

SYNTHESIS OF NOVEL C4-MODIFIED SIALIC ACID ANALOGUES

by

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ABSTRACT

Sialic acids are a family of structurally-unique 9-carbon α -keto acid sugars that are often found on the termini of oligosaccharide chains on glycoproteins and cell surfaces. These sugars can be removed through sialidases-catalyzed hydrolysis and play a number of roles in biological recognition events, serving as receptor sites for sialic acid-recognizing proteins and masking other antigenic oligosaccharides. The influenza virus recognizes sialic acids on cell surfaces of the upper respiratory tract and produces a sialidase that is critical for viral infectivity. Inhibitors of influenza sialidase have been developed as therapeutic agents that will be useful in the event of an influenza pandemic; however, it is important to have a wide array of such drugs to counter viral resistance.

The main objective of the research described in this thesis was to develop synthetic routes to 4-modified sialic acid analogues. These were intended as general routes, allowing the synthesis of many analogues. This initially involved coupling the nitronate of 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol **2.1** with alkyl α -(bromomethyl)acrylate esters **2.6** and **2.12**. These reaction products were subjected to ozonolysis, leading to 4-deoxy-4-nitrosialic acid esters **2.10** and **2.14**. The synthetically versatile nitro group should allow the synthesis of a number of 4-derivatives. Glycosylation of 4-deoxy-4-nitrosialosyl donor sugars was attempted with phenol in order to generate substrates for sialidase-catalyzed hydrolysis; however, only 1-adamantyl thiosialoside **2.21** led to practical

quantities of the desired phenyl α -sialoside product **2.25**. An oxabicyclo[3.1.0]hexane-based sialic acid analogue **4.3** was isolated when the β -sialosyl chloride donor sugar **2.18** was treated with base. This potential sialidase inhibitor had a novel ring structure but the protecting groups could not be removed without destroying the bicyclic system.

Isopropylidene-protected nitromannitol **2.6** was also reacted with ethyl α -(bromomethyl)acrylate to afford an enoate ester product that upon ozonolysis and chromatographic purification led to isolation of a β,γ -unsaturated α -keto ester **3.1**. This enone, resulting from elimination of HNO_2 , was used in copper-catalyzed conjugate addition reactions of dialkylzinc reagents to synthesize 4-alkyl-4-deoxy-4-*epi*-sialic acid analogues that could be converted into analogues of DANA, the glycal of sialic acid. Unfortunately, only Me_2Zn and Et_2Zn underwent conjugate addition to give the less desirable 4*R*-configured products.

Keywords: sialic acids; sialidases; inhibitors; synthesis; influenza

Subject Terms: carbohydrates – synthesis; carbohydrates; biochemistry; carbohydrate drugs

DEDICATION

To my family

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LIST OF ABBREVIATIONS

| | |
|-------|---|
| AIBN | 2,2'-azobis(isobutyronitrile) |
| Bn | benzyl |
| Bz | benzoyl |
| Cbz | carbobenzyloxy |
| CMP | cytidine 5'-monophosphate |
| COSY | correlation spectroscopy |
| CSA | camphorsulfonic acid |
| DANA | 2-deoxy-2,3-didehydro- <i>N</i> -acetylneuraminic acid |
| DAST | diethylaminosulfur trifluoride |
| DBU | 1,8-diazabicycloundec-7-ene |
| dd | doublet of doublet |
| DDQ | dichlorodicyanoquinone |
| DEAD | diethyl azidodicarboxylate |
| DIBAL | diisobutylaluminum hydride |
| DMAP | 4-dimethylaminopyridine |
| DMF | <i>N,N</i> -dimethylformamide |
| DMS | dimethyl sulfide |
| DMSO | dimethyl sulfoxide |
| DMTST | dimethyl(methylthio)sulfonium trifluoromethanesulfonate |

| | |
|--------|---|
| dt | doublet of triplet |
| DTAD | di- <i>t</i> -butyl azidodicarboxylate |
| EDIC | 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide |
| GlcNAc | <i>N</i> -acetylglucosamine |
| H | hemagglutinin |
| HFIP | 1,1,1,3,3,3-hexafluoroisopropanol |
| HMBC | heteronuclear multiple bond correlation |
| HMQC | heteronuclear multiple quantum correlation |
| HMPT | hexamethylphosphoric triamide |
| Kdn | 3-deoxy-D- <i>glycero</i> -D- <i>galacto</i> -non-2-ulopyranosonic acid |
| Kdo | 3-deoxy-D- <i>manno</i> -oct-2-ulopyranosonic acid |
| KHMDS | potassium hexamethyldisilazane |
| m | Multiplet |
| ManNAc | <i>N</i> -acetylmannosamine |
| mCPBA | <i>m</i> -chloroperbenzoic acid |
| MOM | methoxymethyl |
| Ms | methanesulfonyl |
| MS3Å | 3 Angstrom molecular sieves |
| N | neuraminidase |
| NBS | <i>N</i> -bromosuccinimide |
| NIS | <i>N</i> -iodosuccinimide |
| Neu5Ac | <i>N</i> -acetylneuraminic acid |

| | |
|--------|--|
| Neu5Gc | <i>N</i> -glycolylneuraminic acid |
| NMR | nuclear magnetic resonance |
| NOE | nuclear Overhauser effect |
| PLE | pig liver esterase |
| PMP | <i>p</i> -methoxyphenyl |
| PPTS | pyridinium <i>p</i> -toluenesulfonate |
| t | triplet |
| TASF | tris(dimethylamino)sulfonium difluorotrimethylsilicate |
| TBAB | tetra- <i>n</i> -butylammonium bromide |
| TBAF | tetra- <i>n</i> -butylammonium fluoride |
| TBAI | tetra- <i>n</i> -butylammonium iodide |
| TBDMS | <i>t</i> -butyldimethylsilyl |
| TBDPS | <i>t</i> -butyldiphenylsilyl |
| TEMPO | 2,2,6,6,-tetramethylpiperidine-1-oxyl |
| TES | triethylsilyl |
| TFA | trifluoroacetic acid |
| TFE | 1,1,1-trifluoroethanol |
| TfOH | trifluoromethanesulfonic acid |
| THF | tetrahydrofuran |
| TIPS | triisopropylsilyl |
| TLC | thin-layer chromatography |
| TMS | trimethylsilyl |

Ts *p*-toluenesulfonyl

CHAPTER 1: INTRODUCTION

1.1 Carbohydrates

Introductory biochemistry textbooks describe four main classes of biomolecules: carbohydrates, proteins, nucleotides, and lipids. Both the monomeric and polymeric forms of each of these four classes of biomolecules play many important roles in maintaining and regulating the functions of living organisms. Although nucleic acids and proteins have both received a great deal of attention over many years from scientists, it has only been recently that carbohydrates have begun to command the same sort of attention with the advent of new tools for carbohydrate analysis and manipulation.¹

Carbohydrates have long been thought of as sources of fuel and energy storage for living organisms. They provide a direct link between solar energy and chemical energy as plants use light energy from the sun to combine carbon dioxide with water through the process of photosynthesis. This results in generation of carbohydrates and oxygen, both of which can be used by organisms to harvest the converted solar energy for reactions necessary for life.² Carbohydrate monomers can be linked together in numerous ways to form polymers that have several roles. Glycogen and starch are both polymers of glucose that are used for energy storage, synthesized by animals and plants, respectively.³ Cellulose, another glucose polymer, is a major structural component of plant cell walls, while chitin, a polymer of *N*-acetylglucosamine, is a

major structural component of the exoskeleton of arthropods.² Carbohydrates are also industrially important, being used in the food industry as sweeteners and preservatives, in the textiles industry, in plastics, packaging, and in the pharmaceutical industry.³

In addition to their energy storage and structural roles, carbohydrates are now known to play a myriad of roles in many biological processes ranging from intracellular communication to the immune response. Carbohydrates are known to decorate the surfaces of cells and glycoproteins and are thus the first point of contact between different cells and different microorganisms. As such, carbohydrates: (1) mediate interactions between cells/organisms, (2) direct traffic, (3) label cells/proteins as self or non-self, and (4) influence a great number of biological processes.

Carbohydrates are a diverse class of molecules. Initially described as hydrates of carbon with the general formula $C_n(H_2O)_n$, carbohydrates now include several structures that do not conform to this formula but show the same chemical properties such as polyhydroxy aldehydes, alcohols, ketones, acids, and derivatives.³ These diverse monomers (monosaccharides) can be linked together in a number of ways to form oligosaccharides (containing 2-10 monomers) and polysaccharides (containing 10 or more monomers). Thus, a large number of oligosaccharides can be synthesized from a small number of monosaccharides owing to the large number of positional linkages and combinations that can be made. This allows generation of a great number of

biological recognition sites, as different parts (epitopes) of oligosaccharides can have interactions with different proteins or other biomolecules.⁴

Many new research areas have opened up, the goals of which are to understand the relationships between the carbohydrates presented on cell surfaces and the many proteins and other biomolecules that interact with them. These studies have been made possible with the advent of new tools and technologies through advances in analytical chemistry, molecular biology, and genetics to spawn the research field known as glycobiology.^{1,5} These carbohydrate units are often linked to peptides and proteins through oxygen and nitrogen atoms of amino acid side chains to form glycoconjugates that are often presented on cell surfaces.^{3,4}

In addition to proteins that recognize carbohydrate epitopes, there are many enzymes that process carbohydrates in several ways. These include linking carbohydrates to and hydrolysing them from oligosaccharide chains, epimerizing their stereocentres, adding molecules or groups to them, and carrying out functional group interconversions. These carbohydrate-processing enzymes often bind specifically to certain carbohydrates, while some are more promiscuous. The roles of these carbohydrates and enzymes are varied, but many are involved in causing, detecting, and controlling diseases. It has been stated that virtually every major disease afflicting mankind directly involves glycoconjugates.¹ Therefore, it is important to understand what these proteins are doing (function), how they are doing it (mechanism), and what might be done to disrupt it, if desired. Inhibitors of carbohydrate-processing enzymes as well as

altered substrates can be used as probes of function and mechanism and can also be used to treat disease states.

Synthesis of carbohydrates and carbohydrate derivatives and analogues is important in order to generate modified substrates as probes for proteins and enzymes that recognize and process carbohydrates. These probes can lead to information regarding the part of the carbohydrate that is being recognized (the epitope) as well as the mechanism of action. This can lead to the development of better inhibitors for use as drugs. However, synthetic carbohydrate chemistry is somewhat challenging given that carbohydrates are basically polyhydroxylated aldehydes and ketones with multiple chiral centres and the differences in reactivity between various hydroxyl groups is generally not large.⁶ In addition, it is often difficult to separate the many possible regio- and stereoisomeric products from reactions and to identify the products. Synthesis of carbohydrates often involves many protection and deprotection steps to allow selective manipulation of one functional group in particular. In some cases these protection/deprotection steps and tedious product separations can be avoided by using enzymes to effect transformations regio- and stereoselectively via chemoenzymatic synthesis.⁷

1.2 Sialic Acids and Sialidases

1.2.1 Sialic Acids

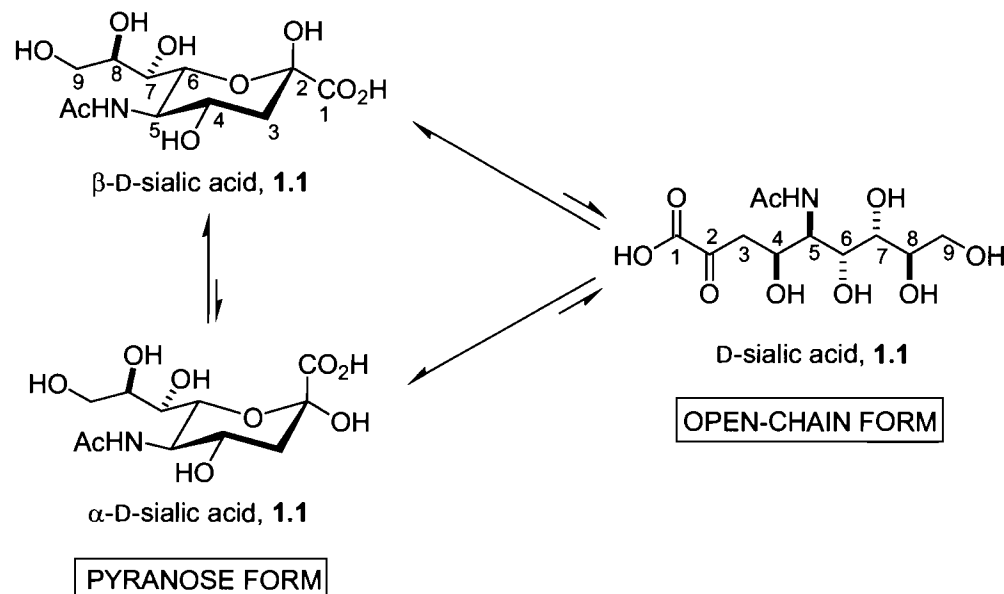


Figure 1.1 Pyranose and open-chain forms of sialic acid (Neu5Ac, 1.1)

Sialic acids are a family of 9-carbon α -ketoacid sugars that are often present on the ends of oligosaccharide chains.⁸ Sialic acid, also known as *N*-acetylneuraminic acid (Neu5Ac, 1.1), is the most abundant member of the family and has an interesting structure as compared with most other carbohydrates (Figure 1.1). Sialic acid has a carboxylate moiety at the anomeric centre (the 2-position) rather than a proton, making the anomeric centre a hemiketal rather than a hemiacetal; this carboxylate is negatively charged at physiological pH. Sialic acid also lacks a substituent at the 3-position and bears an acetamido group at the 5-position. As well, a glycerol side chain is present at the 6-position. Free sialic acid normally exists in solution in the pyranose form as a 95:5 mixture of β : α anomers that interconvert through the open-chain form (Figure 1.1).⁹

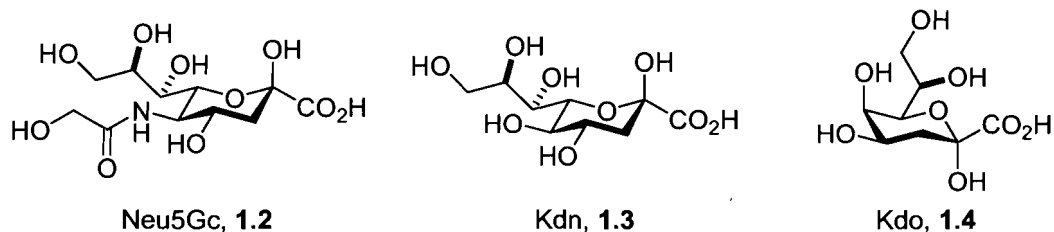


Figure 1.2 Three prominent members of the sialic acid family: Neu5Gc, Kdn, and Kdo

The sialic acid family of carbohydrates contains more than 50 members that are all α -keto acids and have 8 or 9 carbon backbones.¹⁰ The distribution of sialic acids in nature is species-specific, distributed among organisms of different evolutionary origins. The most prevalent sialic acid is sialic acid itself (Neu5Ac, 1.1) but another major subtype is *N*-glycolylneuraminic acid (Neu5Gc, 1.2). Neu5Gc is structurally similar to Neu5Ac but contains a hydroxyl group on the 5-acetamido group (Figure 1.2). This sugar is found in many mammals but is not common in humans.¹⁰ Two other prevalent members of the sialic acid family are 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (Kdn, 1.3) and 3-deoxy-D-*manno*-oct-2-ulopyranosonic acid (Kdo, 1.4). These sugars have a hydroxyl group at the 5-position in place of the acetamido group and Kdo has a truncated 8-carbon backbone (Figure 1.2). Kdn is typically found in vertebrates and bacteria while Kdo is found in plants and Gram-negative bacteria.¹⁰ The majority of the other sialic acid family members are derived from modifications of Neu5Ac, Neu5Gc, and Kdn by forming acetate, lactate, sulfate, and phosphate esters as well as methyl ethers with various hydroxyl groups and forming intramolecular lactones between a hydroxyl group and the carboxylate moiety.

All naturally-occurring sialosides have the α -anomeric configuration with the exception of the CMP-sialoside (CMP = cytidine 5'-monophosphate), the activated donor sugar used by sialyltransferases to install sialic acid residues on other sugars. Sialic acid residues are generally present at ends of oligosaccharide chains, usually α -2,6 or α -2,3 linked with galactose or *N*-acetylgalactosamine. Due to their terminal location on glycoconjugates, sialic acids play a number of important biological roles in cellular and molecular recognition events. These roles are likely enhanced by the species-specific structural diversity of sialic acids. Sialic acids function as targets for recognition by sialic acid-binding proteins; for instance, sialic acids on endothelial cells initiate adhesion of white blood cells to damaged tissue at inflammation sites. They also function as targets for viral and bacterial pathogens.⁸ In contrast, sialic acids also function as masks of other target epitopes such as galactose and can be responsible for labelling cells as "self" toward the immune system.⁸ For instance, red blood cells display sialic acid-terminated glycoproteins on their surfaces that over time lose the sialic acid moiety and become unmasked. The unmasked galactose residues cause these red blood cells to be targeted by macrophages for destruction.⁸ Tumour cells are often over-sialylated, protecting them from the immune system and increasing their malignancy. Terminal sialic acid residues can also influence the clearance rate of glycoproteins from the bloodstream; once a glycoprotein becomes de-sialylated, it is taken into the liver and destroyed.¹¹ As well, pathogens such as trypanosomes are capable of

decorating themselves with sialic acid residues taken from host cells in order to evade the immune system of the host.⁸

1.2.2 Sialidases

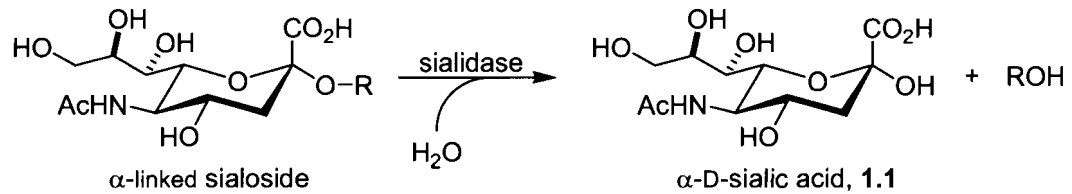


Figure 1.3 Sialidase-catalyzed hydrolysis of α -linked sialosides leads to α -sialic acid

Sialidases (EC 3.2.1.18, also known as neuraminidases) are a family of enzymes that are responsible for hydrolyzing the glycosidic linkage between sialic acid and its glycoconjugate, usually a galactose residue. The vast majority of sialidases are *exo*-sialidases as they hydrolyze terminal sialic acid residues from the ends of oligosaccharide chains. Sialidases hydrolyze α -ketosidically linked sialic acid residues from oligosaccharide chains with retention of configuration, releasing α -sialic acid (Figure 1.3). The newly formed α -sialic acid then undergoes spontaneous mutarotation to give the equilibrium mixture, which is enriched in the β -anomer (see Figure 1.1).^{12,13}

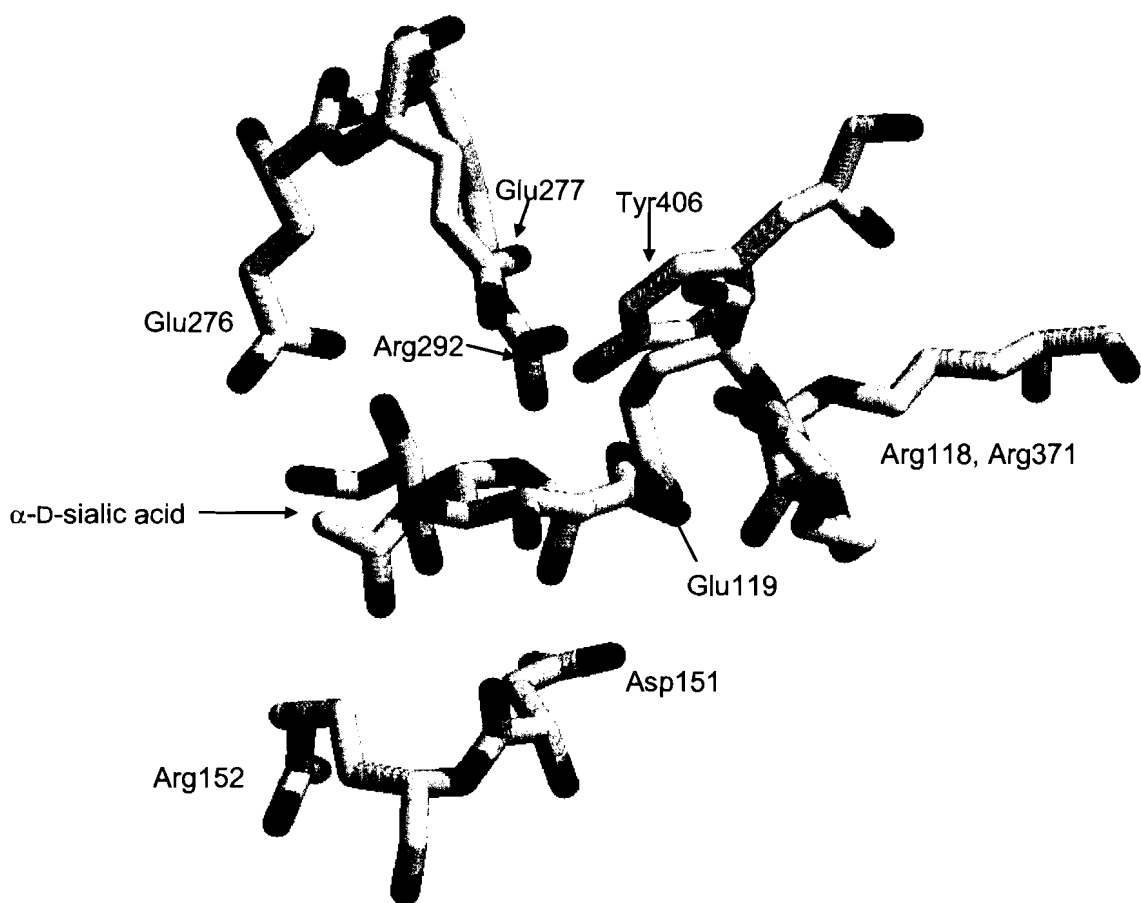


Figure 1.4 X-ray crystal structure of influenza A N2 sialidase active site containing α -Neu5Ac showing important residues (PDB code: 2BAT)

The active sites of all known sialidases contain a conserved tyrosine residue, a pair of acidic residues (glutamic and aspartic acid), and a positively-charged arginine triad that is responsible for binding to the negatively-charged sialic acid carboxylate moiety.¹⁴ X-ray crystal structures of several sialidases with different molecules bound in their active sites have been solved and used to examine interactions between the bound molecule and active site amino acid residues. Figure 1.4 shows the important amino acids in the active site of the N2

sialidase from the influenza A virus that contains an α -sialic acid residue bound in its active site.¹⁵ Analysis of this structure shows the conserved tyrosine and glutamate residues (Tyr406, Glu277) sitting above the sialic acid ring and the conserved aspartate residue (Asp151) situated below; these are the catalytic residues. The structure also shows important interactions between other amino acid residues and substituents on the sialic acid ring. On one end of the sialic acid ring, a triad of positively-charged arginine residues (Arg118, Arg371, Arg292) forms a strong charge-charge interaction with the negatively-charged carboxylate moiety. On the opposite end, the 5-acetamido group interacts with an arginine residue (Arg152) through its carbonyl oxygen and with tryptophan and isoleucine residues through its methyl group. As well, the 8- and 9-hydroxyl groups form hydrogen bond networks with a glutamate residue (Glu276) and the 4-hydroxyl group interacts with another distant glutamate residue (Glu119).¹⁶

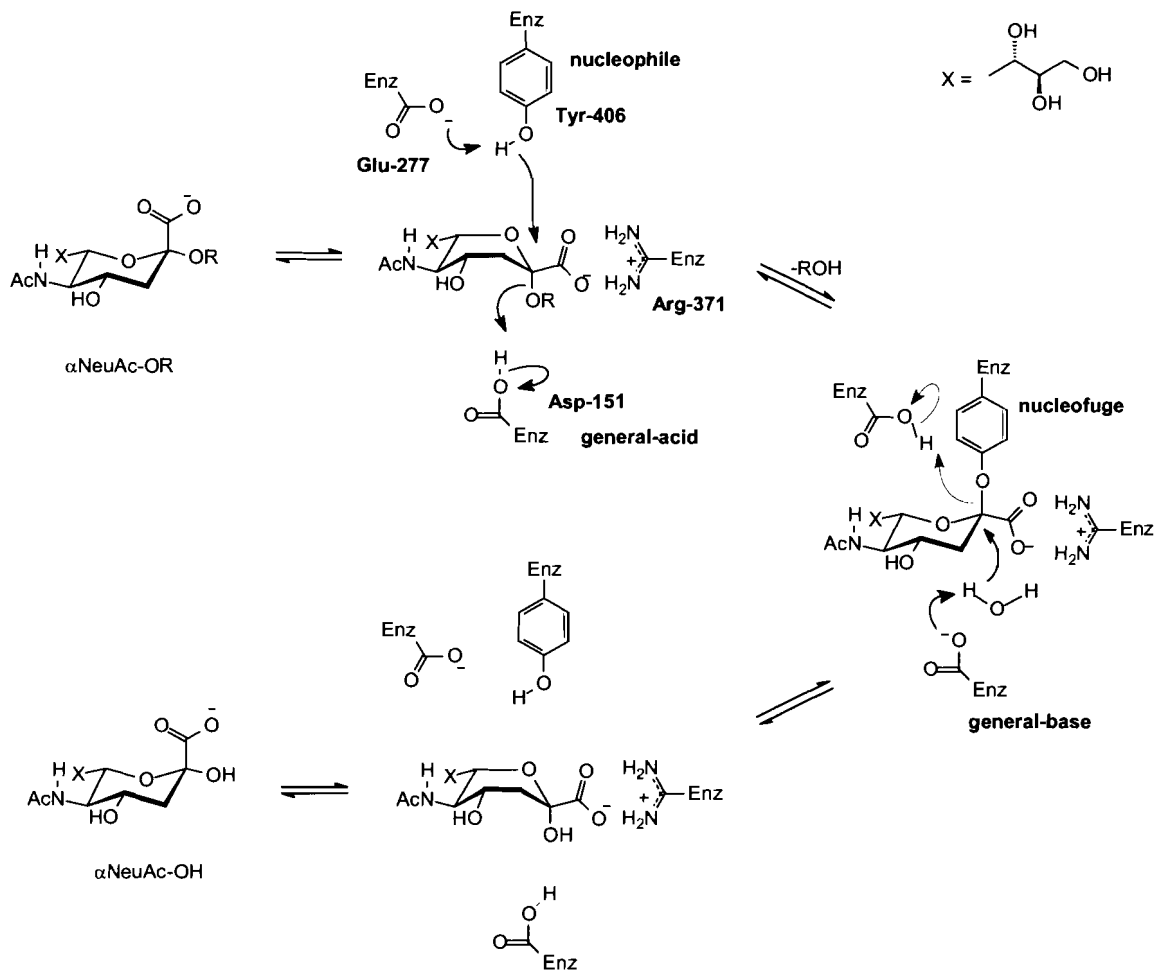


Figure 1.5 Proposed mechanism for sialidase-catalyzed hydrolysis of α -linked sialosides (influenza A N2 sialidase numbering of amino acid residues)

The currently accepted mechanism for sialidase-catalyzed hydrolysis of sialosides is shown in Figure 1.5.^{12,17} The conserved tyrosine residue is in the proper position to serve as the catalytic nucleophile, attacking the anomeric centre of the α -linked sialoside to invert the stereochemistry and form a β -linked sialosyl-enzyme intermediate. This attack likely occurs with assistance from the neighbouring conserved glutamate residue that aids in deprotonation of the tyrosine. The other conserved acidic residue, the aspartate sitting below the

sialic acid ring in Figure 1.4 (Asp151), is the acid/base catalytic residue that protonates the leaving group oxygen (the aglycon), aiding its departure. At this point, the aglycon diffuses from the active site and a water molecule takes its place. The mechanism of deglycosylation is reversed as now water attacks the anomeric carbon of the sialosyl-enzyme intermediate with the aid of the catalytic acid/base Asp151 residue, hydrolyzing the intermediate and resetting the sialidase for another reaction.^{12,17,18} The second nucleophilic attack on the anomeric centre of the sialoside inverts the stereochemistry again, resulting in the generation of α -sialic acid. Since the anomeric configuration of the starting sialoside was retained, the sialidase is a retaining enzyme. In contrast, most glycosidases (enzymes that hydrolyze the glycosidic linkage between sugars) that hydrolyze the more common hexose sugars (which have acetal centres) do not contain a conserved tyrosine residue in their active sites but instead use a conserved pair of acidic residues to effect hydrolysis.^{19,20}

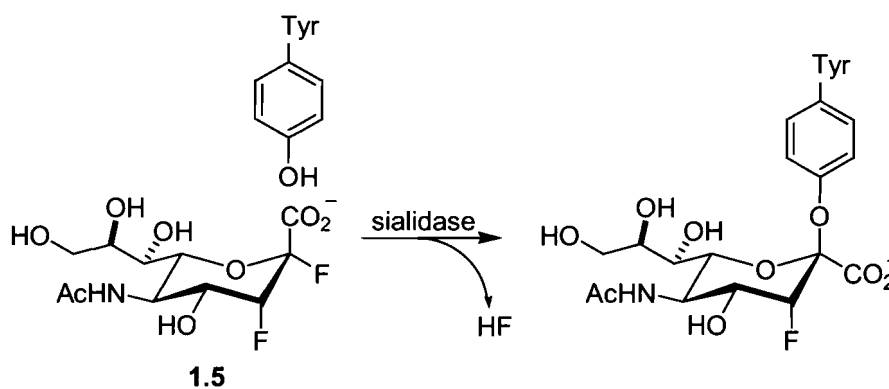


Figure 1.6 Trapping the covalent sialosyl-enzyme intermediate with 2,3-difluorosialoside 1.5

The proposal of the conserved tyrosine as the catalytic nucleophile was supported by the trapping of a sialosyl-enzyme intermediate by Withers and co-workers using 2,3-difluorosialoside **1.5** as a substrate with a sialidase from *Trypanosoma rangeli* (Figure 1.6).¹⁷ The electron-withdrawing properties of the 3-fluoro substituent destabilize the formation of an oxacarbenium ion-like transition state in which the anomeric centre bears a partial positive charge. This destabilization slows down attack at the anomeric centre. Incorporation of a good leaving group, the 2-fluoro substituent, enhances attack by the tyrosine of the sialidase, but hydrolysis of the resulting covalent intermediate is very slow, allowing this intermediate to be characterized by many techniques including mass spectrometry and X-ray crystallography.^{17,21} In addition to these crystallographic studies, Bennet and co-workers produced several mutant sialidases from *Micromonospora viridifaciens* in which the catalytic tyrosine was removed. While many of these mutants retained hydrolytic activity, they were much less efficient than the wild-type enzyme, highlighting the importance of the tyrosine as the catalytic nucleophile.¹² The catalytic acid/base aspartate residue was also mutated, resulting in catalytically-compromised sialidases and highlighting its importance for efficient hydrolysis of natural sialoside linkages.¹⁸

Given the importance of sialic acids in biological processes, it is no surprise that sialidases also play a number of important biological roles. As described in a previous section (Chapter 1.2.1), sialic acids are important masking agents for other carbohydrate epitopes. Sialidases are responsible for removing these terminal sialic acid residues to unmask the underlying antigen for

recognition by the immune system. For example, sialidases remove the terminal sialic acid residues from red blood cell surface glycoconjugates to mark them for destruction and recycling by macrophages. They also remove terminal sialic acid residues from oligosaccharides of glycoproteins circulating in the bloodstream, causing their degradation in the liver. Many species of bacteria produce sialidases to obtain sialic acid from their surroundings for use as a carbon source. Thus, many species use a balance of sialic acid and sialidase production to regulate many biological processes.²²

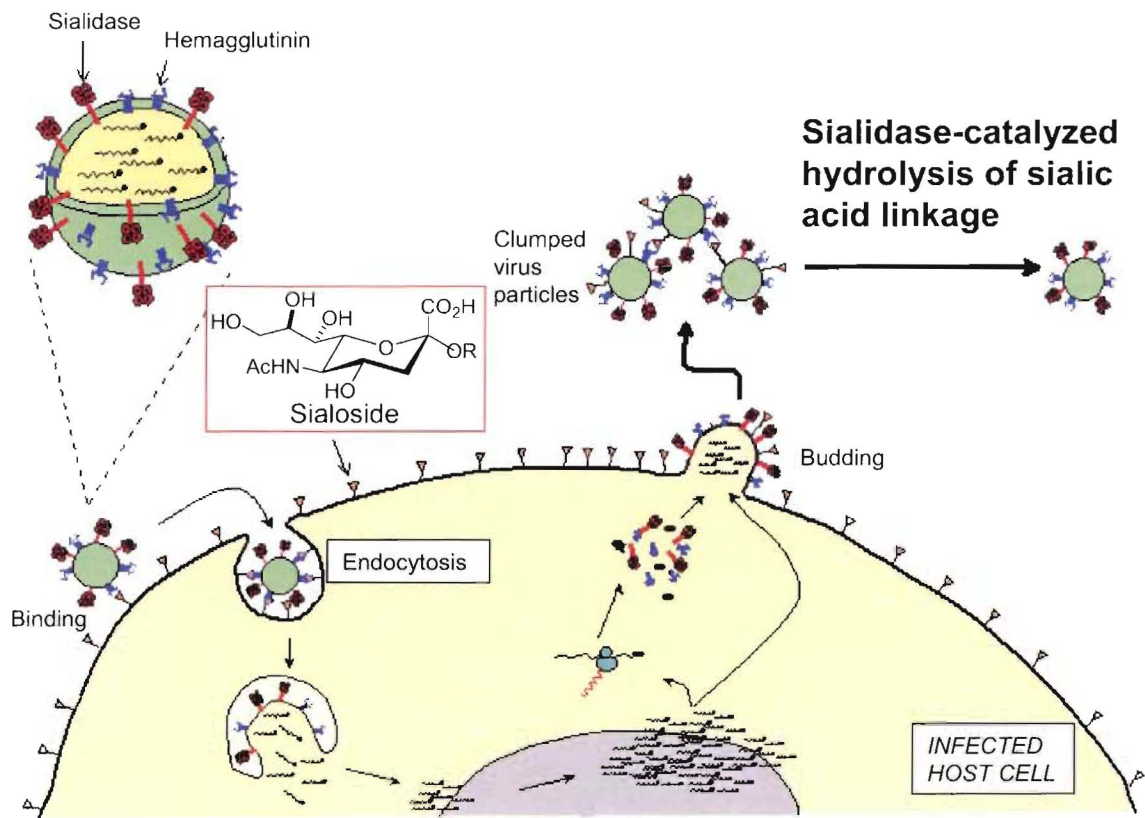


Figure 1.7 Roles of hemagglutinin and sialidase in influenza infection

One of the more prominent examples of the relationship between sialic acids and sialidases is their involvement in influenza infectivity (Figure 1.7). The influenza virus displays two major glycoproteins on its surface, hemagglutinin and a sialidase. The hemagglutinin recognizes and binds to sialic acid residues on the ends of oligosaccharide chains present on cells of the upper respiratory tract.²³ The virus particle is then taken into the host cell via endocytosis where it takes over the genetic machinery of the host cell and is reproduced. As the newly-formed virus particles bud through the host cell membrane, they become coated in host cell sialic acid residues. The hemagglutinin from one virus particle binds to the sialic acid on the host cell and also on neighbouring virus particles. These clumped virus particles cannot infect more cells and present attractive targets for the immune system. The sialidase on the virus particle cleaves the sialic acid residue from the host cell and/or neighbouring virus particles, freeing the progeny virus particles to go on and infect more cells (Figure 1.7).^{16,22}

There are currently 16 hemagglutinin (H) subtypes and 9 sialidase (or neuraminidase, N) subtypes known to be expressed by influenza A virus particles.²⁴ Each of these subtypes has different antigenic properties and the combination of these subtypes gives rise to antigenically-distinct viruses. While numerous combinations exist that give rise to influenza subtypes infecting many animals, influenza pandemics have been caused in humans in the 20th century by the H1N1 subtype in 1918, the H2N2 subtype in 1957, and the H3N2 subtype in 1968. Slight antigenic changes in these subtypes give rise to different influenza strains for which the only prevention is a vaccine that must be

synthesized against every individual strain.²⁵ If an influenza A virus subtype that has never previously infected humans suddenly becomes able to do so (e.g. H5N1 avian influenza), it is likely that many deaths will occur before a vaccine can be produced and distributed to protect the population. Since sialidase action is crucial to influenza infectivity and the active sites of these viral enzymes are all similar, it has been proposed that an inhibitor of influenza sialidase may be an effective treatment for influenza infection.²⁶ This has indeed been shown to be the case, as will be described in Chapter 1.5. These drugs could therefore be of use in the event of another influenza pandemic to provide some measure of protection for the public until a vaccine becomes available.

1.3 Synthesis of Sialic Acid and Positionally-Modified Analogues

The following section (Chapter 1.3) is a reproduction of a review article,²⁷ reprinted with permission from: Hemeon, I.; Bennet, A. J. *Synthesis* **2007**, 1899-1926. Copyright 2007 Georg Thieme Verlag Stuttgart – New York.

This section describes the various synthetic routes taken to synthesize sialic acid as well as sialic acid analogues that have been modified at each of the 9 positions. The figures have been modified from their original versions in order to match the style guidelines of this thesis and the compounds have been re-numbered.

1.3.1 Synthesis of Sialic Acid

The synthesis of sialic acid and its derivatives is often carried out by extending the carbon chain of a stereochemically complex 6-carbon fragment by

3 carbon atoms, often by coupling with a simple 3-carbon unit to form the 9-carbon backbone.^{28,29} Enzymatic syntheses of sialic acid often use a derivative or analogue of *N*-acetylmannosamine (ManNAc) as an electrophilic 6-carbon unit and a pyruvate derivative as a nucleophilic 3-carbon unit and many chemical syntheses emulate this strategy. Thus, the challenge often lies in the synthesis of the ManNAc derivative, commonly using carbohydrate precursors to avoid lengthy synthetic routes from non-carbohydrate/achiral precursors.

1.3.1.1 Enzymatic Syntheses

In mammalian systems, sialic acid biosynthesis involves the phosphorylation of ManNAc at the 6-position using ManNAc 6-kinase.³⁰ The resulting *N*-acetylmannosamine-6-phosphate (ManNAc-6-P) is then coupled with phosphoenolpyruvate by sialic acid 9-P synthetase to afford sialic acid following removal of the 9-phosphate with sialic acid 9-P phosphatase. Another enzyme, sialic acid aldolase (*N*-acetylneuraminate lyase), is responsible for the biodegradation of sialic acid by breaking the C3-C4 bond to generate ManNAc and pyruvate.

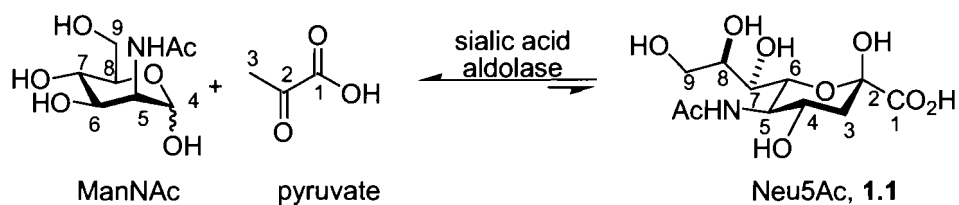


Figure 1.8 Sialic acid synthesis using sialic acid aldolase. Numbers reflect positions of carbon atoms in the sialic acid product.

This aldolase can be used to produce sialic acid chemoenzymatically from pyruvate and ManNAc in good yields by using high reagent concentrations and one of the two components in excess (Figure 1.8).³¹ The group of Serge David pioneered the immobilization of the aldolase on agarose gel, facilitating its separation from other reaction components and its re-use in sialic acid synthesis.³² The aldolase had long been thought to accept only pyruvate as the nucleophilic 3-carbon unit; however, it has recently been shown to accept 3-fluoropyruvate, which can be used for the synthesis of 3-fluorosialic acids.^{33,34} On the other hand, the aldolase has been shown accept a wide range of aldose electrophilic units, allowing a number of sialic acid analogues modified at various positions to be synthesized using this method pioneered by the groups of David³⁵ and Wong.³⁶ The enzyme is selective for the *manno*-configuration (2*S*) at C2 of the aldose since sugars with the *gluco*-configuration at C2 are either poor substrates or are not accepted at all. However, a large number of substitutions at the 2-position are tolerated; for example, aldoses bearing various amido groups at the 2-position are accepted, resulting in the generation of *N*-substituted sialic acids;^{37,38} as well, an amido functionality is not necessary for reactivity. A free hydroxyl group at the 3-position of the aldose is essential for reactivity, not allowing this method to be used for substitution of the ring oxygen of sialic acid with another atom. The configuration at the 3-position influences the stereoselectivity of pyruvate attack on the aldehyde, determining the configuration at C4 of the sialic acid product. Sugars with 3*S*-configuration are attacked to give 4*S*-configured products while 3*R*-configured aldoses are

attacked partially or fully from the opposite face to give 4*R*-configured products. The substitution pattern at other positions of accepted aldoses is highly flexible and a number of D- and L-tetroses, pentoses, and hexoses are accepted.³⁶

1.3.1.2 Chemical Syntheses

Prior to the 1990s, many chemical syntheses of sialic acid emulated the biosynthetic pathway by using a nucleophilic pyruvate derivative to attack an electrophilic aldose.²⁸ Early attempts at the straightforward base-promoted coupling of pyruvate with ManNAc to afford sialic acid were unsuccessful. Replacing pyruvate with oxobutanedioic acid afforded sialic acid following decarboxylation, albeit in 1-2% yield;³⁹ as well, since the stereochemistry of sialic acid had not yet been determined, *N*-acetylglucosamine was used as the electrophile which happened to epimerize to ManNAc under the reaction conditions.³⁹ This procedure was improved by the use of the potassium salt of di-*t*-butyl oxobutanedioate (and ManNAc as the electrophile), followed by treatment with base to induce decarboxylation and afford sialic acid in 34% yield (Figure 1.9, pathway A).⁴⁰ This procedure has been used effectively to generate sialic acid analogues, including its unnatural L-enantiomer in 28% yield as a single isomer at C4 by coupling L-ManNAc with pyruvate and using NiCl₂ to effect the decarboxylation.⁴¹

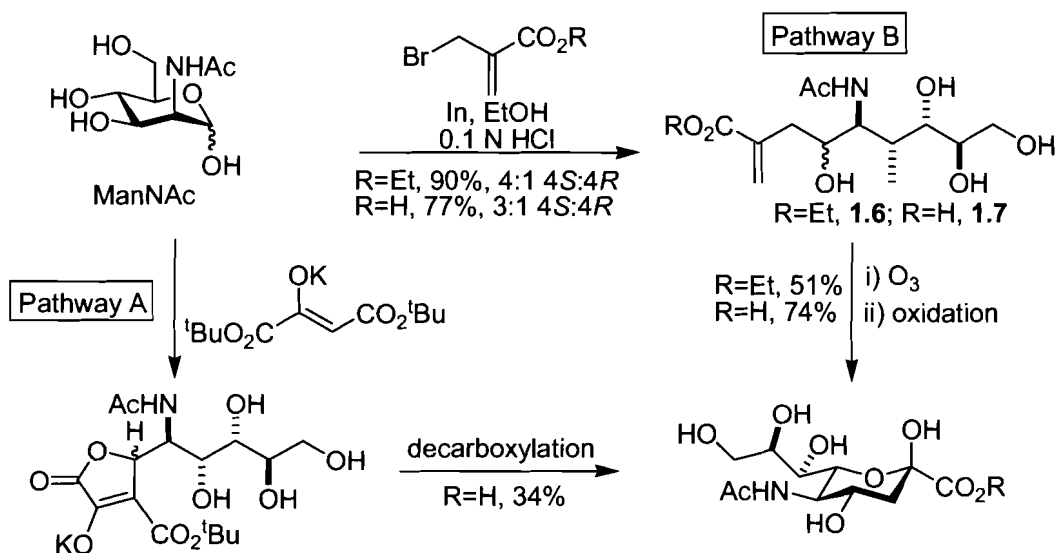


Figure 1.9 Synthesis of sialic acid by coupling ManNAc with di-*t*-butyl oxobutanedioate salts (Pathway A) or alkyl α -(bromomethyl)acrylate esters (Pathway B)

Several other synthetic routes have used α -(bromomethyl)acrylic acid and its esters as pyruvate synthons in nucleophilic zinc- or indium-promoted reactions with ManNAc. The addition products require ozonolytic cleavage of the methylene group at C2 followed by reduction of the ozonides to install the desired α -keto ester moiety. Vasella et al. used Zn/Ag couple to promote the attack of *t*-butyl α -(bromomethyl)acrylate on protected ManNAc which afforded the product in over 90% yield with 80% selectivity for the desired 4*S*-diastereomer, leading to sialic acid after de-esterification followed by ozonolysis and reduction.⁴² Indium has been used to promote coupling of ethyl α -(bromomethyl)acrylate with unprotected ManNAc to afford enoate ester **1.6** as a 4:1 mix of the 4*S*:4*R* isomers in 90% yield, leading to sialic acid ethyl ester following ozonolysis and oxidative decomposition of the ozonides (Figure 1.9, pathway B).⁴³ This procedure has also been used with α -(bromomethyl)acrylic

acid to afford acid **1.7** in 77% yield as a 3:1 4*S*:4*R* diastereomeric mixture which leads directly to following ozonolysis (Figure 1.9, pathway B).⁴⁴ Attack of the allyl indium reagent to form the 4*S*-diastereomer has been proposed to proceed through a Cram chelate wherein the C1-carbonyl and the C2-acetamido nitrogen of ManNAc are held in the same plane by indium, resulting in attack on the less hindered *si*-face to generate the 4*S*-diastereomer preferentially.⁴³

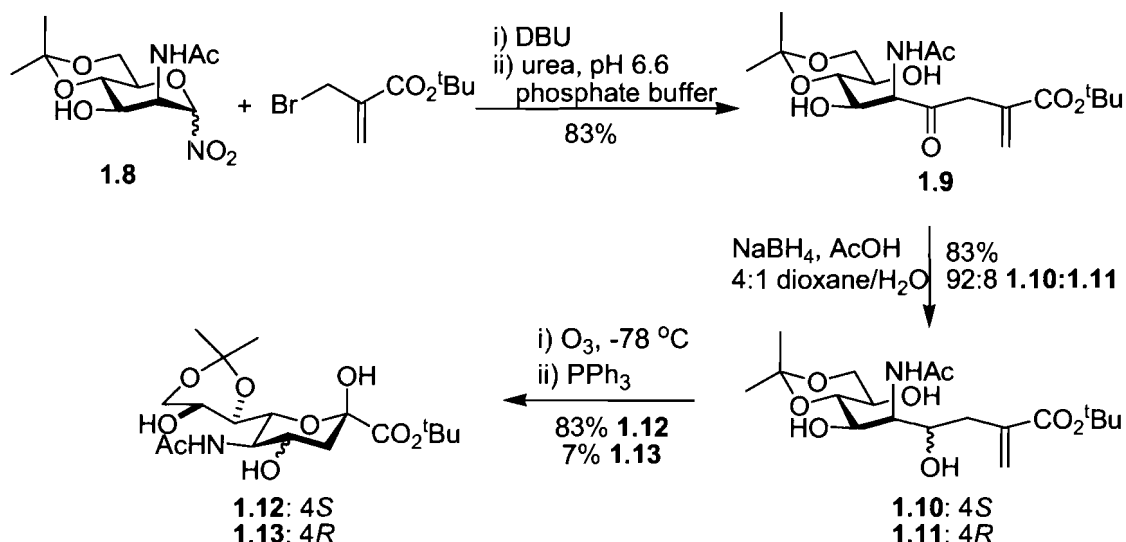


Figure 1.10 Coupling nitroaldopyranose **1.8** with *t*-butyl α -(bromomethyl)acrylate

The same pyruvate synthon has been used as an electrophile in the synthesis of sialic acid, reversing the approach toward the coupling of three- and six-carbon fragments to form the sialic acid backbone (Figure 1.10). Vasella et al. have developed a route involving deprotonation of nitromannosamine derivative **1.8** with the resulting anion performing a Michael addition to *t*-butyl α -(bromomethyl)acrylate.⁴⁵ Following hydrolysis of the nitro group, the resulting ketone **1.9** could be reduced with NaBH₄ and 2 eq. AcOH in 4:1 dioxane/water to give a 92:8 mixture of alcohols **1.10** and **1.11** in 83% yield. In the absence of

AcOH, the reduction proceeded to give the opposite stereoselectivity (15:85 4*S*:4*R*). The authors rationalized these observations by the potential formation of a hydrogen bond between the acetamido group and the carbonyl being reduced in the absence of AcOH.⁴⁵ This synthetic route allows for variation of the substituents from carbons 5-9 depending on the nitroalditol used and has also been used to generate L-sialic acid.⁴⁶ A complementary route has been developed by Vasella et al. wherein the 6-carbon fragment becomes carbons 1-6 of sialic acid and the 3-carbon unit becomes the glycerol chain in the product (Figure 1.11).⁴⁷ The anion generated from deprotonation of nitroglucosamine derivative **1.14** was reacted with cyclohexylidene glyceraldehyde to install the glycerol side chain of sialic acid. The resulting nitroalcohol **1.15** was obtained as a single diastereomer in 85% yield, possessing the desired chirality at both newly-formed stereocentres (carbons 6 and 7 in the final sialic acid product). Radical denitration and acetylation gave **1.16** that was oxidized at the primary OH-group after removal of the benzylidene acetal, installing the carboxylate group at C1. Elimination of HOAc between C2 and C3 gave glycal **1.17** that was brominated with NBS in methanol to give compound **1.18**. Radical debromination and deprotection afforded sialic acid.

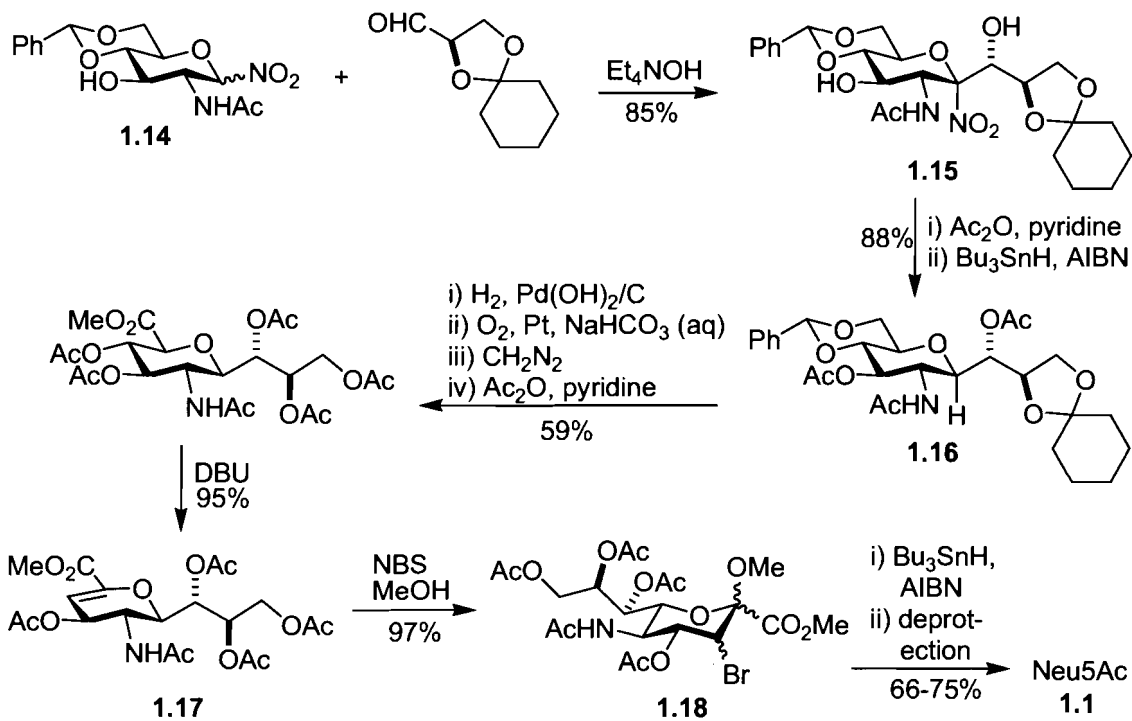


Figure 1.11 Coupling nitroaldopyranose 1.14 with cyclohexylidene glyceraldehyde to synthesize Neu5Ac

Several less conventional syntheses of sialic acid analogues have been developed that do not involve the coupling of 3- and 6-carbon units. One such route involved a cyclocondensation between an aldehyde and the complex diene **1.19** to form a substituted dihydropyranone ring, eventually leading to sialic acid *via* a synthetic route developed by Danishefsky and co-workers (Figure 1.12); this is one of the few routes to use non-carbohydrate-based starting materials. Their initial route used achiral benzyloxyacetaldehyde to generate the substituted tetrahydropyran ring as a pair of enantiomers, resulting in the preparation of racemic sialic acid.⁴⁸ A modified synthesis used a chiral aldehyde, *S*-2-(phenylseleno)propionaldehyde (**1.20**), in the cyclocondensation to prepare a diastereomeric mixture of substituted six-membered rings from which the desired

isomer **1.21** could be separated as the chirality of the 2-phenylseleno moiety imparted diastereofacial selectivity to the cyclocondensation.⁴⁹ By using $\text{BF}_3 \cdot \text{OEt}_2$ in CH_2Cl_2 to promote the cyclocondensation at $-78\text{ }^\circ\text{C}$, the desired cis-isomer **1.21** was obtained in 63% yield with 95% optical purity (13% of the trans-isomer **1.22** was also produced). Luche reduction of ketone **1.21** to the 4S alcohol was followed by the acid-catalyzed addition of methanol across the double bond to give methyl glycoside **1.23**. After protecting the 4-OH as a TBDMS ether, the phenylseleno group was oxidatively eliminated with H_2O_2 to afford alkene **1.24**. This was dihydroxylated with OsO_4 and the diol was cleaved with $\text{Pb}(\text{OAc})_4$ to give aldehyde **1.25**. The aldehyde was subjected to a Horner-Wadsworth-Emmons reaction using $(\text{CF}_3\text{CH}_2\text{O})_2\text{P}(=\text{O})\text{CH}_2\text{CO}_2\text{Me}$ to afford (*Z*)-alkene **1.26** that possesses a terminal methyl carboxylate moiety in 80% yield. Dihydroxylation with OsO_4 in pyridine afforded diol **1.27** with 20:1 selectivity for the desired 7*S*,8*S*-configuration, presumably through OsO_4 -attack on the face of the double bond that was *anti* to the pyranose ring. The methyl ester was reduced with LiEt_3BH and the product was perbenzoylated. At this point, the furyl moiety at the anomeric centre (a masked carboxylate) was oxidized with RuO_4 and treated with CH_2N_2 to afford **1.28** as a methyl ester. The OH-group at C5 was then deblocked by treating the TBDMS ether with HF in methanol which resulted in benzoyl migration from C5 to C4. The 5-OH group was activated as a triflate ester that was reacted with tetra-*n*-butylammonium azide. The azido-substituted product **1.29** was reduced, acetylated, de-benzoylated, and de-esterified to afford sialic acid.

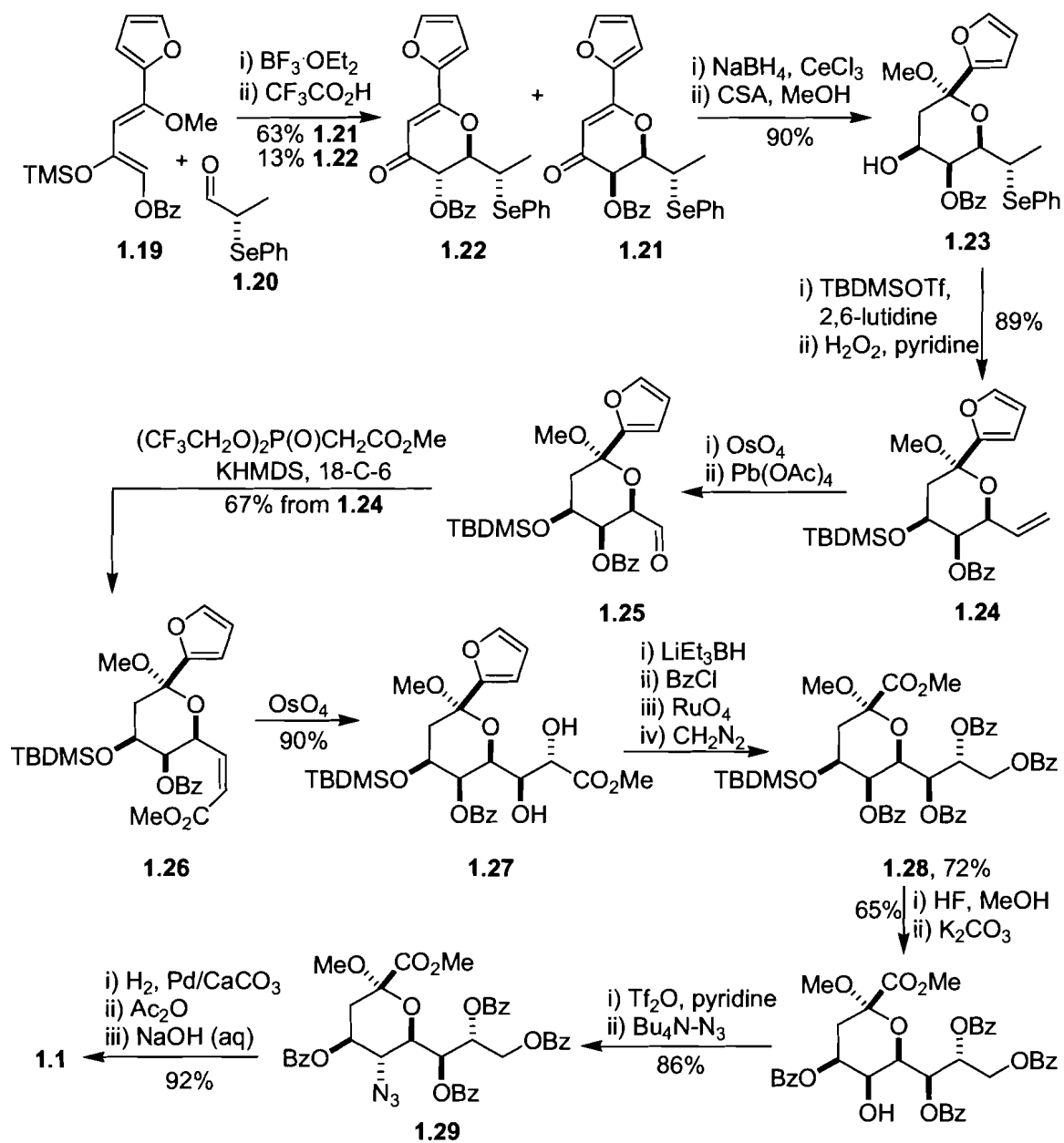


Figure 1.12 Synthesis of Neu5Ac via a cyclocondensation between diene 1.19 and aldehyde 1.20

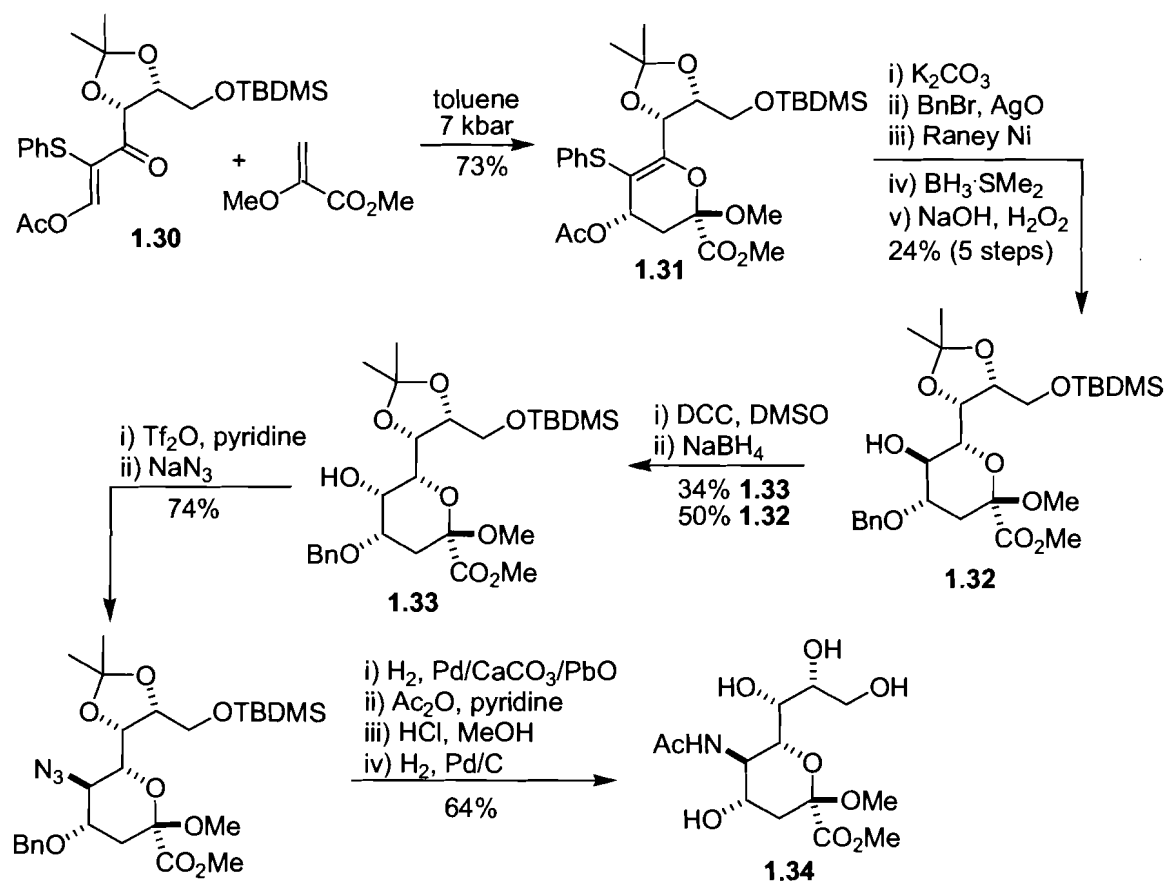


Figure 1.13 Synthesis of Neu5Ac via cycloaddition of heterodiene **1.30** and methyl α -methoxyacrylate

Another synthesis that makes use of a cycloaddition to form a pyranose ring was developed by Schmidt and co-workers (Figure 1.13).⁵⁰ The α,β -unsaturated ketone **1.30** was prepared from mannose and used as a heterodiene in an inverse electron demand hetero-Diels-Alder reaction with methyl α -methoxyacrylate under pressure (7 kbar) to construct a six-membered dihydropyran ring. The diastereofacial selectivity was 9:1 in favour of the desired isomer **1.31**. The product was carried through a number of synthetic steps involving deacetylation and benzyl protection, removal of the 5-thiophenyl group, and hydroboration to give alcohol **1.32**. As the hydroboration occurred from the

top face of the dihydropyran ring, the stereochemistry was correct at C6 but it was necessary to invert the new 5-OH group *via* an oxidation/reduction sequence. The resultant OH-group of **1.33** could be activated as a triflate ester that was displaced with azide to install the acetamido moiety with the proper stereochemistry after reduction of the azide and acetylation. Removal of the TBDMS, isopropylidene, and benzyl protecting groups then led to **1.34**, the methyl glycoside of sialic acid, methyl ester.

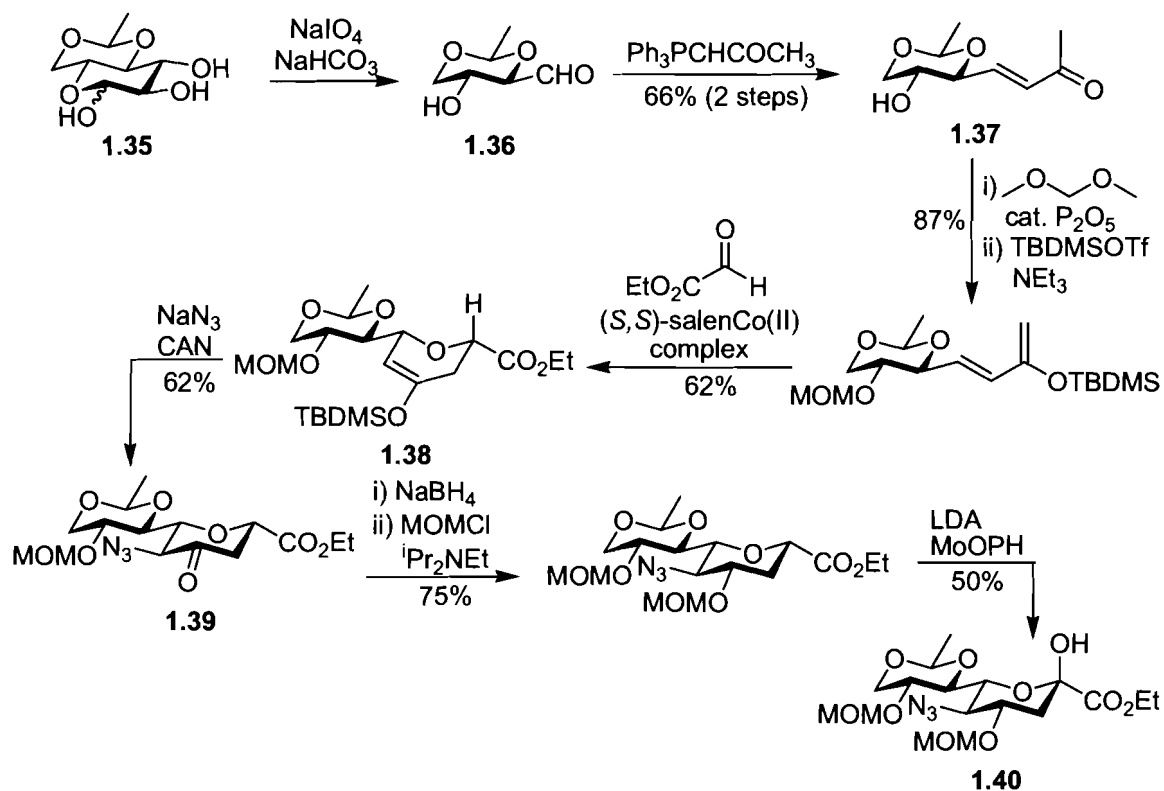


Figure 1.14 Cycloaddition of D-glucose-derived diene and ethyl glyoxylate leading to advanced intermediate **1.40** en route to Neu5Ac

D-Glucose has been used as a starting material to synthesize a diene for another route to sialic acid involving a cycloaddition (Figure 1.14).⁵¹ The 4- and 6-OH groups were protected with acetaldehyde to afford acetal **1.35** that was

treated with NaIO_4 to give aldehyde **1.36** on which a Wittig reaction was performed with $\text{Ph}_3\text{P}=\text{CHCOCH}_3$ to generate enone **1.37**. An enolate generated from this ketone was trapped as its TBDMS ether that was subsequently used as a diene in an (*S,S*)-salenCo(II)-catalyzed hetero-Diels-Alder reaction with ethyl glyoxylate. The 6*R*-dihydropyran **1.38** (sialic acid numbering) was obtained in 62% yield along with <5% of another isomer. The TBDMS enol ether was then treated with NaN_3 and CAN to generate α -azido ketone **1.39** with 5*S*-stereochemistry in 62% yield (the 5*R*-diastereomer was isolated in 8% yield). 2-Deoxysialic acid derivatives were synthesized from this intermediate; of note, after stereoselective reduction of the 4-keto group and protection of the resulting OH-group as a MOM ether, oxidation of the lithium enolate at C2 with $\text{MoO}_5\cdot\text{Py}\cdot\text{HMPA}$ (MoOPH) installed an OH-group at the anomeric centre to give **1.40** that was converted to protected sialic acid after reduction and acetylation of the 5-azido group.

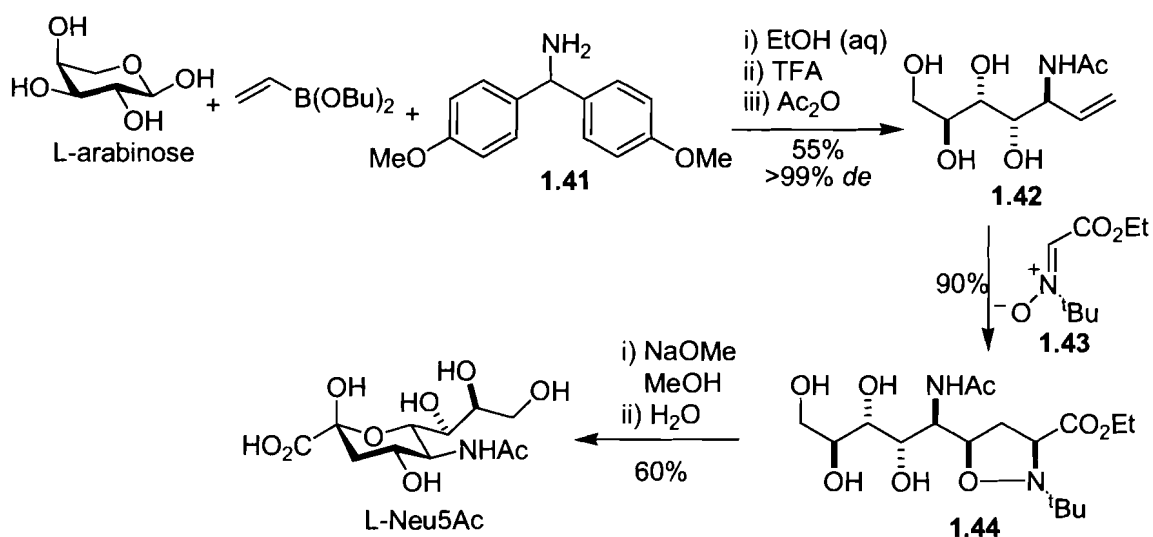


Figure 1.15 1,3-Dipolar cycloaddition on alkene **1.42** leading to L-sialic acid

A route leading to L-sialic acid has been developed which involves a diastereoselective 1,3-dipolar cycloaddition.⁵² This fairly straightforward route started with a three-component reaction involving imine formation between amine **1.41** and the aldehyde group of L-arabinose followed by addition of a vinylboronate ester to the imine group (Figure 1.15). Addition of the vinyl group afforded the desired *S*-stereochemistry at the amine centre, attacking exclusively from the *si*-face of the imine presumably due to coordination of the boronate ester with the neighbouring OH-group. After conversion of the *bis-p*-methoxybenzylamino-substituted product to acetamide **1.42**, the vinyl group was used in a 1,3-dipolar cycloaddition with *N-t*-butyl-substituted nitron **1.43** to afford a 9-carbon sugar containing a 5-membered ring as a 10:1 mixture of diastereomers, the desired *4R*-isomer **1.44** being the major product. Deprotonation at C2 formed an imino group at this position with cleavage of the N-O bond. Following hydrolysis of the *N-t*-butylimino group to a ketone, the product cyclized to the pyranose form of L-sialic acid. This route has also been used to synthesize D-sialic acid using D-arabinose as a starting material as well as other sialic acid analogues including the 7-carbon truncated analogue from D-glyceraldehyde, the 10-carbon analogue from D-galactose, and *N*-glycolylneuraminic acid (Neu5Gc) by acylating the precursor of **1.42** with a 2-acetoxyacetate ester instead of with acetic anhydride.⁵²

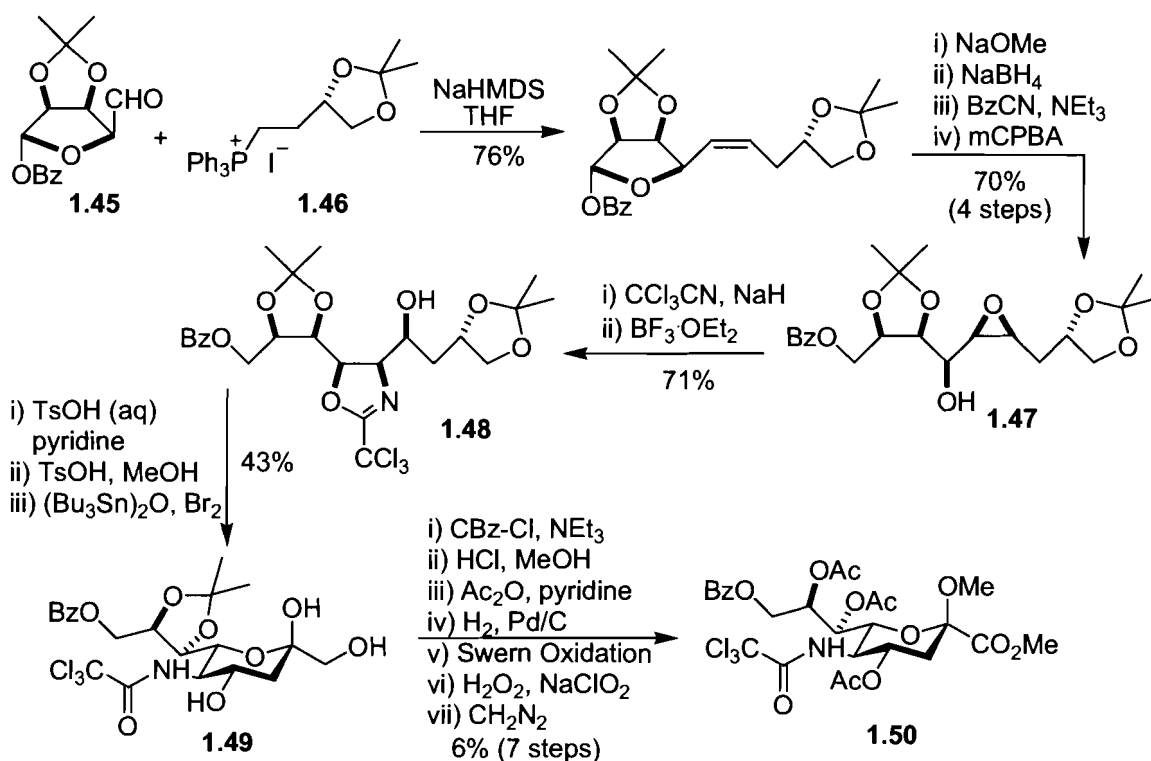


Figure 1.16 Wittig reaction between aldehyde **1.45** and phosphonium ylide **1.46** leads to advanced intermediate en route to Neu5Ac

In addition to their cycloaddition route, Schmidt and co-workers have developed a route to sialic acids involving a *cis*-selective Wittig reaction between the *D-arabino*-configured 5-carbon aldehyde **1.45** and the 4-carbon phosphonium ylide **1.46** using NaHMDS (Figure 1.16).⁵³ Following removal of the anomeric benzoyl group, reduction to the alditol, and protection of the new primary OH-group using benzoyl cyanide, the alkene was treated with mCPBA to generate epoxide **1.47** as a single isomer. The remaining OH-group was transformed into a trichloroacetimidate moiety, the nitrogen of which was used to effect a regioselective *exo*-opening of the epoxide to form oxazoline **1.48**. The oxazoline ring was opened with inversion of configuration at C6 and the 1,2-O-isopropylidene was selectively removed, after which the secondary hydroxyl

group was selectively oxidized using $(\text{Bu}_3\text{Sn})_2\text{O}$ and bromine. The spontaneously-formed pyranose **1.49** was then CBz-protected at the primary OH-group and turned into the methyl glycoside which was then peracetylated and deprotected at C1 *via* hydrogenolysis. The carboxylate moiety was then installed at C1 through a Swern oxidation to an aldehyde followed by a second oxidation to the acid with $\text{NaClO}_2/\text{H}_2\text{O}_2$ and this group was subsequently esterified with diazomethane to give **1.50**. Radical dechlorination with Bu_3SnH and AIBN installed the 5-acetamido group, giving the sialic acid structure as an acetylated/benzoylated methyl glycoside, methyl ester.

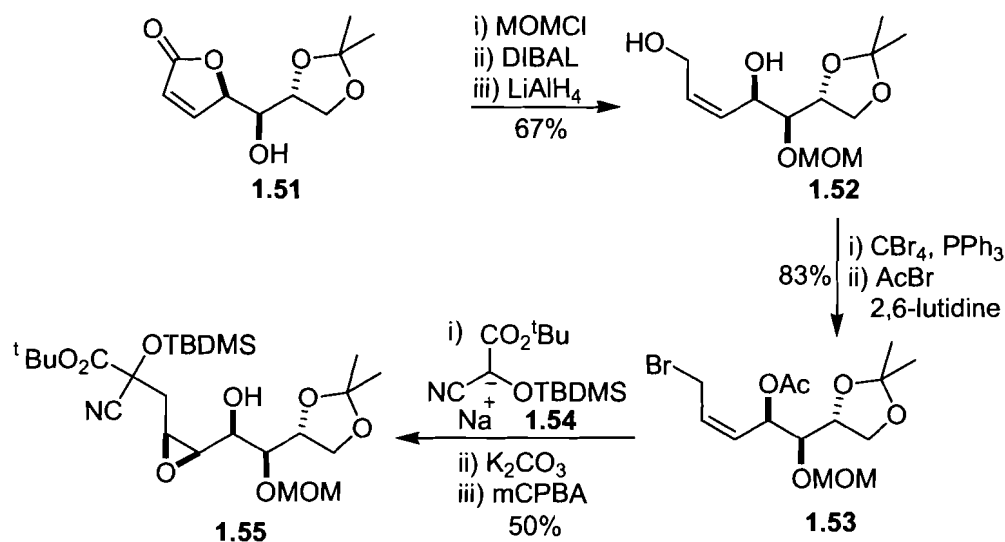


Figure 1.17 Synthesis of advanced intermediate **1.55** en route to Neu5Ac

Another synthesis of sialic acid using a similar strategy to install the 4, 5, and 6-substituents has been reported by Takahashi et al.; however, the alkene precursor was made by a different protocol (Figure 1.17).⁵⁴ Beginning with MOM-protection of the α,β -unsaturated lactone **1.51** (derived from *D-glycero-D-gulo*-heptose-1,4-lactone), the lactone moiety was reduced to an allylic 1,4-diol

1.52 using DIBAL followed by LiAlH₄. After conversion to the allylic bromide **1.53**, the carbon chain was extended to 9 atoms by treatment with the anion of *t*-butyl 2-*t*-butyldimethylsiloxy-2-cyanoacetate **1.54**. After deacetylation, the introduction of the 4-OH and 5-acetamido groups followed the same strategy of Schmidt (see Figure 1.16)⁵⁰ involving stereoselective formation of epoxide **1.55** from the *Z*-olefin with MCPBA in 53% yield. The 6-OH group was converted into a trichloroacetimidate moiety, which was used to regio- and stereoselectively open the epoxide to install the 4*S*-OH group which was protected as a MOM ether. The *cis*-2-oxazoline ring was opened with acid to give a trichloroacetamido group which after reduction with Bu₃SnH and AIBN gave the 5-acetamido and 6-OH groups with the desired stereochemistries in 64% yield (see Figure 1.16). Desilylation at C2 with TBAF afforded the α-keto ester moiety, allowing cyclization to the pyranose to give a protected form of sialic acid.

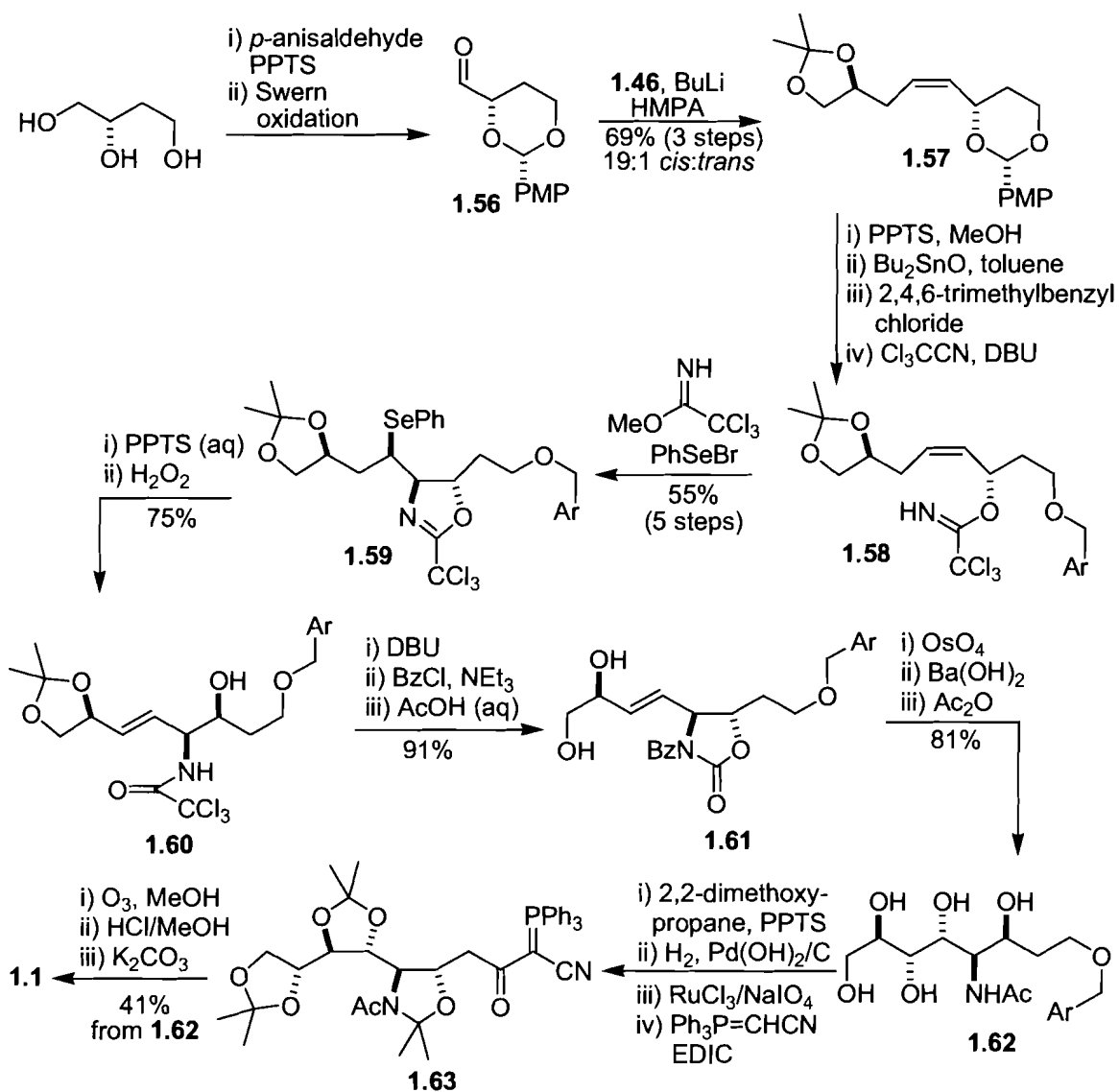


Figure 1.18 Synthesis of Neu5Ac from (*S*)-butane-1,2,4-triol

A recent synthetic route to sialic acid using non-carbohydrate precursors was developed that involved synthetic manipulations on an 8-carbon unit containing an internal *cis*-double bond (**1.57**, Figure 1.18).⁵⁵ This compound had been prepared from (*S*)-butane-1,2,4-triol forming a 2,4-*O*-acetal linkage with *p*-anisaldehyde followed by oxidation under Swern conditions to aldehyde **1.56** that was subsequently used in a *cis*-selective Wittig reaction with phosphonium salt

1.46 (see Figure 1.16).⁵⁶ After hydrolysis of the acetal moiety in **1.57** and differential protection of the OH-groups gave **1.58**, this alkene was treated with phenylselenenylbromide in the presence of methyl trichloroacetimidate, resulting in the addition across the double bond giving *trans*-oxazoline **1.59** in 67% yield along with a small amount (9%) of by-product resulting from formation of the *cis*-oxazoline ring.⁵⁵ The oxazoline ring was hydrolyzed to form trichloroacetamido and phenylseleno groups; the phenylseleno group was oxidatively eliminated to generate *trans*-alkene **1.60**. Cyclization of the 4-OH group onto the trichloroacetamido substituent followed by benzoylation, then removal of the isopropylidene group generated alkene **1.61**. This was stereoselectively dihydroxylated with OsO₄ to give an 81% yield of an 18:1 diastereomeric mixture of products, the major isomer of which was converted to acetamide **1.62**. Installation of the α -keto ester moiety was accomplished by triacetone protection, hydrogenolysis of the primary benzyl ether followed by RuCl₃/NaIO₄ oxidation to the acid, then coupling the acid with Ph₃P=CHCN. The resulting phosphorane **1.63** was treated with ozone in methanol to install a carboxylate moiety; the compound then cyclized to the pyranose after removal of the protecting groups.

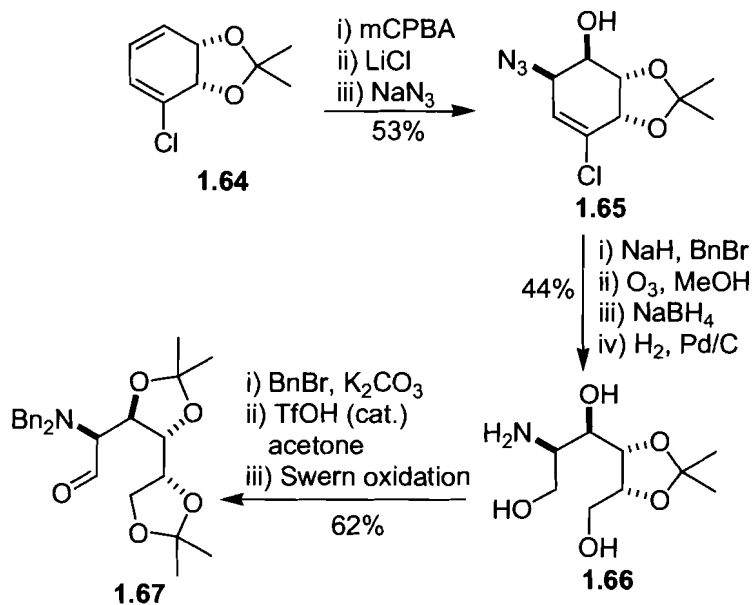


Figure 1.19 Synthesis of protected ManNAc derivative **1.67** from dihydrocatechol **1.64**

A protected ManNAc analogue has been prepared from an enantiopure *cis*-1,2-dihydrocatechol that was obtained from microbial oxidation of chlorobenzene (Figure 1.19).⁵⁷ Protection of the diol to give **1.64** was followed by conversion to an epoxide *trans* to the diol which was subsequently opened with lithium chloride. The chloro-substituent was displaced using sodium azide, inverting the stereochemistry at this centre to afford azido alcohol **1.65**.⁵⁸ The free OH-group was benzylated and the remaining C-C double bond was ozonolyzed to generate a linear diol that was treated with hydrogen to cleave the benzyl ether and to reduce the azido group to an amino group, giving **1.66**. After benzyl protection of the amino group, treatment with a catalytic amount of TfOH in acetone resulted in reassignment of the isopropylidene group to give the 3,4-5,6-di-*O*-isopropylidene-protected derivative. The free primary OH-group was oxidized under Swern conditions to ManNAc analogue **1.67**. This was

elaborated to the sialic acid structure using ethyl α -(bromomethyl)acrylate with zinc dust to afford the desired 9-carbon backbone as described previously (see Figure 1.9). Although the yield of the coupling reaction was good (90%), the major product was the 4*R*-diastereomer (85%). As a consequence, the 4-OH group was inverted *via* Swern oxidation followed by NaBH₄ reduction and was immediately protected as a TMS ether to give the desired 4*S*-diastereomer in 60% yield with 7% of the 4*R*-diastereomer. Sialic acid was then obtained by ozonolysis of the methylene unit at C2 followed by removal of the isopropylidene and benzyl protecting groups and acetylation of the amino group.

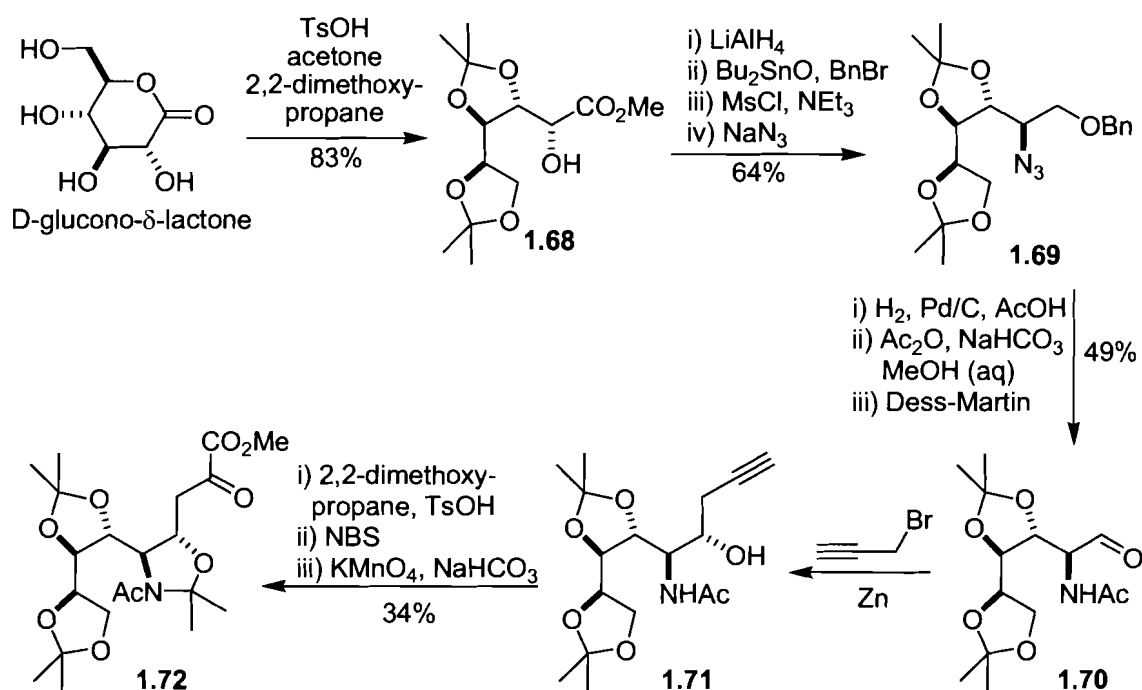


Figure 1.20 Synthesis of protected ManNAc 1.70 from D-glucono- δ -lactone

Synthesis of a protected ManNAc has also been carried out from D-glucono- δ -lactone and was used to synthesize sialic acid *via* stereoselective propargylation and oxidation of the terminal alkyne (Figure 1.20).⁵⁹ The

protected ManNAc was synthesized by converting D-glucono- δ -lactone to the protected derivative **1.68** which was reduced to a 1,2-diol. The primary alcohol was protected as a benzyl ether while the secondary alcohol was converted to a mesylate and displaced with sodium azide, thus inverting the stereochemistry at C2 to give the *manno*-configured azide **1.69**. Reduction of the azide to an amine with hydrogen also cleaved the benzyl ether, unmasking the primary OH-group which was oxidized to aldehyde **1.70** following conversion of the amino group to an acetamido group. The protected ManNAc was then elaborated to the sialic acid structure beginning with treatment with propargyl bromide in the presence of zinc dust, affording **1.71** in 74% yield and the *4R*-diastereomer in 11% yield. Installation of a third isopropylidene protecting group across the 4-OH group and the acetamido nitrogen allowed bromination of the terminal alkyne with NBS which was followed by a basic KMnO_4 oxidation of the alkyne unit to the α -keto methyl ester **1.72**. Removal of the isopropylidene protecting groups resulted in cyclization to the pyranose ring, affording the methyl ester of sialic acid.

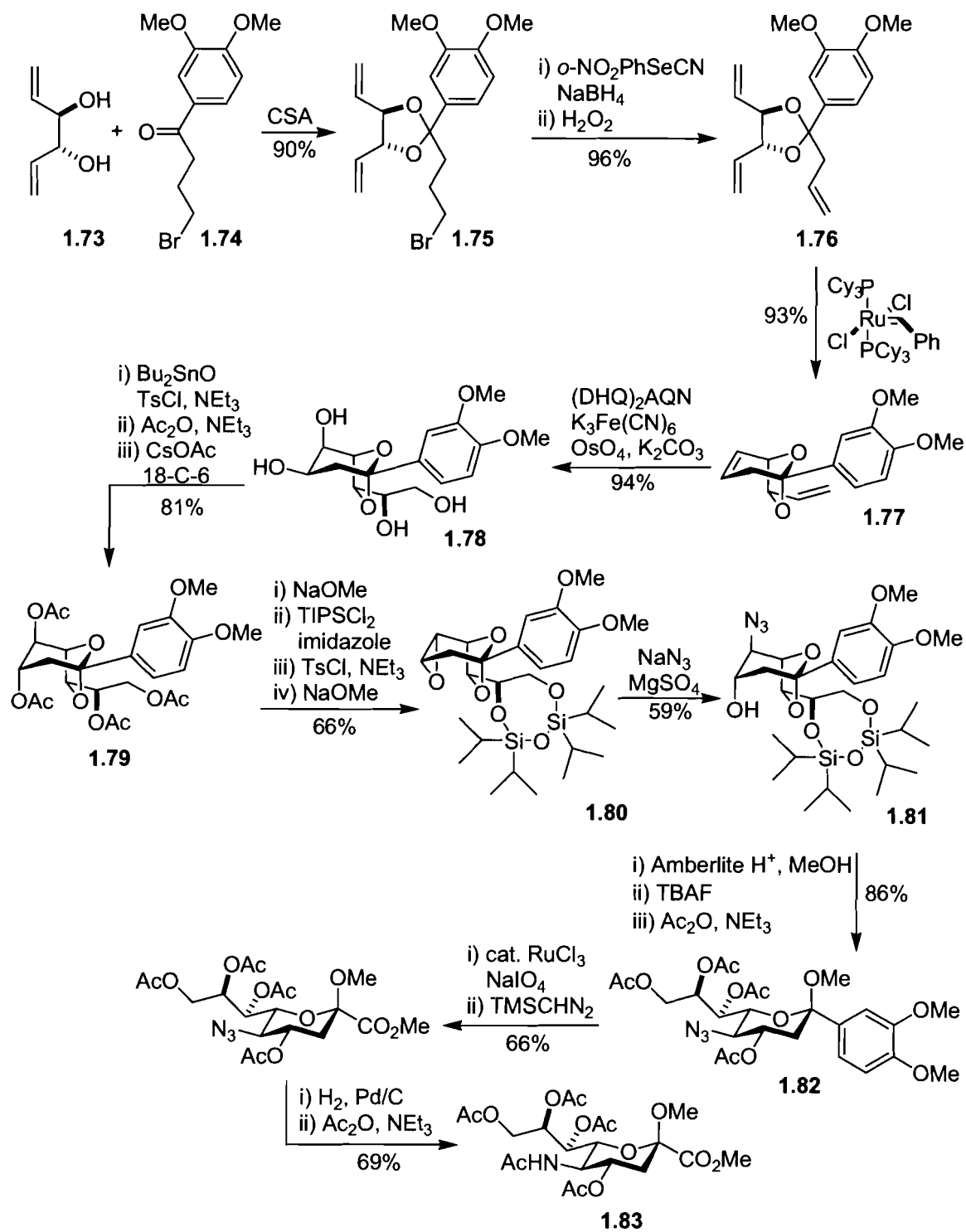


Figure 1.21 Synthetic route to Neu5Ac involving formation of intramolecular ketal **1.75** and ring-closing metathesis of triene **1.76**

An interesting route was developed involving intermolecular formation of a ketal moiety followed by intramolecular ring-closing metathesis to form a 6,8-dioxabicyclo[3.2.1]octane ring system that was further functionalized to sialic acid (Figure 1.21).^{60,61} The intermolecular ketal was formed between diene diol **1.73** (synthesized in four steps from D-mannitol)⁶² and γ -bromoketone **1.74** (synthesized *via* a Friedel-Crafts reaction between veratrole and 4-bromobutyryl chloride).⁶¹ The γ -bromo substituent of ketal **1.75** was then displaced with *o*-nitrophenylselenide which was then oxidized and eliminated to form triene **1.76**. Ring-closing metathesis gave bicyclic **1.77** containing a 3,4-dimethoxyphenyl group as a surrogate for a carboxylate moiety. Two Sharpless asymmetric dihydroxylation reactions were carried out with OsO₄ using (DHQ)₂AQN as a chiral ligand to afford tetrol **1.78** as a single diastereomer in 94% yield. The stereochemistry of the 4-OH group was inverted first by treating the compound with dibutyltin oxide and *p*-toluenesulfonyl chloride, selectively turning the more reactive 9-OH and the equatorial 4-OH groups into tosylate esters, followed by peracetylation then treatment with cesium acetate and 18-crown-6 to displace both tosylate esters with acetates, affording **1.79**. After deacetylation, the 8- and 9-OH groups were protected as a cyclic silyl ether using TIPSCl₂. The more reactive *exo*-5-OH group was converted into a tosylate ester that was displaced by the *endo*-4-OH group under basic conditions to form epoxide **1.80**. Ring opening with sodium azide gave **1.81**, resulting from attack at C5 from the axial position in the presence of MgSO₄ (presumably due to the formation of a chair-like transition state). The 2,7-anhydro linkage was opened using Amberlite H⁺

resin in methanol to afford the methyl glycoside and after removal of the silyl protecting groups and subsequent peracetylation, **1.82** was obtained. The 3,4-dimethoxyphenyl substituent at C1 was converted into a methyl carboxylate moiety by oxidative cleavage with $\text{RuCl}_3/\text{NaIO}_4$ and the 5-azido group was transformed into an acetamido group to afford the protected sialic acid **1.83**.

1.3.2 Substituted Sialic Acid Analogues

Development of synthetic routes to sialic acid is important; however, sialic acid is available commercially at reasonable cost. Synthetic routes to sialic acid are perhaps more important for their potential versatility in synthesizing variously-substituted analogues for a number of purposes simply by modifying the functionality of the synthetic precursors. For example, syntheses that involve chain-extension of a ManNAc analogue can be used with other aldehyde-containing substrates to give products containing the α -keto ester moiety and an unsubstituted carbon at the 3-position. In addition, sialic acid itself can be used as a starting material for the synthesis of many derivatives.

1.3.2.1 Substitution at C1

The carboxylate moiety at C1 is essential for binding to occur to numerous sialic acid-specific proteins and is partially responsible for many of the biological functions of sialosides due to the negative charge present under physiological conditions. Derivatives at C1 that remove the negative charge of the carboxylate are useful for evaluating its contributions to enzyme binding and receptor interactions. The carboxylate moiety has been derivatized as a carboxamido

group (**1.84**) through ammonolysis of the methyl ester and it has also been reduced to a hydroxymethyl group (**1.85**) by treating the methyl ester with borohydride salts, both reactions being performed on alkyl glycosides (Figure 1.22).^{63,64} Such derivatizations have also been performed on sialic acid-containing glycoconjugates; for example, the sialic acid present on human ganglioside GM1 has been derivatized by converting the C1-carboxylate into various C1-amido groups in order to determine the importance of the carboxylate for receptor binding.⁶⁵ These amido linkages have been prepared by reacting methyl esters with ammonia, methylamine, ethylamine, propylamine, and benzylamine; the methyl ester of GM1 has also been reduced with NaBH₄ to the alcohol.⁶⁵

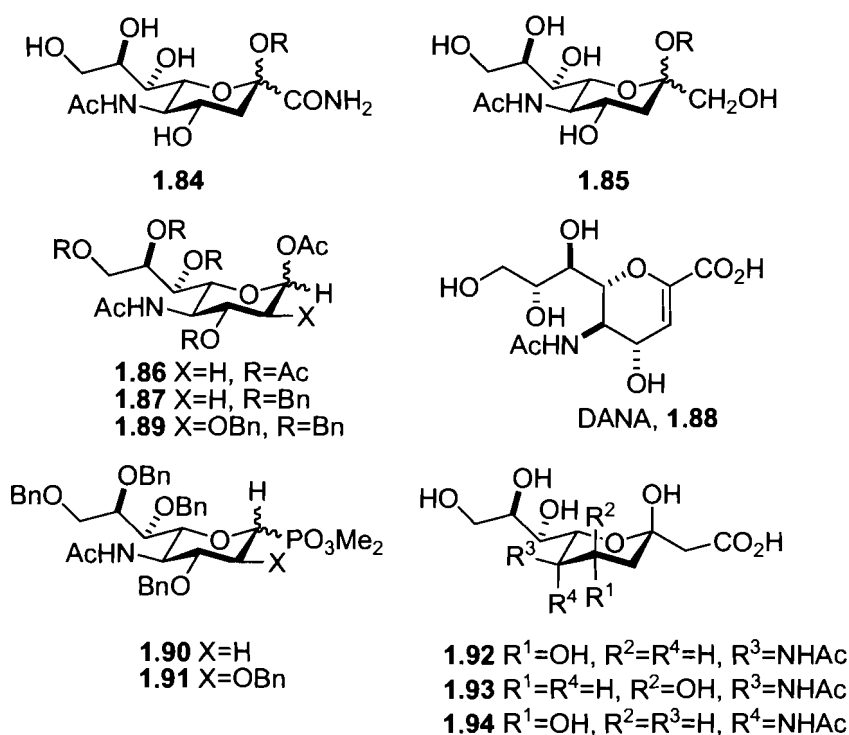


Figure 1.22 C1-modified sialic acid derivatives and the glycol of sialic acid, DANA (1.88)

The carboxylate moiety has also been removed entirely and replaced with a proton to afford an 8-carbon sugar (Figure 1.22). The treatment of the pentaacetate of sialic acid with $\text{Pb}(\text{OAc})_4$ led to the isolation of an anomeric mixture of 8-carbon sugars **1.86** bearing a proton and an acetyl group at the anomeric centre, albeit in low yield (13%).⁶⁶ The decarboxylation of the 2-deoxy analogue of sialic acid using $\text{Pb}(\text{OAc})_4$ gives the same 8-carbon sugar **1.87** but in much higher yield (65%) as a 9:1 mixture of anomers in which the acetate is axial and equatorial, respectively.⁶⁷ The 2-deoxysialic acid used in this synthesis was obtained by the Pd-catalyzed hydrogenation of the protected glycal of sialic acid (2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid, DANA **1.88**) in aqueous methanol, which afforded the pseudo- β anomer exclusively. The decarboxylation of protected pseudo- β -2-deoxy-3-benzyloxysialic acid also proceeds smoothly using $\text{Pb}(\text{OAc})_4$ to give decarboxylated product **1.89** in 74% yield as a 3.3:1.0 $\text{OAc}_{\text{ax}}:\text{OAc}_{\text{eq}}$ anomeric mixture.⁶⁸

Sialic acid analogues bearing a phosphonate group at the anomeric centre in place of the carboxylate moiety have been synthesized to investigate the effects of replacing the anionic carboxylate moiety with another negatively-charged functional group (Figure 1.22). The decarboxylated **1.87** with an axial anomeric acetate was reacted with $\text{P}(\text{OMe})_3$ in the presence of TMSOTf, giving a 68% yield of dimethyl phosphonates **1.90** bearing equatorial and axial dimethyl phosphonate groups in a 1.3:1.0 ratio, respectively, which could be hydrolyzed to the free acids.⁶⁷ A similar compound bearing a 3-benzyloxy (**1.91**) group has been synthesized en route to a DANA-analogue bearing a phosphonate group in

place of the carboxylate moiety.⁶⁸ This compound was synthesized from the 3-alkoxy decarboxylated analogue **1.89** in the same fashion as **1.90** was synthesized from **1.87** except that the anomeric acetyl group of **1.89** required conversion to a trichloroacetimidate moiety for reaction with P(OMe)₃ and TMSOTf, affording **1.91** as a 1:1 anomeric mixture in 44% yield. These products would eventually lead to the DANA analogue after replacing the benzyl protecting groups with acetyl groups, installing an anomeric bromo substituent *via* photobromination, then performing a reductive elimination using Zn/Cu in ethanol to form the glycal.

A chain-extended 10-carbon sialic acid analogue **1.92** has also been synthesized in which a methylene unit has been inserted between the carboxylate group and the anomeric centre.⁶⁹ This compound was made by reacting isopropylidene-protected ManNAc with disodium acetonedicarboxylate in the presence of Ni²⁺ at pH 7, (similar to the reaction with potassium di-*t*-butyloxaloacetate for sialic acid synthesis, mentioned above in Figure 1.9 Pathway A).⁴⁰ The products of this reaction undergo decarboxylation, which following removal of the isopropylidene protecting group affords sialic acid analogues with a CH₂CO₂H group at the anomeric centre. The products were obtained in 27% yield as a 1.8:1.7:1.0 mixture of **1.92:1.93:1.94**, the 5-epimer **1.93** resulting from attack on the *re*-face of ManNAc rather than the *si*-face and the 6-epimer **1.94** resulting from epimerization of the acetamide carbon under the reaction conditions. Unfortunately, **1.92** readily decarboxylated to give a sialic

acid analogue bearing a methyl group in place of the carboxylate moiety; however, **1.93** and **1.94** are less labile, as is the ammonium salt of **1.92**.⁶⁹

1.3.2.2 Variation at C3

The impact of a 3-substituent on binding to various enzymes has been investigated, often using protected versions of DANA as the starting material for electrophilic addition reactions across the C2/C3 double bond (Figure 1.23). 3-Fluorosialic acids are particularly useful as they can be used to gather structural information since the electron-withdrawing 3-fluoro substituent deactivates the anomeric centre towards nucleophilic substitution reactions. These compounds can be used to capture nucleophilic residues in enzyme active sites, allowing identification of the catalytic nucleophile since the enzyme-sialosyl intermediate species is relatively inert to hydrolysis due to the 3-fluoro substituent.^{17,21} As mentioned previously in Chapter 1.3.1.1, 3-fluorosialic acid **1.95** can be made enzymatically using sialic acid aldolase to couple 3-fluoropyruvate with ManNAc to give the 3-fluoro substituent in the axial position.^{33,34} The same compound can be chemically synthesized, initially accomplished by reacting 3-fluoropyruvate with ManNAc in aqueous solution at pH 11 to give **1.95** in 3% yield.⁷⁰ Since then, conditions have been developed that give higher yields that often involve addition of an electrophilic fluorinating agent across the C2/C3 double bond of protected DANA. The direct addition of fluorine across the alkene moiety gave the β -*cis*-2,3-difluorinated product **1.96** as fluorine approached from the less-hindered top face of DANA;^{71,72} this also occurred when XeF₂ was used in a BF₃·OEt₂-catalyzed fluorination reaction to give the same thermodynamic product.^{72,73}

Similarly, addition of acetyl hypofluorite (AcOF) afforded a compound containing an anomeric β -OAc, giving **1.97** after hydrolysis of the acetyl groups.⁷¹

Selectfluor can be reacted with DANA in the presence of a nucleophile to give an axial 3-fluoro substituent; with water as the nucleophile, **1.95** was obtained in 80% yield with a 3:1 ratio of 3-F_{ax}:3-F_{eq}.⁷⁴

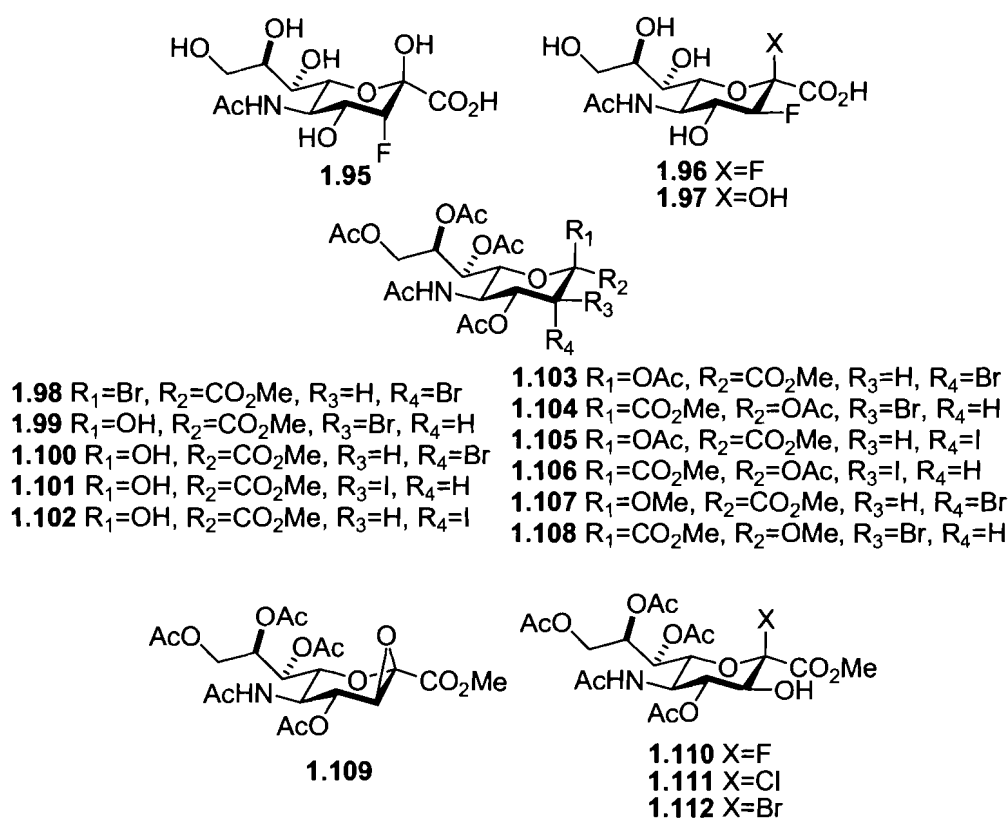


Figure 1.23 3-Halogenated sialic acid derivatives

DANA has been used as a starting material to introduce other halides at the 3-position (Figure 1.23).⁷⁵ Treatment with bromine gave the 2,3-dibromide **1.98**. Treatment with NBS in aqueous acetonitrile gave bromohydrin products **1.99** and **1.100** in >95% yield, the stereoselectivity of which was found to be temperature-dependent. At room temperature, the 3-Br_{eq} product **1.99** was

formed preferentially in a 1.5:1.0 ratio while at 80 °C the 3-Br_{ax} product **1.100** was formed preferentially in a 3.2:1.0 ratio; the same results were found using NIS to give 3-iodosialic acid derivatives **1.101** and **1.102**. Treatment of DANA with NBS in sodium acetate/acetic acid gave equivalent amounts of *trans*-diaxial and *trans*-diequatorial 2-acetoxy-3-bromosialic acid derivatives **1.103** and **1.104** while the use of NIS gave an 80% yield of the *trans*-diaxial product **1.105** and an 11% yield of the *trans*-diequatorial isomer **1.106**. As well, the 3-bromo-2-methoxy *trans*-addition products **1.107** and **1.108** were isolated from NBS reactions with DANA in methanol in 35% and 57% yields, respectively. The bromo- and iodohydrins could be converted into the β-2,3-epoxide **1.109** by treatment with either DBU or *N,N*-diisopropylethylamine. Opening of the epoxide with BF₃·OEt₂, TiCl₄, or TiBr₄ afforded the β-2-halo-3-hydroxysialic acid derivatives **1.110**, **1.111**, and **1.112** in >95% yield. These compounds were useful donor sugars for glycosylation reactions, after which the equatorial 3-OH group could be further functionalized.⁷⁶ By reacting with trifluoromethanesulfonic anhydride, the 3-OH group was turned into a trifloxy leaving group that was displaced by fluoride using tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) to give an axial 3-fluoro substituent. The trifloxy group has also been displaced using cesium acetate to give, after hydrolysis, an axial 3-OH group. These compounds were then tested as inhibitors of various sialic acid-processing enzymes.^{77,78}

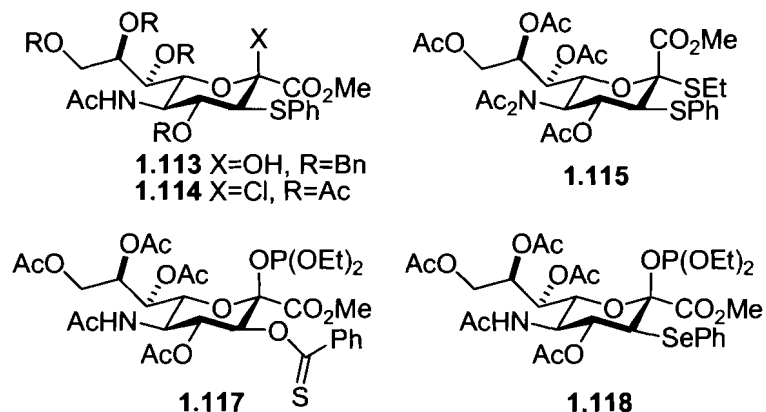


Figure 1.24 Sialic acid derivatives bearing sulfur- and selenium-containing 3-substituents

The 3-position can be substituted in a temporary fashion by a number of functionalities that enhance the selectivity of reactions at other centres within the molecule before these substituents are removed later in the synthesis (Figure 1.24). These substituents can also be used to suppress elimination across carbons 2 and 3 during glycosylation reactions, as the formation of DANA generally occurs concomitantly with glycosylation. An equatorial 3-thiophenyl substituent is commonly used as it affords a high degree of desired α -selectivity during glycosylation and is easily removed by reduction with $\text{Ph}_3\text{SnH/AIBN}$. Initial syntheses to install the 3-thiophenyl group involved treatment of the benzyl-protected version of bromohydrin **1.100** with thiophenol in the presence of $t\text{BuOK}$.⁷⁹ These addition reactions proceeded with retention of configuration at C3, but the axial 3-SPh group could be epimerized with DBU to the desired equatorial isomer **1.113** in an 83% yield. These products were eventually converted into 2-thioalkyl donor sugars which gave high glycosylation yields and exclusive selectivity for the natural α -anomer.⁸⁰ Shorter synthetic routes to 3-thiophenyl-substituted sialosides have been developed involving the addition of

PhSCI across the C2/C3 double bond of DANA, giving **1.114** in 57% yield and the 3-epimer in 19% yield.⁸¹ **1.114** was transformed into the α -thioethyl glycoside which, when used as a donor sugar, again gave reasonable glycosylation yields (28-77%) and complete α -selectivity. The glycosylation yields were further enhanced (44-83%) by using the *N,N*-diacetylated imide **1.115**.⁸² Selectivity for formation of the 3_{eq} isomer was enhanced by addition of the bulkier 2,4-dimethylbenzenesulfenyl chloride to DANA, giving the 3_{eq} isomer in 85% yield and the 3_{ax} isomer in 4% yield, the anomeric chloride of which could be directly transformed into the α -SMe donor sugar.⁸³ The stereodirecting effect of the 3-SPh_{eq} group has been explained by the possibility of sulfur attacking the anomeric centre intramolecularly to form a thiiranium ion intermediate such as **1.116** that is then opened by the glycosyl acceptor, a process that can only generate the α -anomer (if concerted) (Figure 1.25).^{84,85} An axial substituent in the 3-position enhances β -glycosylation, presumably by blocking the bottom face of the donor sugar toward nucleophilic attack, exemplified by the use of 2,3-dibromide **1.98** as a donor sugar which gave good to excellent yields of β -configured products.⁸⁶

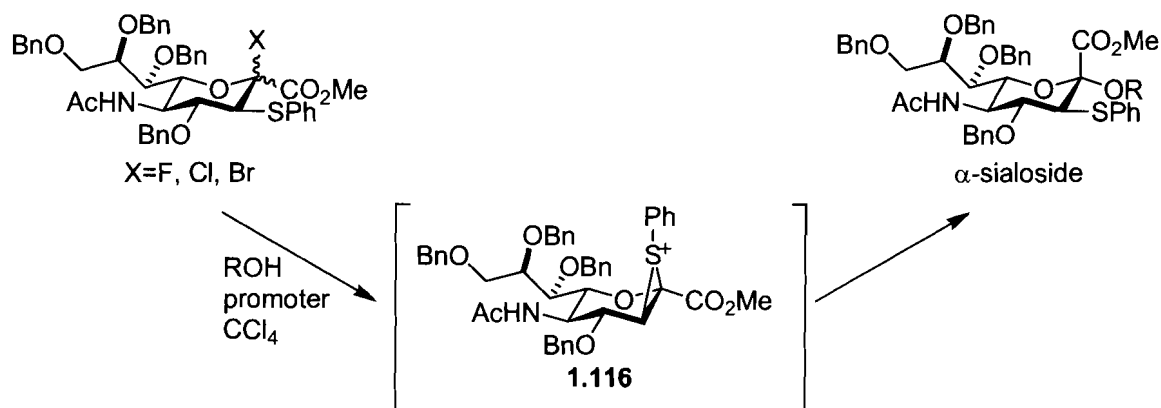


Figure 1.25 Proposed thiiranium ion intermediate 1.116 directing α -glycosylation

Schmidt and co-workers have developed dialkylphosphite donor sugars such as **1.117** that contain an equatorial thiobenzoyloxy substituent in the 3-position that can be reductively removed with $\text{Bu}_3\text{SnH/AIBN}$ (Figure 1.24).⁸⁷ When used in glycosylation reactions, these donors exclusively afforded α -sialosides in high yields (83-88%) also through anchimeric assistance of the 3-substituent; moreover, these compounds were easily prepared from protected DANA by cis-dihydroxylation with OsO_4 selectively from the top face followed by conversion of the 3_{eq}-OH to a 3-OC(=S)Ph and installation of an anomeric diethylphosphite leaving group to give **1.117**. Phosphite donor sugars containing an equatorial 3-phenylseleno substituent such as **1.118** have also been used in glycosylation reactions, their straightforward syntheses involving addition of PhSeOTf (generated *in situ* from PhSeCl and AgOTf in aqueous THF) to protected DANA ($3_{\text{eq}}\text{-SePh}$ 51%, $3_{\text{ax}}\text{-SePh}$ 35%) followed by reaction of the hemiketal with CIP(OEt)_2 and base.⁸⁸ These donors afforded complete α -selectivity in good yields with reactive acceptors (76%) and the 3-SePh group

could easily be reduced with $\text{Ph}_3\text{SnH/AIBN}$; however, no appreciable reactivity was observed with less reactive acceptors.

The CH_2 group at the 3-position of sialic acid is relatively acidic, as evidenced by the ease of glycal formation, and its protons can be readily exchanged for deuterium atoms. That is, stirring in basic D_2O (pD 12.4) results in both protons on C3 being exchanged for deuterium to obtain labelled compounds that can be used for kinetic isotope effect studies to gain mechanistic information.⁸⁹ By stirring in D_2O at a lower pD value (9.0), the *pro-R* axial proton was shown to be selectively exchanged with no noticeable exchange of the equatorial proton; naturally, this exchange process also occurs with tritium.⁹⁰ These labelled compounds were shown to be stable once linked to glycoconjugates, giving useful compounds bearing radioactive labels.

1.3.2.3 Modification at C4

The synthesis of 4-modified sialic acid analogues has been of interest for investigating the binding requirements of these materials with various sialic acid-processing enzymes, perhaps most importantly with influenza sialidase. This enzyme has emerged as a target for therapeutics as it is critical for proliferation of the influenza virus.¹⁶ Studies of the sialic acid binding pocket have highlighted the 4-position as a site for modification leading to influenza sialidase inhibitors. For example, two currently marketed influenza sialidase inhibitors take advantage of this enzyme's tolerance for variation at the 4-position by substituting the 4-OH group with an amino group (Tamiflu, **1.119**)²⁶ and with a guanidinyll group (Relenza, **1.120**, Figure 1.26).⁹¹ Both of these substituents are

positively charged under physiological conditions and likely enhance binding through a charge-charge interaction with the distant glutamate residue.¹⁶ Sialic acid analogues modified at the 4-position are also of synthetic interest because they cannot be readily synthesized enzymatically using sialic acid aldolase, as this enzyme mandatorily installs a 4-OH group.

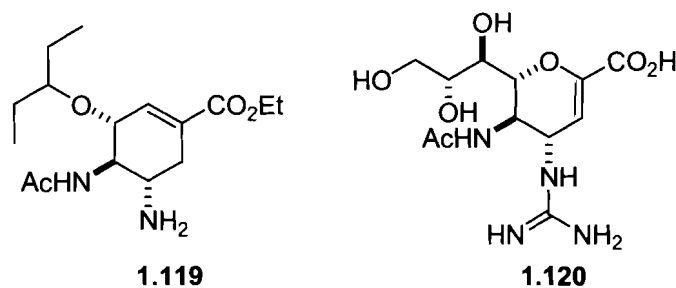


Figure 1.26 Influenza sialidase inhibitors Tamiflu (1.119) and Relenza (1.120)

Sialic acid itself is often used as a starting material to generate 4-modified derivatives. Many of these routes begin with the selectively-protected sialoside **1.121** as the more reactive 4-OH group can be selectively manipulated in the presence of the 7-OH group.⁹² The 4-OH group can be oxidized to ketone **1.122** with a variety of oxidizing agents, including PCC,⁹² PDC/Ac₂O,⁹³ and RuO₄⁹⁴ (Figure 1.27). Zbiral and co-workers have carried out a number of functional group manipulations on the carbonyl moiety of this intermediate. Addition of ZrMe₄ resulted in the isolation of a 3:2 mixture of methyl-addition products **1.123** and **1.124** in 93% yield.⁹⁵ The addition became completely specific for **1.123** when the methylating agent was changed to (BuO)₃ZrMe. In contrast, this reaction did not proceed with other alkyl nucleophiles such as alkyl lithium or Grignard reagents due to enolization of the keto group. Zbiral and co-workers

were also able to replace the keto oxygen with a methylene substituent using $\text{CH}_2\text{I}_2/\text{Zn}/\text{Cp}_2\text{ZrCl}_2$ to give **1.125** in 75% yield.^{95,96} Of note, these authors report that Wittig and Peterson olefination conditions failed to yield **1.125**.^{95,96} This 4-methylene-substituted compound was then hydrogenated to give a 3:2 epimeric mixture of 4-deoxy-4-methyl sialic acid analogues **1.126** and **1.127**.^{95,96} Interestingly, upon removal of the 8,9-*O*-isopropylidene protecting group under acidic conditions, **1.127** formed a 2,7-anhydro linkage to give the bicyclic product **1.128** while deprotection of **1.126** gave the expected product **1.129**. The 2-deoxy analogues of compounds **1.123-1.127** bearing an equatorial hydrogen at the 2-position have also been synthesized *via* the same techniques using the 2-deoxy-4-oxo analogue of **1.122** with the exception that upon deprotection the 2-deoxy analogue of **1.127** did not form the 2,7-anhydro linkage⁹⁷ (the precursor used to synthesize the 2-deoxy analogue of **1.121** was obtained by reduction of the β -2-chlorosialoside with H_2 , Pd/C⁹⁸).

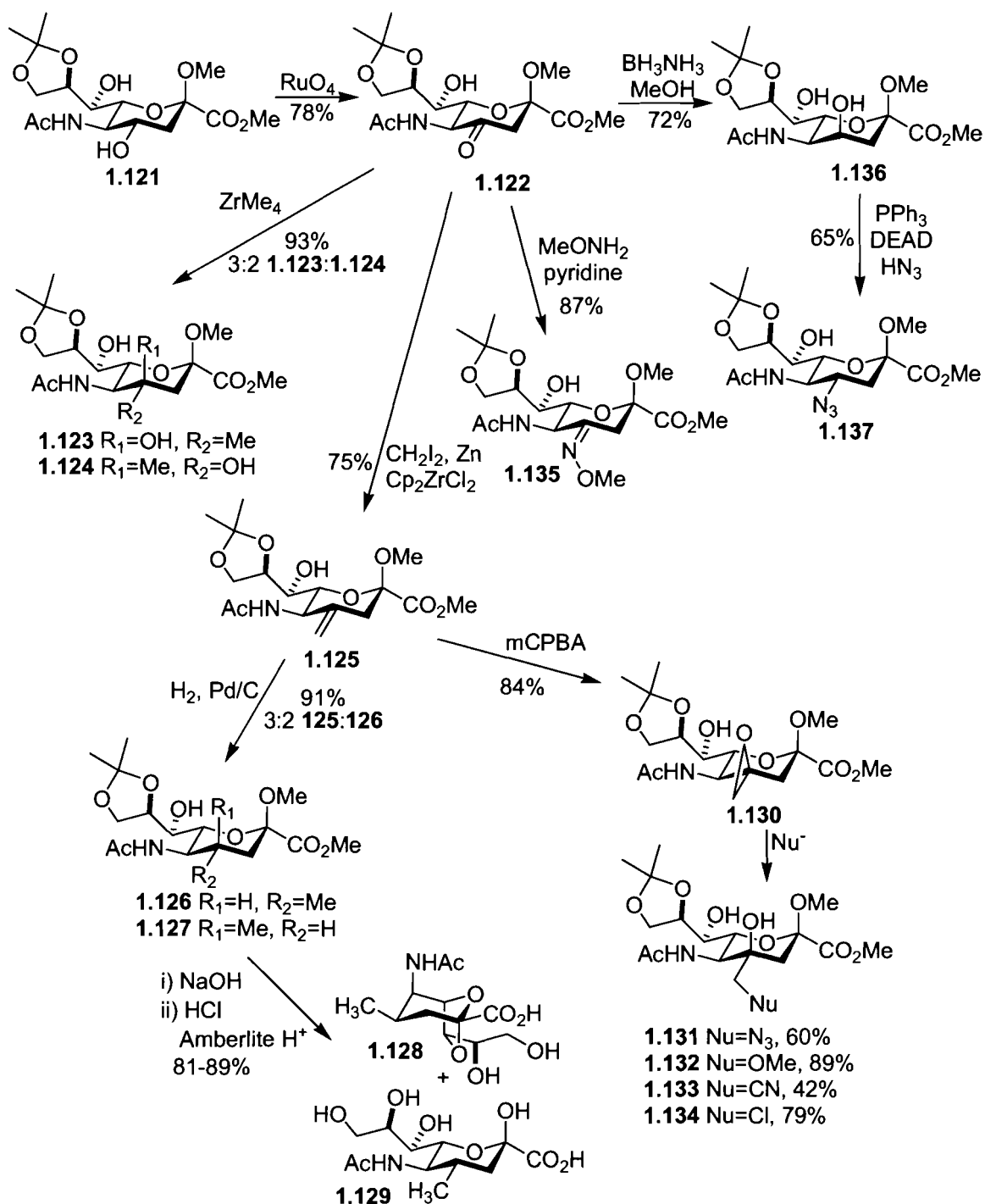


Figure 1.27 4-Substituted sialic acid analogues from common 4-oxo intermediate 1.122

Groves and von Itzstein reacted the 4-methylene-substituted intermediate 1.125 with mCPBA to generate epoxide 1.130, in 84% yield, in which the oxygen

was delivered from the top face.⁹⁹ The epoxide ring was opened with several nucleophiles (azide, methoxide, cyanide, chloride) which attacked exclusively at the less-hindered *exo*-methylene carbon atom to give products **1.131-1.134** in 42-89% yield. These authors have also synthesized oxime **1.135** from **1.122** by treatment with methoxylamine in pyridine.⁹³ **1.122** has also been used to synthesize the 4-epimeric sialic acid **1.136** by reduction with borane-ammonia complex in methanol, affording a 10:1 mixture of 4*R*:4*S* isomers.⁹⁴ **1.136** was then carried on to synthesize a sialic acid derivative bearing a 4-acetamido substituent *via* a Mitsunobu reaction using azide as a nucleophile, affording the 4-azido derivative **1.137** in 65% yield which was later reduced and acetylated.¹⁰⁰ Reduction of a 2-deoxy-4-oxo sialic acid analogue **1.39** (see Figure 1.14) gave the 4-OH group in the equatorial position in 85% yield, a product that was carried on to make sialic acid.⁵¹ Reduction of **1.39** with the bulky LiAl[OC(CH₃)₃]₃H afforded the 4*R*-isomer in 80% yield.

Several 4-modified sialic acid derivatives have also been synthesized using the free 4-OH group as a reaction centre. An analogue of **1.121** bearing a 2-SPh group has been reacted with bromoacetonitrile in the presence of Ag₂O and TBAI to give a 4-cyanomethyl-substituted derivative that was transformed into the glycal and carried on to make DANA analogues with 4-cyanomethyl, 4-carbamoylmethyl, and 4-amidinomethyl substituents.¹⁰¹ **1.121** was also reacted with methanesulfonyl chloride and pyridine to afford mesylate **1.138**, which when reacted with sodium iodide gave equatorial 4-iodo-substituted compound **1.139** in 20% yield which was presumed to have been generated through oxazoline

intermediate **1.140** that was also isolated in 25% yield (Figure 1.28), giving **1.139** with retention of configuration at C4.¹⁰² Subsequent reduction of the C-I bond with H₂ and Pd/C gave 4-deoxy sialic acid **1.141**.

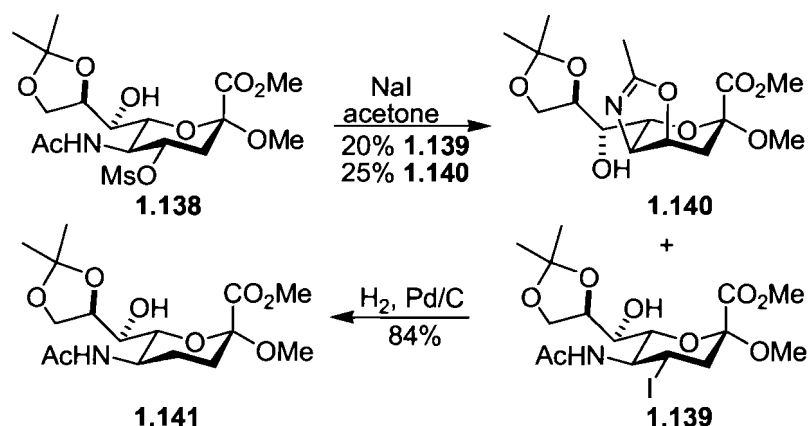


Figure 1.28 Synthesis of 4-deoxysialoside **1.141** through 4-iodo intermediate **1.139**

4-Azido-DANA has been used as a starting material to generate 4-substituted sialic acid analogues; this starting material was obtained by treating peracetylated DANA with BF₃·OEt₂ to generate a 4,5-oxazoline ring similar to compound **1.140** followed by ring-opening with lithium azide or azidotrimethylsilane.¹⁰³ 4-Azido-DANA was reacted with NBS in methanol to form a 1:1 mixture of **1.107** and **1.108** analogues bearing 4-azido groups in 87% yield.¹⁰⁴ The 4-azido-**1.108** analogue was reduced with Bu₃SnH to replace the 3-bromo substituent with hydrogen and to reduce the 4-azido group to an amino group concurrently; the amino group was then transformed into a guanidinyll group. 4-Azido-DANA has also been reacted with HCl in acetonitrile or acetic acid to afford a β-2-chlorosialic acid analogue that was glycosylated with various acceptor units to generate 4-azidosialosides; these azidosialosides were also

reduced with H₂ and Pd/C to afford 4-amino-substituted sialosides that were also acetylated to give 4-acetamido-substituted derivatives.^{105,106}

Although a large proportion of synthetic routes to 4-substituted sialosides use sialic acid as a starting material, several routes from non-sialic acid precursors have been developed. Vasella and co-workers synthesized 4-*epi*-sialic acid and 4-deoxysialic acid by coupling nitroaldopyranose **7** with *t*-butyl α -(bromomethyl)acrylate (described earlier in Chapter 1.3.1.2, Figure 1.10).^{45,107} As described previously, the diastereomeric ratio of alcohols **1.10** and **1.11** obtained following hydrolysis of the nitro group and reduction of the resulting ketone **1.9** could be controlled by choosing the proper reducing conditions and was selective for **1.11** when NaBH₄ was used in 4:1 dioxane:water in the absence of AcOH.⁴⁵ Ozonolysis and reduction of **1.11** led to 4-*epi*-sialic acid. To generate 4-deoxysialic acid, these authors acetylated the diastereomeric mixture of alcohols **1.10** and **1.11** and subsequent ozonolysis in the presence of sodium bicarbonate installed the α -keto ester moiety and induced elimination of HOAc across carbons 3 and 4.¹⁰⁷ The resulting alkene was reduced with Pd/C in ethyl acetate to give, after deprotection, 4-deoxysialic acid (a deprotected version of **1.141**).

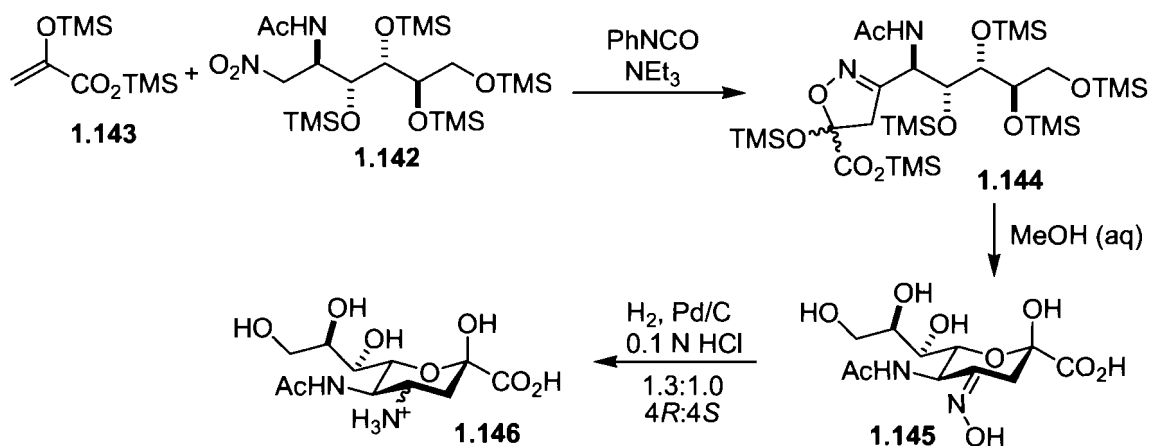


Figure 1.29 Synthesis of 4-amino-4-deoxysialic acid **1.146** through nitrile oxide cycloaddition between **1.142** and **1.143**

The persilylated nitromannitol **1.142** has also been used to generate several 4-substituted sialic acid derivatives through nitrile oxide cycloaddition with silylenolpyruvate **1.143** (Figure 1.29).¹⁰⁸ Deprotection of the cyclic intermediate **1.144** afforded **1.145**, a sialic acid analogue bearing an oxime unit in the 4-position. This could be acetylated to an acetyloxime-substituted derivative or reduced with H₂ and Pd/C to give an 1.3:1.0 4*R*:4*S* mixture of amines **1.146**. The 4*S*-amine was also converted into acetamido- and benzamido-substituted derivatives.¹⁰⁸

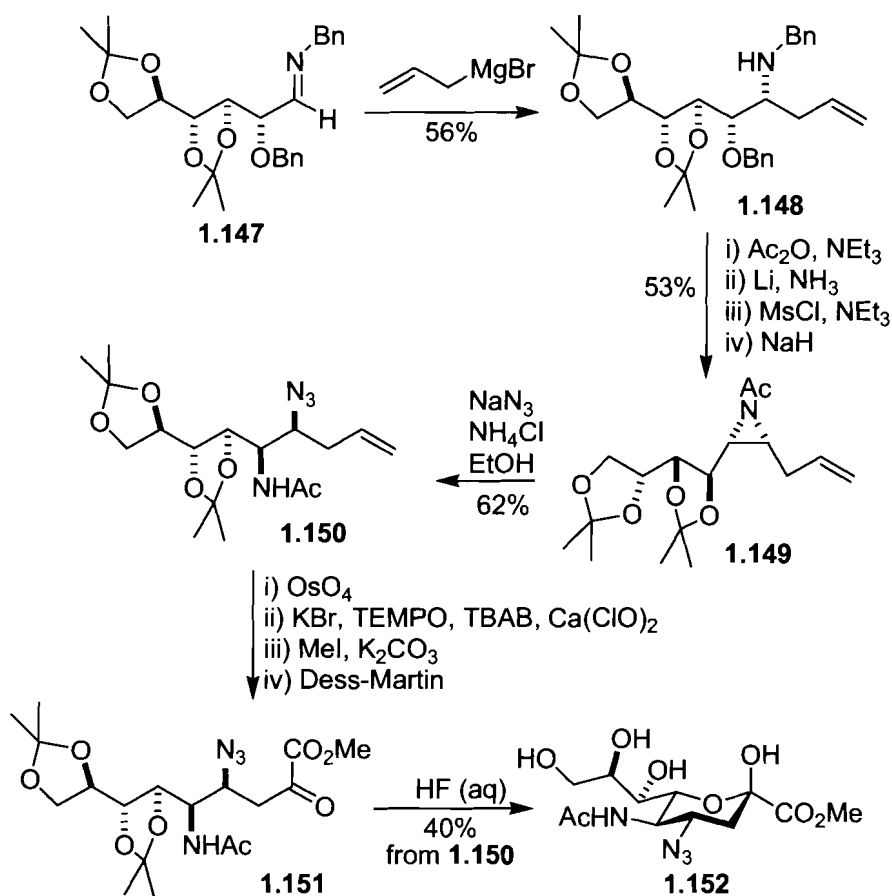


Figure 1.30 Synthesis of 4-azido-4-deoxysialic acid **1.152** from *N*-benzylimine **1.147**

4-Azido-4-deoxysialic acid has also been synthesized from D-glucono- δ -lactone (Figure 1.30).¹⁰⁹ After protection of the hydroxyl groups and formation of an *N*-benzylimino substituent, **1.147** was reacted with allylmagnesium bromide to give **1.148** as a single diastereomer. The benzylamino group was acetylated and the benzyl groups were removed from the 4- and 5-positions with Li/NH₃. The free 5-OH group was derivatized as a mesylate ester and displaced intramolecularly in the presence of sodium hydride to afford aziridine **1.149**. After trying several reagents, the aziridine ring was opened to give **1.150** via attack at the less-hindered 4-position by sodium azide in the presence of NH₄Cl in

refluxing aqueous ethanol. The terminal alkene moiety was then dihydroxylated with OsO₄ to afford a vicinal diol that was selectively oxidized to α -keto ester **1.151**, which following deprotection led to 4-azido-4-deoxysialic acid **1.152**.

1.3.2.4 Amide Variation at C5

The 5-position of sialic acid (*N*-acetylneuraminic acid) bears an acetamido group that is important in several biological recognition events and is often necessary to facilitate binding to sialic acid-recognizing proteins. Two other major naturally-occurring members of the sialic acid family are modified at the 5-position: Neu5Gc **1.2**, which contains an OH group on the acetamide carbon, and KDN **1.3**, which has a hydroxyl group in place of the 5-acetamide (see Chapter 1.2.1, Figure 1.2).^{10,110}

The majority of the C5-variants of sialic acid that have been synthesized involve installation of various amido groups at the 5-position, retaining the nitrogen atom. Some of these substitutions are performed in order to enhance yields or α/β selectivities of glycosylation reactions while other substitutions are made to probe binding interactions with sialic acid-recognizing proteins. A number of C5-variants have been synthesized using sialic acid aldolase to couple pyruvate with 2-modified ManNAc analogues enzymatically, since this enzyme does not require an acetamido function at C2, only that the substituent be in the *manno*-configuration for practical yields (as previously described in Chapter 1.3.1.1).^{29,36} Mannose analogues lacking an amino group at the 2-position that have been used with the aldolase include such 2-substituents as azido,¹¹¹⁻¹¹³ vinyl,²⁹ phenyl,¹¹⁴ acetyl,²⁹ thioalkyl,²⁹ and halide groups (Figure

1.31).²⁹ Several *N*-acylmannosamines have been prepared by acylating mannosamine with various succinimide esters, acyl chlorides, or acid anhydrides; the resulting amides were then coupled with pyruvate using sialic acid aldolase to generate 5-*N*-acylated sialic acid derivatives containing *N*-propionyl, *N*-butanoyl, *N*-pivaloyl, *N*-phenylacetyl, *N*-benzoyl, and *N*-trifluoropropanoyl derivatives,³⁸ as well as *N*-acetoxycetamido¹¹⁵ and carbobenzyloxyamido (NHCBz) derivatives,¹¹⁶ for example (Figure 1.31). Biotin, dansyl groups, and short peptides have also been incorporated into 5-amido substituents using this method.³⁷

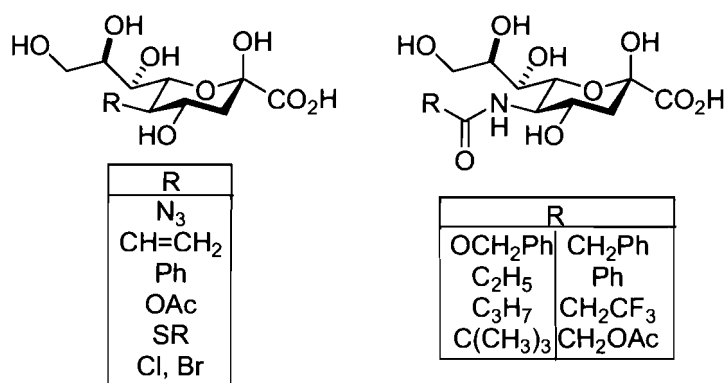


Figure 1.31 Examples of C5-modified sialic acid derivatives synthesized using sialic acid aldolase

A methylene unit was also installed between the nitrogen and C2 of a ManNAc derivative by oxidizing C2 of a protected glucopyranoside to ketone **1.153**, reacting this with nitromethane under basic conditions, and acetylating the resulting nitroalcohols to give **1.154** (Figure 1.32).¹¹⁷ Elimination of HOAc using NaBH₄ provided a nitroalkene that was also reduced under these conditions to give exclusively the mannose derivative **1.155**. This compound was then

reduced and acetylated to give the C2-chain extended ManNAc analogue which after deprotection was converted to the sialic acid analogue **1.156** with the aldolase.

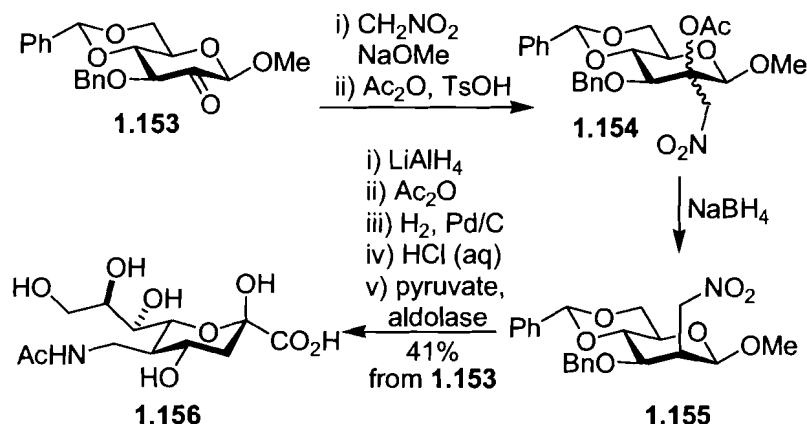


Figure 1.32 Synthesis of sialic acid analogue **1.156** containing methylene-extended 5-acetamido substituent

Some of these aldolase products have been further derivatized at the 5-position. After synthesizing 5-deoxy-5-azidosialic acid using the aldolase (Figure 1.31), Whitesides and co-workers reduced the azido moiety to an amino group with H_2 and Pd/C after protection and conversion to the α -methyl glycoside.¹¹⁸ The amino group was then acylated to form *N*-cyclopropanoyl and *N*-myristoyl substituted sialic acid derivatives. As well, the 5-NHCBz sialic acid derivative (Figure 1.31) was converted to the free amine *via* hydrogenation and was acylated to give *N*-carbomethoxy, *N*-formyl, *N*-cyclopropanoyl, and *N*-butanoyl sialic acid derivatives.¹¹⁹ Whitesides and co-workers also took a chemical approach to amido-modified sialic acids by *N*-acylating mannosamine with various reagents and performing and indium-mediated coupling with ethyl α -(bromomethyl)acrylate (see Figure 1.9).¹²⁰ These reactions proceeded with good

yields (68-90%) and although they were always selective for the desired 4*S*-diastereomer, the product ratios ranged from 4:1 to 1:1 4*S*:4*R*. The 4*S*-addition products were then subjected to ozonolysis and deprotection to lead to 5-substituted sialic acids. As well, the 5-NHBoc sialic acid derivative could be used to synthesize derivatives bearing functional groups that were incompatible with the ozonolysis conditions through removal of the Boc group and re-acylation of the free amine following ozonolysis. The high selectivity of the indium-mediated coupling observed with some amides has been ascribed to formation of a Cram-type chelate in which a five-membered ring is formed involving simultaneous coordination of the indium with the amide nitrogen and the aldehyde oxygen atoms, with the result that nucleophilic attack preferentially occurs from the less-hindered side of this complex. It is possible that a reaction displaying lower selectivity could result from reduced efficiency of complexation with bulkier amide substrates.

Sialic acid itself has commonly been used as a starting material to make various amido-substituted derivatives. The acetamido group has been acetylated to form a diacetylrimido group with isoprenyl acetate, enhancing yield and selectivity of glycosylation reactions (similar to **1.115** but lacking the 3-substituent).^{82,121,122} The acetamido group has also been hydrolyzed to an amino group under acidic conditions using methanesulfonic acid¹²²⁻¹²⁵ and under basic conditions using barium hydroxide,^{126,127} potassium hydroxide,¹²⁸ or tetramethylammonium hydroxide.⁶³ The amino group was then acylated with acid anhydrides/chlorides or esters to give a variety of *N*-alkyl amides^{63,123,124} and

N-thioalkyl amides.¹²⁶ The *N*-trifluoroacetyl and *N*-2,2,2-trichloroethoxycarbonyl (NTroc) substituents introduced *via* this method are commonly used in sialic acid donor sugars to enhance yield and α -selectivity of glycosylation reactions. In addition, many of these groups are relatively easy to remove following the various glycosylation reactions.^{123,124,129} Similarly, fluorescent tags have been introduced at the 5-amido group by coupling the free 5-amino group with CBz-glycine *p*-nitrophenyl ester, followed by attachment of a fluorescein dye after removal of the CBz group.^{127,130} Other transformations include converting the amino group into the azido group in **1.157** by diazo transfer from trifluoromethanesulfonyl azide (Figure 1.33).^{122,125} **1.157** has also been prepared from protected sialic acid by *N*-nitrosation with N₂O₄ in the presence of sodium acetate followed by treatment of the *N*-nitroso derivative **1.158** with sodium isopropoxide in trifluoroethanol (TFE) and addition of hydrazoic acid (Figure 1.33).^{128,131} The conversion of **1.158** to the azide proceeds through a 5-diazo intermediate **1.159** that could not be isolated but could be transformed into **1.157** in 43-65% yield as described or into the 5-OAc derivative **1.160** as a single isomer in 53% yield by reaction with tetrabutylammonium acetate in acetic acid. The *N*-nitroso intermediate can also be converted into a mixture of elimination products **1.161** and **1.162** which can be hydrogenated to give the 5-unsubstituted sialic acid derivative **1.163** (5-deoxy-Kdn).¹³¹

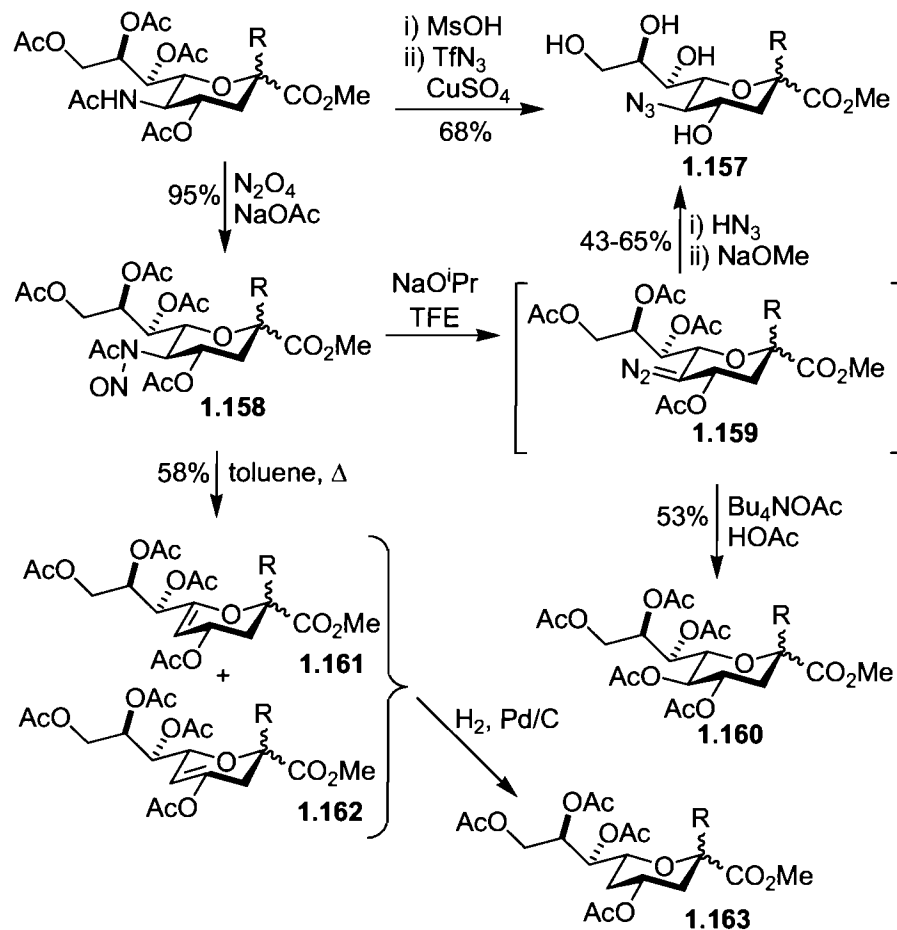


Figure 1.33 Derivatization of the 5-acetamido group of sialosides

1.3.2.5 Modifications at C6

Sialic acid analogues modified at C6 can be sub-divided into two categories depending on which substituent has been replaced or modified. One category involves replacing the ring oxygen with another atom (N, S, or C) by changing the 6-OH group for another group. The other category involves modifications at C6 itself while leaving the 6-OH group intact, such as removing the glycerol side chain or introducing another substituent in place of the C6 hydrogen atom.

Substitution of the Ring Oxygen

Nitrogen, sulfur, and carbon have all replaced the ring oxygen of sialic acid. These compounds have altered biological properties due to the formation of a positive charge in the ring in the case of nitrogen, and increasing hydrophobic interactions and hydrolytic stability in the case of carbon. Chemoenzymatic synthesis of these analogues using sialic acid aldolase with 3-substituted ManNAc derivatives has proven difficult as the enzyme appears to require the 3-OH group on ManNAc; thus, these analogues must be synthesized chemically.^{29,36,132}

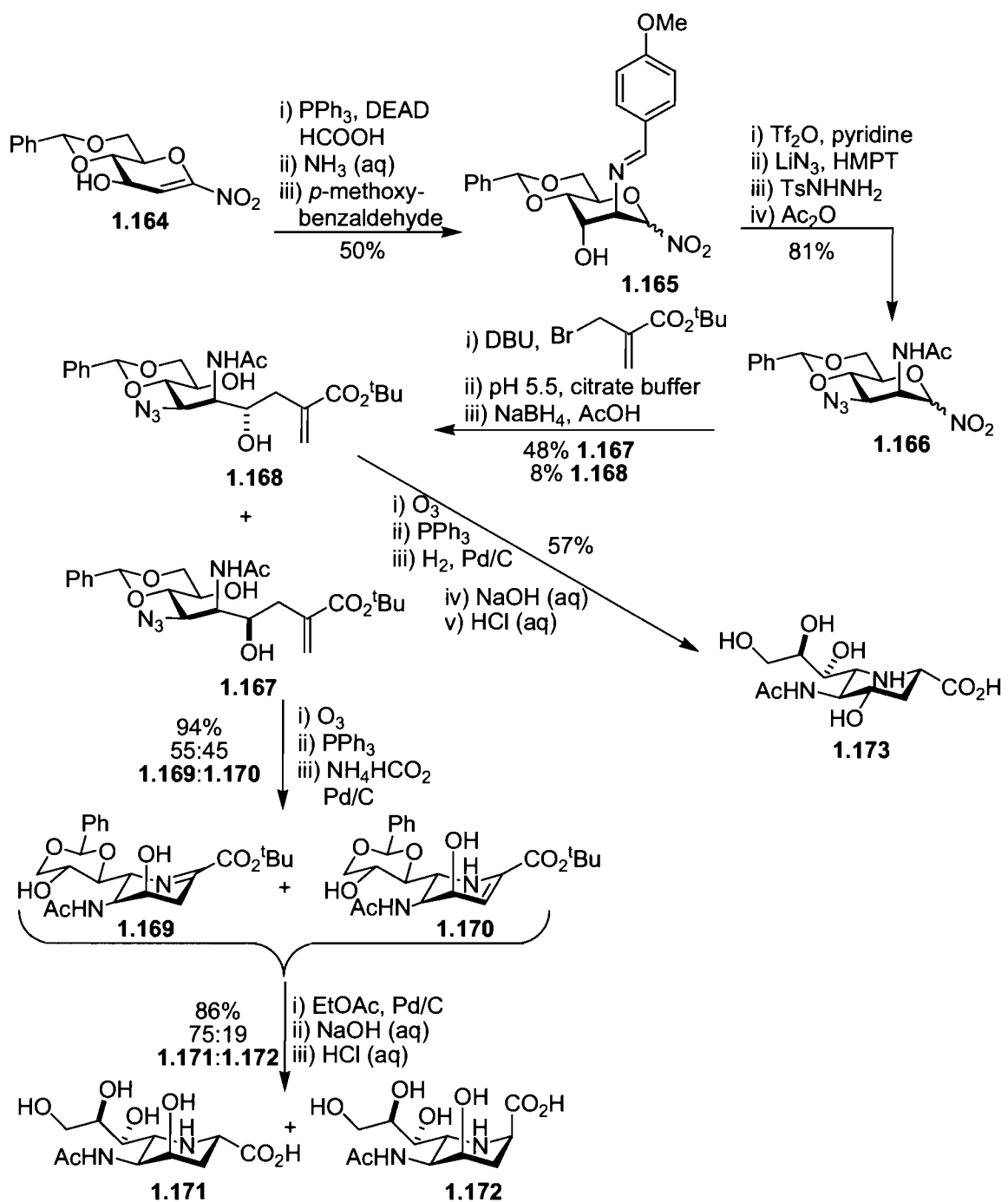


Figure 1.34 Synthesis of sialic acid analogues in which the ring oxygen is replaced by nitrogen

Vasella and co-workers synthesized several 2-deoxy-6-aminosialic acid analogues in which the ring oxygen is replaced with an NH group.¹³³ Starting with nitroglycal **1.164** (a compound derived from 1-deoxy-1-nitroglucose), Mitsunobu inversion of the 3-OH group *via* a formate intermediate followed by addition of ammonia to the nitroglycal and imine formation gave the *D-altro* compound **1.165** (Figure 1.34). The free 3-OH group was converted to a trifloxy group that was displaced with lithium azide to return the stereochemistry at the 3-position to the *D-manno* configuration. The imine at C2 was hydrolyzed and the resultant amine acetylated to give **1.166**, which was then reacted with *t*-butyl α -(bromomethyl)acrylate using DBU and the nitro group was hydrolyzed (the same series of reactions on ManNAc led to sialic acid, see Figure 1.10). The resulting 4-keto group was reduced with NaBH₄ to give a mixture of epimeric alcohols **1.167** and **1.168** in an 84:16 ratio. This reduction could not be made more selective for **1.168**, which contains the 4*S*-stereochemistry of sialic acid. Functional group manipulation of **1.167** by ozonolysis of the 2-methylene group and reduction of the amino group resulted in a 55:45 mixture of imine **1.169** and enamine **1.170** in 94% yield, both of which were reduced with Pd/C in EtOAc to a 75:19 anomeric mixture of 6-amino-2-deoxy-4-*epi*-sialic acid derivatives **1.171** and **1.172**, following deprotection. Ozonolysis of **1.168** and reduction of the azido group with H₂ and Pd/C resulted in exclusive formation of the pseudo- β anomer of 6-amino-2-deoxysialic acid **1.173**. In addition, the azidoketone obtained following chain extension of **1.166** could be induced to undergo an intramolecular cycloaddition between the azido group and the alkene moiety to

form a piperidine ring system fused with an aziridine between the endocyclic nitrogen, the anomeric centre, and an exocyclic CH₂ group and was manipulated to give both anomers of a 6-amino-2-deoxysialic acid homolog possessing a CH₂OH group in place of the anomeric OH group.¹³⁴

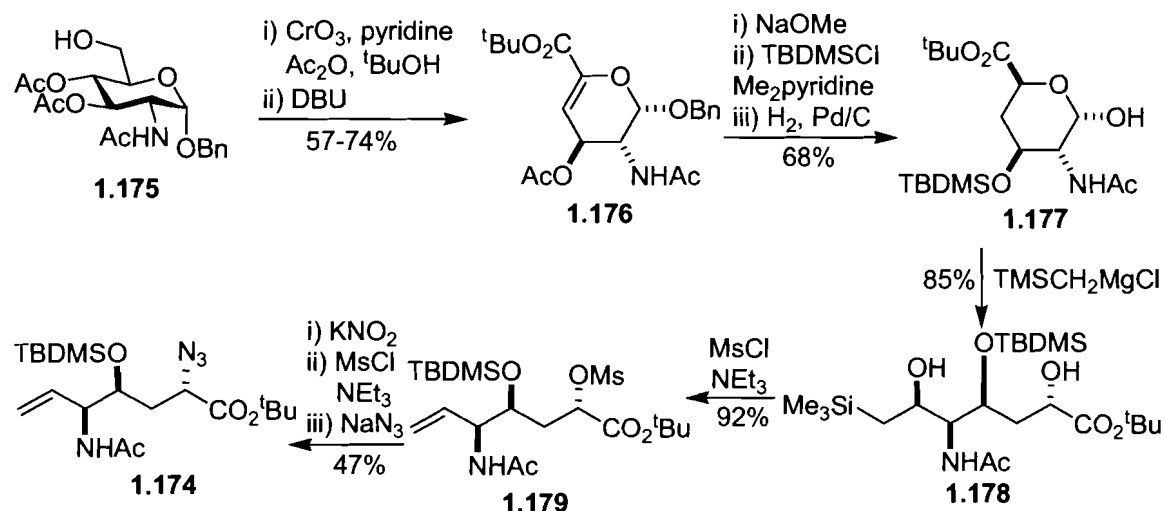


Figure 1.35 Synthesis of azidoalkene intermediate **1.174** en route to truncated 6-deoxy-6-aminosialic acid analogues

Several 6- and 7-carbon 6-aminosialic acid analogues have been synthesized from a common azidoalkene intermediate **1.174**, which was synthesized from the protected *N*-acetylglucosamine (GlcNAc) **1.175**, obtained through tritylation of the primary OH-group, acetylation of the remaining OH-groups, and removal of the trityl group.⁶⁸ Oxidation of the free OH-group of **1.175** to a carboxylate was followed by esterification and elimination to afford α,β -unsaturated ester **1.176** (Figure 1.35). After switching the 3-acetyl group for a silyl ether linkage, **1.176** was reduced with H₂ and Pd/C to give **1.177** as a single diastereomer. **1.177** was reacted with TMSCH₂MgCl to give open-chain addition product **1.178**. This was treated with MsCl and triethylamine, inducing

elimination of the TMS and mesylate groups to give alkene **1.179**. Inversion of the 2-position with KNO_2 was followed by mesylation of the resulting OH-group and displacement with sodium azide to afford **1.174**. This intermediate was then used to synthesize several 6-amino-2-deoxysialic acid derivatives (Figure 1.36), including **1.180** through ozonolysis followed by intramolecular reductive amination to form the piperidine ring, which was also converted to the *N*-alkylated version **1.181**. The double bond of **1.174** was also dihydroxylated which, following oxidation of the secondary OH-group to a keto group, was reductively aminated to afford the 7-carbon derivative **1.182**. Derivatives bearing a CH_2F group (**1.183**) and a CH_3 group (**1.184**) on C6 have also been synthesized from **1.174**.⁶⁸ The pseudo- α -anomer of **1.180** has also been synthesized *via* a route using glucosamine as the starting material that involves intramolecular nucleophilic attack of an amino group on an alkyl bromide moiety to form the piperidine ring system.¹³⁵

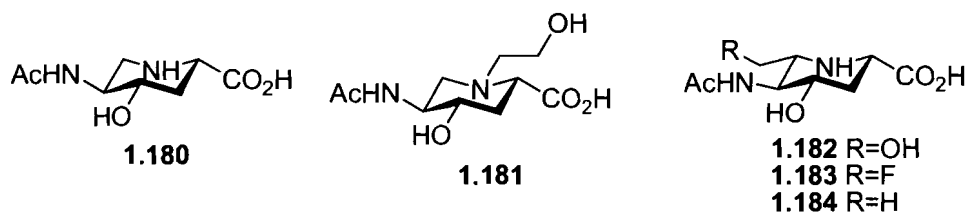


Figure 1.36 6-Deoxy-6-aminosialic acid derivatives synthesized from 1.174

Pyrrolidine compounds have also been synthesized as the five-membered ring analogues of 6-amino-2-deoxysialic acid, contracting the ring to remove C2 and replace it with the ring nitrogen, installing the carboxylate as an *N*-substituent (Figure 1.37).¹³⁶ The synthesis of these compounds began with ozonolysis of

protected DANA, removing carbons 1 and 2 to afford diol **1.185**. After selective protection steps to afford **1.186**, the primary OH was converted to an azide through a Mitsunobu reaction using HN_3 that was then deacetylated and oxidized to azidoketone **1.187**. Reduction of the azide resulted in formation of an imine with the ketone at C4 that was further reduced to give the substituted pyrrolidine **1.188** as its borane adduct. The ring nitrogen could then be substituted with CH_2COOH , $\text{C}(\text{O})\text{COOH}$, and $\text{CH}_2\text{PO}_3\text{H}_2$ groups.

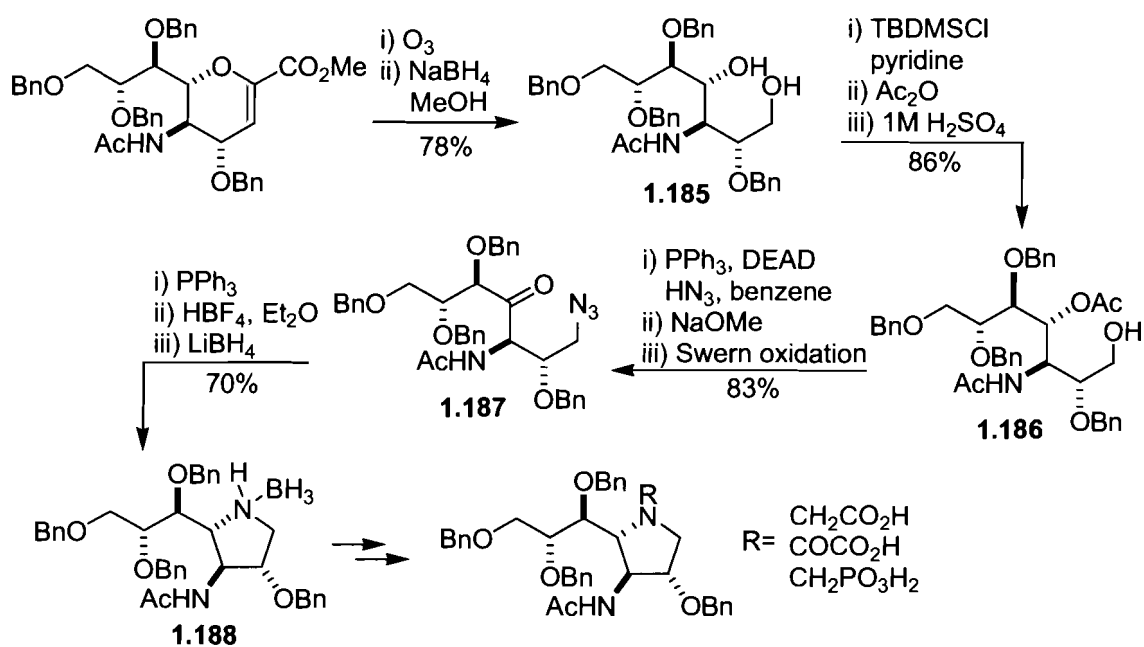


Figure 1.37 Synthesis of pyrrolidine-based sialic acid analogues

Sulfur has been incorporated into the ring of sialic acid *via* a route that involved the synthesis of 3-thio-substituted ManNAc derivatives and then extension of the carbon backbone with a pyruvate moiety (Figure 1.38).¹³⁷⁻¹³⁹ The 3-thioacetyl-substituted *manno*-configured sugar **1.189** was initially converted to thiazoline **1.190** and reacted with potassium di-*t*-butyloxaloacetate

to extend the carbon framework (as can be done with ManNAc to prepare sialic acid, Figure 1.9).^{137,138} However, none of the intended product was formed as under the reaction conditions (pH 9-10) a mixture of epimers had been formed. In order to obtain 6-thiosialic acid, **1.190** was converted to the more reactive furanose form, **1.191**, which was used in a Ni²⁺-promoted chain extension reaction with potassium di-*t*-butyloxaloacetate under less basic conditions (pH 7.5-8.0). This afforded 6-thiosialic acid **1.192** after deprotection and decarboxylation in 24% yield. The yield could be improved to 52% using oxalacetic acid on a multi-gram scale and the products were converted to substituted 6-thioglycals as potential sialidase inhibitors.¹³⁹

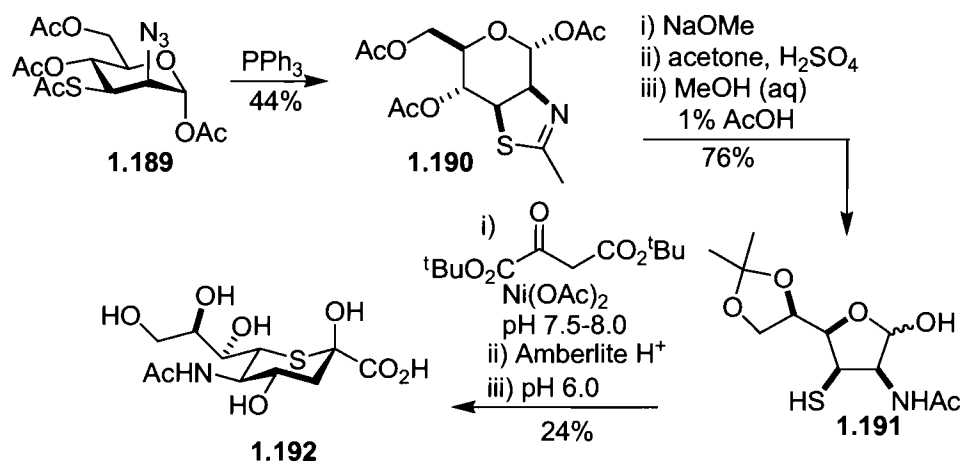


Figure 1.38 Synthesis of 6-deoxy-6-thiosialic acid **1.192**

Substitution of the ring oxygen for carbon results in the formation of carbosialic acid derivatives. These compounds are used to mimic enzymatic substrates as they are hydrolytically stable and can incorporate functional groups such as carbon-carbon double bonds that hold the ring in non-chair conformations.¹⁴⁰ For instance, the potent influenza sialidase inhibitor Tamiflu

(Figure 1.26) is a carbocyclic derivative of DANA. The carbocyclic analogue of sialic acid has been synthesized in a multistep synthesis beginning with *endo*-adduct **1.193** resulting from a Diels-Alder reaction between furan and acrylic acid (Figure 1.39).¹⁴¹ Iodolactonization of the adduct followed by reduction and acetylation gave diester **1.194**. In acetic acid, the titanium tetrachloride-promoted intramolecular attack of the 2-OAc group on C1 opened the 1,4-anhydro ring to afford **1.195** after hydrolysis. Selective protection/deprotection steps led to **1.196** from which the benzoyl ester was removed and the resulting OH-group was oxidized to an aldehyde moiety that was then subjected to a Horner-Wadsworth-Emmons reaction with trimethyl phosphonoacetate, affording the *Z* alkene **1.197** in 68% yield along with 18% of the *E* isomer. Alkene **1.197** was treated with diisobutylaluminum hydride to reduce the ester moiety to an OH-group that was protected as a TBDMS ether. The alkene unit was then *cis*-dihydroxylated with OsO₄ to afford a mixture of D- and L-*erythro*-configured diols **1.198** and **1.199** in 44% and 48% yields, respectively; the diastereoselectivity of this reaction could not be improved. The desired diol **1.198** was protected with MOM ethers, the benzyl ether was cleaved using H₂ and Pd/C, and the resulting OH-group was oxidized to a keto functionality. This product was treated with vinylmagnesium chloride to give **1.200** and **1.201** in 45% and 7% yields from **1.198**. After protection of the new OH-group as a MOM ether, the vinyl group was cleaved with ozone, followed by oxidative work-up and esterification to produce a protected α -anomeric form of carbasialic acid. The silyl groups were removed with TBAF, then the primary OH-group was benzoylated and the

secondary OH-group was converted to the inverted azide **1.202**. The azido group was reduced with H₂ over Raney nickel and the resultant amino group was acetylated, which following removal of the protecting groups afforded α -carbasialic acid **1.203**. Presumably the β -anomer could also be obtained from **1.201** but this was not reported.¹⁴¹

methoxybenzyl protecting group on the 3-OH group was reacted with *t*-butyl α -(bromomethyl)acrylate using indium (see Figure 1.9) to afford the desired 4*S*-addition product **1.204**, obtained in 60-70% yield, >90% d.e. when performed in 20:1 CH₃CN:0.1 N HCl with 0.1 eq. TBAI. The OH-groups were protected as MOM ethers and then the *p*-methoxybenzyl ether moiety was cleaved and the resulting OH-group was then oxidized to ketone **1.204** (Figure 1.40). Radical cyclization of the 2-methylene group onto the keto group using SmI₂ formed the carbocyclic 6-membered ring, giving **1.206** in 56% yield along with 37% of another diastereomer. Elimination of the 6-OH group from the major isomer was accomplished using Martin's sulfurane to form an alkene moiety that was reduced with H₂ and Pd/C to afford a single isomer containing the desired equatorial glycerol side chain in 78% yield, resulting in a protected pseudo- β -anomer of 2-deoxycarbasialic acid. This structure was further elaborated to form cyclohexene-based compounds as potential sialidase inhibitors.¹⁴²

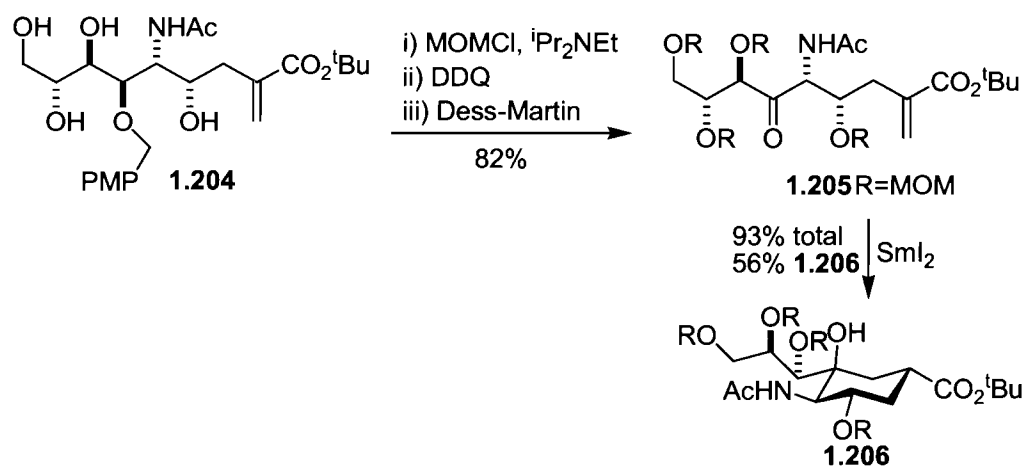


Figure 1.40 Synthesis of 2-deoxycarbasialic acid analogue **1.206** from advanced intermediate **1.204**

Another carbocyclic sialic acid analogue that has been synthesized, **1.207**, lacks a 4-substituent and bears a dialkylamido moiety at the 6-position instead of a glycerol side chain.¹⁴³ The 6-membered ring was formed through a Diels-Alder reaction between **1.208** and **1.209** which afforded **1.210** in 40% yield along with 20% of the regioisomer (Figure 1.41). The nitro group of **1.210** was reduced to an amino group and acetylated, after which the TBDMS group was removed to afford ketone **1.211** after tautomerization. Deprotonation of ethylvinyl ether with *t*-butyllithium followed by addition to ketone **1.211** and subsequent ozonolysis afforded **1.207** as a 3:1 ratio of pseudo- α - and pseudo- β -carbasialic acid derivatives in 53% yield. The configuration of the major isomer was not determined as both isomers were converted to the same cyclohexene product following dehydration.

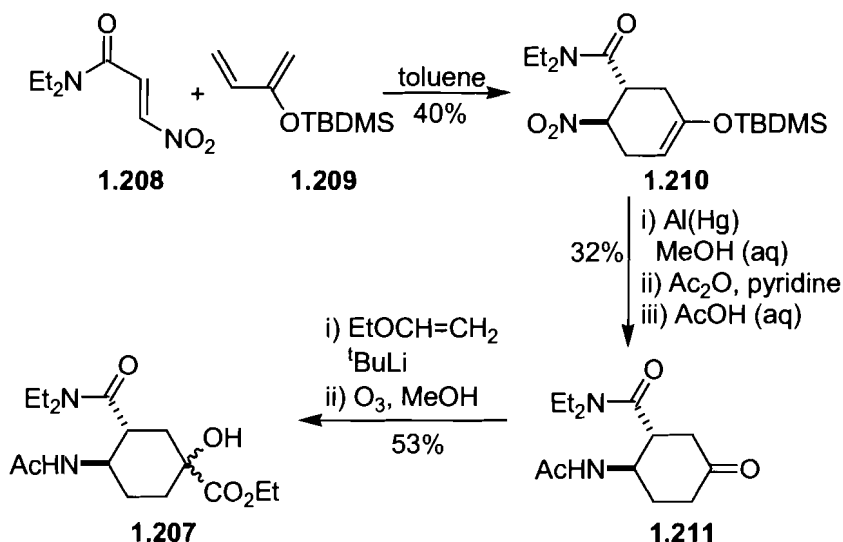


Figure 1.41 Synthesis of 6-amido-substituted carbasialic acid analogue **1.207**

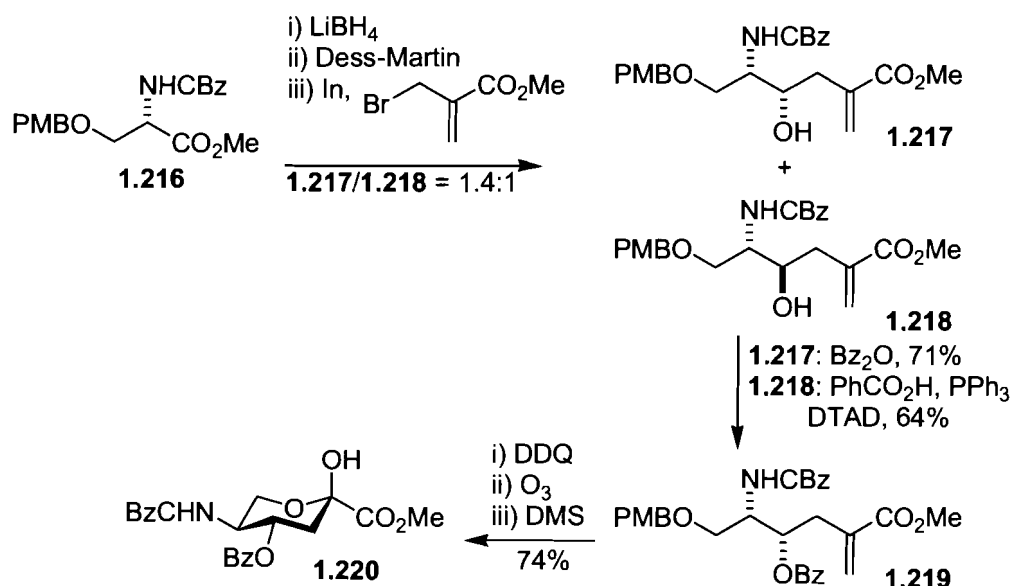


Figure 1.43 Synthesis of the truncated 6-carbon sialic acid analogue **1.220**

Truncated sialic acid analogues that either lack or have a shortened glycerol side chain are frequently synthesized using a zinc- or an indium-mediated addition of α -(bromomethyl)acrylic esters to 3- or 4-carbon aldehydes followed by ozonolysis to install the α -keto ester moiety. In one case, a 3-carbon aldehyde was prepared from the protected version **1.216** of D-serine (Figure 1.43).¹⁴⁵ The ester moiety was converted to an aldehyde *via* a standard reduction/oxidation protocol that was then chain-extended with methyl α -(bromomethyl)acrylate, giving a 1.4:1.0 mix of **1.217** and **1.218**. Both epimers were converted to the same 4S-benzoate **1.219** through the use of benzoic anhydride with **1.217** and a Mitsunobu reaction involving benzoate as the nucleophile with **1.218**. **1.219** was then converted to a 6-carbon sialic acid homolog **1.220** by cleavage of the *p*-methoxybenzyl ether followed by ozonolysis of the 2-methylene group to form the pyranose ring.

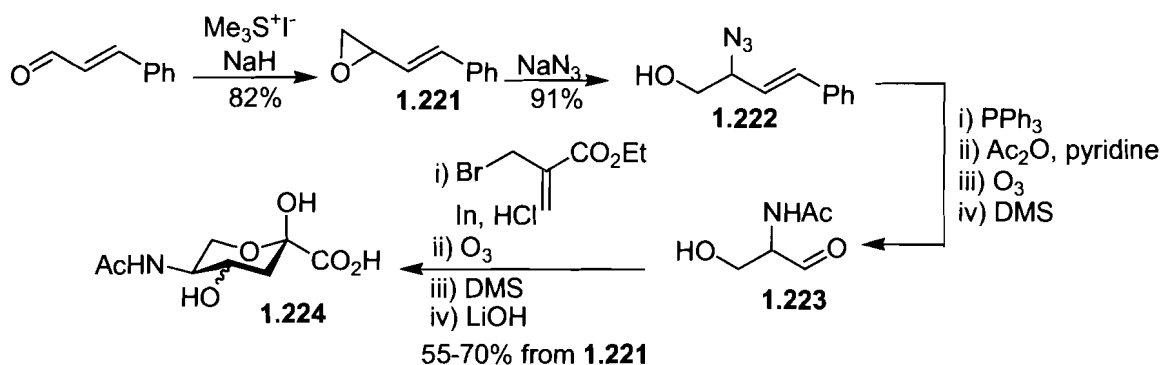


Figure 1.44 Synthesis of truncated 6-carbon sialic acid analogue **1.224** from cinnamaldehyde

An alternate route to this compound involved a more complex synthesis of the 3-carbon aldehyde electrophile.¹⁴⁶ Specifically, the synthesis began by reacting cinnamaldehyde with trimethylsulfonium iodide and sodium hydride to form epoxide **1.221** that was opened with sodium azide to afford α -azidoalcohol **1.222** (Figure 1.44). The azido group was reduced to an amino group and acetylated, after which treatment with ozone followed by reduction cleaved the alkene moiety to afford the desired 3-carbon aldehyde **1.223** which was then reacted with ethyl α -(bromomethyl)acrylate and indium. In neutral ethanol/water, the addition product was isolated as a 60:40 $4S:4R$ diastereomeric mixture; this ratio could be improved to 83:17 by using acidic ethanol/water. Following ozonolysis, reduction, and deprotection, this product led to a sialic acid analogue **1.224**, which lacks a glycerol side chain.

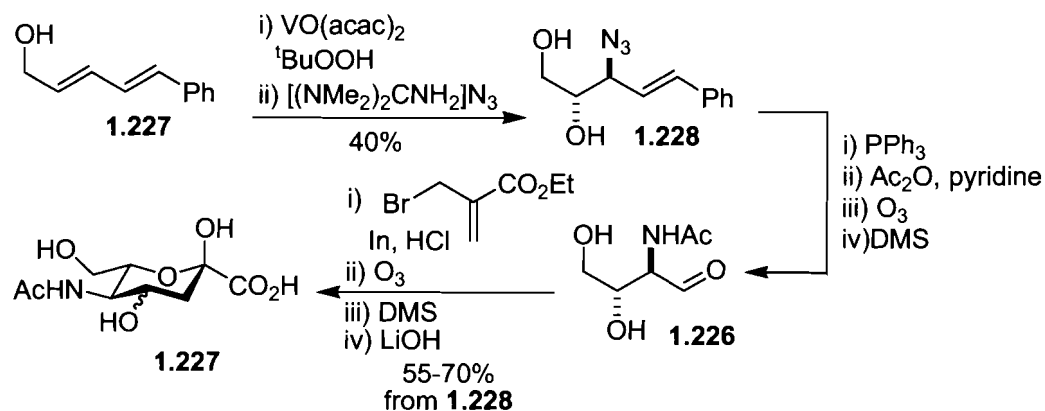


Figure 1.45 Synthesis of truncated 7-carbon sialic acid analogue 1.225

This strategy also led to the synthesis of the truncated 7-carbon sialic acid analogue **1.225** (Figure 1.45). This involved chain extension of a 4-carbon aldehyde **1.226** which was synthesized from **1.227** through allyl-selective epoxidation using *t*-butyl hydroperoxide in the presence of vanadate followed by opening of the epoxide ring with azide to give **1.228**, which was ozonized, reduced, and acetylated to **1.226**. Indium-mediated chain extension of **1.226** with ethyl α -(bromomethyl)acrylate under acidic conditions afforded the same diastereomeric ratio as above which after ozonolysis, reduction, and deprotection led to **1.225**. The same 4-carbon aldehyde, isopropylidene-protected and bearing an *N,N*-dibenzylamino group, was synthesized in several steps from vitamin C; however, this aldehyde coupled with ethyl α -(bromomethyl)acrylate to afford only the undesired *4R*-diastereomer, requiring inversion at this centre through an oxidation/reduction sequence before conversion to **1.225**.¹⁴⁷

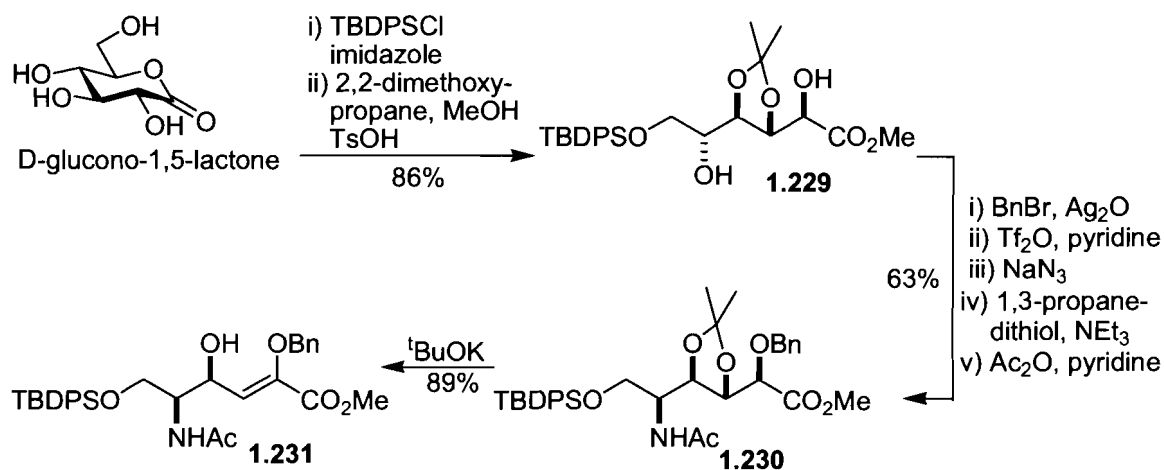


Figure 1.46 Synthesis of advanced intermediate **1.231** from **D-glucono-1,5-lactone** en route to the truncated 6-carbon sialic acid analogue **1.224**

The 6-carbon sialic acid **1.224** has also been prepared from another 6-carbon sugar, **D-glucono-1,5-lactone** (Figure 1.46).¹⁴⁸ The synthetic route began with protection of the 6-OH group as a silyl ether followed by reaction with 2,2-dimethoxypropane in MeOH under acidic conditions to give **1.229**. The 2-OH group was selectively protected as a benzyl ether in 84% yield, leaving the 5-OH group free to be activated as a triflate ester and this was displaced with azide, which was then transformed into an acetamido group to give **1.230**. The proton of the relatively acidic CH-group at C2 was removed with *t*-BuOK, resulting in a stereospecific 3-O-elimination with loss of the isopropylidene group giving enol ether **1.231**. After removal of the silyl and benzyl ethers **1.231** tautomerized to the ketone that spontaneously cyclized to the pyranose ring of **1.224**. Truncated sialic acid derivatives have also been synthesized from sialic acid itself through periodate oxidation of the glycerol side chain followed by borohydride reduction, affording 7- and 8-carbon sialic acid derivatives.^{149,150} Oxidation of the 7-carbon

formyl intermediate with bromine-water in the presence of barium carbonate afforded the 7-carbon sialic acid derivative bearing a carboxylate moiety at C7.¹⁵¹

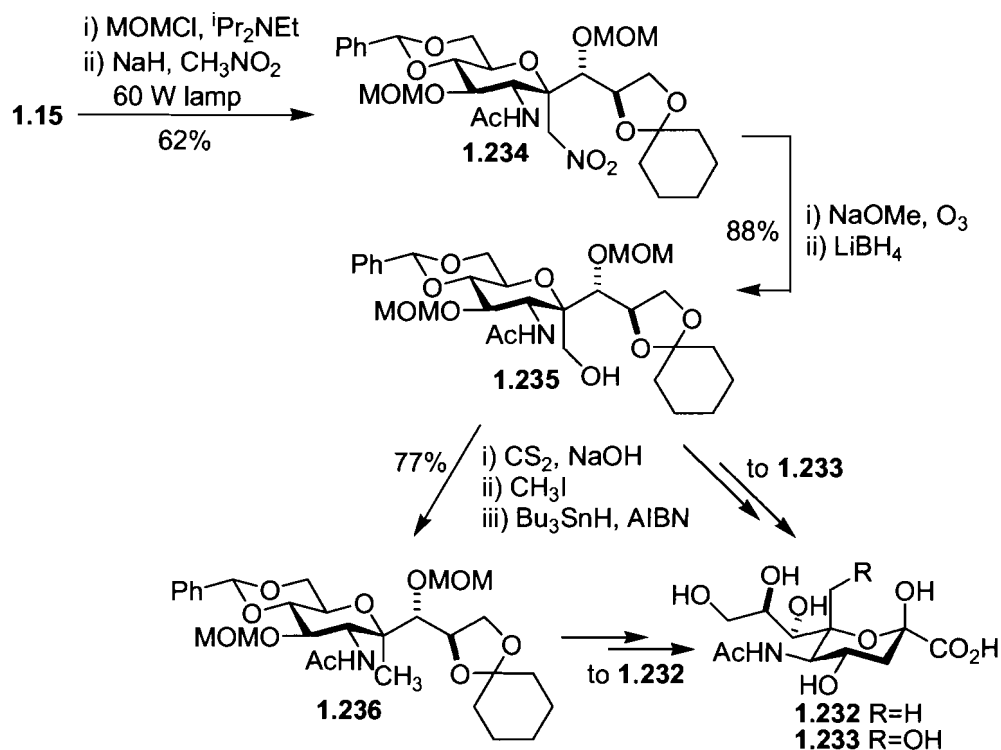


Figure 1.47 Synthesis of 6-substituted sialic acid analogues 1.232 and 1.233

Sialic acid derivatives in which the hydrogen atom at the 6-position has been replaced by either a methyl (1.232) or a hydroxymethyl group (1.233) have been synthesized by Vasella and co-workers (Figure 1.47).¹⁵² The synthesis of these compounds uses intermediate 1.15 from one of their sialic acid syntheses (see Figure 1.11). The quaternary nitro group was replaced by an axial nitromethyl group photochemically in 94% yield using nitromethane and sodium hydride in DMSO, giving 1.234. This was then converted to the hydroxymethyl derivative 1.235 by ozonolysis of the nitronate moiety followed by reduction of the resulting aldehyde group. Conversion of 1.235 to 1.233 mirrored the synthetic

route to sialic acid (see Figure 1.11). As well, the OH-group of **1.235** could be removed by conversion to a methyl xanthate moiety followed by reduction with $\text{Bu}_3\text{SnH/AIBN}$ to give the deoxygenated derivative **1.236** which led to the synthesis of **1.232**.

1.3.2.6 Modification of the Glycerol Side Chain

The glycerol side chain of sialic acid has important interactions with many sialic acid-recognizing proteins; this has been shown through studies using truncated homologues and glycerol side chain-modified derivatives.¹⁵³ Many of these analogues have been synthesized chemoenzymatically using sialic acid aldolase, which is tolerant of substitutions below the 3-position on the ManNAc chain, to couple various 4-, 5-, and 6-substituted ManNAc analogues with pyruvate.^{29,35,36} Such ManNAc analogues include 4-deoxy, 5-deoxy, 6-deoxy, 4-OMe, 5-OMe, 6-OMe, 6-OAc, 6-O-lactyl, 4-N₃, 6-N₃, 6-F, and 6-Br to synthesize the corresponding 7-, 8-, and 9-modified sialic acid analogues.

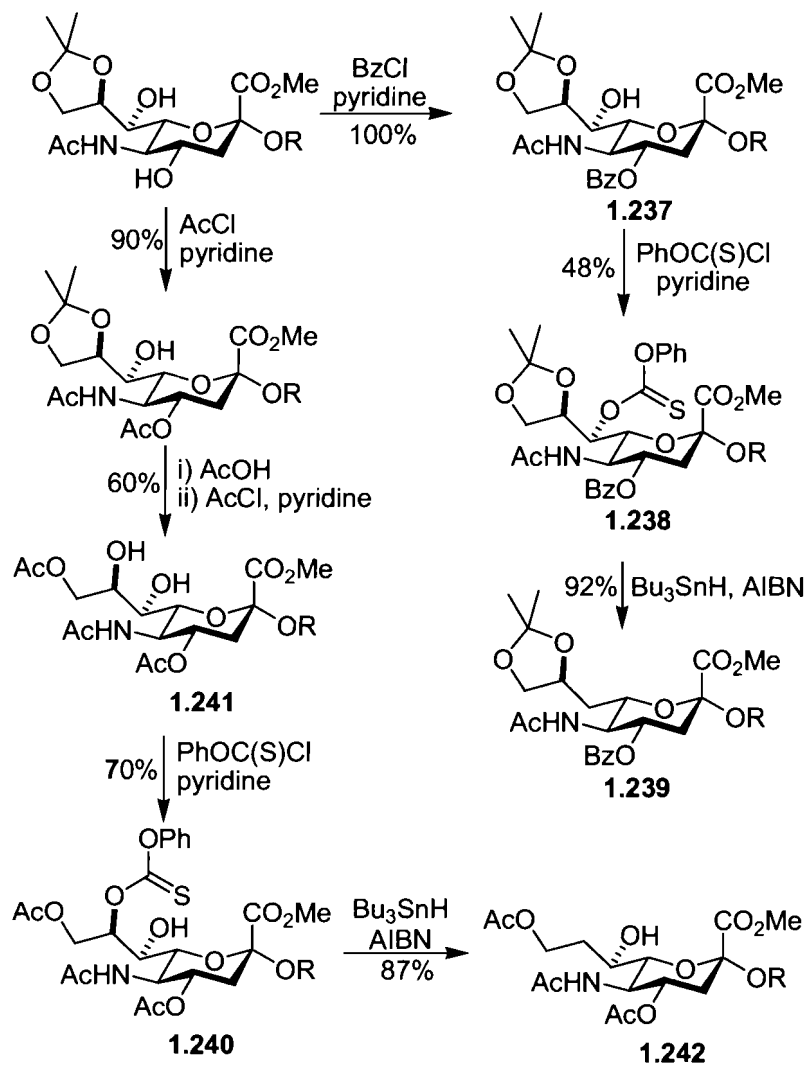


Figure 1.48 Synthesis of 7-deoxy- and 8-deoxysialosides 1.239 and 1.242

The majority of chemically-synthesized glycerol-modified sialic acid analogues use sialic acid itself as a starting material, with most of these syntheses involving selective protection of various OH-groups followed by oxidation, epimerization, or deoxygenation of unprotected OH-groups. Many of these selective protection strategies take advantage of the differential reactivity of the four OH-groups on sialic acid, the relative order being: 9 > 8 > 4 > 7 (as mentioned in Chapter 1.3.2.3). The 7-, 8-, and 9-deoxysialic acid derivatives

have been synthesized by $\text{Bu}_3\text{SnH/AIBN}$ reduction of their 7- and 8-*O*-phenoxythiocarbonyl (Figure 1.48) or 9-chloro precursors (Figure 1.49).¹⁵⁴

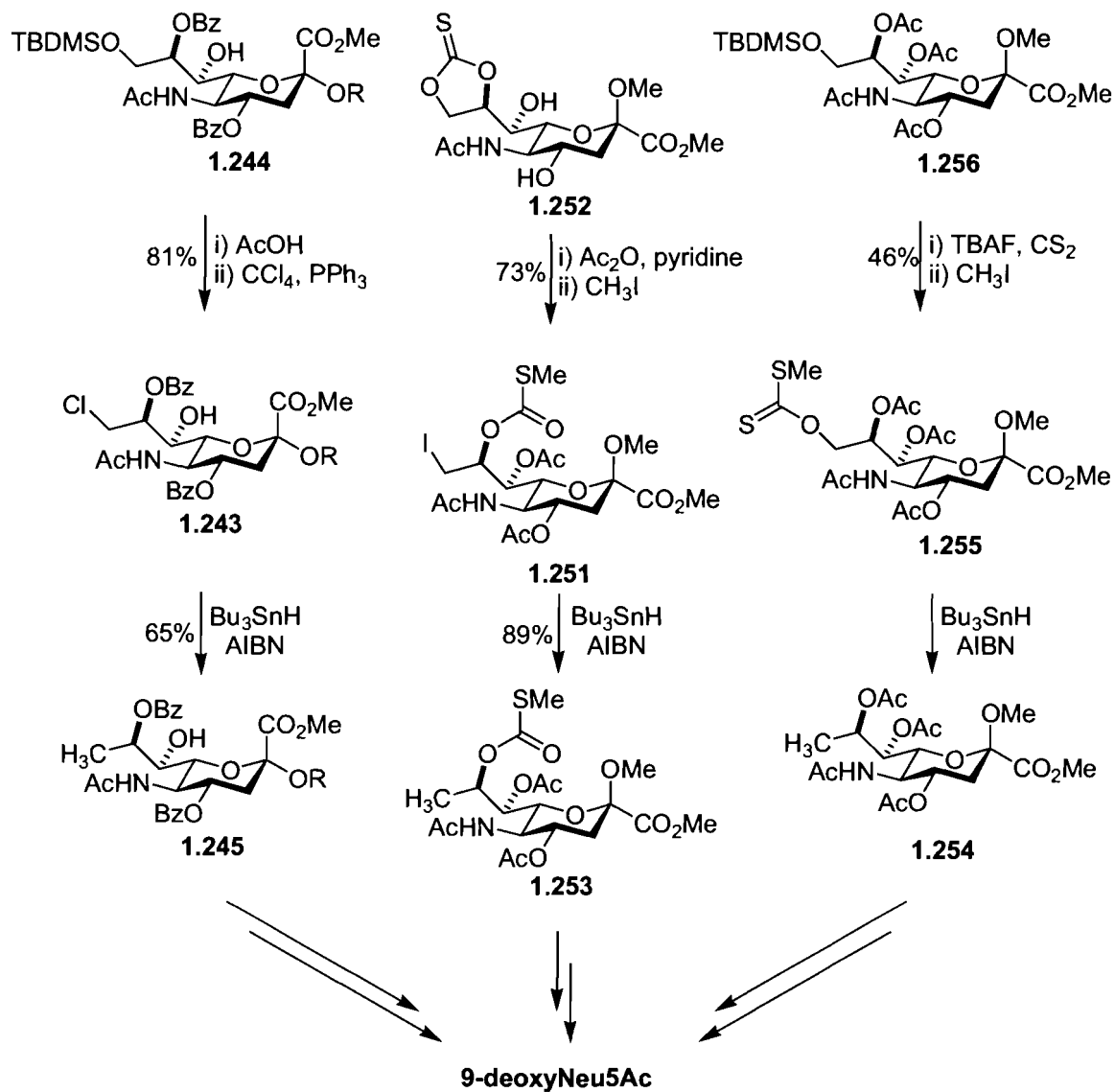


Figure 1.49 Synthetic routes to 9-deoxysialic acid

Selective installation of an 8,9-*O*-isopropylidene group and a 4-*O*-benzoyl group giving **1.237** allowed formation of the 7-*O*-phenoxythiocarbonyl derivative **1.238** that was then reduced to the 7-deoxy derivative **1.239** (Figure 1.48). The 8-phenylthiocarbonyl derivative **1.240** was formed by a similar route involving

removal of the 8,9-*O*-isopropylidene group after 4-*O*-acetylation, followed by 9-*O*-acetylation to give **1.241**, after which the 8-*O*-phenylthiocarbonyl linkage was formed and reduced to **1.242** (Figure 1.48).

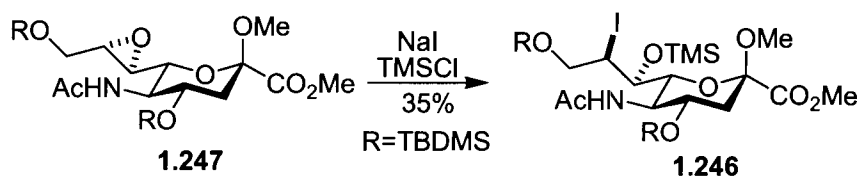


Figure 1.50 Synthesis of 8-deoxy-8-iodosialic acid analogue **1.246**

The 9-chloro derivative **1.243** was formed from a sialoside by installing a silyl ether at the 9-OH group, protecting the 4- and 8-OH groups to afford dibenzoate **1.244**, removing the silyl group and reacting the 9-OH group with CCl₄ and PPh₃; this could then be reduced to the 9-deoxy derivative **1.245** (Figure 1.49). The 7-, 8-, and 9-deoxy sialic acids have also been prepared by reduction of their iodo- and xanthate ester precursors.^{131,155} Formation of the 8-iodo sialic acid derivative **1.246** was accomplished through opening of 7,8-epoxide **1.247** with sodium iodide in the presence of chlorotrimethylsilane (Figure 1.50).^{92,155} The 7-iodo and 8-iodo derivatives **1.248** and **1.249** were formed by treating the 9-OTBDMS ether with *N,N*-dimethylformamide dimethylacetal, forming the 5-membered ring intermediate **1.250** that was opened with iodide from methyl iodide to give a mixture of **1.248** and **1.249** (Figure 1.51).⁹² The 9-iodo derivative **1.251** could be synthesized by reacting sialic acid methyl ester, methyl glycoside with thiophosgene to form the cyclic 8,9-thiocarbonate **1.252** (Figure 1.49).¹³¹ After protecting the 4- and 7-OH groups as acetate esters, the thiocarbonate ring was opened with methyl iodide to afford **1.251** that was

reduced to the 9-deoxy derivative **1.253**. A regioselective Mitsunobu reaction with methyl iodide also affords the 9-iodide.¹⁵⁵ The 7-deoxysialic acid can also be obtained by reduction of the 7-methylxanthate which was synthesized by reacting **1.237** with carbon disulfide and methyl iodide.¹⁵⁵ The 9-deoxysialic acid **1.254** was also obtained by reduction of the 9-methylxanthate **1.255**, formed by regioselective installation of a 9-OTBDMS ether moiety and peracetylation to give **1.256** followed by cleavage of the silyl ether then conversion to **1.255** (Figure 1.49). The 2,7- and 2,8-dideoxysialic acids have also been synthesized by reduction of the 7- and 8-thiocarbonate esters as described above except 2-*H*_{eq}-deoxysialic acid was used as a starting material.¹⁵⁶

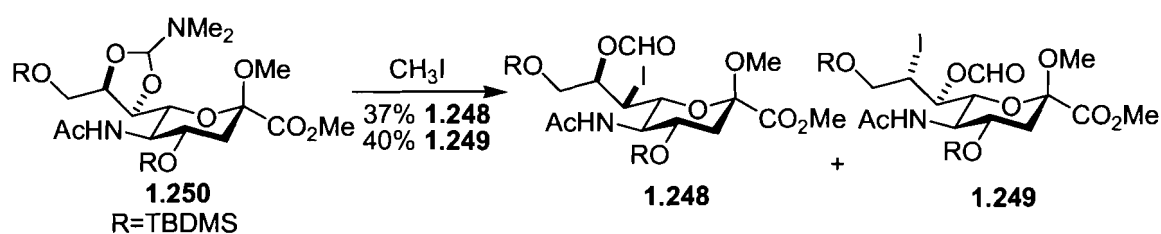


Figure 1.51 Synthesis of 7-deoxy-7-*epi*-7-iodo- and 8-deoxy-8-*epi*-8-iodosialic acid analogues **1.248** and **1.249**

The 7-*epi*, 8-*epi*, and 7,8-*bis-epi*-sialic acids have been synthesized *via* a number of intermediates that were made from sialic acid. One method involved ring-opening of various stereoisomeric 7,8-epoxysialic acids, both isomers being formed by treatment of **1.257** with triphenylphosphine and DEAD to induce an intramolecular Mitsunobu reaction (Figure 1.52).¹⁵⁷ Epoxide **1.258**, resulting from attack of the 7-OH group, was formed in 41% yield while **1.259** was formed in 16% yield, presumably due to the greater reactivity of the 8-OH group toward the

Mitsunobu reagent. These epoxide rings could be opened by treatment with acetic acid followed by acetic anhydride and pyridine to give a mixture of sialic acid and the 7,8-*bis-epi* derivative **1.260**.¹⁵⁸ The 7-*epi*- and 8-*epi*-sialic acid derivatives could be formed by the same ring-opening procedure performed on an epoxide moiety generated from iodide **1.249**.^{92,158} Epoxide **1.258** could also be opened with sodium azide to afford the 8-azido derivative **1.261** (Figure 1.52).¹⁵⁷ As well, epoxide **1.259** could be formed by protection of the 4, 8, and 9-OH groups as TBDMS ethers followed by activation of the 7-OH group as a mesylate ester and displacement by the anion of the 8-OH group during desilylation with TBAF.¹⁵⁹

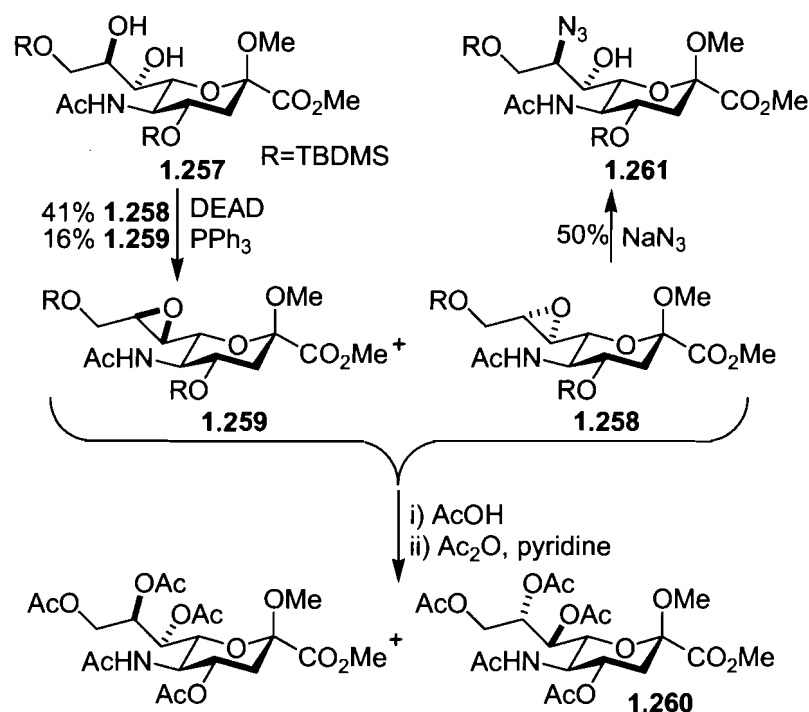


Figure 1.52 Synthesis of several 7- and 8-modified sialic acid analogues via 7,8-epoxides

The 7-*epi*- and 8-*epi*-sialic acid derivatives have also been synthesized by reduction of their keto precursors. The 7-oxo derivative was formed by oxidation of a 4-OTBDMS-substituted variant of **1.237** with RuO₄.¹⁶⁰ Reduction of this keto group with borane:ammonia complex afforded a 3:2 mixture of reduction products, the 7-*epi*-derivative being the major isomer.¹⁶¹ The 8-oxo derivative **1.262** was synthesized by peracetylation of the 9-OTBDMS-substituted sialoside followed by desilylation which caused migration of an acetyl group from the 8- to the 9-position, giving **1.263** (Figure 1.53). This was oxidized with RuO₄ to **1.262** and then reduced with borane:ammonia complex, giving the 8-*epi* derivative **1.264** in 20:1 excess.⁹⁴

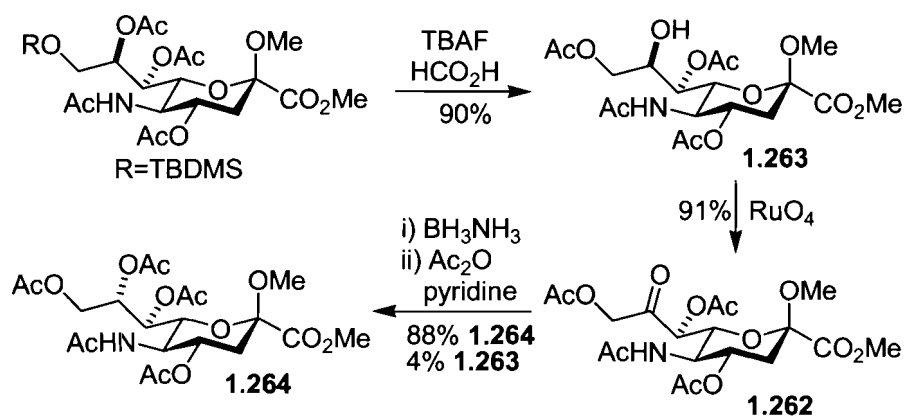


Figure 1.53 Synthesis of 8-*epi*-sialoside **1.264** via reduction of the 8-oxo intermediate **1.262**

Many investigations of the biological properties of sialic acid have involved derivatization of the primary 9-OH group for the attachment of fluorescent tags *via* the installation of an amido moiety. Analogues of this nature have generally been synthesized from a 9-amino-9-deoxy derivative which was prepared by selective activation of the 9-OH group as a tosylate ester followed by

displacement with azide and reduction of the resultant azido group.^{101,126,127}

Many analogues were subsequently made by acylation of the resulting amino group with a variety of alkyl acyl groups,^{101,127} fluorescent acyl groups,¹³⁰ and alkyl thioacyl groups.¹²⁶ The 9-OH group has also been derivatized as a methanesulfonyl ester, a methyl ether, and a methoxymethyl ether.¹⁶² A fluoro substituent was installed to replace the 9-OH group by selective tritylation followed by benzylation of the remaining OH-groups, removal of the trityl group, and finally treatment with DAST.¹⁶³

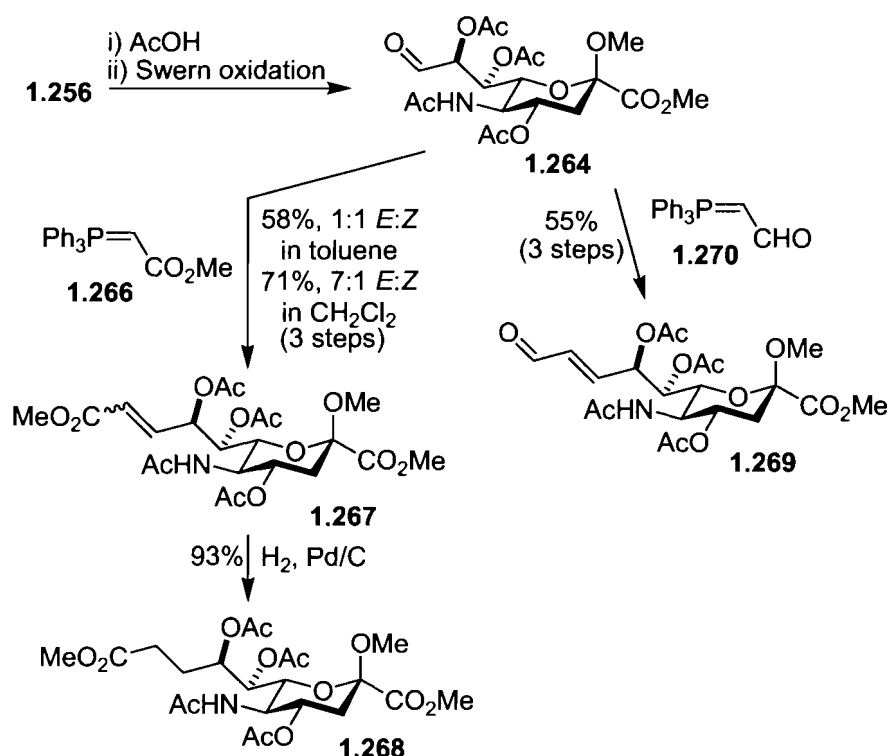


Figure 1.54 Wittig reactions on 9-oxosialoside 1.265 leading to 11-carbon derivatives

Other sialic acid analogues have been reported in which the glycerol side chain has been modified. For instance, a lactone ring has been formed between the 1-carboxylate unit and the 7-OH group via the installation of an 8,9-O-

isopropylidene group followed by treatment with pivaloyl chloride in pyridine, a reaction sequence that formed the 1,7-lactone in 67% yield which was used as an acceptor sugar in glycosylation reactions.¹⁶⁴ An 11-carbon sialic acid derivative has also been synthesized from **1.256** by removing the silyl group, then oxidizing the 9-OH group to aldehyde **1.265** under Swern conditions and performing a Wittig reaction with phosphorane **1.266** (Figure 1.54).¹⁶⁵ The 11-carbon alkene **1.267** was formed as a 1:1 mixture of *E* and *Z* isomers when CH₂Cl₂ was used as a solvent but was isolated as a 7:1 *E:Z* mixture when toluene was used. Both diastereomers were reduced to alkane **1.268**. As well, the 11-carbon aldehyde-containing homolog **1.269** was synthesized via a Wittig reaction using phosphorane **1.270** (Figure 1.54).¹⁶⁵

1.4 Glycosylation with Sialic Acid Donors

It is often desirable to convert sialic acid and its derivatives and analogues into substrates for sialidase-catalyzed hydrolysis or other purposes. The process by which this is accomplished is known as glycosylation, involving the coupling of a donor sugar with an acceptor. The sialic acid is first converted into a donor sugar by installing a good leaving group at the anomeric centre, and the acceptor is often a molecule with a free hydroxyl group such as a substituted phenol or another sugar. These two are then mixed, generally in the presence of a promoter, to generate the glycosylated sialoside (Figure 1.55).

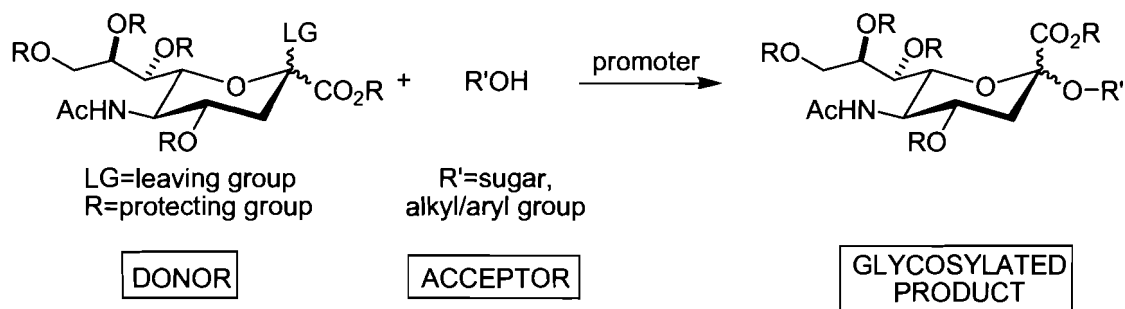


Figure 1.55 Reaction of a general sialic acid donor sugar with an acceptor to generate glycosylated products.

Many factors influence the yield and the anomeric configuration of the products, including the identity of the donor, the solvent used, and the reaction temperature. In many cases the stereochemical outcome is difficult to predict. The sterically crowded tertiary anomeric centre of sialic acid makes glycosylation of this carbohydrate especially difficult and the presence of the anomeric carboxylate destabilizes build-up of positive charge that is often required during glycosylation. The lack of a substituent at the 3-position often leads to low stereoselectivity due to the absence of anchimeric assistance and also leads to a competing elimination of HX across carbons 2 and 3 to generate the glycal whose double bond is in conjugation with the carboxylate.¹⁶⁶ In addition, the less stable α -anomer is often the desired anomer and is often more difficult to synthesize.

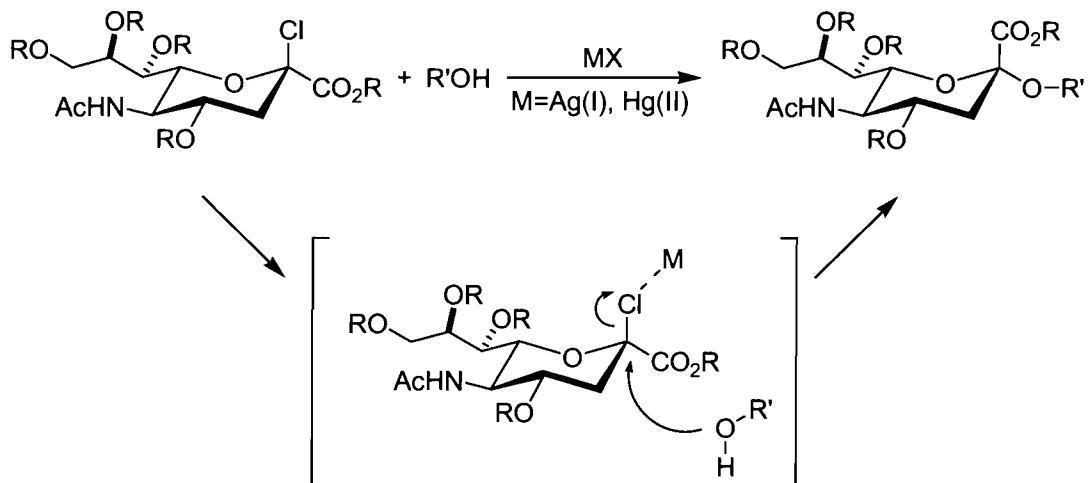


Figure 1.56 Synthesis of α -sialosides by direct attack on a β -sialosyl chloride donor

The halogenated sialosides have been used extensively as donor sugars for glycosylation reactions. Sialosyl chlorides were the most commonly used as they are relatively easy to synthesize and are stable enough to allow purification and storage for a limited time. Sialosyl bromides are not commonly used due to their instability and their propensity for glycal formation and fluorides tend to afford the unnatural β -sialoside.¹⁶⁶ Sialosyl chlorides are easily synthesized from peracetylated sialic acid methyl ester in a number of ways, commonly by stirring in acetyl chloride/acetic acid mixtures.¹⁶⁷ This donor typically gives good yields with simple or primary sugar alcohols as acceptors. Common promoters for glycosylation of sialosyl chlorides are silver(I) or mercury(II) salts as these metals are thought to coordinate with the chloride and enhance its departure, allowing either direct attack of the acceptor at the anomeric centre (Figure 1.56) or facilitating the formation of an oxacarbenium ion that is subsequently attacked by the acceptor.¹⁶⁶ Direct attack in non-polar solvents affords α -sialosides by inverting the configuration of the anomeric centre (Figure 1.56), while in polar

solvents formation of an intimate ion pair blocking the top face of the oxacarbenium ion has been proposed as an explanation for the predominantly α -linked sialosides that are produced. Common promoters include Ag_2CO_3 ,¹⁶⁸ AgOTf ,¹⁶⁹ silver(I) salicylate, silver(I) zeolite,¹⁷⁰ $\text{Hg}(\text{CN})_2$, HgBr_2 , and ZnBr_2 ,¹⁷¹ with silver salts affording better α -selectivity and mercury salts affording higher yields.¹⁶⁶ The sialosyl chlorides have also been glycosylated under nucleophilic conditions in the presence of bases without promoters to afford α -linked products, using Hünig's base in CH_3CN ¹⁷² or aqueous sodium hydroxide in CHCl_3 with BnEt_3NCl as a phase-transfer catalyst,¹⁶⁷ for example. These reactions can also be performed by direct attack of alkoxides on the sialosyl chloride.¹⁷³ All of these nucleophilic conditions tend to result in significant quantities of the glycal elimination product.

Peracetylated sialic acid methyl ester can also be glycosylated using similar promoters as described above but tends to give anomeric mixtures of products. However, this may be acceptable given the relative ease with which these stable donor sialoside can be generated. The use of AgClO_4 with SiCl_4 or Me_2SiCl_2 has been reported to give good yields of α -sialosides with minimal elimination to the glycal when performed in EtCN at $0\text{ }^\circ\text{C}$.¹⁷⁴ Use of SiCl_4 or $\text{BF}_3\cdot\text{OEt}_2$ in the absence of the silver salt gives β -anomeric products from either anomer of the peracetylated donor sugar.¹⁷¹

Sulfur-based donor sialosides have emerged as useful reagents for glycosylation. Aryl and alkyl 2-thiosialosides are stable and easy to synthesize, commonly made by treating an alkyl or aryl thiol with the sialosyl chloride or with

peracetylated sialic acid methyl ester in the presence of $\text{BF}_3 \cdot \text{OEt}_2$.¹⁷⁵ These donor sugars are stable enough to allow further derivatization of the molecule before glycosylation is attempted and anomeric mixtures of these donors can be used. Glycosylation of 2-thiosialosides is often promoted by the use of thiophilic reagents such as dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST), *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) or trimethylsilyl trifluoromethanesulfonate (TMSOTf), and phenylselenyl trifluoromethanesulfonate (PhSeOTf).¹¹⁰ 2-Xanthates have also been synthesized and effectively used as donor sugars. These compounds are also easily synthesized from the sialosyl chloride by stirring with *O*-ethyl-*S*-potassium dithiocarbonate in ethanol, are quite stable, and can be activated toward glycosylation using the same thiophilic promoters described above, sometimes giving higher yields than their 2-alkylthio counterparts.^{175,176}

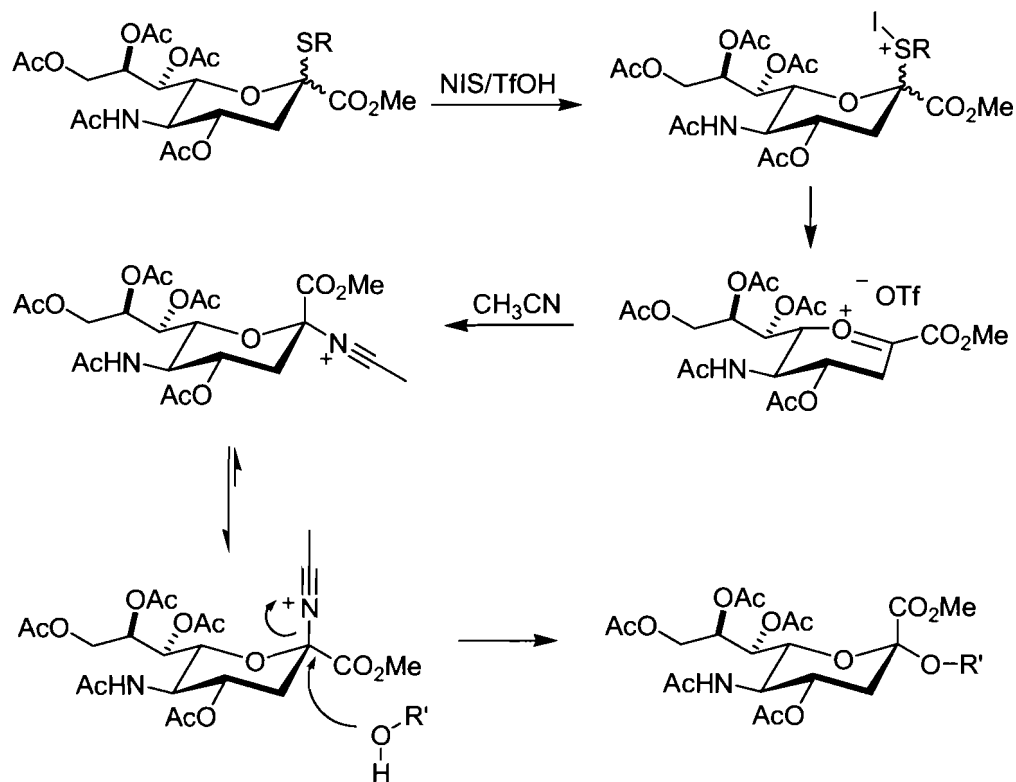


Figure 1.57 Proposed mechanism for glycosylation of 2-thiosialosides promoted by NIS/TfOH in CH₃CN

Glycosylation reactions using 2-thiosialosides tend to be α -selective when performed in CH₃CN. The proposed explanation for this phenomenon involves activation of the sulfur leaving group to form an oxocarbenium ion to which the nitrogen of acetonitrile coordinates or binds to give a nitrilium ion. This nitrilium ion preferentially adopts the more stable β -configuration, allowing attack by the acceptor sugar from the bottom face to form the α -linked sialoside (Figure 1.57).¹¹⁰

Sialyl phosphites have also found use in glycosylation. These can be synthesized from a protected sialic acid bearing a free 2-hydroxyl group by reaction with chloro diethylphosphite in the presence of Hünig's base, giving the

β -configured donor sugar. These 2-phosphite donor sugars can then be glycosylated in the presence of catalytic amounts of TMSOTf to give predominantly the α -linked sialoside product.¹⁷⁷

The incorporation of a 3-substituent in donor sialosides to act as an auxiliary has been found to enhance formation of one anomer over the other in glycosylation reactions as well as to suppress the elimination across carbons 2 and 3.¹¹⁰ Several sulfur-based and selenium-based 3-substituents have been incorporated into the sialosyl donor that can be removed under mild conditions after glycosylation and these have been described in the previous section (Chapter 1.3.2.2).²⁷

The removal of the proton from the acetamido group of sialic acid donor sugars has been shown to increase reactivity and α -selectivity during glycosylation reactions. Several of these derivatives have been described in the previous section (Chapter 1.3.2.4, last paragraph), including *N*-acetylacetamido sialosides and 5-azido sialosides.¹⁶⁶ Recently, the work of Crich and co-workers showed that the use of a 1-adamantyl thiosialoside that contained an *N*-acetyl-5-*N*,4-*O*-oxazolidinone moiety (**1.271**) could be glycosylated in the presence of NIS/TfOH in CH₂Cl₂/CH₃CN solvent mixtures at -78 °C to afford products in greater than 90% yield with near complete selectivity for the α -anomer (Figure 1.58).¹⁷⁸ These conditions are some of the best reported to date for the efficient α -glycosylation of sialic acids.

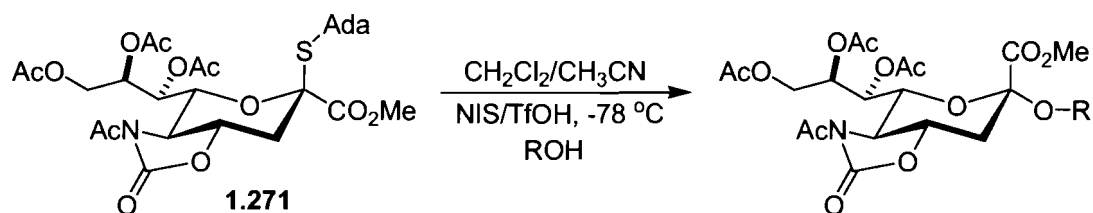


Figure 1.58 1-Adamantyl thiosialoside **1.271** affords excellent yields and α -selectivity during glycosylation in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ at $-78\text{ }^\circ\text{C}$.

1.5 Influenza Sialidase Inhibitors

As alluded to in Chapter 1.2.2, the synthesis of influenza sialidase inhibitors is a research area of great interest as researchers attempt to synthesize more potent and selective compounds as potential therapeutic agents. As stated previously in Chapter 1.3.2.3, currently there are two influenza sialidase inhibitors marketed as anti-influenza therapeutics, Tamiflu (**1.119**)⁹¹ and Relenza²⁶ (**1.120**, Figure 1.26). These compounds take advantage of interactions that are important for binding of sialosides in the active site of sialidases by retaining the carboxylate and acetamido moieties. They also both contain an endocyclic double bond to distort the shape of the pyranose ring to resemble the distortion present in DANA. DANA, the glycal of sialic acid, is a sialidase inhibitor, albeit not a very potent one ($K_i = 4 \times 10^{-6}$ M with influenza A N2 sialidase).¹⁷⁹

The structure of Relenza is similar to that of DANA except it contains a positively-charged guanidinyll group in the 4-position in place of the hydroxyl group. This guanidinyll group forms a charge-charge interaction with a nearby glutamate residue, increasing its binding to the sialidase ($K_i = 1 \times 10^{-9}$ M).¹⁷⁹ The binding constant for the 4-amino-substituted DANA analogue **1.272** was also

measured but was diminished slightly ($K_i = 4 \times 10^{-8} \text{ M}$).¹⁷⁹ Tamiflu retains some of the features of Relenza, containing an amino group at the 4-position that forms a charge-charge interaction with a nearby glutamate residue. Tamiflu, however, is based on a carbocyclic structure in which the ring oxygen of the sialic acid pyranose is replaced with a carbon atom. Tamiflu also contains a double bond but its position has been shifted one position around the ring, being between the pseudo-anomeric centre and the position formerly occupied by the ring oxygen atom. The position of the double bond was shifted in order to better mimic the partial double bond present in the oxacarbenium ion-like species proposed to be present at the transition state during enzyme-catalyzed hydrolysis of sialosides. In addition, the glycerol side chain of sialosides has been replaced in Tamiflu by an isopentyl ether at the 6-position that has been proposed to form favourable contacts with a hydrophobic pocket in the sialidase active site.⁹¹ The binding constant for Tamiflu is similar to that of Relenza ($K_i = 1 \times 10^{-9} \text{ M}$).¹⁸⁰ Several research groups have been working to design more efficient synthetic routes to Tamiflu due to its importance as a front-line drug in the event of an influenza pandemic.¹⁸¹⁻¹⁸³

These two compounds have been marketed as drugs that can be taken to prevent or reduce the severity of influenza infection but have also been sold as frontline drugs to be used in the event of an influenza pandemic; Tamiflu is taken orally (as an ethyl ester pro-drug) and Relenza must be inhaled intranasally. These drugs will be useful in saving lives until a vaccine against the pandemic influenza strain can be synthesized and distributed. However, these compounds

will eventually induce the appearance of influenza strains that are resistant to their actions as the influenza sialidase mutates, either naturally or under pressure from overuse of these antiviral compounds.¹⁸⁴ Indeed, a strain of the H5N1 avian influenza has already been isolated from a human patient that is resistant to Tamiflu (His274→Tyr mutation).¹⁸⁵ Therefore it is important to develop a wide array of anti-influenza compounds that can be used against the mutating target.

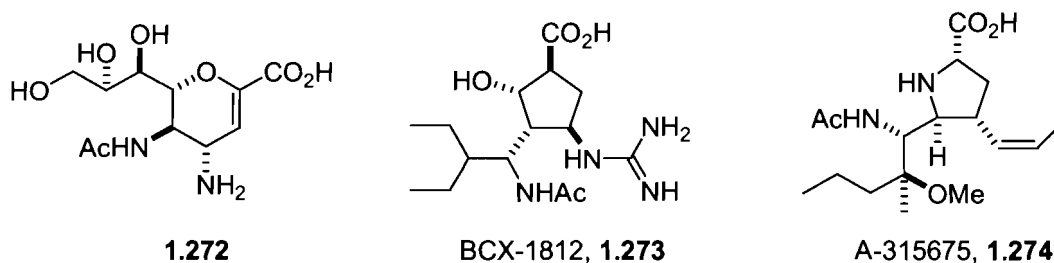


Figure 1.59 Potent influenza sialidase inhibitors in addition to Relenza and Tamiflu

Two other potent influenza sialidase inhibitors are currently under clinical trials and are both based on cyclopentane skeletons; these compounds are BCX-1812 (**1.273**)¹⁸⁶ and A-315675 (**1.274**, Figure 1.59).¹⁸⁷ While it might be said that Tamiflu bears little resemblance to DANA or sialic acid, the structures of the two cyclopentane-based inhibitors are even further removed from the structures of the natural sialidase substrates; however, these structures retain the carboxylate and acetamido moieties necessary for sialidase binding. BCX-1812 was designed based on analysis of X-ray crystal structures of sialidasases containing DANA in their active sites. It was determined that the functional groups making important contacts in the sialidase active site would be better oriented on a cyclopentane scaffold. After several iterations, BCX-1812 was designed to take

advantage of hydrophobic pockets by containing the isopentyl group that is present in Tamiflu, and the charge-charge interactions afforded by the guanidinyll group that is present in Relenza. BCX-1812 was designed as an orally-administered anti-influenza therapeutic, has a range of IC₅₀ values from 1 x 10⁻¹⁰ to 1.4 x 10⁻⁹ M when tested with 15 different influenza A viruses,¹⁸⁶ and has a K_i = 1.1 x 10⁻⁹ M as measured with the influenza N2 sialidase.¹⁸⁰ A-315675 was also designed based on analysis of sialidase X-ray crystal structures containing various inhibitors bound in their active sites and was based on a D-proline scaffold. One major difference A-315675 has compared with Relenza, Tamiflu, and BCX-1812 is the incorporation of a *cis*-propenyl group instead of a basic amino or guanidinyll group as it was determined that the *cis*-propenyl group formed a beneficial hydrophobic contact with the aliphatic glutamate chain.¹⁸⁷ A-315675 has a K_i = 1.9 x 10⁻¹⁰ M when measured with the influenza N2 sialidase.¹⁸⁰

1.6 Objectives

The objectives of the research described in this thesis all centre around the development of synthetic routes leading to 4-substituted sialic acid analogues in the hope that these will lead to new substrates and inhibitors of sialidases. These new compounds could be used to gain valuable insight into the mechanisms and specificities of sialidases, allowing the generation of better drugs to treat sialidase-related disorders and generating novel sialosides as probes and for incorporation into glycoconjugates.

Chapter 2 describes the initial research project, the goal of which was to synthesize 4-deoxy-4-nitrosialic acid. This compound has not been synthesized previously and it was of interest to determine the effects of substituting the 4-hydroxyl group for a nitro group. As well, this compound would be a useful synthetic intermediate since the nitro group is synthetically versatile and should allow a number of 4-modified sialic acid analogues to be generated. When the synthesis of the 4-deoxy-4-nitrosialoside was accomplished, functional group modification of the nitro group would be carried out and glycosylation of these derivatives would be attempted in order to generate substrates for sialidase-catalyzed hydrolysis. This would allow comparison of the effects of the various 4-substituents on sialidase binding and hydrolysis.

Chapter 3 begins with the isolation of an unexpected β,γ -unsaturated α -keto ester from ozonolysis of a protected enoate ester precursor, describing possible pathways for its formation. This enone was then used in conjugate additions to install various groups at the 4-position of the enone, allowing the synthesis of many more 4-modified sialic acid analogues. Dialkylzinc reagents were able to be added in the presence of a copper catalyst and triethylphosphite and attempts were made to convert the addition products into 4-modified sialic acid analogues.

Chapter 4 describes the isolation of another unexpected compound, one with an oxabicyclo[3.1.0]hexane-based scaffold. This product was a result of an intramolecular substitution reaction and had a novel bicyclic structure that could form the basis for a new class of sialidase inhibitors. The product would have to

be deprotected without destroying the bicyclic ring system, something that might be accomplished by reduction of the nitro group.

CHAPTER 2: SYNTHESIS OF 4-DEOXY-4-NITROSIALIC ACID AND DERIVATIVES

2.1 Introduction

Sialic acid derivatives and analogues containing variation at every one of the nine carbons in the carbon skeleton have been synthesized, as described in the previous chapter.²⁷ These compounds have been used: (1) to probe the effects of substituent variation on binding to sialic acid-recognizing proteins; (2) to incorporate additional functionality such as fluorescent groups for use in probes of sialic acid-binding proteins; and (3) to take advantage of substitutions that result in improved binding to design better inhibitors, for example.

Variation at the 4-position of sialic acid is particularly interesting as some important sialidases accept such modifications, influenza sialidases being particularly tolerant. As described in the previous chapter, the two major structural requirements of an influenza sialidase inhibitor are a carboxylate moiety and an amido group on opposite sides of the molecule. The carboxylate forms important charge-charge interactions with an arginine triad in the enzyme's active site while the amido group interacts with an arginine residue through its carbonyl oxygen and it has hydrophobic interactions with tryptophan and isoleucine residues through its methyl group. Therefore, these substituents are often included in sialidase inhibitors. Modification at the 4-position, however, tends to increase sialidase binding with the inclusion of positively-charged

substituents (e.g. Tamiflu, Relenza). The 4-position may be the most exploitable position for inhibitor development; however, there are no general synthetic routes leading to an intermediate that could be widely derivatized at the 4-position.

Many sialic acid analogues have been synthesized chemoenzymatically using sialic acid aldolase (see Chapter 1.3). The normal catabolic function of this enzyme is to break the 9-carbon sialic acid molecule into pyruvate and *N*-acetylmannosamine (ManNAc) (3- and 6-carbon fragments, respectively) but it can be made to act predominantly in a synthetic direction, that is to synthesize sialic acid, by greatly increasing the concentration of either pyruvate or ManNAc (see Figure 1.8). This aldolase displays promiscuous substrate specificity for the aldose fragment and is therefore very useful in synthesizing sialic acid analogues that contain a variety of functional groups at positions 5-9. However, this enzyme mandatorily installs a hydroxyl group at the 4-position as a result of pyruvate attack on the aldehyde carbon of the aldose and therefore this enzyme cannot be used to synthesize readily 4-modified sialic acid analogues. Several 4-modified sialic acid analogues have been synthesized chemically but the majority of these variants used sialic acid as the starting material and were derived from a common 4-oxo intermediate in which the 4-OH group was oxidized to a keto group (see Figure 1.27).

The synthetic strategy described herein was designed to lead to a sialic acid analogue in which the hydroxyl group at the 4-position was replaced by a nitro group. This would be accomplished by reversing the polarity of the chain extension step. In most chemical syntheses of sialic acid and its analogues, a

nucleophilic pyruvate synthon attacks an electrophilic aldoose to extend the carbon framework, forming the sialic acid backbone (see Chapter 1.3.1). The strategy described herein used a nitroalditol that was deprotonated next to the nitro group and the resulting nitronate anion was employed as a nucleophile to attack an electrophilic pyruvate synthon, thus installing a nitro group at the 4-position (Figure 2.1). Of note, a similar strategy was used by Vasella and co-workers to synthesize sialic acid from a nitroaldopyranose but the nitro group was hydrolyzed to allow their coupling product to isomerize to the sialic acid pyranose structure (Figure 1.10).⁴⁵

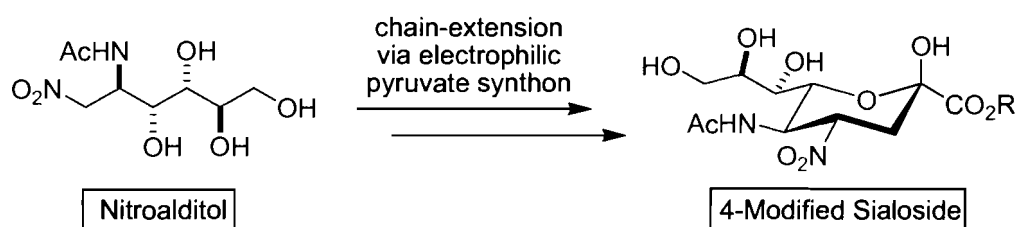


Figure 2.1 Synthetic strategy leading to 4-modified sialosides from a nitroalditol

The first reason to synthesize sialosides containing a nitro group in the 4-position is that they have not been made previously. It would therefore be interesting to investigate the effects of such a substitution on sialidase binding. The second reason to synthesize such compounds is that the nitro group is synthetically versatile, a subject covered in a book by Noboru Ono in 2001.¹⁸⁸ Due to the electron-withdrawing nature of the nitro group, neighbouring protons are rendered relatively acidic, allowing epimerization at this centre via deprotonation/reprotonation and generation of a nucleophile for further alkylation at this centre. The nitro group can also be treated with a variety of reducing

agents to produce oximes, hydroxylamines, and amines. All of these nitrogen-containing derivatives are themselves synthetically versatile. The nitro group can also be hydrolyzed to a ketone, giving the 4-oxo-sialoside intermediate from which many 4-modified sialic acid analogues have been made. In addition, the nitro group can be removed and replaced with a hydrogen atom under radical conditions. Thus, a sialoside containing a 4-nitro group would be a useful synthetic intermediate.

These 4-modified sialic acid analogues could then be glycosylated to form substrates for sialidase-catalyzed hydrolysis reactions. Comparison of the kinetic parameters of hydrolysis of several analogues would allow determination of the contribution of the 4-substituent to binding. This information could then lead to the development of potent sialidase inhibitors.

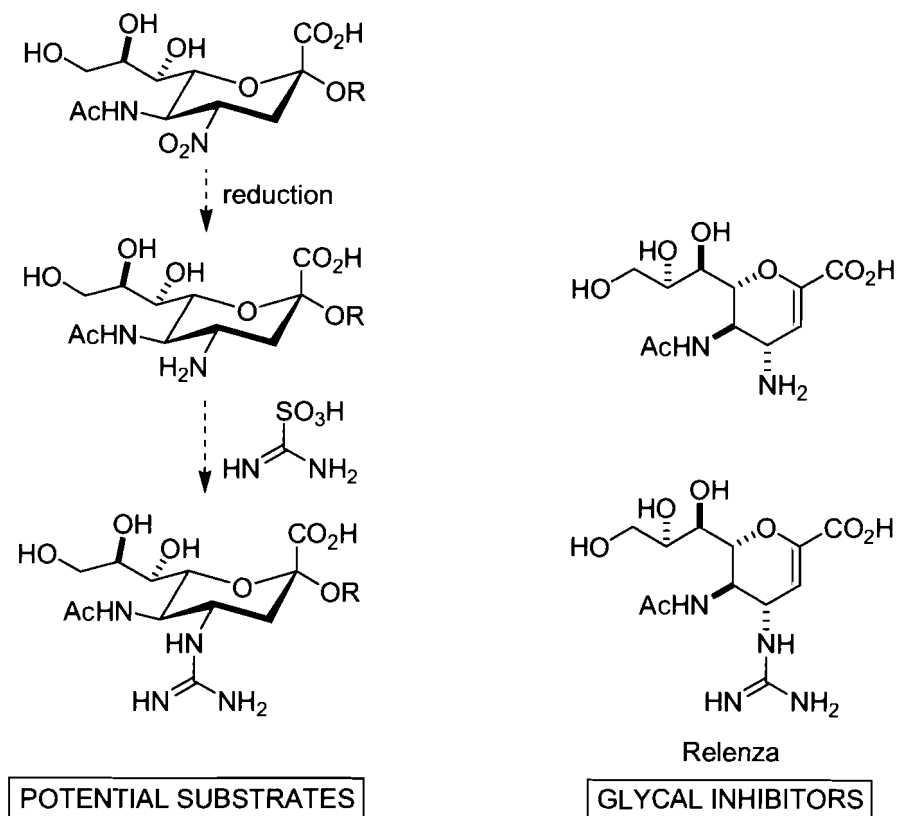


Figure 2.2 4-Modified sialosides as potential sialidase substrates and their glycal counterparts as sialidase inhibitors

If the 4-deoxy-4-nitrosialoside could be glycosylated to form a substrate, the nitro group could be reduced to an amino group that could then be converted into a guanidinyl group (Figure 2.2). These substrates could then be considered as ground state versions of compounds that have been labelled as transition state analogues. Glycals of sialic acid analogues such as DANA and Relenza are often described as transition state analogues based on their potency and on the non-chair conformation of the pyranose ring induced by the double bond. However, there are several conditions that must be met in order to label an inhibitor as a transition state analogue, conformation of the pyranose ring being only one such condition.¹⁸⁹ Although compounds such as Relenza and Tamiflu

contain positively charged groups, these charges are not located in positions necessary to mimic the proposed oxacarbenium ion-like species present at the transition state during sialidase-catalyzed hydrolysis. It is likely that these compounds are simply fortuitous binders, taking advantage of favourable contacts with active site amino acid residues to enhance their inhibitory potency.

Enzymes are presumed to catalyze reactions by binding the transition state during the reaction very tightly in preference to the substrate ground state. By stabilizing the structure of the transition state, enzymes are capable of providing the enormous rate accelerations for which they are known.¹⁹⁰ Thus, true transition state analogue inhibitors can take advantage of this extraordinarily tight binding by attempting to mimic the structural features of the transition state, a difficult feat to accomplish since transition states contain partial bonds. When an inhibitor is labelled as a transition state analogue, the compound is often used as the structural basis for modifications in the search for more potent inhibitors. Therefore, it is important to determine what feature(s) of the inhibitor actually contributes to its potency.

Whether an inhibitor is a transition state analogue or simply a fortuitous binder can be determined by examining the linear free energy relationship between inhibitor binding and transition state stabilization.¹⁹¹ This rigorous test compares the binding constant of an inhibitor with the kinetic parameters for enzyme-catalyzed hydrolysis (k_{cat}/K_m) of a structurally similar substrate. A series of inhibitors and substrates are synthesized that are structurally varied at some position that does not affect binding of unmodified regions to the enzyme. If this

variation affects inhibitor binding to the same degree as it affects substrate hydrolysis, the inhibitor is a transition state analogue since the substituent variation is also affecting the energy of the transition state during catalysis to the same degree (as seen through the kinetic parameters of substrate hydrolysis). A poor correlation between these two values and/or a correlation that does not show equal effects resulting from substituent variation indicates the inhibitor is not a transition state analogue but simply a fortuitous binder since the reaction would proceed through a transition state that is not adequately mimicked by the inhibitor. In order to determine whether glycals such as DANA and Relenza are transition state analogues, a series of inhibitors (glycals) could be made in which the 4-substituent is varied. The same variation must be made in a series of substrates. The compounds shown in Figure 2.2 represent two such variants.

This chapter describes the development of the synthetic route leading to 4-deoxy-4-nitrosialic acid, including difficulties encountered at various steps. It then goes on to describe efforts to glycosylate this material in order to lead to substrates for sialidase-catalyzed hydrolysis. This required the synthesis of several donor sugars and the testing of many different protocols for the notoriously difficult glycosylation of these sugars. Attempts to determine the effect of the 4-nitro group on sialidase binding will also be discussed.

2.2 Synthesis of 4-Deoxy-4-nitrosialic Acid

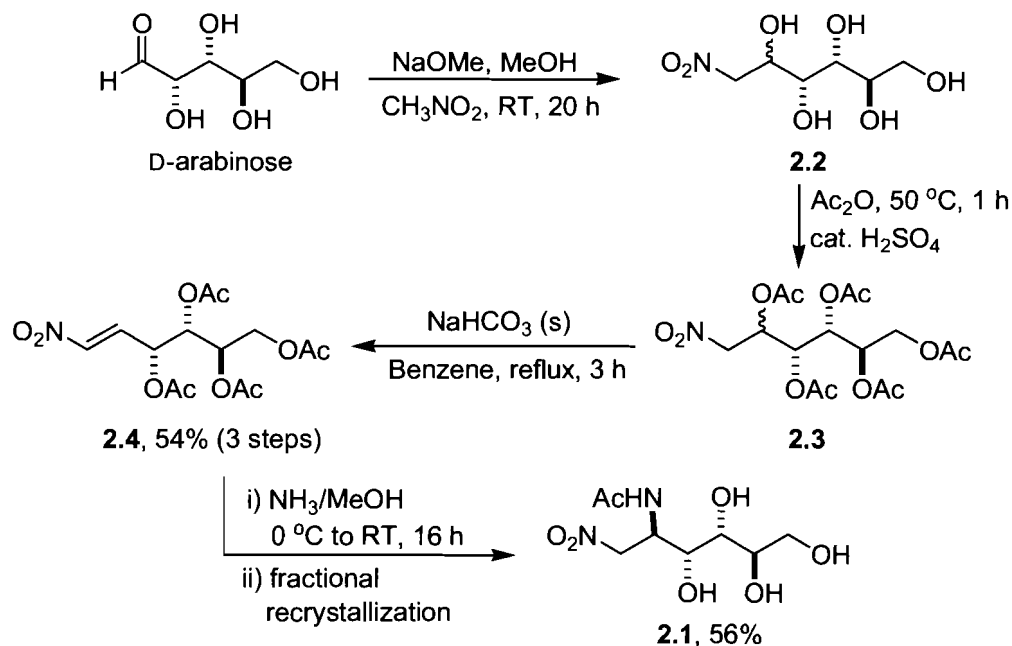


Figure 2.3 Synthesis of nitromannitol 2.1 in four steps from D-arabinose

The route to 4-deoxy-4-nitrosialic acid began with the synthesis of 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol (**2.1**) as a key intermediate that would be deprotonated and coupled with an appropriate 3-carbon electrophilic pyruvate synthon. The synthesis of nitromannitol **2.1** was accomplished in five steps from D-arabinose using a route developed by Sowden and co-workers (Figure 2.3).¹⁹² This involved a Henry reaction between D-arabinose and nitromethane promoted by sodium methoxide to provide a mixture of 1-deoxy-1-nitromannitol and 1-deoxy-1-nitroglucitol **2.2** after neutralization. The syrupy nitroalditols were then peracetylated under acidic conditions to give acetates **2.3**, which were treated with solid sodium bicarbonate in refluxing benzene to induce elimination to give nitrohexene **2.4**. No purification was necessary to this point, allowing the routine

synthesis of multigram quantities of nitrohexene **2.4** which could be recrystallized from ethanol to give a 54% yield of light yellow flakes over the three steps. These reactions typically started with 30 g of D-arabinose; on occasion, 50 g reactions were carried out but led to lower yields of nitrohexene **2.4** and were less practical in terms of the required volumes of solvent.

The final step in the synthesis of the nitromannitol intermediate **2.1** was a Michael addition of ammonia to nitrohexene **2.4**, which also caused deacetylation and migration of an acetyl group to the newly-installed amino group. Initially this was performed according to Sowden's procedure that involved bubbling ammonia through a methanol suspension of **2.4** overnight. Fractional crystallization in ethanol of the nitromannitol product **2.1** from the nitroglucitol isomer and acetamide gave **2.1** in 43% yield (Sowden reported 51%). The yield of **2.1** was modestly improved to 56% using conditions developed by O'Neill in which nitrohexene **2.4** was added portionwise to a cooled (0 °C) saturated ammonia solution in methanol (O'Neill reported an 82% yield).¹⁹³

With nitromannitol **2.1** in hand, the next step was to extend the carbon framework from 6 carbons to 9 carbons by deprotonating **2.1** and reacting the nitronate with a suitable electrophilic pyruvate synthon. Slight difficulties were encountered at this chain-extension step (Figure 2.4). Initially, **2.1** was deprotonated with sodium methoxide and reacted with ethyl bromopyruvate; this would install the pyruvate moiety directly and the product would cyclize to the sialic acid pyranose structure. Unfortunately no reaction was observed after 1 d. The reaction was repeated in the presence of AgBF₄ to enhance the

with or without the addition of AgBF_4 . **2.5** was even treated with LDA, which did not lead to coupling with ethyl bromopyruvate. Allyl bromide was tried as an electrophile with **2.5** as well. Using sodium methoxide as a base induced decomposition of the starting material (although this may have been a result of the acidic resin used to neutralize the base) while the use of aqueous NaOH in THF seemed to provide a small amount of coupled product but not enough to be synthetically useful.

Vasella and co-workers had been successful in coupling a nitronate with alkyl α -(bromomethyl)acrylate esters.^{45,107,133} These reactions proceed as a Michael-type addition rather than an $\text{S}_{\text{N}}2$ -type displacement in which the nitronate attacks the enone rather than the bromomethyl group.¹⁹⁵ This shifts the position of the double bond, eliminating bromide to generate a coupled enoate ester that could be treated with ozone to cleave the methylene moiety and install the desired α -keto ester. Thus, ethyl α -(bromomethyl)acrylate **2.6** was synthesized in three steps from formaldehyde and diethyl malonate (Figure 2.5).¹⁹⁶ Diethylmalonate was coupled with formaldehyde in the presence of NaHCO_3 to give diethyl bis(hydroxymethyl)malonate **2.7**. This product was saponified, decarboxylated, dibrominated, and eliminated in one pot by heating with 48% HBr overnight to afford α -(bromomethyl)acrylic acid **2.8**. This was converted to the ethyl ester **2.6** by refluxing in a benzene/ethanol mixture with catalytic H_2SO_4 in a Soxhlet extractor containing a thimble of Na_2SO_4 to remove the water by-product that was carried into the upper chamber of the extractor as an azeotrope. The product was then purified via Kugelrohr distillation. The

yields on these reactions are not high but all of the materials involved are inexpensive and readily available.

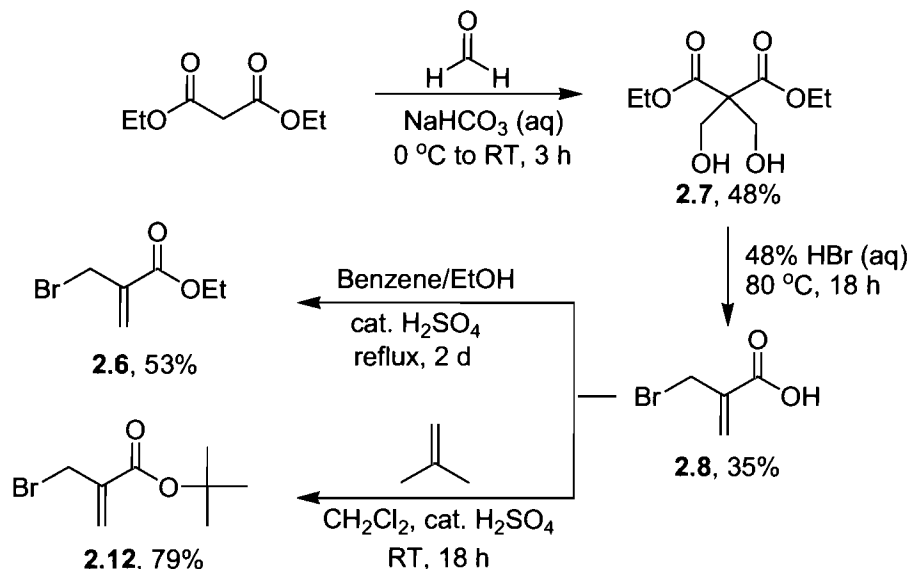


Figure 2.5 Synthesis of alkyl α -(bromomethyl)acrylate esters **2.6** and **2.12**

Addition of nitromannitol **2.1** to ethyl α -(bromomethyl)acrylate **2.6** proceeded smoothly when promoted by sodium methoxide in methanol. After stirring overnight, the reaction afforded two diastereomers of the enoate ester product **2.9** in 75% yield with a 1.3:1.0 4*S*:4*R* ratio (Figure 2.6). The diastereomers of **2.9** could not be separated chromatographically and attempts to crystallize the products from ethanol/Et₂O or ethanol/hexanes mixtures failed. However, ozonolytic cleavage of the methylene moiety in 1:1 methanol/CH₂Cl₂ followed by reduction of the ozonides with DMS afforded the desired α -keto ester moiety and the products spontaneously cyclized to the sialic acid pyranose structures with the major 4*S*-epimer selectively precipitating from the reaction mixture, leaving the minor undesired 4*R*-epimer in solution. Pure 4-deoxy-4-

nitrosialic acid, ethyl ester **2.10** was obtained in 55% yield following filtration and washing with CH₂Cl₂ to remove the DMSO by-product while a 30% yield of impure 4-*epi*-4-deoxy-4-nitrosialic acid, ethyl ester **2.11** was obtained. The stereochemistry at the 4-position of **2.10** was determined by observation of the large coupling constants of proton 4 with proton 3_{ax} and proton 5 ($J_{3_{ax},4} = J_{4,5} = 11.2$ Hz), indicative of trans-diaxial coupling. These values contrast sharply with the small coupling constants observed from the 4-epimer **2.11** between proton 4 with proton 3_{ax}, proton 3_{eq}, and proton 5 ($J_{3_{eq},4} = 3.2$ Hz, $J_{3_{ax},4} = 5.0$ Hz, $J_{4,5} = 4.0$ Hz), indicating that proton 4 was equatorial in **2.11**, thus it is the 4*R*-isomer. As the ratio of **2.10** to **2.11** was approximately 1.8:1.0, it can be assumed that the major isomer of the enoate ester precursor **2.9** was indeed the 4*S* isomer.

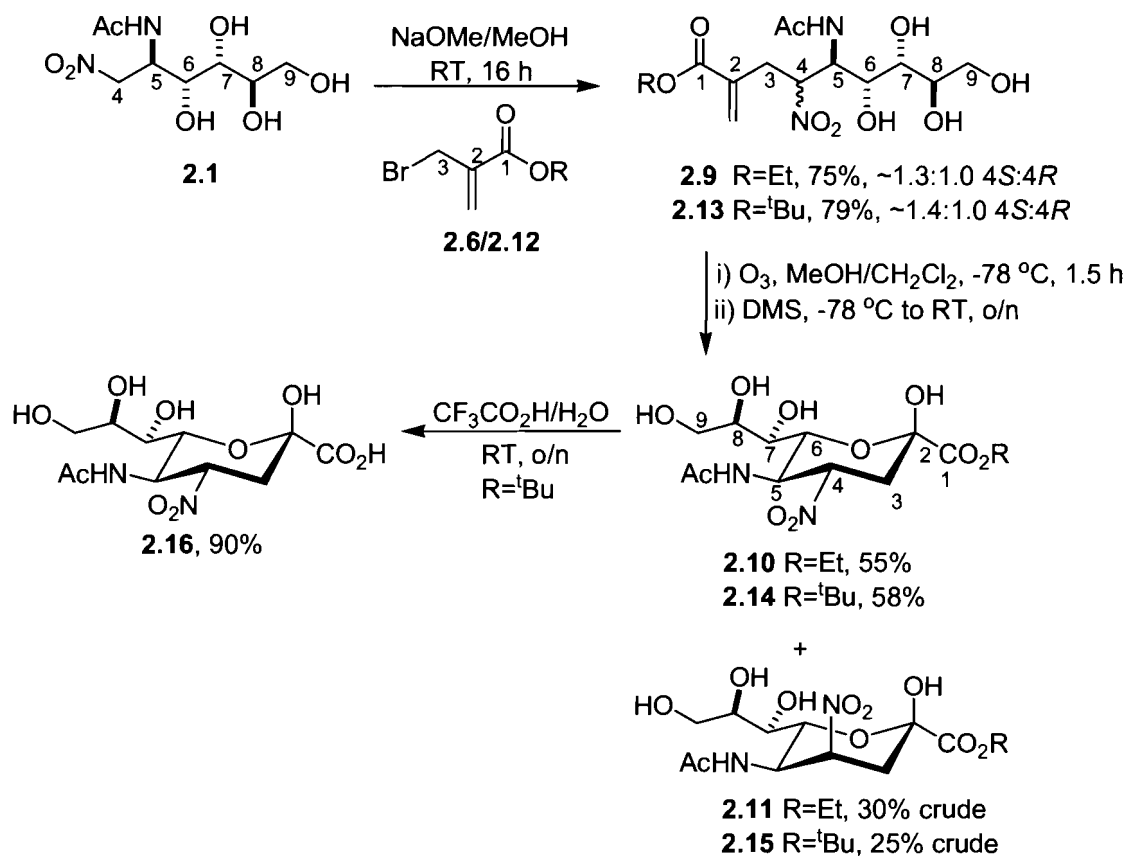


Figure 2.6 Synthesis of 4-deoxy-4-nitrosialic acid by coupling of nitromannitol **2.1** with alkyl α -(bromomethyl)acrylate esters. Numbers reflect positions of carbon atoms in sialic acid analogue products.

Hydrolysis of the ethyl ester of **2.10** did not proceed as expected. Such hydrolyses are commonly carried out using lithium hydroxide in THF/H₂O mixtures without complication. When **2.10** was treated under these conditions at 0 °C, the product lacked the ethyl ester but did not exhibit the expected spectral characteristics. In the ¹H NMR spectrum, the signals for the protons on carbon 3 had shifted downfield slightly to 2.7 and 2.9 ppm from 2.4 and 2.5 ppm and their coupling constants with proton 4 had decreased to 4.4 and 8.3 Hz, showing a lack of trans-diaxial coupling. As well, these peaks disappeared over time as the sample remained dissolved in D₂O. These data suggest that the hydrolysis

product was no longer in the pyranose ring structure but was a linear compound. Supporting this was the fact that the peak for carbon 2 had disappeared from its normal frequency around 94 ppm, a characteristic frequency of the hemiketal carbon of the pyranose ring, and the HMBC spectrum showed a correlation between proton 3 and a carbon at 200 ppm, indicating that carbon 2 was a ketone. The IR spectrum confirmed this as a peak was present at the characteristic ketone carbonyl stretching wavenumber of 1712 cm^{-1} . In addition, the two prominent peaks for nitro group stretching at 1370 and 1550 cm^{-1} were absent from the IR spectrum and the frequency of proton 4 had shifted upfield from 5.0 to 4.0 ppm in the ^1H NMR spectrum. It was apparent that the nitro group had undergone a reaction of some kind during the de-esterification; however, the identity of this product remains unknown. The same product was observed when **2.10** was treated with 2 eq. aqueous sodium hydroxide in ethanol overnight and with 0.02 M aqueous sodium hydroxide (2 eq.) for 30 min. Acidic conditions were employed for ethyl ester hydrolysis on one occasion as **2.10** was stirred in a mix of acetic acid, H_2SO_4 , and water for 2 h; however, no product could be separated from the H_2SO_4 -containing medium.¹⁹⁷

Synthesis of the *t*-butyl ester analogue of **2.10** would allow removal of the ester under acidic conditions in which the nitro group should not interfere. This required the synthesis of *t*-butyl α -(bromomethyl)acrylate **2.12** which was accomplished by treating α -(bromomethyl)acrylic acid **2.8** with 2-methyl-2-propene under acid catalysis to give the product in 79% yield (Figure 2.5).^{198,199} The *t*-butyl ester **2.12** was then coupled with **2.1** under the same conditions as

was its ethyl ester analogue to give the *t*-butyl enoate ester **2.13** in 79% yield as a 1.4:1.0 mixture of 4*S*:4*R* diastereomers (Figure 2.6). This inseparable mixture was treated with ozone to cleave the methylene unit and, as with the ethyl ester analogue, the 4*S* isomer precipitated from solution upon reduction of the ozonides to afford 4-deoxy-4-nitrosialic acid, *t*-butyl ester **2.14** as a white solid after filtration in 33% yield. A further 25% of **2.14** was isolated following flash chromatographic purification of the concentrated filtrate that also gave the impure 4*R*-epimer **2.15** in a crude yield of 25%. Again, the stereochemistry at the 4-position was determined following analysis of the large coupling constants from proton 4 with proton 3_{ax} and proton 5 ($J_{3_{ax},4} = 12.9$ Hz, $J_{4,5} = 10.6$ Hz) for **2.14**, indicative of trans-diaxial coupling. The much smaller coupling constants between proton 4 and the protons on carbon 3 ($J_{3,4} = 3.2, 5.0$ Hz) for **2.15** supported the 4*R* assignment. The final de-esterification step proceeded smoothly by stirring **2.14** in aqueous CF₃CO₂H overnight followed by evaporation of the solvent to give pure 4-deoxy-4-nitrosialic acid **2.16** in 90% yield as a tan solid (Figure 2.6). Attempts to purify **2.16** via chromatography, recrystallization, and decolourization with charcoal resulted in partial decomposition; however, purification was not necessary since the compound gave excellent results from all analytical techniques.²⁰⁰

A sample of 4-deoxy-4-nitrosialic acid **2.16** was sent to the lab of Prof. Garry Taylor at the University of St. Andrews in order to soak the compound into their crystals of influenza N8 sialidase. If **2.16** were to occupy the sialidase active site for any length of time, this would allow an X-ray crystal structure of the

complex to be obtained. From the crystal structure, the effects of substituting the 4-hydroxyl group for a nitro group could be evaluated by observing the contacts made between the nitro group and amino acid residues in the active site. Unfortunately a structure could not be obtained as **2.16** did not bind with sufficient affinity to the sialidase active site. It is possible that **2.16** could not bind to the influenza N8 sialidase since the nitro group is larger than the normal hydroxyl group; however, the binding constant of sialic acid binding with sialidases is very low (in the mM range) and it is therefore likely that the binding constant for **2.16** is also weak.

Since 4-deoxy-4-nitrosialic acid **2.16** had been made successfully, as had the ethyl ester **2.10** and the *t*-butyl ester **2.14**, it was time to attempt glycosylation and derivatization reactions.

2.3 Derivatization of 4-Deoxy-4-nitrosialosides

2.3.1 Synthesis of 4-Deoxy-4-nitrosialoside Donor Sugars

Following the synthesis of 4-deoxy-4-nitrosialic acid, glycosylation was attempted in order to synthesize potential sialidase substrates. Once accomplished, the 4-position could then be derivatized by taking advantage of the synthetic versatility of the nitro group. The kinetic parameters of sialidase-catalyzed hydrolysis could then be measured for these novel substrates allowing comparison with those of known substrates to determine the effects of introducing various substituents in the 4-position.

In order to glycosylate 4-deoxy-4-nitrosialic acid, donor sugars were required. Initially, two donor sugars were synthesized by stirring ethyl ester **2.10** with acetyl chloride in a sealed flask for 3 d (Figure 2.7). The first step of this reaction peracetylated the compound to give **2.17** while over time the anomeric acetate was replaced with chloride with the aid of the HCl that is generated in situ, giving **2.18**. Installation of the anomeric chloride was a slow process as determined by withdrawing an aliquot after 2 d, at which time the ^1H NMR spectrum showed a 67:33 mix of **2.17**:**2.18** while after 3 d and subsequent work-up the final ratio was 85:15. These data highlight the electron-withdrawing effects of the 4-nitro group as it deactivated the anomeric centre since acid-catalyzed replacement of the anomeric acetate with a chloride ion likely proceeds via a $\text{D}_\text{N}^*\text{A}_\text{N}$ mechanism. This mechanism involves oxacarbenium ion pairs that would be destabilized by the electron-withdrawing nature of the 4-nitro group. The fact that **2.17** was isolated from this reaction supports this since the peracetylated sialoside is not obtained from the reaction of sialic acid, methyl ester under the same conditions which has a 4-acetoxy group that is much less electron-withdrawing than a nitro group.

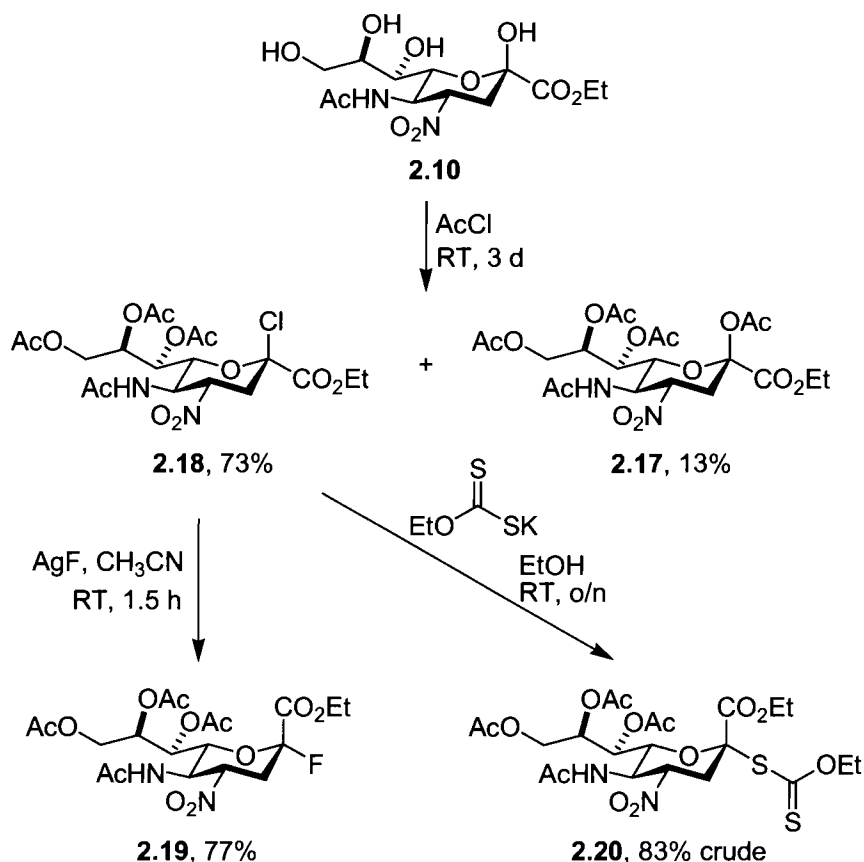


Figure 2.7 Synthesis of 4-deoxy-4-nitrosialoside donor sugars

Two other donor sugars were synthesized from the β -sialosyl chloride **2.18**. By stirring with silver(I) fluoride in CH₃CN for 1.5 h, the α -sialosyl fluoride **2.19** was obtained in 77% yield (Figure 2.7).²⁰¹ As this reaction likely proceeded via an S_N2 type mechanism, the α -anomer was predicted to be the major product. The stereochemistry at the anomeric centre was supported by the low values of the coupling constants between the fluorine and the protons on carbon 3 ($J_{3ax,F} = 6.1$ Hz, $J_{3eq,F} = 10.8$ Hz). Had the product been the β -sialosyl fluoride, the fluorine-proton 3_{ax} coupling constant would have been around 25 Hz. A second donor sugar was obtained by stirring the β -sialosyl chloride **2.18** with O-ethyl-S-potassium dithiocarbonate overnight in ethanol to afford the crude α -ethyl

xanthate donor sugar **2.20** in a yield of 83% (Figure 2.7).¹⁷⁵ The crude product may have been unstable to column chromatography as only a 40% yield of **2.20** was recovered following purification; thus, **2.20** was often used without purification. A series of 1D NOE difference ¹H NMR spectra were acquired in an attempt to assign the anomeric configuration of the product; however, no signal enhancements were observed upon irradiation of the SCH₂ protons of the xanthate moiety as these protons were not close enough to any on the pyranose ring to show contacts. The α-configuration of **2.20** was assigned based on literature precedent.¹⁷⁵

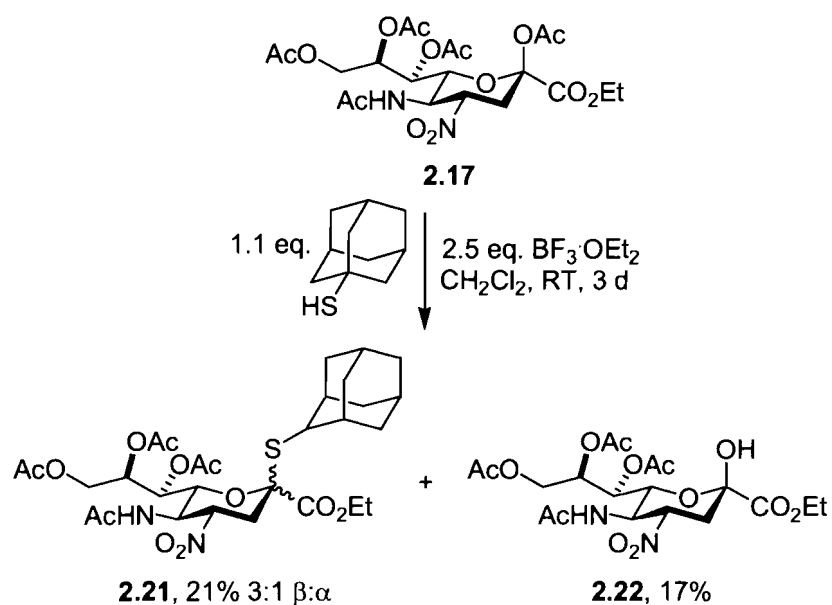


Figure 2.8 Synthesis of 1-adamantyl thiosialoside **2.21** from pentaacetate **2.17**

A fifth donor sugar was synthesized by stirring the pentaacetate **2.17** with 1-adamantanethiol in the presence of BF₃·OEt₂ (Figure 2.8).¹⁷⁸ After 3 d, this afforded a 23% yield of the 1-adamantyl thiosialoside **2.21** as a 3:1 β/α anomeric mixture as well as a 17% yield of the hemiacetal **2.22** resulting from hydrolysis of

the anomeric acetyl group. In addition, 41% of unreacted **2.17** was recovered. It has been reported that peracetylated sialic acid, methyl ester gave an 81% yield of the 1-adamantyl thiosialoside product under these conditions after 1 d.¹⁷⁸ Thus, the reaction of **2.17** was initially performed for 1 d but this afforded a 14% yield of **2.21**. A repeat of the reaction was allowed to proceed for 3 d in an attempt to overcome the low reactivity of **2.17**, likely stemming from the electron-withdrawing nitro group in the 4-position, to afford the aforementioned 23% yield of **2.21**. A third reaction was allowed to proceed for 11 d, but the major product of this was the hemiacetal **2.22**, indicating that longer reaction times simply result in hydrolysis of any donor sugars.

The anomeric configuration of **2.21** was assigned based on a series of 1D NOE difference ¹H NMR spectra. When proton 3_{eq} of the minor anomer was irradiated, enhancement of the signal for proton 2 of the adamantyl group was enhanced, indicating the α-configuration. No enhancement of the adamantyl protons were observed upon irradiation of a number of protons on the pyranose ring of the major anomer, indicating the β-configuration as the axial orientation of the adamantyl group puts it too far away from the pyranose ring to have any contacts. As well, literature precedent shows that the β-anomer should be the major anomer.¹⁷⁸

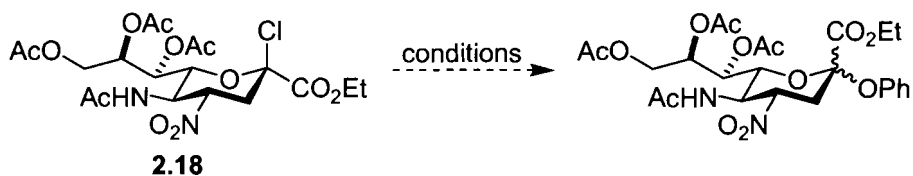
It may be more efficient to use the α-sialosyl fluoride **2.19** to synthesize the 1-adamantyl thiosialoside **2.21** for reasons that will be discussed in the next section of this chapter. The β-sialosyl chloride **2.18** was reacted with 1-

adamantanethiol in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ to try to enhance the yield of **2.21**, but after 1 d there was no reaction.

2.3.2 Glycosylation Attempts on 4-Deoxy-4-nitrosialosides

All naturally-occurring sialidases hydrolyse α -linked sialosides. Therefore it was necessary to find conditions that would allow α -selective glycosylation of one of the five donor sialosides described in the section above. The β -sialosyl chloride **2.18** was the first donor used in this endeavour. Initial glycosylation attempts used standard conditions in which the donor and phenol acceptor were mixed under basic conditions, using Hünig's base in CH_3CN in this case (Table 2.1, entry 1).¹⁷² These reactions proceed via an $\text{S}_{\text{N}}2$ -type reaction, inverting the anomeric configuration to afford the phenyl α -sialoside; although, a competing reaction is often elimination of HCl to form the glycal via deprotonation at the 3-position. In the case of β -sialosyl chloride **2.18**, proton 4 is the most acidic and under basic conditions an intramolecular substitution reaction occurred to form a bicyclic compound (see Figure 4.2). This reaction is the focus of Chapter 4 and will not be discussed further here.

Table 2.1 Conditions used for attempted glycosylation of β -sialosyl chloride **2.18**



| <i>Entry</i> | <i>Conditions</i> | <i>Results</i> |
|--------------|--|--|
| 1 | 10 eq. phenol, 8 eq. EtN ⁱ Pr ₂ , CH ₃ CN, 1 d | Elimination (35% isolated) |
| 2 | 10 eq. KOPh, CH ₃ CN, 1 d | Decomposed |
| 3 | 10 eq. KOPh, DMF, 1 d | Decomposed |
| 4 | 5 eq. phenol, 5 eq. NaOH, CH ₂ Cl ₂ /H ₂ O, Bu ₄ NHSO ₄ , 2 h | Elimination (19% isolated) |
| 5 | 5 eq. phenol, 2 eq. Na ₂ CO ₃ , CHCl ₃ /H ₂ O, Bu ₄ NHSO ₄ , 1 h | No reaction |
| 6 | 5 eq. phenol, 2 eq. Na ₂ CO ₃ , CHCl ₃ /H ₂ O, Bu ₄ NHSO ₄ , 1 d | Elimination (36% isolated) |
| 7 | 5 eq. KOPh, CHCl ₃ /H ₂ O, Bu ₄ NHSO ₄ , 1.5 h | Elimination (20% isolated) |
| 8 | 10 eq. phenol, 3 eq. ZnBr ₂ , MS3Å, CH ₃ CN, 1 d | 51% Hydrolysis to 2.22 |
| 9 | 10 eq. phenol, 3 eq. AgOTf, MS3Å, CH ₃ CN, 2 d | 32% Hydrolysis to 2.22 , 17% 2.18 recovery |

A number of conditions were then tested for glycosylation of β -sialosyl chloride **2.18**, summarized in Table 2.1. Since Hünig's base promoted elimination rather than glycosylation, potassium phenoxide was used to directly attack **2.18** in CH₃CN and in DMF (Table 2.1, entries 2 and 3).¹⁷³ Both reactions resulted in decomposition of the sialoside, however. Phase-transfer conditions are commonly used to glycosylate sialosides wherein the base is often in the aqueous phase and the donor and/or acceptor are in an organic phase and the reaction is mediated by a phase-transfer catalyst.¹⁶⁷ When **2.18** was reacted in CH₂Cl₂ under these conditions with aqueous NaOH in the presence of Bu₄NHSO₄, only elimination was observed after 2 h (Table 2.1, entry 4). When

Na₂CO₃ was used as a weaker base, after 1 h no reaction was observed while after 1 d only the elimination product was isolated (Table 2.1, entries 5 and 6).²⁰² The use of potassium phenoxide under phase-transfer conditions also resulted in isolation of the elimination product only after 1.5 h (Table 2.1, entry 7). Since basic conditions induced elimination of **2.18** rather than glycosylation, two sets of non-basic conditions were tried. The use of ZnBr₂ as a promoter in CH₃CN with powdered 3Å molecular sieves was tested but led simply to hydrolysis of the chloride to give hemiacetal **2.22** (Table 2.1, entry 8).¹⁷¹ Similarly, the use of AgOTf led to the hemiacetal **2.22** in addition to recovery of unreacted β-sialosyl chloride **2.18** (Table 2.1, entry 9).¹⁶⁹

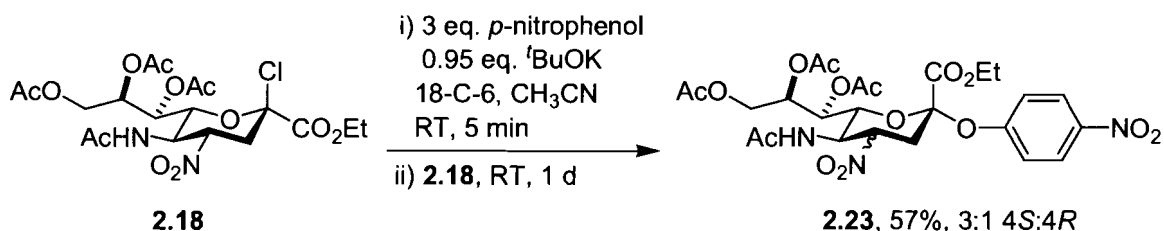


Figure 2.9 Synthesis of *p*-nitrophenyl α-sialoside 2.23

In contrast, the *p*-nitrophenyl α-sialoside **2.23** was synthesized from β-sialosyl chloride **2.18** via direct attack of the *p*-nitrophenoxide anion (Figure 2.9). The lower pK_a of *p*-nitrophenol as compared with phenol (7.18 vs 9.92, respectively) was thought to favour nucleophilic attack at the anomeric carbon of **2.18** over deprotonation at the 4-position. Thus, *p*-nitrophenol was stirred with a sub-stoichiometric amount of ^tBuOK (0.95 eq.) in CH₃CN for 5 min on the assumption that all of the base would be consumed in generating the phenoxide. Upon addition of **2.18** and stirring overnight, no elimination product was observed

and the *p*-nitrophenyl α -sialoside **2.23** was isolated in 57% yield; however, it was isolated as an inseparable mix of 4*S*:4*R* isomers in a 3:1 ratio (Figure 2.9). Thus, deprotonation at the 4-position must have occurred at some point to result in epimerization at this centre but did not result in elimination of whatever leaving group was present at the anomeric centre. The α -anomeric configuration of **2.23** was assigned based on a series of 1D NOE difference ^1H NMR spectra that showed enhancement of the signal for the *ortho*-protons of the *p*-nitrophenyl ring when either of the 3-protons were irradiated (Figure 2.10).

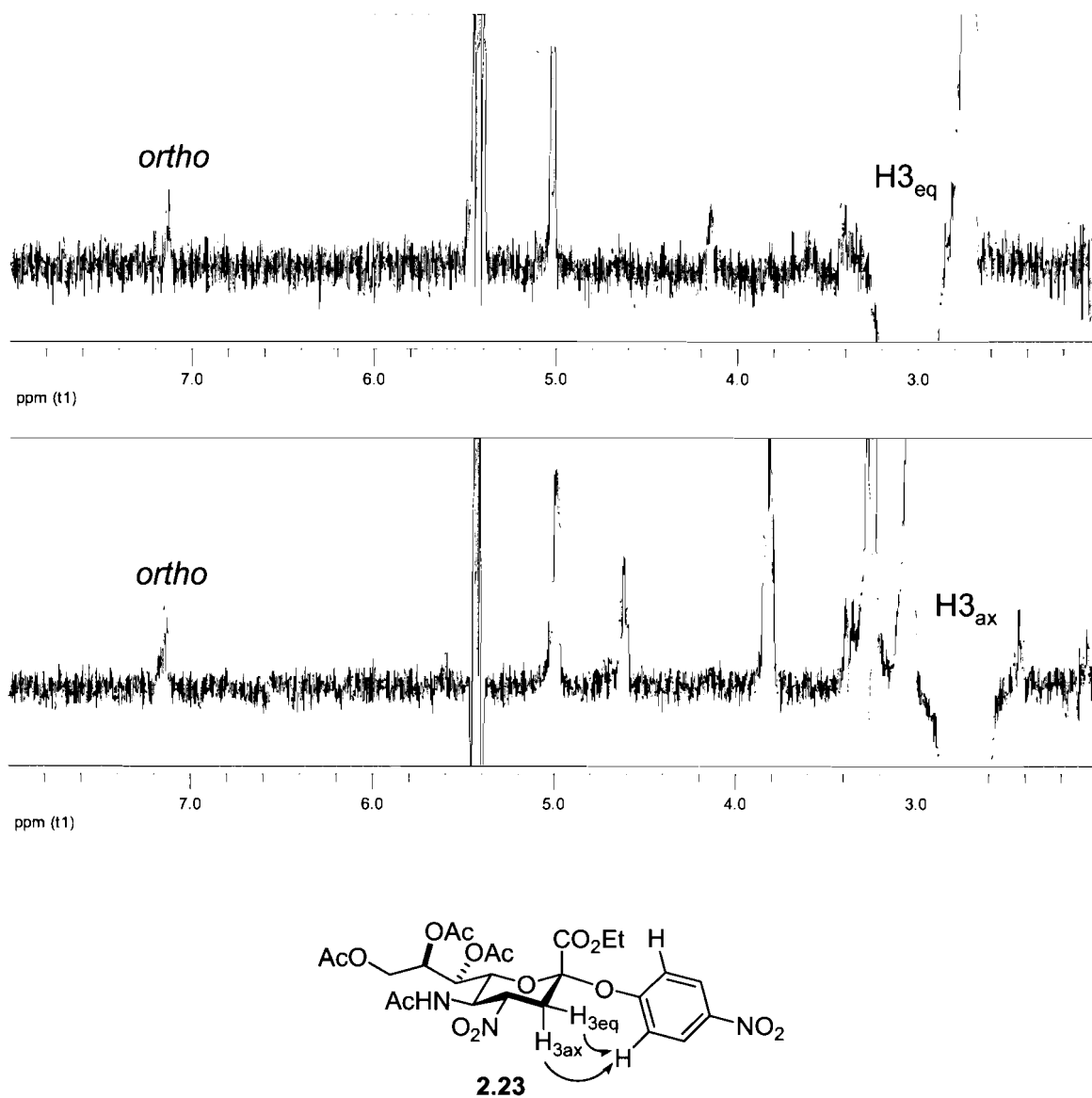


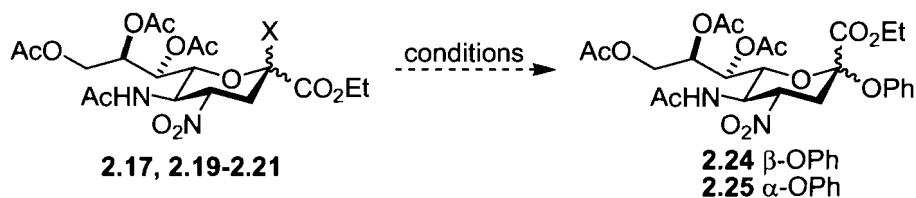
Figure 2.10 1D NOE difference ¹H NMR spectra showing enhancement of *ortho* ArH signals upon irradiation of H_{3eq} and H_{3ax}, indicating α-anomeric configuration of **2.23**

Although these conditions resulted in generation of a desired aryl α-sialoside, the product was isolated as a mix of inseparable 4-epimers. While it may be possible to separate these epimers at a later stage, the larger issue with **2.23** stems from its limited synthetic utility as the next step in the synthesis of substrates would be derivatization of the 4-nitro group. This would require

achieving differential reactivity between the alkyl nitro group and the more reactive aryl nitro group which could be very difficult. The search for α -selective glycosylation conditions was therefore continued using phenol as the acceptor.

Mitsunobu reactions have been used on sialoside hemiacetals to generate aryl sialosides in good yields with moderate α -selectivity.²⁰³ Thus, hemiacetal **2.22** was stirred with 2.0 eq. phenol in CH₃CN containing 1.5 eq. triphenylphosphine and 1.5 eq. diethyl azidodicarboxylate (DEAD) at 0 °C. However, after 2.5 h only starting material was recovered.

Table 2.2 Attempted glycosylation reactions on donor sugars 2.17 and 2.19-2.21



| Entry | Donor ^a | Conditions | Results |
|-------|--------------------|--|---|
| 1 | β-OAc | 10 eq. phenol, 5 eq. BF ₃ OEt ₂ , CH ₂ Cl ₂ , 1 d | 23% β-OPh, 17% 2.22 , 37% β-OAc recovery |
| 2 | α-F | 10 eq. phenol, 5 eq. BF ₃ OEt ₂ , CH ₂ Cl ₂ , 1 d | 72% β-OPh, 8% α-OPh |
| 3 | α-F | 10 eq. phenol, 5 eq. ZnCl ₂ , MS3Å, CH ₃ CN, 2 d | No reaction |
| 4 | α-F | 5 eq. KOPh, CHCl ₃ /H ₂ O, Bu ₄ NHSO ₄ , 14 d | After 2 d, no reaction After 14 d, decomposed |
| 5 | α-Xan | i) 1.6 eq. phenol, MS3Å, CH ₃ CN, 1 d ii) 1.5 eq. NIS, 0.2 eq. TMSOTf, -40 °C to RT, 7 h | 15% β-OPh, 17% α-OPh contaminated with 9% decomposition product |
| 6 | β/α-SAda | i) 1.5 eq. phenol, MS3Å, CH ₂ Cl ₂ /CH ₃ CN, 45 min ii) 2.5 eq. NIS, 1.0 eq. TMSOTf, -78 °C, 1.5 h | 25% β-OPh, 38% α-OPh |

a) β-OAc: **2.17**; α-F: **2.19**; α-Xan: **2.20**; β/α-SAda: **2.21**

Several glycosylation conditions were then attempted with the other four donor sialosides **2.17** and **2.19-2.21** and are summarized in Table 2.2. BF₃OEt₂ was used as a promoter with the peracetylated sialoside **2.17** and with the α-sialosyl fluoride **2.19** (Table 2.2, entries 1 and 2).¹⁷⁵ In both cases, the glycosylation was highly β-selective but the α-sialosyl fluoride **2.19** was much more reactive, affording a 72% yield of the undesired phenyl β-sialoside **2.24** in addition to a small quantity (8%) of the phenyl α-sialoside **2.25**. Under the same conditions, only a 23% yield of phenyl β-sialoside **2.24** was isolated from glycosylation of **2.17** along with 17% of the hydrolysis product **2.22** and 37%

recovered starting material. These results indicate that use of the α -sialosyl fluoride **2.19** in the synthesis of the 1-adamantyl thiosialoside **2.21** may result in greater yields than were obtained when using the peracetylated sialoside **2.17** as a donor sugar, as shown in the previous section of this chapter. The use of a weaker Lewis acid, ZnCl_2 , in CH_3CN with 3Å molecular sieves on the α -sialosyl fluoride **2.19** resulted in no reaction after 2 d (Table 2.2, entry 3). An attempt at using phase-transfer conditions with potassium phenoxide to displace the anomeric fluoride was also fruitless (Table 2.2, entry 4). After 2 d, TLC indicated no reaction had occurred and after 14 d the material had decomposed.

The use of sulfur-based donor sialosides **2.20** and **2.21** gave more promising results. These donor sugars are often activated through the use of *N*-iodosuccinimide (NIS) in the presence of TfOH or TMSOTf under non-basic conditions. It is proposed that the sulfur becomes iodinated, as NIS is a source of I^+ , and departs to generate an oxacarbenium ion pair that can be attacked by the acceptor.¹¹⁰ When glycosylated in CH_3CN , these donor sugars also react in an α -selective manner due to the α -directing effect of this solvent (see Figure 1.57).

Use of the ethyl xanthate **2.20** for the synthesis of phenyl α -sialoside **2.25** was promising but the difficulty in obtaining pure **2.20** was a complicating factor. In this glycosylation reaction, **2.20** was stirred with 1.6 eq. phenol overnight in CH_3CN containing 3Å molecular sieves that had been flame-dried and heated under vacuum at 200 °C for 8 h. The next day, the suspension was cooled to -40 °C and a solution of 1.5 eq. NIS and 20% TMSOTf was added and the reaction

was stirred for 7 h while slowly warming to room temperature.²⁰⁴ The first attempt with these conditions used crude **2.20** and although some phenyl α -sialoside **2.25** was formed, it was heavily contaminated with compounds from the crude preparation of **2.20**. A quantity of **2.20** that was 90% pure as judged by ¹H NMR spectroscopy was used in the next attempt which provided a 15% yield of clean phenyl β -sialoside **2.24** and a 26% yield of phenyl α -sialoside **2.25**, a third of which was contaminated with the impurity from **2.20** (Table 2.2, entry 5).

Since **2.20** was difficult to purify and the impurity was complicating purification of the desired product **2.25**, the 1-adamantyl thiosialoside **2.21** was used as the donor sugar instead. Crich and co-workers found that 1-adamantyl thiosialosides function as excellent donor sugars due to the electron donating properties of the adamantyl group, giving high yields and α -selectivity especially when the 5-acetamido group was converted into an imide.¹⁷⁸ They also conducted a solvent study and obtained the best yield and α -selectivity using a 2:1 v/v mix of CH₂Cl₂/CH₃CN. Thus, 1-adamantyl thiosialoside **2.21** was stirred with 1.5 eq. phenol in a 2:1 v/v mix of CH₂Cl₂/CH₃CN containing 3Å molecular sieves that had been flame-dried and heated under vacuum at 200 °C for 4 h. The suspension was stirred at room temperature for 45 min, then cooled to -78 °C and a solution of 2.5 eq. NIS and 1.0 eq. TMSOTf in 1:1 v/v CH₂Cl₂/CH₃CN was added. After stirring for 1.5 h at -78 °C, the reaction was quenched by the addition of triethylamine and afforded, after column chromatography, 25% of the phenyl β -sialoside **2.24** and 38% of clean phenyl α -sialoside **2.25** (Table 2.2, entry 6). The donor sugar **2.21** was used as a 3:1 β/α anomeric mixture but

since both anomers should lead to the same oxacarbenium ion intermediate, if formed, it was not necessary to separate them.

The anomeric configuration of the phenyl sialosides **2.24** and **2.25** was assigned after analysis of a series of 1D NOE difference ^1H NMR spectra. Irradiation of protons 4 and 6 of **2.24** resulted in enhancement of the signals for the *ortho* protons of the phenyl ring (Figure 2.11, top two spectra) while no enhancement of this signal was observed upon irradiation of protons 3_{ax} (Figure 2.11, bottom spectrum) or 5, indicating that the phenyl ring was in the axial β -configuration. Irradiation of the *ortho*-protons on **2.25** showed enhancements of the signals for both protons on carbon 3, indicating that the phenyl ring was in the equatorial α -configuration.

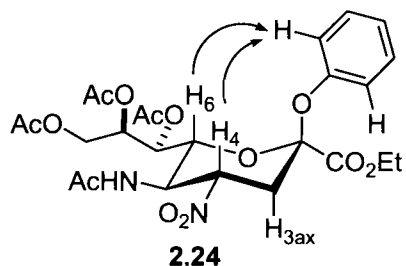
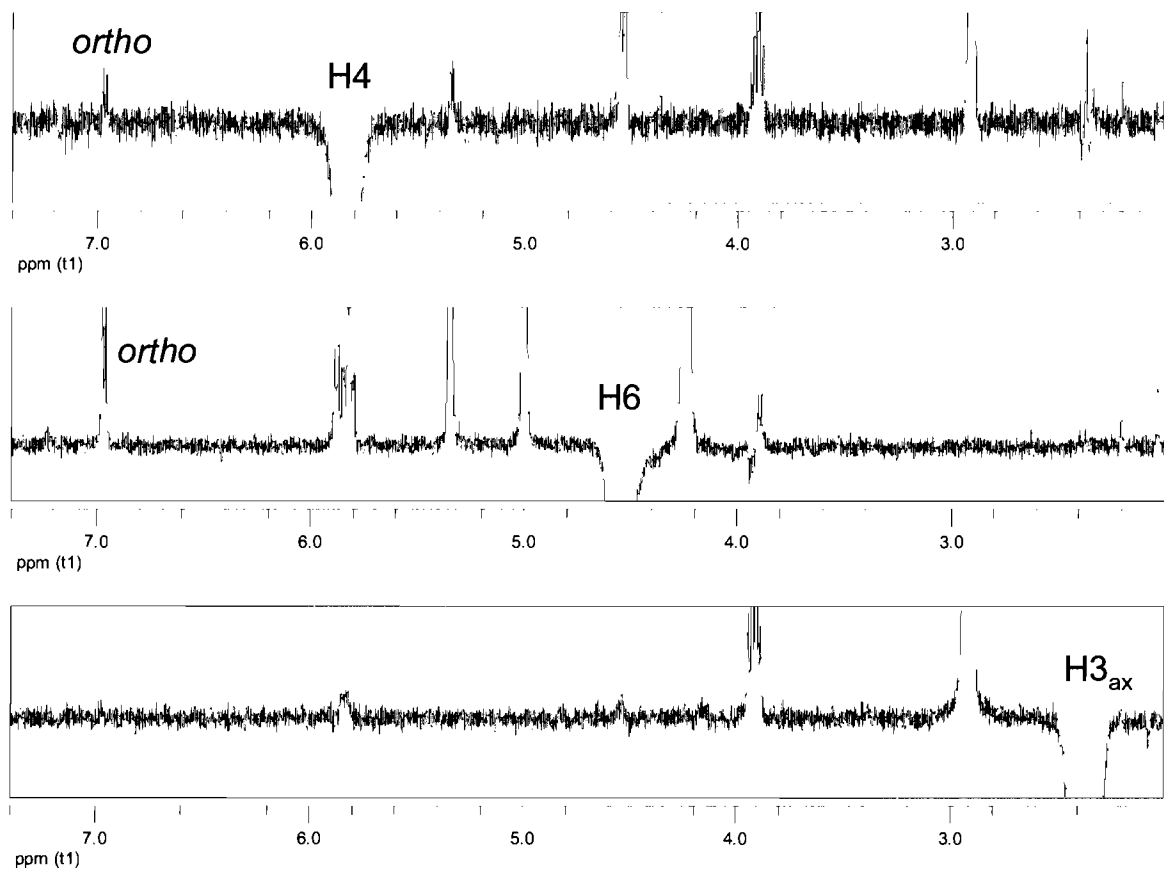


Figure 2.11 1D NOE difference ^1H NMR spectra showing *ortho* ArH contacts upon irradiation of H4 and H6 (top two spectra) and absence of *ortho* ArH contacts upon irradiation of H3_{ax} (bottom spectrum), showing β -configuration for **2.24**

Use of the 1-adamantyl thiosialoside **2.21** as a donor sugar under the glycosylation conditions described above provided acceptable yields of phenyl α -sialoside **2.25**. However, the yield of **2.21** as synthesized from the peracetylated sialoside **2.17** was quite low (21%, Figure 2.8) and must be improved in order to

make this a viable route to phenyl 4-modified α -sialosides. This might be accomplished by using the α -sialosyl fluoride **2.19** to synthesize **2.21** as the fluorosugar appears more reactive to glycosylation (Table 2.2, entry 1 vs entry 2).

2.3.3 Derivatization of the 4-Nitro Group

Several attempts were made to convert the 4-nitro group of various 4-deoxy-4-nitrosialosides into other functional groups, focussing mainly on hydrolysis to a ketone and reduction to an amine. Hydrolysis to the keto group would allow the synthetic route leading to 4-deoxy-4-nitrosialosides to be generalized to include all of the chemistry that has been performed on the 4-oxosialoside intermediate (see Chapter 1.3.2.3). Reduction to the amino group would open up further synthetic possibilities for this route as well.

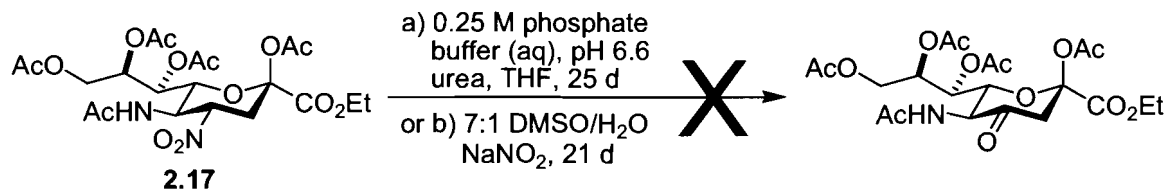


Figure 2.12 Attempted nitro group hydrolyses of **2.17** to a keto group

Two sets of reaction conditions were used to attempt hydrolysis of the nitro group of peracetylated sialoside **2.17** (Figure 2.12). Vasella and co-workers were able to hydrolyze a nitro group to a ketone en route to sialic acid by stirring their compound with urea in THF and aqueous phosphate buffer at pH 6.6 (Figure 1.10).⁴⁵ This was attempted on the peracetylated sialoside **2.17** but gave no reaction after 3 d or 25 d (Figure 2.12). The hydrolysis was also attempted on the unprotected sialoside **2.10** without THF, but the large excess of phosphate

buffer salts complicated product isolation and made it difficult to assess the results of the reaction since the crude mixture was not very soluble in D₂O. A second set of conditions used on **2.17** involved stirring in aqueous DMSO in the presence of NaNO₂.²⁰⁵ After 21 d, however, no reaction was observed.

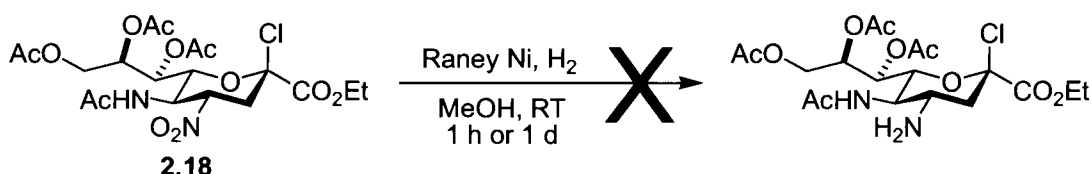


Figure 2.13 Attempted nitro group reduction of β -sialosyl chloride **2.18**

Reduction of the 4-nitro group of β -sialosyl chloride **2.18** to a 4-amino group was attempted (Figure 2.13). This would allow the synthesis of *p*-nitrophenyl 4-amino- α -sialosides under nucleophilic conditions such as those depicted in Figure 2.9 since the 4-nitro group on the sialoside will have already been derivatized, eliminating the problem of having two nitro groups in the same molecule and needing to modify the intrinsically less reactive one. This may also allow synthesis of other aryl sialosides as the acidity of proton 4 would no longer complicate basic glycosylation and it would prevent the problem of 4-epimerization during basic deprotection reactions. However, when **2.18** was stirred with Raney nickel in methanol under a hydrogen atmosphere for 1 h, it was difficult to determine what had occurred. The ¹H NMR spectrum of the crude product showed many broad peaks, but showed the protons on carbon 3 were still doublets of doublets, indicating that the anomeric chloride had not been reduced and replaced with a proton. However, they had shifted upfield from 3.05 and 2.70 ppm only to 2.85 and 2.30 ppm, respectively. As well, proton 4 had

only shifted upfield from 5.40 to 4.60 ppm rather than up to 4.00 ppm. A ^{13}C NMR spectrum also showed that the peak for carbon 4 had not shifted upfield from its original frequency at 83 ppm to where it should appear around 43 ppm if the amino group was present. A singlet integrating to 3 protons had appeared at 3.35 ppm in the ^1H NMR spectrum that correlated with a carbon peak at 51 ppm, raising the possibility that this could be a methoxylamine resulting from incomplete hydrogenation. Glycosylation of this product was attempted with *p*-nitrophenoxide under the conditions described in Figure 2.9 but no change was observed, indicating that the anomeric chloride was no longer present. Thus, the reduction of **2.18** was repeated for 1 d to effect complete nitro reduction to the amine but this gave a mixture of products as determined by TLC analysis and it was difficult to locate the protons on carbon 3 in the ^1H NMR spectrum of the crude product mixture. Glycosylation of this material was also attempted with *p*-nitrophenoxide but resulted in no reaction. It therefore appeared as though reduction of the 4-nitro group of β -sialosyl chloride **2.18** to an amino group would be problematic and this reaction was not pursued further.

Reduction of the 4-nitro group of several 4-deoxy-4-nitrosialosides was attempted using hydrogen in the presence of Raney nickel as a catalyst. A test reaction was performed on peracetylated sialoside **2.17** which was stirred in methanol with Raney nickel under a hydrogen atmosphere for 45 min. The ^1H NMR spectrum of the crude product showed the signal for proton 4 had shifted upfield from 5.7 to 3.2 ppm, consistent with the decreased electron-withdrawing capability of the amino group, and the IR spectrum showed an amine NH stretch

at 3357 cm^{-1} and absence of the nitro stretching bands at 1370 and 1560 cm^{-1} . It was decided to reduce the phenyl β -sialoside **2.24** since a significant quantity had been synthesized to this point and following deprotection the phenyl 4-amino-4-deoxy- β -sialoside **2.26** would be a potential substrate for a mutant sialidase produced in the Bennet lab that is capable of hydrolyzing the unnatural glycosidic linkage in phenyl β -sialoside **2.27** (see Figure 2.15).²⁰⁶ This mutant sialidase will be discussed in greater detail in the next section.

In order to synthesize the phenyl 4-amino-4-deoxy- β -sialoside **2.26**, reduction, de-acetylation, and de-esterification reactions had to be performed on **2.24**. It was likely that de-esterification of **2.24** would cause epimerization of the 4-position resulting from treatment of the mildly acidic proton next to the 4-nitro group with lithium hydroxide. It was therefore decided to reduce the 4-nitro group first, then deprotect the product. The phenyl β -sialoside **2.24** was stirred with Raney nickel in methanol under a hydrogen atmosphere for 45 min, after which time TLC indicated that the reduction had occurred. The product was treated with sodium methoxide in methanol to remove the acetyl groups but following neutralization with Amberlite H^+ resin, no product was obtained after filtration. It was likely that the newly-formed amino group was causing the reduced, de-acetylated product to bind to the resin.

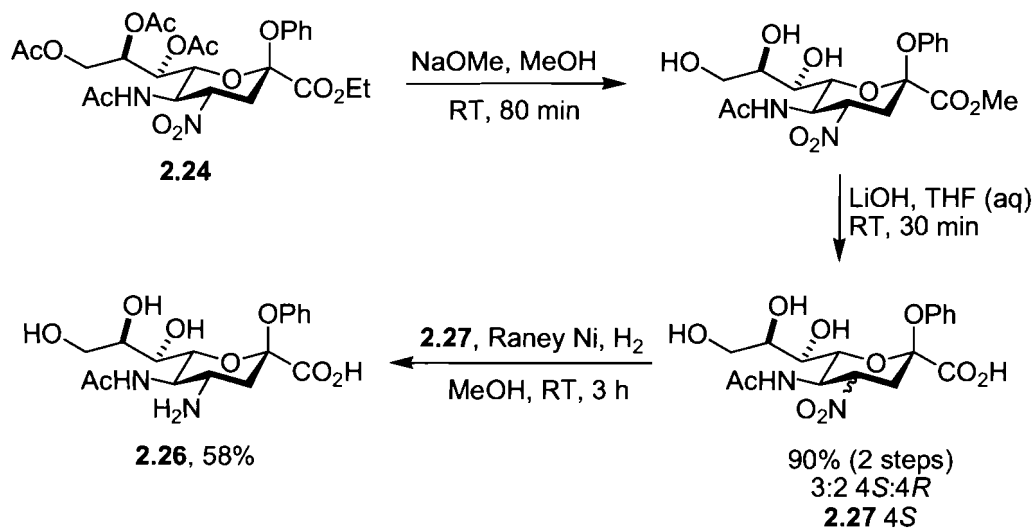


Figure 2.14 Synthesis of phenyl 4-amino-4-deoxy- β -sialoside **2.26**

Since the de-esterification reaction would also require neutralization, it was decided to perform the reduction after deprotection rather than attempt to elute the reduced product from the resin or try to protect the newly-formed amino functionality. Thus, phenyl β -sialoside **2.24** was treated with sodium methoxide in methanol for 80 min (Figure 2.14). After workup and isolation, the de-acetylated product was de-esterified by stirring with 4.7 eq. lithium hydroxide in 3:2 v/v THF/H₂O for 30 min. As expected, this reaction epimerized the 4-position, giving a 90% yield of deprotected products over two steps in a 3:2 4S:4R ratio (Figure 2.14). Approximately half of the 4S-epimer **2.27** could be separated from the mixture via careful column chromatography while the remaining mixture could be epimerized to give more of the 4S-epimer by further treatment with lithium hydroxide. Reduction of the deprotected phenyl β -sialoside **2.27** required a longer reaction time than of the protected precursor **2.24**. After stirring with Raney nickel under a hydrogen atmosphere for 40 min,

some reduction to **2.26** had occurred but the majority of the crude material was still unreacted **2.27**. After 2 h, the reduction was mostly complete but attempts to purify the product by column chromatography led to isolation of a compound whose ^1H NMR spectrum showed no signals for proton 4 or the protons on carbon 3. However, the reduction proceeded to completion after 3 h to give **2.26** in 58% yield, which did not require purification (Figure 2.14).

To summarize, limited attempts to hydrolyse the nitro group to a keto group failed, as did reduction of the nitro group to a keto group in the presence of an anomeric chloride. However, reduction of the nitro group occurred efficiently when performed in the final step in a synthetic sequence, as observed in the synthesis of the phenyl 4-amino-4-deoxy- β -sialoside **2.26** (Figure 2.14). It is likely that the nitro group could be reduced earlier in the synthesis and the resulting amine be protected, giving more synthetic diversity to this route. As well, it should be possible to apply these deprotection/reduction techniques to the phenyl α -sialoside **2.25**, allowing the synthesis of substrates for sialidases. Conversion of the 4-amino group into a guanidinyll group should be possible by stirring with aminoiminomethanesulfonic acid to generate the desired substrate analogue of Relenza.

2.4 Attempted Hydrolysis of β -Phenyl Sialoside 2.24 with Y370G Mutant Sialidase

All known naturally-occurring sialidases hydrolyse sialosides containing the α -glycosidic linkage. This is accomplished via nucleophilic attack of an active site tyrosine residue on the anomeric centre of the sialoside substrate to form a

covalent enzyme-sialosyl intermediate that is later hydrolyzed by water to give retained α -sialic acid as the hydrolysis product (see Chapter 1.2.2, Figure 1.5). In the Bennet lab, a sialidase from *Micromonospora viridifaciens* was recombinantly produced in which the catalytic tyrosine (Y) residue at position 370 had been mutated into a glycine (G) residue, replacing the phenol side chain of the amino acid at position 370 with a proton. This Y370G mutant sialidase was still able to hydrolyse activated α -sialosides (albeit with reduced efficiency) but gave inverted β -sialic acid as the hydrolysis product.^{12,207} This likely resulted from attack on the sialoside by a water molecule occupying the space in the enzyme active site that was created by removal of the phenol moiety of the tyrosine residue. Interestingly, this mutant sialidase showed good activity on phenyl β -sialosides, giving inverted α -sialic acid as the product.²⁰⁶ This was likely a result of the axial phenyl ring of the substrate being able to fit in the hole generated by removal of the phenol ring of the tyrosine residue (Figure 2.15). This allowed the anomeric centre to be attacked from the bottom face by water much in the same way the covalent enzyme-sialosyl intermediate is hydrolyzed in wild-type sialidases. A panel of aryl β -sialosides was screened for activity with the Y370G mutant sialidase but only phenyl β -sialic acid was a substrate for this mutant enzyme.²⁰⁶

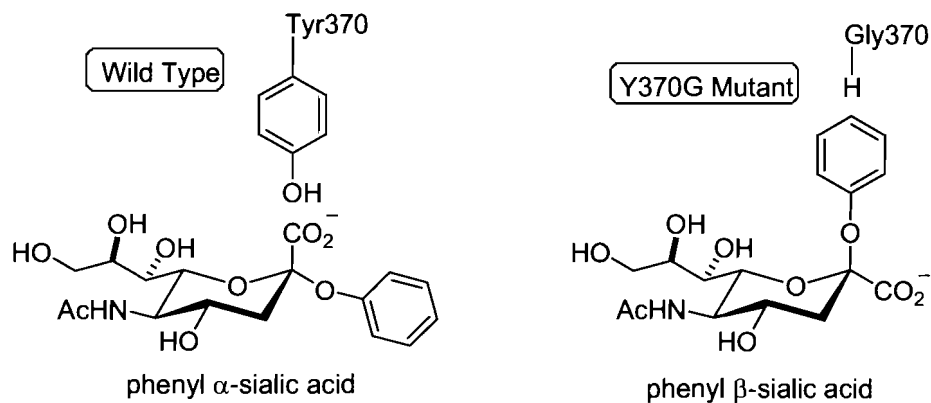


Figure 2.15 Depiction showing phenol ring of Tyr370 sitting above anomeric centre of phenyl α -sialic acid in the wild-type sialidase, then showing the phenyl ring of phenyl β -sialic acid occupying the site vacated by mutating the Tyr370 residue to a glycine residue

It was decided to attempt Y370G sialidase-catalyzed hydrolysis of the phenyl 4-deoxy-4-nitro- β -sialic acid **2.27**. It was assumed that the axial phenyl ring would fit in the enzyme active site and this would give some indication as to the effects of substituting the 4-hydroxyl group for a nitro group, since at this point no aryl 4-deoxy-4-nitro- α -sialoside had been synthesized for use with a wild-type sialidase. The phenyl β -sialoside **2.27** was dissolved in 500 μ L D₂O in order to monitor the reaction by ¹H NMR spectroscopy. Wild-type sialidase from *M. viridifaciens* was added first in order to hydrolyze any possible phenyl α -sialoside contaminant that would complicate evaluation of the mutant sialidase for hydrolysis of the β -anomer **2.27**. As no change in the ¹H NMR spectrum was observed after 13 min, the Y370G mutant sialidase was added. ¹H NMR spectra were acquired over time and analyzed for the disappearance of peaks from **2.27** and appearance of peaks for 4-deoxy-4-nitrosialic acid which would initially be produced as the α -anomer but would quickly mutarotate to the β -anomer **2.16** (Figure 2.16). However, no change was observed after 15 min. The NMR tube

containing the reaction was incubated at 37 °C for 3 h, but the ^1H NMR spectrum still showed no change. After 3 d at 37 °C, the protein had precipitated out of solution and there was still no change in the ^1H NMR spectrum.

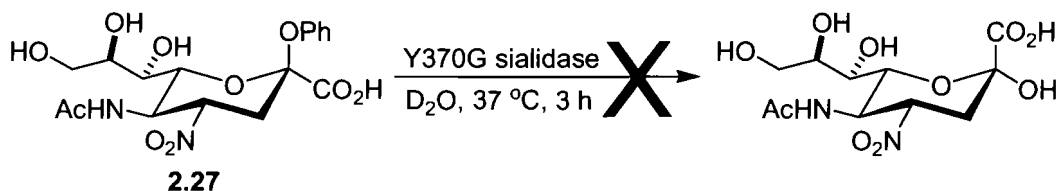


Figure 2.16 Attempted Y370G mutant sialidase-catalyzed hydrolysis of the phenyl 4-deoxy-4-nitro- β -sialoside 2.27

The fact that no hydrolysis of the phenyl 4-deoxy-4-nitro- β -sialoside **2.27** was observed in the presence of the Y370G mutant sialidase could be a result of several possibilities. The enzyme may not be capable of hydrolyzing **2.27** as it has been shown to be inactive toward several aryl sialosides, being very specific for phenyl β -sialic acid.²⁰⁶ However, since the aglycon of **2.27** is still a phenyl group this is unlikely. It is more likely that **2.27** could not bind to the enzyme, or at least not bind well enough to be hydrolyzed. This could be a result of the larger size of the nitro group as compared to the natural hydroxyl group. It could also be a result of unfavourable electrostatic interactions between the nitro group, whose resonance structures put negative charges on the oxygen atoms, and negatively-charged glutamate residues in the enzyme active site near the 4-position of the substrate. If nonproductive binding is occurring, a binding constant for **2.27** could be measured by treating it as a competitive inhibitor of the Y370G mutant sialidase. If such a binding constant cannot be measured, it is likely that **2.27** is not binding to the sialidase. It is more likely that the phenyl 4-

amino-4-deoxy- β -sialoside **2.26** would be able to be hydrolyzed by the Y370G mutant sialidase and these studies are currently underway.

2.5 Conclusions

A new synthetic route to 4-modified sialic acid analogues was developed. Starting from readily available D-arabinose, 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol **2.1** was synthesized en route to 4-deoxy-4-nitrosialic acid. Difficulties were encountered during the chain-extension step while attempting to couple the nitronate of **2.1** with several electrophilic pyruvate synthons, but these were overcome by the use of alkyl α -(bromomethyl)acrylate esters. Following ozonolysis and reduction of the chain-extended products, 4-deoxy-4-nitrosialic acid esters **2.10** and **2.14** could be obtained in multigram quantities. Hydrolysis of the ethyl ester **2.10** did not proceed as expected, affording an unidentified open-chain product, but hydrolysis of the *t*-butyl ester **2.14** proceeded smoothly by stirring in aqueous CF₃CO₂H to afford 4-deoxy-4-nitrosialic acid **2.16**. Attempts were made to soak this compound into influenza sialidase crystals but no X-ray crystal structure was able to be obtained, indicating that the binding of **2.16** with the sialidase was of too low affinity and/or that the 4-nitro group was too big to fit into the enzyme active site or had unfavourable interactions.

Conversion of 4-deoxy-4-nitrosialic acid, ethyl ester **2.10** into a substrate for sialidase-catalyzed hydrolysis was attempted. Several donor sugars were synthesized for use in glycosylation reactions but most did not result in generation of the desired phenyl α -sialosides. Use of a β -sialosyl chloride **2.18** under basic conditions resulted in an intramolecular substitution reaction rather

than glycosylation. Use of peracetylated sialoside **2.17** and the α -sialosyl fluoride **2.19** under $\text{BF}_3 \cdot \text{OEt}_2$ -promoted conditions afforded mainly phenyl β -sialoside **2.24**. The use of sulfur-based donor sugars, ethyl xanthate **2.20** and 1-adamantyl thiosialoside **2.21**, with NIS and TMSOTf afforded both phenyl sialoside anomers **2.24** and **2.25**, with the 1-adamantyl thiosialoside **2.21** affording cleaner products in better yields and greater α -selectivity than the ethyl xanthate **2.20**. A route to 4-modified phenyl α -sialosides has been developed, but the efficiency of formation of the 1-adamantyl thiosialoside **2.21** must be improved; this can likely be achieved by using α -sialosyl fluoride **2.19** rather than the peracetylated sialoside **2.17** in its synthesis.

Attempts at hydrolyzing the 4-nitro group to a 4-keto group failed, as did reduction of the 4-nitro group in the presence of an anomeric chloride. In fact, the majority of the reduction reactions gave products that were unstable to chromatography. These results indicate that the reduction step should be introduced late in the synthesis of 4-modified sialosides or should be followed by a protection step for the new 4-amino moiety.

Conditions were developed to generate a potential substrate for a mutant sialidase from *Micromonaspora viridifaciens* in which the catalytic tyrosine at position 370 has been mutated into a glycine residue. This mutant sialidase is capable of hydrolyzing phenyl β -sialic acid, so the synthesis of phenyl 4-deoxy-4-nitro- β -sialic acid **2.27** was achieved for use as a potential substrate. However, this substrate was not hydrolyzed by the mutant sialidase as monitored by ^1H NMR spectroscopy, supporting the idea that the nitro group is either too big or

has unfavourable interactions with negatively-charged residues in the sialidase active site. This idea could be investigated further by measurement of a binding constant between **2.27** and the sialidase. The 4-nitro group of **2.27** could be reduced to an amino group to give **2.26**, a substrate that should be capable of being hydrolyzed by the mutant sialidase as the amino group would be positively charged at the pH of the reaction.

2.6 Experimental

General

All chemicals were purchased from Aldrich Chemical Company and were used as received, with the exception of D-arabinose which was purchased from V-Labs. Solvents for anhydrous reactions were dried and distilled immediately prior to use. Methanol was dried and distilled over magnesium turnings. CH₂Cl₂ and CH₃CN were dried and distilled over calcium hydride. Glassware used for anhydrous reactions was flame-dried and cooled under a N₂ atmosphere immediately prior to use. TLC was performed on aluminum-backed TLC plates pre-coated with Merck silica gel 60 F₂₅₄. Compounds were visualised with UV light and/or staining with phosphomolybdic acid (5% solution in ethanol). Flash chromatography was performed using Avanco silica gel 60 (230-400 mesh). Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian Unity 500 MHz spectrometer, a Bruker AMX 400 MHz spectrometer, or a Bruker TCI 600 MHz spectrometer. Chemical shifts (δ) are listed in ppm downfield from TMS using the residual solvent peak as an internal reference. ¹H and ¹³C NMR peak

assignments were made based on ^1H - ^1H COSY and ^1H - ^{13}C HMQC experiments. IR spectra were recorded on a Bomem IR spectrometer and samples were prepared as cast evaporative films on NaCl plates from CH_2Cl_2 or methanol. Optical rotations were measured using a Perkin-Elmer 341 polarimeter and are reported in units of $\text{deg cm}^2 \text{g}^{-1}$ (concentrations reported in units of $\text{g}/100 \text{ cm}^3$). 2-Acetamido-1,2-dideoxy-1-nitro-D-mannitol **2.1**,^{192,193} α -(bromomethyl)acrylic acid **2.8**,¹⁹⁶ and ethyl α -(bromomethyl)acrylate **2.6**¹⁹⁶ were synthesized according to literature procedures and their spectral characteristics matched those reported in the literature.

2-Acetamido-1,2-dideoxy-3,4:5,6-di-O-isopropylidene-1-nitro-D-mannitol (2.5)

Nitromannitol **2.1** (19.6 g, 77.7 mmol) was suspended in acetone (750 mL) to which anhydrous CuSO_4 (13.6 g, 85.5 mmol) and concentrated H_2SO_4 (1.0 mL) were added. The suspension was stirred vigorously under N_2 at room temperature for 3 d. The light green suspension was then filtered through celite that was then washed with acetone (100 mL). The filtrate was diluted with Et_2O (700 mL) and washed with saturated aqueous sodium chloride (3 x 700 mL); the aqueous layer was pH neutral after the third washing. The organic layer was then dried (MgSO_4), filtered, and concentrated under reduced pressure to afford **2.5** as a white solid (21.7 g, 65.3 mmol, 84%). The material exhibited identical NMR spectral data to that reported in the literature.¹⁰⁸ The purity was deemed adequate for further use (mp 131–133 °C) but this could be improved by recrystallization from CH_2Cl_2 /hexanes (1:4) (mp 134.0–134.5 °C).

Ethyl 5-acetamido-2,3,4,5-tetra-deoxy-2-methylene-4-nitro-D-glycero-D-galacto-nononate and ethyl 5-acetamido-2,3,4,5-tetra-deoxy-2-methylene-4-nitro-D-glycero-D-falo-nononate (2.9-(S/R))

2-Acetamido-1,2-dideoxy-1-D-nitromannitol **2.1** (13.9 g, 55.1 mmol) was suspended in dry methanol (150 mL). To this suspension was added a solution of sodium methoxide in methanol that had been prepared by reacting sodium metal (1.4 g, 60.9 mmol) with dry methanol (75 mL). The resulting clear light brown solution was stirred for 10 min, after which time ethyl α -(bromomethyl)acrylate **2.6** (15.0 g, 60.6 mmol) was added. After 15 h, the solution was concentrated under reduced pressure to give a yellow foam which was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 5:1 v/v to 3:1 v/v) to afford the enoate ester product **2.9** as a light yellow foam (15.0 g, 41.2 mmol, 75%) in a 1.3:1.0 4S:4R diastereomeric mixture. In addition, a quantity of unreacted nitromannitol **2.1** was recovered and could be re-used. IR (cm⁻¹): 1374 (NO₂), 1549 (NO₂), 1666 (amide C=O), 1711 (unsaturated ester C=O), 3369 (OH). ¹H NMR (D₂O, 500 MHz) **2.9-(S)** δ : 1.29 (t, 3H, $J = 7.1$ Hz, CH₃CH₂O), 2.07 (s, 3H, NHCOCH₃), 2.90 (dd, 1H, $J_{3a,3b} = 14.8$ Hz, $J_{3a,4} = 4.9$ Hz, H-3a), 2.98 (dd, 1H, $J_{3a,3b} = 14.8$ Hz, $J_{3b,4} = 9.8$ Hz, H-3b), 3.45 (d, 1H, $J_{7,8} = 9.2$ Hz, H-7), 3.60 (dd, 1H, $J_{8,9a} = 6.3$ Hz, $J_{9a,9b} = 11.9$ Hz, H-9a), 3.69 (ddd, 1H, $J_{7,8} = 9.0$ Hz, $J_{8,9a} = 6.2$ Hz, $J_{8,9b} = 2.7$ Hz, H-8), 3.81 (dd, 1H, $J_{8,9b} = 2.7$ Hz, $J_{9a,9b} = 11.9$ Hz, H-9b), 3.86 (d, 1H, $J_{5,6} = 10.3$ Hz, H-6), 4.24 (q, 2H, $J = 7.2$ Hz, CH₃CH₂O), 4.55 (dd, 1H, $J_{4,5} = 3.3$ Hz, $J_{5,6} = 10.3$ Hz, H-5), 5.35 (ddd, 1H, $J_{3a,4} = 5.0$ Hz, $J_{3b,4} = 9.6$ Hz, $J_{4,5} = 3.4$ Hz, H-4), 5.79 (s, 1H, C=CH_aH_b), 6.32 (s, 1H, C=CH_aH_b); **2.9-(R)** δ : 1.29 (t, 3H, $J = 7.1$ Hz, CH₃CH₂O), 2.02 (s, 3H, NHCOCH₃), 2.91 (dd, 1H, $J_{3a,3b} = 15.0$ Hz, $J_{3a,4} = 3.9$ Hz, H-3a), 2.96 (dd, 1H,

$J_{3a,3b} = 14.9$ Hz, $J_{3b,4} = 10.6$ Hz, H-3b), 3.47 (d, 1H, $J_{7,8} = 9.3$ Hz, H-7), 3.61 (dd, 1H, $J_{8,9a} = 6.3$ Hz, $J_{9a,9b} = 11.8$ Hz, H-9a), 3.70 (ddd, 1H, $J_{7,8} = 9.0$ Hz, $J_{8,9a} = 6.2$ Hz, $J_{8,9b} = 2.7$ Hz, H-8), 3.82 (dd, 1H, $J_{8,9b} = 2.7$ Hz, $J_{9a,9b} = 11.8$ Hz, H-9b), 3.97 (d, 1H, $J_{5,6} = 10.4$ Hz, H-6), 4.19-4.28 (m, 2H, CH₃CH₂O), 4.84 (dd, 1H, $J_{4,5} = 4.8$ Hz, $J_{5,6} = 10.4$ Hz, H-5), 5.23 (ddd, 1H, $J_{3a,4} = 4.4$ Hz, $J_{3b,4} = 10.4$ Hz, $J_{4,5} = 4.4$ Hz, H-4), 5.78 (s, 1H, C=CHaHb), 6.30 (s, 1H, C=CHaHb). ¹³C NMR (D₂O, 125 MHz) **2.9-(S)** δ: 13.4 (CH₃CH₂O), 22.0 (NHCOCH₃), 33.1 (C-3), 51.2 (C-5), 62.4 (CH₃CH₂O), 63.28 (C-9), 68.4 (C-6), 69.2 (C-7), 70.61 (C-8), 86.3 (C-4), 130.6 (C=CH₂), 134.5 (C=CH₂), 168.1 (C=O), 174.5 (C=O); **2.9-(R)** δ: 13.4 (CH₃CH₂O), 21.9 (NHCOCH₃), 30.4 (C-3), 52.4 (C-5), 62.4 (CH₃CH₂O), 63.30 (C-9), 69.08 (C-6/7), 69.11 (C-6/7), 70.57 (C-8), 87.2 (C-4), 130.3 (C=CH₂), 134.6 (C=CH₂), 168.2 (C=O), 174.4 (C=O). Anal. calcd. for C₁₄H₂₄N₂O₉: C 46.15, H 6.64, N 7.69; found: C 46.51, H 6.96, N 7.47.

Ethyl 5-acetamido-3,4,5-trideoxy-4-nitro-D-glycero-β-D-galacto-non-2-ulopyranosonate (2.10)

Ozone was bubbled through a cooled (−78 °C) solution of enoate ester **2.9** (10.0 g, 27.4 mmol, ~2:1 4*S*:4*R* diastereomeric mixture) in a 1:1 v/v mixture of dry CH₂Cl₂ and dry methanol (300 mL) for 1 h, after which time the reaction became light blue in colour. Addition of dimethyl sulfide (5 mL) caused the product to precipitate. The resulting mixture was allowed to warm to room temperature over 16 h while stirring, after which time the cloudy white suspension was cooled in ice then filtered. The white flocculent solid was washed with CH₂Cl₂ (100 mL) and dried under vacuum to give 4-deoxy-4-nitrosialic acid, ethyl ester **2.10** (4.8 g, 13.1 mmol, 48%). A second crop was obtained by concentrating the filtrate

under reduced pressure to give a yellow syrup and following the addition of methanol (20 mL) a further portion of white flocculent solid was isolated as above to give 0.68 g of the ester **2.10** (1.9 mmol, 7%). mp 175–177 °C (dec.). $[\alpha]_D^{20}$ –28.9 (c 0.63, DMSO). IR (cm⁻¹): 1373 (NO₂), 1544 (NO₂), 1658 (amide C=O), 1735 (ester C=O), 3330 (OH). ¹H NMR (D₂O, 500 MHz) δ: 1.15 (t, 3H, *J* = 7.1 Hz, CH₃CH₂O), 1.84 (s, 3H, NHCOCH₃), 2.40-2.50 (m, 2H, H-3_{ax}, H-3_{eq}), 3.44 (d, 1H, *J*_{7,8} = 9.3 Hz, H-7), 3.46 (dd, 1H, *J*_{9a,9b} = 11.7 Hz, *J*_{9a,8} = 6.1 Hz, H-9a), 3.59 (ddd, 1H, *J*_{7,8} = 9.2 Hz, *J*_{8,9a} = 6.2 Hz, *J*_{8,9b} = 2.7 Hz, H-8), 3.68 (dd, 1H, *J*_{9a,9b} = 11.9 Hz, *J*_{8,9b} = 2.7 Hz, H-9b), 4.11 (d, 1H, *J*_{5,6} = 10.5 Hz, H-6), 4.10-4.21 (m, 2H, CH₃CH₂O), 4.44 (t, 1H, *J*_{4,5} + *J*_{5,6} = 21.3 Hz, H-5), 5.00 (dt, 1H, *J*_{3eq,4} = 5.0 Hz, *J*_{3ax,4} + *J*_{4,5} = 22.7 Hz, H-4). ¹³C NMR (CD₃OD, 125 MHz) δ: 13.1 (CH₃CH₂O), 21.3 (NHCOCH₃), 35.9 (C-3), 49.0 (C-5), 62.1 (CH₃CH₂O), 63.5 (C-9), 68.6 (C-7), 70.1 (C-6), 70.4 (C-8), 83.3 (C-4), 94.3 (C-2), 169.1 (C=O), 172.8 (C=O). Anal. calcd. for C₁₃H₂₂N₂O₁₀: C 42.62, H 6.05, N 7.65; found: C 42.77, H 6.12, N 7.51.

***t*-Butyl α-(bromomethyl)acrylate (2.12)**

2-Methylpropene (25.6 g, 0.456 mol) was condensed in a Schlenk tube at –78 °C. To this was added a solution of α-(bromomethyl)acrylic acid (26.2 g, 0.159 mol) in dry CH₂Cl₂ (120 mL) along with conc. H₂SO₄ (0.5 mL). The Schlenk tube was sealed and the reaction mixture was allowed to warm to room temperature and it was stirred overnight. The solution was then concentrated to half its volume under reduced pressure to remove excess 2-methylpropene and this was diluted with CH₂Cl₂ (300 mL) and washed with saturated aqueous NaHCO₃ (2 ×

400 mL). The organic layer was dried (MgSO_4), filtered, and concentrated under reduced pressure to afford the product **2.12** as a light yellow oil (27.6 g, 0.125 mmol, 79%). The material exhibited identical spectral data to those reported in the literature.¹⁹⁹

***t*-Butyl 5-acetamido-2,3,4,5-tetradecoxy-2-methylene-4-nitro-D-glycero-D-galacto-nononate and *t*-butyl 5-acetamido-2,3,4,5-tetradecoxy-2-methylene-4-nitro-D-glycero-D-talo-nononate (**2.13**-(*S/R*))**

2-Acetamido-1,2-dideoxy-4-nitro-D-mannitol **2.1** (12.0 g, 47.6 mmol) was suspended in dry methanol (150 mL). A solution of sodium methoxide was added that had been prepared by reacting sodium metal (1.5 g, 65 mmol) with dry methanol (75 mL). The resulting clear, brown solution was stirred for 10 min, after which time *t*-butyl α -(bromomethyl)acrylate (12.6 g, 39.2 mmol) was added. The solution was stirred under nitrogen for 20 h and was concentrated under reduced pressure to give a dark brown foamy syrup. This material was purified via flash chromatography (CH_2Cl_2 -MeOH gradient solvent system from 5:1 v/v to 3:1 v/v) to afford enoate ester **2.13** as a tan foam (12.2 g, 31.1 mmol, 79%) in a 1.4:1.0 4*S*:4*R* diastereomeric ratio. IR (cm^{-1}): 1152 (C-O-C), 1370 (NO_2), 1552 (NO_2), 1659 (amide C=O), 1707 (unsaturated ester C=O), 3316 (OH). ^1H NMR (D_2O , 500 MHz) **2.13**-(**S**) δ : 1.49 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.07 (s, 3H, NHCOCH_3), 2.86 (dd, 1H, $J_{3a,3b} = 14.5$ Hz, $J_{3a,4} = 4.8$ Hz, H-3a), 2.94 (dd, 1H, $J_{3a,3b} = 14.6$ Hz, $J_{3b,4} = 9.8$ Hz, H-3b), 3.44 (d, 1H, $J_{7,8} = 9.2$ Hz, H-7), 3.60 (dd, 1H, $J_{9a,9b} = 11.8$ Hz, $J_{8,9b} = 6.2$ Hz, H-9a), 3.67-3.71 (m, 1H, H-8), 3.81 (dd, 1H, $J_{9a,9b} = 11.8$ Hz, $J_{8,9b} = 2.8$ Hz, H-9b), 4.53 (dd, 1H, $J_{4,5} = 3.3$ Hz, $J_{5,6} = 10.2$ Hz, H-5), 5.36 (ddd, 1H, $J_{3a,4} = 4.8$ Hz, $J_{3b,4} = 9.8$ Hz, $J_{4,5} = 3.5$ Hz, H-4), 5.71 (s, 1H, $\text{C}=\text{CHaHb}$), 6.22 (s,

1H, C=CHaHb); **2.13-(R)** δ : 1.49 (s, 9H, C(CH₃)₃), 2.02 (s, 3H, NHCOCH₃), 2.84-2.94 (m, 2H, H-3a, H-3b), 3.48 (d, 1H, $J_{7,8}$ = 9.2 Hz, H-7), 3.61 (dd, 1H, $J_{9a,9b}$ = 11.7 Hz, $J_{8,9b}$ = 6.22 Hz, H-9a), 3.67-3.71 (m, 1H, H-8), 3.82 (dd, 1H, $J_{9a,9b}$ = 11.8 Hz, $J_{8,9b}$ = 2.8 Hz, H-9b), 3.97 (d, 1H, $J_{5,6}$ = 10.4 Hz, H-6), 4.83 (dd, 1H, $J_{4,5}$ = 5.0 Hz, $J_{5,6}$ = 10.5 Hz, H-5), 5.21 (dt, 1H, $J_{3a,4} + J_{3b,4} + J_{4,5}$ = 19.8 Hz, H-4), 5.71 (s, 1H, C=CHaHb), 6.21 (s, 1H, C=CHaHb). ¹³C NMR (D₂O, 125 MHz) **2.13-(S)** δ : 22.0 (NHCOCH₃), 27.3 (C(CH₃)₃), 33.4 (C-3), 51.2 (C-5), 63.3 (C-9), 68.4 (C-6), 69.2 (C-7), 70.6 (C-8), 83.6 (C(CH₃)₃), 86.5 (C-4), 129.8 (C=CH₂), 136.0 (C=CH₂), 167.3 (C=O), 174.4 (C=O); **2.13-(R)** δ : 21.9 (NHCOCH₃), 27.3 (C(CH₃)₃), 30.7 (C-3), 52.3 (C-5), 63.3 (C-9), 69.10 (C-6), 69.14 (C-7), 70.6 (C-8), 83.6 (C(CH₃)₃), 87.5 (C-4), 129.5 (C=CH₂), 136.1 (C=CH₂), 167.5 (C=O), 174.5 (C=O). Anal. calcd. for C₁₆H₂₈N₂O₉: C 48.97, H 7.19, N 7.14; found: C 49.04, H 6.97, N 7.06.

***t*-Butyl 5-acetamido-3,4,5-trideoxy-4-nitro-D-glycero- β -D-galacto-non-2-ulopyranosonate (2.14)**

A solution of enoate ester **2.13** (10.7 g, 27.3 mmol, 1.4:1.0 4*S*:4*R* diastereomeric mixture) was prepared in a 1:1 v/v mix of dry CH₂Cl₂ and dry methanol (300 mL), which was cooled to -78 °C. Ozone was bubbled through the cold solution for 1 h 45 min, after which time the colour was light green. The ozonides were reduced with dimethyl sulfide (5 mL), causing the product to precipitate, and the reaction was allowed to warm to room temperature while stirring overnight. The mixture was then cooled in an ice bath and filtered. The white solid was washed with CH₂Cl₂ (75 mL) and dried to afford the product **2.14** as a white solid (3.58 g, 9.08 mmol, 33%). The filtrate, which contained a mixture of DMSO, **2.14**

and its 4-epimer **2.15**, was concentrated and purified via flash chromatography (CH₂Cl₂/methanol gradient solvent system from 10:1 v/v to 3:1 v/v) to afford a further 2.70 g of *t*-butyl ester **2.14** (6.8 mmol, 25%) as a white foamy solid. mp. 147-149 °C (dec). $[\alpha]_D^{20}$ -25.9 (*c* 0.695, DMSO). IR (cm⁻¹): 1371 (NO₂), 1556 (NO₂), 1652 (amide C=O), 1724 (ester C=O), 3358 (OH). ¹H NMR (D₂O, 500 MHz) δ: 1.50 (s, 9H, C(CH₃)₃), 1.98 (s, 3H, NHCOCH₃), 2.52 (dd, 1H, $J_{3ax,3eq} + J_{3ax,4} = 25.3$ Hz, H-3_{ax}), 2.61 (dd, 1H, $J_{3ax,3eq} = 13.0$ Hz, $J_{3eq,4} = 4.6$ Hz, H-3_{eq}), 3.59 (d, 1H, $J_{7,8} = 8.2$ Hz, H-7), 3.63 (dd, 1H, $J_{9a,9b} = 11.8$ Hz, $J_{8,9a} = 6.3$ Hz, H-9a), 3.75 (ddd, 1H, $J_{7,8} = 8.9$ Hz, $J_{8,9a} = 6.2$ Hz, $J_{8,9b} = 2.6$ Hz, H-8), 3.83 (dd, 1H, $J_{9a,9b} = 11.8$ Hz, $J_{8,9b} = 2.7$ Hz, H-9b), 4.23 (d, 1H, $J_{5,6} = 10.5$ Hz, H-6), 4.57 (dd, 1H, $J_{4,5} + J_{5,6} = 21.3$ Hz, H-5), 5.13 (dt, 1H, $J_{3eq,4} = 4.6$ Hz, $J_{3ax,4} + J_{4,5} = 23.1$ Hz, H-4). ¹³C NMR (CD₃OD, 125 MHz) δ: 22.6 (NHCOCH₃), 28.1 (C(CH₃)₃), 50.3 (C-5), 64.8 (C-9), 70.0 (C-7), 71.4 (C-6), 71.8 (C-8), 84.3 (C(CH₃)₃), 84.6 (C-4), 95.6 (C-2), 169.6 (C-1), 174.1 (NHCOCH₃). Anal. calcd. for C₁₅H₂₆N₂O₁₀: C 45.68, H 6.65, N 7.10; found: C 45.81, H 6.71, N 7.09.

5-Acetamido-3,4,5-trideoxy-4-nitro-D-glycero-β-D-galacto-non-2-ulopyranosonic acid (2.16)

Trifluoroacetic acid (6.0 mL) was added to a suspension of *t*-butyl ester **2.14** (148 mg, 0.375 mmol) in water (12 mL). The clear, colourless solution was stirred at room temperature for 16 h, after which time the solution took on a light pink tinge. The solution was concentrated under reduced pressure to afford the de-esterified product **2.16** as an off-white powder (114 mg, 0.338 mmol, 90%). mp. 175-177 °C (dec). $[\alpha]_D^{20}$ -23 (*c* 0.21, DMSO). IR (cm⁻¹): 1372 (NO₂), 1557 (NO₂), 1664

(amide C=O), 1721 (acid C=O), 3338 (OH/NH). ^1H NMR (D_2O , 500 MHz) δ : 1.98 (s, 3H, NHCOCH_3), 2.50 (dd, 1H, $J_{3\text{ax},3\text{eq}} = J_{3\text{ax},4} = 12.8$ Hz, H-3_{ax}), 2.58 (dd, 1H, $J_{3\text{ax},3\text{eq}} = 12.9$ Hz, $J_{3\text{eq},4} = 4.6$ Hz, H-3_{eq}), 3.56 (d, 1H, $J_{7,8} = 9.2$ Hz, H-7), 3.60 (dd, 1H, $J_{9\text{a},9\text{b}} = 11.8$ Hz, $J_{8,9\text{a}} = 6.3$ Hz, H-9a), 3.75 (ddd, 1H, $J_{7,8} = 9.2$ Hz, $J_{8,9\text{a}} = 6.3$ Hz, $J_{8,9\text{b}} = 2.6$ Hz, H-8), 3.83 (dd, 1H, $J_{8,9\text{b}} = 2.6$ Hz, $J_{9\text{a},9\text{b}} = 11.8$ Hz, H-9b), 4.21 (d, 1H, $J_{5,6} = 10.3$ Hz, H-6), 4.56 (dd, 1H, $J_{4,5} + J_{5,6} = 21.3$ Hz, H-5), 5.13 (dt, 1H, $J_{3\text{ax},4} + J_{4,5} = 23.1$ Hz, $J_{3\text{eq},4} = 4.6$ Hz). ^{13}C NMR (DMSO-d_6 , 125 MHz) δ : 22.5 (NHCOCH_3), 35.4 (C-3), 48.5 (C-5), 63.3 (C-9), 68.4 (C-7), 69.3 (C-6), 69.9 (C-8), 83.7 (C-4), 93.5 (C-2), 170.06 (C=O), 170.13 (C=O). Anal. calcd. for $\text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_{10}$: C 39.06, H 5.36, N 8.28; found: C 39.32, H 5.24, N 8.36.

Ethyl 5-acetamido-7,8,9-tri-O-acetyl-2-chloro-2,3,4,5-tetra-deoxy-4-nitro-D-glycero- β -D-galacto-non-2-ulopyranosonate (2.18) and ethyl 5-acetamido-2,7,8,9-tetra-O-acetyl-3,4,5-trideoxy-4-nitro-D-glycero- β -D-galacto-non-2-ulopyranosonate (2.17)

Ethyl 2-acetamido-3,4,5-trideoxy-4-nitro-D-glycero- β -D-galacto-non-2-ulopyranosonate **2.10** (6.30 g, 17.2 mmol) was suspended in acetyl chloride (120 mL) in a flask sealed with a glass stopper. The mixture was stirred at room temperature for 3 d, over which time the white solid slowly dissolved as it reacted. The yellow solution was concentrated under reduced pressure, to which toluene (100 mL) was added and the solution was again concentrated under reduced pressure to give 9.5 g of a yellow foam. This material was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to give the β -sialosyl chloride **2.18** (6.44 g, 12.6 mmol, 73%) as well as the peracetylated sialoside **2.19** (1.24 g, 2.32 mmol, 13%), both as light

yellow foamy solids. **2.18:** $[\alpha]_D^{20}$ -30.9 (c 1.66, CHCl_3). IR (cm^{-1}): 1215 (C-O-C antisymmetric stretch), 1371 (NO_2), 1561 (NO_2), 1667 (amide C=O), 1750 (ester C=O), 3282 (N-H). ^1H NMR (CDCl_3 , 500 MHz) δ : 1.35 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 1.98 (s, 3H, COCH_3), 2.05 (s, 3H, COCH_3), 2.07 (s, 3H, COCH_3), 2.16 (s, 3H, COCH_3), 2.69 (dd, 1H, $J_{3\text{ax},3\text{eq}} = 13.9$ Hz, $J_{3\text{ax},4} = 12.1$ Hz, H-3_{ax}), 3.04 (dd, 1H, $J_{3\text{ax},3\text{eq}} = 13.9$ Hz, $J_{3\text{eq},4} = 4.4$ Hz, H-3_{eq}), 4.02 (dt, 1H, $J_{4,5} = 10.7$ Hz, $J_{5,6} = 10.7$ Hz, $J_{5,\text{NH}} = 8.4$ Hz, H-5), 4.16 (dd, 1H, $J_{8,9\text{a}} = 5.3$ Hz, $J_{9\text{a},9\text{b}} = 12.7$ Hz, H-9a), 4.29-4.38 (m, 3H, H-9b, OCH_2CH_3), 4.78 (dd, 1H, $J_{5,6} = 10.7$ Hz, $J_{6,7} = 2.1$ Hz, H-6), 5.20 (ddd, 1H, $J_{7,8} = 7.7$ Hz, $J_{8,9\text{a}} = 5.3$ Hz, $J_{8,9\text{b}} = 2.6$ Hz, H-8), 5.45 (dd, 1H, $J_{6,7} = 2.1$ Hz, $J_{7,8} = 7.7$ Hz, H-7), 5.59 (dt, 1H, $J_{3\text{eq},4} = 4.4$ Hz, $J_{3\text{ax},4} + J_{4,5} = 22.8$ Hz, H-4), 5.86 (d, 1H, $J_{5,\text{NH}} = 8.4$ Hz, NH). ^{13}C NMR (CDCl_3 , 100 MHz) δ : 13.6 (OCH_2CH_3), 20.6 (OCOCH_3), 20.7 (OCOCH_3), 20.8 (OCOCH_3), 23.0 (NHCOCH_3), 39.6 (C-3), 48.6 (C-5), 61.8 (C-9), 63.4 (OCH_2CH_3), 67.0 (C-7), 69.6 (C-8), 72.2 (C-6), 81.7 (C-4), 95.2 (C-2), 164.4, 169.5, 170.2, 170.6, 170.9 (C=O x 5). Anal. calcd. for $\text{C}_{19}\text{H}_{27}\text{N}_2\text{O}_{12}\text{Cl}$: C 44.67, H 5.33, N 5.48; found: C 44.41, H 5.41, N 5.23.

2.17: $[\alpha]_D^{20}$ $+0.96$ (c 1.10, CHCl_3). IR (cm^{-1}): 1221 (C-O-C antisymmetric stretch), 1371 (NO_2), 1561 (NO_2), 1666 (amide C=O), 1748 (ester C=O), 3278 (N-H). ^1H NMR (CDCl_3 , 500 MHz) δ : 1.28 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 1.98 (s, 3H, COCH_3), 2.03 (s, 3H, COCH_3), 2.06 (s, 3H, COCH_3), 2.17 (s, 3H, COCH_3), 2.19 (s, 3H, COCH_3), 2.44 (dd, 1H, $J_{3\text{ax},3\text{eq}} + J_{3\text{ax},4} = 25.8$ Hz, H-3_{ax}), 2.84 (dd, 1H, $J_{3\text{eq},3\text{ax}} = 13.4$ Hz, $J_{3\text{eq},4} = 4.6$ Hz, H-3_{eq}), 3.67 (dt, 1H, $J_{4,5} = 10.7$ Hz, $J_{5,6} = 10.6$ Hz, $J_{5,\text{NH}} = 7.5$ Hz, H-5), 4.22 (dd, 1H, $J_{8,9\text{a}} = 5.6$ Hz, $J_{9\text{a},9\text{b}} = 12.5$ Hz, H-9a), 4.25

(q, 2H, $J_{\text{CH}_2, \text{CH}_3} = 7.2$ Hz, OCH_2CH_3), 4.40 (dd, 1H, $J_{8,9b} = 2.4$ Hz, $J_{9a,9b} = 12.6$ Hz, H-9b), 4.66 (dd, 1H, $J_{5,6} = 10.6$ Hz, $J_{6,7} = 1.7$ Hz, H-6), 5.17 (ddd, 1H, $J_{7,8} = 6.8$ Hz, $J_{8,9a} = 5.6$ Hz, $J_{8,9b} = 2.4$ Hz, H-8), 5.33 (dd, 1H, $J_{6,7} = 1.7$ Hz, $J_{7,8} = 6.8$ Hz, H-7), 5.70 (ddd, 1H, $J_{3ax,4} = 12.3$ Hz, $J_{3eq,4} = 4.6$ Hz, $J_{4,5} = 10.7$ Hz, H-4), 5.85 (d, 1H, $J_{5, \text{NH}} = 7.5$ Hz, NH). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 13.7 (OCH_2CH_3), 20.57 (COCH_3), 20.66 (COCH_3), 20.74 (COCH_3), 20.8 (COCH_3), 23.2 (COCH_3), 35.0 (C-3), 49.9 (C-5), 61.9 (C-9), 62.7 (OCH_2CH_3), 67.9 (C-7), 69.9 (C-8/6), 70.3 (C-8/6), 80.4 (C-4), 96.3 (C-2), 165.2, 168.1, 169.9, 170.5, 170.8, 171.2 (C=O x 6). Anal. calcd. for $\text{C}_{21}\text{H}_{30}\text{N}_2\text{O}_{14}$: C 47.19, H 5.66, N 5.24; found: C 46.93, H 5.74, N 5.01.

Ethyl 5-acetamido-7,8,9-tri-O-acetyl-2,3,4,5-tetra-deoxy-2-fluoro-4-nitro-D-glycero- α -D-galacto-non-2-ulopyranosonate (2.19)

Sialosyl chloride **2.18** (0.785 g, 1.54 mmol) was dissolved in acetonitrile (7 mL) that had been passed over alumina. Silver(I) fluoride (0.210 g, 1.66 mmol) was added and the reaction stirred under N_2 at room temperature in the dark. After 1.5 h, the reaction was filtered through celite that was then washed with CH_2Cl_2 (100 mL). The filtrate was concentrated under reduced pressure to give a light brown foam (0.729 g) that was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the α -sialosyl fluoride **2.19** as a colourless syrup (0.581 g, 1.18 mmol, 77%). $[\alpha]_D^{20} +0.45$ (c 2.20, CHCl_3). IR (cm^{-1}): 1214 (C-O-C antisymmetric stretch), 1372 (NO_2), 1563 (NO_2), 1666 (amide C=O), 1752 (ester C=O), 3279 (NH). ^1H NMR (CDCl_3 , 500 MHz) δ : 1.33 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 1.97 (s, 3H, COCH_3),

2.01 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.16 (s, 3H, COCH₃), 2.73 (ddd, 1H, $J_{3\text{eq},3\text{ax}} = 14.3$ Hz, $J_{3\text{eq},4} = 8.3$ Hz, $J_{3\text{eq},\text{F}} = 10.8$ Hz, H-3_{eq}), 2.97 (dt, 1H, $J_{3\text{eq},3\text{ax}} + J_{3\text{ax},4} = 28.7$ Hz, $J_{3\text{ax},\text{F}} = 6.1$ Hz, H-3_{ax}), 3.98 (br dt, 1H, $J_{4,5} + J_{5,6} + J_{5,\text{NH}} = 27.7$ Hz, H-5), 4.22 (dd, 1H, $J_{8,9\text{a}} = 4.8$ Hz, $J_{9\text{a},9\text{b}} = 12.6$ Hz, H-9a), 4.26-4.31 (m, 2H, OCH₂CH₃), 4.35 (dd, 1H, $J_{8,9\text{b}} = 2.2$ Hz, $J_{9\text{a},9\text{b}} = 12.6$ Hz, H-9b), 4.58 (dd, 1H, $J_{5,6} = 10.8$ Hz, $J_{6,7} = 3.3$ Hz, H-6), 5.25-5.32 (m, 2H, H-7, H-8), 5.41 (br dt, 1H, $J_{3\text{eq},4} + J_{3\text{ax},4} + J_{4,5} = 23.5$ Hz, H-4), 6.38 (d, 1H, $J_{5,\text{NH}} = 7.7$ Hz, NH). ¹³C NMR (CDCl₃, 125 MHz) δ: 13.8 (OCH₂CH₃), 20.6 (COCH₃), 20.7 (COCH₃), 20.8 (COCH₃), 23.2 (COCH₃), 33.7 (d, $^2J_{3,\text{F}} = 27.3$ Hz, C-3), 49.3 (C-5), 61.8 (C-9), 63.1 (OCH₂CH₃), 67.3 (C-7), 69.1 (C-8), 70.8 (C-6), 79.8 (d, $^3J_{4,\text{F}} = 6.8$ Hz, C-4), 106.1 (d, $^1J_{2,\text{F}} = 223.5$ Hz, C-2), 164.8 (d, $^2J_{1,\text{F}} = 33.6$ Hz, C-1), 169.7, 170.5, 170.8, 171.2 (COCH₃ x 4). Anal. calcd. for C₁₉H₂₇N₂O₁₂F: C 46.16, H 5.50, N 5.67; found: C 46.42, H 5.57, N 5.63.

***O*-Ethyl *S*-(ethyl 5-acetamido-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-4-nitro-*D*-glycero- α -*D*-galacto-non-2-ulopyranosonate) dithiocarbonate (**2.20**)**

Sialosyl chloride **2.18** (491 mg, 0.961 mmol) was dissolved in anhydrous ethanol (60 mL) to which *O*-ethyl-*S*-potassium dithiocarbonate (235 mg, 1.47 mmol) was then added. The resulting yellow suspension was stirred overnight at room temperature. After 22 h, the mixture was diluted with water (40 mL) and extracted with CH₂Cl₂ (150 mL). The organic layer was dried (MgSO₄), filtered, and concentrated under reduced pressure to yield a light brown foam (475 mg, 0.796 mmol, 83% crude yield). The crude product was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc), affording the xanthate **2.20** as a white foamy syrup (231 mg, 0.388

mmol, 40%). $[\alpha]_D^{20} +46.2$ (c 2.25, CHCl_3). IR (cm^{-1}): 1370 (NO_2), 1561 (NO_2), 1666 (amide $\text{C}=\text{O}$), 1746 (ester $\text{C}=\text{O}$), 3271 (NH). ^1H NMR (CDCl_3 , 500 MHz) δ : 1.30 (t, 3H, $J = 7.1$ Hz, $\text{C}(\text{O})\text{OCH}_2\text{CH}_3$), 1.37 (t, 3H, $J = 7.1$ Hz, $\text{C}(\text{S})\text{OCH}_2\text{CH}_3$), 1.95 (s, 3H, COCH_3), 2.01 (s, 3H, COCH_3), 2.10 (s, 3H, COCH_3), 2.14 (s, 3H, COCH_3), 2.50 (t, 1H, $J_{3\text{eq},3\text{ax}} + J_{3\text{ax},4} = 25.4$ Hz, H-3_{ax}), 2.98 (dd, 1H, $J_{3\text{eq},3\text{ax}} = 12.9$ Hz, $J_{3\text{eq},4} = 4.4$ Hz, H-3_{eq}), 3.80 (dt, 1H, $J_{4,5} + J_{5,6} = 21.4$ Hz, $J_{5,\text{NH}} = 8.3$ Hz, H-5), 4.21-4.29 (m, 3H, H-9_a, $\text{C}(\text{O})\text{CH}_2\text{CH}_3$), 4.34 (dd, 1H, $J_{8,9\text{b}} + J_{9\text{a},9\text{b}} = 14.6$ Hz, H-9_b), 4.57 (dq, 1H, $J_{\text{CHaCH}_3} = 7.1$ Hz, $J_{\text{CHaCHb}} = 10.6$ Hz, $\text{C}(\text{S})\text{OCH}_a\text{CH}_b\text{CH}_3$), 4.73 (dq, 1H, $J_{\text{CHbCH}_3} = 7.1$ Hz, $J_{\text{CHaCHb}} = 10.6$ Hz, $\text{C}(\text{S})\text{OCH}_a\text{H}_b\text{CH}_3$), 4.82 (dd, 1H, $J_{5,6} = 10.7$ Hz, $J_{6,7} = 1.3$ Hz, H-6), 5.23-5.33 (m, 3H, H-8, H-7, H-4), 6.27 (d, 1H, $J_{5,\text{NH}} = 8.2$ Hz, NH). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 13.2 ($\text{C}(\text{S})\text{OCH}_2\text{CH}_3$), 13.9 ($\text{C}(\text{O})\text{OCH}_2\text{CH}_3$), 20.6 (COCH_3), 20.8 (COCH_3), 20.9 (COCH_3), 23.2 (COCH_3), 36.1 (C-3), 49.9 (C-5), 61.7 ($\text{C}(\text{O})\text{OCH}_2\text{CH}_3$), 63.0 (C-9), 67.8 (C-7/8), 69.7 (C-7/8), 70.5 ($\text{C}(\text{S})\text{OCH}_2\text{CH}_3$), 72.8 (C-6), 81.2 (C-4), 86.1 (C-2), 167.0, 170.1, 170.5, 170.7, 170.9 (C=O x 5), 206.5 (C=S).

Ethyl (1-adamantyl 5-acetamido-7,8,9-tri-O-acetyl-3,4,5-trideoxy-4-nitro-2-thio-D-glycero- α/β -D-galacto-non-2-ulopyranoside)onate (2.21) and ethyl 5-acetamido-7,8,9-tri-O-acetyl-3,4,5-trideoxy-4-nitro-D-glycero- β -D-galacto-non-2-ulopyranosonate (2.22)

A solution of peracetylated 4-deoxy-4-nitrosialoside **2.17** (523 mg, 0.979 mmol) and 1-adamantanethiol (181 mg, 1.08 mmol) in dry CH_2Cl_2 (10 mL) was prepared to which $\text{BF}_3 \cdot \text{OEt}_2$ (0.31 mL, 2.5 mmol) was injected via needle and syringe. The resulting solution was stirred under N_2 at room temperature for 3 d. TLC analysis showed the presence of products in addition to remaining starting material. The

reaction was diluted with CH₂Cl₂ (200 mL) and washed with saturated aqueous NaHCO₃ (50 mL). The organic layer was dried (MgSO₄), filtered, and concentrated under reduced pressure to give a tan foamy syrup (634 mg). The crude mixture of compounds was separated via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the 1-adamantyl thiosialoside **2.21** as a 3:1 β:α anomeric mixture (144 mg, 0.225 mmol, 23%); a small quantity of pure β-anomer was obtained for measurement of its optical rotation. In addition, a quantity of hemiacetal **2.22** resulting from hydrolysis of the anomeric acetyl group was isolated (80 mg, 0.162 mmol, 17%) and a further quantity of unreacted starting material **2.17** was recovered (217 mg, 0.406 mmol, 41%). **β-2.21** [α]_D²⁰ +25.2 (c 1.12, CHCl₃). IR (cm⁻¹): 1220 (C-O-C antisymmetric stretch), 1371 (NO₂), 1561 (NO₂), 1666 (amide C=O), 1748 (ester C=O), 2911 (CH aliphatic stretch), 3270 (NH). **β-2.21** ¹H NMR (CDCl₃, 500 MHz) δ: 1.36 (t, 3H, *J* = 7.1 Hz, OCH₂CH₃), 1.66 (br s, 6H, Ada), 1.89-1.96 (m, 9H, Ada, COCH₃), 1.99-2.02 (m, 6H, Ada, COCH₃), 2.15 (s, 3H, COCH₃), 2.17 (s, 3H, COCH₃), 2.29 (t, 1H, *J*_{3eq,3ax} = *J*_{3ax,4} = 12.7 Hz, H-3_{ax}), 2.98 (dd, 1H, *J*_{3eq,3ax} = 12.7 Hz, *J*_{3eq,4} = 4.3 Hz, H-3_{eq}), 3.49 (dt, 1H, *J*_{4,5} = *J*_{5,6} = 10.8 Hz, *J*_{5,NH} = 7.4 Hz, H-5), 4.19-4.27 (m, 2H, H-9a, OCH_aH_bCH₃), 4.32-4.41 (m, 2H, H-9b, OCH_aH_bCH₃), 4.57 (dd, 1H, *J*_{5,6} = 10.8 Hz, *J*_{6,7} = 0.9 Hz, H-6), 5.24 (dd, 1H, *J*_{6,7} = 1.0 Hz, *J*_{7,8} = 9.2 Hz, H-7), 5.28 (ddd, 1H, *J*_{7,8} = 9.2 Hz, *J*_{8,9a} + *J*_{8,9b} = 5.5 Hz, H-8), 5.40 (ddd, 1H, *J*_{3eq,4} = 4.3 Hz, *J*_{3ax,4} + *J*_{4,5} = 23.5 Hz, H-4), 5.95 (d, 1H, *J*_{5,NH} = 7.4 Hz, NH). **α-2.21** ¹H NMR (CDCl₃, 500 MHz) δ: 1.35 (t, 3H, *J* = 7.2 Hz, OCH₂CH₃), 1.67 (br s, 6H, Ada), 1.88-1.92 (m, 6H, Ada, COCH₃), 2.01-2.03 (m, 9H, Ada, COCH₃),

2.07 (s, 3H, COCH₃), 2.16 (s, 3H, COCH₃), 2.44 (t, 1H, $J_{3ax,3eq} + J_{3ax,4} = 26.3$ Hz, H-3_{ax}), 2.80 (dd, 1H, $J_{3ax,3eq} = 13.6$ Hz, $J_{3eq,4} = 4.1$ Hz, H-3_{eq}), 4.16 (br q, 1H, $J_{4,5} + J_{5,6} + J_{5,NH} = 30.6$ Hz, H-5), 4.24-4.37 (m, 3H, H-9a, OCH₂CH₃), 4.80 (dd, 1H, $J_{5,6} = 10.4$ Hz, $J_{6,7} = 2.5$ Hz, H-6), 4.85 (dd, 1H, $J_{8,9b} = 1.7$ Hz, $J_{9a,9b} = 12.4$ Hz, H-9b), 5.17 (td, 1H, $J_{7,8} + J_{8,9a} + J_{8,9b} = 12.1$ Hz, H-8), 5.29 (br dt, 1H, $J_{3eq,4} = 4.1$ Hz, $J_{3ax,4} + J_{4,5} = 23.6$ Hz, H-4), 5.47 (t, 1H, $J_{6,7} + J_{7,8} = 4.6$ Hz, H-7), 5.56 (d, 1H, $J_{5,NH} = 9.2$ Hz, NH). **β-2.21** ¹³C NMR (CDCl₃, 125 MHz) δ: 14.1 (OCH₂CH₃), 20.7 (COCH₃), 20.97 (COCH₃), 21.00 (COCH₃), 23.5 (COCH₃), 29.9 (Ada), 35.9 (Ada), 39.1 (C-3), 43.5 (Ada), 50.7 (C-5), 51.7 (Ada), 61.6 (C-9/OCH₂CH₃), 62.4 (C-9/OCH₂CH₃), 67.8 (C-7), 68.7 (C-8), 70.7 (C-6), 80.9 (C-4), 84.0 (C-2), 168.2, 170.0, 170.5, 170.8, 171.2 (C=O x 5). **α-2.21** ¹³C NMR (CDCl₃, 125 MHz) δ: 13.9 (OCH₂CH₃), 20.7 (COCH₃), 20.8 (COCH₃), 21.0 (COCH₃), 23.1 (COCH₃), 29.8 (Ada), 35.8 (Ada), 38.9 (C-3), 43.5 (Ada), 49.1 (C-5), 51.1 (Ada), 62.5 (C-9/OCH₂CH₃), 62.9 (C-9/OCH₂CH₃), 69.4 (C-7), 71.3 (C-8), 73.4 (C-6), 82.2 (C-4), 85.0 (C-2), 168.7, 170.1, 170.3, 170.5, 171.1 (C=O x 5). Anal. calcd. for C₂₉H₄₂N₂O₁₂S: C 54.19, H 6.59, N 4.36; found: C 54.31, H 6.49, N 4.21.

2.22: $[\alpha]_D^{20} +22.4$ (c 1.62, CHCl₃). IR (cm⁻¹): 1372 (NO₂), 1558 (NO₂), 1666 (amide C=O), 1747 (ester C=O), 3285 (NH), 3360 (OH). ¹H NMR (CDCl₃, 500 MHz) δ: 1.32 (t, 3H, $J = 7.1$ Hz, OCH₂CH₃), 1.94 (s, 3H, COCH₃), 2.01 (s, 3H, COCH₃), 2.08 (s, 3H, COCH₃), 2.15 (s, 3H, COCH₃), 2.45 (dd, 1H, $J_{3ax,3eq} = 12.8$ Hz, $J_{3eq,4} = 4.5$ Hz, H-3_{eq}), 2.66 (t, 1H, $J_{3ax,3eq} = J_{3ax,4} = 12.7$ Hz, H-3_{ax}), 4.06 (dd, 1H, $J_{8,9a} = 7.3$ Hz, $J_{9a,9b} = 12.4$ Hz, H-9a), 4.18 (br q, 1H, $J_{4,5} + J_{5,6} + J_{5,NH} = 30.1$ Hz, H-5), 4.26-4.33 (m, 2H, OCH₂CH₃), 4.48-4.52 (m, 2H, H-6, H-9b), 4.84 (s,

1H, OH), 5.21 (ddd, 1H, $J_{7,8} = 5.3$ Hz, $J_{8,9a} = 7.4$ Hz, $J_{8,9b} = 2.3$ Hz, H-8), 5.28 (br dt, 1H, $J_{3eq,4} = 4.5$ Hz, $J_{3ax,4} + J_{4,5} = 23.1$ Hz, H-4), 5.34 (dd, 1H, $J_{6,7} = 1.8$ Hz, $J_{7,8} = 5.2$ Hz, H-7), 6.39 (d, 1H, $J_{5,NH} = 8.8$ Hz, NH). ^{13}C NMR (CDCl₃, 125 MHz) δ : 13.9 (OCH₂CH₃), 20.7 (COCH₃), 20.8 (COCH₃), 20.9 (COCH₃), 23.1 (COCH₃), 35.3 (C-3), 49.3 (C-5), 62.5 (C-9), 63.0 (OCH₂CH₃), 68.1 (C-7), 69.4 (C-6), 71.4 (C-8), 82.2 (C-4), 94.0 (C-2), 167.9, 170.5, 170.7, 170.8, 171.1 (C=O x 5).

Ethyl (4'-nitrophenyl 5-acetamido-7,8,9-tri-O-acetyl-3,4,5-trideoxy-4-nitro-D-glycero- α -D-galacto-non-2-ulopyranoside)onate and ethyl (4'-nitrophenyl-5-acetamido-7,8,9-tri-O-acetyl-3,4,5-trideoxy-4-nitro-D-glycero- α -D-talo-non-2-ulopyranoside)onate (2.23-(S/R))

To a solution of *p*-nitrophenol (330 mg, 2.37 mmol) in CH₃CN (22 mL) potassium *t*-butoxide (83 mg, 0.74 mmol) was added and the resulting bright yellow mixture was stirred under N₂ at room temperature for 5 min and then 18-crown-6 (250 mg, 0.946 mmol) was added. After stirring the yellow solution for a further 5 min, β -sialosyl chloride **2.18** (398 mg, 0.779 mmol) was added. The reaction was stirred overnight under N₂ at room temperature. After 17 h, TLC showed the absence of starting material and the solution was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate (4 x 100 mL). The aqueous layers were combined and washed with ethyl acetate (100 mL), then the combined organic layers were dried (MgSO₄), filtered, and concentrated under reduced pressure to give a yellow syrup (636 mg). This material was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the *p*-nitrophenyl α -sialoside **2.23** as an inseparable 3:1 mixture of 4S:4R epimers (257 mg, 0.420 mmol, 57%). IR (cm⁻¹): 1347 (aromatic NO₂), 1371 (aliphatic NO₂), 1493 (aromatic NO₂), 1562 (aliphatic NO₂), 1667

(amide C=O), 1749 (ester C=O), 2984 (aliphatic CH), 3061 (aromatic CH), 3273 (NH). ^1H NMR (CDCl_3 , 500 MHz) δ : 1.04 (t, 3H-R, $J = 7.1$ Hz, OCH_2CH_3 -R), 1.07 (t, 3H-S, $J = 7.1$ Hz, OCH_2CH_3 -S), 1.98 (s, 3H-R, COCH_3 -R), 2.01 (s, 3H-S, COCH_3 -S), 2.04 (s, 3H-R, COCH_3 -R), 2.05 (s, 3H-S, COCH_3 -S), 2.07 (s, 3H-R, COCH_3 -R), 2.10 (s, 3H-S, COCH_3 -S), 2.17 (s, 3H-S, COCH_3 -S), 2.20 (s, 3H-R, COCH_3 -R), 2.69 (dd, 1H-R, $J_{3a,3b} = 15.6$ Hz, $J_{3a,4} = 5.9$ Hz, H-3a-R), 2.72 (dd, 1H-S, $J_{3ax,3eq} = 13.3$ Hz, $J_{3ax,4} = 11.8$ Hz, H-3ax-S), 3.05 (dd, 1H-S, $J_{3ax,3eq} = 13.3$ Hz, $J_{3eq,4} = 4.7$ Hz, H-3eq-S), 3.27 (dd, 1H-R, $J_{3a,3b} = 15.6$ Hz, $J_{3b,4} = 2.6$ Hz, H-3b-R), 3.77 (dt, 1H-S, $J_{4,5} + J_{5,6} = 21.0$ Hz, $J_{5,\text{NH}} = 7.6$ Hz, H-5-S), 4.03-4.28 (m, 4H-S, 4H-R, OCH_2CH_3 -S, H-9a-S, H-9b-S, OCH_2CH_3 -R, H-9a-R, H-9b-R), 4.62 (ddd, 1H-R, $J_{4,5} = 4.4$ Hz, $J_{5,6} = 10.9$ Hz, $J_{5,\text{NH}} = 9.8$ Hz, H-5-R), 4.96-4.99 (m, 1H-R, H-4-R), 5.03 (dd, 1H-S, $J_{5,6} = 10.9$ Hz, $J_{6,7} = 1.3$ Hz, H-6-S), 5.04 (d, 1H-R, $J_{5,6} = 11.0$ Hz, H-6-R), 5.31 (dd, 1H-S, $J_{6,7} = 1.3$ Hz, $J_{7,8} = 9.2$ Hz, H-7-S), 5.38-5.50 (m, 2H-S, 2H-R, H-8-S, H-8-R, H-7-R, H-4-S), 5.96 (d, 1H, $J_{5,\text{NH}} = 9.8$ Hz, NH-R), 6.05 (d, 1H-S, $J_{5,\text{NH}} = 7.6$ Hz, NH-S), 7.13-7.19 (m, 2H-S, 2H-R, *ortho* Ar-H-S, *ortho* Ar-H-R), 8.16-8.20 (m, 2H-S, 2H-R, *meta* Ar-H-S, *meta* Ar-H-R). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 13.5 (OCH_2CH_3 -R), 13.6 (OCH_2CH_3 -S), 20.4 (COCH_3 -R), 20.55 (COCH_3 -S), 20.62 (COCH_3 -S), 20.8 (COCH_3 -S), 20.91 (COCH_3 -R), 20.92 (COCH_3 -R), 22.8 (COCH_3 -R), 23.2 (COCH_3 -S), 35.9 (C-3-R), 37.2 (C-3-S), 44.0 (C-5-R), 49.6 (C-5-S), 61.7 (C-9-R), 61.8 (C-9-S), 62.7 (OCH_2CH_3 -R), 63.0 (OCH_2CH_3 -S), 66.9 (C-7-R), 67.2 (C-7-S), 67.9 (C-8-R), 68.0 (C-8-S), 70.5 (C-6-R), 71.7 (C-6-S), 80.5 (C-4-S), 81.7 (C-4-R), 98.0 (C-2-R), 98.7 (C-2-S), 118.6 (*ortho* Ar-C-S), 119.1 (*ortho* Ar-C-R), 125.4 (*meta* Ar-C-R), 125.5 (*meta* Ar-C-S),

143.3 (*ipso* Ar-C-S), 143.4 (*ipso* Ar-C-R), 158.6 (*para* Ar-C-R), 158.7 (*para* Ar-C-S), 165.7 (C=O-R), 166.4 (C=O-S), 169.5 (C=O-R), 170.0 (C=O-S), 170.1 (C=O-R), 170.43, 170.46 (C=O-S x 2), 170.5, 170.6 (C=O-R x 2), 171.0 (C=O-S). Anal. calcd. for C₂₅H₃₁N₃O₁₅: C 48.94, H 5.09, N 6.85; found: C 49.23, H 5.28, N 6.71.

Ethyl (phenyl 5-acetamido-7,8,9-tri-O-acetyl-3,4,5-trideoxy-4-nitro-D-glycero-β-D-galacto-non-2-ulopyranoside)onate (2.24)

Sialosyl fluoride **2.19** (0.284 g, 0.575 mmol) and phenol (0.537 g, 5.71 mmol) were dissolved in dry CH₂Cl₂ (30 mL) to which BF₃·OEt₂ (0.36 mL, 2.9 mmol) was injected via needle and syringe. The resulting yellow solution was stirred under N₂ overnight at room temperature. After 22 h, the red-brown solution was diluted with CH₂Cl₂ (70 mL) and washed with saturated aqueous NaHCO₃ (100 mL). The aqueous layer was washed with CH₂Cl₂ (50 mL) and the combined organic layers were dried (MgSO₄), filtered, and concentrated under reduced pressure to give a red-brown syrup (0.621 g). This syrup was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the phenyl β-sialoside **2.24** as a colourless syrup (0.215 g, 0.413 mmol, 72%). This material was deemed of adequate purity for further use but could be crystallized from EtOAc/hexanes (1:2) to obtain an analytically pure sample. mp. 161-162 °C. [α]_D²⁰ -29.3 (c 3.48, CHCl₃). IR (cm⁻¹): 1222 (C-O-C aliphatic ester antisymmetric stretch), 1371 (NO₂), 1560 (NO₂), 1666 (amide C=O), 1748 (ester C=O), 3061 (aromatic C-H stretch), 3270 (NH). ¹H NMR (CDCl₃, 500 MHz) δ: 1.07 (t, 3H, *J* = 7.1 Hz, OCH₂CH₃), 1.61 (s, 3H, COCH₃), 1.92 (s, 3H, COCH₃), 2.02 (s, 3H, COCH₃), 2.18 (s, 3H, COCH₃), 2.37 (t, 1H,

$J_{3\text{eq},3\text{ax}} + J_{3\text{ax},4} = 25.1$ Hz, H-3_{ax}), 2.90 (dd, 1H, $J_{3\text{eq},3\text{ax}} = 12.8$ Hz, $J_{3\text{eq},4} = 4.5$ Hz, H-3_{eq}), 3.96 (dt, 1H, $J_{4,5} = 10.6$ Hz, $J_{5,6} = 10.6$ Hz, $J_{5,\text{NH}} = 8.4$ Hz, H-5), 4.12-4.19 (m, 2H, OCH₂CH₃), 4.23 (dd, 1H, $J_{8,9\text{a}} = 5.0$ Hz, $J_{9\text{a},9\text{b}} = 12.7$ Hz, H-9a), 4.52 (dd, 1H, $J_{5,6} = 10.5$ Hz, $J_{6,7} = 1.8$ Hz, H-6), 4.54 (dd, 1H, $J_{8,9\text{b}} = 2.2$ Hz, $J_{9\text{a},9\text{b}} = 12.7$ Hz, H-9b), 4.99 (ddd, 1H, $J_{7,8} + J_{8,9\text{a}} = 11.7$ Hz, $J_{8,9\text{b}} = 2.2$ Hz, H-8), 5.35 (dd, 1H, $J_{6,7} = 1.8$ Hz, $J_{7,8} = 6.7$ Hz, H-7), 5.78 (dt, 1H, $J_{3\text{eq},4} = 4.5$ Hz, $J_{3\text{ax},4} + J_{4,5} = 23.1$ Hz, H-4), 5.99 (d, 1H, $J_{5,\text{NH}} = 8.3$ Hz, NH), 6.96 (br d, 2H, *ortho* Ar-H), 7.02 (br t, 1H, *para* Ar-H), 7.22 (br t, 2H, *meta* Ar-H). ¹³C NMR (CDCl₃, 125 MHz) δ: 13.6 (OCH₂CH₃), 20.5 (COCH₃), 20.7 (COCH₃), 20.9 (COCH₃), 23.2 (COCH₃), 37.7 (C-3), 49.8 (C-5), 61.5 (C-9), 62.6 (OCH₂CH₃), 67.8 (C-7), 69.5 (C-6), 70.5 (C-8), 80.7 (C-4), 97.8 (C-2), 116.6 (*ortho* Ar-C), 123.2 (*para* Ar-C), 129.8 (*meta* Ar-C), 153.6 (*ipso* Ar-C), 166.1, 170.2, 170.4, 170.6, 170.8 (C=O x 5). Anal. calcd. for C₂₅H₃₂N₂O₁₃: C 52.81, H 5.67, N 4.93; found: C 52.69, H 5.79, N 5.06.

Ethyl (phenyl 5-acetamido-7,8,9-tri-O-acetyl-3,4,5-trideoxy-4-nitro-D-glycero- α -D-galacto-non-2-ulopyranoside)onate (2.25)

Powdered 3Å molecular sieves were flamed-dried under vacuum, then heated to 200 °C under vacuum for 4 h. After cooling to room temperature, a solution of 1-adamantyl thiosialoside **2.21** (157 mg, 0.251 mmol, 3:1 β : α) and phenol (36 mg, 0.38 mmol, 1.5 eq.) in dry CH₂Cl₂/CH₃CN (2:1, 6 mL) was injected. The resulting slurry was stirred under N₂ at room temperature for 45 min and was then cooled to -78 °C in a dry ice/acetone bath. A solution of NIS (143 mg, 0.635 mmol, 2.5 eq.) and TMSOTf (0.046 mL, 0.25 mmol, 1.0 eq.) in dry CH₂Cl₂/CH₃CN (1:1, 2 mL) was injected and the yellow suspension was stirred under N₂ at -78 °C. After 1.5 h, the reaction was quenched by the addition of triethylamine (0.05 mL,

0.4 mmol) and warmed to room temperature. The mixture was filtered through celite that was then washed with CH₂Cl₂ (50 mL). The filtrate was diluted with CH₂Cl₂ (100 mL) and washed with saturated aqueous sodium thiosulfate (75 mL). The organic layer was dried (MgSO₄), filtered, and concentrated under reduced pressure to yield a brown syrup (230 mg). This crude product was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the phenyl α -sialoside **2.25** as a light yellow syrup (49.7 mg, 0.0955 mmol, 38%) as well as the phenyl β -sialoside **2.24** as a light yellow syrup (32.9 mg, 0.0632 mmol, 25%). IR (cm⁻¹): 1371 (NO₂), 1561 (NO₂), 1668 (amide C=O), 1747 (ester C=O), 2924 (aliphatic CH), 2961 (aliphatic CH), 2982 (aliphatic CH), 3066 (aromatic CH), 3278 (NH). ¹H NMR (CDCl₃, 500 MHz) δ : 1.09 (t, 3H, $J = 7.1$ Hz, OCH₂CH₃), 1.99 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.13 (s, 6H, COCH₃ x 2), 2.61 (t, 1H, $J_{3ax,3eq} + J_{3ax,4} = 25.1$ Hz, H-3_{ax}), 3.04 (dd, 1H, $J_{3ax,3eq} = 13.1$ Hz, $J_{3eq,4} = 4.7$ Hz, H-3_{eq}), 3.69 (dt, 1H, $J_{4,5} + J_{5,6} = 21.2$ Hz, $J_{5,NH} = 7.5$ Hz, H-5), 4.15 (q, 2H, $J = 7.1$ Hz, OCH₂CH₃), 4.29 (dd, 1H, $J_{8,9a} = 4.4$ Hz, $J_{9a,9b} = 12.6$ Hz, H-9a), 4.35 (dd, 1H, $J_{8,9b} = 2.2$ Hz, $J_{9a,9b} = 12.6$ Hz, H-9b), 4.87 (d, 1H, $J_{5,6} = 10.9$ Hz, H-6), 5.31 (d, 1H, $J_{7,8} = 8.5$ Hz, H-7), 5.38-5.41 (m, 1H, H-8), 5.48 (dt, 1H, $J_{3eq,4} = 4.7$ Hz, $J_{3ax,4} + J_{4,5} = 22.2$ Hz, H-4), 5.81 (d, 1H, $J_{5,NH} = 7.5$ Hz, NH), 7.04 (d, 2H, $J_{o,m} = 8.1$ Hz, *ortho* Ar-H), 7.08 (t, 1H, $J = 14.6$ Hz, *para* Ar-H), 7.25-7.28 (m, overlaps with CHCl₃ peak, *meta* Ar-H). ¹³C NMR (CDCl₃, 125 MHz) δ : 13.7 (OCH₂CH₃), 20.7 (COCH₃), 20.8 (COCH₃), 20.9 (COCH₃), 23.4 (COCH₃), 37.0 (C-3), 50.2 (C-5), 61.8 (C-9/OCH₂CH₃), 62.6 (C-9/OCH₂CH₃), 67.9 (C-6/7/8), 69.0 (C-6/7/8), 70.7 (C-6/7/8), 80.6 (C-4), 99.2 (C-

2), 120.0 (*ortho/meta* Ar-C), 124.2 (*para* Ar-C), 129.3 (*ortho/meta* Ar-C), 153.5 (*ipso* Ar-C), 166.4, 170.1, 170.5, 170.9, 171.0 (C=O x 5). HRMS (ESI) for C₂₅H₃₂N₂O₁₃: (MH⁺) calcd: 569.1982, found: 569.1971.

Phenyl (5-acetamido-3,4,5-trideoxy-4-nitro-D-glycero-β-D-galacto-non-2-uloopyranosyl)onic acid (2.27)

Phenyl β-sialoside **2.24** (0.200 g, 0.384 mmol) was dissolved in dry methanol (15 mL) to which a solution of sodium methoxide in methanol (1.3 M, 0.44 mL, 0.57 mmol) was injected via needle and syringe. The resulting solution was stirred under N₂ at room temperature and after 70 min TLC showed no remaining starting material. After 80 min, the solution was neutralized with Amberlite H⁺ resin which was filtered and washed with methanol (50 mL). The filtrate was concentrated under reduced pressure to give a white foamy solid (0.164 g) that was then dissolved in aqueous THF (20 mL, 60% v/v). Lithium hydroxide monohydrate (0.0764 g, 1.82 mmol) was added and the resulting solution was stirred at room temperature. After 25 min, TLC indicated complete reaction and after 30 min the solution was neutralized with Amberlite H⁺ resin. The mixture was filtered and the resin washed with water (30 mL). The filtrate was then concentrated under reduced pressure to a white solid that was purified via flash chromatography (EtOAc-methanol-water gradient solvent system from 10:3:1 to 5:3:1 v/v) to afford a white solid that was a 3:2 mixture of 4*S*:4*R* epimers (0.143 g, 0.346 mmol, 90%). A fraction of pure 4*S*-isomer **2.27** was obtained after a second flash column (0.0462 g, 0.111 mmol, 29%). As well, the remaining 4*R*-isomer could be epimerized to a 1.3:1 4*S*:4*R* mixture by further treatment with

lithium hydroxide as described above. mp. 215-218 °C (dec). $[\alpha]_D^{20}$ -59.5 (c 1.14, H₂O). IR (cm⁻¹): 1376 (NO₂), 1555 (NO₂), 1638 (amide C=O), 1655 (carboxylic acid C=O), 2956 (aliphatic C-H stretch), 3094 (aromatic C-H stretch), 3376 (NH, OH). ¹H NMR (D₂O, 500 MHz) δ: 1.97 (s, 3H, COCH₃), 2.49 (t, 1H, $J_{3ax,3eq} + J_{3ax,4} = 25.3$ Hz, H-3_{ax}), 2.85 (dd, 1H, $J_{3eq,3ax} = 13.0$ Hz, $J_{3eq,4} = 4.5$ Hz, H-3_{eq}), 3.43 (dd, 1H, $J_{8,9a} = 5.8$ Hz, $J_{9a,9b} = 11.9$ Hz, H-9a), 3.55-3.58 (m, 2H, H-7, H-9b), 3.76 (ddd, 1H, $J_{7,8} = 8.8$ Hz, $J_{8,9a} = 5.8$ Hz, $J_{8,9b} = 3.2$ Hz, H-8), 3.93 (d, 1H, $J_{5,6} = 10.5$ Hz, H-6), 4.69 (t, 1H, $J_{4,5} + J_{5,6} = 21.4$ Hz, H-5), 5.40 (dt, 1H, $J_{3eq,4} = 4.5$ Hz, $J_{3ax,4} + J_{4,5} = 23.1$ Hz, H-4), 7.05-7.10 (m, 3H, *ortho* Ar-H, *para* Ar-H), 7.32 (dd, 2H, $J_{o,m} = 8.4$ Hz, $J_{p,m} = 7.5$ Hz, *meta* Ar-H). ¹³C NMR (D₂O, 150 MHz) δ: 21.9 (COCH₃), 36.9 (C-3), 48.0 (C-5), 62.6 (C-9), 67.5 (C-7), 70.5 (C-8), 70.8 (C-6), 82.7 (C-4), 98.8 (C-2), 116.6 (*ortho* Ar-C), 122.5 (*para* Ar-C), 129.6 (*meta* Ar-C), 153.6 (*ipso* Ar-C), 173.5, 174.4 (C=O x 2). HRMS (ESI) for C₁₇H₂₁N₂O₁₀: (M-H⁻) calcd: 413.1196, found: 413.1209.

Phenyl (5-acetamido-4-amino-3,4,5-trideoxy-D-glycero-β-D-galacto-non-2-ulopyranosyl)onic acid (2.26)

Deprotected phenyl β-sialoside **2.27** (0.0364 g, 0.0878 mmol) was dissolved in dry MeOH (75 mL) to which a slurry of Raney nickel in water (0.5 mL) was added. The flask was evacuated and released to a hydrogen atmosphere and the reaction stirred under hydrogen at room temperature. After 2.5 h, TLC indicated complete reaction and after 3 h the reaction was filtered through celite. The celite was washed with MeOH (100 mL) and the filtrate was concentrated under reduced pressure to a white solid that was lyophilized to afford the 4-amino

sialoside **2.26** as an off-white powder (0.0196 g, 0.0510 mmol, 58%). mp. 209-212 °C (dec). $[\alpha]_D^{20}$ -53.3 (c 1.20, H₂O). IR (cm⁻¹): 1636 (amide C=O), 1653 (carboxylic acid C=O), 3384 (NH, OH). ¹H NMR (D₂O, 500 MHz) δ: 1.99-2.06 (m, 4H, H-3_{ax}, COCH₃), 2.63 (dd, 1H, $J_{3ax,3eq} = 13.1$ Hz, $J_{3eq,4} = 4.5$ Hz, H-3_{eq}), 3.49 (d, 1H, $J_{7,8} = 9.4$ Hz, H-7), 3.55 (dd, 1H, $J_{8,9a} = 5.6$ Hz, $J_{9a,9b} = 12.3$ Hz, H-9a), 3.68-3.73 (m, 2H, H-8, H-9b), 3.89 (d, 1H, $J_{5,6} = 10.4$ Hz, H-6), 4.01 (dt, 1H, $J_{3eq,4} = 4.5$ Hz, $J_{3ax,4} + J_{4,5} = 23.2$ Hz, H-4), 4.28 (t, 1H, $J_{4,5} + J_{5,6} = 21.3$ Hz, H-5), 7.04-7.08 (m, 3H, *para* Ar-H, *ortho* Ar-H), 7.32 (t, 2H, $J_{o,m} + J_{m,p} = 15.9$ Hz, *meta* Ar-H). ¹³C NMR (D₂O, 150 MHz) δ: 22.1 (COCH₃), 39.9 (C-3), 47.2 (C-4), 51.6 (C-5), 62.4 (C-9), 67.6 (C-7), 71.1 (C-6/8), 71.7 (C-6/8), 99.7 (C-2), 116.7 (*ortho* Ar-C), 122.3 (*para* Ar-C), 129.5 (*meta* Ar-C), 153.9 (*ipso* Ar-C), 174.9, 175.1 (C=O x 2). HRMS (ESI) for C₁₇H₂₃N₂O₈: (M-H⁻) calcd: 383.1454, found: 383.1459.

CHAPTER 3: ISOLATION AND SYNTHETIC APPLICATIONS OF AN UNEXPECTED ENONE

3.1 Introduction

As described earlier in Chapter 2.2, a number of experiments were conducted in order to find conditions allowing the coupling of nitromannitol **2.1** with a pyruvate synthon to form the desired 9-carbon backbone. In order to prevent possible interference from the hydroxyls of **2.1** during the deprotonation of the nitromethyl carbon and subsequent nucleophilic attack, these groups were protected as isopropylidene acetals to give **2.5**. However, this protection step was found not to be necessary as alkyl α -(bromomethyl)acrylate esters **2.6** and **2.12** coupled efficiently with the unprotected nitromannitol **2.1**.

Since a sizeable quantity of **2.5** had been synthesized, it was decided to react this material with ethyl α -(bromomethyl)acrylate as was similarly described for **2.1** in Chapter 2.2. The protected enoate ester product should have led to 4-deoxy-4-nitrosialic acid, ethyl ester **2.10** following ozonolytic cleavage of the 2-methylene unit and removal of the isopropylidene acetals. However, the ozonolytic cleavage did not proceed as planned, resulting instead in the isolation of the β,γ -unsaturated α -keto ester **3.1** which led to the development of the project described in this chapter.

The objective of this chapter was to use enone **3.1** to synthesize 4-modified sialic acid analogues. This would be accomplished by finding

conditions to perform conjugate addition reactions to the enone, thus installing a nucleophile in the 4-position. The addition products could then be deprotected, allowing cyclization to the sialic acid pyranose structure. Conjugate addition reactions involving alkylcopper and alkylzinc reagents can be used to add a number of nucleophiles and some are highly functional group tolerant. Thus, depending on the type of conjugate addition used, this route to 4-modified sialic acid analogues could be even more general than that originally envisioned in Chapter 2 that involved derivatization of 4-deoxy-4-nitrosialosides.

3.2 Isolation of β,γ -Unsaturated α -Keto Ester **3.1**

The chain extension of the isopropylidene-protected nitromannitol **2.5** was accomplished by stirring with ethyl α -(bromomethyl)acrylate **2.6** in THF containing 0.5 M aqueous sodium hydroxide (Figure 3.1). This afforded protected enoate ester products, giving the desired 4*S* isomer **3.2** in 55% yield and the 4*R* isomer **3.3** in 29% yield as these isomers were chromatographically separable. The 4*R*-isomer **3.3** could be epimerized by treatment with DBU in THF to give again the same mixture of 4*S*:4*R* isomers (Figure 3.1) On one occasion, triethylamine was used instead of DBU but did not induce epimerization. The stereochemistry of the products was confirmed by removing the isopropylidene protecting groups by stirring in aqueous CF₃CO₂H overnight to afford the deprotected enoate esters **2.9-(*R/S*)**, showing that the major isomer **3.2** was converted to **2.9-(*S*)** and the minor isomer **3.3** was converted to **2.9-(*R*)**.

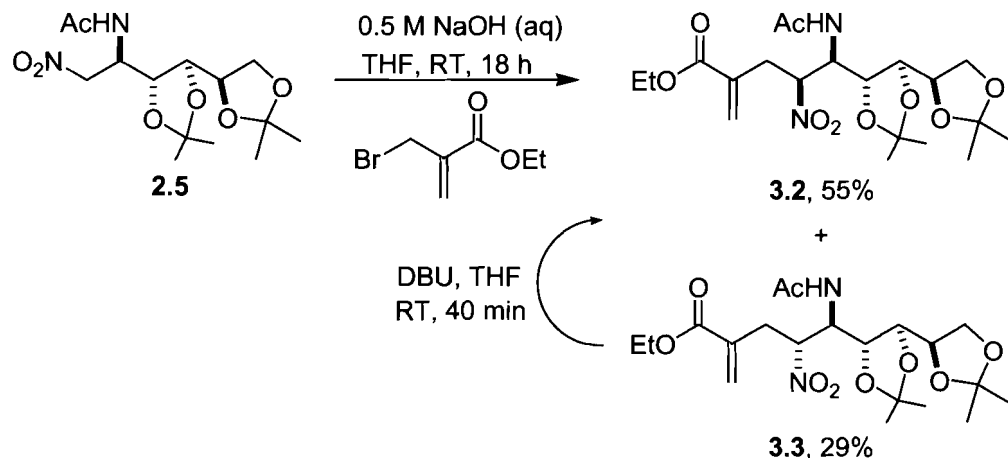


Figure 3.1 Chain extension of protected nitromannitol **2.5** to give enoate esters **3.2** and **3.3**

The next step involving ozonolysis and deprotection to afford the 4-deoxy-4-nitrosialoside was initially performed on the 4*S*-isomer **3.2** rather than on the diastereomeric mixture of enoate esters. This would make it easier to assess the efficiency of these reactions on the protected nitromannitol **2.5** as only the desired diastereomer would be followed through the process, reducing the complexity of product analyses. As described previously, it was envisioned that ozonolysis of **3.3** would give the protected α -keto ester **3.4**, which after removal of the isopropylidene protecting groups would lead simply to a further supply of 4-deoxy-4-nitrosialic acid, ethyl ester **2.10** (Figure 3.2). However, following ozonolysis and chromatographic purification of the product, only the β,γ -unsaturated α -keto ester **3.1** was obtained (Figure 3.2). This enone was obtained in excellent yield (91%), indicating that ozonolysis and elimination of HNO₂ across carbons 3 and 4 was the major reaction pathway taken by enoate ester **3.2**.

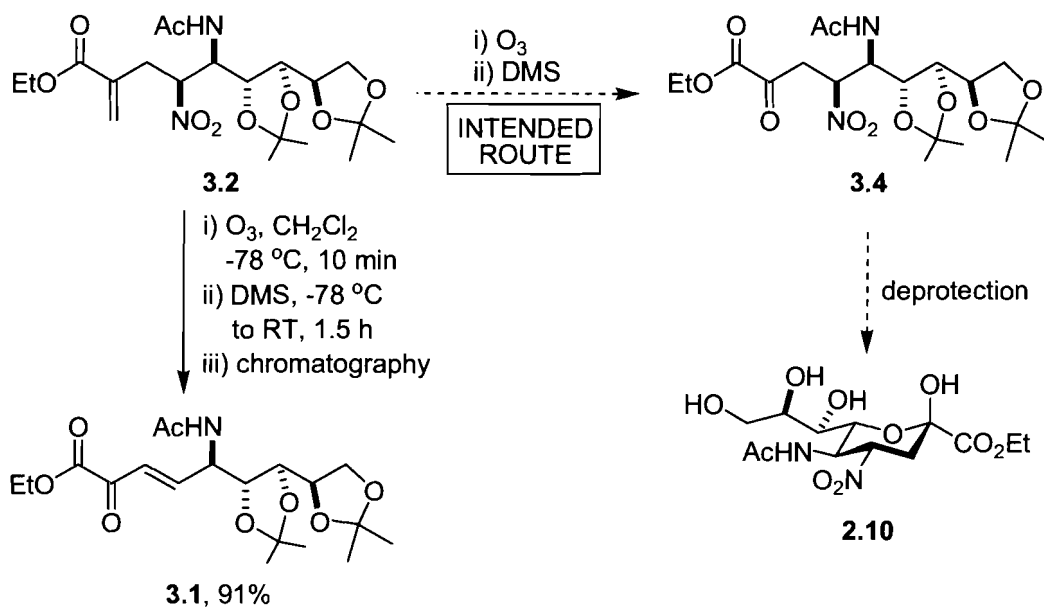


Figure 3.2 Ozonolysis of enoate ester **3.2** lead to enone **3.1** instead of intended α-keto ester **3.4** en route to sialoside **2.10**

Isolation of enone **3.1** was surprising given that alkenes are generally cleaved quite readily by ozone. It was clear that the enone was isolated due to the presence of doublets of doublets at 6.85 and 7.15 ppm in the ¹H NMR spectrum where conjugated alkene protons would be expected to resonate. As well, the peak for carbon 2 in the ¹³C NMR spectrum had shifted downfield to 182.6 ppm from 134 ppm, indicating it had been converted into a carbonyl carbon, and peaks for carbons 3 and 4 resonated at frequencies characteristic of alkene carbons at 126.1 and 147.9 ppm, respectively. The IR spectrum showed absence of a nitro group as the characteristic strong bands at 1375 and 1560 cm⁻¹ were absent. The *trans*-geometry of the alkene was assigned based on the large coupling constant between protons 3 and 4 ($J_{3,4} = 15.9$ Hz).

Due to the reactivity of alkenes toward ozone, it was clear that the elimination of HNO_2 occurred after the ozonolysis step but the source of this reaction was unknown as no base was present to induce such an elimination. The ozonolysis was repeated in the presence of 20 drops of AcOH to neutralize any possible base that might be generated in the reaction to promote elimination of HNO_2 ; however, this had no effect as enone **3.1** was again isolated in 82% yield following chromatography. This elimination was not observed during ozonolysis and reduction of the unprotected enoate ester **2.9** where this reaction was performed in a 1:1 v/v $\text{CH}_2\text{Cl}_2/\text{MeOH}$ solvent mixture. Thus, ozonolysis of **3.2** was carried out in 1:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$ but this also resulted in isolation of enone **3.1** as the sole product. Different reducing agents were tried in the event that DMS was the cause of the elimination. However, when triphenylphosphine was used as a reducing agent, the enone **3.1** was again isolated in 86% yield.²⁰⁸ Enoate ester **3.2** was again treated with ozone under the normal conditions but was reduced with 10 eq. zinc dust and 1.0 mL AcOH.²⁰⁹ Unfortunately, no identifiable products were isolated from this reaction as the materials were likely decomposed by the large amount of AcOH.

At this point it was noticed that very little enone was present in the crude reaction mixture as seen from its ^1H NMR spectrum. In fact, the ozonolysis of pure **3.2** seemed to produce the desired α -keto ester **3.4** along with DMSO and very little else (Figure 3.3). The ^1H NMR spectrum of **3.4** showed no peaks below 5.96 ppm, indicating no alkene protons were present. The peak for proton 4 appeared as a doublet of a triplet at 5.49 ppm where it should appear if the 4-

nitro group was intact and the IR spectrum showed the presence of a nitro group with two strong bands at 1373 and 1557 cm^{-1} . The ^{13}C NMR spectrum showed that carbons 3 and 4 appeared at their expected frequencies at 38.4 and 80.7 ppm, respectively, and that carbon 2 was a carbonyl carbon appearing at 188.7 ppm. Thus, the elimination of HNO_2 to give enone **3.1** was induced by silica gel chromatography (Figure 3.3). Indeed, when this reaction was performed on larger scales, the elimination during chromatography was not efficient enough to convert all of the α -keto ester **3.4** to enone **3.1**. Thus, the elimination could be effected by treatment of **3.4** with DBU in EtOAc for 1-2 min followed by quenching with saturated aqueous ammonium chloride and extraction of the enone with Et_2O (Figure 3.3). The DBU-induced elimination had to be quenched very quickly as decomposition of the enone product occurred very rapidly for unknown reasons; total decomposition was observed after 15 min on one occasion. Of note, Yao and co-workers had synthesized an α -keto ester very similar to **3.4** which contained a 4-azido group instead of a 4-nitro group en route to 4-azido-4-deoxysialic acid (see Figure 1.30) and they also observed elimination of HN_3 to give the β,γ -unsaturated α -keto ester upon silica chromatography.¹⁰⁹ To circumvent this problem, these researchers simply used their α -keto ester without purification.

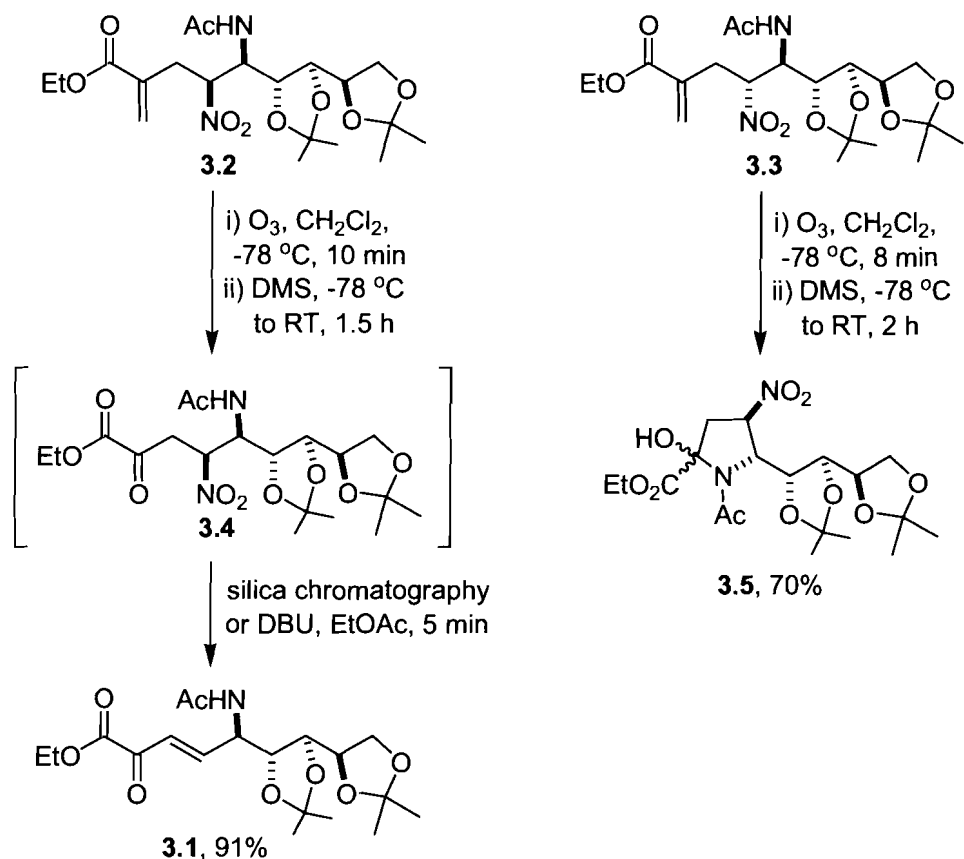


Figure 3.3 Ozonolysis of 4*S* enoate ester 3.2 afforded enone 3.1 after chromatography while ozonolysis of 4*R* enoate ester 3.3 afforded mainly 4-nitro hemiaminal 3.5

On several occasions, ozonolysis reactions were performed on mixtures of the enoate ester 4-epimers 3.2 and 3.3. Lower yields of enone 3.1 were obtained when the fraction of 3.3 in the starting material was increased, indicating that only the 4*S*-isomer 3.2 was undergoing the elimination reaction. This was supported when pure 4*R* enoate ester 3.3 was subjected to ozonolysis as the ^1H NMR of the crude product mixture showed absence of α -keto ester. The major product isolated from this reaction was the 5-membered ring 4-nitro hemiaminal 3.5, obtained in 70% yield along with 18% of enone 3.1 (Figure 3.3). The ^1H NMR spectrum of the crude ozonolysis product showed mainly 4-nitro

hemiaminal **3.5**, indicating that cyclization of the ozonolysis product likely occurred in solution and was not promoted by chromatography.

4-Nitrohemiaminal **3.5** was obtained as a mixture of interconverting anomers at carbon 2 and only the major anomer could be adequately characterized. Several pieces of spectral evidence supported the 5-membered ring hemiaminal structure. The ^1H NMR spectrum of **3.5** lacked the doublet that normally appeared around 6.0 ppm, indicating the amide proton was missing, and it contained a singlet at 4.8 ppm that disappeared when a drop of D_2O was added to the CDCl_3 solution, indicating the presence of a hydroxyl group. As well, the IR spectrum did not contain an NH stretch but did contain an OH stretching band at 3492 cm^{-1} . The nitro group was intact as evidenced by the chemical shift of proton 4 at 4.8 ppm in the ^1H NMR spectrum as well as by the presence of strong nitro stretching bands at 1372 and 1562 cm^{-1} in the IR spectrum. One of the more convincing pieces of spectral evidence of the 5-membered ring was observation of a correlation between proton 5 and carbon 2 in an HMBC spectrum, which shows 2- and 3-bond coupling correlations between protons and carbons. This H5/C2 coupling could only exist through the acetamido nitrogen atom, indicating that it was bonded to carbon 2 forming the 5-membered hemiaminal ring. Since the 4-nitro hemiaminal **3.5** was not synthetically useful, remaining **3.3** was epimerized to the 3:2 **3.2/3.3** mixture and synthetically useful **3.2** was separated.

It is interesting that ozonolysis of **3.2** led to **3.4** that did not cyclize to a 5-membered ring hemiaminal structure, allowing it to undergo elimination of HNO_2 ,

while ozonolysis of **3.3** led directly to 4-nitro hemiaminal **3.5**. While the reasons for this difference in reactivity are not apparent, it appears as though the ozonolysis product of **3.3** cyclizes readily while something prevents cyclization of **3.4** (resulting from ozonolysis of **3.2**), allowing it to exist in a conformation that enhances elimination of HNO₂ to generate enone **3.1**.

Enone **3.1** could be synthesized reliably from the 4*S* enoate ester **3.2** by ozonolysis and reduction followed by chromatography or treatment with DBU to induce elimination of HNO₂. 4*R* enoate ester **3.3** did not eliminate HNO₂ after ozonolysis, leading instead to the 5-membered hemiaminal ring **3.5**; however, **3.3** could be epimerized to give a 3:2 mixture of **3.2/3.3**, allowing its conversion into synthetically useful **3.2**.

3.3 Conjugate Addition Attempts to Enone 3.1

Initially, isolation of enone **3.1** was disappointing since it was produced in such high yields from enoate ester **3.2** and the original goal was simply to convert **3.2** into 4-deoxy-4-nitrosialic acid, ethyl ester **2.10** to increase the supply of this intermediate. However, it was quickly realized that the potential existed to perform a conjugate addition to enone **3.1** where the site of nucleophilic attack would be the 4-position (Figure 3.4). Thus, depending on the nucleophile and conditions chosen, this route could lead to vast numbers of 4-modified sialic acid analogues, possibly eclipsing the originally intended route in terms of its generality.

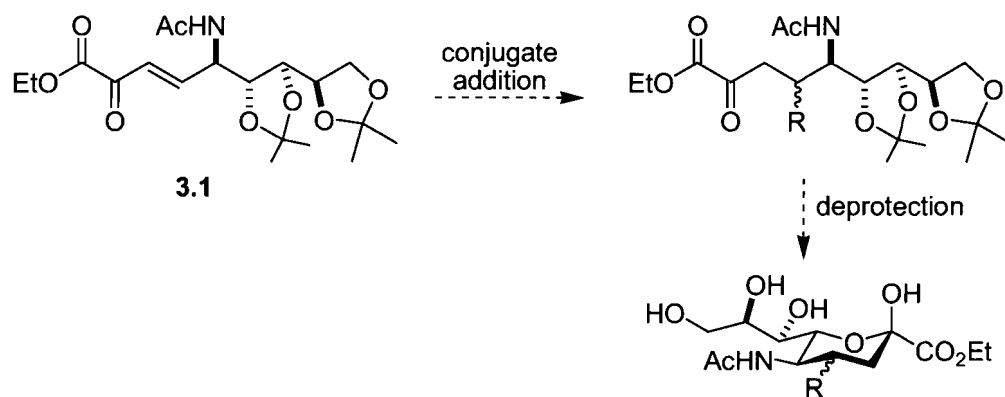


Figure 3.4 Conjugate addition to enone 3.1 followed by deprotection could lead to 4-modified sialic acid analogues

β,γ -Unsaturated α -keto esters similar to enone **3.1** have been synthesized previously for various synthetic purposes. Vasella and co-workers synthesized a similar enone from an open-chain protected enoate ester similar to **3.2/3.3** that contained a 4-hydroxyl group that they acetylated, then eliminated across carbons 3 and 4 using sodium bicarbonate during ozonolysis (described in Chapter 1.3.2.3).¹⁰⁷ They then hydrogenated the double bond of the resulting β,γ -unsaturated α -keto ester to obtain 4-deoxysialic acid. Another β,γ -unsaturated α -keto ester was synthesized by Shing via a Wittig reaction between an aldose and a phosphorus ylide that contained an α -keto ester moiety. The double bond of this enone was hydrogenated as well en route to 4-deoxy-Kdo.²¹⁰ The same enone was also used in alkoxymercuration reactions to install an alkoxy group in the 4-position after demetalation of the organomercury intermediate.²¹¹

Little evidence could be found for conjugate addition reactions being performed on such β,γ -unsaturated α -keto esters. Conjugate addition of

thiophenol to methyl 2-oxopent-3-enoate was achieved in the presence of triethylamine.²¹² Addition of nitromethane to the same enone occurred in the presence of triethylamine, affording a mixture of 1,2- and 1,4-addition products.²¹³ Friedel-Crafts alkylation reactions have also been performed on this enone, using substituted indoles as the alkylating reagent to install an aryl group in the 4-position.²¹⁴ It was puzzling, however, that no reports of alkylcopper reagents being added to these types of enones had been found, indicating that the reactivity of β,γ -unsaturated α -keto esters may be different than that of more traditional α,β -unsaturated carbonyl compounds.

The first attempts at conjugate additions to enone **3.1** used alkylcopper reagents as these are commonly used to perform conjugate additions of alkyl groups to α,β -unsaturated carbonyl compounds.²¹⁵ Unfortunately, all sets of conditions that were tested resulted in isolation of complex product mixtures. Although in some cases conjugate addition products were observed in the mix, they were impossible to separate and characterize and the yields were not practical. Initial conditions used 5 eq. CuI and 10 eq. MeLi to generate 5 eq. Me₂CuLi in THF/Et₂O that was stirred with enone **3.1** while warming from -78 °C to room temperature overnight. A repeat of these conditions with the addition of 5 eq. TMSCl or 5 eq. BF₃·OEt₂ did not change the results, nor did the use of MeMgBr instead of MeLi. The use of BuLi to generate Bu₂CuLi also did not provide useful results. On one occasion, equimolar amounts of CuI, MeLi, and BF₃·OEt₂ were mixed to generate MeCuBF₃ but this reagent also did not afford appreciable quantities of conjugate addition products. 1 eq. of MeLi was added

to enone **3.1** to see if 1,2-addition was possible, but mostly starting material was re-isolated after stirring in THF/Et₂O at -78 °C for 1.5 h indicating the low reactivity of **3.1** toward these sorts of nucleophiles.

There was a possibility that the amide proton of enone **3.1** was somehow interfering with these conjugate additions. Thus, protection of the 5-acetamido group was attempted. Benzyl protection was chosen as the benzyl group should be easily removed by hydrogenation or with lithium and naphthalene in THF.²¹⁶ However, when enone **3.1** was stirred with benzyl chloride and DBU in DMF for 1 d, very little potential product was recovered.²¹⁷ The reaction was attempted again using K₂CO₃ instead of DBU but this resulted mainly in decomposition of the starting material. Sodium hydride was also used as a base in THF/hexanes, but total decomposition was observed after 2.5 h.²¹⁸ Installation of a TBDMS protecting group was attempted by stirring enone **3.1** with TBDMSCl and imidazole in DMF but this also resulted in isolation of a complex product mixture.²¹⁹ On one occasion acetylation of the 5-acetamido group of enone **3.1** was attempted by stirring with 20 eq. of acetyl chloride and 5 eq. Hünig's base in CH₂Cl₂ overnight (Figure 3.5).²²⁰ A low yield (28%) of *N*-acetylacetamido enone **3.6** was obtained but the compound decomposed very rapidly when left exposed to the atmosphere and only IR, ¹H, and ¹H-¹H COSY NMR spectra of the compound were obtained. The IR spectrum of **3.6** showed absence of an NH stretch and the ¹H NMR spectrum showed absence of the amide proton while the frequency for proton 4 had shifted slightly downfield and the singlet for the acetamido group had doubled in size, integrating to 6 protons. However, this

compound was unstable as the next day all spectra showed complete reversion to enone **3.1** after the product was left in a flask exposed to the atmosphere overnight. Thus, protection of the acetamido group was not pursued further.

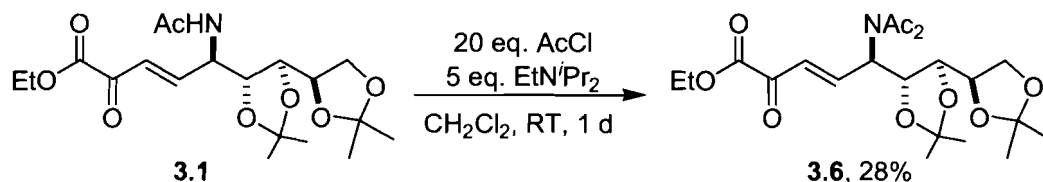


Figure 3.5 Attempted acetylation of 5-acetamido group of enone 3.1

As it seemed the conditions used for conjugate addition of alkylcopper reagents to enone **3.1** were too harsh, addition of alkyl halides under radical conditions was attempted. These reactions are often performed with Bu₃SnH as a hydrogen atom source using catalytic amounts of AIBN as a radical initiator in refluxing benzene to generate the radical thermally, although α,β -unsaturated carbonyl compounds often require an electron withdrawing group to be active toward radical conjugate addition reactions.^{221,222} Initially, benzyl bromide was used as the alkylating agent in hopes that a stable benzyl radical would be formed. Thus benzyl bromide was refluxed in toluene with enone **3.1** and 3 eq. Bu₃SnH and 0.2 eq. AIBN but this reaction afforded a complex mixture of products, none of which contained a benzyl group. The reaction was repeated using a 100 W incandescent light source to initiate radical generation at room temperature, but this reaction also failed. The alkylating agent was switched to isopropyl iodide and allyl iodide in attempts to generate stable radicals, but these reactions also resulted in decomposition of the starting enone. In a final attempt, allyl iodide and enone **3.1** were heated to 60 °C in toluene while a solution

containing Bu_3SnH and AIBN was added over 10 h via syringe pump in hopes that slow radical generation would result in addition, but this reaction also failed.

Addition of nitromethane to enone **3.1** was attempted based on a report that in the presence of triethylamine with catalytic copper(II) triflate and a chiral bisoxazoline ligand, nitromethane gave a 1,2-addition product with ethyl 2-oxobut-3-enoate but gave a mix of 1,2- and 1,4-addition products in the absence of the catalyst, as described earlier.²¹³ However, when enone **3.1** was stirred in nitromethane with 0.2 eq. triethylamine, only the 1,2-addition product **3.7** was obtained in 55% yield as a mix of diastereomers (Figure 3.6).

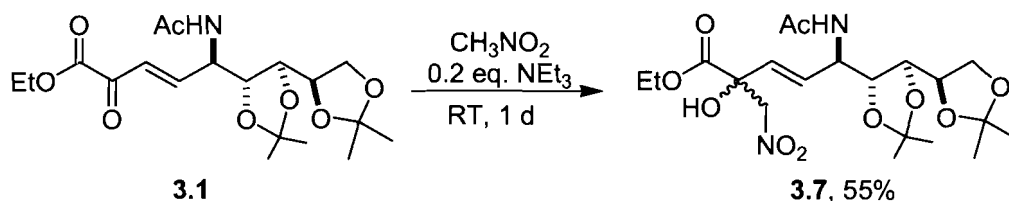


Figure 3.6 Addition of nitromethane to enone **3.1** with triethylamine afforded only 1,2-addition products

The copper-catalyzed conjugate addition of dialkylzinc reagents to enones is similar to the addition of alkylcopper reagents but due to the lower reactivity of alkylzinc reagents, these reactions are milder and tolerate a wide array of functional groups.²²³ Ultimately it was the use of these conditions that led to the isolation of conjugate addition products from enone **3.1** (Figure 3.7). Initially, the conjugate addition was performed in toluene with 1.2 eq. Et_2Zn in the presence of 4% $\text{Cu}(\text{OTf})_2$, stirring overnight while warming from $-20\text{ }^\circ\text{C}$ to room temperature to give a 29% yield of ethyl-addition products. Alexakis and co-workers have screened several copper salts for conjugate additions of dialkylzinc

reagents and found $\text{Cu}(\text{OTf})_2$ to be one of the most active but found that addition of a phosphorus ligand, generally PBU_3 or $\text{P}(\text{OEt})_3$, greatly accelerated the rate of reaction.^{224,225} Thus, the reaction of 1.5 eq. Et_2Zn with enone **3.1** was repeated in degassed toluene using 5% $\text{Cu}(\text{OTf})_2$ and 10% $\text{P}(\text{OEt})_3$ to give a 53% yield of ethyl-addition products (Figure 3.7).

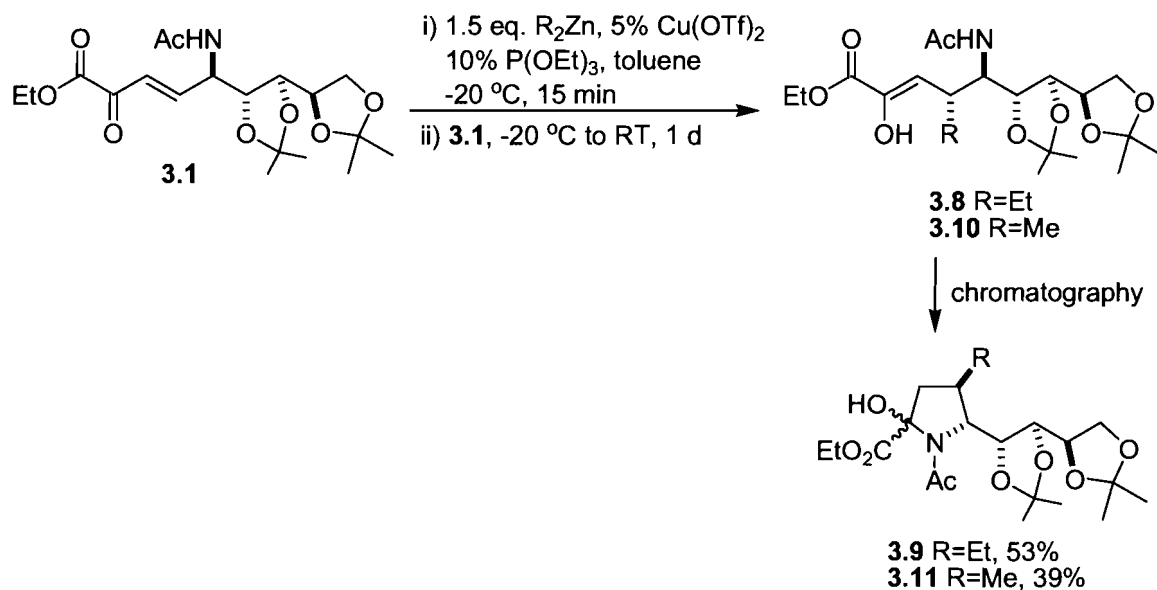


Figure 3.7 Copper-catalyzed conjugate addition of $\text{Et}_2\text{Zn}/\text{Me}_2\text{Zn}$ to enone **3.1** gave enol tautomers of addition products that isomerized to 5-membered ring hemiaminals upon chromatography

The conjugate addition of Et_2Zn to enone **3.1** did not afford the expected tautomeric form of the product. In most conjugate addition reactions, attack of the nucleophile generates an enolate anion that is protonated upon aqueous workup to give the enol form of the addition product that quickly tautomerizes to the more stable keto form. In the case of addition to enone **3.1**, the enol form of the ethyl addition product **3.8** was isolated (Figure 3.7). The enol form **3.8** is likely stabilized by the fact that the double bond is in conjugation with the ester

carbonyl group, again forming an α,β -unsaturated carbonyl compound. NMR spectral evidence supports isolation of the enol tautomer as the initial reaction product. Specifically it was clear that addition of an ethyl group had occurred due to the presence of a second triplet at 0.86 ppm in the ^1H NMR spectrum for the new CH_3 -group and new peaks at 12.0 and 24.6 ppm in the ^{13}C NMR spectrum for the CH_3 and CH_2 carbons of the new ethyl group, respectively; as well, proton 4 had shifted far upfield from 7.15 to 2.85 ppm. However, proton 3 appeared at 5.50 ppm and was a doublet integrating to one proton rather than appearing at the expected frequency around 2.5 ppm as two doublets of doublets. Also, a singlet was present at 6.54 ppm that disappeared when a drop of D_2O was added to the CDCl_3 solution, indicating this was a vinylic hydroxyl group. In addition, the ^{13}C NMR spectrum showed carbons 2 and 3 at 142 and 113 ppm, respectively, indicating they were alkene carbons. These spectral data supported assignment of **3.8** as the enol tautomer.

Unfortunately, the enol tautomer could not be purified for proper characterization as it cyclized to the 5-membered ring hemiaminal **3.9** upon chromatography, giving another unexpected structure (Figure 3.7). This ring-closure was similar to that observed following ozonolysis of the 4*R* enoate ester **3.3** which formed a 5-membered ring 4-nitro hemiaminal **3.5**. The 4-ethyl hemiaminal **3.9** shared many spectral characteristics with the 4-nitro hemiaminal **3.5**, showing disappearance of the amide proton and appearance of a hydroxyl group in both the ^1H NMR spectrum and the IR spectrum. The HMBC spectrum also showed a correlation between proton 5 and carbon 2, indicating that the

acetamido nitrogen was bonded to carbon 2 to form the 5-membered ring hemiaminal. Also similar to the 4-nitro hemiaminal **3.5**, two interconverting anomers were obtained and only the major one was able to be purified for characterization. Initially, it was hoped that these two compounds were not anomers but instead were two 4-epimers resulting from attack of the ethyl nucleophile on both faces of enone **3.1**. However, a CDCl₃ solution of the minor anomer was observed to interconvert to the major anomer after 1 d, a process that would not be possible if these compounds were 4-epimers. The fact that only one enol was observed in ¹H NMR spectra of the crude product also indicated that only one diastereomer at the 4-position was synthesized. Since the addition product was in a 5-membered ring, assignment of the stereochemistry at the 4-position was now possible by analysis of a series of 1D NOE difference ¹H NMR spectra. These spectra showed that the addition had occurred to give the 4*R*-diastereomer since irradiation of the CH₂ of the new ethyl group (Figure 3.8, bottom spectrum) caused enhancement of the signals for proton 5 and proton 3a (on the same face of the ring) while irradiation of proton 3b (on the opposite face) showed only enhancement of proton 4 (Figure 3.8, top spectrum). These spectra indicate that proton 5 and the new ethyl group are on the same face of the 5-membered ring and thus 4-ethyl hemiaminal **3.9** has the 4*R*-configuration. This is unfortunate since natural sialosides have the 4*S*-configuration; thus, the generation of the 4*R*-isomer as the sole conjugate addition product limits the versatility of enone **3.1** for making 4-modified sialic acid analogues.

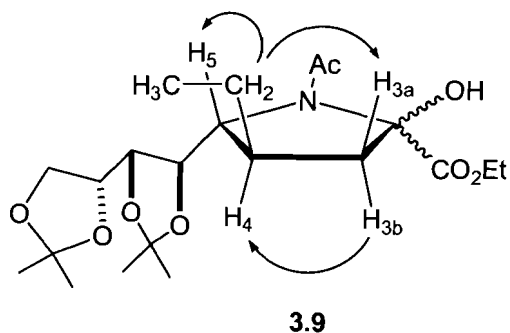
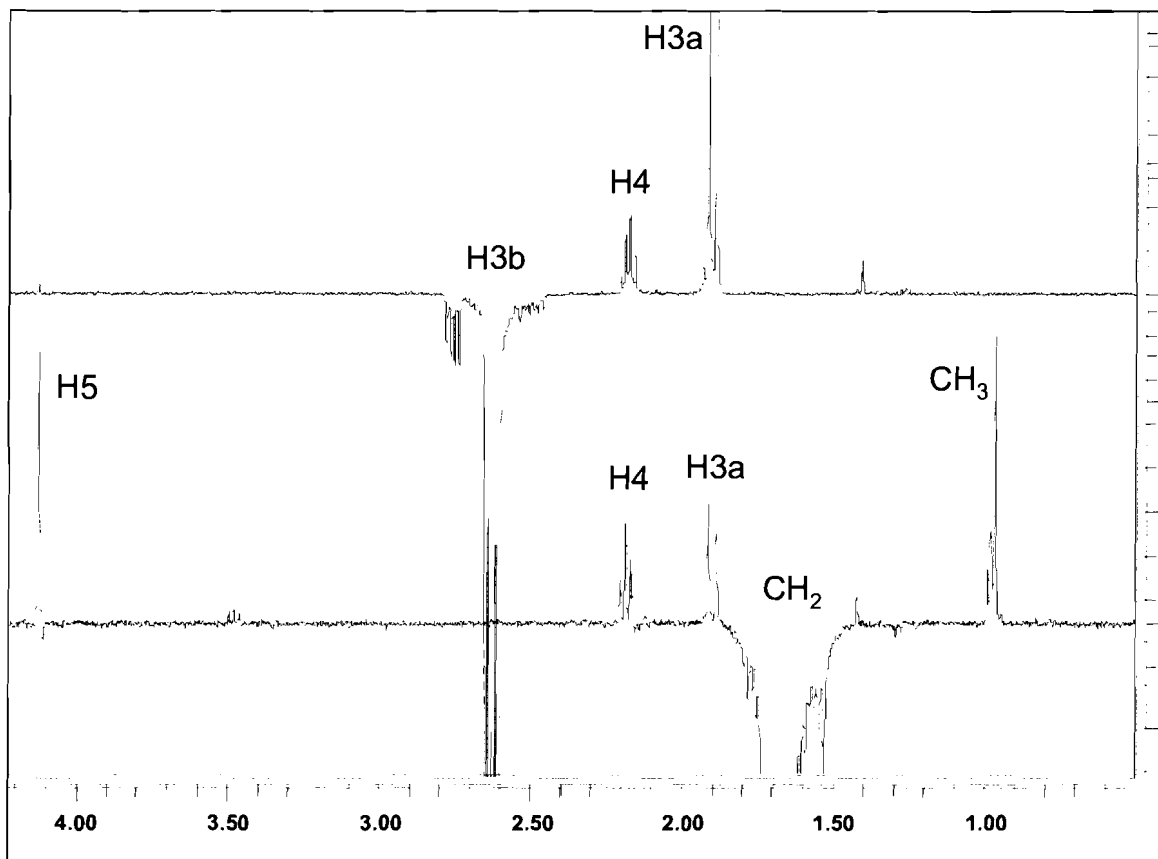


Figure 3.8 1D NOE difference ¹H NMR spectra showing 4*R*-configuration of 4-ethyl hemiaminal 3.9

These conjugate addition reactions are typically performed in non-coordinating solvents such as toluene and CH₂Cl₂, although coordinating solvents such as Et₂O and THF have been used but often reduce the rate of

reaction.²²⁵ When the conjugate addition of Et₂Zn was attempted on enone **3.1** in THF instead of toluene, however, the crude product mixture after 1 d consisted mainly of starting material. A second reaction in THF was performed using BF₃·OEt₂ instead of P(OEt)₃ but also afforded no products after 1 d. This Lewis acid was used again in toluene and resulted in no reaction, indicating that BF₃·OEt₂ is not a good promoter for these conjugate additions and that toluene is a much better solvent. The 53% yield of 4-ethyl hemiaminal is not ideal, but it was the best that could be obtained as longer reaction times induced the formation of other unidentified products. Often unreacted enone **3.1** was also recovered from some fractions after chromatography in 20-25% yields. Use of larger amounts of Et₂Zn did not result in increased yields. It has been noted that α,β-unsaturated esters are less reactive toward dialkylzinc conjugate additions;²²⁴ thus, the 53% yield of 4-ethyl hemiaminal **3.9** was considered to be satisfactory.

The conjugate addition of commercially available Me₂Zn to enone **3.1** was also performed (Figure 3.7). Of note, as Me₂Zn is known to be less reactive than Et₂Zn so lower yields of methyl-addition products were expected.²²⁶ The conjugate addition of Me₂Zn was performed under the same conditions as those used for the ethyl analogue (1.5 eq. Me₂Zn, 5% Cu(OTf)₂, 10% P(OEt)₃, toluene, -20 °C to room temperature, 1 d) and these reaction conditions also afforded the enol tautomer **3.10** of the crude methyl addition product as a single diastereomer. Following chromatography, this product also isomerized to the 5-membered ring hemiaminal structure, giving **3.11** in 39% yield (Figure 3.7). The enol and

hemiaminal tautomers of the methyl-addition products **3.10** and **3.11** exhibited similar spectral characteristics as their ethyl analogues. In particular, a series of 1D NOE difference ^1H NMR spectra showed that 4-methyl hemiaminal **3.11** was the *4R*-diastereomer in the same way as was noted for the ethyl analogue. That is, when the 4-methyl group was irradiated, enhancement of the signals for proton 5 and proton 3a (on the same face of the ring) were observed while when proton 3b was irradiated, only proton 4 was enhanced, indicating that the methyl group was on the same face of the ring as protons 5 and 3a and that **3.11** was the *4R* isomer (see Figure 3.8). Also as with the ethyl analogues, two interconverting anomers of 4-methyl hemiaminal **3.11** were obtained and only the major anomer could be purified for adequate characterization; although, ^1H and ^{13}C NMR spectra were obtained on the minor anomer.

Thus, conjugate addition of a methyl group and an ethyl group to enone **3.1** had been achieved through copper-catalyzed dialkylzinc additions, although the yields were rather low and the reaction appeared to be completely stereospecific for the less desirable *4R*-isomer. The yields could potentially be enhanced by use of a more reactive copper catalyst, copper(I) thiophenecarboxylate, which has been shown to be more efficient than many other copper catalysts.²²⁵ The use of other solvents may provide more favourable yields and may also influence the stereoselectivity of the addition by altering coordination patterns; however, THF was shown to be a poor solvent for conjugate addition to enone **3.1** as no products were isolated from these reactions.

Several chiral phosphorus-based ligands have been developed for asymmetric dialkylzinc additions, most being phosphoramidites in which the phosphorus is complexed with a biphenol or binaphthol moiety and a chiral amine,^{225,227,228} others being based on triphenylphosphine in which one of the phenyl groups contains a chiral dipeptide substituent.²²⁶ It may be that use of such a chiral phosphorus-based ligand could influence the diastereoselectivity of dialkylzinc additions to enone **3.1**. It may also be that the acetamido group was influencing the diastereoselectivity of the addition through the amide proton. Thus, protection of the acetamido group as an imide may allow conjugate addition to form both isomers; although, this protection has been attempted and found to be very difficult. One other possibility is that the isopropylidene protecting groups were holding enone **3.1** in a particular conformation that only allowed attack from one face. Removal of the isopropylidene groups and possibly re-protection with another group such as acetyl groups that allow more conformational freedom may also allow attack from the other face. Removal of the isopropylidene groups was attempted by stirring enone **3.1** in 90% CF₃CO₂H (aq) but this resulted in decomposition of the enone. This strategy was attempted on a 4-epimeric mixture of the enoate ester precursors **3.2/3.3** as these compounds were converted into their deprotected derivatives **2.9-(R/S)** as described previously in 93% yield by stirring in 90% CF₃CO₂H (aq). Acetylation of the diastereomeric mixture of enoate esters **2.9** was attempted in hopes that ozonolysis of the acetylated products would give a compound that could eliminate HNO₂ to afford an acetylated analogue of enone **3.1** on which to try a

dialkylzinc conjugate addition. However, stirring **2.9** in pyridine with acetic anhydride and DMAP at 0 °C for 1.5 h resulted in isolation of a complex mixture of compounds in low mass recovery, possibly resulting from incomplete acetylation, and this strategy was not pursued any further.

3.4 Synthesis of 4-Alkyl-4-deoxy-4-*epi*-DANA Analogues

With 4-ethyl and 4-methyl conjugate addition products **3.9** and **3.11** in hand, attention was turned to developing a route to convert these compounds into sialosides, since the goal was to develop a route to 4-modified sialic acid analogues. This route would be useful if and when conditions can be found to add a variety of functional groups to enone **3.1** and in such a way as to generate the 4*S*-diastereomer. Conversion of the hemiaminals to DANA analogues would allow measurement of the sialidase inhibition constants of these analogues. Comparison of their binding constants with those of other glycals of sialic acid and analogues such as DANA and Relenza would allow evaluation of each new 4-substituent introduced.

Conversion of 4-alkyl hemiaminals **3.9** and **3.11** into sialosides would require removal of the isopropylidene protecting groups, after which the molecule should isomerize to the sialic acid pyranose structure. This would then be converted to the glycal using conditions developed by von Itzstein and co-workers by stirring in acetic anhydride and acetic acid in the presence of a catalytic amount of H₂SO₄ to acetylate the compound and to induce subsequent elimination of HOAc across carbons 2 and 3 in one pot.²²⁹

Removal of the isopropylidene protecting groups was accomplished by stirring the 4-alkyl hemiaminals **3.9** and **3.11** in 90% CF₃CO₂H (aq) overnight (Figure 3.9). While these conditions allowed the compounds to cyclize to the sialic acid pyranose structure, they also induced formation of a 2,7-anhydro linkage to give the 2,7-anhydro-4-ethylsialoside **3.12** and the 2,7-anhydro-4-methylsialoside **3.13** as indicated by some interesting characteristics in their ¹H NMR spectra. Proton 6 showed very small or zero coupling constants to all other protons, as did proton 5, indicating that these two protons were no longer trans-diaxial to one another. As well, the chemical shift of proton 6 was perturbed downfield to 4.60 ppm from its expected position near 4.20 ppm. In the ¹³C NMR spectrum, the frequency of carbon 2 had also shifted downfield to 105 ppm from its expected position around 94 ppm. These spectral discrepancies had been observed previously for such 2,7-anhydrosialosides since the free acid analogue of **3.13** had been synthesized by Zbiral and co-workers by acidic deprotection of a 4-deoxy-4-*epi*-4-methylsialic acid analogue (see Figure 1.27).⁹⁵ Thus, it appears as though 4-alkyl-4-deoxy-4-*epi*-sialic acid analogues preferentially form the 2,7-anhydro linkage under acidic conditions while their 4-alkyl-4-deoxysialoside counterparts do not (see Figure 1.27). A series of 1D NOE difference ¹H NMR spectra on the 2,7-anhydro-4-methylsialoside **3.13** showed enhancement of the signals for both protons on carbon 3 as well as for protons 4 and 5 upon irradiation of the 4-methyl group while irradiation of proton 4 showed enhancement of the signals for protons 3, 5, and 7 and not for proton 6, supportive of the structure shown in Figure 3.9. A key piece of spectral evidence

for the formation of the 2,7-anhydro linkage was obtained from the HMBC spectra of **3.12** and **3.13**. These spectra showed a correlation between proton 7 and carbon 2 that is not possible without the 2,7-anhydro linkage, since HMBC spectra show 2- and 3-bond coupling between protons and carbons and without the 2,7-anhydro linkage the H7/C2 coupling would be through 4 bonds. Incidentally, these spectra also showed correlations between proton 6 and carbon 2, indicating that the molecule had also cyclized to form the sialic acid pyranose ring structure.

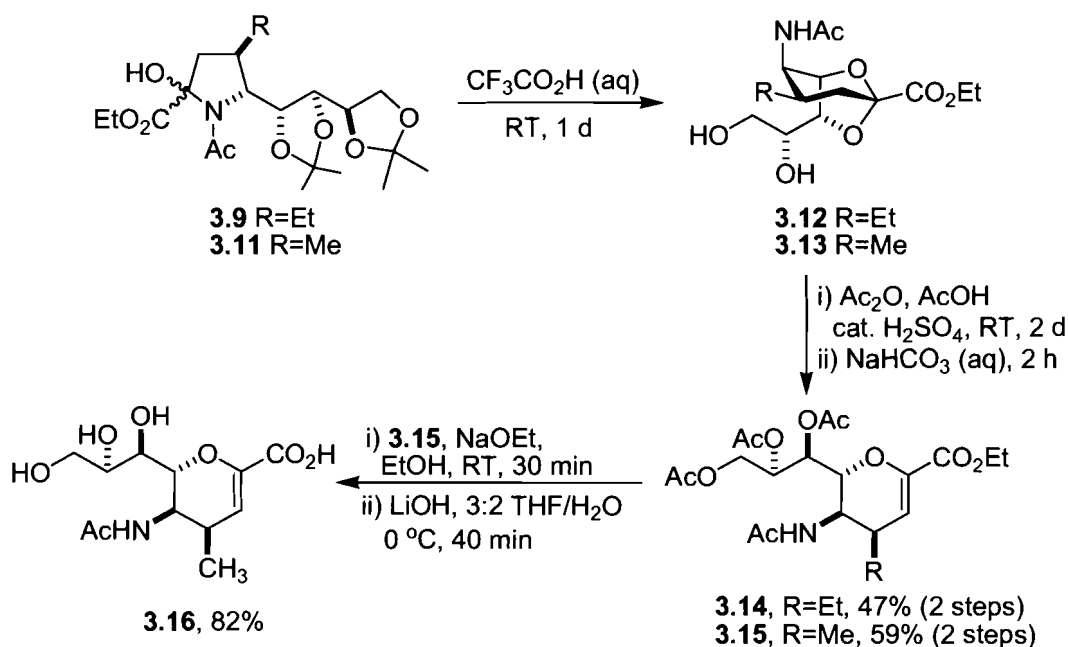


Figure 3.9 Conversion of 4-alkyl hemiaminals **3.9** and **3.11** to 4-alkyl-4-deoxy-4-*epi*-DANA analogues **3.14** and **3.15** through a 2,7-anhydrosialoside intermediate

On one occasion, deprotection of the 4-ethyl hemiaminal **3.9** was attempted by stirring with Amberlite H⁺ resin in methanol. After 2 d, the reaction was filtered and the ¹H NMR spectrum of the filtrate showed some product but

also showed peaks indicative of isopropylidene groups. The material was then re-subjected to the reaction conditions and after a further 3 d much of the resin had disappeared. ^1H NMR spectra of the products showed no isopropylidene peaks, but these conditions were abandoned in favour of $\text{CF}_3\text{CO}_2\text{H}$.

Deprotection of both major and minor anomers of the 4-alkyl hemiaminals led to the same 4-alkyl-2,7-anhydrosialosides, as did deprotection of the enol tautomer of the ethyl addition product on one occasion. Attempts were made to purify the 4-alkyl-2,7-anhydrosialosides by column chromatography but this generally resulted in low product recovery. Crystallization of the 2,7-anhydro-4-ethylsialoside **3.12** was attempted by dissolving the crude material in 5:1 CHCl_3 /methanol and adding Et_2O to turbidity. A fine white flocculent solid was formed but upon filtration the white solid turned brown and became a syrup again. Acetylation of **3.12** was attempted using acetic anhydride in pyridine in hopes that the acetylated product would be easier to chromatograph; however, no identifiable products were obtained. Thus, the crude product mixture resulting from deprotection of the 4-alkyl hemiaminals was often used directly in the next step and yields were calculated over two steps.

The next reaction involved peracetylation and elimination of HOAc across carbons 2 and 3 to form the protected 4-alkyl-4-deoxy-4-*epi*-DANA analogues. It was reasoned that the 2,7-anhydro linkage should re-open under the acidic conditions, allowing generation of the glycal without difficulty. Thus, crude preparations of the 4-alkyl-2,7-anhydrosialosides **3.12** and **3.13** were dissolved in a 1:1 v/v mix of acetic anhydride and acetic acid containing a catalytic amount of

conc. H₂SO₄ and stirred for 2 d (Figure 3.9). The reaction was then neutralized by stirring with excess saturated aqueous NaHCO₃ for 1.5-2.0 h and the products were extracted. This afforded 4-deoxy-4-*epi*-4-ethyl-DANA **3.14** in 47% yield over the two steps from the hemiaminal and gave 4-deoxy-4-*epi*-4-methyl-DANA **3.15** in 59% yield over the two steps (Figure 3.9). As was observed with the 4-alkyl-2,7-anhydrosialosides, the 4-alkyl-DANA analogues **3.14** and **3.15** could be purified via flash chromatography but their yields significantly decreased despite the crude preparations of these compounds being composed mainly of the desired products. On one occasion 4-methyl hemiaminal **3.11** was treated with acetic anhydride in acetic acid with catalytic H₂SO₄ in order to perform the deprotection, acetylation, and elimination reactions all in one pot but this did not afford any identifiable products.

The final deprotection steps for removal of the acetyl groups and the ethyl ester were performed on the 4-deoxy-4-*epi*-4-methyl-DANA analogue **3.15**. This was stirred with sodium ethoxide in ethanol for 30 min to remove the acetyl groups, followed by treatment with lithium hydroxide in 3:2 THF/H₂O at 0 °C for 40 min, affording the fully deprotected 4-deoxy-4-*epi*-4-methyl-DANA **3.16** in 82% yield (Figure 3.9).²³⁰ The efficiency of these reactions did not necessitate purification of **3.16**; however, crystallization was attempted by dissolving the product in methanol and adding Et₂O to turbidity. Although this did afford a small amount of white solid, its ¹H NMR spectrum showed broad peaks, perhaps indicating that some decomposition or contamination had taken place.

A synthetic route had therefore been developed for conversion of the conjugate addition products **3.9** and **3.11** into protected 4-alkyl-4-deoxy-4-*epi*-DANA analogues **3.14** and **3.15**, proceeding through a 2,7-anhydrosialoside intermediate. Although the reaction yields are not exceptional, they are useful enough to generate sufficient quantities of 4-substituted DANA analogues for measurement of sialidase binding constants. It is unlikely that the 4-deoxy-4-*epi*-4-methyl-DANA analogue **3.16** would have a very low binding constant with a sialidase due to the inverted stereochemistry and hydrophobic nature of the 4-substituent and was therefore not measured. However, this synthetic route would be useful if methods could be found to perform conjugate additions to enone **3.1** with moieties other than ethyl or methyl groups and with the desired 4*S*-configuration.

These DANA analogues could also be useful synthetic intermediates for generating other interesting sialic acid analogues. For instance, treatment with NBS in methanol could lead to a sialoside with a methoxy substituent at the anomeric centre and a bromide at the 3-position (Figure 1.23)⁷⁵ which when treated with a strong base could lead to elimination of HBr across carbons 3 and 4, generating a sialic acid analogue containing a double bond that has been moved one position around the pyranose ring in comparison to DANA. This could be a useful scaffold for a new class of sialidase inhibitors. Treatment of the 4-alkyl-4-deoxy-4-*epi*-DANA analogues with NBS in water could lead to the 3-bromohemiacetal that could be converted into the 2,3-epoxide and opened with TiCl₄ to generate a 3-hydroxy-2-chlorosialoside donor sugar bearing a 4-alkyl

group that could be glycosylated to generate substrates for sialidase-catalyzed hydrolysis (Figure 1.23).⁷⁶ The resulting 3-OH group could be used to install an auxiliary group to aid selectivity of the glycosylation (e.g. a thiobenzoyl group, OC(S)Ph) that could then be removed by radical techniques (see Figure 1.24).⁸⁷

3.5 Further Alkylation Attempts

As described in the previous section, Et₂Zn and Me₂Zn were successfully used in copper-catalyzed conjugate addition reactions to enone **3.1**. These two dialkylzinc reagents were chosen due to their commercial availability as solutions in hexanes or toluene. It is possible, however, to synthesize other alkylzinc reagents that contain a wide array of functional groups.^{223,231} These alkylzinc reagents could then be used in copper-catalyzed conjugate addition reactions with enone **3.1** to synthesize more exotic 4-substituted sialic acid analogues.

Alkylzinc halides were the first reagents chosen for conjugate addition attempts as they can be synthesized by insertion of metallic zinc into the carbon-halogen bond of an alkyl halide. These reactions occur best when zinc dust is reacted with alkyl iodides or allylic or benzylic halides.²³¹ Zinc dust was activated by heating to reflux in THF for 1 min in the presence of 5% 1,2-dibromoethane, then cooled and 1 eq. benzyl bromide was added to generate benzyl zinc bromide (BnZnBr).^{232,233} After stirring for 6 h at room temperature, the mixture was cooled to -20 °C and the 5% Cu(OTf)₂/10% P(OEt)₃ catalytic system was added, stirred for 15 min, then 2-cyclohexen-1-one was added. The reaction warmed to room temperature while stirring overnight, but very little product was isolated. This reaction was repeated using dry, degassed toluene as the solvent

since it was known that conjugate additions to enone **3.1** occur more efficiently in toluene (see Chapter 3.3). The use of toluene afforded a 26% yield of 3-benzylcyclohexanone (Figure 3.10). 2-Cyclohexen-1-one was used as a model enone to determine whether the alkylzinc halide had in fact been generated on the assumption that its addition products would be easier to identify than those from enone **3.1**. Since BnZnBr seemed to have been generated under conditions outlined above (Figure 3.10), these were used with enone **3.1**; however, no addition products were obtained after stirring overnight and a moderate quantity of unreacted enone was re-isolated. The reaction was repeated with zinc dust that had been activated by washing three times with 5% HCl, twice with water, twice with acetone, then twice with Et₂O but this also resulted only in isolation of unreacted enone.²²³

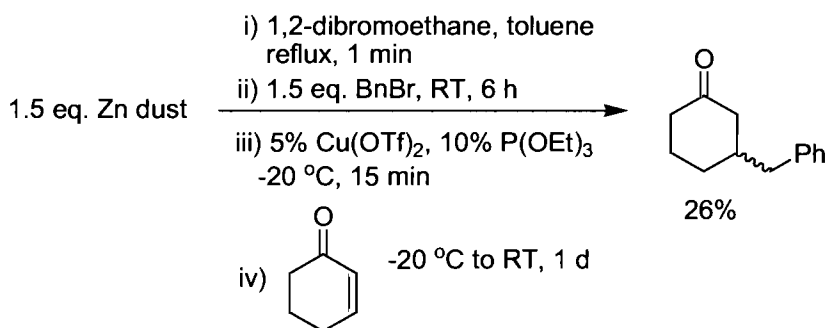


Figure 3.10 Synthesis of BnZnBr and its copper-catalyzed conjugate addition to 2-cyclohexen-1-one

It appeared as though alkylzinc halides were not able to add to enone **3.1**, or that their synthesis was not very efficient as evidenced by the 26% yield obtained from the model enone 2-cyclohexen-1-one. The synthesis of dialkylzinc reagents was attempted next since Et₂Zn and Me₂Zn were able to be added to

enone **3.1**. These reagents could be synthesized by zinc-halogen exchange in which a dialkylzinc reagent is reacted with 2 eq. of an alkyl halide to induce a switch of R groups, forming a new dialkylzinc reagent and a new alkyl halide.^{223,231} The synthesis of dibenzylzinc (Bn_2Zn) was attempted by stirring 2 eq. benzyl bromide with 1 eq. Me_2Zn in toluene at 0 °C but when this solution was warmed to room temperature, the reagent decomposed. Thus, the synthesis of Bn_2Zn was carried out at -30 °C for 45 min, to which was added the 5% $\text{Cu}(\text{OTf})_2/10\%$ $\text{P}(\text{OEt})_3$ catalyst system. After stirring for 15 min, 2-cyclohexen-1-one was added and after being allowed to stir overnight while warming to room temperature afforded a 22% yield of 3-benzyl-2-methylcyclohexanone (Figure 3.11). This product resulted from a copper-catalyzed conjugate addition of the benzyl group from dibenzylzinc but the resulting enolate had attacked the methyl bromide that was generated in situ during the synthesis of dibenzylzinc, installing a methyl group at the 2-position. The electrophilic trapping of zinc enolates resulting from conjugate addition of dialkylzinc reagents has been used as a synthetic strategy to generate α,β -disubstituted carbonyl compounds;²³⁴ however, it was not intended in this case. The synthesis of Bn_2Zn was repeated while the solution was placed under a partial vacuum for 1 h in an attempt to evaporate methyl bromide as it was formed; however, this again resulted in generation of 3-benzyl-2-methylcyclohexanone in 15% yield. Even though these conditions resulted in double alkylation, they were used on enone **3.1** but this resulted in low recovery of unreacted **3.1** and a small amount of 4-methyl hemiaminal **3.11**, likely resulting

from conjugate addition of a methyl group from unreacted Me_2Zn or a mixed zinc reagent such as BnZnMe .

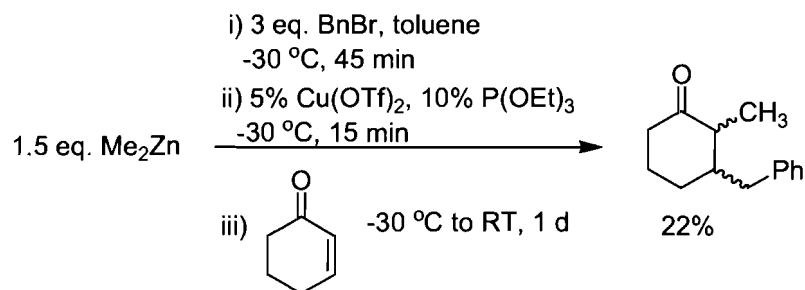


Figure 3.11 Synthesis of Bn_2Zn from Me_2Zn and its copper-catalyzed conjugate addition to 2-cyclohexen-1-one

The synthesis of a dialkylzinc reagent was next attempted by transmetallation since the synthesis of Bn_2Zn by zinc-halogen exchange resulted in generation of undesired methyl bromide. This involved reaction of a zinc(II) halide with 2 eq. of an alkyllithium or alkyl Grignard reagent in which the alkyl group transmetallates from lithium/magnesium to zinc.^{223,231} This method for generating alkylzinc reagents has less functional group tolerance than the previous two methods owing to the use of reactive organolithium or Grignard reagents as precursors but this may allow generation of a useful dialkylzinc reagent with which to test addition to enone **3.1**. Thus, 1.5 eq. ZnCl_2 was fused under vacuum, then suspended in toluene and cooled to -30 °C. 3.0 eq. phenyl magnesium bromide in THF was added, stirred at -30 °C for 2 h and the diphenylzinc was added to 2-cyclohexen-1-one in the presence of 5% $\text{Cu}(\text{OTf})_2$ and 10% $\text{P}(\text{OEt})_3$, giving 3-phenylcyclohexanone in 68% yield (Figure 3.12). When these conditions were used on enone **3.1**, however, no conjugate addition products were isolated as only small amounts of unreacted **3.1** and possible 1,2-

fact that Me_2Zn obtained from commercial sources does give rise to conjugate addition products. Thus, it may be advisable to attempt to purify lab-generated dialkylzinc reagents, perhaps through distillation, or to find more commercially-available dialkylzinc reagents that are free from impurities.

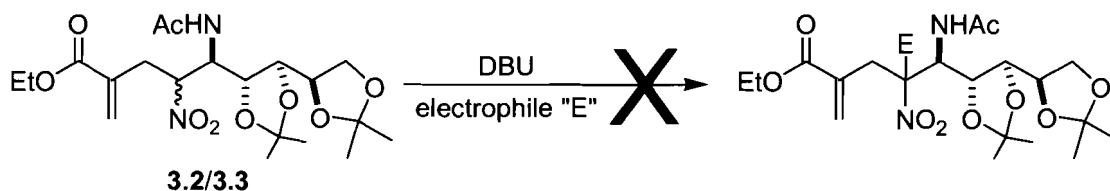


Figure 3.13 Deprotonation of enoate esters **3.2/3.3** and attempted reactions with several electrophiles

A different strategy was tested in order to generate 4-substituted sialic acid analogues using enoate esters **3.2/3.3**. This strategy involved further alkylation at the 4-position by generating the nitronate and attempting to react this with various electrophiles. Alkylation of the nitronate of enone **3.2/3.3** would generate 4,4-disubstituted sialic acid analogues in which the nitro group could be removed under radical conditions, replacing it with a proton. Another possible use of this intermediate would be elimination of HNO_2 following ozonolysis of the alkylated product to generate a 4-alkyl substituted β,γ -unsaturated α -keto ester that would also be synthetically versatile. Vasella and co-workers have shown that tertiary nitrosugars could be deprotonated and reacted with Michael acceptors and aldehydes, so this was attempted with enoate esters **3.2/3.3**.²³⁵ A mix of these substrates was stirred with 1.2 eq. DBU in THF for 15-45 min to effect deprotonation, then various electrophiles were added and the reactions were stirred overnight at room temperature (Figure 3.13). However, no alkylated

products were observed when using methyl iodide, benzyl bromide, allyl bromide, or benzaldehyde as electrophiles. A small amount of possible alkylation products may have been formed from the use methyl iodide and allyl bromide as sterically-unhindered electrophiles but the yields would have been much too low to be of use. These results suggest that the 4-position of the enoate esters **3.2/3.3** may be sterically congested, preventing attack of the nitronate on electrophiles since it was clear that deprotonation had occurred as the ratio of **3.2** to **3.3** of the recovered starting materials did not match the starting ratio. Thus, synthesis of 4-substituted sialic acid analogues via alkylation of the enoate ester did not appear to be a viable route. This was not altogether surprising since overalkylated products were not observed during the coupling of protected nitromannitol **2.5** with alkyl α -(bromomethyl)acrylate esters (Figure 3.1).

3.6 Conclusions

An unexpected β,γ -unsaturated α -keto ester **3.1** was isolated following chromatographic purification of the products resulting from ozonolysis of the isopropylidene-protected enoate ester **3.2**. It was determined that the initial ozonolysis product from the 4*S*-enoate ester **3.2** retained the nitro group but upon chromatography or treatment with DBU, elimination of HNO₂ occurred to generate enone **3.1**. Ozonolysis of the 4*R*-enoate ester **3.3** led mainly to isolation of a 5-membered ring hemiaminal **3.5** resulting from cyclization of the 5-acetamido nitrogen onto the newly-generated carbonyl carbon at the 2-position. Treatment of the 4*R*-enoate ester **3.3** with DBU resulted in epimerization of the 4-position to give a 3:2 mixture of **3.2:3.3** which was chromatographically

separable, allowing the undesired *4R*-diastereomer to be converted into the useful *4S*-diastereomer.

Since the conjugate addition of a nucleophile to enone **3.1** would generate a sialic acid analogue bearing the new substituent at the 4-position, several conditions were attempted to accomplish this. The copper-catalyzed addition of commercially available Et₂Zn and Me₂Zn solutions in the presence of P(OEt)₃ was found to afford conjugate addition products that were initially isolated as their enol tautomers but upon chromatography cyclized to 5-membered ring hemiaminal structures **3.9** and **3.11**. Unfortunately these conditions resulted solely in the synthesis of the less desirable *4R*-diastereomers. The diastereoselectivity may be able to be influenced by protection of the 5-acetamido group to remove possible coordinating effects of the amide proton; however, the 5-acetamido group of enone **3.1** has proven difficult to protect with synthesis of the *N*-acetylacetamido derivative being the most promising. The use of a chiral phosphorus-based ligand may also induce a change in the diastereoselectivity of the dialkylzinc addition.

The 4-alkyl hemiaminal structures **3.9** and **3.11** were able to be converted into 4-alkyl-4-deoxy-4-*epi*-DANA analogues in order to generate molecules whose sialidase binding constants could be measured and compared with DANA and other 4-substitued sialic acid glycal analogues as benchmark compounds. Removal of the isopropylidene groups from **3.9** and **3.11** using CF₃CO₂H (aq) allowed the products to isomerize to the sialic acid pyranose structures but the products also contained a 2,7-anhydro linkage that has been observed previously

following acidic deprotections of 4-alkyl-4-deoxy-4-*epi*-sialic acid analogues. These 2,7-anhydrosialosides were then converted into glycals in one pot by stirring in acetic anhydride and acetic acid with H₂SO₄. The 2,7-anhydrosialoside intermediates did not appear to be stable to chromatography and were used without purification for generation of the glycals, but chromatographic purification of the glycals also resulted in a decrease in product yields. It may therefore be more efficient to carry crude products through from the 4-alkyl hemiaminal stage until the final deprotection of the DANA analogue.

It would be useful to be able to add nucleophiles to enone **3.1** other than ethyl and methyl groups. This should be possible since copper-catalyzed conjugate additions of dialkylzinc reagents are known to be tolerant of a variety of functional groups. Thus, generation of benzyl- and phenyl-containing zinc species was attempted in order to show that non-commercial alkylzinc reagents could be added to enone **3.1**. Although modest yields of conjugate addition products from 2-cyclohexen-1-one were obtained, no conjugate addition was observed with enone **3.1**, indicating that benzyl and phenyl groups may be too large to add to the sterically-crowded 4-position of enone **3.1** and/or that some by-product from the synthesis of the alkylzinc reagents impeded the capricious conjugate addition to **3.1**. It may be of use to attempt purification of lab-generated dialkylzinc reagents, perhaps via distillation, or find commercial sources of pure dialkylzinc solutions. It may also be useful to use dialkylzinc reagents with smaller alkyl groups such as allyl or vinyl groups which may add

more efficiently to the sterically-crowded 4-position of **3.1** and whose functionality would allow further derivatization at the 4-position of the addition products.

3.7 Experimental

General

All chemicals were purchased from Aldrich Chemical Company and were used as received. Solvents for anhydrous reactions were dried and distilled immediately prior to use. Methanol and ethanol were dried and distilled over magnesium turnings. CH₂Cl₂ and toluene were dried and distilled over calcium hydride. Toluene was degassed by repeated freezing, then thawing while under vacuum. Glassware used for anhydrous reactions was flame-dried and cooled under a N₂ atmosphere immediately prior to use. TLC was performed on aluminum-backed TLC plates pre-coated with Merck silica gel 60 F₂₅₄. Compounds were visualised with UV light and/or staining with phosphomolybdic acid (5% solution in ethanol). Flash chromatography was performed using Avanco silica gel 60 (230-400 mesh). Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer, on a Varian Unity 500 MHz spectrometer, or on a Bruker TCI 600 MHz spectrometer. Chemical shifts (δ) are listed in ppm downfield from TMS using the residual solvent peak as an internal reference. ¹H and ¹³C NMR peak assignments were made based on ¹H-¹H COSY and ¹H-¹³C HMQC experiments. IR spectra were recorded on a Bomem IR spectrometer and samples were prepared as cast evaporative films on NaCl plates from CH₂Cl₂ or methanol. Optical rotations were measured using a

Perkin-Elmer 341 polarimeter and are reported in units of $\text{deg cm}^2 \text{g}^{-1}$ (concentrations reported in units of $\text{g}/100 \text{ cm}^3$). Ethyl α -(bromomethyl)acrylate **2.6** was synthesized according to literature procedures and its spectral characteristics matched those reported in the literature.¹⁹⁶

Ethyl 5-acetamido-2,3,4,5-tetradecoxy-6,7:8,9-di-O-isopropylidene-2-methylene-4-nitro-D-glycero-D-galacto-nononate (3.2) and ethyl 5-acetamido-2,3,4,5-tetradecoxy-6,7:8,9-di-O-isopropylidene-2-methylene-4-nitro-D-glycero-D-talo-nononate (3.3)

2-Acetamido-1,2-dideoxy-3,4:5,6-di-O-isopropylidene-1-nitro-D-mannitol **2.5** (10.6 g, 31.9 mmol) was dissolved in THF (400 mL) along with ethyl α -(bromomethyl)acrylate **2.6** (8.0 g, 41 mmol). An aqueous solution of NaOH (0.5 M; 82 mL, 41 mmol) was added dropwise via an addition funnel over 15 min and the turbid yellow solution was stirred at room temperature. After 18 h, the reaction was diluted with water (500 mL) and extracted with CH_2Cl_2 (1 x 500 mL, 2 x 250 mL). The combined organic layers were dried (MgSO_4), filtered, and concentrated under reduced pressure to give a dark yellow syrup (16.9 g). This was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:1 to 1:2 v/v) to afford the 4*S* enoate ester **3.2** as a sticky white solid (7.8 g, 17.5 mmol, 55%) and the 4*R* enoate ester **3.3** as a colourless foamy syrup (4.1 g, 9.2 mmol, 29%).

3.3 could be epimerized by stirring with DBU (1.4 mL, 9.2 mmol) in THF (150 mL) for 40 min. The reaction was quenched by the addition of saturated aqueous ammonium chloride (50 mL) and was then diluted with water (200 mL) and extracted with Et_2O (2 x 100 mL). The combined organic layers were dried

(MgSO₄), filtered, and concentrated under reduced pressure to give a yellow syrup (3.9 g). Following chromatographic purification (hexanes-EtOAc gradient solvent system from 1:1 to 1:2 v/v), the 4*S* enoate ester **3.2** (2.0 g, 4.5 mmol, 49%) was separated from recovered 4*R* enoate ester **3.3** (1.2 g, 2.7 mmol, 29%). **3.2** [α]_D²⁰ -29.4 (*c* 1.14, CHCl₃); **3.3** [α]_D²⁰ +28.5 (*c* 2.32, CHCl₃). IR (cm⁻¹): 1372 (NO₂), 1553 (NO₂), 1634 (C=C), 1666 (amide C=O), 1714 (unsaturated ester C=O), 3295 (amide NH). ¹H NMR (CDCl₃, 500 MHz) **3.2** δ : 1.31 (t, 3H, *J* = 7.1 Hz, CH₃CH₂O), 1.34 (s, 6H, CMe₂), 1.38 (s, 3H, CMe₂), 1.39 (s, 3H, CMe₂), 2.07 (s, 3H, NHCOCH₃), 2.88-2.90 (m, 2H, H-3_{ax}, H-3_{eq}), 3.828 (dd, 1H, *J*_{9a,9b} = 8.8 Hz, *J*_{8,9a} = 12.2 Hz, H-9a), 3.829 (d, 1H, *J*_{7,8} = 8.8 Hz, H-7), 3.93-3.98 (m, 2H, H-6, H-8), 4.13 (dd, 1H, *J*_{9a,9b} = 8.8 Hz, *J*_{8,9b} = 6.3 Hz, H-9b), 4.23 (q, 2H, *J* = 7.1 Hz, CH₃CH₂O), 4.56 (dt, 1H, *J*_{4,5} = 2.9 Hz, *J*_{5,6} = *J*_{5,NH} = 9.8 Hz, H-5), 5.26 (ddd, 1H, *J*_{3a,4} = 5.9 Hz, *J*_{3b,4} = 8.8 Hz, *J*_{4,5} = 2.9 Hz, H-4), 5.71 (s, 1H, C=CHaHb), 6.15 (d, 1H, *J*_{5,NH} = 9.8 Hz, NHCOCH₃), 6.30 (s, 1H, C=CHaHb); **3.3** δ : 1.32 (t, 3H, *J* = 7.1 Hz, CH₃CH₂O), 1.37 (s, 6H, CMe₂), 1.41 (s, 3H CMe₂), 1.42 (s, 3H, CMe₂), 2.01 (s, 3H, NHCOCH₃), 2.94 (dd, 1H, *J*_{3a,3b} = 14.9 Hz, *J*_{3a,4} = 3.7 Hz, H-3a), 3.02 (dd, 1H, *J*_{3a,3b} = 14.9 Hz, *J*_{3b,4} = 10.5 Hz, H-3b), 3.80-3.84 (m, 2H, H-7, H-9a), 3.95-4.01 (m, 1H, H-8), 4.10 (dd, 1H, *J*_{5,6} = 9.5 Hz, *J*_{6,7} = 5.8 Hz, H-6), 4.18 (dd, 1H, *J*_{9a,9b} + *J*_{8,9b} = 14.9 Hz, H-9b), 4.24 (q, 2H, *J* = 7.1 Hz, CH₃CH₂O), 4.66 (dt, 1H, *J*_{4,5} = 4.8 Hz, *J*_{5,6} + *J*_{5,NH} = 17.4 Hz, H-5), 5.10 (dt, 1H, *J*_{3b,4} = 10.7 Hz, *J*_{3a,4} + *J*_{4,5} = 8.5 Hz, H-4), 5.71 (s, 1H, C=CHaHb), 6.11 (d, 1H, *J*_{5,NH} = 8.3 Hz, NHCOCH₃), 6.28 (s, 1H, C=CHaHb). ¹³C NMR (CDCl₃, 125 MHz) **3.2** δ : 13.9 (CH₃CH₂O), 23.2 (NHCOCH₃), 25.1, 26.4, 27.0, 27.4 (C(CH₃)₂ x 2), 34.0 (C-3),

51.9 (C-5), 61.0 (CH₃CH₂O), 67.6 (C-9), 76.7 (C-8), 79.2 (C-6), 80.5 (C-7), 85.4 (C-4), 109.6, 110.7 (C(CH₃)₂ × 2), 129.7 (C=CH₂), 134.0 (C=CH₂), 165.5 (C=O), 170.2 (C=O); **3.3** δ: 13.9 (CH₃CH₂O), 23.0 (NHCOCH₃), 25.1, 26.4, 26.5, 27.2 (C(CH₃)₂ × 2), 31.8 (C-3), 53.7 (C-5), 61.0 (CH₃CH₂O), 67.9 (C-9), 77.0 (C-8), 79.3 (C-6), 80.5 (C-7), 87.1 (C-4), 109.8, 110.9 (C(CH₃)₂ × 2), 128.9 (C=CH₂), 134.5 (C=CH₂), 165.8 (C=O), 170.2 (C=O). **3.2/3.3** Anal. calcd. for C₂₀H₃₂N₂O₉: C 54.04, H 7.26, N 6.30; found: C 54.01, H 7.21, N 6.31.

Ethyl 5-acetamido-2,3,4,5-tetradecoxy-2-methylene-4-nitro-D-glycero-D-galacto-nononate and ethyl 5-acetamido-2,3,4,5-tetradecoxy-2-methylene-4-nitro-D-glycero-D-talo-nononate (2.9-(S/R))

A 60/40 mixture of isopropylidene-protected enoate esters **3.2** and **3.3** (2.04 g, 4.59 mmol) was dissolved in CF₃CO₂H (5 mL) and water (0.5 mL) and stirred at room temperature overnight. After 1 d, the solution was concentrated under reduced pressure to afford a purple syrup that was suspended in CHCl₃ and concentrated under reduced pressure (3 × 5 mL), to give a purple foamy syrup. The crude product was purified via flash chromatography (CH₂Cl₂-methanol gradient solvent system from 20:1 to 3:1 v/v) to afford the deprotected enoate ester **2.9** as a light brown foam in a 2.6:1 mixture of 4S:4R isomers (1.55 g, 4.25 mmol, 93%). The spectral characteristics of the product matched those reported earlier (Chapter 2.6).

Ethyl 5-acetamido-3,4,5-trideoxy-6,7:8,9-di-O-isopropylidene-4-nitro-D-glycero-D-galacto-non-2-ulosonate (3.4)

The 4S enoate ester **3.2** (494 mg, 1.11 mmol) was dissolved in dry CH₂Cl₂ (50 mL) and cooled in a dry ice/acetone bath to -78 °C. The flask was fitted with a

CaCl₂ drying tube and ozone was bubbled through the solution until a blue colour persisted (10 min). The solution was purged with oxygen and dimethyl sulfide (1 mL) was added. The colourless solution was allowed to warm to room temperature while stirring for 1.5 h, after which time it was concentrated under reduced pressure to yield a colourless syrup (588 mg) that consisted mainly of DMSO and α -keto ester **3.4**. The crude material was unstable and could not be fully characterized. IR (cm⁻¹): 1373 (NO₂), 1557 (NO₂), 1673 (amide C=O), 1733 (α -keto ester C=O, one band), 3267 (NH). ¹H NMR (CDCl₃, 500 MHz) δ : 1.35 (s, 3H, CMe₂), 1.377 (t, 3H, J = 7.1 Hz, CH₃CH₂O), 1.382 (s, 6H, CMe₂), 1.43 (s, 3H, CMe₂), 2.01 (s, 3H, NHCOCH₃), 3.46 (dd, 1H, $J_{3a,3b}$ = 19.2 Hz, $J_{3a,4}$ = 6.8 Hz, H-3a), 3.63 (dd, 1H, $J_{3a,3b}$ = 19.2 Hz, $J_{3b,4}$ = 7.3 Hz, H-3b), 3.76 (dd, 1H, $J_{6,7}$ = 5.2 Hz, $J_{7,8}$ = 8.8 Hz, H-7), 3.81 (dd, 1H, $J_{8,9a}$ = 6.7 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9a), 3.98 (dt, 1H, $J_{7,8}$ = 8.8 Hz, $J_{8,9a} + J_{8,9b}$ = 12.9 Hz, H-8), 4.13 (dd, 1H, $J_{5,6}$ = 9.8 Hz, $J_{6,7}$ = 5.2 Hz, H-6), 4.15 (dd, 1H, $J_{8,9b}$ = 6.2 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9b), 4.35 (q, 2H, J = 7.1 Hz, CH₃CH₂O), 4.43 (dt, 1H, $J_{4,5}$ = 2.3 Hz, $J_{5,6} + J_{NH,5}$ = 18.5 Hz, H-5), 5.49 (dt, 1H, $J_{3a,4} + J_{3b,4}$ = 14.1 Hz, $J_{4,5}$ = 2.3 Hz, H-4), 5.96 (d, 1H, $J_{NH,5}$ = 8.7 Hz, NHCOCH₃). ¹³C NMR (CDCl₃, 125 MHz) δ : 13.2 (CH₃CH₂O), 22.2 (NHCOCH₃), 24.7, 25.8, 26.4, 26.9 (CMe₂ x 2), 38.4 (C-3), 52.3 (C-5), 62.1 (CH₃CH₂O), 66.4 (C-9), 75.9 (C-8), 77.5 (C-6), 80.5 (C-7), 80.7 (C-4), 109.0, 110.2 (CMe₂ x 2), 158.9 (C-1), 170.3 (NHCOCH₃), 188.7 (C-2).

Ethyl (*E*)-5-acetamido-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-D-mannon-3-en-2-ulosonate (3.1)

The crude α -keto ester **3.4** from ozonolysis of enoate ester **3.2** was subjected to flash chromatography (hexanes-EtOAc gradient solvent system from 1:1 v/v to

100% EtOAc), inducing elimination of HNO₂ to afford enone **3.1** as a colourless syrup (403 mg, 1.01 mmol, 91%). The elimination could also be effected by stirring **3.4** with 1 eq. DBU in EtOAc for 2 min, followed by quenching with saturated aqueous ammonium chloride and extraction with Et₂O to afford crude enone **3.1** that could be purified as described above. $[\alpha]_D^{20} +18.8$ (*c* 1.10, CHCl₃). IR (cm⁻¹): 1073 (aliphatic ether C-O-C), 1654 (amide C=O), 1733 (α-keto ester C=O, one band), 3292 (NH). ¹H NMR (CDCl₃, 500 MHz) δ: 1.36-1.38 (m, 12H, CH₃CH₂O, CMe₂ x 2), 1.44 (s, 3H, CMe₂), 2.04 (s, 3H, NHCOCH₃), 3.70 (dd, 1H, *J*_{6,7} = 7.2 Hz, *J*_{7,8} = 8.7 Hz, H-7), 3.89 (dd, 1H, *J*_{8,9a} = 5.8 Hz, *J*_{9a,9b} = 8.7 Hz, H-9a), 3.99-4.04 (m, 2H, H-6, H-8), 4.17 (dd, 1H, *J*_{8,9b} = 6.2 Hz, *J*_{9a,9b} = 8.7 Hz, H-9b), 4.34 (q, 2H, *J* = 7.1 Hz, CH₃CH₂O), 4.80-4.85 (m, 1H, H-5), 6.16 (d, 1H, *J*_{NH,5} = 7.9 Hz, NHCOCH₃), 6.85 (dd, 1H, *J*_{3,4} = 15.9 Hz, ⁴*J*_{3,5} = 1.2 Hz, H-3), 7.15 (dd, 1H, *J*_{3,4} = 15.9 Hz, *J*_{4,5} = 6.4 Hz, H-4). ¹³C NMR (CDCl₃, 125 MHz) δ: 13.8 (CH₃CH₂O), 23.0 (NHCOCH₃), 24.9, 26.5, 26.7, 27.0 (CMe₂ x 2), 52.5 (C-5), 62.3 (CH₃CH₂O), 67.7 (C-9), 76.8 (C-8), 79.0 (C-7), 81.3 (C-6), 109.8, 110.3 (CMe₂ x 2), 126.1 (C-3), 147.9 (C-4), 161.5 (C-1), 169.4 (NHCOCH₃), 182.6 (C-2). Anal. calcd. for C₁₉H₂₉NO₈: C 57.13, H 7.32, N 3.51; found: C 56.82, H 7.17, N 3.76.

Ethyl *N*-acetyl-5-amino-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-4-nitro-*D*-glycero-*D*-talo-non-2-ulofuranosonate (3.5)

The 4*R* enoate ester **3.3** (168 mg, 0.377 mmol) was dissolved in dry CH₂Cl₂ (30 mL) and cooled to -78 °C in a dry ice/acetone bath. The reaction vessel was fitted with a CaCl₂ drying tube and ozone was bubbled through the solution until a blue colour persisted (8 min). The solution was purged with oxygen and

dimethyl sulfide (0.5 mL) was added. The reaction was removed from the dry ice/acetone bath and allowed to warm to room temperature while stirring for 2 h and was then concentrated under reduced pressure to give a colourless syrup (228 mg). The crude material was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:1 v/v to 100% EtOAc) to afford the 4-nitro hemiaminal **3.5** as a colourless syrup (118 mg, 0.265 mmol, 70%). This product forms two anomers at C2 that interconvert in solution; only the major anomer could be isolated in sufficient quantities for characterization. A quantity of enone **3.1** was also obtained (26.5 mg, 0.066 mmol, 18%). IR (cm⁻¹): 1372 (NO₂), 1562 (NO₂), 1658 (amide C=O), 1753 (ester C=O), 3492 (OH). ¹H NMR (CDCl₃, 500 MHz) δ: 1.25 (t, 3H, *J* = 7.1 Hz, CH₃CH₂O), 1.33 (s, 3H, CMe₂), 1.35 (s, 3H, CMe₂), 1.39 (s, 3H, CMe₂), 1.45 (s, 3H, CMe₂), 2.20 (s, 3H, NHCOCH₃), 2.97-2.98 (m, 2H, H-3a, H-3b), 3.58 (t, 1H, *J*_{6,7} + *J*_{7,8} = 17.3 Hz, H-7), 3.99 (dd, 1H, *J*_{8,9a} = 4.5 Hz, *J*_{9a,9b} = 8.9 Hz, H-9a), 4.06-4.11 (m, 1H, H-8), 4.14-4.26 (m, 3H, CH₃CH₂O, H-9b), 4.29 (d, 1H, *J*_{6,7} = 8.3 Hz, H-6), 4.81-4.83 (m, 2H, H-4, 2-OH), 5.40 (s, 1H, H-5). ¹³C NMR (CDCl₃, 125 MHz) δ: 13.9 (CH₃CH₂O), 22.5 (NHCOCH₃), 25.0, 26.5, 26.6, 26.9 (CMe₂ x 2), 41.5 (C-3), 62.2 (C-5), 62.3 (CH₃CH₂O), 68.0 (C-9), 77.3 (C-8), 78.4 (C-7), 81.8 (C-6), 84.1 (C-4), 89.2 (C-2), 110.2, 110.7 (CMe₂ x 2), 169.9 (C=O), 171.1 (C=O). Anal. calcd. for C₁₉H₃₀N₂O₁₀: C 51.12, H 6.77, N 6.27; found: C 51.24, H 6.81, N 6.01.

Ethyl (*E*)-5-*N*-acetylacetamido-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-*D*-manno-non-3-en-2-ulosonate (3.6**)**

Enone **3.1** (62.9 mg, 0.157 mmol) was dissolved in dry CH₂Cl₂ (8 mL) to which *N,N*-diisopropylethylamine (0.14 mL, 0.88 mmol) and acetyl chloride (0.22 mL,

3.1 mmol) were injected. The solution was stirred under N₂ at room temperature for 19 h. The solution was diluted with CH₂Cl₂ (50 mL) and washed with saturated aqueous NaHCO₃ (50 mL) and saturated aqueous NaCl (50 mL). The aqueous layers were combined and washed with CH₂Cl₂ (50 mL), then the combined organic layers were dried (MgSO₄), filtered, and concentrated under reduced pressure to give a yellow syrup (68.5 mg). The crude product was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 3:1 v/v to 100% EtOAc), affording the imide **3.6** as a colourless film (19.6 mg, 0.044 mmol, 28%). IR (cm⁻¹): 1674 (C=C trans vinylidene stretch), 1707 (imide C=O, α-keto ester C=O). ¹H NMR (CDCl₃, 500 MHz) δ: 1.31 (s, 3H, C(CH₃)₂), 1.34 (s, 3H, C(CH₃)₂), 1.36-1.39 (m, 6H, C(CH₃)₂, OCH₂CH₃), 1.42 (s, 3H, C(CH₃)₂), 2.42 (s, 6H, N(COCH₃)₂), 3.69 (dd, 1H, J_{6,7} = 6.2 Hz, J_{7,8} = 8.5 Hz, H-7), 3.83 (dd, 1H, J_{8,9a} = 6.9 Hz, J_{9a,9b} = 8.6 Hz, H-9a), 3.94 (dt, 1H, J_{7,8} + J_{8,9a} + J_{8,9b} = 21.5 Hz, H-8), 4.11 (dd, 1H, J_{8,9b} = 6.2 Hz, J_{9a,9b} = 8.6 Hz, H-9b), 4.35 (q, 2H, J = 7.2 Hz, OCH₂CH₃), 4.64 (dd, 1H, J_{5,6} = 8.2 Hz, J_{6,7} = 6.2 Hz, H-6), 5.07 (ddd, 1H, ⁴J_{3,5} = 2.1 Hz, J_{4,5} = 4.3 Hz, J_{5,6} = 8.2 Hz, H-5), 6.74 (dd, 1H, J_{3,4} = 16.3 Hz, ⁴J_{3,5} = 2.0 Hz, H-3), 7.47 (dd, 1H, J_{3,4} = 16.3 Hz, J_{4,5} = 4.3 Hz, H-4).

Ethyl (*E*)-5-acetamido-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-2-nitromethyl-*D*-glycero-*D*-galacto-non-3-enonate and ethyl (*E*)-5-acetamido-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-2-nitromethyl-*D*-glycero-*D*-talono-3-enonate (3.7**)**

Enone **3.1** (84.9 mg, 0.212 mmol) was dissolved in nitromethane (2.0 mL) that had been passed through alumina. Triethylamine (6.0 μL, 0.043 mmol) was injected and the dark yellow solution was stirred overnight under N₂ at room temperature. After 24 h, the solution was concentrated under reduced pressure

to give a light brown foamy syrup that was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:1 v/v to 100% EtOAc). This afforded a 1:1 diastereomeric mix (**a/b**) of the 1,2-addition product **3.7** as a colourless syrup (53.5 mg, 0.116 mmol, 55%). ^1H NMR (CDCl_3 , 500 MHz) δ : 1.31-1.43 (m, 24 H, $\text{C}(\text{CH}_3)_2\text{-a} \times 2$, $\text{C}(\text{CH}_3)_2\text{-b} \times 2$), 1.98 (s, 3H, $\text{COCH}_3\text{-a}$), 1.99 (s, 3H, $\text{COCH}_3\text{-b}$), 3.53 (t, 1H, $J_{6,7} + J_{7,8} = 16.2$ Hz, H-7-a), 3.64 (t, $J_{6,7} + J_{7,8} = 15.8$ Hz, H-7-b), 3.84-3.90 (m, 2H, H-9a-a, H-9b-b), 3.95-4.02 (m, 4H, H-6-a, H-6-b, H-8-a, H-8-b), 4.11-4.16 (m, 2H, H-9b-a, H-9b-b), 4.26-4.41 (m, 4H, $\text{OCH}_2\text{CH}_3\text{-a}$, $\text{OCH}_2\text{CH}_3\text{-b}$), 4.48 (d, 1H, $J = 13.9$ Hz, $\text{CH}_a\text{CH}_b\text{NO}_2\text{-a}$), 4.49 (d, 1H, $J = 13.8$ Hz, $\text{CH}_a\text{CH}_b\text{NO}_2\text{-b}$), 4.66-4.71 (m, 2H, H-5-a, H-5-b), 4.87 (d, 1H, $J = 13.8$ Hz, $\text{CH}_a\text{CH}_b\text{NO}_2\text{-b}$), 4.89 (d, 1H, $J = 13.8$ Hz, $\text{CH}_a\text{CH}_b\text{NO}_2\text{-a}$), 5.71 (d, 1H, $J_{3,4} = 15.4$ Hz, H-3-b), 5.76 (d, 1H, $J_{3,4} = 15.3$ Hz, H-3-a), 5.96 (d, 1H, $J_{5,\text{NH}} = 8.4$ Hz, NH-b), 6.00 (d, 1H, $J_{5,\text{NH}} = 8.3$ Hz, NH-a), 6.15-6.21 (m, 2H, H-4-a, H-4-b). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 13.9 ($\text{OCH}_2\text{CH}_3\text{-a}$, $\text{OCH}_2\text{CH}_3\text{-b}$), 23.37, 23.40, 25.0, 25.1, 26.58, 26.69, 26.77, 26.84, 27.0, 27.1 ($\text{C}(\text{CH}_3)_2\text{-a} \times 2$, $\text{C}(\text{CH}_3)_2\text{-b} \times 2$, $\text{COCH}_3\text{-a}$, $\text{COCH}_3\text{-b}$), 51.82 (C-5-b), 51.85 (C-5-a), 63.5 ($\text{OCH}_2\text{CH}_3\text{-a}$, $\text{OCH}_2\text{CH}_3\text{-b}$), 67.8 (C-9-a, C-9-b), 75.06 (C-2-a), 75.14 (C-2-b), 76.9 (C-8-a, C-8-b), 78.6 (C-6-a), 78.9 (C-6-b), 79.79 ($\text{CH}_2\text{NO}_2\text{-b}$), 79.82 ($\text{CH}_2\text{NO}_2\text{-a}$), 81.74 (C-7-a), 81.78 (C-7-b), 109.78 ($\text{C}(\text{CH}_3)_2\text{-a}$), 109.83 ($\text{C}(\text{CH}_3)_2\text{-b}$), 110.0 ($\text{C}(\text{CH}_3)_2\text{-a}$), 110.1 ($\text{C}(\text{CH}_3)_2\text{-b}$), 128.6 (C-3/4-b), 129.1 (C-3/4-a), 130.4 (C-3/4-a), 130.7 (C-3/4-b), 169.0, 169.1, 171.29, 171.3 (C=O-a $\times 2$, C=O-b $\times 2$).

Ethyl 5-acetamido-3,4,5-trideoxy-4-ethyl-6,7:8,9-di-O-isopropylidene-D-glycero-D-talo-non-3-enonate (3.8)

Copper(II) trifluoromethanesulfonate (10 mg, 0.028 mmol) was suspended in dry, degassed toluene (7 mL) and triethylphosphite (10 μ L, 0.058 mmol) was added dropwise. The resulting colourless solution was stirred under N₂ at room temperature for 15 min, after which it was cooled to -20 °C and a solution of diethylzinc in hexanes (1.0 M, 0.90 mL, 0.90 mmol) was added dropwise. After stirring under N₂ for 10 min, a solution of enone **3.1** (236 mg, 0.59 mmol) in toluene (3 mL) was injected dropwise. The solution was stirred under N₂ overnight, warming to room temperature. After 18 h, the reaction was quenched by the addition of saturated aqueous NH₄Cl (5 mL). The mixture was diluted with water (15 mL) and extracted with Et₂O (2 x 50 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated under reduced pressure to give a mixture of compounds that contained mainly the enol tautomer of the ethyl addition product **3.8** as colourless syrup (217 mg). The enol addition product could not be purified for characterization as it rearranged to the 5-membered ring hemiaminal **3.9** upon chromatography. ¹H NMR (CDCl₃, 500 MHz) δ : 0.86 (t, 3H, $J = 7.3$ Hz, CH₂CH₃), 1.29-1.41 (m, 16H, CMe₂ x 2, OCH₂CH₃, CH_aH_bCH₃), 1.51-1.58 (m, 1H, CH_aCH_bCH₃), 1.95 (s, 3H, COCH₃), 2.82-2.88 (m, 1H, H-4), 3.78-3.83 (m, 3H, H-6, H-7, H-9a), 3.89-3.95 (m, 1H, H-8), 4.06-4.09 (m, 1H, H-9b), 4.23-4.28 (m, 3H, OCH₂CH₃, H-5), 5.50 (d, 1H, $J_{3,4} = 10.2$ Hz, H-3), 5.73 (d, 1H, $J_{5,NH} = 9.4$ Hz, NH), 6.54 (s, 1H, OH). ¹³C NMR (CDCl₃, 125 MHz) δ : 12.0 (CH₂CH₃), 14.1 (OCH₂CH₃), 23.4 (COCH₃), 24.6 (CH₂CH₃), 25.3, 26.5, 27.2, 27.5 (C(CH₃)₂ x 2), 39.1 (C-4), 54.1 (OCH₂CH₃/C-5), 61.8 (OCH₂CH₃/C-5), 67.8 (C-9),

77.0 (C-8), 80.5 (C-6/C-7), 80.7 (C-6/C-7), 109.6, 110.3 (C(CH₃)₂ x 2), 113.1 (C-3), 142.3 (C-2), 164.4, 170.1 (C=O x 2).

Ethyl *N*-acetyl-5-amino-3,4,5-trideoxy-4-ethyl-6,7:8,9-di-*O*-isopropylidene-*D*-glycero-*D*-talo-non-2-ulofuranosonate (3.9)

The crude enol **3.8** was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 2:1 to 1:2 v/v) to afford the hemiaminal **3.9** as a colourless syrup (134 mg, 0.31 mmol, 53%). IR (cm⁻¹): 1648 (amide C=O), 1747 (ester C=O), 3490 (OH). ¹H NMR (CDCl₃, 500 MHz) δ: 0.97 (t, 3H, *J* = 7.4 Hz, CH₂CH₃), 1.25 (t, 3H, *J* = 7.1 Hz OCH₂CH₃), 1.34, 1.35, 1.40, 1.41 (s, 12H, C(CH₃)₂ x 2), 1.65-1.71 (m, 2H, CH₂CH₃), 1.89 (d, 1H, *J*_{3a,3b} = 13.2 Hz, H-3a), 2.14-2.19 (m, 4H, COCH₃, H-4), 2.62 (ddd, 1H, *J*_{3a,3b} = 13.2 Hz, *J*_{3b,4} = 7.6 Hz, *J*_{3b,OH} = 1.6 Hz, H-3b), 3.47 (t, 1H, *J*_{6,7} = *J*_{7,8} = 8.5 Hz, H-7), 3.93 (dd, 1H, *J*_{8,9a} = 5.8 Hz, *J*_{9a,9b} = 8.7 Hz, H-9a), 4.04 (dt, 1H, *J*_{7,8} + *J*_{8,9a} + *J*_{8,9b} = 20.8 Hz, H-8), 4.12 (s, 1H, H-5), 4.14-4.26 (m, 4H, H-9b, H-6, OCH₂CH₃), 4.87 (d, 1H, *J*_{3b,OH} = 1.5 Hz, OH). ¹³C NMR (CDCl₃, 100 MHz) δ: 12.6 (CH₂CH₃), 14.0 (OCH₂CH₃), 22.5 (COCH₃), 25.1, 26.6, 26.8, 27.0 (C(CH₃)₂ x 2), 27.3 (CH₂CH₃), 40.5 (C-4), 41.1 (C-3), 61.7 (OCH₂CH₃), 65.3 (C-5), 68.5 (C-9), 77.5 (C-8), 78.9 (C-7), 83.4 (C-6), 90.6 (C-2), 109.8, 110.2 (C(CH₃)₂ x 2), 171.4, 171.8 (C=O x 2).

Ethyl *N*-acetyl-5-amino-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-4-methyl-*D*-glycero-*D*-talo-non-2-ulofuranosonate (3.11)

Triethylphosphite (43 μL, 0.25 mmol) was added dropwise to a suspension of copper(II) trifluoromethanesulfonate (47 mg, 0.13 mmol) in dry, degassed toluene (10 mL). The resulting colourless solution was stirred for 5 min at room temperature under N₂. The solution was then cooled to -20 °C and a solution of

dimethylzinc in toluene (2.0 M, 1.9 mL, 3.8 mmol) was added dropwise. The resulting bright yellow solution was stirred for 5 min under N₂, after which time a solution of enone **3.1** (1.02 g, 2.56 mmol) in dry, degassed toluene (15 mL) was added dropwise over 5 min. The dark yellow reaction was allowed to warm to room temperature as it stirred overnight under N₂. After 17 h the reaction was quenched by the addition of saturated aqueous NH₄Cl (10 mL), it then was diluted with water (50 mL) and extracted with Et₂O (2 x 75 mL). The organic layer was dried (MgSO₄), filtered, and concentrated under reduced pressure to give a green syrup (974 mg). The crude material was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:1 v/v to 100% EtOAc) to afford the conjugate addition product as a pair of 5-membered ring hemiaminal anomers **3.11** (1.4:1.0 anomeric ratio, 373 mg, 0.898 mmol, 35%), the major anomer of which was purified to analytical standards for complete characterization. Major anomer [α]_D²⁰ +16.5 (c 1.95, CHCl₃). IR (cm⁻¹): 1648 (amide C=O), 1746 (ester C=O), 3482 (OH). ¹H NMR (CDCl₃, 500 MHz) δ : 1.25 (t, 3H, *J* = 7.1 Hz, CH₃CH₂O), 1.32 (d, 3H, *J*_{CH₃,4} = 7.3 Hz, 4-CH₃), 1.33, 1.34, 1.39, 1.42 (s, 12H, C(CH₃)₂ x 2), 1.78 (d, 1H, *J*_{3a,3b} = 13.1 Hz, H-3a), 2.16 (s, 3H, COCH₃), 2.44-2.53 (m, 1H, H-4), 2.66 (dd, 1H, *J*_{3a,3b} = 13.0 Hz, *J*_{3b,4} = 7.6 Hz, H-3b), 3.49 (t, 1H, *J*_{6,7} + *J*_{7,8} = 17.2 Hz, H-7), 3.99 (dd, 1H, *J*_{8,9a} = 4.9 Hz, *J*_{9a,9b} = 8.7 Hz, H-9a), 4.03 (s, 1H, H-5), 4.03-4.07 (m, 1H, H-8), 4.12-4.28 (m, 4H, H-6, H-9b, CH₃CH₂O), 4.91 (s, 1H, OH). ¹³C NMR (CDCl₃, 125 MHz) δ : 14.0 (CH₃CH₂O), 21.3 (4-CH₃), 22.6 (COCH₃), 25.1, 26.8 (x 2), 27.0 (C(CH₃)₂ x 2), 33.0 (C-4), 43.0 (C-3), 61.8 (CH₃CH₂O), 67.6 (C-5), 68.2 (C-9), 77.5 (C-8), 78.8

(C-7), 83.0 (C-6), 90.8 (C-2), 109.8, 110.1 (C(CH₃)₂ x 2), 171.4, 171.7 (C=O x 2).
Anal. calcd. for C₂₀H₃₃NO₈: C 57.82, H 8.01, N 3.37; found: C 57.52, H 7.90, N 3.59.

Minor anomer: ¹H NMR (CDCl₃, 500 MHz) δ: 1.23 (d, 3H, *J*_{CH₃,4} = 7.5 Hz, 4-CH₃), 1.30 (t, 3H, *J* = 7.1 Hz, CH₃CH₂O), 1.34, 1.37, 1.42, 1.44 (s, 12H, C(CH₃)₂ x 2), 1.97 (d, 1H, *J*_{3a,3b} = 12.5 Hz, H-3a), 2.14 (s, 3H, COCH₃), 2.50-2.60 (m, 2H, H-3b, H-4), 3.57 (t, 1H, *J*_{6,7} + *J*_{7,8} = 17.0 Hz, H-7), 3.95 (s, 1H, H-5), 3.98 (dd, 1H, *J*_{8,9a} = 4.9 Hz, *J*_{9a,9b} = 8.8 Hz, H-9a), 4.04-4.08 (m, 1H, H-8), 4.17-4.30 (m, 4H, H-6, H-9b, CH₃CH₂O), 4.47 (s, 1H, OH). ¹³C NMR (CDCl₃, 125 MHz) δ: 14.0 (CH₃CH₂O), 21.4 (4-CH₃), 22.1 (COCH₃), 25.0, 26.8, 26.90, 26.93 (C(CH₃)₂ x 2), 31.9 (C-4), 45.0 (C-3), 62.0 (CH₃CH₂O), 67.6 (C-5), 68.1 (C-9), 77.6 (C-8), 78.8 (C-7), 83.1 (C-6), 90.0 (C-2), 109.8, 110.6 (C(CH₃)₂ x 2), 170.5 (COCH₃), 171.8 (C-1).

Ethyl 5-acetamido-2,7-anhydro-3,4,5-trideoxy-4-ethyl-D-glycero-D-talo-non-2-ulopyranosonate (3.12)

4-Ethyl hemiaminal **3.9** (239 mg, 0.557 mmol) was dissolved in trifluoroacetic acid (4.0 mL) to which water (0.4 mL) was added. The resulting solution was stirred at room temperature for 15 h and was then concentrated under reduced pressure to give a dark brown syrup (249 mg) that was used directly in the next reaction. A sample for characterization could be purified by flash chromatography (EtOAc-MeOH-H₂O gradient solvent system from 20:3:1 to 10:3:1 v/v/v), giving the 2,7-anhydro-4-ethylsialoside **3.12** as a light brown foamy syrup. IR (cm⁻¹): 1645 (amide C=O), 1746 (ester C=O), 3328 (OH). ¹H NMR

(D₂O, 600 MHz) δ : 0.82 (t, 3H, $J = 7.4$ Hz, CH₂CH₃), 1.24-1.33 (m, 5 H, CH₂CH₃, OCH₂CH₃), 1.65 (t, 1H, $J_{3a,3b} = J_{3a,4} = 13.1$ Hz, H-3a), 2.02-2.06 (m, 4H, H-3b, COCH₃), 2.12-2.17 (m, 1H, H-4), 3.52-3.58 (m, 2H, H-8, H-9a), 3.71 (br d, 1H, $J_{9a,9b} = 11.5$ Hz, H-9b), 4.05 (dd, 1H, $J_{4,5} = 2.0$ Hz, $J_{5,6} = 4.7$ Hz, H-5), 4.21 (d, 1H, $J_{7,8} = 7.4$ Hz, H-7), 4.29 (q, 2H, $J = 7.1$ Hz, OCH₂CH₃), 4.59 (d, 1H, $J_{5,6} = 1.9$ Hz, H-6). ¹³C NMR (D₂O, 150 MHz) δ : 10.2 (CH₂CH₃), 13.1 (OCH₂CH₃), 21.7 (COCH₃), 23.5 (CH₂CH₃), 32.3 (C-4), 34.3 (C-3), 47.9 (C-5), 61.9 (C-9), 63.5 (OCH₂CH₃), 71.3 (C-8), 77.9 (C-7), 79.9 (C-6), 105.0 (C-2), 168.2 (C-1), 173.9 (COCH₃). HRMS (ESI) for C₁₅H₂₅NO₇: (MNa⁺) calcd: 354.1528, found: 354.1532.

Ethyl 5-acetamido-2,7-anhydro-3,4,5-trideoxy-4-methyl-D-glycero-D-talono-2-ulopyranosonate (3.13)

4-Methyl hemiaminal **3.11** (228 mg, 0.548 mmol) was dissolved in trifluoroacetic acid (4.0 mL) and water (0.4 mL) was added. The clear brown solution stirred overnight at room temperature, after which time it was concentrated under reduced pressure to yield a light brown foamy syrup (263 mg) that was of adequate purity to be used directly in the next reaction. An analytical sample was purified via flash chromatography (20:3:1 EtOAc-methanol-water v/v/v) to afford the product as a tan solid, mp 117-120 °C (dec). $[\alpha]_D^{20} +48$ (c 0.13, H₂O). IR (cm⁻¹): 1647 (amide C=O), 1741 (ester C=O), 3333 (OH). ¹H NMR (D₂O, 500 MHz) δ : 0.87 (d, 3H, $J_{CH_3,4} = 6.8$ Hz, 4-CH₃), 1.28 (t, 3H, $J = 7.1$ Hz, CH₃CH₂O), 1.65 (dd, 1H, $J_{3a,3b} = 14.0$ Hz, $J_{3a,4} = 12.3$ Hz, H-3a), 1.97 (dd, 1H, $J_{3a,3b} = 14.1$ Hz, $J_{3b,4} = 5.6$ Hz, H-3b), 2.06 (s, 3H, COCH₃), 2.34-2.43 (m, 1H, H-4), 3.52-3.59 (m, 2H, H-8, H-9a), 3.71 (dd, 1H, $J_{8,9b} = 2.4$ Hz, $J_{9a,9b} = 11.5$ Hz, H-9b), 3.99 (dd,

^1H , $J_{4,5} = 4.7$ Hz, $J_{5,6} = 2.0$ Hz, H-5), 4.22 (d, 1H, $J_{7,8} = 7.4$ Hz, H-7), 4.29 (q, 2H, $J = 7.1$ Hz, $\text{CH}_3\text{CH}_2\text{O}$), 4.60 (d, 1H, $J_{5,6} = 1.8$ Hz, H-6). ^{13}C NMR (D_2O , 125 MHz) δ : 13.2 ($\text{CH}_3\text{CH}_2\text{O}$), 15.5 (4- CH_3), 21.9 (COCH_3), 26.0 (C-4), 36.0 (C-3), 49.7 (C-5), 62.1 (C-9), 63.7 ($\text{CH}_3\text{CH}_2\text{O}$), 71.5 (C-8), 78.2 (C-7), 80.0 (C-6), 105.1 (C-2), 168.4 (C-1), 174.2 (COCH_3). HRMS (ESI) for $\text{C}_{14}\text{H}_{23}\text{NO}_7$: (MH^+) calcd: 318.1552, found: 318.1564.

Ethyl 5-acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-3,4,5-trideoxy-4-ethyl-D-glycero-D-talo-non-2-enonate (3.14)

The crude preparation of 2,7-anhydro-4-ethylsialoside **3.12** from the previous reaction (249 mg) was dissolved in acetic anhydride (2.0 mL) and glacial acetic acid (2.0 mL). Concentrated H_2SO_4 (15 drops) was added and the resulting solution was stirred at room temperature. After 3 d, the reaction was poured into saturated aqueous NaHCO_3 (100 mL) which bubbled vigorously and the resulting basic mixture was stirred for 2 h. The mixture was then extracted with EtOAc (2 x 100 mL) and the combined organic layers were dried (MgSO_4), filtered, and concentrated under reduced pressure to give the 4-ethyl-substituted glycal **3.14** as a light brown syrup (119 mg, 0.261 mmol, 47%). The glycal could be further purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford a sample for characterization. $[\alpha]_D^{20} +3$ (c 0.24, CHCl_3). IR (cm^{-1}): 1656 (amide C=O), 1747 (ester C=O). ^1H NMR (CDCl_3 , 600 MHz) δ : 1.04 (t, 3H, $J = 7.4$ Hz, CH_2CH_3), 1.31 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 1.34-1.39 (m, 1H, $\text{CH}_a\text{H}_b\text{CH}_3$), 1.48-1.56 (m, 1H, $\text{CH}_a\text{H}_b\text{CH}_3$), 1.97 (s, 3H, COCH_3), 2.06 (s, 3H, COCH_3), 2.07 (s, 3H, COCH_3), 2.11 (s, 3H, COCH_3), 2.56-

2.60 (m, 1H, H-4), 4.20-4.31 (m, 4H, H-6, H-9a, OCH₂CH₃), 4.40 (dd, 1H, $J_{8,9b} = 4.4$ Hz, $J_{9a,9b} = 12.0$ Hz, H-9b), 4.55-4.58 (m, 1H, H-5), 5.19 (ddd, 1H, $J_{7,8} + J_{8,9b} = 11.4$ Hz, $J_{8,9a} = 3.2$ Hz, H-8), 5.40 (dd, 1H, $J_{6,7} = 8.5$ Hz, $J_{7,8} = 3.1$ Hz, H-7), 5.54 (d, 1H, $J_{5,NH} = 9.4$ Hz, NH), 6.03 (br d, 1H, $J_{3,4} = 2.6$ Hz, H-3). ¹³C NMR (CDCl₃, 150 MHz) δ: 11.4 (CH₂CH₃), 14.1 (OCH₂CH₃), 20.66 (COCH₃), 20.72 (COCH₃), 20.9 (COCH₃), 23.2 (CH₂CH₃), 23.3 (COCH₃), 33.9 (C-4), 43.7 (C-5), 61.1 (C-9), 61.5 (OCH₂CH₃), 68.7 (C-7), 69.3 (C-8), 76.7 (C-6), 112.8 (C-3), 141.1 (C-2), 161.8, 169.7, 170.0, 170.3, 170.6 (C=O x 5). HRMS (ESI) for C₂₁H₃₁NO₁₀: (MH⁺) calcd: 458.2026, found: 458.2020.

Ethyl 5-acetamido-6,7,8-tri-O-acetyl-2,6-anhydro-3,4,5-trideoxy-4-methyl-D-glycero-D-falo-non-2-enonate (3.15)

The crude 2,7-anhydrosugar **3.11** from the previous reaction (263 mg) was dissolved in acetic anhydride (2.0 mL) and glacial acetic acid (2.0 mL). Concentrated sulfuric acid was added (0.1 mL) and the solution was stirred under N₂ at room temperature for 2 d. The reaction was neutralized by adding saturated aqueous NaHCO₃ (100 mL) and stirring for 1.5 h, after which time the product was extracted with EtOAc (2 x 100 mL) which was then dried (MgSO₄), filtered, and concentrated under reduced pressure to afford the glycal product **3.15** as a light brown syrup (0.144 mg, 0.324 mmol, 59% over 2 steps). This product could be further purified *via* flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) giving the pure glycal as a colourless syrup (58 mg, 0.13 mmol, 24% over 2 steps). $[\alpha]_D^{20} +1.2$ (c 0.995, CHCl₃). IR (cm⁻¹): 1653 (amide C=O), 1683 (trisubstituted C=C), 1748 (ester

C=O), 3291 (NH). ^1H NMR (CDCl_3 , 500 MHz) δ : 1.08 (d, 3H, $J_{\text{CH}_3,4} = 7.1$ Hz, 4- CH_3), 1.30 (t, 3H, $J = 7.1$ Hz, $\text{CH}_3\text{CH}_2\text{O}$), 1.98 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.77-2.83 (m, 1H, H-4), 4.20 (dd, 1H, $J_{5,6} = 5.0$ Hz, $J_{6,7} = 7.1$ Hz, H-6), 4.20-4.27 (m, 2H, $\text{CH}_3\text{CH}_2\text{O}$), 4.28 (dd, 1H, $J_{8,9a} = 7.1$ Hz, $J_{9a,9b} = 12.1$ Hz, H-9a), 4.48 (dd, 1H, $J_{8,9b} = 4.3$ Hz, $J_{9a,9b} = 12.0$ Hz, H-9b), 4.48-4.51 (m, 1H, H-5), 5.21 (br quintet, 1H, $J_{7,8} + J_{8,9a} + J_{8,9b} = 14.5$ Hz, H-8), 5.43 (dd, 1H, $J_{6,7} = 7.1$ Hz, $J_{7,8} = 3.3$ Hz, H-7), 5.55 (d, 1H, $J_{\text{NH},5} = 9.6$ Hz, NHCOCH_3), 5.96 (d, 1H, $J_{3,4} = 3.2$ Hz, H-3). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 14.1 ($\text{CH}_3\text{CH}_2\text{O}$), 15.6 (4- CH_3), 20.61, 20.67, 20.8, 23.2 ($\text{COCH}_3 \times 4$), 27.8 (C-4), 45.1 (C-5), 61.36 ($\text{CH}_3\text{CH}_2\text{O}$), 61.38 (C-9), 68.6 (C-7), 70.0 (C-8), 75.8 (C-6), 114.7 (C-3), 141.3 (C-2), 161.8, 169.7, 170.0, 170.3, 170.5 (C=O $\times 5$). HRMS (ESI) for $\text{C}_{20}\text{H}_{29}\text{NO}_{10}$: (MH^+) calcd: 444.1869, found: 444.1870.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-methyl-D-glycero-D-talo-non-2-enonic acid (3.16)

A 1.2 M solution of sodium ethoxide in ethanol (0.080 mL, 0.096 mmol) was added to a solution of peracetylated 4-methyl-substituted glycal **3.15** (29 mg, 0.065 mmol) in dry ethanol (3.0 mL). This solution was stirred at room temperature for 30 min, after which time TLC analysis indicated the absence of starting material. The reaction was neutralized with Amberlite H^+ resin and filtered. The resin was washed with methanol (30 mL) and the filtrate was concentrated under reduced pressure to give a light yellow syrup (21.1 mg, 0.065 mmol). The de-acetylated product was then dissolved in THF/water (3:2 v/v, 5 mL) and cooled in an ice bath. Lithium hydroxide monohydrate was added (23.7 mg, 0.565 mmol) and the solution stirred in ice for 30 min, after which time TLC

analysis indicated complete de-esterification. The reaction was removed from the cooling bath, diluted with water (5 mL) and neutralized with Amberlite H⁺ resin to pH 5, which was then filtered and washed with water (30 mL). The combined filtrates were concentrated under reduced pressure to afford the deprotected glycal **3.16**, as the lithium salt, as a colourless syrup (16.0 mg, 0.0542 mmol, 82%). $[\alpha]_D^{20}$ -100 (c 0.48, H₂O). IR (cm⁻¹): 1645 (amide C=O), 1712 (acid C=O), 3287 (NH), 3340 (OH). ¹H NMR (D₂O, 500 MHz) δ : 1.04 (d, 3H, $J_{\text{CH}_3,4}$ = 7.2 Hz, 4-CH₃), 2.02 (s, 3H, NHCOCH₃), 2.70-2.76 (m, 1H, H-4), 3.64 (dd, 1H, $J_{8,9a}$ = 6.3 Hz, $J_{9a,9b}$ = 11.9 Hz, H-9a), 3.67 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 8.8 Hz, H-7), 3.86 (dd, 1H, $J_{8,9b}$ = 2.8 Hz, $J_{9a,9b}$ = 11.9 Hz, H-9b), 3.90 (ddd, 1H, $J_{7,8}$ = 8.9 Hz, $J_{8,9a}$ = 6.3 Hz, $J_{8,9b}$ = 2.8 Hz, H-8), 4.20 (dd, 1H, $J_{5,6}$ = 8.8 Hz, $J_{6,7}$ = 2.2 Hz, H-6), 4.30 (dd, 1H, $J_{4,5}$ = 5.8 Hz, $J_{5,6}$ = 8.8 Hz, H-5), 6.14 (d, 1H, $J_{3,4}$ = 5.0 Hz, H-3). ¹³C NMR (D₂O, 500 MHz) δ : 15.1 (4-CH₃), 21.9 (NHCOCH₃), 28.9 (C-4), 46.4 (C-5), 63.0 (C-9), 69.0 (C-7), 70.5 (C-8), 72.6 (C-6), 116.7 (C-3), 141.8 (C-2), 166.6, 174.1 (C=O x 2). HRMS (ESI) for C₁₂H₁₈NO₇: (M⁻) calcd: 288.1083, found: 288.1091.

3-Benzylcyclohexanone

Zinc dust (98 mg, 1.5 mmol) was suspended in dry, degassed toluene (1.0 mL). 1,2-Dibromoethane (6.4 μ L, 0.075 mmol) was injected and the mixture was heated to reflux for 1 min under N₂. After cooling to room temperature, a solution of benzyl bromide (0.18 mL, 1.5 mmol) in toluene (2.0 mL) was injected dropwise over 5 min and the mixture was stirred at room temperature under N₂. After 6 h, the suspension was cooled to -20 °C and Cu(OTf)₂ (18 mg, 0.050 mmol) and

P(OEt)₃ (17 μL, 0.096 mmol) were added. The mixture was stirred for 15 min, after which time a solution of 2-cyclohexen-1-one (100 μL, 1.03 mmol) in toluene (2.0 mL) was injected dropwise over 2 min. The mixture was stirred under N₂ overnight as it warmed from –20 °C to room temperature. After 15 h, the reaction was poured into a mix of saturated aqueous NH₄Cl (50 mL) and Et₂O (50 mL). The mixture was extracted and the aqueous layer was washed with Et₂O (2 x 50 mL). The combined organic layers were washed with water (100 mL) and saturated aqueous NaCl (100 mL) and were dried (MgSO₄), filtered, and concentrated under reduced pressure to give a yellow oil (149 mg). The crude product mixture was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 10:1 to 5:1 v/v) to afford 3-benzylcyclohexanone as a colourless oil (49.9 mg, 0.265 mmol, 26%). The spectral data for this compound matched those reported in the literature.²³⁶

3-Benzyl-2-methylcyclohexanone

A toluene solution of Me₂Zn (2.0 M, 0.75 mL, 1.5 mmol) was injected into a flask containing dry, degassed toluene (1.0 mL) and was cooled to –30 °C under N₂. Benzyl bromide (0.36 mL, 3.0 mmol) was injected and the resulting clear, colourless solution was stirred under N₂ at –30 °C for 45 min, after which time Cu(OTf)₂ (18 mg, 0.050 mmol) and P(OEt)₃ (17 μL, 0.096 mmol) were added. The mixture was stirred for 15 min, then a solution of 2-cyclohexen-1-one (100 μL, 1.03 mmol) in toluene (1.0 mL) was injected dropwise over 1 min. The reaction was then allowed to stir under N₂ overnight as it warmed from –30 °C to room temperature. After 17 h, the reaction was quenched by the addition of

saturated aqueous NH_4Cl (5 mL), then was diluted with water (50 mL) and extracted with Et_2O (2 x 75 mL). The combined organic layers were dried (MgSO_4), filtered, and concentrated under reduced pressure to give a yellow oil (421 mg). This was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 10:1 to 5:1 v/v) to afford 3-benzyl-2-methylcyclohexanone as a colourless oil (46.2 mg, 0.228 mmol, 22%). The spectral data for this compound matched those reported in the literature.²³⁷

3-Phenylcyclohexanone

ZnCl_2 (110 mg, 0.807 mmol) was fused by heating under vacuum with a propane torch. After cooling to room temperature, this was suspended in dry, degassed toluene (2.0 mL) and cooled to $-30\text{ }^\circ\text{C}$ under N_2 . A THF solution of phenyl magnesium bromide (1.0 M, 1.5 mL, 1.5 mmol) was injected and the resulting mixture was stirred under N_2 at $-30\text{ }^\circ\text{C}$ for 2 h. At this point, $\text{Cu}(\text{OTf})_2$ (8.5 mg, 0.024 mmol) and $\text{P}(\text{OEt})_3$ (9.0 μL , 0.052 mmol) were added, stirred for 15 min, then a solution of 2-cyclohexen-1-one (50 μL , 0.52 mmol) in toluene (1.0 mL) was injected and the mixture was stirred under N_2 overnight while warming from $-30\text{ }^\circ\text{C}$ to room temperature. After 17 h, the reaction was quenched by the addition of saturated aqueous NH_4Cl (5 mL). The reaction was then diluted with water (50 mL) and extracted with Et_2O (2 x 50 mL). The combined organic layers were dried (MgSO_4), filtered, and concentrated under reduced pressure to give a yellow oil (100 mg). The crude product was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 5:1 to 3:1 v/v) to afford 3-

phenylcyclohexanone as a colourless oil (60.8 mg, 0.349 mmol, 68%) The spectral data for this compound matched those reported in the literature.²³⁸

CHAPTER 4: OXABICYCLO[3.1.0]HEXANE-BASED SIALIC ACID ANALOGUES

4.1 Introduction

The development of glycosidase inhibitors that contain a cyclopropane moiety has been a subject of interest in the Bennet research group for a number of years. The bicyclo[4.1.0]heptane framework represented a novel structure for glycosidase inhibitors. In 2001, Tanaka et al. published the synthesis of a bicyclo[4.1.0]heptane-based compound having the *gluco*-configuration (Figure 4.1, compound **4.1**) that is a potent yeast α -glucosidase inhibitor, having a K_i value of 107 nM.²³⁹ Recently, Wang and Bennet reported the synthesis of a similar compound having the *galacto*-configuration (Figure 4.1, compound **4.2**) that was also a potent inhibitor of the corresponding *galacto*-sugar-processing enzyme, inhibiting the coffee bean α -galactosidase with a K_i of 541 nM.²⁴⁰

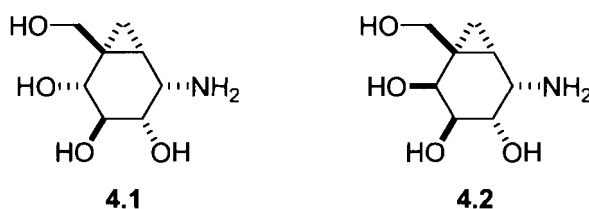


Figure 4.1 Bicyclo[4.1.0]heptane-based glycosidase inhibitors

These inhibitors contain two important features that have been proposed as the basis for their potency.^{239,240} The first is the incorporation of a basic nitrogen atom that will be protonated at physiological pH and is proposed to have

favourable interactions with negatively-charged residues in the enzyme's active site in an attempt to mimic the positively-charged nature of the glycosyl oxacarbenium ion-like species present at the transition state during enzyme-catalyzed hydrolysis of glycosidic linkages. The second common structural feature is the cyclopropane ring. The cyclopropane ring distorts the conformation of the cyclohexane ring to which it is fused, forcing it into a non-chair conformation that also mimics the ring distortion of the glycosyl oxacarbenium ion-like species present at the transition state during enzyme-catalyzed hydrolysis. It is also possible that the methylene group of the cyclopropane ring has favourable interactions with hydrophobic residues in the enzyme active site, although no X-ray crystal structures of these compounds bound to their respective enzymes have been obtained to substantiate such a possibility.

Many glycosidase inhibitors with 6-membered ring-based structures contain a feature that distorts the ring into a non-chair conformation, including sialidase inhibitors. Both Relenza and Tamiflu contain a double bond in the ring to induce a non-chair conformation and BCX-1812 and A-315675 have a 5-membered ring carbon frameworks, showing the enhancement in inhibitory potency afforded by non-chair conformations. It would therefore be prudent to synthesize a sialidase inhibitor based on a bicyclo[4.1.0]heptane skeleton or a similar moiety containing a fused cyclopropane ring and such efforts are currently underway in the Bennet group.

The work described in this chapter involves the isolation of a product resulting from an intramolecular substitution reaction. The formation of this

product was predicted as an intriguing possibility but it was not expected to occur as easily as it did. This product has an oxabicyclo[3.1.0]hexane ring system and although the placement of the substituents around the rings may not be ideal for maximizing contacts in the sialidase active site, it still represents the first potential sialidase inhibitor in this structural class of fused cyclopropane-containing molecules. Therefore, synthesis of it and its derivatives was deemed a worthwhile pursuit. The work would involve removing the acetyl and ester protecting groups and modification to the nitro group.

4.2 Isolation of the Oxabicyclo[3.1.0]hexane Analogue

The serendipitous discovery of the oxabicyclo[3.1.0]hexane-based compound **4.3** occurred during an attempt to synthesize glycal **4.4** by elimination of the β -sialosyl chloride **2.18** under basic conditions (Figure 4.2).¹⁰⁹ When glycosylating sialosides that do not contain a 4-nitro group under basic conditions, a competing reaction that ubiquitously occurs is the elimination of HX across carbons 2 and 3. This forms the glycal due to the acidity of the proton on carbon 3 and activated donor sugars can be eliminated to form glycals intentionally by treatment with a strong base. In the case of the 4-deoxy-4-nitrosialoside **2.18**, the most acidic proton in the molecule is on carbon 4 next to the nitro group. As a result, the glycal was not formed under basic conditions but instead an intramolecular substitution reaction occurred in which the nitronate at the 4-position attacked carbon 2, displacing the anomeric chloride to form the bicyclic system **4.3** (Figure 4.2).

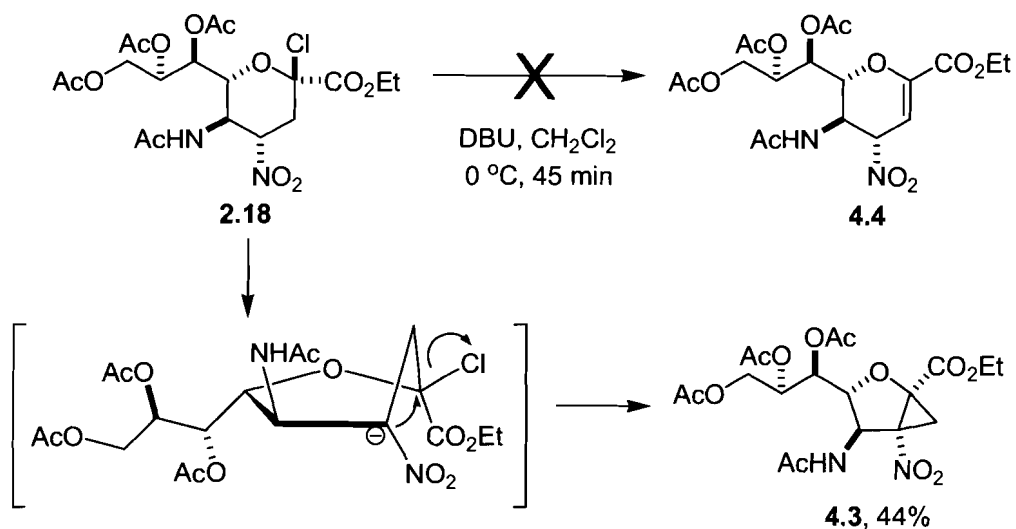


Figure 4.2 Synthesis of the oxabicyclo[3.1.0]hexane-based compound **4.3**

This intramolecular substitution reaction occurred readily in the presence of any base strong enough to deprotonate carbon 4; generally DBU was used as the base to synthesize **4.3**. Thus, as described in Chapter 2.3.2, glycosylation attempts under basic conditions using **2.18** resulted mainly in the isolation of **4.3** rather than of any glycosylated product or glycol **4.4**. Reaction of the β -sialosyl chloride precursor **2.18** with DBU was a clean process when being performed to synthesize **4.3** intentionally, going from spot to spot as monitored by TLC. However, isolated yields of **4.3** were always less than 50% and the fate of the remaining material is not known.

The structure of **4.3** was supported through a variety of spectroscopic experiments. In the ^1H NMR spectrum, the signals for the protons on carbon 3 (sialic acid numbering) appeared as two doublets rather than as two doublets of doublets, indicating the absence of a proton on carbon 4 whose characteristic signal near 5.5 ppm was absent. A small coupling ($^4J_{3\text{anti},5} = 1.5$ Hz) was

observed between proton 5 and proton 3_{anti}, presumably a result of W-coupling with the bonds between these atoms being in the same plane. The IR spectrum showed the nitro group was intact due to the presence of nitro stretching bands at 1373 and 1552 cm⁻¹. In the ¹³C NMR spectrum, carbon 4 had shifted only slightly upfield from 81.7 to 77.2 ppm, but the frequency of carbon 3 had shifted upfield from 39.6 ppm in **2.18** to 25.5 ppm in **4.3**; cyclopropane methylene carbons often appear at low frequencies. The signal for carbon 2 had also shifted upfield from 95.2 ppm to 72.6 ppm, indicating it was no longer part of an acetal moiety. Several HMBC spectra of **4.3** were acquired using various coupling constants in an attempt to see a correlation between proton 5 and carbon 2, as these spectra show correlations for two- and three-bond proton-carbon coupling. The only possibility for observation of a proton 5/carbon 2 correlation would be through a bond between carbons 2 and 4. However, this correlation was not observed although these spectra were helpful in the assignment of the ¹³C NMR. These results do not mean a carbon 2/carbon 4 bond does not exist; it may be that the proton 5/carbon 2 coupling constant is far outside the range of those used during the HMBC experiments. Acquisition of an INADEQUATE spectrum was attempted to delineate the connectivity of the carbon atoms and to further support the existence of a bond between carbons 2 and 4; however, this was not possible due to the amount of material needed to give sufficient sensitivity for this 2D NMR ¹³C-¹³C coupling experiment. As well, the compound is a syrup, making an X-ray crystal structural determination impossible.

The substitution reaction shown in Figure 4.2 exclusively gave **4.3** as a single diastereomer. The stereochemistry of **4.3** was deduced by examining a series of 1D-NOE difference ^1H NMR spectra (Figure 4.3). When the proton at 2.31 ppm was irradiated, an NOE enhancement of the signals at 4.25 and 6.06 ppm was observed, indicating that H-3_{syn} was near H-6 and the acetamide NH, respectively. The absence of an NOE enhancement of any signal besides that of H3_{syn} upon irradiation at 2.93 ppm indicated that H-3_{anti} was not near H-5, further supporting the stereochemical assignment. This isomer likely resulted from an S_N2-type attack of the nitronate on the anomeric carbon, displacing the chloride from the back side (Figure 4.2). Synthesis of the other diastereomer in which the cyclopropane ring is “down” would therefore not be possible from **2.18** since the nitronate would not be able to attack the anomeric centre from the back side to result in this configuration.

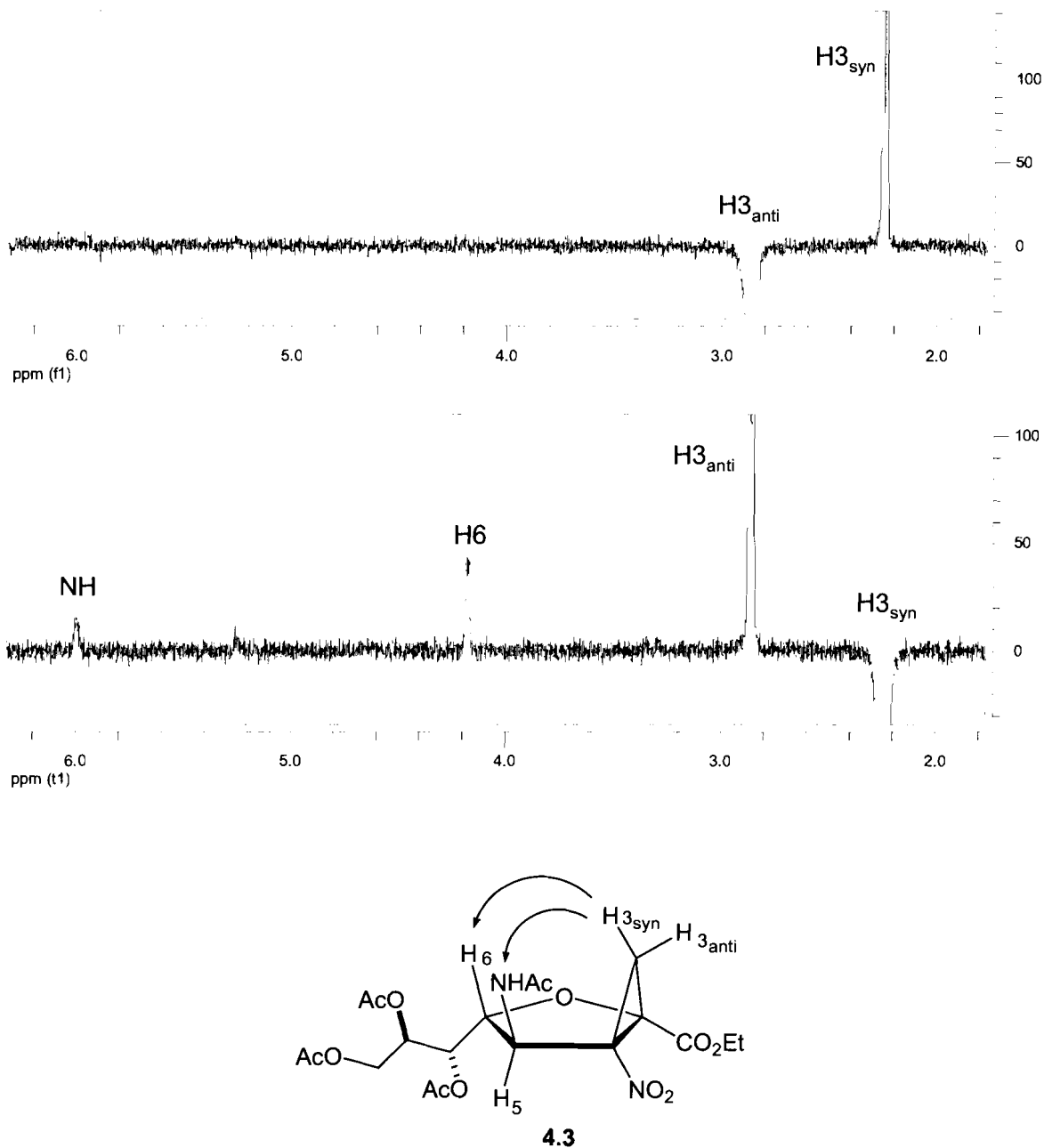
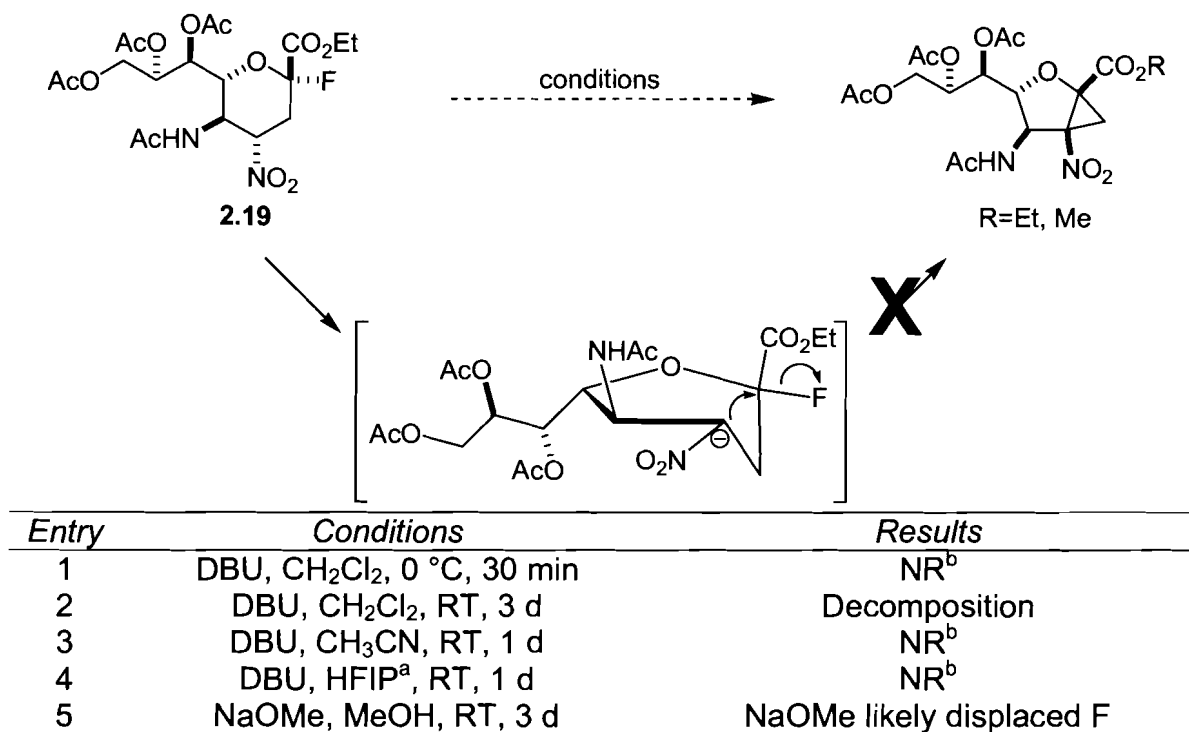


Figure 4.3 1D NOE difference ¹H NMR spectra of 4.3 showing H₃_{syn}-H₆/NH contacts (bottom spectrum) and an absence of any relevant H₃_{anti} contacts (top spectrum)

The α-fluoro donor sugar **2.19** was used to attempt the intramolecular substitution in hopes that the inverted stereochemistry at the anomeric centre would result in generation of the other diastereomer of **4.3** in which the

cyclopropane ring is “down”. This reaction would have to occur via a transition state similar to that depicted in Figure 4.2 for the synthesis of **4.3**, but in the present case the methylene moiety at the 3-position would have to point “down” in order to allow the S_N2-type backside displacement of the α-fluoro leaving group (see the figure above Table 4.1). Synthesis of this isomer was desirable as it would give a wider variety of compounds to test as potential sialidase inhibitors. A variety of solvents was chosen for these attempts that generally employed DBU as a base; however, no bicyclic products were obtained from these reactions that are summarized in Table 4.1. It is unlikely that DBU was incapable of deprotonating the α-sialosyl fluoride **2.19**; thus, it seems that the nitronate was not capable of displacing the anomeric fluoride. Reasons for this may be that the molecule could not adopt the necessary conformation for displacement of the α-fluoro leaving group or that this is a poor leaving group requiring the use of conditions that encourage fluoride departure. When sodium methoxide was used as a base (Table 4.1, entry 5) no starting material was isolated although the ¹H NMR spectrum of the crude reaction mixture showed several doublets of doublets between 2 and 3 ppm, indicating that the anomeric fluoride had been displaced but that no intramolecular substitution product was present in this mixture.

Table 4.1 Elimination attempts on α -sialosyl fluoride 2.19



a) HFIP = 1,1,1,3,3,3-hexafluoroisopropanol

b) Only identifiable compound was unreacted **2.19**

4.3 Oxabicyclo[3.1.0]hexane Deprotection Attempts

Deprotection of the oxabicyclo[3.1.0]hexane-based compound **4.3** is a critical step before being able to measure sialidase inhibition constants of this structure and its derivatives. The deprotection required removal of the acetyl protecting groups as well as the ethyl ester. Normally these are straightforward reactions that proceed without complication, using sodium methoxide to remove the acetyl groups followed by lithium hydroxide to remove the ester (as seen in Chapters 2.4 and 3.4). However, the reactivity of the functional group that allowed the facile synthesis of **4.3** (the nitro group) also prevented its deprotection.

The first deprotection step, removal of the acetyl groups, proceeded relatively smoothly. Initial de-acetylations were performed using sodium ethoxide in ethanol to prevent trans-esterification, making it easier to identify reaction products. When these reactions were performed at room temperature, complete de-acetylation was observed after 20 minutes to give **4.5** while at -20 °C the reaction took slightly longer (70 min). Attack of sodium ethoxide at the bridgehead carbon bearing the carboxylate was a competing reaction, giving larger amounts of **4.6** as the reaction time increased (Figure 4.4). The electron-withdrawing effect of the nitro group increased the electrophilicity of the opposite bridgehead carbon, making it more susceptible to nucleophilic attack. Ethoxide-addition product **4.6** was never isolated in large quantities but was suspected to be the byproduct due to the resemblance of its ¹H NMR spectrum, which contained a second set of ethyl peaks, to that of 4-deoxy-4-nitrosialic acid ethyl ester (**2.10**), corresponding to the ethyl glycoside. Sodium methoxide in methanol was used for all subsequent de-acetylation reactions for practical reasons which gave the trans-esterified product **4.7** cleanly when used at 0 °C for 30 min; the formation of the methoxide-addition product **4.8** was barely observable in these reactions. The de-acetylated product was not stable to silica gel chromatography, always giving ring-opened products as evidenced by the appearance of a multiplet at 5.2 ppm corresponding to a proton on carbon 4 next to the nitro group and by the fact that the signals from the protons on carbon 3 had expanded from doublets to doublets of doublets. Thus, the de-acetylated compound could not be purified.

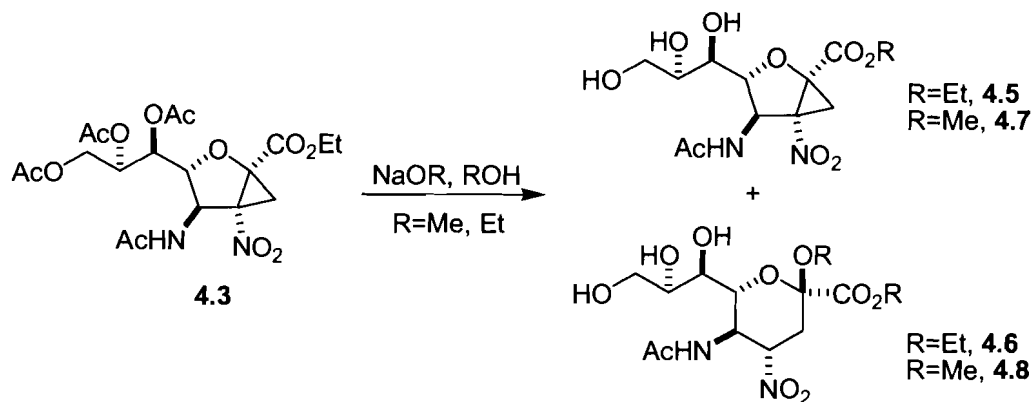
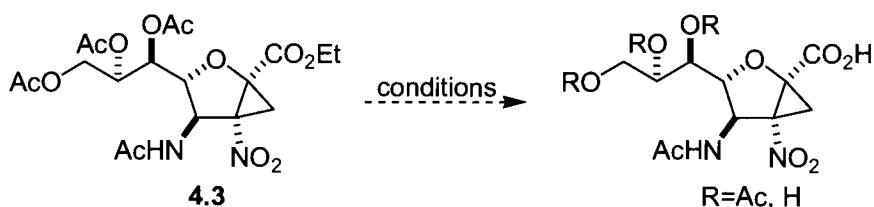


Figure 4.4 De-acetylation of **4.3** gave mainly **4.5/4.7** with increasing amounts of **4.6/4.8** as reaction time increased

After developing protocols for the de-acetylation of **4.3**, hydrolysis of the ester was attempted. These reactions are normally carried out with lithium hydroxide, a protocol that was problematic due to the electrophilicity of the bridgehead carbon atom bearing the carboxylate. Although the ester could be hydrolyzed via this method, the bicyclic system was also destroyed to afford 4-deoxy-4-nitrosialic acid (**2.16**) (Table 4.2, entry 4). A variety of techniques were then tested in order to remove the ester without destroying the bicyclic ring system; these methods are summarized in Table 4.2.

Table 4.2 Attempted de-esterification reactions on 4.3



| <i>Entry</i> | <i>Conditions</i> | <i>Results</i> |
|--------------|--|---|
| 1 | NaOH (aq), MeOH, RT, 30 min | Deprotected, ring opened by OH |
| 2 | NaOH (aq), THF, 0 °C, 1.5 h | Decomposed |
| 3 | i) NaOMe/MeOH ii) H ₂ O, 1 d | Deprotected, ring opened by OMe or OH |
| 4 | i) NaOMe/MeOH ii) 4 eq. LiOH, THF/H ₂ O, 10 min | Deprotected, ring opened by OH |
| 5 | i) NaOMe/MeOH ii) 0.9 eq. LiOH, THF/H ₂ O, 45 min | Ring opened, ester may be intact |
| 6 | i) NaOMe/MeOH ii) 0.9 eq. LiOH, MeOH, 40 min | Ring opened, likely by OMe |
| 7 | CF ₃ COOH, 2d | Decomposed |
| 8 | i) NaOMe/MeOH ii) ^t BuOK, DMSO, 2 h | Several ring-opened deprotected products |
| 9 | i) NaOMe/MeOH ii) ^t BuOK, 18-C-6, THF, 3.5 h | Decomposed |
| 10 | PLE, ^a MeOH/50 mM phosphate buffer, pH 8.1, 2 d | Acetyl groups intact, ester removed |
| 11 | i) NaOMe/MeOH ii) PLE, ^a MeOH/50 mM phosphate buffer, pH 7.0, 1 d | Inconclusive, Me-ester intact |
| 12 | i) NaOEt/EtOH ii) PLE, ^a MeOH/50 mM phosphate buffer, pH 7.0, 2 d | Deprotected, NMR spectra difficult to interpret |

a) PLE = pig liver esterase

Initial de-esterification reactions were attempted using sodium hydroxide on fully-protected **4.3** in order to remove simultaneously the acetyl groups and the ester (Table 4.2, entries 1 and 2).^{241,242} Although these conditions removed the protecting groups, the bicyclic ring system was also destroyed. Similar results were obtained when the acetyl groups were removed with sodium methoxide followed by addition of water to generate sodium hydroxide in situ²⁴³

(Table 4.2, entry 3) or by treatment with lithium hydroxide (Table 4.2, entry 4). Use of sub-stoichiometric amounts of lithium hydroxide on de-acetylated **4.3** also gave ring-opened products, indicating that hydroxide attack at the ring carbon may be faster than attack at the ester carbonyl moiety (Table 4.2, entries 5 and 6). Less conventional techniques were then attempted, but use of trifluoroacetic acid²⁴⁴ and potassium *t*-butoxide²⁴⁵ both resulted in decomposition (Table 4.2, entries 7-9).

Promising results were obtained using pig liver esterase (PLE) to remove the ester.²⁴⁶ When fully-protected **4.3** was treated with PLE, it appeared as though the ethyl ester had been hydrolyzed but the acetyl groups were intact (Table 4.2, entry 10). De-acetylation of this product was attempted using sodium methoxide but was very slow (no reaction after 2.5 h) and only ring-opened products were obtained. PLE did not appear to hydrolyze the methyl ester following de-acetylation and trans-esterification of **4.3** (Table 4.2, entry 11). Although PLE appeared to hydrolyze the ethyl ester of de-acetylated **4.3**, the NMR spectra of the products were difficult to interpret as many of the peaks were broad and some disappeared over time as the sample remained dissolved in D₂O (Table 4.2, entry 12). The use of PLE appeared to provide the most encouraging results; thus, it may be beneficial to test a panel of esterases for their efficiency at removing the ethyl ester of **4.3**, both acetylated and de-acetylated.

4.3.1 Synthesis of *t*-Butyl Ester Analogues

Since hydrolysis of the ethyl and methyl esters of **4.3** under basic, nucleophilic conditions was not possible, it was decided to adopt the strategy used in the preparation of 4-deoxy-4-nitrosialic acid by synthesizing the *t*-butyl ester analogue of **4.3**. This would allow ester hydrolysis of the ester under acidic conditions, hopefully preserving the bicyclic ring system.

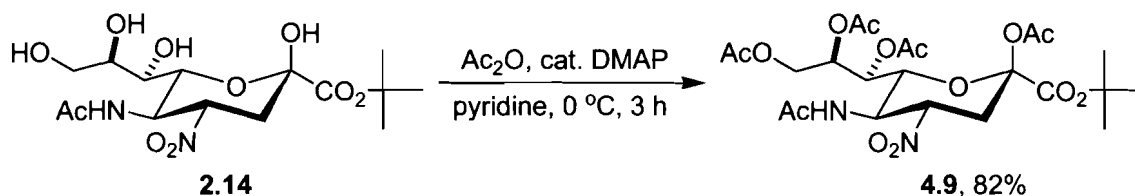
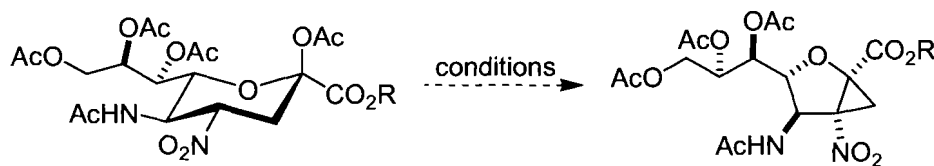


Figure 4.5 Peracetylation of **2.14**

The synthesis began by peracetylating **2.14** with acetic anhydride in pyridine to give **4.9** in 82% yield (Figure 4.5) along with a small quantity (4%) of the α -anomer. Synthesis of the β -chloro donor sugar for use in the intramolecular substitution reaction was attempted by stirring **2.14** in acetyl chloride but this resulted in removal of the *t*-butyl ester and generation of a complex product mixture. It was therefore decided to attempt the intramolecular substitution reaction on **4.9**, using the acetate as a leaving group in order to avoid development of a route to the β -sialosyl chloride (summarized in Table 4.3).

Table 4.3 Attempts at intramolecular substitution on β -acetate donor sugars



| Entry | R | Conditions | Results |
|-------|-------------|--|----------------------|
| 1 | <i>t</i> Bu | DBU, CH ₂ Cl ₂ , RT, 1 d | 4-epimerization |
| 2 | Et | DBU, HFIP, ^a RT, 1 d | No reaction |
| 3 | <i>t</i> Bu | NaH, THF, RT, 4 d | No reaction |
| 4 | <i>t</i> Bu | Pyridine, DMAP, RT, 10 d | Some 4-epimerization |
| 5 | <i>t</i> Bu | Pyridine, DMAP, reflux, 2 d | No reaction |
| 6 | Et | AgOTf, Ph ₂ SiCl ₂ , CH ₃ CN, RT, 3 d | No reaction |
| 7 | Et | AgClO ₄ , Ph ₂ SiCl ₂ , CH ₃ CN, RT, 5 h | No reaction |
| 8 | Et | AgClO ₄ , Me ₂ SiCl ₂ , CH ₃ CN, RT, 1 d | No reaction |
| 9 | <i>t</i> Bu | TMSOTf, CH ₃ CN, RT, 1 d | Decomposed |

a) HFIP = 1,1,1,3,3,3-hexafluoroisopropanol

Initial attempts used basic conditions in a variety of solvents but at best this only resulted in epimerization of the 4-position (Table 4.3, entries 1-5). It was then decided to use conditions that result in elimination to the glycal when used on conventional sialosides bearing an anomeric acetyl group on the assumption that these conditions would lead instead to intramolecular substitution when used on a 4-deoxy-4-nitrosialoside. Treating ethyl ester **2.17** with silver salts and dialkyldichlorosilanes in acetonitrile proved fruitless despite the fact that these conditions generally lead to elimination of conventional sialosides.¹⁷⁴ Use of AgOTf with Ph₂SiCl₂ for 3 d led to low starting material recovery (22%, Table 4.3, entry 6) while higher recoveries were obtained under similar conditions with shorter reaction times (Table 4.3, entries 7 and 8), indicating that decomposition of the starting material was likely occurring slowly over time. Use of TMSOTf on both the ethyl and *t*-butyl ester β -acetate

precursors resulted in decomposition of the material as no starting material or bicyclic products were isolated (Table 4.3, entry 9).

It was clear that a better leaving group was required at the anomeric centre in order for a facile intramolecular substitution reaction to occur. Attempts were made to install an anomeric bromide directly from the β -acetate precursor using TMSBr in CH_2Cl_2 .²⁴⁷ No reaction was observed after using 1.5 eq. TMSBr for 4 h but increasing the amount of TMSBr to 6.0 eq. caused disappearance of starting material after 1.5 h, although nothing was obtained following a purification attempt via flash chromatography indicating that the reaction products were unstable. The bromination was repeated and after evaporation of the volatile materials the crude compound was treated with DBU in CH_2Cl_2 to induce the intramolecular substitution immediately; however, only a small amount of starting material was isolated following aqueous workup. Repeating the reaction in the presence of TMSBr and DBU simultaneously also gave no identifiable products and changing the solvent to CH_3CN had no effect. Reagents with electrophilic trimethylsilyl groups such as TMSI ²⁴⁸ and TMSOTf ²⁴⁹ are known to promote cleavage of *t*-butyl esters by forming a TMS-ester that is then hydrolyzed upon aqueous workup, possibly contributing to the difficulties encountered in these reactions using TMSBr. Indeed, after stirring β -acetate **4.9** with 6.0 eq. TMSBr in CH_3CN overnight, the ^1H NMR spectrum of the products after chromatography indicated absence of the *t*-butyl ester as did the fact that this spectrum had to be acquired in D_2O . However, treatment of the ethyl ester **2.17** with TMSBr in CH_2Cl_2 for 7 h followed by DBU overnight resulted mainly in

re-isolation of starting material, indicating that the sialosyl bromide may not be able to be generated via this route.

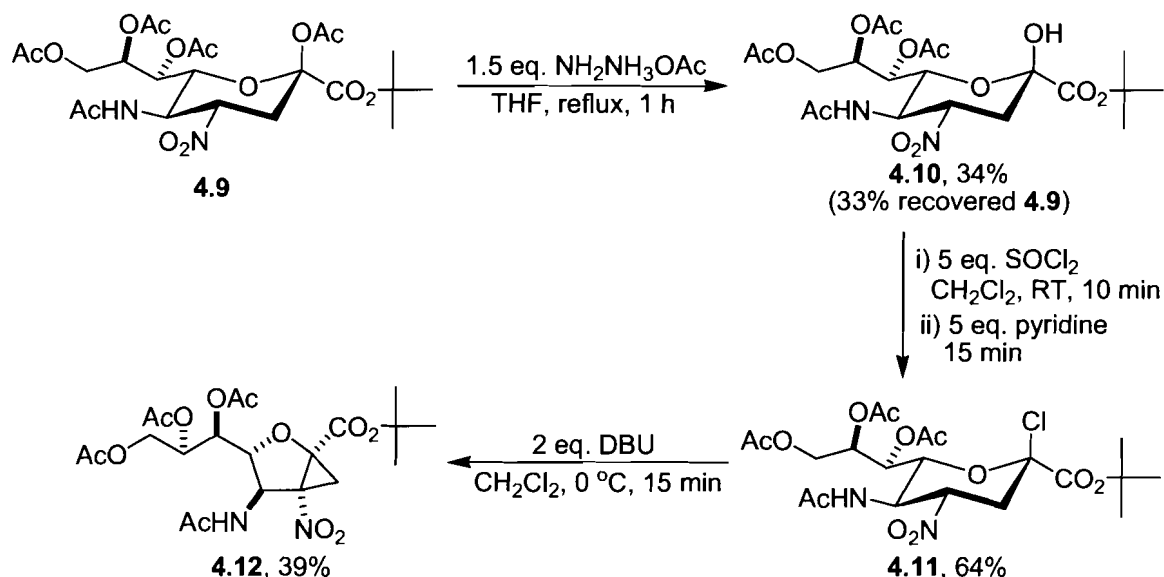


Figure 4.6 Synthetic route to bicyclic *t*-butyl ester **4.12** from pentaacetate **4.9**

As the synthesis of a β -sialosyl chloride *t*-butyl ester now seemed unavoidable, the rather lengthy and low-yielding synthetic route depicted in Figure 4.6 was developed. This route involved anomeric de-acetylation, conversion of hemiacetal **4.10** into sialosyl chloride **4.11**, then treatment with DBU to form the oxabicyclo[3.1.0]hexane ring system bearing a *t*-butyl ester (**4.12**).

Removal of the anomeric acetyl group was accomplished via treatment with hydrazinium acetate although this was a delicate reaction.³⁴ The reaction proceeded quite slowly, leading to decomposition products before the reaction was complete. Initial attempts used excess amounts of hydrazinium acetate (3.0-5.7 eq.) in CH₂Cl₂/MeOH mixtures at room temperature, giving very small

amounts of hemiacetal **4.10** and leading to decomposition with longer reaction times and increased amounts of reagent. Using smaller amounts of hydrazinium acetate (1.5 eq.) and using MeOH or DMF as solvents did not afford any improvement. Use of 1.1 eq. hydrazinium acetate in refluxing THF for 1 h provided acceptable yields, giving **4.10** in 34% yield and allowing 33% recovery of **4.9** before the decomposition proceeded too far (Figure 4.6). The recovered **4.9** was re-subjected to the reaction conditions to afford a further 11% of **4.10** along with 8% recovered **4.9** for a total yield of 45% **4.10** over two reaction cycles.

Before converting the hemiacetal **4.10** into the sialosyl chloride **4.11**, attempts were made to synthesize the bicyclic compound **4.12** directly from **4.10**. An intramolecular Mitsunobu reaction was attempted by stirring **4.10** with triphenylphosphine and diethyl azidodicarboxylate (DEAD) in THF as these conditions have been used to synthesize α -nitrocyclopropanes from γ -nitroalkanols.²⁵⁰ However, these reactions have generally been performed on primary nitroalkanes in unstrained systems giving trans-addition products; thus, only starting material was recovered from the reaction using **4.10** after 2 h. Mesylation and elimination of **4.10** was attempted by stirring with 3.0 eq. methanesulfonyl chloride and 4.5 eq. DBU in CH₂Cl₂ as this procedure has been used to generate nitroalkenes from β -nitroalkanols.^{251,252} Interestingly, this reaction gave no mesylate intermediate nor did it give any bicyclic products but instead gave a 17% yield of the β -sialosyl chloride **4.11** along with 18% recovered **4.10**. It is therefore likely that the mesylate intermediate formed but

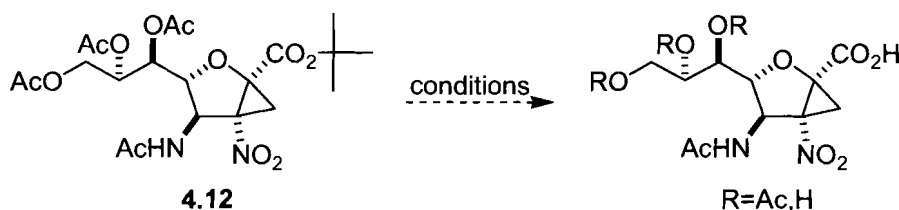
was somehow displaced by the chloride ion by-product and it is unclear how the excess amount of DBU was unable to induce the intramolecular substitution to form **4.12**.

In order to obtain higher yields of β -sialosyl chloride **4.11**, more conventional techniques were employed for its synthesis. **4.10** was stirred with thionyl chloride in CH_2Cl_2 for 10 min followed by the addition of pyridine and stirring for a further 15 min to afford **4.11** in 64% yield (Figure 4.6); addition of excess pyridine did not induce the formation of **4.12**. Interestingly, no reaction occurred between thionyl chloride and **4.10** until the addition of pyridine. As well, **4.11** was relatively stable but slowly hydrolyzed back to **4.10**; after being stored at $-20\text{ }^\circ\text{C}$ for two months, approximately half of the material had hydrolyzed although this would likely not have occurred if the material was stored under anhydrous conditions. However, the bicyclic compound **4.12** was easily synthesized in 39% yield from **4.11** by treatment with DBU in CH_2Cl_2 at $0\text{ }^\circ\text{C}$ for 15 min (Figure 4.6). As with the synthesis of the ethyl ester analogue **4.3**, the yield of **4.12** was not exceptional and the fate of the remaining material is unclear as the reaction proceeded from spot to spot by TLC. The stereoselectivity of the intramolecular substitution reaction was again supported by a series of 1D-NOE difference ^1H NMR spectra, showing a contact between H3_{syn} and H6 and an absence of contacts between H5 and any other protons as was observed for ethyl ester **4.3** (see Figure 4.3).

4.3.2 Deprotection of *t*-Butyl Ester Analogue 4.12

As with ethyl ester 4.3, the acetyl groups of 4.12 could be removed by treatment with sodium methoxide in methanol for 30 min at room temperature. Similarly, prolonged reaction times with sodium methoxide led to ring-opened products likely resulting from methoxide attack at the carboxylate-bearing bridgehead carbon. Also, the de-acetylated product was unstable to chromatography, afforded ring-opened products afterward.

Table 4.4 Conditions used in attempted *t*-butyl ester hydrolysis of 4.12



| Entry | Conditions | Results |
|-------|--|---|
| 1 | CF ₃ CO ₂ H, CH ₂ Cl ₂ , RT, 1 d | Ring-opened, no ester |
| 2 | i) NaOMe, MeOH, RT, 30 min ii) CF ₃ COOH, H ₂ O, 2.5 h | Ring-opened, partial ester hydrolysis |
| 3 | i) TsOH·H ₂ O, THF, RT, 2.5 h ii) reflux 1 d | No reaction at RT, decomposed at reflux |
| 4 | CF ₃ SO ₃ H, CH ₂ Cl ₂ , RT, 1 h | Decomposed |
| 5 | 88% HCO ₂ H (aq), RT, 1 d | Ring-opened, no ester |
| 6 | HCl/MeOH, 0 °C to RT, 1 d | Several compounds, ester intact |
| 7 | 2 drops H ₂ SO ₄ , CH ₂ Cl ₂ , RT, 1 h | Decomposed |
| 8 | K ₂ CO ₃ , MeOH, H ₂ O, RT, 2 h | Ring-opened, ester intact, no acetates |

Removal of the *t*-butyl ester proved to be a more significant challenge. Various acidic conditions were tested in attempts to hydrolyze the *t*-butyl ester as summarized in Table 4.4. Since trifluoroacetic acid was able to hydrolyze the *t*-butyl ester of 2.14 to give 4-deoxy-4-nitrosialic acid (Chapter 2.2), it was the first

reagent tried on compound **4.12**.²⁵³ Although trifluoroacetic acid did to remove the ester, it also induced a ring-opening reaction after stirring overnight in CH₂Cl₂ (Table 4.4, entry 1). Ring-opening also occurred when de-acetylated **4.12** was treated with aqueous trifluoroacetic acid after only 2.5 h (Table 4.4, entry 2). Partial hydrolysis of the *t*-butyl ester had occurred as the integration of its singlet in the ¹H NMR spectrum showed only 3 protons rather than 9, but this indicates that the acid-promoted ring-opening is likely faster than de-esterification. Treatment of **4.12** with *p*-toluenesulfonic acid in THF²⁵⁴ had no effect at room temperature after 2.5 h but induced decomposition after refluxing overnight, while treatment with trifluoromethanesulfonic acid in CH₂Cl₂²⁵⁵ induced decomposition after only 1 h at room temperature (Table 4.4, entries 3 and 4). Use of 88% aqueous formic acid overnight was able to remove the *t*-butyl ester but also induced ring-opening (Table 4.4, entry 5). Methanolic HCl generated by the reaction of acetyl chloride with methanol was used to attempt removal of the *t*-butyl ester from **4.12** but this afforded several unidentified compounds that still contained the ester (Table 4.4, entry 6).²⁵⁶ CH₂Cl₂ solutions containing H₂SO₄ have been reported to cleave *t*-butyl esters, but treatment of a CH₂Cl₂ solution of **4.12** with 2 drops of conc. H₂SO₄ for 1 h resulted in decomposition (Table 4.4, entry 7).²⁵⁷ One set of basic conditions was tried as potassium carbonate has been reported to remove *t*-butyl esters in aqueous methanol.⁴⁵ However, when **4.12** was reacted under these conditions for 2 h, only de-acetylated ring-opened products were obtained that still contained the *t*-butyl ester (Table 4.4, entry 8).

In the majority of the ring-opening reactions it was difficult to identify the products and to determine what groups were present at the anomeric centre, although analysis of the ^1H NMR spectra clearly showed when the bicyclic system had undergone a ring opening reaction since the two doublets for proton 3_{syn} and proton 3_{anti} became doublets of doublets as they coupled to a new proton on carbon 4, which also appeared as a doublet of a triplet at around 5.0 ppm. Since acidic hydrolysis of *t*-butyl esters proceeds via a *t*-butyl cation, it is likely that these conditions also enable opening of the bicyclic system to generate an oxacarbenium ion that is then attacked by any available nucleophile. Thus, synthesis of **4.12** was an unrewarding effort as the *t*-butyl ester could not be removed, preventing overall deprotection of the bicyclic compound and measurement of its inhibition constants with various sialidases.

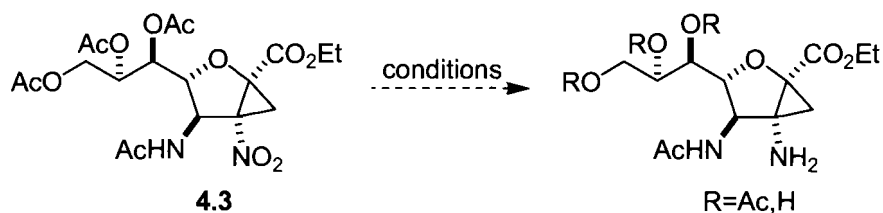
4.4 Oxabicyclo[3.1.0]hexane Reduction Attempts

Attempts at nucleophilic hydrolysis of the ethyl ester of **4.3** had failed due to the electrophilicity of the carboxylate-bearing bridgehead carbon. The electrophilicity at this centre was enhanced by the electron-withdrawing nitro group on the opposite bridgehead carbon. If the nitro group of **4.3** could be reduced to an amino group, it was reasoned that this would decrease the electrophilicity of the carboxylate-bearing bridgehead carbon and allow hydrolysis of the ethyl ester while keeping the bicyclic ring system intact. Reduction to the amino group would also likely enhance sialidase binding since the majority of the potent sialidase inhibitors contain a positively-charged group in the 4-position that is proposed to form favourable interactions with a glutamate residue in the

enzyme active site (e.g. Tamiflu, Relenza). Results of the reduction attempts are summarized in Table 4.5.

Charette and co-workers have developed conditions to reduce nitrocyclopropane carboxylates to cyclopropylamines in which both substituents are on the same carbon atom, leaving the cyclopropyl moiety intact.²⁵⁸ The researchers screened several reduction techniques and found that palladium-catalyzed reductions using hydrogen or ammonium formate as reducing agents resulted in reduction of both the nitro group and the cyclopropane ring. The use of Raney Ni, Fe/HCl, and Zn/Ac₂O led to mixtures of unidentifiable products as these compounds were prone to efficient nucleophilic ring opening and rearrangements and many of the reaction products were unstable to chromatography. After screening several acid sources, however, they found that using 20 eq. zinc dust in isopropanol containing 10 eq. 1 N HCl (aq) afforded the desired reduction products. Thus, these were the first conditions employed for attempting to reduce the nitro group in **4.3** (Table 4.5, entry 1).

Table 4.5 Reduction attempts on bicyclic compound 4.3



| Entry | Conditions | Results |
|-------|---|---|
| 1 | i) NaOEt, EtOH, -5 °C, 30 min ii) 20 eq. Zn, 10 eq. 1.0 N HCl, <i>i</i> PrOH, 2 h | Nitro reduced, ring opened |
| 2 | 20 eq. Zn, 10 eq. 1.0 N HCl, <i>i</i> PrOH, 2 h | Nitro reduced, ring opened |
| 3 | 20 eq. Zn, AcOH, 2.5 h | 42% 4.3 recovery, some nitro reduced, ring-opened products |
| 4 | H ₂ , Pd/C, MeOH, 1 d | Two nitro reduced, ring-opened compounds |
| 5 | H ₂ , Raney Ni, MeOH, 40 min | Nitro intact, ring-opened |
| 6 | H ₂ , Raney Ni, MeOH, 5 min | Mostly 4.3 , some ring opening |
| 7 | H ₂ , Raney Ni, MeOH, 25 min | Nitro intact, ring-opened |
| 8 | 6.0 eq. NaBH ₄ , 0.7 eq. NiCl ₂ , MeOH, 30 min | No 4.3 , inconclusive |

Initially, de-acetylated **4.3** was reduced under Charetté's conditions, adding the zinc dust portionwise over 15 min then stirring for 2 h. The crude ¹H NMR spectrum showed two doublets of doublets at the chemical shifts expected for the cyclopropyl methylene carbons, indicating the ring had opened. Also, the lack of any signals above 5.0 ppm was consistent with nitro group reduction. The reaction was repeated on fully-protected **4.3** but gave the same results (Table 4.5, entry 2). A zinc dust reduction using acetic acid as the solvent was then attempted but after 2.5 h this afforded starting material (42% recovery) as well as the same mix of ring-opened reduction products obtained previously (Table 4.5, entry 3).²⁵⁹ Attempts at chromatographic purification of products from these

reactions did not afford cleaner material; thus, it was difficult to determine how the ring was opened whether it be from reduction or an acid-promoted process.

Since acidic conditions seemed to be promoting ring-opening reactions, metal-catalyzed hydrogenations were attempted despite the observations of Charette and co-workers that these conditions tended to lead to over-reduction.²⁵⁸ The use of hydrogen and Pd/C in methanol had been reported to reduce nitro groups on cyclopropane rings but these rings had only alkyl substituents and were not activated.²⁶⁰ These conditions afforded the same reduced ring-opened compound when tried on **4.3** as was obtained using Charette's protocol (Table 4.5, entry 4). Analysis of the compound's IR spectrum showed absence of a nitro group, indicating that it had been reduced. A second unidentifiable compound was also isolated from the Pd/C-catalyzed reduction of **4.3** that was ring-opened due to the appearance of a doublet of a triplet (presumed to be H-5) at 3.8 ppm and several doublets of doublets and a doublet of a triplet between 2.0-3.0 ppm possibly resulting from reduction of the cyclopropane moiety.

Hydrogenation of **4.3** using Raney Ni was explored, as these conditions were successful in reducing the nitro group of phenyl β -sialoside **2.27**. After 40 min, TLC showed the absence of **4.3** but only ring-opened products were obtained (Table 4.5, entry 5). It was difficult to determine whether the nitro group had been reduced since a peak in the ^1H NMR spectrum presumed to be H4 appeared at 3.0 ppm but the IR spectrum showed peaks at 1372 and 1550 cm^{-1} indicative of NO_2 stretching. The reaction was repeated twice, being allowed to

proceed for 5 min and 25 min (Table 4.5, entries 6 and 7). After 5 min, mostly unreacted **4.3** and a small amount of ring-opened products were isolated. After 25 min, no **4.3** remained and only ring-opened products were isolated that still contained a nitro group, showing that reduction of the cyclopropyl ring had occurred faster than reduction of the nitro group.

After hydrogen failed to reduce selectively the nitro group with several catalysts, a mix of sodium borohydride and nickel(II) chloride was used to generate nickel boride in methanol (Table 4.5, entry 8), a reagent which has been reported to reduce aliphatic nitro compounds to amines.²⁶¹ Sodium borohydride (3.0 eq.) was added to a solution of nickel(II) chloride (0.7 eq.) in methanol. **4.3** was then added, followed by another 3.0 eq. of sodium borohydride. After 30 min, the reaction was worked up but the crude product was not soluble in CDCl_3 . ^1H NMR spectra in CD_3OD and D_2O were very noisy but did not show peaks above 4.8 ppm, indicating either that the cyclopropane ring was intact or that both the cyclopropane ring and the nitro group had been reduced; although, no discernable signals for the protons on carbon 3 could be found between 2.0 and 3.0 ppm. These conditions may be worth repeating, at least to determine their effect on the nitro group. Also, it may be worth trying the in situ-generated Ni_2B with hydrazine hydrate as a reducing agent as it has been reported that double bonds are inert to these conditions.²⁶²

Unfortunately, it appears as though the cyclopropane ring of **4.3** is more reactive toward reduction than the nitro group based on the results of the experiments described above. The conditions developed by Charette and co-

workers using zinc dust in methanol containing aqueous HCl were found to reduce selectively the nitro group of nitrocyclopropane carboxylates without reducing the cyclopropane ring; thus, it is possible that some variant of these conditions would lead to nitro reduction of **4.3**, perhaps by decreasing the reaction time or using highly activated zinc dust. Other reducing agents should be tested as well, such as tin(II) chloride or samarium(II) iodide.²⁶³

4.5 Conclusions

Isolation of the protected oxabicyclo[3.1.0]hexane-based sialic acid analogue **4.3** was an intriguing event as it represented the first such bicyclic compound that could be a potent sialidase inhibitor, as syntheses of cyclopropane-containing glycosidase inhibitors have been underway for some time in the Bennet laboratory. While the acetyl protecting groups could be removed fairly easily, cleavage of the ester proved much more difficult. It seems the reactivity imparted to the molecule by the nitro group that allowed the synthesis of **4.3** also prevented its deprotection and evaluation as a sialidase inhibitor.

Hydroxide-based de-esterification techniques on **4.3** led to nucleophilic attack at the carboxylate-bearing bridgehead carbon. This reaction appeared to occur faster than attack at the ester carbonyl group as evidenced by the use of sub-stoichiometric quantities of hydroxide. As well, the deprotected products were unstable to chromatography, making their purification extremely difficult. Synthesis of the *t*-butyl ester analogue **4.12** in 4 steps from 4-deoxy-4-nitrosialic acid, *t*-butyl ester turned out to be in vain as the acidic conditions employed to

remove the *t*-butyl ester also promoted ring-opening reactions. Again, ring-opening reactions were often faster than *t*-butyl ester hydrolysis. The most promising results came from the use of PLE on **4.3**. It appeared as though this enzyme could catalyze hydrolysis of the ethyl ester but removal of the acetyl groups proved to be a problem. A panel of esterases should be screened for their ability to hydrolyze the ethyl ester and perhaps the acetate esters as well to fully deprotect **4.3** in a one pot reaction.

Reduction of the nitro group of **4.3** to an amino group was attempted for two reasons. Firstly, reduction to an amino group would greatly reduce the electrophilicity of **4.3**, possibly allowing the de-esterification to proceed using standard techniques. Secondly, incorporation of a positively-charged amino group in this position would also likely lead to better sialidase inhibition. However, no conditions could be found that would allow the reduction of the nitro group without inducing a ring-opening reaction through nucleophilic attack or reduction of the cyclopropane ring. As well, many of these reactions gave products whose ¹H NMR spectra were difficult to interpret, showing multiple broad peaks in many different solvents. Varying the conditions developed by Charette and co-workers would be worthwhile, such as decreasing the reaction time and using highly active zinc, as well as trying other reducing agents such as sodium borohydride, tin(II) chloride, or samarium(II) iodide. Deprotection and/or reduction of **4.3** should provide a useful sialidase inhibitor that could form the basis for a new class of structural derivatives.

4.6 Experimental

General

All chemicals were purchased from Aldrich Chemical Company and were used as received. Solvents for anhydrous reactions were dried and distilled immediately prior to use. CH_2Cl_2 was dried and distilled over calcium hydride. THF was dried and distilled over sodium/benzophenone. Methanol and ethanol were dried and distilled over magnesium turnings. Glassware used for anhydrous reactions was flame-dried and cooled under a N_2 atmosphere immediately prior to use. TLC was performed on aluminum-backed TLC plates pre-coated with Merck silica gel 60 F₂₅₄. Compounds were visualised with UV light and/or staining with phosphomolybdic acid (5% solution in ethanol). Flash chromatography was performed using Avanco silica gel 60 (230-400 mesh). Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian Unity 500 MHz spectrometer. Chemical shifts (δ) are listed in ppm downfield from TMS using the residual solvent peak as an internal reference. ^1H and ^{13}C NMR peak assignments were made based on ^1H - ^1H COSY and ^1H - ^{13}C HMQC experiments. IR spectra were recorded on a Bomem IR spectrometer and samples were prepared as cast evaporative films on NaCl plates from CH_2Cl_2 or methanol. Optical rotations were measured using a Perkin-Elmer 341 polarimeter and are reported in units of $\text{deg cm}^2 \text{g}^{-1}$ (concentrations reported in units of $\text{g}/100 \text{ cm}^3$). Sialic acid numbering has been used for assignment of ^1H and ^{13}C NMR spectra of oxabicyclo[3.1.0]hexane-based compounds.

Ethyl (1*S*,3*R*,4*R*,5*R*)-3-[(1*R*,2*R*)-1,2,3-triacetoxypentyl]-4-acetamido-5-nitro-2-oxabicyclo[3.1.0]hexane-1-carboxylate (4.3)

Sialosyl chloride **2.18** (1.01 g, 1.97 mmol) was dissolved in dry CH₂Cl₂ (150 mL) and cooled to 0 °C. DBU (0.59 mL, 4.0 mmol) was injected, causing the solution to turn dark yellow. After stirring for 45 min under N₂ at 0 °C, TLC indicated that the reaction was complete. Saturated aqueous ammonium chloride was added (25 mL) and the mixture was stirred for 5 min, which was then concentrated under reduced pressure to half its volume, diluted with water (100 mL) and extracted with EtOAc (2 x 100 mL). The organic extracts were dried (MgSO₄), filtered, and concentrated under reduced pressure to give a brown syrup (0.687 g) that was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford **4.3** as a yellow syrup (0.408 g, 0.859 mmol, 44%). $[\alpha]_D^{20}$ -8.99 (c 2.28, CHCl₃). IR (cm⁻¹): 1373 (NO₂), 1552 (NO₂), 1659 (amide C=O), 1749 (ester C=O), 3287 (N-H). ¹H NMR (CDCl₃, 500 MHz) δ: 1.28 (t, 3H, *J* = 7.1 Hz, OCH₂CH₃), 2.02 (s, 3H, COCH₃), 2.06 (s, 3H, COCH₃), 2.09 (s, 3H, COCH₃), 2.15 (s, 3H, COCH₃), 2.31 (d, 1H, *J*_{3syn,3anti} = 8.3 Hz, H-3_{syn}), 2.93 (dd, 1H, *J*_{3syn,3anti} = 8.4 Hz, ⁴*J*_{3anti,5} = 1.5 Hz, H-3_{anti}), 4.19 (dd, 1H, *J*_{8,9a} = 6.3 Hz, *J*_{9a,9b} = 12.6 Hz, H-9a), 4.22-4.27 (m, 3H, OCH₂CH₃, H-6), 4.55 (dd, 1H, *J*_{8,9b} = 2.5 Hz, *J*_{9a,9b} = 12.6 Hz, H-9b), 5.27-5.34 (m, 2H, H-5, H-8), 5.41 (dd, 1H, *J*_{7,6/8} = 2.1 Hz, *J*_{7,6/8} = 5.7 Hz, H-7), 6.01 (d, 1H, *J*_{5,NH} = 5.6 Hz, NH). ¹³C NMR (CDCl₃, 125 MHz) δ: 14.0 (OCH₂CH₃), 20.7 (OCOCH₃), 20.8 (OCOCH₃), 20.9 (OCOCH₃), 22.9 (NHCOCH₃), 25.5 (C-3), 57.0 (C-5), 61.9 (C-9), 62.2 (OCH₂CH₃), 69.9 (C-7), 70.7 (C-8), 72.6 (C-2), 77.2 (C-4), 83.1 (C-6), 164.3

(C-1), 170.1, 170.2, 170.77, 170.83 (COCH₃ x 4). Anal. calcd. for C₁₉H₂₆N₂O₁₂:
C 48.10, H 5.52, N 5.90; found: C 48.22, H 5.69, N 5.58.

Methyl (1*S*,3*R*,4*R*,5*R*)-4-acetamido-3-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5-nitro-2-oxabicyclo[3.1.0]hexane-1-carboxylate (4.7)

A solution of **4.3** (40 mg, 0.084 mmol) in dry methanol (3.0 mL) was cooled in an ice bath, to which was injected a solution of sodium methoxide in methanol (2.3 M, 0.036 mL, 0.083 mmol) that had been freshly prepared by reacting sodium chips washed in hexanes with dry methanol. After 25 min, TLC analysis indicated complete reaction and after 30 min the solution was neutralized by the addition of Amberlite H⁺ resin. The mixture was filtered, the resin was washed with methanol (20 mL), and the filtrate was concentrated under reduced pressure to a light brown syrup (27 mg, 0.081 mmol, 96%). The product was deemed of adequate purity by ¹H NMR spectroscopy for further use given that the product could not be purified without decomposition. ¹H NMR (CD₃OD, 500 MHz) δ: 1.97 (s, 3H, NHCOCH₃), 2.50 (d, 1H, *J*_{3anti,3syn} = 8.5 Hz, H-3_{syn}), 2.71 (dd, 1H, *J*_{3anti,3syn} = 8.5 Hz, ⁴*J*_{3anit,5} = 1.9 Hz, H-3_{anti}), 3.46 (dd, 1H, *J*_{6,7} = 1.2 Hz, *J*_{7,8} = 9.1 Hz, H-7), 3.63-3.70 (m, 2H, H-8, H-9a), 3.76 (s, 3H, CO₂CH₃), 3.79-3.81 (m, 1H, H-9b), 4.04 (dd, 1H, *J*_{5,6} = 8.5 Hz, *J*_{6,7} = 1.3 Hz, H-6), 5.85 (dd, 1H, ⁴*J*_{3anti,5} = 1.8 Hz, *J*_{5,6} = 8.5 Hz, H-5).

***t*-Butyl 5-acetamido-2,7,8,9-tetra-*O*-acetyl-3,4,5-trideoxy-4-nitro-*D*-glycero-β-*D*-galacto-non-2-ulopyranosonate (4.9)**

t-Butyl 5-acetamido-3,4,5-trideoxy-4-nitro-*D*-glycero-β-*D*-galacto-non-2-ulopyranosonate **2.14** (0.480 g, 1.22 mmol) was dissolved in pyridine (5.0 mL) and cooled in an ice/salt bath. Acetic anhydride (0.90 mL, 8.6 mmol) and a

catalytic amount of DMAP were added to the solution that was then stirred under N_2 in an ice/salt bath for 3 h. The solution was then poured into a mixture of CH_2Cl_2 (75 mL) and 10% aqueous H_2SO_4 (75 mL) and extracted. The aqueous layer was washed with a further amount of CH_2Cl_2 (75 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a yellow solid (0.753 g). This crude material was dissolved in EtOAc (30 mL) to which hexanes (70 mL) was added to the point of turbidity. The flask was scratched to encourage precipitation then the mixture was cooled at $-20\text{ }^\circ\text{C}$. After 1 h, the mixture was filtered and the resulting yellow powder was washed with hexanes (40 mL), affording pure **4.9** (0.352 g, 0.626 mmol, 51%). The filtrate was concentrated under reduced pressure to a yellow solid that was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford a further quantity of **4.9** (0.212 g, 0.377 mmol, 31%) as well as a small quantity of the peracetylated α -anomer as a yellow syrup (0.027 g, 0.048 mmol, 4%). β -anomer: mp. 151-154 $^\circ\text{C}$ (dec). $[\alpha]_D^{20} +1.3$ (c 1.48, $CHCl_3$). IR (cm^{-1}): 1260 (C-O-C antisymmetric stretch), 1371 (NO_2), 1561 (NO_2), 1665 (amide C=O), 1749 (ester C=O), 3268 (NH). 1H NMR (500 MHz, $CDCl_3$) δ : 1.47 (s, 9H, $OC(CH_3)_3$), 1.98 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 2.19 (s, 3H, $COCH_3$), 2.44 (t, 1H, $J_{3ax,3eq} + J_{3ax,4} = 25.9$ Hz, H-3_{ax}), 2.82 (dd, 1H, $J_{3ax,3eq} = 13.4$ Hz, $J_{3eq,4} = 4.6$ Hz, H-3_{eq}), 3.70 (dt, 1H, $J_{4,5} + J_{5,6} = 21.4$ Hz, $J_{5,NH} = 7.6$ Hz, H-5), 4.22 (dd, 1H, $J_{8,9a} = 5.7$ Hz, $J_{9a,9b} = 12.6$ Hz, H-9a), 4.40 (dd, 1H, $J_{8,9b} = 2.4$ Hz, $J_{9a,9b} = 12.6$ Hz, H-9b), 4.63 (dd, 1H, $J_{5,6} = 10.6$ Hz, $J_{6,7} = 1.7$ Hz, H-6), 5.15 (ddd, 1H, $J_{7,8} = 6.6$ Hz, $J_{8,9a} = 5.8$ Hz, $J_{8,9b}$

= 2.4 Hz, H-8), 5.33 (dd, 1H, $J_{6,7} = 1.7$ Hz, $J_{7,8} = 6.7$ Hz, H-7), 5.66 (ddd, 1H, $J_{3ax,4} = 12.4$ Hz, $J_{3eq,4} = 4.6$ Hz, $J_{4,5} = 10.8$ Hz, H-4), 5.84 (d, 1H, $J_{5,NH} = 7.6$ Hz, NH). ^{13}C NMR (125 MHz, CDCl_3) δ : 20.65 (OCOCH₃), 20.70 (OCOCH₃), 20.8 (OCOCH₃), 20.9 (OCOCH₃), 23.4 (NHCOCH₃), 27.5 (OC(CH₃)₃), 34.9 (C-3), 50.1 (C-5), 61.8 (C-9), 67.9 (C-7), 69.6 (C-6), 70.1 (C-8), 80.3 (C-4), 84.0 (OC(CH₃)₃), 96.6 (C-2), 163.8 (CO₂^tBu), 168.0 (2-OCOCH₃), 169.7 (8-OCOCH₃), 170.5 (9-OCOCH₃), 171.0 (7-OCOCH₃, NHCOCH₃). Anal. calcd. for C₂₃H₃₄N₂O₁₄: C 49.11, H 6.09, N 4.98; found: C 49.30, H 6.11, N 4.85.

α -Anomer: ^1H NMR (CDCl_3 , 500 MHz) δ : 1.51 (s, 9H, OC(CH₃)₃), 1.97 (s, 3H, COCH₃), 2.04 (s, 3H, COCH₃), 2.08 (s, 3H, COCH₃), 2.18 (s, 3H, COCH₃), 2.45 (dd, 1H, $J_{3ax,3eq} = 13.1$ Hz, $J_{3ax,4} = 11.7$ Hz, H-3_{ax}), 2.97 (dd, 1H, $J_{3ax,3eq} = 13.2$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3_{eq}), 3.93 (dt, 1H, $J_{4,5} + J_{5,6} = 20.9$ Hz, $J_{5,NH} = 8.2$ Hz, H-5), 4.19 (dd, 1H, $J_{8,9a} = 5.3$ Hz, $J_{9a,9b} = 12.6$ Hz, H-9a), 4.38 (dd, 1H, $J_{8,9b} = 2.4$ Hz, $J_{9a,9b} = 12.6$ Hz, H-9b), 4.85 (dd, 1H, $J_{5,6} = 10.7$ Hz, $J_{6,7} = 1.8$ Hz, H-6), 5.20 (ddd, 1H, $J_{7,8} + J_{8,9a} = 12.3$ Hz, $J_{8,9b} = 2.4$ Hz, H-8), 5.32 (dd, 1H, $J_{6,7} = 1.8$ Hz, $J_{7,8} = 7.1$ Hz, H-7), 5.41 (dt, 1H, $J_{3eq,4} = 4.8$ Hz, $J_{3ax,4} + J_{4,5} = 21.7$ Hz, H-4), 5.72 (d, 1H, $J_{5,NH} = 8.1$ Hz, NH). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 20.71 (COCH₃), 20.73 (COCH₃), 20.85 (COCH₃), 20.89 (COCH₃), 23.4 (COCH₃), 27.7 (OC(CH₃)₃), 35.3 (C-3), 49.8 (C-5), 61.9 (C-9), 67.8 (C-7), 69.8 (C-8), 71.2 (C-6), 81.0 (C-4), 84.5 (OC(CH₃)₃), 96.1 (C-2), 165.7, 168.0, 169.9, 170.56, 170.58, 170.8 (C=O x 6).

***t*-Butyl 5-acetamido-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-4-nitro-*D*-glycero- β -*D*-galacto-non-2-ulopyranosonate (4.10)**

t-Butyl 5-acetamido-2,7,8,9-tetra-*O*-acetyl-3,4,5-trideoxy-4-nitro-*D*-glycero- β -*D*-galacto-non-2-ulopyranosonate (**4.9**, 662 mg, 1.18 mmol) was dissolved in dry THF (50 mL). Hydrazinium acetate (123 mg, 1.34 mmol) was added and the mixture was heated to reflux under N₂. After 45 min, TLC analysis showed a spot for the hemiacetal product as well as a spot for starting material and a spot on the baseline. After 50 min, the reaction was cooled and concentrated under reduced pressure to a light yellow foam (691 mg) that was shown to be a 1.0 : 0.9 mix of hemiacetal : starting material by ¹H NMR. This was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc), affording the hemiacetal product **4.10** as a colourless syrup (208 mg, 0.400 mmol, 34%). A quantity of unreacted **4.9** was also isolated (218 mg, 0.388 mmol, 33%) and was re-subjected to the de-acetylation conditions by dissolving in dry THF (50 mL), adding hydrazinium acetate (49 mg, 0.53 mmol) and heating to reflux for under N₂ for 1 h. TLC analysis showed similar results as before and the reaction was cooled and concentrated under reduced pressure to give a light yellow foamy syrup (253 mg). This was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the hemiacetal **4.10** as a colourless syrup (66 mg, 0.13 mmol, 11%) as well as a small quantity of unreacted **4.9** (53 mg, 0.094 mmol, 8%). $[\alpha]_D^{20}$ +24.6 (c 1.43, CHCl₃). IR (cm⁻¹): 1372 (NO₂), 1559 (NO₂), 1667 (amide C=O), 1746 (ester C=O), 3355 (NH), 3458 (OH). ¹H NMR (500 MHz, CDCl₃) δ : 1.46 (s, 9H, C(CH₃)₃), 1.90 (s, 3H, COCH₃), 1.98 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 2.12

(s, 3H, COCH₃), 2.37 (dd, 1H, $J_{3_{\text{eq}},3_{\text{ax}}} = 12.8$ Hz, $J_{3_{\text{eq}},4} = 4.4$ Hz, H-3_{eq}), 2.60 (t, 1H, $J_{3_{\text{eq}},3_{\text{ax}}} = J_{3_{\text{ax}},4} = 12.7$ Hz, H-3_{ax}), 4.05 (dd, 1H, $J_{8,9a} = 7.1$ Hz, $J_{9a,9b} = 13.8$ Hz, H-9a), 4.28 (br q, 1H, $J_{4,5} + J_{5,6} + J_{5,\text{NH}} = 30.3$ Hz, H-5), 4.41-4.46 (m, 2H, H-6, H-9b), 4.82 (br s, 1H, 2-OH), 5.12-5.18 (m, 2H, H-4, H-8), 5.35 (dd, 1H, $J_{6,7} = 2.0$ Hz, $J_{7,8} = 4.5$ Hz, H-7), 6.73 (d, 1H, $J_{5,\text{NH}} = 9.2$ Hz, NH). ¹³C NMR (CDCl₃, 125 MHz) δ : 20.6 (COCH₃), 20.7 (COCH₃), 20.8 (COCH₃), 22.9 (COCH₃), 27.6 (OC(CH₃)₃), 35.2 (C-3), 48.9 (C-5), 62.5 (C-9), 68.1 (C-7), 69.7 (C-6), 71.4 (C-8), 82.6 (C-4), 84.2 (OC(CH₃)₃), 94.0 (C-2), 166.7, 170.4, 170.68, 170.73, 170.9 (C=O x 5). Anal. calcd. for C₂₁H₃₂N₂O₁₃: C 48.46, H 6.20, N 5.38; found: C 48.23, H 6.43, N 5.59.

***t*-Butyl 5-acetamido-7,8,9-tri-*O*-acetyl-2-chloro-2,3,4,5-tetra-deoxy-4-nitro-*D*-glycero- β -*D*-galacto-non-2-ulopyranosonate (4.11)**

Hemiacetal **4.10** (743 mg, 1.43 mmol) was dissolved in dry CH₂Cl₂ (75 mL) to which thionyl chloride (0.26 mL, 3.5 mmol) was injected. This solution was stirred under N₂ at room temperature for 10 min, after which time pyridine (0.29 mL, 3.5 mmol) was added. After a further 15 min, TLC showed no remaining starting material and the reaction was quenched by the addition of saturated aqueous NH₄Cl (10 mL). After stirring the mixture for 5 min, water was added (50 mL) and the mixture was extracted. The aqueous layer was washed with a further portion of CH₂Cl₂ (50 mL) and the organic layers were combined, dried (MgSO₄), filtered, and concentrated under reduced pressure to give a yellow foamy solid (758 mg). The crude product was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the pure chlorinated product **4.11** as a white foamy syrup (497 mg, 0.922 mmol,

64%). $[\alpha]_D^{20}$ -38 (c 0.69, CHCl₃). IR (cm⁻¹): 1372 (NO₂), 1559 (NO₂), 1669 (amide C=O), 1749 (ester C=O), 3275 (NH). ¹H NMR (CDCl₃, 500 MHz) δ : 1.49 (s, 9H, OC(CH₃)₃), 1.94 (s, 3H, COCH₃), 2.01 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 2.12 (s, 3H, COCH₃), 2.67 (dd, 1H, $J_{3\text{eq},3\text{ax}} = 13.9$ Hz, $J_{3\text{ax},4} = 12.2$ Hz, H-3_{ax}), 2.94 (dd, 1H, $J_{3\text{eq},3\text{ax}} = 13.9$ Hz, $J_{3\text{eq},4} = 4.4$ Hz, H-3_{eq}), 4.15 (dd, 1H, $J_{8,9\text{a}} = 5.6$ Hz, $J_{9\text{a},9\text{b}} = 12.6$ Hz, H-9a), 4.21 (br q, 1H, $J_{4,5} + J_{5,6} + J_{5,\text{NH}} = 30.4$ Hz, H-5), 4.33 (dd, 1H, $J_{8,9\text{b}} = 2.6$ Hz, $J_{9\text{a},9\text{b}} = 12.6$ Hz, H-9b), 4.63 (dd, 1H, $J_{5,6} = 10.6$ Hz, $J_{6,7} = 2.1$ Hz, H-6), 5.14 (ddd, 1H, $J_{8,9\text{b}} = 2.6$ Hz, $J_{7,8} + J_{8,9\text{a}} = 12.6$ Hz, H-8), 5.37 (dt, 1H, $J_{3\text{ax},4} + J_{3\text{eq},4} + J_{4,5} = 27.3$ Hz, H-4), 5.44 (dd, 1H, $J_{6,7} = 2.1$ Hz, $J_{7,8} = 7.1$ Hz, H-7), 6.53 (d, 1H, $J_{5,\text{NH}} = 8.9$ Hz, NH). ¹³C NMR (CDCl₃, 125 MHz) δ : 20.6 (COCH₃), 20.7 (COCH₃ x 2), 23.0 (COCH₃), 27.5 (OC(CH₃)₃), 39.6 (C-3), 48.6 (C-5), 61.7 (C-9), 66.9 (C-7), 69.5 (C-8), 72.2 (C-6), 81.9 (C-4), 84.8 (OC(CH₃)₃), 95.9 (C-2), 163.1, 169.4, 170.2, 170.6, 170.8 (C=O x 5). Anal. calcd. for C₂₁H₃₁N₂O₁₂Cl: C 46.80, H 5.80, N 5.20; found: C 46.65, H 6.03, N 5.43.

***t*-Butyl (1*S*,3*R*,4*R*,5*R*)-3-[(1*R*,2*R*)-1,2,3-triacetoxypopyl]-4-acetamido-5-nitro-2-oxabicyclo[3.1.0]hexane-1-carboxylate (4.12)**

A solution of β -sialosyl chloride **4.11** (124 mg, 0.230 mmol) in dry CH₂Cl₂ (10 mL) was cooled in an ice bath while stirring under N₂. DBU was injected (0.070 mL, 0.47 mmol, 2.0 eq.) and after stirring under N₂ in the ice bath for 10 min, TLC indicated complete reaction. After 15 min, the reaction was quenched by the addition of saturated aqueous ammonium chloride (5 mL). The mixture was then diluted with water (40 mL) and extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated under reduced

pressure to a yellow syrup (75 mg). This was purified via flash chromatography (hexanes-EtOAc gradient solvent system from 1:2 v/v to 100% EtOAc) to afford the bicyclic compound **4.12** as a colourless syrup (45 mg, 0.089 mmol, 39%). IR (cm⁻¹): 1372 (NO₂), 1552 (NO₂), 1657 (amide C=O), 1749 (ester C=O), 3288 (NH). ¹H NMR (CDCl₃, 500 MHz) δ: 1.45 (s, 9H, OC(CH₃)₃), 2.02 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.08 (s, 3H, COCH₃), 2.17 (s, 3H, COCH₃), 2.20 (d, 1H, *J*_{3anti,3syn} = 8.3 Hz, H-3_{syn}), 2.86 (dd, 1H, *J*_{3anti,3syn} = 8.2 Hz, ⁴*J*_{3anti,5} = 1.4 Hz, H-3_{anti}), 4.19-4.23 (m, 2H, H-6, H-9a), 4.54 (dd, 1H, *J*_{8,9b} = 2.4 Hz, *J*_{9a,9b} = 12.6 Hz, H-9b), 5.29-5.33 (m, 2H, H-8, H-5), 5.43 (dd, 1H, *J*_{6,7} = 2.1 Hz *J*_{7,8} = 5.9 Hz, H-7), 5.97 (d, 1H, *J*_{5,NH} = 5.6 Hz, NH). ¹³C NMR (CDCl₃, 125 MHz) δ: 20.77 (COCH₃), 20.78 (COCH₃), 20.9 (COCH₃), 22.8 (COCH₃), 25.5 (C-3), 27.8 (OC(CH₃)₃), 57.1 (C-5), 61.8 (C-9), 70.0 (C-7), 70.6 (C-8), 72.9 (C-2), 77.4 (C-4), 83.5 (C-6), 84.2 (OC(CH₃)₃), 163.0, 170.0, 170.3, 170.7, 170.8 (C=O x 5).

***t*-Butyl (1*S*,3*R*,4*R*,5*R*)-4-acetamido-3-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5-nitro-2-oxabicyclo[3.1.0]hexane-1-carboxylate**

4.12 (9.6 mg, 0.019 mmol) was dissolved in dry methanol (2 mL) and a solution of sodium methoxide in methanol (0.89 M, 0.019 mL, 0.017 mmol) was injected that had been freshly prepared by reacting sodium chips cleaned in hexanes with dry methanol. The solution was stirred under N₂ at room temperature for 30 min, after which TLC indicated complete reaction. The reaction was neutralized with Amberlite H⁺ resin that was then filtered and washed with methanol (20 mL). The filtrate was concentrated under reduced pressure to a colourless film (6.9 mg, 0.018 mmol, 95%). The product was deemed of adequate purity by ¹H NMR spectroscopy for further use given that the product could not be purified without

decomposition. ^1H NMR (CD_3OD , 500 MHz) δ : 1.42 (s, 9H, $\text{OC}(\text{CH}_3)_3$), 1.97 (s, 3H, COCH_3), 2.43 (d, 1H, $J_{3\text{anti},3\text{syn}} = 8.5$ Hz, H-3_{syn}), 2.66 (dd, 1H, $J_{3\text{anti},3\text{syn}} = 8.5$ Hz, $^4J_{3\text{anti},5} = 1.9$ Hz, H-3_{anti}), 3.43-3.45 (m, 1H, H-7), 3.63-3.70 (m, 2H, H-9a, H-8), 3.79-3.82 (m, 1H, H-9b), 4.02 (dd, 1H, $J_{5,6} = 8.5$ Hz, $J_{6,7} = 1.4$ Hz, H-6), 5.83 (dd, 1H, $^4J_{3\text{anti},5} = 1.8$ Hz, $J_{5,6} = 8.5$ Hz, H-5).

CHAPTER 5: SUMMARY AND FUTURE WORK

The research described in this thesis centred around the development of synthetic routes to 4-modified sialic acid analogues. These were intended to be general routes, leading to intermediates that could be derivatized and used in a number of synthetic pathways. Due to the lack of general synthetic routes that lead to 4-modified sialic acid analogues, this would be a worthwhile achievement. These routes would allow the generation of compounds that could be used as inhibitors of sialic acid-recognizing proteins and as probes of their binding specificities.

The initial idea was to reverse the polarity of the step leading to formation of the 9-carbon backbone of sialic acid. Normally this chain is synthesized by reacting a nucleophilic pyruvate synthon with an electrophilic aldose, generating a hydroxyl group at the 4-position; this coupling can be accomplished chemoenzymatically using sialic acid aldolase or can be performed chemically. The strategy used herein reversed the polarity of this coupling step by reacting the nitronate of a 1-deoxy-1-nitroalditol with an electrophilic pyruvate synthon, allowing the synthetically versatile 4-nitro group to be retained in the coupled product.

The coupling of 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol **2.1** with ethyl α -(bromomethyl)acrylate **2.6** under basic conditions was achieved in good yield after several coupling attempts failed with ethyl bromopyruvate and allyl bromide.

Following ozonolytic cleavage of the 2-methylene moiety, 4-deoxy-4-nitrosialic acid ethyl ester **2.10** was obtained in multigram quantities. Basic de-esterification attempts resulted in generation of an unknown product; thus, the *t*-butyl ester was synthesized using *t*-butyl α -(bromomethyl)acrylate **2.12**. This could be de-esterified under acidic conditions, leading to 4-deoxy-4-nitrosialic acid **2.16**. This compound was allowed to soak with crystals of influenza N8 sialidase in order to obtain an X-ray crystal structure to analyze active site interactions of the 4-nitro group but no structure was obtained, likely due to low affinity of the compound for the enzyme.

The 4-deoxy-4-nitrosialic acid esters are useful synthetic intermediates for the generation of 4-modified sialic acid analogues due to the synthetic versatility of the 4-nitro group. For instance, nitro groups can be reduced to amines, hydrolyzed to ketones, removed under radical conditions and replaced with a hydrogen atom, and they allow further alkylation by enhancing the acidity of neighbouring protons. Glycosylation of 4-deoxy-4-nitrosialic acid ethyl ester **2.10** was attempted to synthesize substrates for sialidase-catalyzed hydrolysis in hopes that several 4-derivatives could be made and their kinetic parameters of hydrolysis could be analyzed to determine the contribution of various 4-substituents to binding and hydrolysis. This required the synthesis of α -linked sialosides and was attempted using phenol as the acceptor. Several donor sugars were synthesized and gave varying results under different glycosylation conditions. The best results were obtained using a 1-adamantyl thiosialoside **2.21** with NIS/TMSOTf in CH₂Cl₂/CH₃CN solvent mixtures at -78 °C as a 38%

yield of the phenyl α -sialoside **2.25** was obtained in addition to a 25% yield of the β -anomer.

Although the 1-adamantyl thiosialoside **2.21** proved to be the best donor sugar for α -selective glycosylation, its synthesis must be improved. Currently this donor sugar is obtained in 21% yield from the peracetylated 4-deoxy-4-nitrosialic acid ethyl ester **2.17** by treatment with 1-adamantanethiol and $\text{BF}_3 \cdot \text{OEt}_2$. Use of the α -sialosyl fluoride **2.19** to synthesize the thiosialoside may be a solution as it afforded better yields of glycosylated products (80%) when reacted under the same conditions as used for thiosialoside formation. Once significant quantities of the phenyl α -sialoside **2.25** are synthesized, derivatization of the 4-nitro group must be undertaken. This should be reduced to an amine (using Raney nickel and H_2) and can be converted into a guanidinyll group (using aminoiminomethanesulfonic acid) to give substrates for sialidase-catalyzed hydrolysis. The kinetic parameters for these hydrolyses can be compared with the binding constants of the corresponding glycals. After several 4-modified sialosides are synthesized and hydrolyzed, the contributions of the 4-substituent to binding and catalysis can be determined, making it possible to determine whether or not sialidase inhibitors such as DANA and Relenza are actually transition state analogues. This information would hopefully lead to the development of more potent influenza sialidase inhibitors.

A *Micromonospora viridifaciens* sialidase has been recombinantly produced in the Bennet lab in which the catalytic tyrosine nucleophile has been mutated to a glycine. This mutant is capable of hydrolyzing phenyl β -sialic acid

and transferring the sialic acid moiety to lactose. Since a quantity of phenyl 4-deoxy-4-nitro- β -sialic acid **2.27** had been produced, its hydrolysis was attempted using the mutant sialidase. However, no hydrolysis was observed as monitored by ^1H NMR spectroscopy, indicating that the nitro group may be preventing binding to the enzyme. The nitro group was reduced to an amino group which should enhance binding to the sialidase as it should be positively charged and have favourable charge-charge interactions with a nearby glutamate residue. The hydrolysis of phenyl 4-amino-4-deoxy- β -sialic acid **2.26** should be attempted with the mutant sialidase and attempts should be made to transfer this unnatural sugar to lactose.

An isopropylidene-protected version of 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol was synthesized (**2.5**) and coupled with ethyl α -(bromomethyl)acrylate **2.6**. When the 4*S*-isomer of the coupled product (**3.2**) was treated with ozone and the reaction product was purified via chromatography, a β,γ -unsaturated α -keto ester **3.1** was isolated in excellent yield. This resulted from a silica-induced elimination of HNO_2 across carbons 3 and 4; this elimination could also be induced by treating the crude ozonolysis product **3.4** with DBU. This enone was treated with Et_2Zn and Me_2Zn in the presence of 5% $\text{Cu}(\text{OTf})_2$ and 10% $\text{P}(\text{OEt})_3$ to afford conjugate addition products. Since the nucleophile would attack at the 4-position of the enone, this represented another route to 4-modified sialic acid analogues that could possibly eclipse the route initially envisioned in terms of its generality due to the noted functional group tolerance of copper-catalyzed dialkylzinc addition reactions. These reactions afforded products that were

initially isolated as their enol tautomers, which cyclized to give 5-membered ring hemiaminal structures upon chromatographic purification. Unfortunately these reactions gave exclusively the undesired 4*R*-isomer which would lead to 4-alkyl-4-deoxy-4-*epi*-sialic acid analogues. As well, only Et₂Zn and Me₂Zn were able to be used effectively in the conjugate addition reactions as various benzyl and phenylzinc reagents were synthesized and did not add to the enone despite affording conjugate addition products with a model enone, 2-cyclohexen-1-one.

It may be possible to change the diastereoselectivity of the conjugate addition by using a chiral phosphorus-based ligand instead of P(OEt)₃. Several chiral phosphoramidite ligands have been developed that have been used in diastereoselective dialkylzinc conjugate additions and may be worth trying. It may also be that the proton of the 5-acetamido group is playing a role in directing the conjugate addition. Protection of the 5-acetamido group may solve this problem, although this has been found to be difficult. The most promising results were obtained when attempting to synthesize the *N*-acetylacetamido derivative and should be re-investigated. Another possibility is that the 5-membered rings of the two isopropylidene protecting groups are holding the enone in a conformation that only allows conjugate addition from one face. Removal of these groups and replacing them with protecting groups that allow more conformational freedom, such as acetyl groups, may influence the diastereoselectivity of the reaction. It may be possible to enhance the yields of the conjugate addition reactions by using a more reactive copper catalyst, copper(I) thiophenecarboxylate. As well, the synthesis of other dialkylzinc

reagents bearing smaller functional groups such as vinyl or allyl groups should be attempted and added to the enone. It may be beneficial to purify the dialkylzinc reagents through distillation or to find commercial sources of dialkylzinc reagents of acceptable purity since it is possible that addition of dibenzylzinc and diphenylzinc to the enone was inhibited by some by-product of their syntheses.

The conjugate addition products were able to be converted into sialic acid analogues. By deprotecting the hemiaminal products under acidic conditions, the sialic acid pyranose ring was formed in addition to a 2,7-anhydro linkage. These could be converted into 4-alkyl-4-deoxy-4-*epi*-DANA analogues in one pot. Although these DANA analogues may not be particularly interesting in terms of acting as sialidase inhibitors, the methods used to generate them could be used on other conjugate addition products, once synthesized. As well, these 4-alkyl-4-deoxy-4-*epi*-DANA analogues represent useful synthetic intermediates. Treatment with NBS in methanol would lead to a methyl 3-bromosialoside which upon treatment with a strong base might lead to elimination of HBr to form a double bond between carbons 3 and 4 and may lead to a new class of sialidase inhibitor. Treatment of the 4-modified DANA analogues with NBS in water would generate a 3-bromo hemiacetal from which the 2,3-epoxide could be formed and opened with a halide source, affording a potential donor sugar for glycosylation reactions and allowing these DANA analogues to be converted into substrates for sialidase-catalyzed hydrolysis.

A sialic acid analogue with an interesting bicyclic structure (**4.3**) was obtained by treating the β -sialosyl chloride with DBU. The resulting intramolecular elimination formed an oxabicyclo[3.1.0]hexane scaffold that could be a potent sialidase inhibitor. While the acetyl groups could be removed from this molecule, the ethyl ester proved much more challenging to remove as the nucleophilic reagents used attacked the anomeric centre resulting in the destruction of the bicyclic ring system. Reduction of the nitro group to an amine was also attempted in hopes that this would decrease the electrophilicity of the anomeric centre but under the conditions tested the cyclopropyl moiety reduced as fast or faster than the nitro group. The *t*-butyl ester analogue was synthesized and acidic deprotections were attempted but these conditions also induced ring-opening reactions. The most promising results were obtained from the use of pig liver esterase; therefore, a panel of esterases and lipases should be tested for removal of the ethyl ester from the bicyclic system. As well, it is likely that reduction of the nitro group to an amino group would afford a tighter-binding sialidase inhibitor; therefore conditions will have to be found for nitro reduction while keeping the cyclopropyl moiety intact. This may be accomplished by the use of tin(II) chloride or sodium borohydride-based reagents. Varying the conditions developed by Charette and co-workers for reducing nitrocyclopropanes may also be of value; these conditions use zinc dust in isopropanol in the presence of HCl and the use of highly active zinc and short reaction times may allow reduction of the nitro group before the cyclopropane ring is opened.

Several projects were described in this thesis and although the majority of them did not give rise to compounds that were used as substrates or inhibitors of sialidases, there are several promising aspects. A route to phenyl α -sialosides has been developed that can be used to make a number of 4-modified substrates for sialidase-catalyzed hydrolysis. This route could be improved as described earlier by using the α -sialosyl fluoride **2.19** to synthesize the 1-adamantyl thiosialoside donor sugar and conversion of the 5-acetamido group to an imide should enhance the α -selectivity of the glycosylation. A number of sialosides could be made via this route, including the fluorogenic 4-methylumbelliferyl sialoside. The synthesis of these sialosides containing nitrogen substituents in the 4-position should be completed and their rates of sialidase-catalyzed hydrolysis should be measured. This information would hopefully lead to the development of better sialidase inhibitors.

In addition, the 4-modified phenyl β -sialosides that have been synthesized should be tested with the Y370G mutant sialidase for hydrolytic activity, particularly the 4-amino derivative. If the mutant sialidase can hydrolyze the phenyl 4-amino-4-deoxy- β -sialoside, the possibility exists that the transferase activity of this enzyme can be taken advantage of to install this unnatural sialoside on various glycoconjugates with possible medical benefits.

Another highlight of this thesis is the serendipitous synthesis of the oxabicyclo[3.1.0]heptane-based sialoside **4.3**. This compound has an unusual structure that may provide access to a new class of sialidase inhibitors. Deprotection attempts should continue on this compound so that its sialidase

binding constants can be measured. This will most likely be achieved by testing a panel of esterases for their abilities to remove the ethyl ester of **4.3**. As well, the search for effective conditions for reduction of the nitro group that leave the cyclopropyl moiety intact should continue. It would also be of value to attempt the synthesis of the other diastereomer of **4.3** as this isomer may have better contacts between its substituents and active site amino acid residues.

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