the couplings between all the protons associated with changes in stereochemistry are altered when comparing 138 with 137, which support the fact that the double displacement had occurred producing the desired bis-azide compound 138. Together with the aforementioned spectral analysis data, we were certain that we had been successful in creating the bis-azide 138.

4.4 Synthesis of 5,7-diacetamido-pseudaminic acid analogues

Having successfully achieved the synthesis of the bis-azide 138, functional group manipulations were still required in order to create our desired pseudaminic acid analogues. Firstly, a method was needed to reduce the azides in 138 to amines, which could then be acetylated to form acetamides in the first instance. Secondly, C-9 had to be deoxygenated to produce the essential 9-deoxy functionality seen in all pseudaminic acid based compounds. These final functional group changes are summarised in Scheme 67.
Scheme 67: General overview of changes needed (highlighted in blue) to transform the bis-azide 138 into a pseudaminic acid analogue

There are numerous methods described in the literature for the reduction of azides to amines, as the manipulation of azides has been employed routinely in organic synthesis for over 100 years. In particular the reduction of azides to amines has been achieved through use of numerous reducing agents like LiAlH₄, NaBH₄, PPh₃, Zn/HCl, Bu₃SnH just to name a few. Regarding the bis-azide 138, initially we thought the process could be achieved using palladium as a catalyst under hydrogenation conditions. The use of hydrogenation reactions to reduce azide moieties is routinely used in organic synthesis with excellent yields generally produced, providing there are no other reducible functionalities present in the compound. There are numerous palladium catalysts available for this type of transformation, however initially we chose to use a 10% by weight palladium on activated carbon catalyst. In this regard 138 was dissolved in methanol to which 20% weight by mass of catalyst was added and a few drops of acetic acid. The reaction was stirred under an atmosphere of hydrogen gas (H₂(g)) at 1 atm and room temperature for 24 hours, before being checked by TLC to see if the reaction was complete. The TLC analysis (RF = 0.1, EtOAc) showed that the starting material had been consumed and a sole product had been formed, which stained purple using a ninhydrin dip, which is used to identify amine functional groups. From this result the reaction mixture was filtered to remove the palladium catalyst and the filtrate concentrated under reduced pressure before being acetylated using a pyridine and acetic anhydride solution stirring at room temperature overnight. Upon work up of the acetylated product the reaction residue was purified using column chromatography (4:1 EtOAc/MeOH; RF = 0.3) yielding a single compound in 90% yield.
The $^1$H NMR spectrum of the hydrogenation product was unexpected. The spectrum had only the three proton singlet for the methyl glycoside peak present at $\delta$ 3.59 ppm, with the expected three proton singlet of the methyl ester seen in the bis-azide 138 absent in the hydrogenation product. Although confusing at first, after considerable analysis characterisation of this unknown compound was achieved. Firstly, the 8,9-O-isopropylidene ($\delta$ 1.34, 1.46 (2 x s, 3 H, C(CH$_3$)$_2$)) and 4-O-TBDMS ($\delta$ 0.01 (2 x s, 2 x 3 H, Si(CH$_3$)$_2$) and 0.83 (s, 9 H, SiC(CH$_3$)$_3$)) protecting groups were still intact and there were what appeared to be two resonances for the NH proton of the newly formed amide groups at $\delta$ 5.77 ppm and $\delta$ 6.55 ppm (Figure 35). However, there was only one acetamide methyl group peak seen at $\delta$ 2.03 ppm, which indicated that one of the apparent NH peaks seen at either $\delta$ 5.77 ppm or $\delta$ 6.55 ppm was in fact an amide in a very different environment to the other having no methyl group associated with it. This was an important clue to solving the structure of the hydrogenation product. Looking at the couplings of the various protons, and with the aid of the COSY spectrum, it could be seen that the acetamide peak at $\delta$ 5.77 ppm showed correlation with H-7 ($J_{\text{NHAc,7}} = 9.0$ Hz), whilst the peak at $\delta$ 6.55 ppm showed correlation with H-5 with a coupling of $J = 5.7$ Hz. This suggested that the amide at C-5 was in a different environment than that at C-7 due to both the chemical shift and the coupling differences. Further NMR analysis via $^{13}$C NMR showed that there was indeed only one apparent acetamide present in the molecule with a single acetamide methyl group seen at $\delta$ 23.2 ppm (NHCOCH$_3$), supporting the fact that the C-5 amide was not an acetamide. Looking at the IR spectrum we could see that both of the azides had been reduced as there were no azide peaks, whilst the mass spectrum showed a mass of $m/z = 481.1$ [M + Na]$^+$, which suggested a molecular formula of C$_{21}$H$_{38}$N$_2$O$_7$Si and was consistent with the other spectroscopic data.
Considering the fact that the NMR spectrum had no methyl ester peak present, and the spectral data supported the fact that the C-5 amide was not an acetamide, it became obvious what the structure of this unknown compound may be. Based on all of the spectral analysis performed, we hypothesised that what had occurred was that when the azide at C-5 was reduced to form an amine, this newly formed group must have attacked the methyl ester at C-1 displacing methanol and creating a 1,5-lactam ring (Scheme 68). This outcome would explain why there was no methyl ester present and the amide at C-5 was in fact a cyclic amide (lactam) which was supported by the NMR data. Scheme 68 shows our proposed mechanism for the formation of the 1,5-lactam compound 147.

**Figure 35.** $^1$H NMR spectrum of the 1,5-lactam 147
As can be seen in Scheme 68, if the bis-azole compound 138 or the reduced bis-amine compound was to take on a boat conformation, then the C-5 amine would be close enough to the C-1 ester functionality to allow for attack at this position forming the 1,5-lactam 147. Final confirmation of the structure of 147 was gained from a x-ray crystal structure (Figure 36, ORTEP image), which clearly shows that the pyranose ring is in a type of twisted boat confirmation and the lactam ring is a 1,5-lactam rather than the potential 1,7-lactam formation. 

Figure 36. ORTEP image of the 1,5-lactam 147 (carbons in grey) labelled C-1 to C-9, nitrogen atoms in blue, oxygen atoms in red and hydrogen in black.
The 1,5-lactam 147 has the potential to be a valuable intermediate in the synthesis of differentially functionalised C-5 and C-7 pseudaminic acid analogues. Having the C-5 nitrogen functionality associated with the 1,5-lactam moiety, allows direct access to differentiation between the type of nitrogen-based functional groups generated at C-7 or at C-5. The amine at C-7 produced from the reduction of 138 could have an N-acyl-group introduced. Subsequent cleavage of the 1,5-lactam and N-acylation at C-5 would then lead to a derivative with different N-acyl groups at C-5 and C-7 (Scheme 69). This type of differentiation between the types of functional groups at C-5 and C-7 could permit access to the type of pseudaminic acid analogues that are seen commonly within nature.

**Scheme 69.** Possible future use of the 1,5-lactam 147 in producing differentially functionalised C-5 and C-7 pseudaminic acid analogues.

Despite the attractiveness of the 1,5-lactam 147 as a potential synthetic intermediate, we focused our attention on reducing the *bis*-azide 138 to the corresponding *bis*-acetamide 148. Accordingly, we began investigating approaches for the direct reduction and *in situ* acylation of the *bis*-azide 138 using Ac₂O, in order to prevent the formation of the lactam 147. To this end, numerous attempts were made using Pd/C, Ac₂O and AcOH under hydrogenation conditions or Pd(OH)₂/C, Ac₂O and AcOH. The reaction times, amounts of catalyst and concentration of Ac₂O were varied in order to investigate if we could directly transform the *bis*-azide 138 into the desired *bis*-acetamide 148. These reaction attempts resulted in complex mixtures of compounds that were hard to visualise by TLC and difficult to purify. Therefore after considerable effort we decided to abandon this approach for creating the *bis*-acetamide 148.

With the unsuccessful hydrogenation attempts at directly reducing the two azide functionalities in 138, we began investigating alternative methods for the selective
reduction of these two functional groups. Looking at the literature we saw that numerous publications describe such selective reductions utilising various reagents\textsuperscript{217,219}. One of the most well studied synthetic chemistry methods for such a transformation is the Staudinger reaction also known as the Staudinger reduction\textsuperscript{219–224}. The Staudinger reaction utilises an azide and a phosphine or phosphate which are reacted together to form an iminophosphorane intermediate that is hydrolysed to form a phosphine oxide and the desired amine\textsuperscript{220}. In our hands we wanted to use a version of the Staudinger reaction that uses an acetylation agent that reacts with the newly formed iminophosphorane intermediate, followed by a intramolecular rearrangement that allows excess acetylation agent to displace triphenylphosphine oxide producing a transitory anhydride intermediate that is hydrolysed by water in the final step producing the desired acetamide product and a carboxylic acid from the acetylation agent\textsuperscript{224} (Scheme 70).

\textbf{Scheme 70.} The mechanism for a Staudinger reaction using acetic anhydride as an acetylation agent to convert an azide into an acetamide\textsuperscript{224}
With the idea of proceeding via a Staudinger reaction the bis-azide 138 was dissolved in anhydrous THF to which molecular sieves (4Å) were added to aid in the exclusion of water, followed by triphenylphosphine. The reaction mixture was cooled to 0°C under argon gas and stirred for 15 minutes before the addition of acetic anhydride. Following this, the reaction was stirred for 15 minutes at 0°C before being allowed to warm to room temperature and stirred for a further 5 hours; the reaction was monitored via TLC (5:1 hex/EtOAc; R_f = 0.1). After 5 hours a clear purple spot (seen using ninhydrin dip) was apparent near the base line; this was assumed to be the anhydride intermediate product. The reaction was filtered to remove the sieves before being stirred in an equal volume of water for 15 minutes in order to hydrolyse the anhydride intermediate to give the desired bis-acetamide 148. The resulting reaction mixture was concentrated under reduced pressure and purified using column chromatography (4:1 EtOAc/MeOH, R_f = 0.1), yielding a single major component in a 60% yield (Scheme 71). The 1H NMR spectrum of this product interestingly showed only a single acetamide peak, with the three proton singlet seen at δ 1.96 ppm (CH_3 group) and an NH proton at δ 5.88 ppm. The absence of the second acetamide group was confirmed by examination of the IR spectrum, which showed a clear and sharp azide peak at 2105 cm^{-1} which indicated that one of the azide groups had been unaffected by the Staudinger reaction. The mass spectrum of 149 also supported this fact providing a parent ion m/z 539.2 [M + Na]^+. Evidence that this product from the Staudinger reaction was the 7-acetamido-5-azido compound 149 came from careful examination of the 1H and COSY spectra. The key piece of information was that the NH proton of the acetamide (δ 5.88 ppm) showed a clear correlation to the H-7 proton at δ 4.34 ppm (J_{7NHAc,7} = 9.9 Hz).
**Reagents and Conditions:** (a) PPh$_3$, Ac$_2$O, 5.5 hrs, 0ºC to 25ºC followed by H$_2$O, 30 min, 60%.

**Scheme 71.** Outcome of the Staudinger reaction on 138.

The isolation of the mono-acetamide 149 in good yield from the Staudinger reaction was particularly interesting, since it clearly shows that we could readily differentiate between the two nitrogen functionalities we had introduced at C-5 and C-7. Potentially, 149 could permit a synthesis aimed at creating pseudaminic acid analogues that have a different N-acyl functionality at C-5 compared with C-7, providing access to pseudaminic acid analogues similar to those isolated from nature. Despite the attractiveness of using 149 in this way we were still focused on producing our desired bis-acetamide 148 in the first instance.

With the partial conversion of 138 to the mono-acetamide 149 via a Staudinger reaction, we wondered if, in 138, steric hindrance of the 8,9-O-isopropylidene and the 4-O-silyl ether was causing the problems we had encountered. With this in mind, we considered removal of the protecting groups before attempting functionalisation of the azides at C-5 and C-7. Accordingly, treatment of the bis-azide 138 with aqueous 80% AcOH at 80°C, over 2 hours gave a mixture of the partially deprotected 4-O-TBDMS-bis-azide 150 and the fully deprotected compound 151 in 60% and 35% yields, respectively (Scheme 72). In the $^1$H NMR spectrum of 150, the removal of the isopropylidene protecting group was evident by the disappearance of the peaks at δ 1.25 ppm and δ 1.37 ppm seen in 138 compared with 150. The mass spectrum of 150 showed a molecular ion peak at $m/z$ 483.2 [M + Na]$^+$, which is consistent with the molecular formula C$_{17}$H$_{32}$N$_6$O$_7$Si. The $^1$H NMR spectrum of 151 also showed the disappearance of the isopropylidene peaks at δ 1.25 ppm and δ 1.37 ppm along with the silyl methyl peaks at δ 0.02 ppm, δ 0.04 ppm (Si(CH$_3$)$_2$) and δ 0.82 ppm (C(CH$_3$)$_3$), and the mass spectrum showed a molecular ion peak at $m/z$ 369.1 [M + Na]$^+$ which is consistent with
the molecular formula of 151 \((C_{11}H_{18}N_6O_7)\). Having created the partially deprotected compound 150 and the fully deprotected compound 151 from the bis-azide 138, we now looked at various methods to covert the azide functionalities in these compounds to the desired acetamide groups.

Reagents and Conditions: (a) Aqueous 80% acetic acid, 80ºC, 3 hours; 60% 150 and 35% 151.

Scheme 72: Deprotection of the bis-azide 138 to produce 150 and 151.

Table 9 summarises some of the attempts that were made in order to generate the desired 4,8,9-triol-bis-acetamide compound 139 using the fully deprotected compound 151.

<table>
<thead>
<tr>
<th>Reaction Attempt</th>
<th>Reagents</th>
<th>Time/Temp</th>
<th>Yield/Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AcCl, PPh\textsubscript{3}</td>
<td>3 hrs/25ºC</td>
<td>Complex Mixture</td>
</tr>
<tr>
<td>2</td>
<td>AcCl, PPh\textsubscript{3}</td>
<td>4 hrs/25ºC</td>
<td>Complex Mixture</td>
</tr>
<tr>
<td>3</td>
<td>AcCl, PPh\textsubscript{3}</td>
<td>6 hrs/25ºC</td>
<td>50% 152</td>
</tr>
<tr>
<td>4</td>
<td>Ac\textsubscript{2}O, PPh\textsubscript{3}, H\textsubscript{2}O</td>
<td>6 hrs/25ºC</td>
<td>30% 152</td>
</tr>
<tr>
<td>5</td>
<td>Bu\textsubscript{3}SnH, AIBN Zn/AcCl</td>
<td>3 hrs/80ºC</td>
<td>Complex Mixture</td>
</tr>
<tr>
<td>6</td>
<td>Bu\textsubscript{3}SnH, AIBN Zn/AcCl</td>
<td>7 hrs/80ºC</td>
<td>Complex Mixture</td>
</tr>
<tr>
<td>7</td>
<td>Bu\textsubscript{3}SnH, AIBN Zn/AcCl</td>
<td>15 hrs/80ºC</td>
<td>10% 152</td>
</tr>
</tbody>
</table>

Referring to Table 9, it can be seen that our idea that steric hinderance in 138 was a potential problem in our attempted reduction of the two azides was not borne out. In
all instances none of the desired bis-acetamide 139 was obtained, with the final outcome of these different reactions being the yield of the 7-acetamide-5-azido-compound 152. This outcome was practically identical to that seen for the reactions we had attempted on the fully protected bis-azide 138.

![Chemical structures](image)

(139)  (152)

Although the outcome of the reaction attempts summarised in Table 9 were disheartening, we had gained some valuable information regarding the way forward for the transformation of the bis-azide 138 to the bis-acetamide 148. However, before changing direction we wanted to investigate the possibility of functionalising the C-9 position in the fully deprotected compound 151. The rationale behind this approach was that if we could successfully introduce a good activating group at C-9 in 151, we could attempt a triple reduction protocol, whereby we would reduce both of the azides at C-5 and C-7 along with the C-9 group, thereby creating an 8-epi-pseudaminic acid 153 in an efficient two steps from 151 (Scheme 73). In order to achieve this aim we thought that an iodination, reduction process would be efficient, whereby we would firstly introduce an iodine functionality at C-9 in 151, then once introduced we could carry out the final reduction step, which would be the triple reduction step as seen in Scheme 73.

![Scheme 73](image)

Scheme 73. The proposed triple reduction step to produce the target compound 153 from the 4,8,9-triol 151.
There are several methods in the literature for converting a primary alcohol to the corresponding deoxy functionality\textsuperscript{166,226–228}. Based on work we had carried out on the mannose derivatives in our chemoenzymatic synthesis (See Chapter 2, Sections 2.3.1 and 2.3.2), we decided to adopt an iodination-de-iodination sequence to create a 9-deoxy compound. The first step of this process involved treatment of 151 via a Garegg-Samuelsson iodination reaction\textsuperscript{166,227} (using triphenylphosphine, imidazole and iodine) to give the 9-iodo derivative 154, which was obtained in a 70\% yield (Scheme 73). The $^{13}$C NMR spectrum of 154 showed the movement of the C-9 peak from $\delta$ 68.2ppm in 151 to $\delta$ 9.0 ppm in 154, which is consistent with the introduction of an iodo-functionality\textsuperscript{164}. Furthermore, using mass spectrometry, the expected molecular mass of 154 was confirmed as $m/z = 479.1$ [M + Na]$^+$. A preliminary hydrogenation reaction using the 5,7-\textit{bis}-azido-9-iodo derivative 154 and Pd(OH)$_2$ as catalyst, with the addition of acetic anhydride was attempted. This reaction produced a complex mixture of products from which we were able to isolate the 9-deoxy-1,5-lactam derivative 155 (Scheme 74) in relatively low yield (~5\%). The $^1$H NMR spectrum of 155 showed the loss of the three proton methyl-ester peak at around $\delta$ 3.82 ppm being consistent with 1,5-lactam formation. Additionally, the presence of two three proton acetate peaks around $\delta$ 2.0 ppm is consistent with the one acetamide and C-8 acetate group in 155. Furthermore the reaction was successful in reducing the 9-iodo-functionality as the newly formed C-9 methyl group could be clearly seen at $\delta$ 1.06 ppm as a doublet which showed correlation to H-8 ($J_{9,8} = 6.3$ Hz).

![Diagram of 154 and 155](attachment:image)

\textit{Reagents and Conditions:} (a) Pd(OH)$_2$, Ac$_2$O/MeOH, 25$^\circ$C, 24 hrs, 4%.

\textbf{Scheme 74.} Synthesis of the 1,5-lactam of 8-\textit{epi}-pseudaminic acid (155).
The synthesis of the 9-deoxy-1,5-lactam derivative 155 was expected, although the very low yield was disappointing. However, the reaction outcome showed us that under hydrogenation conditions we could deliberately create this significant and novel pseudaminic acid analogue, which contains the essential 9-deoxy-functionality and could be used as a precursor to pseudaminic acid itself and other differentially 5,7-N-acyl analogues. An important structural feature of the lactam derivative 155 is that the two nitrogen functionalities are different. This means that we could, in future work, differentially functionalise these two nitrogens which would be important to generate some of the naturally occurring pseudaminic acid derivatives, as described in Table 1, Chapter 1.

Whilst the ability to differentiate between the C-5 and C-7 nitrogen functionalities is important in the longer term, our primary aim was to convert the bis-azide 138 directly into the bis-acetamide 148. From the reactions carried out thus far, we knew we could successfully introduce a 9-iodo-functionality and reduce this to the desired methyl group via a hydrogenation reaction using palladium hydroxide as catalyst, however we also knew that under those hydrogenation reaction conditions the bis-azide 138 was always converted to a 1,5-lactam. Therefore we needed a protocol that converted the azides in 138 firstly to acetamides and then we could proceed with the iodination-reduction steps, instead of attempting to reduce all three positions at once.

With the knowledge that the presence of the protecting groups in 138 had little effect on the outcome of the reduction of the two azides in this compound, we took a step back and considered a new protocol using the bis-azide 138. The main issue with all the reaction attempts so far was either the formation of the 1,5-lactam or the partial conversion to form the 7-acetamide-5-azido-products. Therefore we considered whether or not it might be possible to reduce the two azides in 138 to amine functionalities and then trap these groups in situ as there ammonium salts, which would in theory, inhibit the C-5 amine from attacking the methyl ester as the process would no longer be electronically favourable. With this type of reaction mechanism in mind we thought that if we could run a hydrogenation reaction in the presence of a suitable amount of acid, this would protonate the newly formed amines at C-5 and C-7 converting them into their ammonium salts which could be maintained as long as the reaction mixture was not subjected to an alkaline environment.
In order to test our hypothesis, the fully protected bis-azide 138 was dissolved in MeOH to which a trace amount of p-toluenesulfonic acid (5% molar equivalents used in the first instance) and Pd(OH)$_2$ (as catalyst) were added, and the resulting reaction mixture was stirred at room temperature for 4 hours in the presence of hydrogen gas at atmospheric pressure. The reaction was monitored by TLC (5:1 Hex/EtOAc; $R_f$ = 0.05) every hour. Using a ninhydrin dip a clear purple spot was visible ($R_f$ = 0.05) after 4 hours and the starting material had been consumed. The reaction mixture was filtered to remove the palladium catalyst and the filtrate was concentrated under reduced pressure without neutralising the p-toluenesulfonic acid present in order to ensure the mixture remained acidic.

The resulting clear oily residue from the reaction was dried under high vacuum for 6 hours to remove as much of the methanol from the residue as possible before acetylation was attempted. Once dry, the clear crystalline material was taken up in acetic anhydride to which pyridine was added followed by a trace amount of DMAP. The resulting reaction mixture was stirred overnight under an atmosphere of nitrogen gas at room temperature before being worked up via an aqueous acid wash (1 N HCl) and purified using column chromatography (EtOAc; $R_f$ = 0.2) producing a sole product in 81% yield over the two steps from 138.

The NMR spectra of this product showed that we had indeed been successful in producing the desired bis-acetamide 148. The $^1$H NMR spectrum of 148 showed two peaks for the acetamide methyl groups at $\delta$ 1.92 ppm and $\delta$ 1.95 ppm, as well as the two NH protons at $\delta$ 5.55 ppm and $\delta$ 5.46 ppm (Figure 37). Furthermore, the $^1$H NMR spectrum of 148 showed the methyl ester was still present ($\delta$ 3.79 ppm, Figure 37) and the mass spectrum showed a molecular ion peak of $m/z$ 555.1 [M + Na]$^+$, which was consistent with the molecular formula C$_{24}$H$_{44}$N$_2$O$_9$Si.
With the successful synthesis and characterisation of the bis-acetamide 148 from our first attempt, we could finally begin work on transforming this important intermediate into our target compound, an 8-epi-pseudaminic acid analogue (153). The next step toward our final compound was to remove the 8,9-isopropylidene protecting group in order to unmask the C-9 hydroxyl group, which was essential, as the final transformation we need to perform was the C-9 deoxygenation.

As we no longer required either the silyl ether at C-4 or the 8,9-isopropylidene group in 148, we looked at employing a deprotection step that would remove both groups simultaneously in order to produce the 4,8,9-triol compound 139. The removal of these two protecting groups was first attempted in aqueous 80% AcOH solution, in the exact same manner that had been used to deprotect the bis-azide compound 138. However, this reaction proved to be surprisingly problematic when carried out on the bis-acetamide compound 148. After considerable experimentation, we were never able to remove the 8,9-isopropylidene, as the reaction always proceeded with the efficient removal of the silyl ether at C-4 instead, whilst the 8,9-isopropylidene protecting group

**Figure 37.** $^1$H NMR spectrum of 148 showing the characteristic amide peaks
remained robust even at elevated temperatures and longer reaction times; therefore, a new strategy was required.

Turning to the literature, Leblanc et al.\textsuperscript{229} used a reaction for the selective removal of acetonides in their total synthesis of the arachidonic acid derivatives 12-HETE and 12,20-DiHETE, which involved trifluoroacetic acid in a mixture of THF/water at room temperature. These reaction conditions were very mild compared with the harsh elevated temperatures we had been using in the AcOH reactions. Therefore, we dissolved the bis-acetamide 148 in THF and water (4:1 ratio) to which 2.4 eq. of trifluoroacetic acid was added. The reaction was monitored by TLC (4:1 EtOAc/MeOH; $R_f = 0.3$) and produced the desired triol 139 in 85\% yield after 3 days (Scheme 75). Although the reaction was quite sluggish, we were happy with the overall procedure as 139 was obtained as a sole product and was easily purified in a good yield. The $^1$H NMR spectrum of 139 showed that compared with 148, all of the accompanying protecting group peaks from the 8,9-isopropylidene and silyl ether seen in 148 had disappeared in the spectra of 139. Furthermore, the removal of these protecting groups was supported by the mass spectrum of 139, which showed a molecular ion peak of $m/z$ 401.1 [M + Na]$^+$. 

\begin{eqnarray*}
\text{Reagents and Conditions: (a) TFA, THF/H}_2\text{O, 72 hrs, 85%}.
\end{eqnarray*}

\textbf{Scheme 75.} Synthesis of the triol compound 139 from 148.

With the successful deprotection and characterisation of the triol 139 we could now investigate our final C-9 modification in order to create our target compound 153. In this regard 139 was reacted under Garegg-Samuelsson\textsuperscript{166} iodination conditions (I\textsubscript{2}, Ph\textsubscript{3}P, imidazole) producing the 9-iodo derivative 156 in 71\% yield. After some optimisation work on this reaction the yield of 71\% was the best that could be achieved. The modest yield is most probably due to difficulties that were encountered during
workup, the major issue being that the product is partially water soluble which made separation from the triphenylphosphine oxide and subsequent purification troublesome. Nonetheless, spectroscopic analysis of 156 clearly showed the presence of an iodine attached to C-9, since the chemical shift of C-9 was $\delta$ 9.4 ppm in 156 compared with $\delta$ 64.8 in 139. Furthermore the mass spectrum supported the formation of 156 providing a molecular ion peak of $m/z$ at 511.1 [M+Na]$^+$ being consistent with the expected mass.

In order to produce the 8-epi-pseudaminic acid anologue 153, the 9-iodo compound 156 was dissolved in MeOH to which Pd(OH)$_2$ and EtN$i$Pr$_2$ were added. The resulting reaction mixture was stirred at room temperature under 1 atmosphere of hydrogen gas overnight before being filtered to remove the palladium catalyst and the filtrate concentrated under reduced pressure. The reduction of the 9-iodo group smoothly afforded the desired 8-epi-pseudaminic acid 153 in 96% yield (Scheme 76).

![Scheme 76](image)

Reagents and Conditions: (a) Pd(OH)$_2$, EtN$i$Pr$_2$, MeOH, 15 hrs, 25$^\circ$C, 96%.

**Scheme 76.** Reduction of 9-iodo compound 156 to produce the target compound 153.

The successful introduction of the target 9-deoxy group was clearly observed in the $^1$H NMR spectrum of 153, which showed a three proton doublet at $\delta$ 1.06 ppm for the C-9 methyl group, along with the associated change in the multiplicity of the signal for H-8. All of the other resonances seen in the spectra of 153 were as expected.
4.5 Conclusion and future directions

This chapter has outlined in detail the procedures that were followed in order to create pseudaminic acid analogues from the sialic acid based precursor KDN (3). The efficient 10 step protocol outlined in this chapter is novel and represents a major step forward in the field of pseudaminic acid and tetradeoxy-nonulosonic acid chemistry. In the end this approach produced two novel pseudaminic acid based analogues, the 8-epi-pseudaminic acid 153 in a 35% yield from KDN (3) and the 1,5-lactam 155.

There is no doubt that further work needs to be undertaken to optimise the synthesis of pseudaminic acid and its analogues from a common precursor as set out in this chapter. However, with more time we are confident that the 9-deoxy-1,5-lactam analogue 155 and the two 7-acetamido-5-azido-compounds 149 and 152 will become important precursors to selectively functionalised N-acyl C-5 and C-7 analogues. Unfortunately, time did not permit me further exploring the potential synthetic utility of these compounds. The real significance of these analogues lies in the ability of being able to efficiently and selectively acylate the two nitrogen functionalities, thus allowing the synthesis of a range of pseudaminic acid analogues that reflect the structural variability found in natural pseudaminic acid compounds.

The synthesis described here represents a major step forward in synthetic approaches towards pseudaminic acid and its analogues. Future work will explore the synthesis of further pseudaminic acid analogues, including derivatives with different acetamides at C-5 and C-7 as well as inverting the C-8 stereochemistry in 153 to produce pseudaminic acid (4) itself.

In summary this thesis describes a an investigation into the synthesis of 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonic acid analogues which is novel in its undertaking, with there being to date no single report excluding those published by us, put forth in this regard. 5,7-diamino-3,5,7,9-tetradeoxy-nonulosonic acids are an important class of bacterial nonulosonic acid being recognised to contribute to the virulence capabilities of numerous clinically relevant Gram-negative species. Currently there are two important parent compounds recognised, these being the pseudaminic acids and legionaminic acids.

Chapter 2 of this thesis outlines our successful design and synthesis of structural and functional group analogues of legionaminic acid using a chemoenzymatic approach.
from the commercially available carbohydrate monomer D-mannose (37). This synthesis protocol utilised the type 1 aldolase Neu5Ac aldolase in order to transform selectively functionalised D-rhamnosides and D-talosides into their corresponding legionaminic acids as best exemplified by compounds 74 and 76. Although, our chemoenzymatic approach was successful in fulfilling the aims of this part of our project, it also served to produce key intermediate compound like 77, which were utilised in other syntheses as seen in Chapter 3, only highlighting the versatility of such compounds to not only enable us to create legionaminic acid-based compounds but also pseudaminic acid like compounds from a common pathway.

Following the success of our chemoenzymatic synthesis of legionaminic acid analogues, we were interested in creating other tetradeoxy-nonulosonic acids, which proved to be difficult due to the stereochemical limitations of the enzyme Neu5Ac aldolase. Therefore, Chapter 3 of this thesis describes our attempts at utilising a chemical aldol condensation reaction to create pseudaminic acid analogues from C-2 gluco-configured sugars. The major success of this part of our project was the synthesis of the N-acetyl-6-deoxy-glucosmaine derivative 135. This compound has important functional group and structural detail required for successful transformation into pseudaminic acid-based analogues.

Having spent a large amount of time refining and optimising the work described in Chapters 2 and 3, we turned our attention to the synthesis of pseudaminic acid analogues starting from the more advance precursor, the nine-carbon carbohydrate KDN (3). This work (described in Chapter 4) was started due to the stereochemical limitations of substrates in the two aldol condensation reactions we had been investigating. The synthesis outlined in Chapter 4 resulted in the creation of three important pseudaminic acid precursors (compounds 147, 152 and 149), along with two pseudaminic acid analogues compounds 155 and 153. Our successful synthesis of the 8-epi-pseudaminic acid analogue 153, resulted in a publication in Organic & Bimolecular Chemistry\textsuperscript{230} and to the best of our knowledge is the most efficient and versatile synthesis of a pseudaminic acid-based compounds reported in the literature to date, and furthermore is the only paper aimed specifically at creating pseudaminic acid analogues.
In closing, my PhD project aimed to investigate methods for the efficient and versatile syntheses of structural and functional group analogues of pseudaminic acid (4) and legionaminic acid (5) as there was an obvious gap in the literature for their creation. Specially, we aimed to create a variety of C-5 and C-7 analogues of pseudaminic acid (4) and legionaminic acid (5), which have the potential to be used as biological probes, enabling research into key tetradeoxy-nonulosonic acid processing enzymes (e.g. PA 2794). With the successful development of more efficient methodologies towards the synthesis of such analogues as described (vide infra), we now hope to be able to use these compounds to gain a better understanding of how these important carbohydrates are utilised by clinically relevant pathogenic Gram-negative bacteria.
CHAPTER 5

EXPERIMENTAL

5.1 General Methods

$^1$H and $^{13}$C spectra were obtained using a Bruker 300 MHz spectrometer at 300 and 75.5 MHz, respectively. Signals are reported in terms of their chemical shift ($\delta$ in ppm) relative to CDCl$_3$ ($^1$H, $\delta$ 7.26 ppm and $^{13}$C, $\delta$ 77.0 ppm), MeOD4 ($^1$H, $\delta$ 3.30 ppm and $^{13}$C, $\delta$ 49.0 ppm), CD$_3$CN ($^1$H, $\delta$ 1.96 ppm and $^{13}$C, $\delta$ 1.39 ppm) and D$_2$O ($^1$H, $\delta$ 4.78 ppm and $^{13}$C external reference used). For $^1$H spectra, multiplicity, integration intensity, coupling constants and assignment values are reported, two dimensional COSY, HSQC and HMBC spectra were used to aid assignment. $^1$H coupling constants are reported in their entirety for each peak seen within a spectrum. Mass spectral analysis was performed using a Bruker esquire 3000 electrospray ionisation mass spectrometer. IR spectral analysis was performed using an ALPHA-P compact FTIR spectrometer. Melting points were recorded on a Gallenkamp MPD350 melting point apparatus, and are uncorrected. Monitoring of reactions was performed by thin layer chromatography using Merck pre-coated aluminium silica plates. Plates were observed under UV light at 245 nm and after charring using H$_2$SO$_4$ unless otherwise stated. ‘Flash’ chromatography using silica gel was performed routinely in order to purify all products.

All nonulosonic acid analogues produced via the chemoenzymatic route (Chapter 2) were purified using Amberlite IRA-400 ion exchange resins as described for Method A, Section 5.3.1.

All nonulosonic acids produced via a synthetic aldol condensation (Chapter 3) were purified using an Amberlite GC-400 ion exchange resin as described in Method 1, Section 5.5.1.

All solvents were distilled prior to use, all reagents were purchased from Sigma Aldrich unless otherwise stated.
5.2 Experimental Procedures

5.2.1 Synthesis of \(p\)-methoxyphenyl \(\alpha\)-D-hexopyranosides

1,2,3,4,6-Penta-\(O\)-acetyl-\(\alpha\)-D-mannopyranose (65)

\(\alpha\)-D-mannose (37) (5.0 g, 27.7 mmol) was dissolved in pyridine (50 mL) under \(N_2(g)\) and stirred for 5 minutes at 0°C, at which time acetic anhydride (25 mL) was added followed by DMAP (200 mg). The reaction was allowed to warm to room temperature and stirred for 15 hours. The resulting solution was concentrated to dryness. The residue was taken up in EtOAc (100 mL) and washed with aqueous HCl (1 N, 50 mL). The organic phase was dried (Na\(2\)SO\(4\)) and concentrated under reduced pressure and recrystallised from EtOAc/Hex to yield 65 (\(\alpha\)-only) (10.70 g, 99%) as a white crystalline solid.

\[^1\]H NMR (CDCl\(3\)) \(\delta\): 2.00, 2.05, 2.09, 2.16, 2.17 (5 \(\times\) OCO\(CH_3\)), 4.04 (dd, 1 H, \(J_{6a,6} = 11.7\) Hz, \(J_{6a,5} = 6.9\) Hz, H-6a), 4.09-4.13 (m, 1 H, H-5), 4.27 (dd, 1 H, \(J_{6,6a} = 11.7\) Hz, \(J_{6,5} = 6.9\) Hz, H-6), 5.25 (dd, 1 H, \(J_{2,3} = 2.7\) Hz, \(J_{2,1} = 1.8\) Hz, H-2), 5.33-5.53 (m, 2 H, H-3 & H-4), 6.07 (d, 1 H, \(J_{1,2} = 1.8\) Hz, H-1).

\[^{13}\]C NMR (CDCl\(3\)) \(\delta\): 21.6-21.8 (5 \(\times\) OCO\(CH_3\)), 62.0 (C-6), 65.4 (C-5), 68.2 (C-2), 68.7 (C-4), 70.5 (C-3), 90.5 (C-1), 168.0-170.6 (5 \(\times\) OCO\(CH_3\)).

\(m/z = 413.1\) [M + Na]+.

mp. 73-75°C (lit. value 74-75°C\(^{163}\))

\(p\)-Methoxyphenyl 2,3,4,6-tetra-\(O\)-acetyl-\(\alpha\)-D-mannopyranose (66)

A solution of 65 (2.10 g, 5.40 mmol) in CH\(_2\)Cl\(_2\) (20 mL) at 0°C was stirred for 30 minutes under \(N_2(g)\) before \(p\)-methoxyphenol (1.33 g, 10.80 mmol) was added followed by TfOH (72 \(\mu\)L, 0.81 mmol). The resulting solution was allowed to warm to room temperature and stirred for 15 hours. The reaction was quenched with Et\(_3\)N, concentrated under reduced pressure and purified using column chromatography (EtOAc/Hex 1:1, \(R_f = 0.5\)) yielding 66 (2.40 g, 98%) as a white foam.
$^1$H NMR (CDCl$_3$) δ: 2.02, 2.04, 2.05, 2.18 (4 x OCOCH$_3$), 3.76 (s, 3 H, Ar-OCH$_3$), 4.11 (d, 1 H, J$_{6a,6} = 11.7$ Hz, H-6a), 4.14 (dd, 1 H, J$_{6,6a} = 11.7$ Hz, J$_{6,5} = 6.9$ Hz, H-5), 4.26 (dd, 1 H, J$_{3,5} = 9.6$ Hz, J$_{3,6} = 6.9$ Hz, H-3), 5.28 (dd, 1 H, J$_{4,5} = 9.6$ Hz, J$_{4,3} = 9.0$ Hz, H-4), 5.41 (d, 1 H, J$_{1,2} = 1.8$ Hz, H-1), 5.43 (dd, 1 H, J$_{2,3} = 3.6$ Hz, J$_{2,1} = 1.8$ Hz, H-2), 5.54 (dd, 1 H, J$_{3,4} = 9.0$ Hz, J$_{3,2} = 3.6$ Hz, H-3), 6.82 (m, 2 H, Ar-H), 7.01 (m, 2 H, Ar-H).

$^{13}$C NMR (CDCl$_3$) δ: 21.7-21.9 (4 x OCOCH$_3$), 55.6 (ArOCH$_3$), 62.2 (C-6), 65.9 (C-5), 68.9 (C-2), 69.0 (C-4), 69.4 (C-3), 96.6 (C-1), 114.6-117.7 (4 x Ar-H), 149.6 (ipso-Ar), 155.3 (C-4’), 169.8-170.6 (4 OCOCH$_3$).

$m/z$ = 477.5 [M + Na]$^+$

$p$-Methoxyphenyl $\alpha$-D-mannopyranose (67)

Aqueous NaOH (1 N) was added dropwise to a solution of 66 (2.40 g, 5.28 mmol) in methanol (30 mL) at room temperature until adjusted to pH 12, the reaction mixture was then stirred for 15 hours. The resulting solution was neutralised to pH 7 by the dropwise addition of glacial acetic acid and then concentrated under reduced before being purified using column chromatography (EtOAc/MeOH 4:1, $R_f = 0.6$) yielding 67 (2.0 g, 98%) as an amorphous solid.

$^1$H NMR (MeOD$_4$) δ: 3.63-3.69 (m, 2 H, H-5, H-6a), 3.75 (s, 3 H, Ar-OCH$_3$), 3.72-3.78 (m, 2 H, H-4, H-6), 3.90 (dd, 1H, J$_{3,4} = 9.0$ Hz, J$_{3,2} = 3.3$ Hz, H-3), 4.05 (dd, 1 H, J$_{2,1} = J_{2,3} = 3.3$ Hz, 1.8 Hz, H-2), 5.35 (d, 1 H, J$_{1,2} = 1.8$ Hz, H-1), 6.84 (m, 2 H, Ar-H), 7.06 (m, 2 H, Ar-H).

$^{13}$C NMR (MeOD$_4$) δ: 54.6 (Ar-OCH$_3$), 61.2 (C-6), 66.9 (C-5), 70.7 (C-2), 71.0 (C-4), 73.8 (C-3), 99.7 (C-1), 114.1-117.7 (4 x Ar-H), 150.5 (ipso-Ar), 155.1 (C-4’).

$m/z$ = 309.1 [M + Na]$^+$
To a stirred solution of 67 (1.0 g, 3.49 mmol) in pyridine (10 mL) at 0°C under N\textsubscript{2}(g) was added p-TsCl (800 mg, 4.18 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 1 hour, after which time more p-TsCl (330 mg, 1.74 mmol) was added. The reaction mixture was allowed to stir for a further 1 hour, before being concentrated to dryness, taken up in EtOAc (50mL) washed with aqueous HCl (1 N, 20mL), and water (2 x 20mL). The organic phase was dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated under reduced pressure before being purified using column chromatography (EtOAc, R\textsubscript{f} = 0.6) to yield 68 (1.03g, 67\%) as a white foam.

\[ 1^1\text{H NMR (CDCl}_3\text{) } \delta: 2.37 (s, 3 H, SO}_2\text{Ar-CH}_3\text{), 3.75 (s, 3 H, Ar-OCH}_3\text{), 3.86 (dd, 1 H, } J_{4,5} = 9.6 \text{ Hz, } J_{4,3} = 9.0 \text{ Hz, H-4), 3.98-4.03 (br. m, 1 H, H-5), 4.04-4.11 (br. m, 1 H, H-6), 4.17 (br. m, 3 H, OH, H-6a & H-3), 4.27 (br. s, 1 H, OH), 4.42 (br. dd, 2 H, Hz, } J_{2,3} = 3.3 \text{ Hz, } J_{2,1} = 1.8 \text{, OH & H-2), 4.42 (dd, 1 H, } J_{3,4} = 9.0 \text{ Hz, } J_{3,2} = 3.3 \text{ Hz, H-3), 5.43 (d, 1 H, } J_{1,2} = 1.8 \text{ Hz, H-1), 6.76 (m, 2 H, Ar-H), 6.92 (m, 2 H, Ar-H), 7.27 (m, 2 H, Ar-H, OTs), 7.75 (m, 2 H, Ar-H, OTs). \]

\[ 1^3\text{C NMR (CDCl}_3\text{) } \delta: 21.6 \text{ (SO}_2\text{PhCH}_3\text{), 55.6 \text{ (ArOCH}_3\text{), 66.7 \text{ (C-5), 70.6 \text{ (C-6), 71.5 \text{ (C-2), 72.7 \text{ (C-4), 69.1 \text{ (C-3), 98.8 \text{ (C-1), 114.5-117.7 \text{ (4 x Ar-H), 125.0-126.7 \text{ (4 x Ar-H, OTs), 128.4 \text{ (Ar-CH}_3\text{), 144.9 \text{ (ipso-SO}_2\text{), 150.0 \text{ (ipso-Ar), 155.0 \text{ (C-4'). m/z } = 459 \text{ [M + Na}]^+}. \]
**p-Methoxyphenyl α-D-rhamnopyranose (64)**

**Approach using LiAlH₄ as a reducing agent from 68**

LiAlH₄ (120 mg, 3.16 mmol) was added to a stirred solution of 68 (1.0 g, 2.26 mmol) in THF (10 mL) at room temperature under N₂(g). The reaction mixture was stirred for 1 hour, after which time more LiAlH₄ (43 mg, 1.13 mmol) was added and stirred for a further 1 hour. The resulting reaction mixture was cooled to 0°C and quenched via the dropwise addition of EtOAc over several minutes and then diluted with water (20 mL). The diluted reaction mixture was washed with aqueous HCl (1 N, 10 mL) and the organic phase was washed with water (20 mL), dried (Na₂SO₄) and concentrated under reduced pressure before being purified using column chromatography (EtOAc/CH₂Cl₂, 5:1, Rᵣ = 0.3) yielding 64 (180 mg, 29%) as a white crystalline solid.

**1H NMR (CDCl₃) δ:** 1.25 (d, 3 H, J₆,₅ = 6.3 Hz, CH₃), 3.75 (s, 3 H, ArOC₃H₃), 3.77 (dd, 1 H, J₅,₄ = 9.6 Hz, J₅,₆ = 6.3 Hz, H-5), 3.65 (dd, 1 H, J₄,₅ = 9.6 Hz, J₄,₃ = 9.3 Hz, H-4), 3.97 (dd, 1 H, J₃,₄ = 9.3 Hz, J₃,₂ = 3.3 Hz, H-3), 4.12 (dd, 1 H, J₂,₃ = 3.3 Hz, J₂,₁ = 1.5 Hz, H-2), 5.37 (d, 1 H, J₁,₂ = 1.5 Hz, H-1), 6.97 (m, 2 H, Ar-H), 7.08 (m, 2 H, Ar-H).

**13C NMR (CDCl₃) δ:** 17.5 (C-6), 55.6 (ArOC₃H₃), 68.6 (C-5), 70.6 (C-2), 71.5 (C-4), 72.8 (C-3), 98.3 (C-1), 114.5-117.7 (4 x Ar-H), 150.0 (ips-o-Ar), 155.0 (C-4').

m/z = 293.1 [M + Na]+.

mp. 100-103°C (lit. value 101-103 °C)⁴⁶²

**p-Methoxyphenyl 6-deoxy-6-ido-α-D-mannopyranose (69)**

To a stirred solution of 67 (1.0 g, 3.49 mmol) in THF (20 mL) at room temperature under N₂(g) was added PPh₃ (1.37 g, 5.23 mmol), I₂ (1.35 g, 5.23 mmol) and Imidazole (475 mg, 6.98 mmol). The resulting reaction mixture was stirred at reflux for 2 hours before being concentrated under reduced pressure, taken up in EtOAc (100 mL) washed with aqueous Sodium thiosulphate (1 N, 20 mL) and water (50 mL). The
organic phase was concentrated under reduced pressure and purified using column chromatography (EtOAc, Rf = 0.5) yielding 69 (1.31 g, 95%) as a white foam.

\(^1\)H NMR (CDCl\(_3\)) \(\delta\): 3.30 (dd, 1 H, \(J_{6,6a} = 10.8\) Hz, \(J_{6,5} = 7.5\) Hz, H-6), 3.52-3.61 (br. m, 2 H, H-5 & H-6), 3.72 (t, 1 H, \(J_{4,5} = J_{4,3} = 9.3\) Hz, H-4), 3.77 (s, 3 H, Ar-OC\(_3\)H\(_3\)), 3.89 (d, 1 H, \(J_{OH,2} = 2.1\) Hz, 2-OH), 4.02 (br. dd, 1 H, \(J_{3,4} = 9.3\) Hz, \(J_{3,2} = 3.3\) Hz, H-3), 4.11 (br. dd, 1 H, \(J_{2,3} = 3.3\) Hz, \(J_{2,1} = 1.5\) Hz, H-2), 5.05 (br. s, 1 H, 3-OH), 5.43 (d, 1 H, \(J_{1,2} = 1.5\) Hz, H-1), 6.82 (m, 2 H, Ar-H), 7.05 (m, 2 H, Ar-H).

\(^{13}\)C NMR (CDCl\(_3\)) \(\delta\): 7.3 (C-6), 55.6 (OCH\(_3\)), 70.6 (C-5), 71.6 (C-2), 71.7 (C-4), 72.0 (C-3), 98.9 (C-1), 114.4-118.0 (4 x Ar-H), 150.3 (ipso-Ar), 154.8 (C-4’).

\(m/z = 419.1\) [M + Na]\(^+\).

**Approaches from 69 using varied reducing agents**

**Attempt 1**

LiAlH\(_4\) (135 mg, 3.53 mmol) was added to a stirred solution of 69 (1.0 g, 2.52 mmol) in THF (10 mL) at room temperature under N\(_2\)(g). The resulting mixture was stirred for 1 hour, after which time more LiAlH\(_4\) (48 mg, 1.26 mmol) was added and stirred for a further 1 hour. The resulting reaction mixture was cooled to 0°C and quenched via the dropwise addition of EtOAc over several minutes and then diluted with water (20 mL). The diluted reaction mixture was washed with aqueous HCl (1 N, 10 mL) and the organic phase was washed with water (20 mL), dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure before being purified using column chromatography (EtOAc/CH\(_2\)Cl\(_2\), 5:1, R\(_f\) = 0.3) yielding 64 (410 mg, 60%) as an oil. 64 prepared in this manner was spectroscopically identical to the material described above.
Attempt 2

To a stirred solution of 69 (1.0 g, 2.52 mmol) dissolved in degassed anhydrous toluene (5 mL) at room temperature under N₂(g) was added Bu₃SnH (1.1 g, 3.78 mmol) and AIBN (41 mg, 0.25 mmol). The resulting mixture was heated to 110°C and stirred for 1 hour. The reaction mixture was concentrated under reduced pressure and purified using column chromatography (EtOAc/CH₂Cl₂ 5:1, Rₜ = 0.3) yielding 64 (338 mg, 48%) as an oil. 86 prepared in this manner was spectroscopically identical to the material described previously.

Attempt 3

To a solution of 69 (1.0 g, 2.52 mmol) in Methanol (10 mL) was added Et₂NPr (5 mL) and Pd(OH)₂/C (20% wt.) (500 mg, 3.56 mmol). The resulting mixture was shaken on a Parr Hydrogenator at 40 psi and room temperature for 15 hours. The resulting mixture was filtered through Celite and concentrated to dryness before being purified using column chromatography (EtOAc/CH₂Cl₂ 5:1, Rₜ = 0.3) yielding 64 (680 mg, 99%) as a white foam. 86 prepared in this manner was spectroscopically identical to the material described previously.

α/β-D-Rhamnopyranose (35)

Compound 64 (220 mg, 0.81 mmol) was dissolved in a two phase solution of acetonitrile and water (4:1, 5 mL) at 0°C under N₂(g) to which cerium ammonium nitrate (444 mg, 8.1 mmol) was added. The resulting reaction mixture was allowed to warm to room temperature over 1 hour before being quenched with Et₃N and concentrated under reduced pressure. The crude reaction residue was purified using column chromatography (EtOAc/MeOH 4:1, Rₜ = 0.3) yielding 35 as a mixture of anomers (131 mg, 98%).

¹H NMR (D₂O, 270 MHz) (α-anomer) δ: 1.16 (d, 3 H, J₆,₅ = 6.3 Hz, CH₃), 3.21-3.27 (m, 2 H, H-4 & H-5), 3.69 (br. d, 1 H, J₃,₂ = 3.3 Hz, H-3), 3.81 (dd, 1 H, J₂,₃ = 3.3 Hz, J₂,₁ = 1.8 Hz, H-2), 5.00 (d, 1 H, J₁,₂ = 1.8 Hz, H-1).
\[^{13}\text{C} \text{ NMR (D}_2\text{O)} \delta: 16.7 \text{ (C-6)}, 68.2 \text{ (C-5)}, 69.8 \text{ (C-3)}, 70.7 \text{ (C-2)}, 72.1 \text{ (C-4)}, 93.9 \text{ (C-1).}\]

\[^{1}\text{H} \text{ NMR (D}_2\text{O)} (\beta\text{-anomer}) \delta: 1.18 \text{ (d, 3 H, J}_6,5 = 6.3 \text{ Hz, CH}_3), 3.34 \text{ (dd, 1 H, J}_4,3 = J_4,5 = 9.6 \text{ Hz, H-4)}, 3.76 \text{ (dd, 1 H, J}_5,4 = 9.6 \text{ Hz, J}_5,6 = 6.3 \text{ Hz, H-5), 3.49 \text{ (dd, 1 H, J}_4,3 = 9.6 \text{ Hz, J}_3,2 = 3.3 \text{ Hz, H-3), 3.81 \text{ (dd, 1 H, J}_2,3 = 3.3 \text{ Hz, J}_2,1 = 0.9 \text{ Hz, H-2), 4.75 \text{ (d, 1 H, J}_1,2 = 0.9 \text{ Hz, H-1).}\n
\[^{13}\text{C} \text{ NMR (D}_2\text{O)} \delta: 16.7 \text{ (C-6)}, 71.2 \text{ (C-2)}, 71.7 \text{ (C-4)}, 71.9 \text{ (C-5)}, 72.6 \text{ (C-3)}, 93.4 \text{ (C-1).}\]

\[m/z = 187.1 \text{ [M + Na]}^+.\]

mp. 90-93 ºC (lit value 90-91 ºC)\[^{231}\]

### 5.2.2 Synthesis of benzyl \(\alpha\)-D-hexopyranosides

**Benzyl \(\alpha\)-D-mannopyranose (70)**

![Benzyl \(\alpha\)-D-mannopyranose (70)]

To a stirred solution of D-mannose (37) (2.0 g, 11.1 mmol) in benzyl alcohol (12 mL, 8.15 mmol) under N\(_2\) (g) at 50°C was added acetyl chloride (2.0 mL, 0.028 mmol) dropwise over several minutes. The resulting mixture was stirred for 1.5 hours at 50°C and then removed from the heat and left to stir at room temperature for a further 15 hours. The resulting mixture was poured into water (100 mL), washed with EtOAc (3 x 25 mL). The combined organic fractions were washed with water (2 x 50 mL) and the combined aqueous phase fractions concentrated under reduced pressure to a clear oily residue which was recrystallised from hot EtOAc/Hex yielding 70 (2.95 g, 98%) as a white crystalline solid.

\[^{1}\text{H} \text{ NMR (D}_2\text{O)} \delta: 3.56-3.59 \text{ (m, 2 H, H-4 & H-5), 3.61-3.72 \text{ (m, 2 H, H-3 & H-6), 3.77 \text{ (dd, 1 H, J}_6a,6 = 11.7 \text{ Hz, J}_6a,5 = 6.3 \text{ Hz, H-6a), 3.84 \text{ (dd, 1 H, J}_2,3 = 3.3 \text{ Hz, J}_2,1 = 1.8 \text{ Hz, H-2), 4.57 \text{ (ABq, 2 H, J = 11.4 Hz, OCH}_2\text{Ph), 4.87 (d, 1 H, J}_1,2 = 1.8 \text{ Hz, H-1), 7.24-7.36 (m, 5 H, Ar-H).}\n
\[^{13}\text{C} \text{ NMR (D}_2\text{O)} \delta: 60.7 \text{ (C-6), 66.6 \text{ (C-5), 69.3 \text{ (OCH}_2\text{Ar), 69.9 \text{ (C-2), 70.5 \text{ (C-3), 72.8 \text{ (C-4), 99.2 \text{ (C-1), 125.4 \text{ (C-4')}), 125.4-125.7 \text{ (4 x Ar-H), 136.5 \text{ (ipso-Ar).}\n
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\[ m/z = 263 \text{ [M + Na]}^+. \]

mp. 131-133 °C (lit. value 130-131 °C)\(^{163}\)

**Benzyl 6-deoxy-6-iodo-α-D-mannopyranose (71)**

To a stirred solution of 70 (2.95 g, 10.9 mmol) in dry THF (30 mL) under \( \text{N}_2(g) \) at reflux was added triphenylphosphine (4.30 g, 16.4 mmol), I\(_2\) (4.16 g, 16.4 mmol) and imidazole (1.49 g, 21.8 mmol). The resulting mixture was stirred at reflux for 2 hrs before being concentrated under reduced pressure, taken up in EtOAc (40 mL) and washed with aqueous thiosulfate solution (1 N, 40 mL) and water (40 mL). The organic phase was dried (\( \text{Na}_2\text{SO}_4 \)) and concentrated under reduced pressure. The resulting oily residue was purified using column chromatography (toluene/acetone 3:1, \( R_f = 0.5 \)) to yield 71 (4.05 g, 98%) as white foam.

\(^1\)H NMR (CDCl\(_3\)) \( \delta: \) 3.19 (dd, 1 H, \( J_{5,4} = 9.3 \text{ Hz}, J_{5,6} = 6.3 \text{ Hz}, \text{H-5} \)), 3.60-3.65 (m, 3 H, H-4, H-6 \& H-6’), 3.83 (dd, 1 H, \( J_{3,4} = 8.4 \text{ Hz}, J_{3,2} = 3.3 \text{ Hz}, \text{H-3} \)), 3.93 (dd, 1 H, \( J_{2,3} = 3.3 \text{ Hz}, J_{2,1} = 1.5 \text{ Hz}, \text{H-2} \)), 4.66 (AB\(_q\), 2 H, \( J = 11.7 \text{ Hz}, \text{OCH}_2\text{Ph} \)), 4.89 (d, 1 H, \( J_{1,2} = 1.5 \text{ Hz}, \text{H-1} \)), 7.31-7.42 (m, 5 H, Ar-H).

\(^{13}\)C NMR (CDCl\(_3\)) \( \delta: \) 7.4 (C-6), 68.8 (OCH\(_2\)Ar), 70.7 (C-5), 71.9 (C-2), 72.0 (C-3), 72.1 (C-4), 98.7 (C-1), 125.3 (C-4’), 125.4-125.7 (4 x Ar-H), 137.2 (ipso-Ar).

\[ m/z = 403.1 \text{ [M + Na]}^+. \]

**Benzyl α-D-rhamnopyranose (72)**

To a solution of 71 (4.05 g, 10.65 mmol) in methanol (40 mL) was added EtN\(^\text{Pr}_2\) (5 mL) and Pd(OH)\(_2\)/C (20% wt.) (1.0 g, 7.12 mmol). The resulting mixture was shaken on a Parr hydrogenator at 40 psi under \( \text{H}_2(g) \) at room temperature for 15 hours. The reaction mixture was filtered through Celite and concentrated to dryness to yield 72 (2.70 g, 99%) as yellow foam.
\[ ^1 \text{H NMR (CDCl}_3 \text{) } \delta: 1.26 (d, 3 \text{ H, } J_{6,5} = 6.3 \text{ Hz, } CH_3), 3.51 (t, 1 \text{ H, } J_{4,5} = 9.6 \text{ Hz, } J_{4,3} = 9.3 \text{ Hz, H-4}), 3.70 (dd, 1 \text{ H, } J_{5,4} = 9.6 \text{ Hz, } J_{5,6} = 6.3 \text{ Hz, H-5}), 3.79 (dd, 1 \text{ H, } J_{3,4} = 9.3 \text{ Hz, } J_{3,2} = 3.3 \text{ Hz, H-3}), 3.94 (dd, 1 \text{ H, } J_{2,3} = 3.3 \text{ Hz, } J_{2,1} = 1.5 \text{ Hz, H-2}), 4.56 (ABq, 2 \text{ H, } J = 12.0 \text{ Hz, OCH}_2\text{Ar}), 4.84 (d, 1 \text{ H, } J_{1,2} = 1.5 \text{ Hz, H-1}), 7.25-7.36 (m, 5 \text{ H, Ar-H}). \]

\[ ^{13} \text{C NMR (CDCl}_3, 75 \text{ MHz) } \delta: 17.6 (C-6), 68.1 (C-5), 68.9 (OCH}_2\text{Ar), 70.7 (C-2), 71.8 (C-3), 72.9 (C-4), 99.0 (C-1), 125.3 (C-4'), 125.5-125.7 (4 x Ar-H), 137.5 (ipso-Ar). \]

\[ m/z = 277 [M + Na]^+ \]

\[ \alpha/\beta-\text{D-Rhamnopyranose (35)} \]

To a solution of 72 (2.70 g, 10.62 mmol) in water (15 mL) was added Dowex 50 H+ resin (5.0 g). The mixture was stirred at 80°C for 24 hours, then filtered, concentrated and lyophilised to yield an anomeric mixture of \( \alpha/\beta-\text{D-rhamnose (35)} \) (1.74 g, 100%) as a white solid.

\[ ^1 \text{H NMR (D}_2\text{O) (}\alpha\text{-anomer) } \delta: 1.16 (d, 3 \text{ H, } J_{6,5} = 6.3 \text{ Hz, CH}_3), 3.21-3.27 (m, 2 \text{ H, H-4 & H-5}), 3.69 (br d, 1 \text{ H, } J_{3,2} = 3.3 \text{ Hz, H-3}), 3.81 (d, 1 \text{ H, } J_{2,3} = 3.3 \text{ Hz, } J_{2,1} = 1.8 \text{ Hz, H-2}), 5.00 (d, 1 \text{ H, } J_{1,2} = 1.8 \text{ Hz, H-1}). \]

\[ ^{13} \text{C NMR (D}_2\text{O) } \delta: 16.7 (C-6), 68.2 (C-5), 69.8 (C-3), 70.7 (C-2), 72.1 (C-4), 93.9 (C-1). \]

\[ ^1 \text{H NMR (D}_2\text{O) (}\beta\text{-anomer) } \delta: 1.18 (d, 3 \text{ H, } J_{6,5} = 6.3 \text{ Hz, CH}_3), 3.34 (dd, 1 \text{ H, } J_{4,3} = J_{4,5} = 9.6 \text{ Hz, H-4}), 3.76 (dd, 1 \text{ H, } J_{5,4} = 9.6 \text{ Hz, } J_{5,6} = 6.3 \text{ Hz, H-5}), 3.49 (dd, 1 \text{ H, } J_{4,3} = 9.6 \text{ Hz, } J_{3,2} = 3.3 \text{ Hz, H-3}), 3.81 (dd, 1 \text{ H, } J_{2,3} = 3.3 \text{ Hz, } J_{2,1} = 0.9 \text{ Hz, H-2}), 4.75 (d, 1 \text{ H, } J_{1,2} = 0.9 \text{ Hz, H-1}). \]

\[ ^{13} \text{C NMR (D}_2\text{O) } \delta: 16.7 (C-6), 71.2 (C-2), 71.7 (C-4), 71.9 (C-5), 72.6 (C-3), 93.4 (C-1). \]

\[ m/z = 187.1 [M + Na]^+ \]

mp. 90-93 ºC (lit value 90-91 ºC)
**Benzyl 2,3-0-isopropylidene-α-D-rhamnopyranose (77)**

To a stirred solution of 72 (2.7 g, 10.63 mmol) in dry acetone (20 mL) under N$_2$(g) at room temperature was added 2,2-dimethoxypropane (20 mL) followed by TsOH•H$_2$O (405 mg, 2.13 mmol) and 4Å molecular sieves. The resulting solution was stirred for 30 minutes and then neutralised with Et$_3$N (~2 mL). The solution was filtered and concentrated under reduced pressure then purified using column chromatography (EtOAc, R$_f$ = 0.7) yielding 77 (3.12 g, 99%) as a white amorphous solid.

$^1$H NMR (CDCl$_3$) δ: 1.28 (d, 3 H, $J_{6,5} = 6.3$ Hz, CH$_3$), 1.36 & 1.54 (2 s, 6 H, C(CH$_3$)$_2$), 2.57 (d, 1 H, $J_{O_{OH,4}} = 4.5$ Hz, OH), 3.42 (ddd, 1 H, $J_{4,5} = 9.3$ Hz, $J_{4,3} = 7.2$ Hz, $J_{4,OH} = 4.5$ Hz, H-4), 3.75 (dd, 1 H, $J_{5,4} = 9.3$ Hz, $J_{5,6} = 6.3$ Hz, H-5), 4.12 (dd, 1 H, $J_{3,4} = 7.2$ Hz, $J_{3,2} = 5.7$ Hz, H-3), 4.21 (d, 1 H, $J_{2,3} = 5.7$ Hz, H-2), 4.63 (AB$_q$, 2 H, $J = 11.7$ Hz, OCH$_2$Ph), 5.07 (s, 1 H, H-1), 7.31-7.43 (m, 5H, Ar-H).

$^{13}$C NMR (CDCl$_3$) δ: 17.4 (C-6), 25.1 & 26.9 (C(CH$_3$)$_2$), 66.0 (C-5), 69.1 (OCH$_2$Ar), 74.5 (C-4), 75.8 (C-2), 78.4 (C-3), 96.2 (C-1), 109.5 (C(CH$_3$)$_2$), 125.0 (C-4'), 125.2-125.5 (4 x Ar-H), 137.0 (ipso-Ar).

m/z = 317.1 [M + Na]$^+$.  

**Benzyl 2,3-0-isopropylidene-4-O-trifluoromethanesulfonate-α-D-rhamnopyranose (78)**

To a stirred solution of 77 (3.12 g, 10.61 mmol) in CH$_2$Cl$_2$ (20 mL) under N$_2$(g) at -78 °C was added anhydrous Pyridine (2.6 mL, 31.8 mmol) follow by triflic anhydride (2.67 mL, 15.9 mmol). The resulting solution was allowed to stir at -78 °C for 10 minutes followed by further stirring at 0 °C for 30 minutes. The mixture was diluted with CHCl$_3$ (20 mL) and washed with aqueous HCl (1 N, 20 mL) and water (20 mL). The organic phase was concentrated under reduced pressure and purified using column chromatography (Hexane/EtOAc 3:1, R$_f$ = 0.8) yielding 78 (3.61 g, 80%) as a yellow foam.
1H NMR (CDCl$_3$) δ: 1.33 (d, 3 H, J$_{6,5}$ = 6.3 Hz, CH$_3$), 1.38, 1.56 (2 s, 6 H, C(CH$_3$)$_2$), 3.98 (dd, 1 H, J$_{5,4}$ = 10.2 Hz, J$_{5,6}$ = 6.3 Hz, H-5), 4.27 (d, 1 H, J$_{2,3}$ = 5.4 Hz, H-2), 4.34 (dd, 1 H, J$_{3,4}$ = 7.5 Hz, J$_{3,2}$ = 5.4 Hz, H-3), 5.11 (s, 1 H, H-1), 7.31-7.43 (m, 5 H, Ar-H).

13C NMR (CDCl$_3$) δ: 16.9 (C-6), 26.2, 27.5 (C(CH$_3$)$_2$), 63.1 (C-5), 69.6 (OCH$_2$Ar), 74.9 (C-3) 76.9 (C-2), 89.2 (C-4), 95.9 (C-1), 110.5 (C(CH$_3$)$_2$), 120.5 (q, CF$_3$, J = 317 Hz), 124.7 (C-4'), 127.8-128.6 (4 x Ar-H), 136.5 (ipso-Ar).

$m/z = 449.5$ [M + Na]$^+$

**Benzyl 4-O-trifluoromethanesulfonate-α-D-rhamnopyranoside (79)**

Crude 78 (3.61 g, 8.74 mmol) was stirred in an aqueous 80% acetic acid solution (20 mL) at 80°C for 2 hrs. The resulting mixture was concentrated under reduced pressure co-evaporating with toluene (3 x 10 mL) before being purified using column chromatography (Hex/EtOAc 3:1, $R_f = 0.3$) yielding 79 (3.81 g, 93% over two steps from 77) as a yellow foam.

1H NMR (CDCl$_3$) δ: 1.36 (d, 3 H, J$_{6,5}$ = 6.3 Hz, CH$_3$), 2.65 (br. s, 2 H, 2,3-OH), 3.98 (dd, 1 H, J$_{5,4}$ = 9.6 Hz, J$_{5,6}$ = 6.3 Hz, H-5), 4.08 (dd, 1 H, J$_{2,3}$ = 3.6 Hz, J$_{2,1}$ =1.8 Hz, H-2), 4.12 (br. d, 1 H, J$_{3,2}$ = 3.6 Hz, H-3), 4.63 (ABq, 2 H, J = 11.7 Hz, OCH$_2$Ph), 4.70 (br. d, 1 H, J$_{4,5}$ = 9.6 Hz, H-4), 4.89 (d, 1 H, J$_{1,2}$ =1.8 Hz, H-1), 7.31-7.42 (m, 5 H, Ar-H).

13C NMR (CDCl$_3$) δ: 17.2 (C-6), 65.2 (C-5), 69.0 (C-3), 69.6 (OCH$_2$Ar), 71.9 (C-2), 88.5 (C-4), 98.3 (C-1), 121.5 (q, CF$_3$, J = 317 Hz), 125.0 (C-4'), 125.2-125.6 (4 x Ar-H), 136.5 (ipso-Ar).

$m/z = 409.1$ [M + Na]$^+$
**Benzyl 2,3-di-O-acetyl-4-azido-4,6-dideoxy-α-D-talopyranose (81) and benzyl 2-O-acetyl-3,4-anhydro-6-dIDEOXY-α-D-talopyranose (80)**

To a stirred solution of 79 (3.81 g, 9.87 mmol) in DMF (60 mL) under N\(_2\) at 0°C was added NaN\(_3\) (1.28 g, 19.7 mmol). The resulting mixture was stirred at 0°C for 1 hour, after which time it was concentrated under reduced pressure. The crude reaction mixture was taken up in pyridine (50 mL) under N\(_2\) at 0°C to which acetic anhydride (20 mL) was added. The mixture was stirred at 0°C for 10 minutes and then at room temperature for 15 hours to yield after column chromatography (Hex/EtOAc 3:1) 81 (2.15 g, 60%) and 80 (960 mg, 35%) as white amorphous solids.

**Spectrum of 81**

\(^1\)H NMR (CDCl\(_3\)) \(\delta\): 1.32 (d, 3 H, \(J_{6,5} = 6.6 \text{ Hz}, \text{CH}_3\)), 2.11 (s, 3 H, COCH\(_3\)), 2.22 (s, 3 H, COCH\(_3\)), 3.73 (dd, 1 H, \(J_{4,3} = 3.9, J_{4,5} = 1.2 \text{ Hz}, \text{H-4}\)), 4.10 (dd, 1 H, \(J_{5,6} = 6.6 \text{ Hz}, J_{5,4} = 1.2 \text{ Hz}, \text{H-5}\)), 4.60 (ABq, 2 H, \(J = 11.7 \text{ Hz}, \text{OCH}_2\text{Ph}\)), 4.91 (d, 1 H, \(J_{1,2} = 1.2 \text{ Hz}, \text{H-1}\)), 5.23 (dd, 1 H, \(J_{2,3} = 3.6 \text{ Hz}, J_{2,1} = 1.2 \text{ Hz}, \text{H-2}\)), 5.39 (dd, 1 H, \(J_{3,4} = 3.9 \text{ Hz} J_{3,2} = 3.6, \text{H-3}\)), 7.32-7.41 (m, 5 H, Ar-H).

\(^{13}\)C NMR (CDCl\(_3\)) \(\delta\): 17.2 (C-6), 20.6 (COCH\(_3\)), 21.0 (COCH\(_3\)), 60.1 (C-3), 65.1 (C-2), 67.1 (C-5), 68.8 (C-4), 69.6 (OCH\(_2\text{Ar}\)), 97.7 (C-1), 128.0 (C-4’), 128.1-128.5 (4 x Ar-H), 136.5 (ipso-Ar), 169.6 (COCH\(_3\)), 170.3 (COCH\(_3\)).

\(m/z = 386.1 \ [\text{M+Na}]^+\)

IR = 2111 cm\(^{-1}\) (4-N\(_3\)), 1747 cm\(^{-1}\) (O\(\text{COCH}_3\))

**Spectrum of 80**

\(^1\)H NMR (CDCl\(_3\)) \(\delta\): 1.41 (d, 3 H, \(J_{6,5} = 6.6 \text{ Hz}, \text{CH}_3\)), 2.16 (s, 3 H, COCH\(_3\)), 3.18 (dd, 1 H, \(J_{4,3} = 4.2, J_{4,5} = 0.9 \text{ Hz}, \text{H-4}\)), 3.60 (dd, 1 H, \(J_{3,4} = 4.5, J_{3,2} = 4.2 \text{ Hz}, \text{H-3}\)), 4.18 (dd, 1 H, \(J_{5,6} = 6.6 \text{ Hz}, J_{5,4} = 0.9 \text{ Hz}, \text{H-5}\)), 4.63 (ABq, 2 H, \(J = 11.7 \text{ Hz}, \text{OCH}_2\text{Ph}\)), 4.72 (d, 1 H, \(J_{1,2} = 1.5 \text{ Hz}, \text{H-1}\)), 4.93 (dd, 1 H, \(J_{2,3} = 4.5 \text{ Hz}, J_{2,1} = 1.5 \text{ Hz}, \text{H-2}\)), 7.32-7.41 (m, 5 H, Ar-H).
\[ ^{13}\text{C} \text{ NMR (CDCl}_3 \text{)} \delta: 17.3 \text{ (C-6), 20.8 (COCH}_3 \text{), 49.5 (C-3), 53.3 (C-4), 61.4 (C-5), 67.3 (C-2), 69.3 (OCH}_2\text{Ar), 95.9 (C-1), 127.9 (C-4'), 128.0-128.5 (4 x Ar-H), 136.8 (ipso-Ar), 170.3 (COCH}_3 \text{).} \]
\[ m/z = 301.1 \text{ [M + Na]}^+ \]

**Benzy1 3,4-anhydro-6-deoxy-\alpha-D-talopyranose (82)**

To a stirred solution of 79 (3.30 g, 8.55 mmol) in anhydrous methanol (20 mL) under N\(_2\)(g) at 0°C was added K\(_2\)CO\(_3\) (1.18 g, 8.55 mmol). The resulting mixture was stirred at 0°C for 30 minutes, after which time it was concentrated under reduced pressure. The resulting clear residue was purified using column chromatography (Hex/EtOAc 3:1, R\(_f\) = 0.4) yielding 82 (2.02 g, 100%) as a white amorphous solid.

\[ ^1\text{H} \text{ NMR (CDCl}_3 \text{)} \delta: 1.41 \text{ (d, 3 H, } J_{6,5} = 6.6 \text{ Hz, CH}_3 \text{), 2.61 \text{ (br. s, 1 H, } 2 \text{-OH), 3.24 \text{ (dd, 1 H, } J_{4,3} = 4.2 \text{ Hz, H-4), 3.57 \text{ (dd, 1 H, } J_{3,2} = 4.5 \text{ Hz, H-3), 3.82 \text{ (dd, 1 H, } J_{2,1} = 1.5 \text{ Hz, H-2), 4.13 \text{ (dd, 1 H, } J_{5,6} = 6.6 \text{ Hz, H-5), 4.62 \text{ (ABq, 2 H, } J = 11.7 \text{ Hz, OCH}_2\text{Ph), 4.69 \text{ (d, 1 H, } J_{1,2} = 1.5 \text{ Hz, H-1), 7.32-7.41 (m, 5 H, Ar-H).} \]

\[ ^{13}\text{C} \text{ NMR (CDCl}_3 \text{)} \delta: 17.3 \text{ (C-6), 49.5 (C-3), 53.3 (C-4), 61.4 (C-5), 67.3 (C-2), 69.3 (OCH}_2\text{Ar), 95.9 (C-1), 127.9 (C-4'), 128.0-128.5 (4 x Ar-H), 136.8 (ipso-Ar).} \]

HRMS: (C\(_{13}\)H\(_{16}\)O\(_4\)Na): Calculated: 259.09463; found: 259.09461

**Benzy1 3,4-anhydro-2-O-tert-butyldimethylsilyl-6-deoxy-\alpha-D-talopyranose (83)**

To a stirred solution of 80 (130 mg, 0.5 mmol) in CH\(_2\)Cl\(_2\) (3 mL) at room temperature under N\(_2\)(g) was added TBDMSOTf (160 \(\mu\)L, 0.70 mmol) followed by 2,6-lutidine (140 \(\mu\)L, 1.17 mmol). The resulting reaction mixture was stirred for 30 minutes at room temperature before being diluted with CHCl\(_3\) (5 mL), washed with aqueous HCl (1 N, 5mL), saturated aqueous NaHCO\(_3\) (5 mL) and brine (10 mL). The organic phase was dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure before being purified using column chromatography (Hex/EtOAc 3:1, R\(_f\) = 0.4) yielding 83 (53 mg, 30%) as a white foam.
\[ ^1H \text{ NMR (CDCl}_3 \delta: 0.12, 0.14 \ (2 \text{ s, 6 H, Si(CH}_3)_2), 0.91 \ (s, 9 \text{ H, SiC(CH}_3)_3), 1.39 \ (d, 3 \text{ H, J}_6,5 = 6.6 \text{ Hz, CH}_3), 3.22 \ (dd, 1 \text{ H, J}_4,3 = 4.2, J_4,5 = 1.2 \text{ Hz, H-4}), 3.37 \ (dd, 1 \text{ H, J}_3,2 = 3.3, J_3,4 = 4.2 \text{ Hz, H-3}), 3.93 \ (dd, 1 \text{ H, J}_{2,3} = 3.3 \text{ Hz, J}_{2,1} = 3.0 \text{ Hz, H-2}), 4.18 \ (dd, 1 \text{ H, J}_{5,6} = 6.6 \text{ Hz, J}_{5,4} = 1.2 \text{ Hz, H-5}), 4.62 \ (\text{ABq}, 2 \text{ H, J} = 11.7 \text{ Hz, OCH}_2\text{Ph}), 4.64 \ (d, 1 \text{ H, J}_{1,2} = 3.0 \text{ Hz, H-1}), 7.32-7.41 \ (m, 5 \text{ H, Ar-H}). \]

\[ ^13C \text{ NMR (CDCl}_3 \delta: -4.7, -4.6 \ (\text{Si(CH}_3)_2), 16.8 \ (\text{C-6}), 18.3 \ (\text{SiC(CH}_3)_3), 25.6 \ (\text{SiC(CH}_3)_3), 53.7 \ (\text{C-4}), 55.0 \ (\text{C-3}), 62.9 \ (\text{C-5}), 68.2 \ (\text{C-2}), 69.6 \ (\text{OCH}_2\text{Ar}), 98.6 \ (\text{C-1}), 127.8 \ (\text{C-4'}), 128.0-128.5 \ (4 \times \text{ Ar-H}), 137.3 \ (\text{ipso-Ar}). \]

\( m/z = 373.1 \ [\text{M + Na}]^+. \)

**Benzyl 2-O-acetyl-4-azido-4-deoxy-α-d-rhamnopyranose (84)**

To a solution of 80 (200 mg, 0.71 mmol) in TMSN\(_3\) (3 mL) under \( \text{N}_2(g) \) at room temperature was added BF\(_3\)\text{EtO}_2 (0.3 mL) dropwise over several minutes. The reaction was allowed to stir at room temperature for 1 hour at which time it was poured over ice and extracted with CH\(_2\)Cl\(_2\). The combined extracts were washed with saturated aqueous Na\(_2\)CO\(_3\), dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure. The resulting clear oil was purified using column chromatography (3:1 Hex/EtOAc, \( R_f = 0.6 \)) to yield 84 (160 mg, 70%) as a clear oil.

\[ ^1H \text{ NMR (CDCl}_3 \delta: 1.36 \ (d, 3 \text{ H, J}_6,5 = 6.3 \text{ Hz, CH}_3), 2.16 \ (s, 3 \text{ H, COCH}_3), 2.50 \ (\text{br. s, 1 H, 3-OH}), 3.36 \ (t, 1 \text{ H, J}_{4,3} = 9.9 \text{ Hz, H-4}), 3.65 \ (dd, 1 \text{ H, J}_{5,4} = 9.9 \text{ Hz, J}_{5,6} = 6.3 \text{ Hz, H-5}), 4.09 \ (dd, 1 \text{ H, J}_{3,4} = 9.9 \text{ Hz, J}_{3,2} = 3.3 \text{ Hz, H-3}), 4.61 \ (\text{ABq, 2 H, J} = 11.7 \text{ Hz, OCH}_2\text{Ph}), 4.87 \ (d, 1 \text{ H, J}_{1,2} = 1.5 \text{ Hz, H-1}), 5.13 \ (dd, 1 \text{ H, J}_{2,3} = 3.3 \text{ Hz, J}_{2,1} = 1.5 \text{ Hz, H-2}), 7.31-7.42 \ (m, 5 \text{ H, Ar-H}). \]

\[ ^13C \text{ NMR (CDCl}_3 \delta: 18.3 \ (\text{C-6}), 20.9 \ (\text{COCH}_3), 65.8 \ (\text{C-4}), 67.0 \ (\text{C-5}), 69.1 \ (\text{C-3}), 69.5 \ (\text{OCH}_2\text{Ar}), 71.6 \ (\text{C-2}), 96.7 \ (\text{C-1}), 128.0-128.6 \ (5 \times \text{ Ar-H}), 136.7 \ (\text{ipso-Ar}), 170.9 \ (\text{COCH}_3). \]

\( m/z = 344.1 \ [\text{M + Na}]^+. \)

IR = 3395 cm\(^{-1}\) (3-OH), 2109 cm\(^{-1}\) (4-N\(_3\)), 1736 cm\(^{-1}\) (C=O).
Benzyl 3,4-anhydro-2-O-benzoyl-6-deoxy-α-D-talopyranose (85)

To a stirred solution of 82 (106 mg, 0.45 mmol) in pyridine (2 mL) under N₂(g) at 0°C was added benzoyl chloride (80 µL, 0.67 mmol) dropwise over 1 hour with stirring. The resulting mixture was stirred for a further 1 hour at 0°C. The solution was concentrated under reduced pressure, taken up in EtOAc (20 mL) and washed with aqueous HCl (1 N, 10 mL) and water (10 mL). The organic phase was dried (Na₂SO₄) and concentrated to yield 85 (170 mg, 98%) as a clear oil.

¹H NMR (CDCl₃) δ: 1.45 (d, 3 H, J₆,₅ = 6.6 Hz, CH₃), 3.24 (dd, 1 H, J₄,₃ = 4.2, J₄,₅ = 1.2 Hz, H-4), 3.74 (dd, 1 H, J₃,₂ = J₃,₄ = 4.2 Hz, H-3), 4.25 (dd, 1 H, J₅,₆ = 6.6 Hz, J₅,₄ = 1.2 Hz, H-5), 4.66 (ABq, 2 H, J = 11.7 Hz, OCH₂Ph), 4.90 (d, 1 H, J₁,₂ = 1.5 Hz, H-1), 5.19 (dd, 1 H, J₂,₃ = 4.2 Hz, J₂,₁ =1.5 Hz, H-2), 7.32-7.41 (m, 5 H, Ar-H), 7.42-7.50 (m, 2 H, Ar-H), 7.56-7.62 (m, 1 H, Ar-H), 8.11-8.15 (m, 2 H, Ar-H);

¹³C NMR (CDCl₃) δ: 17.2 (C-6), 49.6 (C-3), 53.5 (C-4), 61.9 (C-5), 68.2 (C-2), 69.4 (OCH₂Ar), 95.9 (C-1), 127.9 (C-4’), 128.0-133.3 (9 x Ar-H), 129.5 (ipso-Ar), 136.8 (ipso-Ar), 165.9 (OCO-Ar).
m/z = 363.1 [M + Na]⁺.

Benzyl 4-azido-4-deoxy-α-D-rhamnopyranose (72)

To a solution of 82 (300 mg, 1.27 mmol) in TMSN₃ (4 mL) under N₂(g) at room temperature was added BF₃·EtO₂ (0.4 mL) dropwise over several minutes. The reaction was allowed to stir at room temperature for 1 hour at which time it was poured over ice and extracted with CH₂Cl₂. The combined extracts were washed with saturated aqueous Na₂CO₃, dried (Na₂SO₄) and concentrated under reduced pressure. The resulting clear oil was purified using column chromatography (3:1 Hex/EtOAc, Rₚ = 0.3) to yield 72 (92 mg, 26%) as a clear oil.
$^1$H NMR (CDCl$_3$) $\delta$: 1.35 (d, 3 H, $J_{6.5} = 6.3$ Hz, CH$_3$), 3.34 (t, 1 H, $J_{4.5} = J_{4.3} = 9.9$ Hz, H-4), 3.65 (dd, 1 H, $J_{5,4} = 9.9$ Hz, $J_{5,6} = 6.3$ Hz, H-5), 3.88-3.95 (m, 2 H, H-2), 4.59 (AB$_q$, 2 H, $J = 12.0$ Hz, OCH$_2$Ph), 4.88 (d, 1 H, $J_{1,2} = 1.5$ Hz, H-1), 7.31-7.40 (m, 5 H, Ar-$H$).

$^{13}$C NMR (CDCl$_3$) $\delta$: 18.3 (C-6), 65.8 (C-4), 67.0 (C-5), 69.1 (C-3), 69.3 (C-2), 69.5 (OCH$_2$Ar), 96.7 (C-1), 128.0-128.6 (5 x Ar-$H$), 136.7 (ipso-Ar).

$m/z = 302.1$ [M + Na$^+$].

IR = 2109 cm$^{-1}$ (4-N$_3$)

**Benzyl 4-azido-2-O-benzoyl-4-deoxy-$\alpha$-D-rhamnopyranose (86)**

To a solution of 85 (150 mg, 0.44 mmol) in TMSN$_3$ (2 mL) under N$_2$(g) at room temperature was added BF$_3$·EtO$_2$ (0.2 mL) dropwise over several minutes. The reaction was allowed to stir at room temperature for 1 hour at which time it was poured over ice and extracted with CH$_2$Cl$_2$. The combined extracts were washed with saturated aqueous Na$_2$CO$_3$, dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The resulting clear oil was purified using column chromatography (3:1 Hex/EtOAc, $R_f = 0.6$) to yield 86 (101 mg, 60%) as a clear oil.

$^1$H NMR (CDCl$_3$) $\delta$: 1.42 (d, 3 H, $J_{6.5} = 6.3$ Hz, CH$_3$), 2.65 (d, 1 H, $J_{OH,3} = 5.7$ Hz, 3-OH), 3.47 (t, 1 H, $J_{4.5} = J_{4.3} = 9.9$ Hz, H-4), 3.73 (dd, 1 H, $J_{5,4} = 9.9$ Hz, $J_{5,6} = 6.3$ Hz, H-5), 4.22 (ddd, 1 H, $J_{3,4} = 9.9$ Hz, $J_{3,2} = 3.3$ Hz, $J_{3,OH} = 5.7$, H-3), 4.65 (AB$_q$, 2 H, $J = 11.7$ Hz, OCH$_2$Ph), 5.01 (d, 1 H, $J_{1,2} = 1.5$ Hz, H-1), 5.40 (dd, 1 H, $J_{2,3} = 3.3$ Hz, $J_{2,1} = 1.5$ Hz, H-2), 7.34-7.64 (m, 8 H, Ar-$H$), 8.07 (m, 2 H, Ar-$H$).

$^{13}$C NMR (CDCl$_3$) $\delta$: 18.5 (C-6), 66.1 (C-4), 67.1 (C-5), 69.5 (C-3), 69.6 (OCH$_2$Ar), 72.1 (C-2), 96.9 (C-1), 127.8-129.9 (6 x Ar-$H$), 127.9 (C-4’ (OBn), 133.6 (C-4’ (OBz), 136.7 (ipso-Ar (OBn)), 162.3 (ipso-Ar (OBz), 166.3 (COAr).

$m/z = 406.1$ [M + Na$^+$].

IR = 3395 cm$^{-1}$ (3-OH), 2100 cm$^{-1}$ (4-N$_3$), 1735 cm$^{-1}$ (C=O).
**Benzyl 4-azido-4,6-dideoxy-α-D-talopyranose (75)**

To a stirred solution of 81 (2.15 g, 5.92 mmol) in methanol (50 mL) was added a Na\textsuperscript{+} (s) (2.5 mmol) at 0°C under N\textsubscript{2}(g). The resulting reaction mixture was stirred at 0°C until all of the Na\textsuperscript{+} (s) metal had dissolved and then was allowed to warm to room temperature over one hour. The resulting clear reaction mixture was neutralised with glacial acetic acid and concentrated co-evaporating with toluene (3 x 25 mL). The clear residue was purified using column chromatography (EtOAc/Hex 1:1, R\textsubscript{f} = 0.6) yielding 75 (1.64 g, 99%) as a white amorphous solid.

\[ \text{1H NMR (CDCl}_3\text{)} \delta: 1.38 (d, 3 H, J_{6,5} = 6.6 \text{ Hz}, \text{CH}_3), 2.50 (s, 1 H, 2-OH), 2.52 (s, 1 H, 3-OH), 3.25 (dd, 1 H, J_{4,3} = 4.2, J_{4,5} = 1.2 \text{ Hz}, \text{H-4}), 3.58 (ddd, 1 H, J_{3,4} = 3.9 \text{ Hz} J_{3,2} = 3.6, J_{3,OH} = 4.5, \text{H-3}), 3.83 (ddd, 1 H, J_{2,3} = 3.6 \text{ Hz}, J_{2,1} = 1.2, J_{2,OH} = 4.5 \text{ Hz}, \text{H-2}), 4.61 (\text{ABq}, 2H, J = 11.7 \text{ Hz}, \text{ OCH}_2\text{Ph}), 4.65 (d, 1 H, J_{1,2} = 1.2 \text{ Hz}, \text{ H-1}), 7.32-7.41 (m, 5 H, Ar-H). \]

\[ m/z = 302.1 [\text{M + Na}]^+ \]

IR = 2109 cm\textsuperscript{-1} (4-N\textsubscript{3})

**4-Azido-4,6-dideoxy-α/β-D-talopyranose (87)**

To a solution of 75 (1.64 g, 5.43 mmol) in water (20 mL) was added Dowex 50 H\textsuperscript{+} resin (3.5 g). The mixture was stirred at 80°C for 24 hours, then filtered, concentrated and lyophilised to yield 87 as an anomeric mixture of α/β (3:2) (1.03 g, 100%) as a white amorphous solid.

\[ \text{1H NMR (D}_2\text{O)} (\alpha-\text{anomer}) \delta: 1.22 (d, 3 H, J_{6,5} = 6.3 \text{ Hz}, \text{CH}_3), 3.72-79 (m, 2 H, H-2 & H-4), 4.06 (dd, 1 H, J_{3,2} = 3.6 \text{ Hz}, J_{3,4} = 4.2 \text{ Hz}, \text{H-3}), 3.81 (dd, 1 H, J_{3,4} = 0.9 \text{ Hz}, J_{5,6} = 6.3 \text{ Hz}, \text{H-5}), 5.07 (d, 1 H, J_{1,2} = 1.2 \text{ Hz}, \text{H-1}). \]

\[ m/z = 302.1 [\text{M + Na}]^+ \]

IR = 2109 cm\textsuperscript{-1} (4-N\textsubscript{3})
\(^1\)H NMR (D\(_2\)O) (β-anomer) \(\delta\): 1.25 (d, 3 H, \(J_{6.5} = 6.3\) Hz, CH\(_3\)), 3.65-3.71 (m, 3 H, H-4, H-2 & H-5), 3.92 (dd, 1 H, \(J_{3.2} = 3.6\) Hz, \(J_{3.4} = 4.2\) Hz, H-3), 4.65 (d, 1 H, \(J_{1.2} = 0.9\) Hz, H-1).
\(^1\)C NMR (D\(_2\)O) \(\delta\): 16.4 (C-6), 63.5 (C-4), 69.2 (C-3), 69.8 (C-5), 70.0 (C-2), 93.9 (C-1).

\(m/z = 212.0\) [M + Na]\(^+\).

HRMS: (C\(_6\)H\(_{11}\)N\(_3\)O\(_4\)Na): Calculated: 212.06417; found: 212.06372

**4-Azido-4-deoxy-α/β-D-rhamnopyranose (88)**

To a solution of 73 (500 mg, 1.65 mmol) in water (10 mL) was added Dowex 50 H\(^+\) resin (1.2 g). The mixture was stirred at 80°C for 24 hours, then filtered, concentrated and lyophilised to yield 88 as an anomic mixture of α/β (3:2) (313 mg, 100%) as a white amorphous solid.

\(^1\)H NMR (D\(_2\)O) (α-anomer) \(\delta\): 1.28 (d, 3 H, \(J_{6.5} = 6.3\) Hz, CH\(_3\)), 3.35 (dd, 1 H, \(J_{3.2} = 3.3\) Hz, \(J_{3.4} = 9.9\) Hz, H-3), 3.73-3.81 (m, 2 H, H-5 & H-4), 3.85 (dd, 1 H, \(J_{2.3} = 3.3\) Hz, \(J_{2.1} = 1.2\) Hz, H-2), 5.07 (d, 1 H, \(J_{1.2} = 1.2\) Hz, H-1).

\(^1\)C NMR (D\(_2\)O) \(\delta\): 17.5 (C-6), 66.9 (C-4), 69.1 (C-5), 70.0 (C-2), 70.7 (C-3), 93.9 (C-1).

\(^1\)H NMR (D\(_2\)O) (β-anomer) \(\delta\): 1.26 (d, 3 H, \(J_{6.5} = 6.3\) Hz, CH\(_3\)), 3.21-3.32 (m, 2 H, H-4 & H-5), 3.65 (dd, 1 H, \(J_{3.2} = 3.3\) Hz, \(J_{3.4} = 9.9\) Hz, H-3), 3.87 (dd, 1 H, \(J_{2.3} = 3.3\) Hz, \(J_{2.1} = 0.9\) Hz, H-2), 4.76 (d, 1 H, \(J_{1.2} = 0.9\) Hz, H-1).

\(^1\)C NMR (D\(_2\)O) \(\delta\): 17.4 (C-6), 64.5 (C-4), 65.0 (C-5), 70.7 (C-2), 72.1 (C-3), 93.4 (C-1).

\(m/z = 212.0\) [M + Na]\(^+\).

HRMS: (C\(_6\)H\(_{11}\)N\(_3\)O\(_4\)Na): Calculated: 212.06410; found: 212.06373
5.2.3 Synthesis of D-talopyranose (36)

1,2,3,4,6-Penta-O-acetyl-β-D-galactopyranose (95)

D-Galactose (94) (5.0 g, 27.78 mmol) was added to a stirred solution of sodium acetate (2.5 g) in acetic anhydride (50 mL) under N₂(g) and stirred for 3 hours at 110°C. Excess Ac₂O was destroyed by stirring the mixture with Sat. Aq. NaHCO₃ (200 mL). The product was extracted into CH₂Cl₂ (2 x 50 mL) and the organic phase was dried (Na₂SO₄) and concentrated yielding 95 (10.0 g, 92%). A TLC (3.5:1 Hex/Acetone) and ¹H NMR spectrum showed sole product (β-only), which was used in the next reaction without purification.

NMR data consistent with that reported by Descroix et al. 2011.²³² for 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose

m/z = 413.1 [M + Na]⁺.

1,3,4,6-Tetra-O-acetyl-α-D-galactopyranose (96)

A solution of 95 (10.0 g, 25.6 mmol) in aqueous 90% trifluoroacetic acid (60 mL) was stirred for 6 hours at room temperature. The reaction mixture was diluted with CH₂Cl₂ (200 mL), washed with H₂O (2 x 100 mL), Sat. Aq. NaHCO₃ (2 x 100 mL) and brine (2 x 100 mL) before being dried (Na₂SO₄) and concentrated. The residue was taken up in isopropyl ether which caused a white precipitate to form. The white solid was collected via filtration and recrystallised from EtOAc/Et₂O yielding 96 (5.35 g, 60%) as a white solid.

NMR data consistent with that reported by Xiao et al. 2010¹⁹⁶.

¹H NMR (CDCl₃) δ: 2.04, 2.07, 2.15, 2.19 (s, 3 H, COCH₃), 4.06-4.12 (m, 2 H, H-6, H-6a), 4.18 (dd, 1 H, J₂,₃ = 10.5, J₂,₁ = 4.1 Hz, H-2), 4.27-4.29 (m, 1 H, H-5), 5.18 (dd, 1 H, J₃,₂ = 10.5, J₃,₄ = 3.2 Hz, H-3), 5.46 (dd, 1 H, J₄,₅ = 1.8 Hz, J₄,₃ = 3.2 Hz, H-4), 6.31 (d, 1 H, J₁,₂ = 4.1 Hz, H-1). m/z = 371.1 [M + Na]⁺.
1,3,4,6-tetra-O-Acetyl-2-O-trifluoromethanesulfonate-α-galactopyranose (97)

To a solution of 96 (5.35 g, 15.37 mmol) in anhydrous CH₂Cl₂ (50 mL) was added anhydrous pyridine (3.0 mL). The reaction was cooled to -78°C after which Tf₂O (4 mL) was added gradually over several minutes. The resulting reaction mixture was stirred for 15 minutes before being diluted with CHCl₃ (100 mL) and washed with aqueous HCl (1N, 100 mL) and water (100 mL). The Organic phase was dried (Na₂SO₄) and concentrated, before being purified using column chromatography (1:1 Hex/EtOAc, Rf = 0.8) yielding 97 (3.0 g, 40%) as a yellow oil. 97 was never characterised due to poor stability being used directly in the next reaction.

1,3,4,6-tetra-O-Acetyl-α-D-talopyranose (98) and 1,2,4,6-tetra-O-acetyl-α-D-talopyranose (99)

To a solution of 97 (3.0 g, 6.25 mmol) in N,N-DMF (30 mL) was added 2 mL of water, the resulting solution was stirred at 70°C for 48 Hours before being concentrated and purified using column chromatography (4:1 EtOAc/Hex) yielding a mixture of 98 and 99 (880 mg, 40%) as a white amorphous solid.

NMR data consistent with that reported by Xiao et al. 2010¹⁹⁶.

98: ¹H NMR (DMSO-d6) δ: 1.98, 1.99, 2.04, 2.10 (s, 3 H, COCH₃), 3.75-3.77 (m, 1 H, H-2), 4.05-4.15 (m, 2 H, H-6, H-6a), 4.29 (dd, 1 H, J₅₆ = 6.6, J₅₄ = 1.1 Hz, H-5), 5.02 (dd, 1 H, J₃₂ = J₃₄ = 3.9 Hz, H-3), 5.23 (dd, 1 H, J₄₅ = 1.1 Hz, J₄₃ = 3.9 Hz, H-4), 5.85 (d, 1 H, J₁₂ = 1.8 Hz, H-1).

m/z = 371.1 [M + Na]⁺.
99: $^1$H NMR (DMSO-$d_6$) δ: 1.98, 2.06, 2.08, 2.10 (s, 3 H, COCH$_3$), 3.97-4.06 (m, 3 H, H-3, H-6, H-6a), 4.24 (dd, 1 H, $J_{5,6}$ = 6.6 Hz, $J_{5,4}$ = 1.1 Hz, H-5), 4.85 (dd, 1 H, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.9 Hz, H-2), 5.17 (d, 1 H, $J_{4,3}$ = 1.1 Hz, $J_{4,2}$ = 3.9 Hz, H-4), 5.95 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1).

α/β-D-talopyranose (36) To a solution of 98 and 99 (300 mg, 0.86 mmol) in MeOH (10 mL) was added Na$_2$O$_4$$_{(s)}$, the reaction mixture was stirred at 0ºC until all of the metal had dissolved and then stirred for a further 1 hour at room temperature before being neutralised with glacial acetic acid. The neutralised reaction mixture was concentrated under reduced pressure from toluene (3 x 20 mL) to give 5 (158 mg, 100%) as a white crystalline solid.

NMR data consistent with that reported by Xiao et al. 2010.

$^1$H NMR (D$_2$O) (α-anomer) δ: 3.77 (dd, 1 H, $J_{6a,5}$ = 4.5 Hz, $J_{6a,6}$ = 11.7 Hz, H-6a), 3.81 (dd, 1 H, $J_{6,5}$ = 7.7 Hz, $J_{6,6a}$ = 11.7 Hz, H-6), 3.87 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 1.8 Hz, H-2), 3.90 (dd, 1 H, $J_{4,3}$ = 3.3, $J_{4,5}$ = 1.2 Hz, H-4), 3.95 (dd, 1 H, $J_{1,2}$ = 3.3 Hz, H-3), 4.10 (dd, 1 H, $J_{5,6}$ = 4.5 Hz, $J_{5,4}$ = 1.2 Hz, H-5), 5.25 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1).

$^{13}$C NMR (D$_2$O) δ: 63.0 (C-6), 66.5 (C-3), 71.1 (C-4), 72.0 (C-2), 72.5 (C-5), 96.1 (C-1).

$^1$H NMR (D$_2$O) (β-anomer) δ: 3.60 (dd, 1 H, $J_{5,6}$ = 4.5 Hz, $J_{5,4}$ = 1.2 Hz, H-5), 3.78 (dd, 1 H, $J_{6a,5}$ = 4.5 Hz, $J_{6a,6}$ = 11.7 Hz, H-6a), 3.82 (dd, 1 H, $J_{6,5}$ = 7.7 Hz, $J_{6,6a}$ = 11.7 Hz, H-6), 3.85 (dd, 1 H, $J_{4,3}$ = 3.3, $J_{4,5}$ = 1.2 Hz, H-4), 3.89 (dd, 1 H, $J_{1,2}$ = 3.3 Hz, H-3), 3.95 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 3.2 Hz, H-2), 4.80 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1).

$^{13}$C NMR (D$_2$O) δ: 62.7 (C-6), 69.9 (C-3), 70.1 (C-4), 73.0 (C-2), 77.2 (C-5), 95.8 (C-1).

m/z = 203.1 [M + Na]$^+$.

mp. 122-125 ºC (lit. mp. 123-124ºC)
5.3 Aldol condensation reactions catalysed by Neu5Ac aldolase

5.3.1 General procedures

Method A. Enzymatic reactions catalysed by Neu5Ac aldolase

Hexose substrate (1.0 mmol) was dissolved in milli-Q water (10.0 mL/mmol of sugar) to which sodium pyruvate (28) (0.6 M final concentration) was added inside a large quickfit test tube (30 mm diameter). The pH was adjusted to 7.3 ± 0.2 with 0.1 M NaOH and a small amount of NaN₃ (no more than 0.1% of total concentration) was added. Neu5Ac aldolase (3.0 U/mmol of sugar) and bovine serum albumin (2 mg/U of enzyme) were added to a 2000 Mw dialysis tube along with milli-Q water (2.5 mL/U enzyme). The dialysis tubing was tied at either end and placed into the test tube containing the solution of substrate and sodium pyruvate (28). The test tube was sealed with a stopper which was parafilmmed and placed into an oil bath at 37°C and stirred for 4 days. After this time the dialysis tubing was removed from original test tube and placed into fresh milli-Q water (20 mL) and stirred for a further 6 hours. The combined aqueous fractions were concentrated under reduced pressure. The residue was taken up in water (5.0 mL) and purified using ion exchange column chromatography (IRA-400, formate type), eluting with two column volumes of water followed by one column volume of 0.5 M formic acid and one column volume of 1 M formic acid. The aqueous acidic fractions were combined and concentrated under reduced pressure co-evaporating with toluene (3 x 50 mL) and dried using lyophilisation to yield the desired nonulosonic acid.

Method B. Esterification

To a stirred solution of nonulosonic acid obtained from Method A, in anhydrous methanol at room temperature under N₂(g) was added an equal mass of Dowex 50 H⁺ resin. The resulting suspension was stirred at room temperature for 48 hours before being filtered to remove the resin and the filtrate concentrated under reduced pressure producing the methyl ester without purification.
Method C. Glycosylation

The methyl ester obtained from Method B was taken up in anhydrous methanol at room temperature under N$_2$(g) to which Dowex 50 H$^+$ resin (double the mass of the compound) was added. The resulting suspension was heated to 70ºC and stirred for 15 hours before being cooled and filtered to remove the resin. The filtrate was concentrated and placed on a high vac line to remove any traces of methanol.

Method D. Acetylation

Crude Methyl ester Methyl glycoside residue obtained from Method C was taken up in an appropriate volume of pyridine to which acetic anhydride (2:1 ratio py./Ac$_2$O) was added and the resulting reaction mixture was stirred at room temperature under N$_2$(g) for 15 hours. The resulting reaction mixture was concentrated to dryness before being taken up in EtOAc, washed with aqueous HCl (1 N) and water. The organic phase was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude reaction residue was purified using column chromatography to yield the corresponding per-O-acetyl-nonulosonic acid product.

All of the following compounds were created using Methods A through to D unless otherwise stated.

3-Deoxy-d-glycero-d-galacto-non-2-ulopyranosidone (KDN) (3)

KDN (3) was prepared from d-mannose (37) following Method A in a 75% yield.

$^1$H NMR (D$_2$O) $\delta$: 1.67 (dd, 1 H, $J_{3ax,3eq} = 12.9$ Hz, $J_{3ax,4} = 12.0$ Hz, H-3ax), 2.07 (dd, 1 H, $J_{3eq,3ax} = 12.9$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3eq), 3.48 (dd, 1 H, $J_{5,4} = J_{5,6} = 9.6$ Hz, H-5), 3.53-3.59 (m, 2 H, H-8, H-9), 3.67 (dd, 1 H, $J_{6,5} = 9.9$ Hz, $J_{6,7} = 1.2$ Hz, H-6), 3.87 (ddd, 1 H, $J_{4,3ax} = 12.0$ Hz, $J_{4,5} = 9.6$ Hz, $J_{4,3eq} = 4.8$ Hz, H-4).

$^{13}$C NMR (D$_2$O) $\delta$: 38.4 (C-3), 63.1 (C-9), 67.7 (C-5), 68.5, 69.9, 70.3 (C-4,C-7,C-8), 71.5 (C-6), 95.1 (C-2), 165.5 (C-1). $m/z = 291.2$ [M + Na]$^+$. 

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3,9-Dideoxy-9-methyl-D-glycero-D-galacto-non-2-ulopyranosidonate (9-deoxy-KDN) (89)

9-deoxy-KDN (89) was prepared from D-rhamnose (35) following Method A in a 72% yield.

\[
\begin{align*}
\text{1H NMR (D}_2\text{O)} & \; \delta: \; 1.06 \; (d, \; 3 \; H, \; J_{9,8} = 6.9 \; Hz, \; H-9), \; 1.65 \\
& \; (dd, \; 1 \; H, \; J_{3ax,3eq} = 12.9 \; Hz, \; J_{3ax,4} = 11.4 \; Hz, \; H-3ax), \; 2.05 \\
& \; (dd, \; 1 \; H, \; J_{3eq,3ax} = 12.9 \; Hz, \; J_{3eq,4} = 4.8 \; Hz, \; H-3eq), \; 3.44 \\
& \; (dd, \; 1 \; H, \; J_{5,4} = J_{5,6} = 9.9 \; Hz, \; H-5), \; 3.52 \; (dd, \; 1 \; H, \; J_{8,7} = \\
& \; 8.7 \; Hz, \; J_{8,9} = 6.9 \; Hz, \; H-8), \; 3.70 \; (br \; d, \; 1 \; H, \; J_{1,8} = 8.7 \; Hz, \; H-7), \; 3.75-3.84 \; (m, \; 2 \; H, \; H-4/H-6).
\end{align*}
\]

\[m/z = 275.2 \; [M + Na]^+.\]

Methyl 3,9-dideoxy-9-methyl-D-glycero-D-galacto-non-2-ulopyranosidonate (9-deoxy-KDN1Me) (90)

Methyl 9-deoxy-KDN (90) was prepared from 89 following Method B in a 98% yield.

\[
\begin{align*}
\text{1H NMR (MeOD)} & \; \delta: \; 1.16 \; (d, \; 3 \; H, \; J_{9,8} = 6.6 \; Hz, \; H-9), \\
& \; 1.80 \; (dd, \; 1 \; H, \; J_{3ax,3eq} = 12.4 \; Hz, \; J_{3ax,4} = 11.4 \; Hz, \; H-3ax), \\
& \; 2.10 \; (dd, \; 1 \; H, \; J_{3eq,3ax} = 12.4 \; Hz, \; J_{3eq,4} = 4.8 \; Hz, \; H-3eq), \\
& \; 3.33-3.51 \; (m, \; 3 \; H, \; H-5, \; H-7, \; H-8), \; 3.56 \; (ddd, \; 1 \; H, \; J_{4,3ax} = 4.8 \; Hz, \; J_{4,5} = 9.6 \; Hz \; H-4), \; 3.78 \; (s, \; 1 \; H, \; CO_2CH_3), \; 4.01 \; (dd, \; 1 \; H, \; J_{6,7} = 1.8 \; Hz, \; J_{6,5} = 9.9 \; Hz, \; H-6),
\end{align*}
\]

\[m/z = 289.1 \; [M + Na]^+.\]

Methyl (methyl 3,9-dideoxy-9-methyl-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (9-deoxy-KDN1,β2Me2) (91)

9-deoxy-KDN1,β2Me2 (91) was prepared from 90 following Method C in a 70% yield without purification or characterisation being used directly in the next reaction to produce 92.
Methyl (methyl 4,5,7,8-tetra-O-acetyl-3,9-dideoxy-9-methyl-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (Per-O-acetyl-9-deoxy-KDN1,β2Me2) (92)

Per-O-acetyl-9-deoxy-KDN1,β2Me2 (92) was prepared from 91 following Method D in a 98% yield.

\[ \text{\text{H NMR (CDCl}_3, 600 MHz) \delta: 1.27 (d, 3 H, \text{J}_9,8 = 6.6 Hz, H-9), 1.97, 2.01, 2.02, 2.12 (s, 3 H, COCH}_3), 1.83 (dd, 1 H, J_{3ax,3eq} = 12.6 Hz, J_{3ax,4} = 11.4 Hz, H-3ax), 2.49 (dd, 1 H, J_{3eq,3ax} = 12.6 Hz, J_{3eq,4} = 5.4 Hz, H-3eq), 3.18 (s, 3 H, COC}_H}_3), 3.79 (s, 3 H, CO}_2C}_H}_3), 4.03 (dd, 1 H, J_{6,5} = 10.2 Hz, H-6), 4.91 (dd, 1 H, J_{5,4} = 9.6 Hz, J_{5,6} = 10.2 Hz, H-5), 5.15 (dd, 1 H, J_{8,7} = J_{8,9} = 6.6 Hz, H-8), 5.22 (dd, 1 H, J_{7,6} = 2.4 Hz, J_{7,8} = 6.6 Hz, H-7), 5.29 (dd, 1 H, J_{4,3eq} = 5.4 Hz, J_{4,3ax} = 11.4 Hz, J_{4,5} = 9.6 Hz H-4). \]

\[ \text{\text{C NMR (CDCl}_3, 600 MHz) \delta: 15.9 (C-9), 20.6, 20.7, 20.8, 21.2 (COCH}_3), 36.9 (C-3), 50.9 (COCH}_3), 52.6 (CO}_2C}_H}_3), 67.9 (C-5), 68.9 (C-8), 69.1 (C-4), 69.4 (C-6), 69.5 (C-7), 98.6 (C-2), 167.5 (C-1), 169.9, 170.0, 170.1, 170.4 (COCH}_3). \]

\[ m/z = 471.1 \text{ [M + Na]}^+. \]

7-Azido-3,7,9-tri-ddeoxy-9-methyl-D-glycero-D-galacto-non-2-ulopyranosidonate (74) (7-azido-5-hydroxy-legionaminic acid)

7-azido-5-hydroxy-legionaminic acid (74) was prepared from 88 following Method A in a 32% yield.

\[ \text{\text{H NMR (D}_2O) \delta: 1.25 (d, 3 H, J_{9,8} = 6.6 Hz, H-9), 1.85 (dd, 1 H, J_{3ax,3eq} = 12.0 Hz, J_{3ax,4} = 11.1 Hz, H-3ax), 2.10 (dd, 1 H, J_{3eq,3ax} = 12.0 Hz, J_{3eq,4} = 5.1 Hz, H-3eq), 3.51 (dd, 1 H, J_{5,4} = J_{5,6} = 9.9 Hz, H-5), 3.62 (dd, 1 H, J_{8,7} = 8.7 Hz, J_{8,9} = 6.9 Hz, H-8), 3.75 (br. d, 1 H, J_{7,8} = 8.7 Hz, H-7), 3.76-3.87 (m, 2 H, H-4/H-6). \]

\[ m/z = 250.1 \text{ [M + Na]}^+. \]
Methyl (methyl 4,5,8-tri-O-acetyl-3,7,9-trideoxy-9-methyl-d-glycero-β-D-galacto-non-2-ulopyranosidonate (Per-O-acetyl-7-azido-Leg1,β2Me2) (93)

Per-O-acetyl-7-azido-Leg1,β2Me2 (93) was prepared from 74 following Method B through D in a 78% yield over 3 steps.

\[
\text{1}^1\text{H NMR (CDCl}_3\text{) } \delta: 1.31 \text{ (d, } 3 \text{ H, } J_{9,8} = 6.6 \text{ Hz, H-9), 1.97, 2.01, 2.12 \text{ (s, } 3 \text{ H, COCH}_3\text{), 1.87 (dd, 1 H, } J_{3ax,3eq} = 12.6 \text{ Hz, } J_{3ax,4} = 11.4 \text{ Hz, H-3ax), 2.49 (dd, 1 H, } J_{3eq,3ax} = 12.6 \text{ Hz, } J_{3eq,4} = 5.1 \text{ Hz, H-3eq), 3.31 (s, } 3 \text{ H, COCH}_3\text{), 3.82 (s, } 3 \text{ H, CO}_2\text{CH}_3\text{), 3.85 (dd, 1 H, } J_{7,6} = 2.4 \text{ Hz, } J_{7,8} = 6.6 \text{ Hz, H-7), 3.83 (dd, 1 H, } J_{6,7} = 2.4 \text{ Hz, } J_{6,5} = 9.9 \text{ Hz, H-6), 5.05 (dd, 1 H, } J_{5,4} = 9.6 \text{ Hz, } J_{5,6} = 9.9 \text{ Hz, H-5), 5.19 (dd, 1 H, } J_{8,7} = J_{8,9} = 6.6 \text{ Hz, H-8), 5.29 (ddd, 1 H, } J_{4,3eq} = 5.1 \text{ Hz, } J_{4,3ax} = 11.4 \text{ Hz, } J_{4,5} = 9.6 \text{ Hz, H-4).}
\]

\[
\text{13}^1\text{C NMR (CDCl}_3\text{) } \delta: 17.5 \text{ (C-9), 20.6, 20.7, 21.2 \text{ (COCH}_3\text{), 36.9 (C-3), 51.9 (COCH}_3\text{), 52.5 \text{ (CO}_2\text{CH}_3\text{), 67.9 (C-5), 68.9 (C-8), 69.1 (C-4), 69.4 (C-6), 69.5 (C-7), 99.6 (C-2), 168.5 (C-1), 169.9, 170.0, 170.1 \text{ (COCH}_3\text{).}}
\]

HRMS: (C\text{17}H\text{25}N\text{3}O\text{10}Na): Calculated: 454.14376; found: 454.14372

IR = 2109 cm\textsuperscript{-1} (7-N\text{3})

3-Deoxy-d-glycero-1-altro-non-2-ulopyranosidonate (7-epi-KDN) (55)

7-epi-KDN (55) was prepared from d-talose (36) following Method A in a 25% yield.

\[
\text{1}^1\text{H NMR (D}_2\text{O) } \delta: 1.75 \text{ (dd, } 1 \text{ H, } J_{3ax,3eq} = J_{3ax,4} = 12.0 \text{ Hz, H-3ax), 2.21 (dd, 1 H, } J_{3eq,3ax} = 12.0 \text{ Hz, } J_{3eq,4} = 5.4 \text{ Hz, H-3eq), 3.54 (dd, 1 H, } J_{5,4} = J_{5,6} = 9.6 \text{ Hz, H-5), 3.68 (dd, 1 H, } J_{9,9} = 12.0 \text{ Hz, } J_{9,8} = 2.4 \text{ Hz, H-9'), 3.71 (dd, 1 H, } J_{9,9} = 12.0 \text{ Hz, } J_{9,8} = 4.2 \text{ Hz, H-9), 3.84 (dd, 1 H, } J_{6,7} = 9.0 \text{ Hz, } J_{6,5} = 9.6 \text{ Hz, H-6), 3.94 (dd, 1 H, } J_{7,6} = 9.0 \text{ Hz, } J_{7,8} = 4.5 \text{ Hz, H-7), 3.98 (ddd, 1 H, } J_{4,3eq} = 5.4 \text{ Hz, } J_{4,3ax} = 12.0 \text{ Hz, } J_{4,5} = 9.6 \text{ Hz H-4), 4.01 (ddd, 1 H, } J_{8,7} = 4.5 \text{ Hz, } J_{8,9} = 4.2 \text{ Hz, } J_{8,9} = 2.4 \text{ Hz, H-8). m/z = 268.1 [M + Na]^+}.\]
Methyl (methyl 3-deoxy-β-D-glycero-α-L-altro-non-2-ulopyranosid)onate (7-epi-KDN1,α2Me2) (100)

7-epi-KDN1,β2Me2 (100) was prepared from 55 following Methods B and C in a 98% yield over two steps.

$^{1}$H NMR (MeOD$_4$) δ: 1.76 (dd, 1 H, J$_{3ax,3eq}$ = J$_{3ax,4}$ = 12.0 Hz, H-3ax), 2.25 (dd, 1 H, J$_{3eq,3ax}$ = 12.0 Hz, J$_{3eq,4}$ = 5.4 Hz, H-3eq), 3.35 (s, 3 H, COCH$_3$), 3.54 (dd, 1 H, J$_{5,4}$ = J$_{5,6}$ = 9.6 Hz, H-5), 3.68 (dd, 1 H, J$_{9',9}$ = 12.0 Hz, J$_{9',8}$ = 2.4 Hz, H-9'), 3.71 (dd, 1 H, J$_{9,9}$ = 12.0 Hz, J$_{9,8}$ = 4.2 Hz, H-9), 3.78 (s, 3 H, CO$_2$CH$_3$), 3.82 (s, 3 H, CO$_2$CH$_3$) was prepared from 100 following Method D in a 98% yield.

$^{13}$C NMR (CDCl$_3$) δ: 20.6, 20.7, 20.8, 20.9, 21.2 (COCH$_3$), 44.1 (C-3), 52.2 (COCH$_3$), 52.7 (CO$_2$CH$_3$), 62.5 (C-9), 67.4 (C-5), 69.8 (C-8), 71.2 (C-7), 72.0 (C-4), 78.2 (C-6), 99.5 (C-2), 168.5 (C-1), 169.9, 170.0, 2 x 170.1, 170.4 (COCH$_3$).

$m/z = 319.1$ [M + Na]$^+$. 

Methyl (methyl 4,5,7,8,9-penta-O-acetyl-3-deoxy-β-D-glycero-α-L-altro-non-2-ulopyranosid)onate (7-epi-per-O-acetyl-KDN1,α2Me$_2$) (101)

Per-O-acetyl-7-epi-KDN1,α2Me$_2$ (101) was prepared from 100 following Method D in 98% yield.

$^{1}$H NMR (CDCl$_3$) δ: 1.95, 1.97, 2.05, 2.10, 2.12 (s, 3 H, COCH$_3$), 1.85 (dd, 1 H, J$_{3ax,3eq}$ = 12.0 Hz, J$_{3ax,4}$ = 11.1 Hz, H-3ax), 2.51 (dd, 1 H, J$_{3eq,3ax}$ = 12.0 Hz, J$_{3eq,4}$ = 5.7 Hz, H-3eq), 3.33 (s, 3 H, COCH$_3$), 3.82 (s, 3 H, CO$_2$CH$_3$), 4.30 (dd, 1 H, J$_{9',9}$ = 12.9 Hz, J$_{9',8}$ = 2.7 Hz, H-9'), 4.43 (dd, 1 H, J$_{6,7}$ = 9.0 Hz, J$_{6,5}$ = 9.9 Hz, H-6), 4.53 (dd, 1 H, J$_{9,9}$ = 12.9 Hz, J$_{9,8}$ = 3.9 Hz, H-9), 4.91 (dd, 1 H, J$_{5,4}$ = J$_{5,6}$ = 9.9 Hz, H-5), 5.44 (br. ddd, 1 H, J$_{4,3eq}$ = 5.7 Hz, J$_{4,3ax}$ = 11.1 Hz, J$_{4,5}$ = 9.9 Hz H-4), 5.56 (m, 2 H, H-7, H-8).

$^{13}$C NMR (CDCl$_3$) δ: 20.6, 20.7, 20.8, 20.9, 21.2 (COCH$_3$), 44.1 (C-3), 52.2 (COCH$_3$), 52.7 (CO$_2$CH$_3$), 62.5 (C-9), 67.4 (C-5), 69.8 (C-8), 71.2 (C-7), 72.0 (C-4), 78.2 (C-6), 99.5 (C-2), 168.5 (C-1), 169.9, 170.0, 2 x 170.1, 170.4 (COCH$_3$).

$m/z = 529.1$ [M + Na]$^+$. 

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7-Azido-3,7,9-tri-deoxy-9-methyl-D-glycero-1-altro-non-2-ulopyranosidonate (7-epi-7-azido-5-hydroxy-legionaminic acid) (76)

7-azido-7-epi-5-hydroxy-legionaminic acid (76) was prepared from 87 following Method A in a 26% yield.

\[^1\text{H NMR (D}_2\text{O)} \delta: 1.35 \ (d, 3 \text{ H, J}_{9,8} = 6.6 \text{ Hz, H-9}), \ 1.87 \ (dd, 1 \text{ H, J}_{3ax,3eq} = 15.0 \text{ Hz, J}_{3ax,4} = 12.4 \text{ Hz, H-3ax}), \ 2.10 \ (dd, 1 \text{ H, J}_{3eq,3ax} = 15.0 \text{ Hz, J}_{3eq,4} = 6.0 \text{ Hz, H-3eq}), \ 3.68 \ (dd, 1 \text{ H, J}_{7,6} = 4.2 \text{ Hz, J}_{7,8} = 9.3 \text{ Hz, H-7}), \ 4.35 \ (dd, 1 \text{ H, J}_{6,7} = 4.2 \text{ Hz, J}_{6,5} = 9.6 \text{ Hz, H-6}), \ 4.41 \ (dd, 1 \text{ H, J}_{5,4} = 9.6 \text{ Hz, J}_{5,6} = 9.6 \text{ Hz, H-5}), \ 4.43 \ (br. dd, 1 \text{ H, J}_{4,3eq} = 6.0 \text{ Hz, J}_{4,3ax} = 12.4 \text{ Hz H-4}), \ 4.51 \ (dd, 1 \text{ H, J}_{8,7} = 9.3 \text{ Hz, J}_{8,9} = 6.6 \text{ Hz, H-8}). \]

\[m/z = 300.0 \ [M + Na]^+\]

IR = 2109 cm\(^{-1}\) (7-N\(_3\)), 1735 cm\(^{-1}\) (CO\(_2\)Me)

Methyl (methyl 4,5,8-tri-\(O\)-acetyl-7-azido-3,7,9-tri-deoxy-9-methyl-D-glycero-\(\alpha\)-1-altro-non-2-ulopyranosidonate (7-epi-per-\(O\)-acetyl-7-azido-Leg1,\(\alpha\)2Me\(_2\)) (102)

Per-\(O\)-acetyl-7-azido-7-epi-Leg1,\(\alpha\)2Me\(_2\) (102) was prepared from 76 following Method B through D in a 67% yield over 3 steps.

\[^1\text{H NMR (CDCl}_3\} \delta: 1.22 \ (d, 3 \text{ H, J}_{9,8} = 6.6 \text{ Hz, H-9}), \ 1.99, 2.00, 2.06 \ (s, 3 \text{ H, COCH}_3), \ 1.75 \ (dd, 1 \text{ H, J}_{3ax,3eq} = 15.6 \text{ Hz, J}_{3ax,4} = 12.4 \text{ Hz, H-3ax}), \ 2.15 \ (dd, 1 \text{ H, J}_{3eq,3ax} = 15.6 \text{ Hz, J}_{3eq,4} = 6.0 \text{ Hz, H-3eq}), \ 3.33 \ (s, 3 \text{ H, COCH}_3), \ 3.75 \ (dd, 1 \text{ H, J}_{7,6} = 4.2 \text{ Hz, J}_{7,8} = 9.3 \text{ Hz, H-7}), \ 3.83 \ (s, 3 \text{ H, CO}_2\text{CH}_3), \ 4.40 \ (dd, 1 \text{ H, J}_{6,7} = 4.2 \text{ Hz, J}_{6,5} = 9.6 \text{ Hz, H-6}), \ 4.90 \ (dd, 1 \text{ H, J}_{8,7} = 9.3 \text{ Hz, J}_{8,9} = 6.6 \text{ Hz, H-8}), \ 5.43 \ (br. s, 1 \text{ H, H-4}), \ 5.56 \ (dd, 1 \text{ H, J}_{5,4} = 9.6 \text{ Hz, J}_{5,6} = 9.6 \text{ Hz, H-5}). \]

\[^{13}\text{C NMR (CDCl}_3\} \delta: 17.4 \ (C-9), \ 20.5, 20.9, 21.1 \ (COCH}_3), \ 44.8 \ (C-3), \ 51.9 \ (COCH}_3), \ 52.7 \ (CO}_2\text{CH}_3), \ 65.1 \ (C-7), \ 67.0 \ (C-6), \ 68.1 \ (C-8), \ 71.3 \ (C-4), \ 78.9 \ (C-5), \ 99.3 \ (C-2), \ 168.3 \ (C-1), \ 169.0, 2 \times 170.0 \ (COCH}_3). \]

HRMS: (C\(_{17}\)H\(_{25}\)N\(_3\)O\(_{10}\)Na): Calculated: 454.14376; found: 454.14374

IR = 2105 cm\(^{-1}\) (7-N\(_3\))
5.4 Synthesis of C-2 modified hexopyranosides

5.4.1 C-2 modified benzyl α-D-rhamnopyranosides

Benzyl 4-O-acetyl-2,3-O-isopropylidene-α-D-rhamnopyranose (112)

To a stirred solution of 77 (3.12g, 10.61 mmol) in pyridine (20 mL) under N₂(g) at 0°C was added acetic anhydride (10 mL). The reaction was allowed to stir at 0°C for 10 minutes and then warmed to room temperature and stirred for a further 15 hours. The resulting solution was concentrated under reduced pressure, taken up in EtOAc (20 mL) and washed with aqueous HCl (1 N, 20 mL) and water (20 ml), dried (Na₂SO₄) and again concentrated under reduced pressure to yield 112 (3.56g, 100%) as a white amorphous solid.

¹H NMR (CDCl₃) δ: 1.18 (d, 3 H, J₆,₅ = 6.3 Hz, CH₃), 1.35 & 1.58 (2 s, 6 H, C(CH₃)₂), 2.12 (s, 3 H, OCOC₃H₃), 3.81 (dd, 1 H, J₅,₄ = 10.2 Hz, J₅,₆ = 6.3 Hz, H-5), 4.18-4.24 (m, 2 H, H-2 & H-3), 4.62 (AB,q, 2 H, J = 11.7 Hz, OCH₂Ph), 4.90 (ddd, 1 H, J₄,₅ = 10.2 Hz, J₄,₃ = 9.6 Hz, H-4), 5.12 (s, 1 H, H-1), 7.31-7.43 (m, 5H, Ar-H).

¹³C NMR (CDCl₃) δ: 16.9 (C-6), 21.1 (OCOCH₃), 26.4 & 27.6 (C(CH₃)₂), 64.1 (C-5), 69.2 (OCH₂Ar), 74.5 (C-3), 75.8 (C-2), 78.4 (C-4), 96.1 (C-1), 109.7 (C(CH₃)₂), 128.0-128.5 (4 x Ar-H), 136.9 (ipso-Ar), 170.1 (OCOCH₃).

m/z = 359.1 [M + Na]⁺.

Benzyl 4-O-acetyl-α-D-rhamnopyranose (113)

Compound 112 (3.56g, 10.61 mmol) was stirred in an aqueous 80% acetic acid solution (20 mL) at 80°C for 2 hrs. The resulting mixture was concentrated under reduced pressure with Toluene (3 x 10 mL) before being purified using column chromatography (EtOAc, Rₐ = 0.5) yielding 113 (3.14 g, 99%) as a white amorphous solid.

¹H NMR (CDCl₃) δ: 1.20 (d, 3 H, J₆,₅ = 6.3 Hz, CH₃), 2.12 (s, 3 H, OCOCH₃), 3.35 (br. s, 2 H, 2 & 3 OH), 3.83 (dd, 1 H, J₅,₄ = 9.9 Hz, J₅,₆ = 6.3 Hz, H-5), 3.91 (dd, 1 H, J₃,₄ =
9.6 Hz, $J_{3,2} = 3.6$ Hz, H-3), 3.98 (dd, 1 H $J_{2,3} = 3.6$ Hz, $J_{2,1} = 1.5$ Hz, H-2), 4.62 (AB, 2 H, $J = 11.7$ Hz, OCH$_2$Ph), 4.88 (t, 1 H, $J_{4,5} = 9.9$ Hz, $J_{4,3} = 9.6$ Hz, H-4), 4.91 (d, 1 H, $J_{1,2} = 1.5$ Hz, H-1), 7.31-7.43 (m, 5H, Ar-H).

$^{13}$C NMR (CDCl$_3$) δ: 17.4 (C-6), 21.1 (OCOCH$_3$), 65.9 (C-5), 69.2 (OCH$_2$Ar), 70.1 (C-2), 71.0 (C-3), 75.1 (C-4), 98.7 (C-1), 127.9-128.5 (4 x Ar-H), 137.1 (ipso-Ar), 172.0 (OCOCH$_3$).

$m/z = 319.1$ [M + Na]$^+$. 

**Benzyl 4-O-acetyl-3-O-tert-butyldimethylsilyl-α-D-rhamnopyranose (114)**

To a solution of 113 (3.14 g, 10.6 mmol) in anhydrous methanol (30 mL) under N$_2$(g) at 80°C was added Bu$_2$SnO (2.64 g, 10.61 mmol). The resulting suspension was refluxed for 30 minutes, after which the reaction was concentrated under reduced pressure. The residue was taken up in N,N-DMF (20 mL) to which TBDMSiCl (2.0 g, 13.26 mmol) was added under N$_2$(g) at room temperature. The reaction was then heated to 110°C and stirred at this temperature for 1 hour. The reaction mixture was allowed to cool to room temperature before poured into a mixture of EtOAc and NaHCO$_3$ (sat. aq. 100 mL). The organic phase was washed with NaCl (sat. aq. 100 mL) dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified using column chromatography (solid addition; 3:1 Hex/EtOAc; R$_f$ = 0.5) yielding 114 (3.13 g, 72%) as a viscous oil.

$^1$H NMR (CDCl$_3$) δ: 0.05, 0.07 (2 s, 6 H, Si(C$_3$H$_7$)$_2$), 0.84 (s, 9 H, SiC(CH$_3$)$_3$), 1.19 (d, 3 H, $J_{6,5} = 6.3$ Hz, CH$_3$), 2.10 (s, 3 H, OCOCH$_3$), 2.68 (br. s, 1 H, 2-OH), 3.80 (dd, 1 H, $J_{5,4} = 9.9$ Hz, $J_{5,6} = 6.3$ Hz, H-5), 3.86 (dd, 1 H $J_{2,3} = 3.6$ Hz, $J_{2,1} = 1.8$ Hz, H-2), 4.10 (dd, 1 H, $J_{3,4} = 9.6$ Hz, $J_{3,2} = 3.6$ Hz, H-3), 4.61 (AB, 2 H, $J = 11.7$ Hz, OCH$_2$Ph), 4.79 (d, 1 H, $J_{1,2} = 1.8$ Hz, H-1), 5.01 (t, 1 H, $J_{4,5} = 9.9$ Hz, $J_{4,3} = 9.6$ Hz, H-4), 7.31-7.43 (m, 5H, Ar-H).

$^{13}$C NMR (CDCl$_3$) δ: -4.8, -4.6 (Si(CH$_3$)$_2$), 17.4 (C-6), 17.8 (SiC(CH$_3$)$_3$), 21.1 (OCOCH$_3$), 25.6 (SiC(CH$_3$)$_3$), 66.1 (C-5), 69.1 (OCH$_2$Ar), 70.5 (C-3), 71.6 (C-2), 73.7 (C-4), 97.6 (C-1), 127.7 (C-4'), 127.9-128.5 (4 x Ar-H), 137.2 (ipso-Ar), 169.9 (OCOCH$_3$). $m/z = 433.2$ [M + Na]$^+$. 

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To a solution of 114 (3.13 g, 7.63 mmol) in anhydrous methanol (50 mL) under N\textsubscript{2}(g) at 0\textdegree C was added Na\textsuperscript{+}(s). The reaction was stirred at 0\textdegree C 20 minutes before being allowed to warm to room temperature. The reaction was stirred at room temperature for a further 1 hour before being neutralised with glacial acetic acid (2 mL) and concentrated under reduced pressure from toluene (3 x 10 mL). The resulting residue was purified using column chromatography (Hex/EtOAc 3:1, R\textsubscript{f} = 0.3) yielding 115 (2.75 g, 98%) as a white amorphous solid.

\textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\): 0.14, 0.16 (2 s, 6 H, Si(CH\textsubscript{3})\textsubscript{2}), 0.96 (s, 9 H, SiC(CH\textsubscript{3})\textsubscript{3}), 1.34 (d, 3 H, \textit{J}_{6.5} = 6.3 Hz, CH\textsubscript{3}), 2.01 (d, 1 H, \textit{J}_{OH,4} = 3.6 Hz, 4-OH), 2.56 (br. s, 1 H, 2-OH), 3.51 (ddd, 1 H, \textit{J}_{4,5} = 9.0 Hz, \textit{J}_{4,4} = 8.7 Hz, \textit{J}_{4,OH} = 3.6, H-4), 3.75 (dd, 1 H, \textit{J}_{5,4} = 9.0 Hz, \textit{J}_{5,6} = 6.3 Hz, H-5), 3.85 (dd, 1 H \textit{J}_{2.3} = 3.6 Hz, \textit{J}_{2.1} = 1.5 Hz, H-2), 3.90 (dd, 1 H, \textit{J}_{3.4} = 8.7 Hz, \textit{J}_{3.2} = 3.6 Hz, H-3), 4.61 (AB\textsubscript{q}, 2 H, \textit{J} = 11.7 Hz, OCH\textsubscript{2}Ph), 4.90 (d, 1 H, \textit{J}_{1,2} = 1.5 Hz, H-1), 7.31-7.43 (m, 5H, Ar-H).

\textsuperscript{13}C NMR (CDCl\textsubscript{3}) \(\delta\): -4.56, -4.51 (Si(CH\textsubscript{3})\textsubscript{2}), 17.6 (C-6), 18.0 (SiC(CH\textsubscript{3})\textsubscript{3}), 25.6 (SiC(CH\textsubscript{3})\textsubscript{3}), 67.6 (C-5), 68.9 (OCH\textsubscript{2}Ar), 71.4 (C-3), 73.0 (C-2), 73.3 (C-4), 98.1 (C-1), 127.8-128.4 (4 x Ar-H), 137.3 (ipso-Ar).

\textit{m/z} = 391.2 [M + Na\textsuperscript{+}].

To a solution of 115 (210 mg, 0.57 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (3 mL) under N\textsubscript{2}(g) at -78\textdegree C was added anhydrous pyridine (276 \textmu L, 3.42 mmol) and Tf\textsubscript{2}O (290 \textmu L, 1.71 mmol). The reaction was allowed to stir at -78\textdegree C for 10 minutes before being stirred for a further 1 hour. The resulting solution was diluted with CHCl\textsubscript{3} (20 mL) and washed with aqueous HCl (1 N, 20 mL) and water (20 mL). The organic phase was dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated under reduced pressure and purified using column chromatography (5:1 Hex/EtOAc, R\textsubscript{f} = 0.4) yielding 116 (313 mg, 87%), as a yellow oil.
$^1$H NMR (CDCl$_3$) $\delta$: 0.17, 0.19 (2 s, 6 H, Si(CH$_3$)$_2$), 0.98 (s, 9 H, SiC(CH$_3$)$_3$), 1.36 (d, 3 H, $J_{6,5} = 6.3$ Hz, CH$_3$), 4.02 (dd, 1 H, $J_{5,4} = 9.6$ Hz, $J_{5,6} = 6.3$ Hz, H-5), 4.32 (dd, 1 H, $J_{3,4} = 7.2$ Hz, $J_{3,2} = 2.1$ Hz, H-3), 4.61 (AB$_q$, 2 H, $J = 11.7$ Hz, OCH$_2$Ph), 4.72 (dd, 1 H, $J_{4,5} = 9.6$ Hz, $J_{4,3} = 7.2$ Hz, H-4), 4.97 (br. s, 2 H H-2 & H-1), 7.31-7.43 (m, 5 H, Ar-H).

$^{13}$C NMR (CDCl$_3$) $\delta$: -4.8, -4.6 (Si(CH$_3$)$_2$), 18.0 (SiC(CH$_3$)$_3$), 25.8 (SiC(CH$_3$)$_3$) 17.2 (C-6), 66.4 (C-5), 67.9 (C-3), 70.4 (OCH$_2$Ar), 85.3 (C-4), 85.9 (C-2), 95.2 (C-1), 120.0, 120.1 (2 q, 2 CF$_3$, $J = 317.0$ Hz), 127.9-128.7 (4 x Ar-H), 135.6 (ipso-Ar).

$\text{m/z} = 655.1$ [M + Na]$^+$

**Benzyl 4-azido-2,4-dideoxy-3-oxo-$\alpha$-D-talopyranose (118)**

![Structural formula](image)

To a stirred solution of 116 (130 mg, 0.2 mmol) in anhydrous $N,N$-DMF (5 mL) under $N_2$(g) at room temperature was added NaN$_3$ (140 mg, 2.04 mmol). The resulting reaction suspension was stirred at room temperature for 15 hours, after which it was concentrated under reduced pressure before being purified using column chromatography (Hex/EtOAc 3:1, $R_f = 0.3$) yielding 118 (45 mg, 98%) as a white powder.

$^1$H NMR (CDCl$_3$) $\delta$: 1.43 (d, 3 H, $J_{6,5} = 6.0$ Hz, CH$_3$), 2.71 (dd, 1 H, $J_{2ax,1} = 1.2$ Hz, $J_{2ax,2eq} = 14.4$ Hz, H-2ax), 2.79 (dd, 1 H, $J_{2eq,1} = 4.2$ Hz, $J_{2eq,2ax} = 14.4$ Hz, H-2eq), 3.68 (d, 1 H, $J_{4,5} = 1.2$ Hz, H-4), 3.97 (dd, 1 H, $J_{5,4} = 1.2$ Hz, $J_{5,6} = 6.0$ Hz, H-5), 4.61 (AB$_q$, 2 H, $J = 12.3$ Hz, OCH$_2$Ph), 4.97 (br. d, 1 H, $J_{1,2ax} = 1.2$ Hz, $J_{1,2eq} = 4.2$ Hz H-1), 7.29-7.39 (m, 5 H, Ar-Ph).

$^{13}$C NMR (CDCl$_3$) $\delta$: 19.3 (C-6), 46.1 (C-2), 68.6 (C-5), 69.1 (OCH$_2$Ar), 69.9 (C-4), 97.1 (C-1), 127.8-128.5 (4 x Ar-H), 136.6 (ipso-Ar), 199.8 (C-3).

HRMS: (C$_{13}$H$_{15}$N$_3$O$_3$Na): Calculated: 284.10111; found: 284.10110

IR = 2100 cm$^{-1}$ (4-N$_3$), 1735 cm$^{-1}$ (3-C=O)
Benzyl 4-\textit{O}-acetyl-3-\textit{O}-benzyl-\textalpha-\texttextit{D}-rhamnopyranose (120)

To a solution of 113 (1.1 g, 3.71 mmol) in anhydrous methanol (30 mL) under N\texttextit{2(g)} at 80°C was added Bu\textsubscript{2}SnO (1.11 g, 4.46 mmol). The resulting suspension was refluxed for 30 minutes, after which the reaction was concentrated under reduced pressure. The residue was taken up in N,N-DMF (30 mL) to which BnBr (1 mL, 8.16 mmol) was added under N\texttextit{2(g)} at room temperature. The reaction was then heated to 110°C and stirred at this temperature for 1 hour. The reaction mixture was allowed to cool to room temperature before poured into a mixture of EtOAc and NaHCO\textsubscript{3} (sat. aq. 100 mL). The organic phase was washed with NaCl (sat. aq. 100 mL) dried (Na\textsubscript{2}SO\textsubscript{4}), and concentrated under reduced pressure. The residue was purified using column chromatography (solid addition; 5:1 Hex/EtOAc; R\textsubscript{f} = 0.2) yielding 120 (1.14 g, 80%) as a viscous oil.

\textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\): 1.21 (d, 3 H, \(J_{6,5} = 6.3\) Hz, CH\textsubscript{3}), 2.03 (s, 3 H, OCOCH\textsubscript{3}), 2.64 (br. s, 1 H, 2-OH), 3.76-3.85 (m, 2 H, H-3, H-5), 4.10 (dd, 1 H, \(J_{2,1} = 1.8\) Hz, \(J_{2,3} = 3.6\) Hz, H-2), 4.63 (2 AB\textsubscript{q}, 4 H, \(J = 11.7\) Hz, OCH\textsubscript{2}Ph), 4.96 (d, 1 H, \(J_{1,2} = 1.8\) Hz, H-1), 5.10 (t, 1 H, \(J_{4,5} = 9.9\) Hz, \(J_{4,3} = 9.6\) Hz, H-4), 7.30-7.43 (m, 10 H, Ar-H).

\textsuperscript{13}C NMR (CDCl\textsubscript{3}) \(\delta\): 17.4 (C-6), 21.0 (OCOCH\textsubscript{3}), 66.2 (C-5), 68.3 (C-2), 69.2 (OCH\textsubscript{2}Ar), 71.7 (OCH\textsubscript{2}Ar), 72.4 (C-4), 77.0 (C-3), 98.2 (C-1), 127.6-128.5 (10 x Ar-H), 137.1 (ipso-Ar), 137.7 (ipso-Ar), 170.1 (OCOCH\textsubscript{3}).

\textit{m/z} = 409.1 [M + Na\textsuperscript{+}].

Benzyl 3-\textit{O}-benzyl-\textalpha-\texttextit{D}-rhamnopyranose (121)

To a solution of 120 (650 mg, 1.68 mmol) in anhydrous methanol (10 mL) under N\texttextit{2(g)} at 0°C was added Na\textsubscript{(aq)}. The reaction was stirred at 0°C 20 minutes before being allowed to warm to room temperature. The reaction was stirred at room temperature for a further 1 hour before being neutralised with glacial acetic acid (1 mL) and concentrated under reduced pressure from toluene (3 x 10 mL). The resulting residue was purified using column
chromatography (Hex/EtOAc 1:1, Rf = 0.6) yielding 121 (568 mg, 98%) as a white amorphous solid.

$^1$H NMR (CDCl₃) δ: 1.34 (d, 3 H, $J_{6,5} = 6.0$ Hz, CH₃), 2.29 (br. s, 1 H, 4-OH), 2.44 (br. s, 1 H, 2-OH), 3.60 (dd, 1 H, $J_{5,4} = J_{4,3} = 9.3$ Hz, H-4), 3.70-3.81 (m, 2 H, H-3, H-5), 4.08 (br. d, 1 H, $J_{2,1} = 1.5$ Hz, H-2), 4.62 (2 ABq, 4 H, $J = 11.7$ Hz, OCH₂Ph), 4.93 (d, 1 H, $J_{1,2} = 1.5$ Hz, H-1), 7.33-7.43 (m, 10 H, Ar-H).

$^{13}$C NMR (CDCl₃) δ: 17.6 (C-6), 67.8 (C-5), 67.9 (C-2), 69.0 (OCH₂Ar), 71.6 (C-4), 71.7 (OCH₂Ar), 79.9 (C-3), 98.5 (C-1), 127.8-128.7 (10 x Ar-H), 137.2, 137.6 (2 ipso-Ar).

$m/z$ = 367.1 [M + Na]⁺.

**Benzyl 3-O-benzyl-2,4-bis-trifluoromethanesulphonate-α-D-rhamnopyranose (122)**

To a solution of 121 (420 mg, 1.22 mmol) in CH₂Cl₂ (6 mL) under N₂(g) at -78°C was added anhydrous pyridine (592 µL, 7.32 mmol) and Tf₂O (615 µL, 3.66 mmol). The reaction was allowed to stir at -78°C for 10 minutes before being stirred for a further 1 hour. The resulting solution was diluted with CHCl₃ (20 mL) and washed with aqueous HCl (1 N, 20 mL) and water (20 mL). The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure and purified using column chromatography (5:1 Hex/EtOAc, Rf = 0.7) to yield 122 (668 mg, 90%), as a yellow oil.

$^1$H NMR (CDCl₃) δ: 1.38 (d, 3 H, $J_{6,5} = 6.3$ Hz, CH₃), 4.01 (dd, 1 H, $J_{5,4} = 9.6$ Hz, $J_{5,6} = 6.3$ Hz, H-5), 4.11 (dd, 1 H, $J_{3,4} = 9.6$ Hz, $J_{3,2} = 3.0$ Hz, H-3), 4.61 (2 ABq, 4 H, $J = 11.7$ Hz, OCH₂Ph), 4.72 (dd, 1 H, $J_{4,5} = J_{4,3} = 9.6$ Hz., H-4), 5.01 (d, 1 H, $J_{1,2} = 1.8$ Hz, H-1), 5.09 (dd, 1 H, $J_{2,3} = 3.0$ Hz, $J_{2,1} = 1.8$ Hz, H-2), 7.27-7.46 (m, 10 H, Ar-H).

$^{13}$C NMR (CDCl₃) δ: 17.2 (C-6), 66.3 (C-5), 70.4 (OCH₂Ar), 72.8 (OCH₂Ar), 73.3 (C-3), 81.5 (C-2), 84.4 (C-4), 95.1 (C-1), 120.0, 120.1 (2 x CF₃, $J = 317$ Hz), 128.0-128.7 (10 x Ar-H), 135.5 (2 ipso-Ar).

$m/z$ = 631.0 [M + Na]⁺
Benzyl 4-azido-3-O-benzyl-2,4-dideoxy-2,3-unsaturated-α-D-talopyranose (123)

To a stirred solution of 122 (600 mg, 0.98 mmol) in anhydrous N,N-DMF (10 mL) under N₂(g) at room temperature was added NaN₃ (625 mg, 9.61 mmol). The resulting reaction suspension was stirred at room temperature for 15 hours, after which it was concentrated under reduced pressure before being purified using column chromatography (Hex/EtOAc 10:1, Rᵣ = 0.5) yielding 123 (312 mg, 90%) as a white powder.

¹H NMR (CDCl₃) δ: 1.32 (d, 3 H, J₆,₅ = 6.3 Hz, CH₃), 3.43 (d, 1 H, J₄,₅ = 2.4 Hz, H-4), 4.35 (dd, 1 H, J₅,₄ = 2.4 Hz, J₅,₆ = 6.3 Hz, H-5), 4.62 (2 ABq, 4 H, J = 12.3 Hz, OCH₂Ph), 5.10 (d, 1 H, J₅,₁ = 3.3 Hz, H-2), 5.33 (d, 1 H, J₆,₂= 3.3 Hz, H-1), 7.33-7.43 (m, 10 H, Ar-H).

¹³C NMR (CDCl₃) δ: 16.6 (C-6), 59.2 (C-4), 65.9 (C-5), 69.5, 69.7 (2 OCH₂Ar), 94.7 (C-2), 97.6 (C-1), 127.5-128.5 (10 x Ar-H), 135.6, 137.9 (2 ipso-Ar), 153.7 (C-3).

HRMS: (C₂₀H₂₁N₃O₃Na): Calculated: 374.4806; found: 374.4801

IR = 2101 (4-N₃)
5.4.2 Synthesis of N-acetyl-glucosamine derivatives

1,3,4,6-tetra-O-Acetyl-N-acetyl-α-D-glucosamine (124)

D-Glucosamine hydrochloride (107) (2.0 g, 9.27 mmol) was dissolved in pyridine (20 mL) under N₂(g) and stirred for 5 minutes at 0°C, after which time acetic anhydride (10 mL) was added followed by DMAP (100mg). The reaction mixture was allowed to warm to room temperature and stirred for 15 hours. The resulting solution was concentrated to dryness, taken up in EtOAc (50 mL) and washed with cold aqueous HCl (1N, 20 mL) and water (20 mL). The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure yielding 124 (α-only) (2.5 g, 70%) as a white foam.

¹H NMR (CDCl₃) δ: 1.92, 2.02, 2.03, 2.07 (5 x OCOCH₃), 2.17 (NHCOC₂H₃), 3.96-4.03 (m, 1 H, H-5), 4.10 (dd, 1 H, J₆.₆₆ = 12.0 Hz, J₆.₅ = 6.9 Hz, H-6), 4.23 (dd, 1 H, J₆₆.₆ = 12.0 Hz, J₆₆₅ = 6.9 Hz, H-6a), 4.47 (ddd, 1 H, J₂.₃ = J₂NHAc = 9.0 Hz, J₂₁ = 3.6 Hz, H-2), 5.15-5.26 (m, 2 H, H-3, H-4), 5.74 (d, 1 H, J₂NHAc₂ = 9.0 Hz, NHAc), 6.14 (d, 1 H, J₁₂ = 3.6 Hz, H-1).

¹³C NMR (CDCl₃) δ: 20.5, 20.6, 20.6, 21.0 (4 x OCOCH₃), 22.9 (NHCOC₂H₃), 50.9 (C-2), 61.5 (C-6), 67.7 (C-4), 69.6 (C-5), 70.6 (C-3), 90.6 (C-1), 168.6-171.6 (5 x OCOCH₃).

m/z = 412.1 [M + Na]⁺.

Benzyl 3,4,6-tri-O-acetyl-N-acetyl-β-D-glucosamine (125)

To a stirred solution of 124 (6.0 g, 15.38 mmol) in 1,2-dichloroethane (30 mL) at room temperature under N₂(g) was added TMSOTf (3.1 mL, 16.92mmol). The reaction mixture was heated to 50°C and stirred for 24 hours, after which it was cooled to room temperature and 3Å molecular sieves were added and stirred for 30 minutes. Following this benzyl alcohol (5 mL, 46.15 mmol) was added and the reaction mixture was stirred for a further 24 hours. The resulting reaction mixture was adjusted to pH 9 with triethylamine and filtered through Celite using CHCl₃/MeOH (10:1) as an
eluent. The resulting filtrate was concentrated under reduced pressure and purified using column chromatography (EtOAc/MeOH 3:1, Rf = 0.3) to give 125 (3.5 g, 52%) as a white foam.

\[ ^1H \text{ NMR (CDCl}_3) \delta: 1.75, 1.91, 2.02, 2.11 (4 \times \text{OCOC}_3\text{H}_3), 3.68 (\text{ddd}, 1 \text{H}, J_{5,4} = 9.9 \text{ Hz}, J_{5,6} = 4.8 \text{ Hz}, J_{5,6} = 2.7 \text{ Hz}, \text{Hz}, \text{-}5), 3.98 (\text{ddd}, 1 \text{H}, J_{2,3} = 10.2 \text{ Hz}, J_{2,\text{NHAc}} = 8.7 \text{ Hz}, J_{2,1} = 8.4 \text{ Hz}, \text{-}2), 4.17 (\text{dd}, 1 \text{H}, J_{6a,6} = 12.3 \text{ Hz}, J_{6a,5} = 4.8 \text{ Hz}, \text{H-6a}), 4.28 (\text{dd}, 1 \text{H}, J_{6,6a} = 12.3 \text{ Hz}, J_{6,5} = 2.7 \text{ Hz}, \text{-}6), 4.63 (\text{d}, 1 \text{H}, J_{1,2} = 8.4 \text{ Hz}, \text{-}1), 4.75 (\text{AB}_q, 2 \text{H}, J = 12.0 \text{ Hz}, \text{OCH}_2\text{Ph}), 5.10 (\text{dd}, 1 \text{H}, J_{4,5} = 9.9 \text{ Hz}, J_{4,3} = 9.3 \text{ Hz}, \text{-}4), 5.22 (\text{dd}, 1 \text{H}, J_{3,2} = 10.2 \text{ Hz}, J_{3,4} = 9.3 \text{ Hz}, \text{-}3), 5.52 (\text{d}, 1 \text{H}, J_{\text{NHAc,2}} = 8.7 \text{ Hz}, \text{NHAc}), 7.29-7.38 (\text{m}, 5 \text{H}, \text{Ar-}H). \]

\[ ^{13}\text{C NMR (CDCl}_3) \delta: 20.6-20.7 (4 \times \text{OCOC}_3\text{H}_3), 23.2 (\text{NHCOC}_3\text{H}_3), 54.4 (\text{C}-2), 62.1 (\text{C}-6), 68.5 (\text{C}-4), 70.6 (\text{OCH}_2\text{Ar}), 71.8 (\text{C}-5), 72.4 (\text{C}-3), 99.4 (\text{C}-1), 127.5-128.7 (4 \times \text{Ar-}H), 136.8 (\text{ipso-}Ar), 169.4-170.9 (5 \times \text{OCOC}_3\text{H}_3). \]

\[ m/z = 460.1 [\text{M + Na}]^+. \]

**Benzyl N-acetyl-β-D-glucosamine (126)**

Aqueous NaOH (1N, 3mL) was added dropwise to a solution of 125 (1.0 g, 2.28 mmol) in methanol (15mL) at room temperature until adjusted to pH 12, the solution was then stirred for 15 hours. The resulting solution was neutralised to pH 7 by the dropwise addition of glacial acetic acid, concentrated under reduced pressure and purified using column chromatography (Hex/EtOAc 5:1, Rf = 0.3) to yield 126 (710 mg, 100%) as a white foam.

\[ ^1H \text{ NMR (MeOD}_4) \delta: 1.97 (\text{NHCOC}_3\text{H}_3), 3.25-3.38 (\text{br. m}, 2 \text{H}, \text{H-4}, \text{H-5}), 3.45 (\text{dd}, 1 \text{H}, J_{3,4} = 10.2 \text{ Hz}, J_{3,2} = 8.7 \text{ Hz}, \text{-}3), 3.69-3.77 (\text{br. m}, 2 \text{H}, \text{H-2}, \text{H-6}), 3.93 (\text{dd}, 1 \text{H}, J_{6a,6} = 12.0 \text{ Hz}, J_{6a,5} = 6.6 \text{ Hz}, \text{H-6a}), 4.47 (\text{d}, 1 \text{H}, J_{1,2} = 8.4 \text{ Hz}, \text{-}1), 4.75 (\text{AB}_q, 2 \text{H}, J =12.0 \text{ Hz}, \text{OCH}_2\text{Ph}), 7.25-7.36 (\text{m}, 5 \text{H}, \text{Ar-}H). \]

\[ ^{13}\text{C NMR (MeOD}_4) \delta: 22.9 (\text{NHCOC}_3\text{H}_3), 57.3 (\text{C}-2), 62.8 (\text{C}-6), 70.0 (\text{OCH}_2\text{Ph}), 71.5 (\text{C}-4), 75.9 (\text{C}-3), 78.0 (\text{C}-5), 101.8 (\text{C}-1), 128.6-129.4 (\text{Ar-}H), 139.2 (\text{ipso-}Ar), 173.7 (\text{NHCOC}_3\text{H}_3). \]

\[ m/z = 334.1 [\text{M + Na}]^+. \]
**Benzyl N-acetyl-4,6-benzylidene-β-D-glucosamine (129)**

Formic acid (10 mL, 26.52 mmol) was added to a solution of **126** (1.0 g, 3.21 mmol) in benzaldehyde (10 mL, 9.81 mmol) at room temperature under N\(_2\) (g). The reaction mixture was stirred for 24 hours before being diluted with EtOAc (100 mL) and washed with water (100 mL). The organic phase was dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure before being purified using column chromatography (EtOAc/Hex 2:1, R\(_f\) = 0.1) yielding **129** (1.14 g, 89%) as a clear amorphous solid.

\[^1\]H NMR (MeOD\(_4\)) δ: 2.07 (NHCOC\(_2\)H\(_3\)), 3.44 (ddd, 1 H, J\(_{5,6}\) = 4.8 Hz, J\(_{5,6a}\) = 9.0 Hz, J\(_{5,4}\) = 9.3 Hz, H-5), 3.57 (dd, 1 H, J\(_{3,4}\) = 9.3 Hz, J\(_{3,2}\) = 8.7 Hz, H-3), 3.74-3.79 (br. m, 3 H, H-2, H-6, H-6a), 4.34 (dd, 1 H, J\(_{4,3}\) = 9.3 Hz, J\(_{4,5}\) = 9.3 Hz, H-4), 4.60 (d, 1 H, J\(_{1,2}\) = 8.7 Hz, H-1), 4.75 (AB\(_q\), 2 H, J =12.0 Hz, OCH\(_2\)Ph), 7.27-7.52 (m, 5 H, Ar-H).

m/z = 422.1 [M + Na]+.

**Benzyl N-acetyl-4,6-O-isopropylidene-β-D-glucosamine (130)**

4Å molecular sieves were added to a solution of **126** (1.4 g, 4.48 mmol) in 2,2-dimethoxypropane (30 mL) and dried acetone (30 mL) at room temperature under N\(_2\) (g). The resulting reaction mixture was stirred for 10 minutes before the addition of p-toluenesulfonic acid (280 mg, 0.84 mmol) upon which the reaction was stirred for a further 30 minutes. After this time the reaction was neutralised with triethylamine, filtered to remove the sieves and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified using column chromatography (EtOAc/MeOH 100:2, R\(_f\) = 0.2) yielding **130** (1.47 g, 93%) as a white foam.

\[^1\]H NMR (CD\(_3\)CN) δ: 1.34, 1.47 (2 s, 6 H, C(CH\(_3\)\(_2\))\(_2\)), 1.86 (NHCOCH\(_3\)), 3.20 (ddd, 1 H, J\(_{5,6}\) = 5.4 Hz, J\(_{5,6a}\) = 8.6 Hz, J\(_{5,4}\) = 9.6 Hz, H-5), 3.48 (dd, 1 H, J\(_{4,3}\) = 9.3 Hz, J\(_{4,5}\) = 9.6 Hz, H-4), 3.55 (dd, 1 H, J\(_{3,4}\) = 9.3 Hz, J\(_{3,2}\) = 9.6 Hz, H-3), 3.68 (ddd, 1 H, J\(_{2,1}\) = 8.7 Hz, J\(_{2,3}\) = 9.6 Hz, J\(_{2,NHAc}\) = 8.7 Hz, H-2), 3.78 (dd, 1 H, J\(_{6,6a}\) = 10.2 Hz, J\(_{6a,5}\) = 8.6 Hz, H-6a), 3.84 (dd, 1 H, J\(_{6,6a}\) = 10.2 Hz, J\(_{6,5}\) = 5.4 Hz, H-6), 4.50 (d, 1 H, J\(_{1,2}\) = 8.7 Hz, H-1), 4.66 (AB\(_q\),
2 H, J =12.0 Hz, OCH$_2$Ph), 6.53 (d, 1 H, J$_{NHAc,2}$ = 8.7 Hz, NHAc), 7.29-7.35 (m, 5 H, Ar-H).

$^{13}$C NMR (CD$_3$CN) δ: 19.6 (NHCO$_2$CH$_3$), 23.6, 29.5 (C(CH$_3$)$_2$), 57.7 (C-2), 62.8 (C-6), 68.2 (C-4), 71.7 (OCH$_2$Ph), 73.2 (C-3), 74.9 (C-5), 99.0 (C(CH$_3$)$_2$), 102.1 (C-1), 128.8-129.5 (Ar-H), 137.1 (ipso-Ar), 170.1 (NHCOCH$_3$).

$m/z = 374.1$ [M + Na]$^+$

**Benzyl N-acetyl-3-O-tert-butyldimethylsilyl-4,6-O-isopropylidene-β-D-glucosamine (131)**

To a stirred solution of 130 (1.9 g, 5.41 mmol) in pyridine (25 mL) at room temperature under $N_2$(g) was added tert-butyldimethylsilyl chloride (2.1 g, 13.53 mmol). The resulting clear reaction mixture was stirred at room temperature for 48 hours before being concentrated under reduced pressure and purified using column chromatography (EtOAc, R$_f$ = 0.6), yielding 131 (2.0 g, 80%) as an opaque foam.

$^1$H NMR (CDCl$_3$) δ: -0.02, -0.04 (2 s, 6 H, Si(CH$_3$)$_2$)$_2$, 0.81 (s, 9 H, SiC(CH$_3$)$_3$), 1.35, 1.48 (2 s, 6 H, C(CH$_3$)$_2$), 1.86 (NHCOCH$_3$), 3.20-3.36 (m, 2 H, H-2, H-5), 3.42 (dd, 1 H, J$_{4,5}$ = J$_{4,3}$ = 8.4 Hz, H-4), 3.73 (dd, 1 H, J$_{6a,5}$ = 6.5 Hz, J$_{6a,6}$ = 10.8 Hz, H-6a), 3.84 (dd, 1 H, J$_{6,5}$ = 6.5 Hz, J$_{6,6a}$ = 10.8 Hz, H-6), 4.07 (dd, 1 H, J$_{3,2}$ = 8.7 Hz, J$_{3,4}$ = 8.4 Hz, H-3), 4.66 (AB$_q$, 2 H, J = 12.0 Hz, OCH$_2$Ph), 4.85 (d, 1 H, J$_{1,2}$ = 8.8 Hz, H-1), 6.54 (d, 1 H, J$_{NHAc,2}$ = 8.7 Hz, NHAc), 7.29-7.35 (m, 5 H, Ar-H).

$^{13}$C NMR (CDCl$_3$) δ: -5.0, -4.1 (Si(CH$_3$)$_2$)$_2$, 18.1 SiC(CH$_3$)$_3$, 18.8 (NHCOCH$_3$), 23.6, 28.9 (C(CH$_3$)$_2$), 25.7 (SiC(CH$_3$)$_3$), 59.2 (C-2), 62.2 (C-6), 66.8 (C-4), 70.1 (OCH$_2$Ph), 71.6 (C-3), 74.1 (C-5), 99.2 (C(CH$_3$)$_2$), 99.6 (C-1), 127.8-128.5 (Ar-H), 137.3 (ipso-Ar), 170.0 (NHCOCH$_3$).

$m/z = 488.1$ [M + Na]$^+$
**Benzyl N-acetyl-3-O-tert-butyldimethylsilyl-β-D-glucosamine (132)**

Compound 131 (1.50 g, 3.22 mmol) was stirred in an aqueous 80% acetic acid solution (20 mL total) at 100°C for 1 hour, after which the reaction was cooled to room temperature and concentrated under reduced pressure from toluene (3 x 20 mL). The resulting yellow residue was purified using column chromatography (EtOAc, R_f = 0.5) yielding 132 (1.34 g, 98%) as a clear oil.

{eq}{^1}H\ NMR\ (\text{MeOD}_4)\:\delta:\ -0.22,\ -0.44\ (2\ s,\ 6\ H,\ Si(CH_3)_2),\ 0.82\ (s,\ 9\ H,\ SiC(CH_3)_3),\ 1.84\ (NHCOC(CH_3)_2),\ 3.18-3.25\ (m,\ 2\ H,\ H-4,\ H-5),\ 3.55\ (dd,\ 1\ H,\ J_{6,6a} = 12.0\ Hz,\ J_{6,5} = 5.7\ Hz,\ H-6),\ 3.62\ (dd,\ 1\ H,\ J_{2,3} = 8.5\ Hz,\ J_{2,1} = 8.7\ Hz,\ H-2),\ 3.51\ (dd,\ 1\ H,\ J_{6a,6} = 12.0\ Hz,\ J_{6a,5} = 5.7\ Hz,\ H-6a),\ 3.84\ (dd,\ 1\ H,\ J_{3,2} = 8.5\ Hz,\ J_{3,4} = 9.5\ Hz,\ H-3),\ 4.40\ (d,\ 1\ H,\ J_{1,2} = 8.7\ Hz,\ H-1),\ 4.69\ (ABq,\ 2\ H,\ J = 12.0\ Hz,\ OCH_2Ph),\ 7.31-7.44\ (m,\ 5\ H,\ Ar-H).

{eq}{^{13}}C\ NMR\ (\text{MeOD}_4)\:\delta:\ -4.6,\ -3.6\ (Si(CH_3)_2),\ 19.0\ SiC(CH_3)_3),\ 19.1\ (NHCOC(CH_3)_2),\ 26.2\ (SiC(CH_3)_3),\ 57.8\ (C-2),\ 62.9\ (C-6),\ 71.4\ (OCH_2Ph),\ 72.6\ (C-4),\ 77.0\ (C-3),\ 78.0\ (C-5),\ 101.5\ (C-1),\ 128.7-129.3\ (Ar-H),\ 139.1\ (ipso-Ar),\ 173.1\ (NHCOC(CH_3)).

m/z = 448.1 [M + Na]^+

**Benzyl N-acetyl-3-O-tert-butyldimethylsilyl-6-deoxy-6-ido-β-D-glucosamine (133)**

To a stirred solution of 132 (150 mg, 0.35 mmol) in anhydrous toluene (10 mL) at room temperature under N_2(g) was added imidazole (70 mg, 1.02 mmol), triphenylphosphine (130 mg, 0.51 mmol) and iodine (100 mg, 0.40 mmol). The resulting reaction mixture was stirred at reflux for 15 minutes before being allowed to cool to room temperature and concentrated under reduced pressure. The crude reaction mixture was taken up in EtOAc (100 mL), washed with aqueous Na_2S_2O_3 (1 N, 50 mL) and water (50 mL), the organic phase was dried (Na_2SO_4) and concentrated under reduced pressure before being purified using column chromatography (Hex/EtOAc 1:1, R_f = 0.5) yielding 133 (170 mg, 90 %) as a clear oil.

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1H NMR (CDCl₃) δ: -0.02, -0.04 (2 s, 6 H, Si(CH₃)₂), 0.81 (s, 9 H, SiC(CH₃)₃), 1.85 (NHCOCH₃), 3.14-3.27 (br. m, 4 H, H-2, H-4, H-6, H-6a), 3.84 (dd, 1 H, J₃,₂= 8.7 Hz, J₃,₄ = 9.3 Hz, H-3), 3.97 (dd, 1 H, J₅,₄ = 7.8 Hz, J₅,₆ = 9.9 Hz, H-5), 4.71 (ABq, 2 H, J = 12.0 Hz, OCH₂Ph), 4.42 (d, 1 H, J₁,₂ = 8.7 Hz, H-1), 5.39 (d, 1 H, J₂,₃ = 8.1 Hz, NHAc), 7.21-7.27 (m, 5 H, Ar-H).

13C NMR (CDCl₃) δ: -4.4, -4.2 (Si(CH₃)₂), 6.3 (C-6), 18.2 SiC(CH₃)₃, 23.8 (NHCOCH₃), 25.7 (SiC(CH₃)₃), 58.7 (C-2), 70.1 (OCH₂Ph), 74.1 (C-4), 74.2 (C-3), 76.0 (C-5), 98.2 (C-1), 127.9-128.8 (Ar-H), 137.3 (ipso-Ar), 170.3 (NHCOCH₃).

m/z = 558.1 [M + Na]⁺

Benzyl N-acetyl-3-O-tert-butylimethylsilyl-6-deoxy-β-D-glucosamine (134)

To a solution of 133 (100 mg, 0.18 mmol) in anhydrous methanol (10 mL) was added diisopropylethylamine (0.5 mL) and Palladium Hydroxide (on Carbon 20% wt.) (50 mg, 0.35 mmol). The resulting reaction mixture was shaken on a Parr Hydrogenator at room temperature under an atmosphere of H₂(g), at 40 psi for 15 hours. The resulting reaction mixture was filtered through Celite to remove the palladium catalyst and the filtrate was concentrated under reduced pressure before being purified using column chromatography (Hex/EtOAc 1:1, Rf = 0.3) yielding 134 (75 mg, 100%) as a white amorphous solid.

1H NMR (CDCl₃) δ: -0.03, -0.01 (2 s, 6 H, Si(CH₃)₂), 0.88 (s, 9 H, SiC(CH₃)₃), 1.30 (d, 3 H, J₆,₅ = 6.3 Hz, H-6), 1.89 (NHCOCH₃), 3.04 (dd, 1 H, J₄,₃ = J₄,₅ = 8.4 Hz, H-4), 3.25 (dd, 1 H, J₃,₄ = 8.4 Hz, J₃,₅ = 6.3 Hz, H-5), 3.54 (dd, 1 H, J₂,₃ = 9.9 Hz, J₂,₄ = 8.4 Hz, H-3), 3.67 (ddd, 1 H, J₂,₃ = 9.9 Hz, J₂,₁ = J₂,NHAc = 8.4 Hz, H-2), 4.44 (d, 1 H, J₁,₂ = 8.4 Hz, H-1), 4.67 (ABq, 2 H, J = 12.0 Hz, OCH₂Ph), 5.29 (d, 1 H, J₂,NHAc = 8.4 Hz, NHAc), 7.25-7.29 (m, 5 H, Ar-H).

13C NMR (CDCl₃) δ: -3.8, -4.7 (Si(CH₃)₂), 17.9 (C-6), 18.6 SiC(CH₃)₃, 23.2 (NHCOCH₃), 26.1 (SiC(CH₃)₃), 57.6 (C-2), 71.1 (OCH₂Ph), 72.6 (C-4), 75.9 (C-3), 77.8 (C-5), 100.6 (C-1), 128.2-128.9 (Ar-H), 138.3(ipso-Ar), 172.4 (NHCOCH₃).

m/z = 432.1 [M + Na]⁺
**N-acetyl-6-deoxy-glucosamine (135)**

To a stirred solution of 134 (190 mg, 0.46 mmol) in water (15 mL) was added Dowex 50 H+ acidic resin (400 mg), the resulting reaction mixture was stirred at 80°C for 24 hours. The reaction was allowed to cool and then filtered to remove the acidic resin before being concentrated under reduced pressure and purified using column chromatography (10:3 CHCl₃/MeOH, Rₓ = 0.4) yielding 135 (94 mg, 100%) as a fine white amorphous powder.

\(^{1}\)H NMR (D₂O) (α-anomer) δ: 1.31 (d, 3 H, J₆,₅ = 6.3 Hz, H-6), 2.05 (NHCOCH₃), 3.23 (dd, 1 H, J₄,₃ = J₄,₅ = 7.8 Hz, H-4), 3.50 (dd, 1 H, J₅,₄ = 7.8 Hz, J₅,₆ = 6.3 Hz, H-5), 3.71 (dd, 1 H, J₃,₂ = 9.9 Hz, J₃,₄ = 7.8 Hz, H-3), 3.88 (ddd, 1 H, J₂,₃ = 9.9 Hz, J₂,₁ = 3.6 Hz, J₂,NHAc = 8.4 Hz, H-2), 5.15 (d, 1 H, J₁,₂ = 3.6 Hz, H-1).

\(^{13}\)C NMR (CDCl₃) δ: 16.7 (C-6), 21.8 (NHCOCH₃), 54.2 (C-2), 70.3 (C-3), 71.9 (C-5), 75.6 (C-4), 90.6 (C-1), 174.7 (NHCOCH₃).

\(^{1}\)H NMR (D₂O) (β-anomer) δ: 1.28 (d, 3 H, J₆,₅ = 6.3 Hz, H-6), 2.05 (NHCOCH₃), 3.23 (dd, 1 H, J₄,₃ = J₄,₅ = 7.8 Hz, H-4), 3.50 (dd, 1 H, J₃,₂ = 9.9 Hz, J₃,₄ = 7.8 Hz, H-3), 3.67 (ddd, 1 H, J₂,₃ = 9.9 Hz, J₂,₁ = J₂,NHAc = 8.4 Hz, H-2), 3.93 (dd, 1 H, J₅,₄ = 7.8 Hz, J₅,₆ = 6.3 Hz, H-5), 4.69 (d, 1 H, J₁,₂ = 8.4 Hz, H-1).

\(^{13}\)C NMR (CDCl₃) δ: 16.6 (C-6), 22.1 (NHCOCH₃), 56.8 (C-2), 67.5 (C-5), 73.6 (C-3), 75.0 (C-4), 94.7 (C-1), 174.4 (NHCOCH₃).

HRMS: (C₉H₁₅NO₂Na): Calculated: 228.08479; found: 228.08475
5.5 Synthetic Aldol condensation reaction outcomes

5.5.1 General procedures

**Method 1. Synthetic aldol condensation reactions**

The hexose substrate was dissolved in water, followed by the addition of base catalyst (e.g. Na$_2$CO$_3$), then oxaloacetic acid (27) (2 eq. of substrate concentration) was added portion wise, following this the pH was adjusted to 11 with the use of aqueous NaOH (10 M). After stirring for 2-4 hours at room temperature, the reaction mixture was acidified to pH 2 using acid resin (Amberlite IR 120H$^+$) heated to 60ºC and stirred for a further 1 hour. The reaction mixture was allowed to cool to room temperature before being neutralised with concentrated ammonia and then concentrated under reduced pressure. The resulting residue was purified using an Amberlite GC-400 ion-exchange column (HCO$_3^-$) initially eluting with water (~200 mL) followed by NH$_4$HCO$_3$ (0.5 M, 500 mL) to elute the acidic fraction containing the desired nonulosonic acid.

**Method 2. Synthetic aldol condensation reactions**

Starting with a cooled aqueous solution (0ºC) of oxaloacetic acid (27) (1.6 eq. of substrate concentration) adjusted to pH 11 with NaOH (10 M), to this an ice cold aqueous solution of the hexose substrate was then added. The resulting reaction mixture was then stirred for 15 hours allowing it to warm to room temperature. After this, the pH was tested and adjusted to pH 11 again if needed before being heated to 60ºC and stirred for a further 24 hours. The reaction was acidified (pH 6) with acidic resin (Dowex 50 H$^+$ resin) (note: refined Method 2 involves adding trace amount of Ni$^{2+}$ once acidified) before stirring for a further hour. After cooling to room temperature, it was filtered to remove the resin, the pH was adjusted to 7 (if needed) and then the filtrate was concentrated under reduced pressure. The residue was columned on an anion exchange column (HCO$_3^-$), initially eluting with water (200 mL) then aqueous NH$_4$HCO$_3$ (0.5 M).
3-Deoxy-D-glycero-D-galacto-non-2-ulopyranosidate (KDN) (3)

KDN (3) was prepared from D-mannose (37) following Method 1 in a 60% yield.

\[
{^1}H \text{ NMR (D}_2\text{O)} \delta: 1.67 (\text{dd, 1 H, } J_{3ax,3eq} = 12.9 \text{ Hz, } J_{3ax,4} = 12.0 \text{ Hz, } H_{-3ax}), 2.07 (\text{dd, 1 H, } J_{3eq,3ax} = 12.9 \text{ Hz, } J_{3eq,4} = 4.8 \text{ Hz, } H_{-3eq}), 3.48 (\text{dd, 1 H, } J_{5,4} = J_{5,6} = 9.6 \text{ Hz, } H_{-5}), 3.53-3.59 (\text{m, 2 H, } H_{-8}, H_{-9}), 3.67 (\text{dd, 1 H, } J_{9,9} = 11.8 \text{ Hz, } J_{9,8} = 2.7 \text{ Hz, H-9}), 3.75 (\text{dd, 1 H, } J_{7,8} = 7.5 \text{ Hz, } J_{7,6} = 1.2 \text{ Hz, H-7}), 3.82 (\text{dd, 1 H, } J_{6,5} = 9.9 \text{ Hz, } J_{6,7} = 1.2 \text{ Hz, H-6}), 3.87 (\text{dd, 1 H, } J_{4,3ax} = 12.0 \text{ Hz, } J_{4,5} = 9.6 \text{ Hz, } J_{4,3eq} = 4.8 \text{ Hz, H-4}). \\
m/z = 291.2 [M + Na]^{+}.
\]

3,9-Dideoxy-9-methyl-D-glycero-D-galacto-non-2-ulopyranosidate (89)

9-deoxy-KDN (89) was prepared from D-rhamnose (35) following Method 1 in a 50% yield.

\[
{^1}H \text{ NMR (D}_2\text{O)} \delta: 1.06 (\text{d, 3 H, } J_{9,8} = 6.9 \text{ Hz, H-9}), 1.65 (\text{dd, 1 H, } J_{3ax,3eq} = 12.9 \text{ Hz, } J_{3ax,4} = 11.4 \text{ Hz, } H_{-3ax}), 2.05 (\text{dd, 1 H, } J_{3eq,3ax} = 12.9 \text{ Hz, } J_{3eq,4} = 4.8 \text{ Hz, } H_{-3eq}), 3.44 (\text{dd, 1 H, } J_{5,4} = J_{5,6} = 9.9 \text{ Hz, H-5}), 3.52 (\text{dd, 1 H, } J_{8,7} = 8.7 \text{ Hz, } J_{8,9} = 6.9 \text{ Hz, H-8}), 3.70 (\text{br. d, 1 H, } J_{7,8} = 8.7 \text{ Hz, H-7}), 3.75-3.84 (\text{m, 2 H, } H_{-4/H-6}). \\
m/z = 275.2 [M + Na]^{+}.
\]
5.6 Synthesis of Pseudaminic acid analogues from KDN

Methyl (methyl 3-deoxy-4,5,7,8,9-penta-O-acetyl D-glycero-β-D-galacto-non-2-ulopyranosid)onate (142)

To a stirred solution of KDN (3) (5.0 g, 18.65 mmol) in anhydrous methanol (200 mL) at room temperature under N₂(g) was added Dowex 50 H⁺ resin (5.0 g). The resulting suspension was stirred at room temperature for 48 hours before being filtered to remove the resin and the filtrate concentrated under reduced pressure producing (141) (5.15 g, 98%) as a white foam without purification.

Compound 141 (5.15 g, 18.26 mmol) was taken up in anhydrous methanol (200 mL) at room temperature under N₂(g) to which Dowex 50 H⁺ resin (10.3 g) was added. The resulting suspension was heated to 70°C and stirred for 15 hours before being cooled and filtered to remove the resin. The filtrate was concentrated and placed on a high vac line to remove any traces of methanol. The resulting clear crude residue was taken up in pyridine (50 mL) to which acetic anhydride (20 mL) was added and the resulting reaction mixture was stirred at room temperature under N₂(g) for 15 hours. The resulting reaction mixture was concentrated to dryness before being taken up in EtOAc (100 mL), washed with aqueous HCl (1 N, 100 mL) and water (100 mL). The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure. The crude reaction residue was purified using column chromatography yielding 142 (6.47 g, 70%), which was recrystallised from hot EtOAc and Hexane to yield the solely the β-methyl-glycoside.

Assignments of 141 and 142 are in agreement with those published by Nakamura et al. 1992₁⁸⁹.
1H NMR (CDCl$_3$) $\delta$: 1.84 (dd, 1 H, $J_{3ax,3eq} = 12.9$ Hz, $J_{3ax,4} = 12.0$ Hz, H-3ax), 2.00, 2.03, 2.04, 2.09, 2.13 (s, 3 H, OCOC$_3$H$_3$), 2.58 (dd, 1 H, $J_{3eq,3ax} = 12.9$ Hz, $J_{3eq,4} = 5.1$ Hz, H-3eq), 3.26 (s, 3 H, OCH$_3$), 3.82 (s, 3 H, CO$_2$CH$_3$), 4.07 (dd, 1 H, $J_{6,5} = 10.2$ Hz, $J_{6,7} = 2.1$ Hz, H-6), 4.16 (dd, 1 H, $J_{9',9} = 12.6$ Hz, $J_{9',8} = 2.4$ Hz, H-9'), 4.72 (dd, 1 H, $J_{9,9'} = 12.6$ Hz, $J_{9,8} = 2.4$ Hz, H-9), 4.92 (dd, 1 H, $J_{5,6} = 9.9$ Hz, $J_{5,4} = 9.6$ Hz, H-5), 5.28-5.37 (m, 2 H, H-4, H-8), 5.43 (dd, 1 H, $J_{7,8} = 5.4$ Hz, $J_{7,6} = 2.1$ Hz, H-7).

$^{13}$C NMR (CDCl$_3$) $\delta$: 20.6, 20.7, 20.8, 20.9, 21.0 (CO$_3$C$_3$H$_3$), 36.8 (C-3), 51.3 (OCH$_3$), 52.8 (CO$_2$CH$_3$), 62.0 (C-9), 67.3 (C-7), 67.7 (C-5), 69.0 (C-8), 69.9 (C-6), 70.7 (C-4), 98.7 (C-2), 167.7 (C-1), 169.9, 170.0, 170.1, 170.2, 170.6 (OCH$_3$).

$m/z = 529.1$ [M + Na]$^+$.  

mp. 117-120°C (lit. value 117-118°C) 189

Methyl (methyl 3-deoxy-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (140)

To a solution of 142 (5.92 g, 11.7 mmol) in anhydrous methanol (30 mL) under N$_2$(g) at 0°C was added Na(s).

The reaction was stirred at 0°C 20 minutes before being allowed to warm to room temperature. The reaction was stirred at room temperature for a further 1 hour before being neutralised with glacial acetic acid (2 mL) and concentrated under reduced pressure from toluene (3 x 10 mL). The resulting residue of 140 (3.31 g, 99%) was characterised by $^1$H NMR spectroscopy and used in the flowing reaction without purification.

$^1$H NMR (D$_2$O) $\delta$: 1.65 (1H, dd, $J_{3ax,3eq} = 13.5$ Hz, $J_{3ax,4} = 11.5$ Hz, H-3ax), 2.26 (1H, dd, $J_{3eq,3ax} = 13.5$ Hz, $J_{3eq,4} = 5.2$ Hz, H-3eq), 3.19 (3H, s, OCH$_3$), 3.49 (1H, dd, $J_{5,6} = 9.0$ Hz, $J_{5,8} = 10.0$ Hz, H-5), 3.63 (1H, dd, $J_{9,8} = 6.6$ Hz, $J_{9,9'} = 12.3$ Hz, H-9), 3.73 (1H, dd, $J_{6,5} = 10.0$ Hz, $J_{6,7} = 0.8$ Hz, H-6), 3.77 (3H, s, COOCH$_3$), 3.79 (1H, dd, $J_{7,6} = 0.8$ Hz, $J_{7,8} = 5.4$ Hz, H-7), 3.89 (1H, dd, $J_{4,3ax} = 11.5$ Hz, $J_{4,3eq} = 5.2$ Hz, $J_{4,5} = 9.0$ Hz, H-4).
Methyl (methyl 3-deoxy-8,9-\textit{O}-isopropylidene-D-glycero-\textbeta-D-galacto-non-2-ulopyranosid)onate (143) and Methyl (methyl 3-deoxy-4,5-8,9-\textit{O}-di-isopropylidene-D-glycero-\textbeta-D-galacto-non-2-ulopyranosid)onate (144)

2,2-Dimethoxypropane (0.33 mL, 2.68 mmol) and \textit{p}-toluenesulphonic acid monohydrate (73 mg, 0.38 mmol) were added to a stirred solution of (140) (570 mg, 1.92 mmol) in dry acetone (10 mL) under \textit{N}_2(g) at room temperature. The resulting solution was kept at room temperature for 30 minutes before being neutralised with triethylamine and concentrated under reduced pressure. The resulting yellow residue was purified using column chromatography (EtOAc, \textit{R}_f (143) = 0.25, \textit{R}_f (144) = 0.85) yielding 143 (577mg, 90\%) and a small amount of 144 (<10\%) as a white foam.

Spectroscopic data for 143 is in agreement with that published by David \textit{et al.} 1994\textsuperscript{213}.

$^1$H NMR (CDCl$_3$) (143) δ: 1.31, 1.39 (s, 3 H, C(CH$_3$)$_2$), 1.58 (dd, 1 H, $J_{3ax,3eq} = 12.9$ Hz, $J_{3ax,4} = 12.0$ Hz, H-3\textit{ax}), 2.29 (dd, 1 H, $J_{3eq,3ax} = 12.9$ Hz, $J_{3eq,4} = 5.1$ Hz, H-3\textit{eq}), 3.42 (s, 3 H, OCH$_3$), 3.49 (dd, 1 H, $J_{5,6} = 9.6$ Hz, $J_{5,4} = 9.0$ Hz, H-5), 3.67 (dd, 1 H, $J_{6,5} = 9.6$ Hz, $J_{6,7} = 1.2$ Hz, H-6), 3.82 (dd, 1 H, $J_{7,8} = 8.7$ Hz, $J_{7,6} = 1.2$ Hz, H-7), 3.84 (s, 3 H, CO$_2$CH$_3$), 3.89 (ddd, 1 H, $J_{4,3ax} = 12.0$ Hz, $J_{4,5} = 9.0$ Hz, $J_{4,3eq} = 5.1$ Hz, H-4), 3.99 (dd, 1 H, $J_{9,9'} = 8.4$ Hz, $J_{9,8} = 5.7$ Hz, H-9), 4.14 (dd, 1 H, $J_{9',9} = 8.4$ Hz, $J_{9',8} = 5.7$ Hz, H-9), 4.24 (dd, 1 H, $J_{8,7} = 8.4$ Hz, $J_{8,9} = 5.7$ Hz, H-8).

$^{13}$C NMR (CDCl$_3$) δ: 25.7, 27.2 (C(CH$_3$)$_2$), 40.9 (C-3), 51.5 (OCH$_3$), 53.4 (CO$_2$CH$_3$), 68.8 (C-9), 70.1 (C-4), 70.9 (C-7), 71.2 (C-6), 73.7 (C-5), 76.0 (C-8), 100.5 (C-2), 110.4 (C(CH$_3$)$_2$), 171.6 (CO$_2$CH$_3$).

$m/z = 359.1$ [M + Na]$^+$. 

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1H NMR (CDCl₃) (144) δ: 1.32, 1.39, 1.40, 1.44 (4 s, 12 H, 2 C(CH₃)₂), 1.87 (dd, 1 H, J₃ax,3eq = J₃ax,4 = 12.0 Hz, H-3ax), 2.55 (dd, 1 H, J₃eq,3ax = 12.0 Hz, J₃eq,4 = 4.5 Hz, H-3eq), 3.28 (s, 3 H, OCH₃), 3.45 (dd, 1 H, J₅,6 = 9.9 Hz, J₅,4 = 9.0 Hz, H-5), 3.78 (dd, 1 H, J₉,9' = 8.4 Hz, J₉,8 = 5.7 Hz), 3.80 (s, 3 H, CO₂C(CH₃)₃), 3.92 (ddd, 1 H, J₄,3ax = 12.0 Hz, J₄,5 = 9.0 Hz, J₄,3eq = 4.5 Hz, H-4), 3.99 (dd, 1 H, J₉,9' = 8.4 Hz, J₉,8 = 5.7 Hz, H-9), 4.01 (dd, 1 H, J₆,5 = 9.9 Hz, J₆,7 = 1.5 Hz, H-6), 4.11-4.20 (m, 2 H, H-7, H-8).

Methyl (methyl 4-O-(tert-butyldimethylsilyl)-3-deoxy-8,9-O-isopropylidene-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (137)

Imidazole (328 mg, 4.82 mmol) and TBDMSCI (363 mg, 2.41 mmol) were added to a stirred solution of 143 (577 mg, 1.72 mmol) in anhydrous DMF (10 mL) under N₂(g) at room temperature. The resulting reaction mixture was stirred for 16 hours at room temperature before being concentrated under reduced pressure and purified directly using column chromatography (EtOAc, Rₕ = 0.8) yielding 137 (605 mg, 74% and 100% based on recovered 143) as a white amorphous solid. Spectroscopic data for 137 is in agreement with that published by David et al. 1994²¹³.

1H NMR (CDCl₃) δ: 0.10, 0.12 (2 s, 6 H, Si(CH₃)₂), 0.89 (s, 9 H, SiC(CH₃)₃), 1.31, 1.41 (s, 3 H, C(CH₃)₂), 1.66 (dd, 1 H, J₃ax,3eq = 12.9 Hz, J₃ax,4 = 12.0 Hz, H-3ax), 2.30 (dd, 1 H, J₃eq,3ax = 12.9 Hz, J₃eq,4 = 5.4 Hz, H-3eq), 2.65 (d, 1 H, J₀H,5 = 3.3 Hz, OH), 3.00 (br. s, 1 H, 7-OH), 3.25 (s, 3 H, OCH₃), 3.60 (dd, 1 H, J₅,6 = 9.6 Hz, J₅,4 = 9.0 Hz, J₅,OH = 3.3 Hz H-5), 3.76 (dd, 1 H, J₆,5 = 9.6 Hz, J₆,7 = 1.2 Hz, H-6), 3.80 (s, 3 H, CO₂C(CH₃)₃), 3.89 (dd, 1 H, J₇,8 = 8.7 Hz, J₇,6 = 1.2 Hz, H-7), 3.97-4.09 (m, 2 H, H-4, H-9'), 4.11-4.21 (m, 2 H, H-8, H-9).
**13C NMR** (CDCl₃) δ: -4.5 (Si(CH₃)₂), 17.9 (Si(C(CH₃)₃)), 25.4 (C(CH₃)₂), 25.6 (Si(CH₃)₂), 26.8 (C(CH₃)₂), 40.4 (C-3), 50.9 (OCH₃), 52.7 (CO₂CH₃), 67.7 (C-9), 70.2 (C-7), 70.3 (C-4), 70.6 (C-5), 71.8 (C-6), 74.8 (C-8), 99.2 (C-2), 109.2 (C(CH₃)₂), 169.2 (CO₂CH₃). m/z = 473.0 [M + Na]+.

**Methyl (methyl 5,7-di-O-acetyl-4-O-(tert-butyldimethylsilyl)-3-deoxy-8,9-O-isopropylidene-D-glycero-ß-D-galacto-non-2-ulopyranosid)onate (145).**

A small portion (~100 mg) of 137 was acetylated (Ac₂O/pyridine) overnight. The residue was purified using column chromatography (EtOAc/Hex, 1:4, Rf = 0.35) yielding 145 as an amorphous solid.

**1H NMR** (CDCl₃) δ: 0.03, 0.05 (2 s, 6 H, Si(C(H₃)₃)₂), 0.85 (s, 9 H, SiC(CH₃)₃), 1.31, 1.37 (2 s, 6 H, C(CH₃)₂), 1.80 (dd, 1 H, J₃ax,3eq = 12.9 Hz, J₃ax,4 = 11.1 Hz, H-3ax), 2.02, 2.10 (2 s, 6 H, COC(H₃)₂), 2.20 (dd, 1 H, J₃eq,3ax = 12.9 Hz, J₃eq,4 = 5.4 Hz, H-3eq), 3.26 (s, 3 H, OCH₃), 3.81 (s, 3 H, CO₂CH₃), 3.89–3.96 (m, 2 H, H-6, H-9), 4.01–4.09 (m, 2 H, H-4, H-9'), 4.36 (ddd, 1 H, J₈,7 = 8.7 Hz, J₈,9 = J₈,9' = 5.7 Hz, H-8), 4.89 (dd, 1 H, J₅,6 = J₅,4 = 9.6 Hz, H-5), 5.24 (dd, 1 H, J₇,8 = 6.6 Hz, J₇,6 = 2.1 Hz, H-7).

**Methyl (methyl 4-O-(tert-butyldimethylsilyl)-3,5,7-tri-deoxy-8,9-O-isopropylidene-5,7-O-di-(trifluoromethanesufonyl)-D-glycero-ß-D-galacto-non-2-ulopyranosid)onate (146).**

To a stirred solution of 137 (605 mg, 1.27 mmol) in anhydrous CH₂Cl₂ (10 mL) under N₂(g) at -78°C was added pyridine (0.616 mL, 7.62 mmol) and trifluoromethanesulphonic anhydride (0.64 mL, 3.81 mmol). The resulting reaction mixture was stirred at -78°C for 10 minutes, and then kept at 0°C for 1 hour, before being allowed to warm to room temperature for 5 hours. The reaction mixture was diluted with fresh CH₂Cl₂ (10 mL), washed with aqueous 1 M HCl (20 mL) and water (20 mL), the organic phase was dried (Na₂SO₄) and concentrated under reduced pressure before being purified using column chromatography (Hex/EtOAc (5:1), Rf = 0.4) yielding 146 (785 mg, 86%) as a yellow oil. 146 was used directly in the following azide displacement reaction:
Sodium Azide (5 mol. eq. of 137) was added to a stirred solution of 146 (785 mg, 1.1 mmol) in anhydrous DMF (20 mL) under argon\((g)\) at 0°C. The resulting reaction mixture was stirred at 0°C for 24 hours before being concentrated under reduced pressure and columned directly (Hex/EtOAc (5:1), \(R_f = 0.45\)) yielding 138 (610 mg, 93%) as an opaque amorphous solid.

\(^1\)H NMR (CDCl\(_3\)) \(\delta\): 0.10, 0.12 (2 s, 6 H, Si(CH\(_3\))\(_2\)), 0.89 (s, 9 H, SiC(CH\(_3\))\(_3\)), 1.31, 1.45 (s, 3 H, C(CH\(_3\))\(_2\)), 1.99 (m, 2 H, H-3\_ax/H-3\_eq), 3.17 (s, 3 H, OCH\(_3\)), 3.57 (dd, 1 H, \(J_{7,6} = 9.9\) Hz, \(J_{7,8} = 4.5\) Hz, H-7), 3.68 (dd, 1 H, \(J_{6,5} = 1.5\) Hz, \(J_{6,7} = 9.9\) Hz, H-6), 3.78 (s, 3 H, CO\(_2\)C\(_2\)H\(_3\)), 4.83 (dd, 1 H, \(J_{5,4} = 4.8\) Hz, \(J_{5,6} = 1.5\) Hz, H-5), 3.96 (dd, 1 H, \(J_{9',8} = 6.9\) Hz, \(J_{9,9'} = 8.7\) Hz, H-9'), 4.11 (ddd, 1 H, \(J_{9,8} = 6.9\) Hz, \(J_{9,9'} = 8.7\) Hz, H-9), 4.30 (ddd, 1 H, \(J_{4,3ax} = 9.3\) Hz, \(J_{4,3eq} = 5.1\) Hz, \(J_{4,5} = 4.8\) Hz, H-4), 4.40 (ddd, 1 H, \(J_{8,7} = 4.5\) Hz, \(J_{8,9} = 6.9\) Hz, \(J_{8,9'} = 7.2\) Hz, H-8).

\(^13\)C NMR (CDCl\(_3\)) \(\delta\): -4.6 Si(CH\(_3\))\(_2\)), 17.9 (SiC(CH\(_3\))\(_3\)), 25.2 (C(CH\(_3\))\(_2\)), 25.5 (SiC(CH\(_3\))\(_3\)), 26.0 (C(CH\(_3\))\(_2\)), 35.9 (C-3), 51.1 (OCH\(_3\)), 52.7 (CO\(_2\)CH\(_3\)), 61.3 (C-5), 61.5 (C-7), 66.6 (C-9), 67.9 (C-4), 69.5 (C-6), 75.4 (C-8), 99.4 (C-2), 109.4 (C(CH\(_3\))\(_2\)), 167.9 (CO\(_2\)CH\(_3\)).

IR = 2105 cm\(^{-1}\) (5,7-N\(_3\)), 1738 cm\(^{-1}\) (CO\(_2\)Me)

HRMS: (C\(_{20}\)H\(_{36}\)N\(_6\)O\(_7\)SiNa): Calculated: 523.23124; found: 523.23123

Methyl (methyl 7-acetamido-4-O-(tert-butyldimethylsilyl)-3,5,7-tri-deoxy-1,5-lactam-8,9-O-isopropylidene-D-glycero-β-L-manno-non-2-ulyopyranosid)onate (147)

Pd/C (10% wt.) (20% by mass) was added to a solution of 138 (100 mg, 0.2 mmol) in methanol (5 mL) under argon\((g)\) at room temperature. The resulting reaction mixture was degased and flushed with H\(_2\) gas, after which the reaction was run at room temperature at 1 atm of H\(_2\) gas for 12 hours, after which time the reaction mixture was filtered through Celite and the filtrate concentrated under...
reduced pressure. The resulting clear oil was purified using column chromatography (Hex/EtOAc (3:1), R_f = 0.4) yielding 147 (80 mg, 87%) as a white solid.

^1H NMR (CDCl_3) δ: 0.01, 0.03 (2 s, 6 H, Si(CH_3)_2), 0.83 (s, 9 H, Si(CH_3)_3), 1.34, 1.46 (s, 3 H, C(CH_3)_2), 1.70 (dd, 1 H, J_{3ax,3eq} = 14.1 Hz, J_{3ax,4} = 1.8 Hz, H-3_{ax}), 2.03 (s, 3 H, NHCOCH_3), 2.46 (dd, 1 H, J_{3eq,3ax} = 14.1 Hz, J_{3eq,4} = 8.4 Hz, H-3_{eq}), 3.35 (br. dd, 1 H, J_{5,NHCO} = 5.7 Hz, J_{5,NH} = 2.7 Hz, H-5), 3.55 (dd, 1 H, J_{9',9} = 8.4 Hz, J_{9',8} = 6.3 Hz, H-9\text{'}), 3.59 (s, 3 H, OCH_3), 3.63 (dd, 1 H, J_{6,NH} = 2.7 Hz, J_{6,7} = 6.9 Hz, H-6), 4.00 (ddd, 1 H, J_{7,8} = 1.2 Hz, J_{7,6} = 6.9 Hz, J_{7,7NHAc} = 9.0 Hz, H-7), 4.07 (m, 2 H, H-9/H-4), 4.60 (br. dd, 1 H, J_{8,9} = 6.3 Hz, J_{8,7} = 1.2 Hz, H-8), 5.77 (d, 1 H, J_{7NHAc} = 9.0 Hz, 7-NHAc), 6.55 (d, 1 H, J_{5NHCO} = 5.7 Hz, 5-NHCO).

^13C NMR (CDCl_3) δ: -4.7 Si(CH_3)_2, 17.8 (Si(CH_3)_3), 23.2 (NHCOCH_3), 24.1 (C(CH_3)_2), 25.6 (Si(CH_3)_3), 26.2 (C(CH_3)_2), 41.9 (C-3), 50.0 (C-7), 52.4 (OCH_3), 54.1 (C-5), 66.0 (C-9), 67.6 (C-4), 72.0 (C-8), 73.0 (C-6), 96.8 (C-2), 109.3 (C(CH_3)_2), 169.6 (CO_2CH_3), 170.9 (NHCOCH_3 & NHCO).

HRMS: (C_{21}H_{38}N_{2}O_{7}SiNa): Calculated: 481.23460; found: 481.23459

Methyl (methyl 7-acetamido-5-azido-4-O-(tert-butyldimethylsilyl)-3,5,7-tri-deoxy-8,9-O-isopropylidene-D-glycero-α-L-manno-non-2-ulopyranosid)onate (149)

Molecular sieves (4 Å) and triphenylphosphine (231 mg, 0.88 mmol) were added to a stirred solution of the bis-azide 138 (110 mg, 0.22 mmol) in THF (10 mL) under argon at 0°C. The reaction mixture was stirred at 0°C for 30 minutes before the addition of acetic anhydride (208 μL, 2.2 mmol) then stirred for a further 10 minutes at 0°C then 5.5 hours at room temperature. Upon completion (monitored by TLC 5:1 Hex/EtOAc, purple spot in ninhydrin dip, R_f = 0) the reaction mixture was filtered to remove sieves and then stirred in an equal volume of water (10 mL) at room temperature for a further 30 minutes before being concentrated under reduced pressure and purified using column chromatography (EtOAc/MeOH 4:1, R_f = 0.1) yielding 149 (68 mg, 60%) as an amorphous solid.
1H NMR (CDCl3) δ: 0.15, 0.18 (2 s, 6 H, Si(CH3)2), 0.88 (s, 9 H, SiC(CH3)3), 0.91, 1.11 (s, 3 H, C(CH3)2), 1.80 (dd, 1 H, J3ax,3eq = 12.9 Hz, J3ax,4 = 11.4 Hz, H-3ax), 1.93 (s, 3 H, NHOCH3), 2.00 (dd, 1 H, J3eq,3ax = 12.9 Hz, J3eq,4 = 4.5 Hz, H-3eq), 3.17 (s, 3 H, OCH3), 3.22 (dd, 1 H, J6,5 = 1.2 Hz, J6,7 = 9.9 Hz, H-6), 3.40 (dd, 1 H, J9,9y = 9.3 Hz, J9,8 = 6.0 Hz, H-9), 3.70 (s, 3 H, CO2CH3), 4.08 (dd, 1 H, Jy,9 = 9.3 Hz, Jy,8 = 6.0 Hz, H-9), 4.15 (br. s, 1 H, J5,4 = 3.0 Hz, H-5), 4.34 (ddd, 1 H, J7,8 = 1.2 Hz, J7,6 = J7,7NHAc = 9.9 Hz, H-7), 4.45–4.53 (m, 2 H, H-8/H-4), 5.88 (d, 1 H, J7NHAc,7 = 9.9 Hz, 7-NHAc).

IR = 2105 cm⁻¹ (5-N3).

HRMS: (C22H40N3O3SiNa): Calculated: 539.25131; found: 539.25130

Methyl (methyl 5,7-di-azido-4-O-(tert-butyldimethyl-silyl)-3,5,7-tri-deoxy-D-glycero-β-L-manno-non-2-ulopyranosid)onate (150) and Methyl (methyl 5,7-di-azido-3,5,7-tri-deoxy-D-glycero-β-L-manno-non-2-ulopyranosid)onate (151)

Compound 138 (368 mg, 0.73 mmol) was stirred in an aqueous 80% acetic acid solution (10 mL) at 80 °C for 4 hrs. The resulting mixture was concentrated under reduced pressure co-evaporating with toluene (3 x 10 mL) before being purified using column chromatography (Hex/EtOAc 1:1, Rf = 0.4 (150) Rf = 0.1 (151)) yielding 150 (215 mg, 65%) and 151 (81 mg, 32%) as amorphous solids.

1H NMR (CDCl3) (150) δ: 0.12, 0.13 (2 s, 6 H, Si(CH3)2), 0.91 (s, 9 H, SiC(CH3)3), 2.02 (m, 2 H, H-3ax/H-3eq), 2.38 (br. s, 2 H, 8-OH, 9-OH), 3.22 (s, 3 H, OCH3), 3.71 (dd, 1 H, J7,8 = 1.8 Hz, J7,6 = 9.6 Hz, H-7), 3.79 (s, 3 H, CO2CH3), 3.78-3.84 (m, 3 H, H-5, H-9, H-9'), 3.88 (dd, 1 H, J6,5 = 1.5 Hz, J6,7 = 9.6 Hz, H-6), 4.14 (br. s, 1 H, H-8), 4.35 (ddd, 1 H, J4,3ax = 9.3 Hz, J4,3eq = 5.1 Hz, J4,5 = 4.8 Hz, H-4).

13C NMR (CDCl3) (150) δ: -4.6, -4.7 Si(CH3)2), 17.9 (SiC(CH3)3), 25.6 (SiC(CH3)3), 35.8 (C-3), 51.4 (OCH3), 52.8 (CO2CH3), 61.6 (C-5), 62.6 (C-7), 64.6 (C-9), 67.9 (C-4), 68.4 (C-6), 70.1 (C-8), 99.3 (C-2), 168.2 (CO2CH3).

IR = 2109 cm⁻¹ (5,7-N3), 1735 cm⁻¹ (CO2Me)

**H NMR (MeOD) (151)** δ: 1.84 (dd, 1 H, J₃ax,3eq = J₃ax,4 = 12.0 Hz, H-3ax), 2.03 (dd, 1 H, J₃eq,3ax = 12.0 Hz, J₃eq,4 = 4.8 Hz, H-3eq), 3.17 (s, 3 H, OCH₃), 3.58-3.63 (m, 2 H, H-9, H-9'), 3.72 (s, 3 H, CO₂CH₃), 3.88 (br. d, 1 H, J₈b,5 = 4.8 Hz, H-8), 4.00 (br. dd, 1 H, J₈,₇NHAc = 7.8 Hz, J₈,₇ = 1.5 Hz, H-8), 4.23 (ddd, 1 H, J₄,3ax = 12.0 Hz, J₄,3eq = 4.8 Hz, J₄,5 = 4.5 Hz, H-4).

**13C NMR (MeOD) (151)** δ: 35.8 (C-3), 51.7 (OCH₃), 53.0 (CO₂CH₃), 63.3 (C-5), 63.5 (C-7), 64.2 (C-9), 67.4 (C-4), 69.3 (C-6), 70.4 (C-8), 100.5 (C-2), 169.9 (CO₂CH₃).

HRMS (C₁₁H₁₈N₆O₇Na) Calculated: 369.1135, Found: 369.1129

Methyl (methyl 7-acetamido-5-azido-3,5,7-tri-deoxy-D-glycero-α-L-manno-non-2-ulopyranosid)onate (152)

Molecular sieves (4 Å) and triphenylphosphine (302 mg, 1.15 mmol) were added to a stirred solution of the bis-azide 151 (100 mg, 0.23 mmol) in THF (10 mL) under argon at 0°C. The reaction mixture was stirred at 0°C for 30 minutes before the addition of acetic anhydride or Acetyl Chloride (10 eq.) then stirred for a further 10 minutes at 0°C then 5.5 hours at room temperature. Upon completion (monitored by TLC 5:1 Hex/EtOAc, purple spot in ninhydrin dip, Rf = 0) the reaction mixture was filtered to remove sieves and then stirred in an equal volume of water (10 mL) at room temperature for a further 30 minutes before being concentrated under reduced pressure and purified using column chromatography (EtOAc/MeOH 4:1, Rf = 0.1) yielding 152 (50 mg, 60%) as an amorphous solid.

**H NMR (CDCl₃)** δ: 1.82 (dd, 1 H, J₃ax,3eq = J₃ax,4 = 12.0 Hz, H-3ax), 1.93 (s, 3 H, NHOCH₃), 2.00 (dd, 1 H, J₃eq,3ax = 12.0 Hz, J₃eq,4 = 4.5 Hz, H-3eq), 3.17 (s, 3 H, OCH₃), 3.21 (dd, 1 H, J₆,₅ = 1.2 Hz, J₆,₇ = 9.9 Hz, H-6), 3.52 (dd, 1 H, J₉,₉ = 9.3 Hz, J₉,₈ = 6.0 Hz, H-9), 3.70 (s, 3 H, CO₂CH₃), 4.03 (dd, 1 H, J₉,₉ = 9.3 Hz, J₉,₈ = 6.0 Hz, H-9'), 4.10 (br. s, 1 H, J₅,₄ = 3.0 Hz, H-5), 4.29 (ddd, 1 H, J₇,₈ = 1.2 Hz, J₇,₆ = J₇,₉NHAc = 9.6 Hz, H-7), 4.42-4.50 (m, 2 H, H-8/H-4), 5.88 (d, 1 H, J₇NHAc = 9.6 Hz, 7-NHAc).

IR: 2109 cm⁻¹ (5-N₃).
Methyl (methyl 5,7-di-azido-9-iodo-3,5,7,9-tetra-deoxy-D-glycero-ß-L-manno-non-2-ulopyranosid)onate (154)

Triphenylphosphine (175 mg, 0.67 mmol), imidazole (61 mg, 0.90 mmol) and Iodine (170 mg, 0.67 mmol) were added to a stirred solution of 151 (205 mg, 0.45 mmol) in THF (10 mL) under argon at room temperature, followed by stirring at reflux for 2 hours. Upon completion, the reaction was allowed to cool before a stoichiometric amount of Na2S2O5 dissolved in methanol (10 mL) was added. The now clear reaction mixture was subsequently absorbed onto non-flash silica gel and applied directly to a flash silica column for purification (EtOAc/Hex1:1, Rf = 0.8) yielding 154 (146 mg, 71%) as a white amorphous solid.

1H NMR (CDCl3) δ: 1.83 (dd, 1 H, J3ax,3eq = 12.9 Hz, J3ax,4 = 12.3 Hz, H-3ax), 2.16 (br. s, 1 H, 8-OH), 2.21 (dd, 1 H, J3eq,3ax = 12.9 Hz, J3eq,4 = 4.8 Hz, H-3eq), 3.21 (s, 3 H, OCH3), 3.38-3.40 (m, 2 H, H-9/H-9’), 3.80 (s, 3 H, CO2CH3), 3.80-3.89 (br. m, 3 H, H-5, H-6, H-7), 4.21 (br. dd, 1 H, J8,9 = 7.8 Hz, J8,7 = 1.5 Hz, H-8), 4.36 (ddd, 1 H, J4,3ax = 12.9 Hz, J4,3eq = 4.8 Hz, J4,5 = 4.2 Hz, H-4).

13C NMR (CDCl3) δ: 8.8 (C-9), 35.9 (C-3), 51.3 (OCH3), 52.7 (CO2CH3), 61.5 (C-5), 63.7 (C-7), 67.9 (C-4), 69.0 (C-6), 70.2 (C-8), 99.4 (C-2), 167.9 (CO2CH3).

HRMS: (C11H17N6O8INa): Calculated: 479.01519; found: 479.01515

Methyl (methyl 7-acetamido-3,5,7,9-tetra-deoxy-1,5-lactam-D-glycero-ß-L-manno-non-2-ulopyranosid)onate (155)

Pd/C (10% wt.) (20% by mass) was added to a solution of 154 (100 mg, 0.22 mmol) in methanol (5 mL) under argon at room temperature. The resulting reaction mixture was degased and flushed with H2 gas, after which the reaction was run at room temperature at 1 atm of H2 gas for 12 hours, after which time the reaction mixture was filtered through Celite and the filtrate concentrated under reduced pressure. The resulting clear oil was purified using
column chromatography (Hex/EtOAc (3:1), R_f = 0.2) yielding 155 (63 mg, 87%) as a white foam.

^1^H NMR (CDCl_3) δ: 1.10 (d, 3 H, J_{9,9} = 6.3 Hz, H-9), 1.81 (dd, 1 H, J_{3ax,3eq} = 14.1 Hz, J_{3ax,4} = 1.8 Hz, H-3ax), 1.98 (s, 3 H, NHCOC\_CH\_3), 2.51 (dd, 1 H, J_{3eq,3ax} = 14.1 Hz, J_{3eq,4} = 8.4 Hz, H-3eq), 3.39 (br. dd, 1 H, J_{5,NHCO} = 5.7 Hz, J_{5,6} = 2.7 Hz, H-5), 3.61 (s, 3 H, OCH\_3), 3.65 (dd, 1 H, J_{6,5} = 2.7 Hz, J_{6,7} = 6.9 Hz, H-6), 4.08 (ddd, 1 H, J_{7,8} = 1.2 Hz, J_{7,7NHAc} = 9.0 Hz, J_{7,6} = 6.9 Hz, J_{7,7NHAc} = 9.0 Hz, H-7), 4.09 (br. dd, 1 H, J_{4,3eq} = 8.4 Hz, J_{4,3ax} = 1.2 Hz, H-4), 4.58 (br. dd, 1 H, J_{8,9^c} = 6.3 Hz, J_{8,7} = 1.2 Hz, H-8), 5.75 (d, 1 H, J_{7NHAc,7} = 9.0 Hz, 7-NHAc), 6.55 (d, 1 H, J_{5NHCO,5} = 5.7 Hz, 5-NHCO).

^1^C NMR (CDCl_3) δ: 21.2 (C-9), 23.5 (NHCO\_CH\_3), 41.9 (C-3), 50.0 (C-7), 51.5 (OCH\_3), 54.1 (C-5), 67.8 (C-4), 71.6 (C-8), 72.8 (C-6), 96.8 (C-2), 169.6 (CO\_2CH\_3), 170.9 (NHCO\_CH\_3 & NHCO).

HRMS (C\_14\_H\_22\_N\_2\_O\_7\_Na) Calculated: 353.1325, Found: 353.1328

Methyl (methyl 5,7-di-acetamido-4-O-(tert-butyldimethylsilyl)-3,5,7-tri-deoxy-8,9-O-isopropylidene-D-glycero-β-L-manno-non-2-ulopyranosid)onate (148)

To a solution of 138 (610 mg, 1.25 mmol) in methanol under argon\((g)\) at room temperature was added p-toluenesulphonic acid monohydrate (5% mol. eq.) and Pd(OH)\_2/C 20% wt. (20% by mass). The resulting reaction mixture was degased and flushed with H\_2 gas, after which the reaction was run at room temperature at 1 atm of H\_2 gas for 4 hours. The reaction mixture was filtered through Celite concentrated under reduced pressure with the resulting crystalline solid being taken up in acetic anhydride (5 mL) to which pyridine (10 mL) and DMAP (20 mg) were added. The reaction mixture was stirred at room temperature under N\_2\((g)\) for 16 hours, before being concentrated under reduced pressure taken up in EtOAc (50 mL) and washed with 1 M HCl (30 mL) and water (30 mL). The organic phase was dried (Na\_2SO\_4) and concentrated before being purified using column chromatography (EtOAc/MeOH (4:1), R_f = 0.3) yielding 148 (530 mg, 80% over two steps) as an white crystalline solid.
\(^1\)H NMR (CDCl\(_3\)) \(\delta\): 0.048, 0.050 (2 s, 6 H, Si(CH\(_3\))\(_2\)), 0.82 (s, 9 H, Si(C(H\(_3\)))\(_3\)), 1.25, 1.37 (s, 3 H, C(CH\(_3\))\(_2\)), 1.60 (dd, 1 H, J\(_{3ax,3eq}\) = 13.5 Hz, J\(_{3ax,4}\) = 10.4 Hz, H-3\(_{ax}\)), 1.92, 1.95 (s, 3 H, NHOCH\(_3\)), 2.05 (dd, 1 H, J\(_{3eq,3ax}\) = 13.5 Hz, J\(_{3eq,4}\) = 5.1 Hz, H-3\(_{eq}\)), 3.23 (s, 3 H, OCH\(_3\)), 3.52 (dd, 1 H, J\(_{9',9}\) = 8.4 Hz, J\(_{9',8}\) = 7.5 Hz, H-9\(_{'}\)), 3.79 (s, 3 H, CO\(_2\)CH\(_3\)), 3.92 (dd, 1 H, J\(_{6,5}\) = 2.1 Hz, J\(_{6,7}\) = 10.5 Hz, H-6), 3.97 (dd, 1 H, J\(_{9,9'}\) = 8.4 Hz, J\(_{9,8}\) = 6.9 Hz, H-9), 4.12 (dd, 1 H, J\(_{4,5}\) = 4.5 Hz, J\(_{4,3ax}\) = 10.4 Hz, J\(_{4,3eq}\) = 5.1 Hz, H-4), 4.29 (ddd, 1 H, J\(_{7,8}\) = 1.5 Hz, J\(_{7,6}\) = J\(_{7,7NHAc}\) = 10.5 Hz, H-7), 4.38 (ddd, 1 H, J\(_{5,4}\) = 4.5 Hz, J\(_{5,6}\) = 2.1 Hz, J\(_{5,5NHAc}\) = 10.5 Hz, H-5), 4.52 (ddd, 1 H, J\(_{8,9}\) = 6.9 Hz, J\(_{8,9'}\) = 7.5 Hz, J\(_{8,7}\) = 1.5 Hz, H-8), 5.46 (d, 1 H, J\(_{7NHAc,7}\) = 10.5 Hz, J\(_{7NHAc,5}\) = 10.5 Hz, H-5NHAc).

\(^13\)C NMR (CDCl\(_3\)) \(\delta\): -4.9 Si(CH\(_3\))\(_2\), 17.9 (Si(C(CH\(_3\)))\(_3\)), 23.2, 23.3 (2 NHCOCH\(_3\)), 24.7 (C(CH\(_3\))\(_2\)), 25.5 (SiC(CH\(_3\)))\(_3\)), 26.2 (C(CH\(_3\))\(_2\)), 36.6 (C-3), 47.7 (C-5), 48.0 (C-7), 51.1 (OCH\(_3\)), 52.6 (CO\(_2\)CH\(_3\)), 65.2 (C-4), 65.6 (C-9), 70.5 (C-6), 73.6 (C-8), 99.3 (C-2), 108.6 (C(CH\(_3\))\(_2\)), 168.5 (CO\(_2\)CH\(_3\)), 170.2 (NHCOCH\(_3\)), 170.3 (NHCOCH\(_3\)).

m/z = 555.1 [M + Na]\(^+\)

HRMS: (C\(_2\)H\(_4\)\(_4\)N\(_2\)O\(_3\)SiNa): Calculated: 555.270828; found: 555.269614

Methyl (methyl 5,7-di-acetamido-3,5,7-tri-deoxy-D-glycero-β-L-manno-non2-ulopyranosid)onate (139)

Trifluoroacetic acid (0.23 mL, 3 mmol) was added to a stirred solution of 148 (530 mg, 1.0 mmol) in a mixture of THF and water (4:1) at 0°C. The resulting reaction mixture was allowed to warm to room temperature and stirred for 48 hours before being neutralised with concentrated ammonia and concentrated under reduced pressure and columned directly (EtOAc/MeOH (4:1), R\(_f\) = 0.25) yielding 139 (302 mg, 85%) as an white amorphous solid.

\(^1\)H NMR (CD\(_3\)CN) \(\delta\): 1.63 (dd, 1 H, J\(_{3ax,3eq}\) = 12.3 Hz, J\(_{3ax,4}\) = 12.0 Hz, H-3\(_{ax}\)), 1.86, 1.88 (s, 3 H, NHOCH\(_3\)), 1.99 (dd, 1 H, J\(_{3eq,3ax}\) = 12.3 Hz, J\(_{3eq,4}\) = 4.2 Hz, H-3\(_{eq}\)), 3.21 (s, 3 H, OCH\(_3\)), 3.32 (m, 2 H, H-9/H-9\(_{'\})), 3.75 (s, 3 H, CO\(_2\)CH\(_3\)), 3.93 (br. dd, 1 H, J\(_{8,9}\) = J\(_{8,9'}\) = 7.5 Hz, H-8), 4.06 (br. m, 3 H, H-4/H-6/H-7), 4.34 (br. dd, 1 H, J\(_{5,4}\) = 3.9 Hz,
$J_{5,5\text{NHAc}} = 9.9$ Hz, H-5), 6.49 (d, 1 H, $J_{5\text{NHAc},5} = 9.9$ Hz, 5-NHAc), 6.60 (d, 1 H, $J_{7\text{NHAc},7} = 9.9$ Hz, 7-NHAc).

$^{13}$C NMR (MeOD$_4$) δ: 22.6, 22.8 (2 NHCOCH$_3$), 36.5 (C-3), 49.6 (C-5), 50.7 (C-7), 51.9 (OCH$_3$), 53.2 (CO$_2$CH$_3$), 64.8 (C-9), 66.2 (C-4), 70.1 (C-6), 70.3 (C-8), 100.6 (C-2), 170.7 (CO$_2$CH$_3$), 173.8, 174.5 (2 NHCOCH$_3$).

$m/z = 401.1$ [M + Na]$^+$

HRMS: (C$_{15}$H$_{26}$N$_2$O$_9$Na): Calculated: 401.1531; found: 401.1531.

Methyl (methyl 5,7-di-acetamido-9-iodo-3,5,7,9-tetra-deoxy-D-glycero-β-L-manno-non-2-ulopyranosid)onate (156)

![Structure of 156](image)

Triphenylphosphine (315 mg, 1.2 mmol), imidazole (110 mg, 1.6 mmol) and Iodine (305 mg, 1.2 mmol) were added to a stirred solution of 139 (302 mg, 0.8 mmol) in THF (10 mL) under argon at room temperature, followed by stirring at reflux for 2 hours. Upon completion, the reaction was allowed to cool before a stoichiometric amount of Na$_2$S$_2$O$_5$ dissolved in methanol (10 mL) was added. The now clear reaction mixture was subsequently absorbed onto non-flash silica gel and applied directly to a flash silica column for purification (EtOAc/MeOH (4:1), $R_f = 0.4$) yielding 156 (280 mg, 71%) as a clear oil.

$^1$H NMR (CD$_3$CN) δ: 1.63 (dd, 1 H, $J_{3ax,3eq} = 12.9$ Hz, $J_{3ax,4} = 12.3$ Hz, H-3ax), 1.88, 1.92 (s, 3 H, NHOC$_2$H$_3$), 2.01 (dd, 1 H, $J_{3eq,3ax} = 12.9$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3eq), 3.20 (s, 3 H, OCH$_3$), 3.25 (br. m, 2 H, H-9/H-9'), 3.75 (s, 3 H, CO$_2$CH$_3$), 4.06 (br. m, 3 H, H-6/H-4/H-8), 4.25 (br. s, 1 H, H-5), 4.29 (br. s, H-7), 6.69 (d, 1 H, $J_{5\text{NHAc},5} = 9.9$ Hz, 5-NHAc), 6.85 (d, 1 H, $J_{7\text{NHAc},7} = 9.9$ Hz, 7-NHAc).

$^{13}$C NMR (MeOD$_4$) δ: 9.4 (C-9), 22.7, 22.9 (2 NHCOCH$_3$), 36.4 (C-3), 52.0 (C-5), 52.2 (CO$_2$CH$_3$), 52.3 (C-7), 53.3 (OCH$_3$), 66.2 (C-4), 70.9 (C-6), 71.5(C-8), 100.5 (C-2), 170.7 (CO$_2$CH$_3$), 173.4, 174.6 (2 NHCOCH$_3$).

$m/z = 511.1$ [M + Na]$^+$

HRMS: (C$_{15}$H$_{25}$N$_2$O$_8$INa): Calculated: 511.0548; found: 511.0548.
Methyl (methyl 5,7-di-acetamido-3,5,7,9-tetra-deoxy-D-glycero-β-L-manno-non-2-ulopyranosid)onate (PSE1,β2Me₂) (153)

Pd(OH)_2/C (20% wt.) (20% by mass) and Hunig’s base (0.02 mL, 0.1 mmol) were added to a solution of 156 (280 mg, 0.57 mmol) in methanol (5 mL) under argon(g) at room temperature. The resulting reaction mixture was degased and flushed with H₂ gas, after which the reaction was run at room temperature at 1 atm of H₂ gas for 12 hours, after which time the reaction mixture was filtered through Celite and concentrated under reduced pressure. The resulting clear oil was purified using column chromatography (EtOAc/MeOH (4:1), Rf = 0.3) yielding 153 (200 mg, 96%) as a white amorphous solid.

\[ \text{1H NMR (CNCD}_3\text{)} \delta: \ 1.06 \text{ (d, 3 H, } J_{9,8} = 6.3 \text{ Hz, H-9), 1.62 \text{ (dd, 1 H, } J_{3ax,3eq} = 13.2 \text{ Hz, } J_{3ax,4} = 12.0 \text{ Hz, H-3}_{ax}), 1.86, 1.87 \text{ (s, 3 H, NHOCCH}_3\text{), 2.01 \text{ (dd, 1 H, } J_{3eq,3ax} = 13.2 \text{ Hz, } J_{3eq,4} = 5.1 \text{ Hz, H-3}_{eq}), 3.20 \text{ (s, 3 H, OCH}_3\text{), 3.75 \text{ (s, 3 H, CO}_2\text{CH}_3\text{), 3.83 \text{ (br. dd, 1 H, } J_{7,8} = 1.5 \text{ Hz, } J_{7,7NHAc} = 9.6 \text{ Hz, H-7), 4.02 \text{ (br. m, 3 H, H-6/H-4/H-8), 4.24 \text{ (br. m, 1 H, H-5), 6.35 \text{ (d, 1 H, } J_{7NHAc,7} = 9.6 \text{ Hz, 7-NHAc), 6.47 \text{ (d, 1 H, } J_{SNHAc,5} = 9.6 \text{ Hz, 5-NHAc).}}\]

\[ \text{13C NMR (CNCD}_3\text{)} \delta: \ 20.9 \text{ (C-9), 23.2, 23.3 \text{ (2 NHCOCH}_3\text{), 36.6 \text{ (C-3), 49.4 \text{ (C-5), 51.7 \text{ (OCH}_3\text{), 53.0 \text{ (CO}_2\text{CH}_3\text{), 54.2 \text{ (C-7, 65.5 \text{ (C-8), 66.7 \text{ (C-4), 70.5 \text{ (C-6), 100.2 \text{ (C-2), 170.7 \text{ (CO}_2\text{CH}_3\text{), 171.3, 173.2 \text{ (2 NHCOCH}_3\text{).}}\]

\[ m/z = 385.1 \text{ [M + Na]}^+ \]

HRMS: (C_{15}H_{26}N_{2}O_{8}Na): Calculated: 385.1581; found: 385.1581.
References


bacteria: Data from an university hospital over a 36-month period. *Int. J. Hyg. Environ. Health* **211**: 251–257.


218. Preliminary crystallographic analysis of 147 was carried out by Prof Peter Healy, Griffith University, Brisbane, Australia. Unfortunately the crystals contained four molecules in the asymmetric unit that could not be resolved to publication standard, but the data obtained is sufficiently well resolved to support the proposed structure.


APPENDIX 1

A SELECTION OF $^1$H, $^{13}$C DEPT and 2D NMR SPECTRA
$p$-Methoxyphenyl $\alpha$-D-mannopyranose (67)
\textit{p-}\textit{Methoxyphenyl 6-\textit{O}}\textit{-tosyl-\textit{\alpha}}\textit{-D-mannopyranose (68)}
$p$-Methoxyphenyl 6-deoxy-6-iodo-$\alpha$-D-mannopyranose (69)
\( p\)-Methoxyphenyl \( \alpha\)-d-rhamnopyranose (64)
Benzyl α-D-mannopyranose (70)
Benzyl α-D-rhamnopyranose (72)
α/β-D-Rhamnopyranose (35)
Benzyl 2,3-O-isopropylidene-α-d-rhamnopyranose (77)
Benzyl 2,3-di-O-acetyl-4-azido-4,6-dideoxy-α-D-talopyranose (81)
Benzyl 2-O-acetyl-3,4-anhydro-6-deoxy-α-D-talopyranose (80)
4-Azido-4,6-dideoxy-α/β-D-talopyranose (87)
4-Azido-4-deoxy-α/β-D-rhamnopyranose (88)
Benzyl 4-azido-2,4-dideoxy-3-oxo-α-D-talopyranose (118)
Benzyl 4-azido-3-O-benzyl-2,4-dideoxy-2,3-unsaturated-benzyl-α-D-talopyranose (123)
Benzyl \textit{N-acetyl-3-O-tert-butyldimethylsilyl-6-deoxy-6-iodo-\textbeta-D-glucosamine} (133)
\(N\text{-acetyl-6-deoxy-}\alpha-D\text{-glucosamine (135)}\)
Methyl (methyl 4-\textit{O}-(tert-butylidimethylsilyl)-3-deoxy-8,9-\textit{O}-isopropylidene-D-glycero-\textit{\beta}-D-galacto-non-2-ulopyranosid)onate (137)
Methyl (methyl 5,7-di-azido-4-O-(tert-butyldimethylsilyl)-3,5,7-tri-deoxy-8,9-O-isopropylidene-D-glycero-β-L-manno-non-2-ulopyranosid)onate (138)
Methyl (methyl 7-acetamido-4-O-(tert-butyldimethylsilyl)-3,5,7-tri-deoxy-1,5-lactam-8,9-O-isopropylidene-D-glycero-β-L-manno-non-2-ulopyranosid)onate (147)
Methyl (methyl 5,7-di-acetamido-4-O-(tert-butyldimethylsilyl)-3,5,7-tri-deoxy-8,9-O-isopropylidene-D-glycero-β-L-manno-non-2-ulopyranosid)onate (148)
Methyl (methyl 5,7-di-acetamido-3,5,7,9-tetra-deoxy-D-glycero-β-L-manno-non-2-ulo.pyranosid)onate (8-epi-PSE1,β2Me2) (153)