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Synthetic Studies toward 2,3-di-N-Acyl-2,4,6-Trideoxy-L-Altropyranoses as Synthetic Precursors to Pseudaminic Acid

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Abstract

With the growing therapeutic inefficiency of traditional antibiotics by rapidly spreading antimicrobial resistance (AMR) through different mechanisms, and a significant slow-down in the development of novel antimicrobials, especially in the pharmaceutical industry during recent years, it is of utmost importance to maintain research to address this global challenge. The chemical synthesis of carbohydrate antigens that are unique to pathogenic bacteria can benefit the search for antibacterial therapeutics with the development of prophylactic vaccines such as polysaccharide conjugates. Bacterial nonulosonic acids (NonAs) that include pseudaminic (Pse) and legionaminic (Leg) acids are found in important structural components that contribute to certain pathogens' virulence, like *Pseudomonas aeruginosa* and *Campylobacter jejuni*: they have been recently shown to be good candidates for use as antigen epitopes in vaccination, and their biosynthetic precursors can also be used towards the development of other types of antibacterial therapeutics.

The research presented here begins with preliminary investigations into a synthesis from L-arabinose that has the potential to produce 5 different NonA structures with only a few appropriate variations in the scheme. The synthesis of two C5-(*R*)/(*S*) hexose diastereomers was achieved with different selectivity, and those can further undergo an inversion and installation of nitrogen functionalities on C-2 and C-4, before the final three-carbon extension with a phosphoenolpyruvate (PEP) equivalent to produce the target NonA. The work showed promise, justifying future development.

Next, a short, mild and scalable synthetic scheme towards 2,4-di-acetamido-2,4,6-trideoxy-L-altrose (Alt-diNAc), the biosynthetic precursor to Pse, is presented: the desired product was obtained from commercially available L-fucose in 10 steps and 23% overall yield, making it the most efficient synthesis published to-date. A further optimized shorter version of synthesis is described as well through regioselective sulfonyl activation to form a key epoxide intermediate, ultimately giving Alt-diNAc in 7 steps and 27% overall yield. Based on these achievements, a new and elegant methodology for the differentiable functionalization of the N2/N4 amide groups of Alt-diNAc was developed, which relies on a regiospecific O→N migration of acyl groups during a Staudinger reduction of the O-acylated di-azido precursor. The new methodology was proved to have broad scope and provides unprecedented versatility to introduce different N-acyl functionalities to the N5 and N7 positions of Pse.

Finally, preliminary work towards a potentially stereoselective three-carbon extension of hexose precursors to NonAs is described, with the synthesis of a phenol-based cleavable linker containing an α-methyl ketone that can potentially undergo aldol addition intramolecularly, and then ruthenium-catalyzed oxidation to produce the required carboxylic functionalities for Pse. A successful selective coupling of this linker to one of the two amido groups on the L-altro-configuration precursor was then achieved, paving the way to investigate the diastereoselectivity of intramolecular aldol additions with this strategy in the future. Several possible variations to the linker functional groups and length can easily be incorporated in this synthetic plan , and provide an exciting prospect for future developments.

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“Nothing in life is to be feared; it is only to be understood”

- Marie Skłodowska Curie,
first woman to win a Nobel Prize,
twice

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List of Abbreviations

Ace	Acinetaminic Acid
Alt-diN**Ac**	2,4-di-N-acetyl-2,4,6-trideoxy-L-altrose
Alt-diN**Acy**	2,4-di-N-acyl-2,4,6-trideoxy-L-altrose
AMR	Antimicrobial Resistance
APC	Antigen-presenting cell
Cbz	benzyloxycarbonyl
CF	Cystic Fibrosis
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
CPS	Capsular Polysaccharide
CRM	Corynebacterium diphtheriae
DBCO	dibenzocyclooctyne
DC	Dendritic cell
DMP	Dess Martin periodinane
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species
FDA	Food and Drug Administration
Fus	Fusaminic Acid
GN	Gram negative
GP	Gram Positive
Hib	*Haemophilus influenzae* type b
^{1}H NMR	Proton nuclear magnetic resonance
HRMS	High Resolution Mass Spectroscopy
IgM/IgG	Immunoglobin M/G
KDN	2-keto-3-deoxy-D-glycero-D-galactononic acid
KDO	2-keto-3-deoxy-D-manno-oct-2-ulosonic acid

LecB	Lectin B
Leg	Legionaminic acid
LPS	Lipopolysaccharide
mAB	Monoclonal antibody
ManNAc	N-acetyl-D-mannose
mCPBA -	meta-chloroperbenzoic acid
MHC-II	Major histocompatibility complex
MOE	Methoxy ethanol (ethoxy)
NBCD	Non-biological complex drugs
Neu5Ac	N-acetylneuraminic acid
NonA	Nonulosonic acid
OMV	Outer membrane vesicle
PCV	Polysaccharide vaccine
PEP	Phosphoenolpyruvate
Pse	Pseudaminic acid
RT	Room temperature
SARS-COV-2	Severe acute respiratory syndrome coronavirus 2
S-Layer	Surface layer
sRNA	Small ribonucleic acid
TBACl	*tetra*-butyl ammonium chloride
TCCA	trichloroisocyanuric acid
TLC	Thin-layer chromatography
TLR	Toll-like receptor
TS	Transition State
WHO	World Health Organization

Chapter 1 Introduction

1.1 The rise and fall of antibiotics

When penicillin was accidentally discovered in 1928 by Alexander Fleming,[1] it accelerated the coming of a "Golden Age" where humans finally took the upper hand in the ongoing war against our tiniest, yet arguably deadliest, adversaries – bacteria. As much as the discovery of β-lactams and other important antimicrobial treatments proved ground-breaking at the time, we knew from quite early on that this boom would only be temporary.[2] Antimicrobial resistance is a natural process and, coupled with our careless use and misuse of these valuable instruments over the last century, it has unfortunately reached its crescendo: new strategies for the treatment of bacterial infections are direly needed now more than ever.

One of the first instances of antimicrobial resistance was documented in *Streptococcus pyogenes* during World War II, following widespread prophylactic administration of sulfanilamides to soldiers as a way to prevent many types of infections.[3] Soon after the introduction of penicillin, a resistant *Staphylococcus aureus* strain was discovered in London civil hospitals in the 1940's, and a decade later the first multiple-drug resistant strains among *Escherichia coli*, *Shigella* and *Salmonella* were identified.[4] Global antimicrobial resistance has since multiplied exponentially, often referred-to as a resistance "tsunami,"[5] or more recently something even more "fast and furious".[6] Nowadays, terms like "superbugs" and "super-resistance" have become common when referring to bacterial infections, due to the advent of microbes with multidrug resistance that carry an enhanced risk of morbidity and mortality for the infected[7]: *M. tuberculosis* and *C. difficile* are notorious examples of this. An unfortunate

acronym has even been created recently, "ESKAPE" (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*), to group the pathogens able to completely side-step, or escape, the bactericidal activity of most antibiotics.[8]

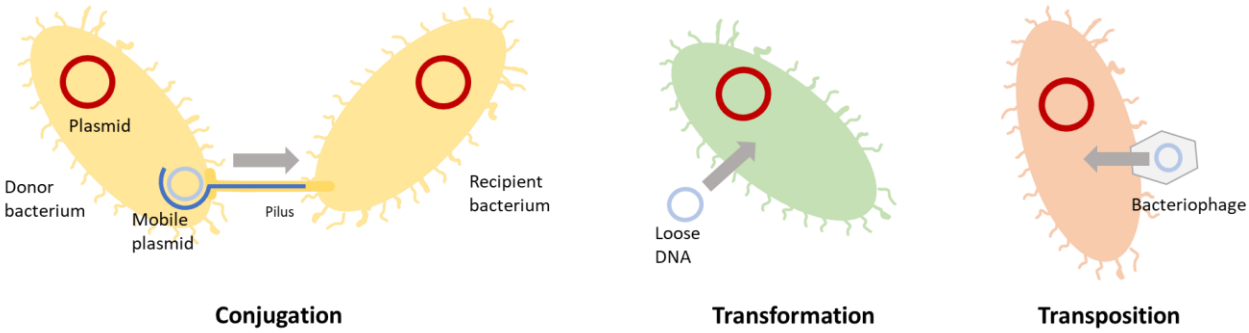


Figure 1.1 *– Horizontal genetic transfer between bacteria can be done through conjugation, transformation and transposition.*

Several factors are generally agreed to be the cause of the rapid rise of bacterial resistance to antibiotics. First, bacteria possess an intrinsic resistance to certain classes of antibiotics, which is quite out of our control, although some of these natural safeguards can be induced by the activation of dormant genes, or mutations following exposure to an antibiotic[9] - an inevitable side-effect of medical treatments. A more salient feature of bacterial processes is their ability to transmit genetic information through horizontal gene transfer, allowing them to spread genetic information through transformation (acquiring loose extracellular DNA), transposition (infection with DNA packaged in a bacteriophage) and conjugation (mating)[9] (**Figure 1.1**): antibiotic-resistant genes can thus be transferred between bacteria of the same strain, or of different taxonomic and ecological groups.[4] It is also important to consider that

traditional antibiotics, generally considered as broad-spectrum medicines, can target many bacterial strains with little discrimination: using these to treat one infection usually results in the transfer of antibiotic-resistant genes to the host gut microbiome, adding many more levels to the antibiotic resistance predicament.[10]

While natural modes of antimicrobial growth resistance play a big role in the currently evolving crisis, there is no denying that human behaviours, more precisely the carelessness with which we have used and abused antibiotics, have accelerated the inevitable by many orders of magnitude. The most notable anthropogenic activities, past and present, that contribute to the widespread antimicrobial resistance are: antibiotic use in agriculture and aquaculture, both therapeutic and prophylactic; household usage of antibiotics for pets (therapeutic and prophylactic), cleaning products and toiletries; their usage towards pest control, cloning in both agriculture and research; and finally, and most staggeringly, the large-scale disposal of waste from their manufacture and usage that inevitably causes the release of antibiotics into the biosphere.[7] While the above mostly apply to developed economies, it is worth mentioning the other socioeconomic determinants related to poverty in developing countries (and some "first-world" ones too) that contribute to antimicrobial resistance: poor access to health-care, coupled with poor hygiene in communities, hospitals and clinics that promote the spread of infections.[9] With the increasing pressures of population growth on these types of human activities, it is not surprising that the World Health Organization (WHO) has declared antimicrobial resistance as one of the top ten threats to global health in 2019[11]: with the recent advent of the SARS-COV-2 pandemic and its additional pressures on our world economy and health systems, many argue that leaving this issues unchecked will put in motion another pandemic.[12–14] Looking at recent

statistics, there were 4.95 million deaths worldwide associated to antimicrobial resistance in 2019, 1.27 million of those being directly caused by bacterial infections.[15] In 2016, the "Review on Antimicrobial Resistance" sponsored by the UK Government estimated that the number of associated deaths will reach 10 million annually by 2050 if the current trends continue.[16]

1.2 Mechanisms of Action of Antibiotics vs How Bacteria Resist Them

To understand the mechanisms of antibiotic resistance among pathogenic bacteria, an overview of the modes of action of major antibiotic families is in order[17] (**Table 1.1**). The mechanisms for antibiotic resistance among pathogens are abundant and are continuously evolving: antibiotics are designed to kill bacteria, allowing the ones that have naturally gained the necessary mutations to gain resistance to survive, flourish and multiply during treatment.

Table 1.1 *– Select examples of antibiotic families and their mechanisms of action against bacteria*

Antibiotic Family	Mechanism of Action
β-lactams	Cell wall synthesis disruption
Glycopeptides	Peptidoglycan synthesis disruption
Quinolones and fluoroquinolones	Block DNA replication
Aminoglycosides	Protein synthesis disruptions
Lincosamides	Translation of protein disruption
Rifamycins	Inhibit transcription and protein synthesis
Sulfonamides and trimethoprims	Disrupt nucleotide synthesis

Cell wall synthesis disruption is the main target of *β*-lactams, a class of antibiotics with a specific core structure capable of inhibiting penicillin-binding proteins, which in turns prevents them from cross-linking peptide chains during cell-wall biosynthesis, leading to bacterial cell lysis

when it is getting ready for division: this mechanism is particularly effective in Gram-negative (GN) bacteria, which have a much thinner peptidoglycan then their Gram-positive (GP) counterparts[18] (**Figure 1.2**). β-lactamases, for one, are bacterial enzymes that emerged in response to antibiotics derived from penicillin: they hydrolyze the amide bond of the β-lactam rings that are included in the structures of this class of drugs (**Figure 1.3**).

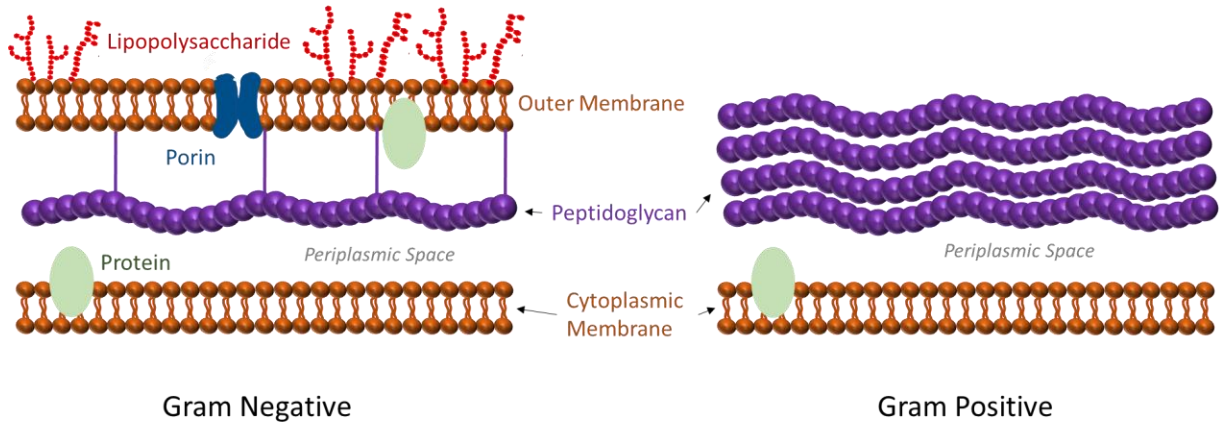


Figure 1.2 *– Structure of cell walls of Gram-positive and Gram-negative bacteria.*

Glycopeptides, which include the drug Vancomycin, are designed to inhibit the synthesis of cell wall peptidoglycans by binding to their building block dipeptide D-alanyl-D-alanine termini, causing deficient cross-linking through a different mechanism than *β*-lactams[18]. The weaker cell walls make the bacteria more sensitive to osmotic damage, as well as ultrasound: this type of antibiotic is used in the treatment of serious infections caused by drug-resistant GP bacteria. Vancomycin, a large cationic molecule, has been losing its potency lately because of a decreased permeability of bacterial cytoplasmic membranes caused by the reduction of the number of bacterial wall porins or the changing of their differential expression (**Figure 1.3**). This mechanism

of resistance has been affecting a wide range of hydrophilic drugs, which depend on porins to access the cell interior.

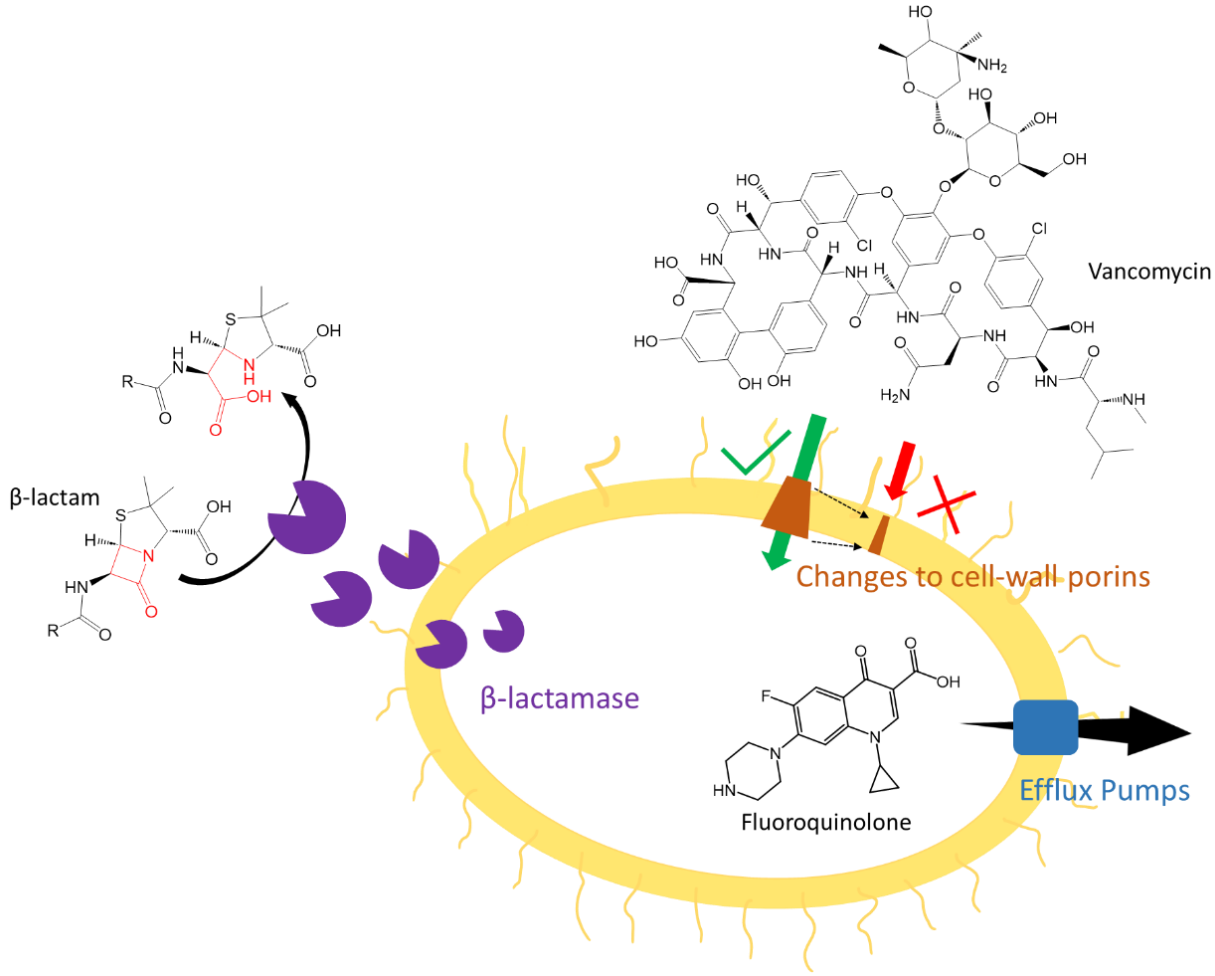


Figure 1.3 *- Select antibiotics and associated resistance mechanisms developed by bacterial cells.*[17]

Quinolones and fluoroquinolones, such as ciprofloxacin, block DNA replication in bacteria by binding to proteins involved in DNA supercoiling, strand-cutting and ligating: at sufficiently consistent exposure to the drug, these complexes become stable and persistent enough to cause cell apoptosis. One resistance mechanism to prevent this thus became the appearance of efflux pumps in bacteria capable of extruding these toxic molecules (**Figure 1.3**).[17]

Aminoglycosides, including gentamicin and streptomycin, are an important class of antibiotics as well, the mechanism of action of which is generally agreed to proceed through interactions of the many amino groups on the drug with bacterial ribosomes, which in turn causes toxic mistranslated proteins and resulting cell death. Mutations to counter this effect consist of gaining new abilities to synthesize aminoglycoside-modifying enzymes such as acetyltransferases and phosphotransferases. Mutations in small RNA (sRNA) genes are also effective resistance mechanisms, and can work to inhibit the action of lincosamides, such as clindamycin, a class a antibiotics that also target the translation of proteins.[17]

Rifamycins target the bacteria's DNA-dependent RNA polymerase by strongly binding to its β-subunit, hence inhibiting transcription and general protein synthesis. Sulfonamides and trimethoprim are used synergistically to inhibit several steps in bacterial nucleotide synthesis, especially thymine, the deficiency of which causes cell death. The effects of both antibiotic treatments have been attenuated by key mutations in the drug bacterial gene targets, in addition to some of the previously mentioned resistance mechanisms.[17]

1.3 The Need for Novel Antibacterial Strategies

The rapid emergence of drug-resistant strains of bacteria to new antibiotics has considerably shortened their clinical lifespans, making it difficult for pharmaceutical companies to recuperate their initial investments for research and clinical trails. As a result interest in the discovery of novel antibiotics by big pharma has significantly decreased during recent years. As a matter of fact, only a few new antibiotic classes have emerged from clinical trials in the last 20

years, and these are only effective against GP bacteria.[19] Oxazolidones, first synthesized in 1987, act as inhibitors for protein synthesis by binding to specific sites on bacterial ribosomes: linezolid was approved by the FDA for the treatment against infections caused by methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium* and penicillin-resistant *S. pneumoniae*. [20] There has already been detection of resistance mechanisms associated with this type of drug, consisting of genetic changes to the domains involved in the binding of the drug to bacterial ribosome.[21] Peptides with looped molecular structures, such as daptomycin, are another type of novel antibiotics that act either via depolarization by inserting the lipophilic tail into bacterial cell wall membranes, or by preventing the synthesis of lipids involved in cell walls.[22] There has not been any significant innovations in drugs against GN bacteria since the discovery of quinolones in 1962.[19] The major advantage of GN over GP bacteria is their second outer cell membrane made up of phospholipids and strain-specific lipopolysaccharides (LPS), also containing porins and efflux pumps (**Figure 1.2**). This extra layer blocks access to the peptides found on the first outer membranes usually targeted by drugs, and the pumps additionally extrude any drugs that make it into the periplasm.[23]

Most antibiotic treatments nowadays fall within the above-described drug families, including ones that are currently undergoing clinical trials, meaning that they will likely become ineffective in the coming years due to already-existing resistance mechanisms.[2] There have been numerous efforts to discover new natural products and their synthetic derivatives as antimicrobial molecules, including target-based high-throughput screening programmes conducted by GlaxoSmithKline and AstraZeneca, but novel therapeutics among these are rare.[24] Despite the discovery of hundreds of bacterial sites that could be potential drug targets, new

drug candidates are usually not passed into clinical trials due to factors like inefficient uptake of the compounds into bacterial cells, toxicity toward human cells, and lack of broad-spectrum activity against bacteria. Nature is a great source of novel antibiotic candidates due to the fierce competition among organisms for survival, but it seems like it can no longer be relied on for new antimicrobials, and we can no longer afford to wait for it to come up with novelty. However, despite the lack of interest of big pharma to continue to invest in antimicrobials due to the so-called "Innovation Gap", important research continues in smaller research institutions and academia.[25] These efforts encompass medicinal chemistry, improved screening and discovery using the more sophisticated tools of this decade, along with the search for "alternative approaches", where treatments do not fit the traditional definition of antibiotics as "direct-acting small molecules". This last category includes monoclonal antibodies, enzymes, virulence inhibitors and immunomodulating agents, and will be covered more thoroughly in the next section.

1.4 Alternative Antimicrobial Treatment Strategies

As a substitute to targeting bacterial processes that stop their multiplication or cause their death, drugs targeting virulence factors to "disarm" bacteria are promising and have been getting increased attention in the last two decades.[24] Targets for these molecules have included bacterial cell-to-cell communication processes, toxin and exotoxin secretions, bacterial abilities to adhere to (pili) or invade (flagella) host cells, form protective biofilms, and finally enzymes that are produced by the pathogens for host invasion, immune evasion and virulence regulation (**Figure**

1.4).[26–28] By targeting non-essential bacterial processes, there is less selective pressure for resistance to evolve in the bacteria: additionally, the targets can be very specific to certain bacterial strains and therefore less toxic to humans and to commensal bacteria populations living in the gut. Disabling these virulence factors can decrease the pathogens' rate of infection and make them more vulnerable to clearance by the host immune system.[27] Some drawbacks of this approach include the narrow scope of developed drugs as they target pathogens that share a certain virulence factor, a potential need to target several virulence factors at once with several different drugs for efficiency, and the probable need to co-administer the treatment with an established antibiotic for certain immunocompromised or high-risk patients.[27]

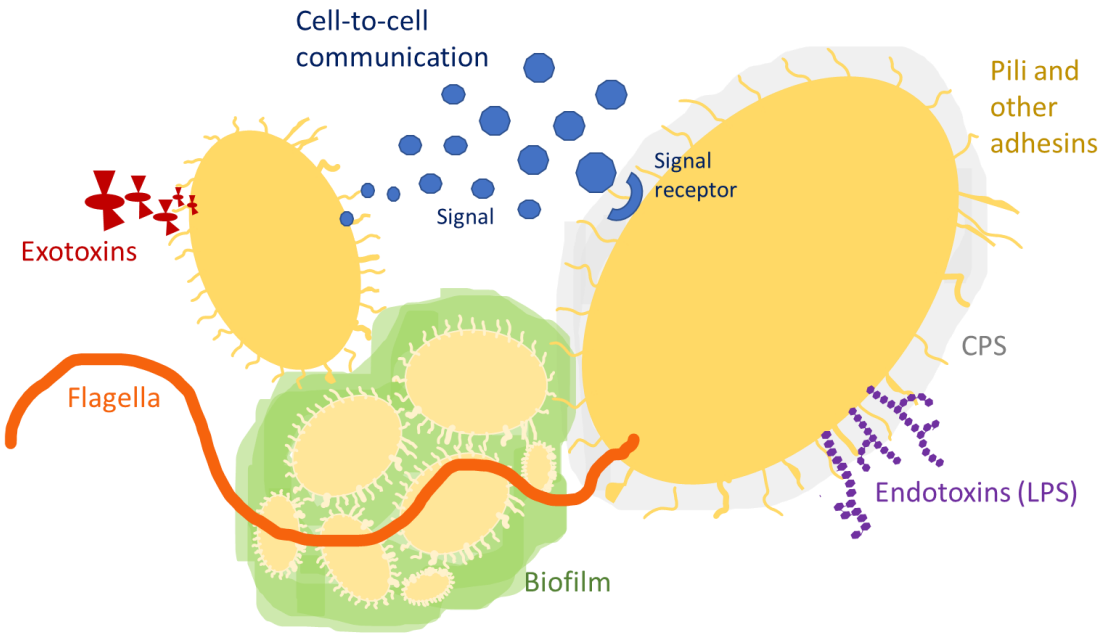


Figure 1.4 *– Select examples of bacterial features that contribute to their virulence*

Several compounds for this type of treatment have recently made it into phase I/II clinical trials: Ftoriazinone, a toxin-secretion system inhibitor that has shown activity against GN bacteria

in murine models[29]; CAL02, an engineered liposome that intercepts bacterial toxins that usually bind to host cell membrane lipids[30]; ALS-4, a small molecule inhibitor of an enzyme that produces a protective pigment in *S. aureus*, causing the bacteria to be exposed to oxidative stress and increases its clearance in animal infection models.[31] Carbohydrate-based small molecules as antibiofilm agents have recently shown promising results as well, especially with regards to inhibiting bacterial carbohydrate-binding protein called lectins. These are believed to be bridging agents between the bacteria and host cells, and between bacteria themselves, allowing the bacterial aggregates needed for robust biofilm formation.[32] Orally bioavailable LecB inhibitors were synthesized and tested *in vitro* by Sommer *et al*[33] for blocking biofilm formation in *P. aeruginosa*, with promising results. Alginate oligosaccharides have also undergone phase III trials as biofilm disruptors when administered along with the antibiotic aztreonam to *Burkholderia cepacia* complex-infected cystic fibrosis patients, showing a favorable safety profile and warranting more research.[34,35]

Monoclonal antibody (mAb) therapies, often used in the treatment of cancers, autoimmune disease and more recently COVID infections are proteins mainly designed to bind to antigens on the surface of pathogens to label them as targets for host immune system clearance. While clinical studies have not produced any promising results to show that this strategy could work with bacteria directly, several mAbs are currently undergoing clinical trials that have shown to bind to their secreted toxins instead, including the *S. aureus* α-toxin, as well as proteins from the DNABII family, causing the disruption of biofilm produced by *S. aureus*, *P. aeruginosa*, and *A. baumanii.*[24]

Another noteworthy strategy lies in non-biological complex drugs (NBCDs), which have high molecular mass, are often nanoparticulate structures and have shown to provide a promising and novel alternative to small-molecule antibiotics.[36] Because of how different they are from traditional antibiotics in size and composition, it is believed that their mechanism of action against infection could be distinct enough to overcome the resistance mechanisms currently circulating among pathogens. On the flip side, the fact that NBCDs are so big and so heterogenous often makes them difficult to characterize, which makes their mode of action against bacteria hard to define. NBCDs include polymers, polymeric nanoparticles, inorganic nanomaterials, carbon-based nanomaterials and combinations of some or all of the above.

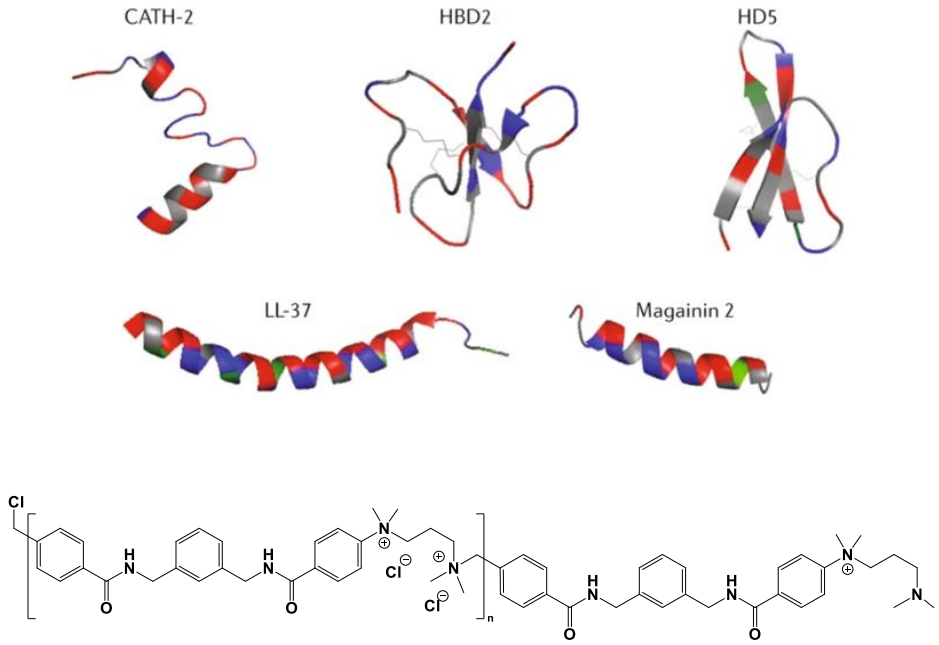


Figure 1.5 *– Top: Examples of cationic host defense peptide structures from vertebrates with amino acid side chains colored as follows: red for hydrophobic, blue for basic and green for acidic (reproduced with permission from Springer Nature). Bottom: Synthesized polyionene containing rigid amine motifs with distinct hydrophobic and hydrophilic sections.*[37]

Antimicrobial polymers that mimic host-defense peptides, for one, are being synthesized and tested with an increasing possibility for structural precision, and therefore specificity, due to

advances in polymer chemistry[38]: just like their natural counterparts, they can be designed to contain a variety of cationic, hydrophilic and hydrophobic groups capable of interacting with anionic bacterial membranes in disruptive ways (**Figure 1.5**).[39] For example, Lou *et al* designed a polyionene with rigid amide bonds that showed activity against the multidrug resistant *K. pneumonia* in murine lung infection models, with negligible toxicity and no sign of the development of resistance.[37]

1.5 Vaccines as a Preventative Strategy

Faced with the limited arsenal of available treatments against bacterial infections, it becomes apparent that a viable strategy for combatting pathogens is one that relies on prevention, with prophylactic vaccination at its helm. Vaccines have been proven to have long-lasting beneficial effects, while resistance to them is relatively rare.[40,41] The key to their success lies in two factors: timing and therapeutic redundancy. First, since vaccines are prophylactic, the response to infection is swift, keeping pathogen population low and giving them less chances to develop mutations that become problematic. The other advantage of vaccines lies in the exposure of the host immune system to multiple antigens and multiple epitopes, giving the response to infection multivalent attack opportunities: pathogens are less likely to mutate all of their antigens at once.[42]

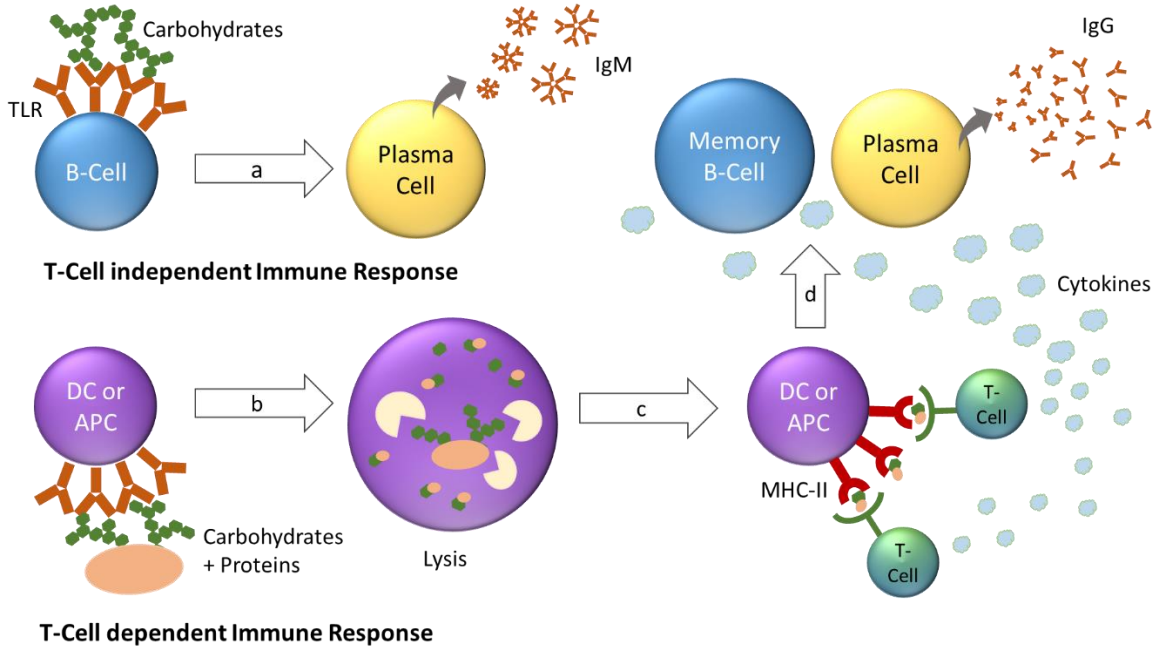
The vaccine advantage in the face of antimicrobial resistance is quite evident, and has been the subject of numerous studies: achieving herd immunity against a disease reduces the circulation of resistant bacterial strains, and the decreased pathogen carriage of people who get

infected means that antibiotic treatment can also be reduced.[43] Another consideration is that administering vaccines to food-producing animals would be a very desirable alternative to antibiotic use, if the appropriate vaccines existed, and would reduce the overall need for the wide-spread prophylactic usage of antibiotics.[44]

Many examples of different types of successful or partially successful antibacterial vaccines exist, including candidates that regularly reach different stages of clinical trials. The *Haemophilus influenzae* type b (Hib) vaccine was introduced into routine infant immunization schedules in the USA in 1987, and was shortly afterwards adopted globally, causing a well-documented dramatic reduction in the incidence of Hib disease, and the associated mortality among children in particular.[45] The global use of this vaccine successfully turned the tide against Hib antimicrobial resistance, which had been observed to steadily grow in the form of β-lactam resistance in the early 1970's. The Hib vaccine is a capsular polysaccharide (CPS) conjugate vaccine: while the protective polysaccharide external layer that compromises many bacteria alone was unable to induce antibody responses in infants or children when used as an antigen in vaccines, it was found that covalently linking, or conjugating, bacterial CPS to a carrier protein produced the desired immunogenic response across all age groups. In the case of the Hib vaccine, the carrier protein used was the outer membrane protein complex of serogroup B meningococcus, which is one of five carrier proteins currently included in conjugate vaccines, the others being: diphteria toxoid, tetanus toxoid, CRM_{197} and *Haemophilus* protein D.

The importance of using carrier proteins in carbohydrate-based conjugate vaccines is well investigated, as carbohydrate antigens alone typically only trigger a short-lived T-cell independent immune response, producing low-affinity antibodies called immunoglobulin M

(IgM), because they are only able to activate B-cells via carbohydrate-specific toll-like receptors (TLR) (**Figure 1.6**). The long-term, more specific and robust IgG antibodies are induced through the T-cell-dependent pathway, which requires the presence of short peptide chains, like the ones found on carrier proteins. In this pathway, detection by dentritic (DCs) or other antigen-presenting cells (APCs) is followed by phagocytosis of the short peptide chains covalently linked to the carbohydrates, which are then presented as T-epitopes in complex with major histocompatibility protein (MHC-II) on the surfaces of DCs and APCs. Only then can T-helper cells recognize these antigens and get activated, releasing the cytokines necessary to stimulate B-cells to in-turn differentiate into IgG-producing plasma cells, as well as into the longer-lasting memory B-cells (**Figure 1.6**).[46]



Steps: (a) B-cell proliferation and differentiation after encountering carbohydrate antigens alone; **(b)** Phagocytosis of carbohydrates conjugated to protein carriers by DCs and APCs; **(c)** Presentation of epitopes in complex with MHC-II; **(d)** Proliferation and differentiation of B-Cells into memory B-cells and IgG-producing plasma cells, catalyzed by cytokines produced by T-cells, among other factors.

***Figure 1.6** - Schematic illustration of T-cell independent and T-cell dependent immune responses during vaccinations using glycoconjugate vaccines.*

Other successful examples of polysaccharide conjugate vaccines (PCVs) against bacteria include the pneumococcal conjugate vaccine, which demonstrated a 90% efficacy against *S. pneumoniae*, some isolates of which had been exhibiting resistance to penicillin and other antibiotics in the 1990's. Advantageously, PCVs offer the possibility of multivalency, where one or more serotypes of one or more different strains of bacteria can be presented: for pneumococcal vaccines, the initial version covered 7 serotypes, and the updated one introduced in 2009 includes 13 serotypes. The revision to pneumococcal PCV has been tremendously successful in reducing the occurrence of the disease and its resistance to antibiotics, and current research continues to incorporate more bacterial serotypes to address infections by the ones not included in the current versions.[45]

Other types of antibacterial vaccines besides PCV had undergone early and late-stage clinical development at the time of writing, a few examples of which are worth detailing. The GP superbug *C. difficile* is an important target for the preventative vaccine strategy, where recent developments have focused on vaccine formulations comprised of genetically and chemically inactivated versions of the large clostridial glucosylating toxins, TcdA and TcdB, responsible for the cytotoxicity, inflammation and terrible diarrhea causes by this bacterium.[47] These modifications were shown to leave the major epitopes undisturbed, allowing neutralizing host antibodies to bind them, with the expectation of inducing an immune response that could potentially prevent the disease in inoculated patients: among others, the patented P-06425090 Pfizer vaccine had undergone phase III clinical trials, showing that the vaccine decreased the severity and time of the disease.[48] As expected with a vaccine containing toxoids alone, initial infection was not averted, thwarting the effort to prevent transmission between patients. A

vaccine to combat *Mycobacterium tuberculosis*, the M72/ASO1$_E$ candidate, was engineered to contain several highly immunogenic protein antigens as well as T-cell stimulating components, the combination of which was chosen following extensive research.[49] This peptide vaccine has shown a lot of promise for efficiency and safety through numerous clinical trials,[50] and is a good example of how a combination of a variety of antigens makes antibacterial vaccines more efficient. *Neisseria gonorrhoeae* is another multidrug resistant bacterium that has been the subject of much research over the last decades, especially towards producing a gonococcal vaccine. The vaccine strategy for this case is complicated by the extreme diversity and phase variation of the bacteria's surface proteins and oligosaccharides, making it highly challenging to find appropriate and usable antigens.[51] Four vaccine candidates that made it to clinical trials did not provide adequate protection in the end: these either contained the whole bacterial cell, a partially autolyzed cell, or were pilus-based or protein I-based.[52] The only promising strategy in this case is through outer membrane vesicle (OMV) based vaccines, which have resulted in some protection against gonorrhea. OMV's are made up of a mixture of outer membrane components that are excreted by the bacteria itself, and can be harvested from bacterial cultures via detergent extraction.[53] Advances in metabolic engineering have enabled more efficient OMV production and collection, in addition to providing a means to modify the OMVs in a way that could improve their immunogenicity, such as "detoxifying" them to contain less reactogenic lipid polysaccharide (LPS) structures, and remodelling the carbohydrate portion of LPS to contain a wider variety of antigens.[54]

1.6 A special case: Pseudomonas aeruginosa

Among today's multidrug resistant bacteria, *Pseudomonas aeruginosa*, part of the infamous ESKAPE group, was declared as one of the WHO 2018 top three critical pathogens to urgently be addressed through research.[40] In addition to the difficulty in treating it, no potential vaccine candidates have made it past early stages of clinical trials, despite substantial effort from the research community. This GN bacterium is known for its high natural immunity to antibiotic treatments, in big part because of its ability to produce biofilm, and is very apt at developing adaptive resistance mechanisms to the antibiotics that do affect it.[55,56] Although aggressive treatment during early colonization has been found to be successful for up to 27 months, with better pulmonary function in the long run,[57,58] total eradication of this bacterial strain is unlikely, especially after the development of chronic pseudomonal infection in people with Cystic Fibrosis (CF).[59] Unfortunately, complications due to lung infection by this pathogen remain the highest cause of mortality in individuals suffering from CF.[60] *P. aeruginosa* is additionally associated with a variety of clinical diseases, medical equipment contamination and colonization, and infections in immunocompromised and vulnerable patients in hospital settings, especially critical burn patients and patients requiring the use of ventilators, catheters or bronchoscopes.[61] In the same year as this thesis was written, the bacterium made headlines in the USA when it was found to have contaminated eye drops, causing vision loss in 8 people, enucleation in 4 and even death in 3 cases.[62]

Several types of vaccines have been synthesized and tested over the last fifty years against *P. aeruginosa*, including LPS conjugates, attenuated whole cells, outer membrane protein formulations and flagellar antigens.[61] Many of these were able to produce good immunogenicity

and safety profiles, but none were shown to make any statistical difference in outcomes for patients treated with the vaccine when compared to placebos. In other words, although the tested vaccines were able to induce the production of antibodies and other related immune responses, it seems that more information is required about the mechanisms of *P. aeruginosa* infection and what biological elements are needed for a protective immune response. The discovery of new potential antigens, adjuvants (boosters of immune response) and combinations of these to stimulate the immune response at all the required levels remain a priority in vaccine research against this tenacious superbug.

1.7 Nonulosonic Acids

Pseudaminic acid (Pse) (**101, Figure 1.7**) was first discovered and characterized by Knirel *et al* in 1984 from the LPS of *P. aeruginosa* and *Shigella boydii* – it was found to be a new type of sialic acid, later confirmed to be unique to bacterial structures.[63,64] As part of the nonulosonic acid (NonA) class of carbohydrates, Pse is an 9-carbon 3-deoxy-α-ketoacid, different from the mammalian N-acetylneuraminic acid (**102**, Neu5Ac) by containing an amido group on C-7 instead of a hydroxyl group, and by lacking the hydroxyl group on C-9 entirely, in addition to different chiralities at C-5, C-7 and C-8 (**Figure 1.7**). Later on, the 5,7,8-epimer of Pse was discovered in 1994 on an LPS from *Legionella pneumophilia*[65] and appropriately called legionaminic acid (**103**, Leg), and since then a variety of prokaryote-specific NonAs have been characterized, including Leg 8- and 4- epimers (**104** and **105**) and the more recently discovered acinetaminic (**106**, Ace) and fusaminic (**107**, Fus) acids (**Figure 1.7**). Derivatives of all of these compounds have been

found on structural components of many pathogenic bacteria, including the flagellin, LPS, CPS and S-layers of *Campylobacter jejuni, Helicobacter pylori, Acinetobacter baumanii,* and many more, with several different variations in their 5,7-amido functionalities.[66]

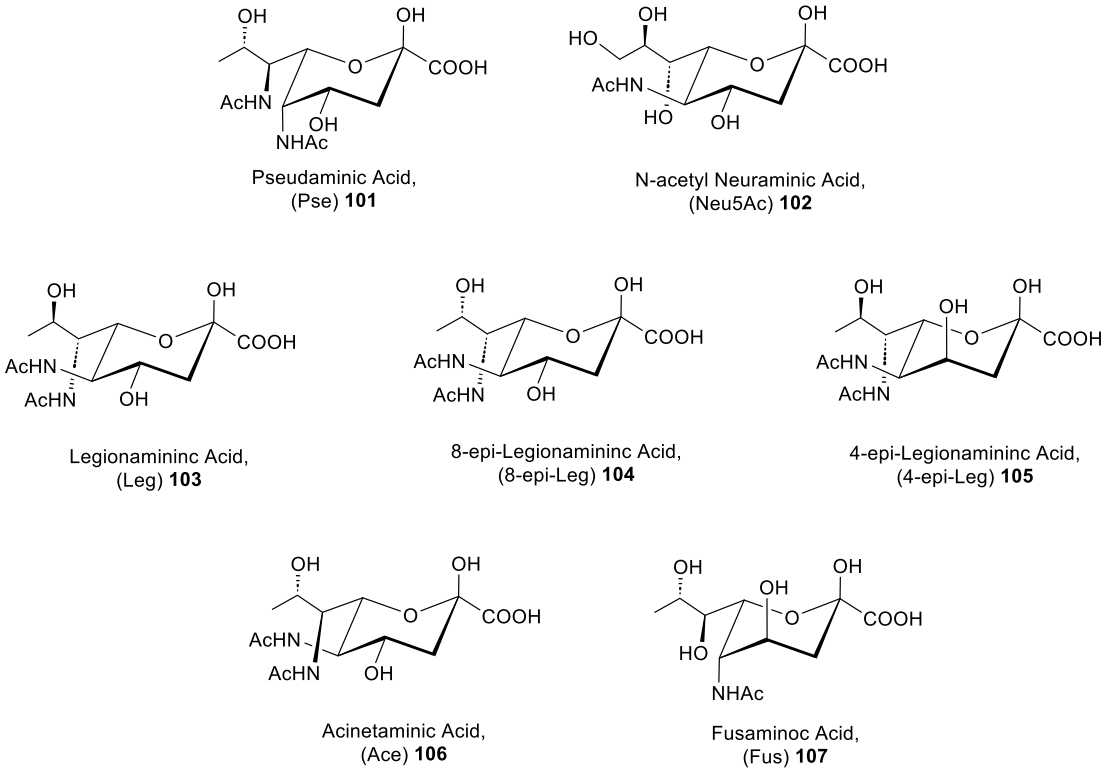


***Figure 1.7** - Structure of selected bacterial nonulosonic acids*

The physiological role of these bacterial NonAs has been studied extensively, but much remains to be discovered. In many pathogens, Pse and Leg residues heavily decorate flagellin proteins in a way that significantly contributes to their motility, adhesion to host cells and general virulence.[66–68] Another interesting potential role of these carbohydrates in glycosylated bacterial S-layers is the downregulation of host immune responses, due to their structural similarities with eukaryotic sialic acids, including the human carbohydrate Neu5Ac. Studies with *Tannerella forsythia*[69] and *C. jejuni*[70] have shown that Pse likely binds to host immune system glycan

receptors siglec-10, which in turn promotes the induction of interleukin-10, an immune response suppressant.

The chemical and chemoenzymatic syntheses of Pse, Leg and other bacterial NonAs towards different goals have been given much consideration since their discovery, with some important breakthroughs in the last decade that will indisputably advance the research for therapeutical applications. While their synthesis is challenging, it is important to achieve in scalable quantities, as it is difficult to extract a sufficient amount of the compound in monodisperse polysaccharides from nature, and the pseudaminic linkage is quite sensitive and labile[71]: relevant synthetic schemes towards these compounds will be provided and discussed in the chapters of this thesis. Once obtained in pure form, these structural components can then be incorporated into oligosaccharides and/or conjugated to protein carriers towards biological and medical investigations. As such, The Li group[72] produced a scalable synthesis of the *P. aeruginosa* 1244 pillin trisaccharide **108**, selectively achieving an α-(2→4)-linked Pse-xylose linkage (**Figure 1.8**), matching the structure of the glycan that coats the surface of the bacterium's pili, and plays significant roles in adhesion to host tissue and colonization.[73] It had previously exhibited some immunomodulating properties in mice[74] and its efficient synthesis can provide homogenous samples of this potential antigen that will facilitate further investigations for vaccine development. Following Yang *et al*[75] reports that polysaccharides containing Pse isolated from *A. baumannii* strain 54149 were highly immunogenic when conjugated to a carrier protein and administered as vaccines to rabbits, the Li group[71] demonstrated that a synthetic Pse molecule alone, conjugated to a carrier protein, could elicit a similar immune response. Their synthesized Pse-CRM_{197} conjugates (**109**) with different antigen loadings containing a novel

ortho-phthalaldehyde-Pse linker stimulated high immune responses in mice, protecting them from infection by *A. baumannii.*

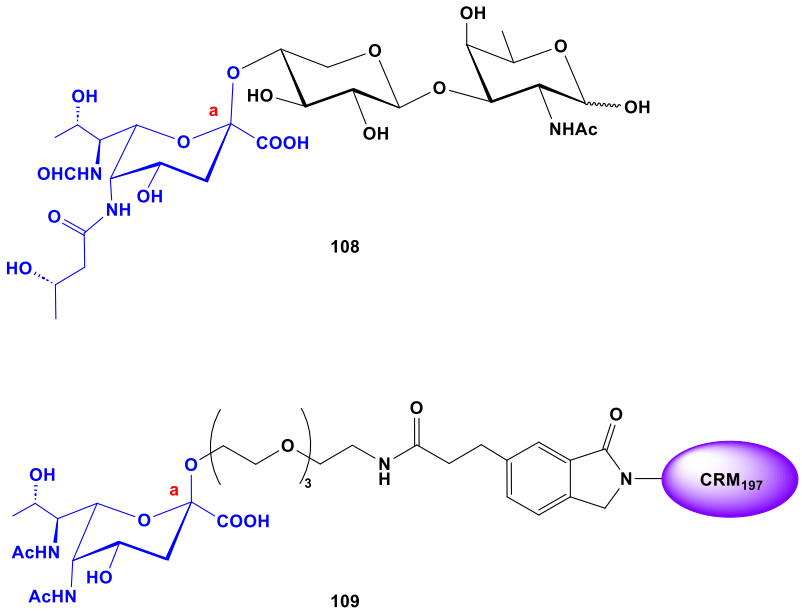
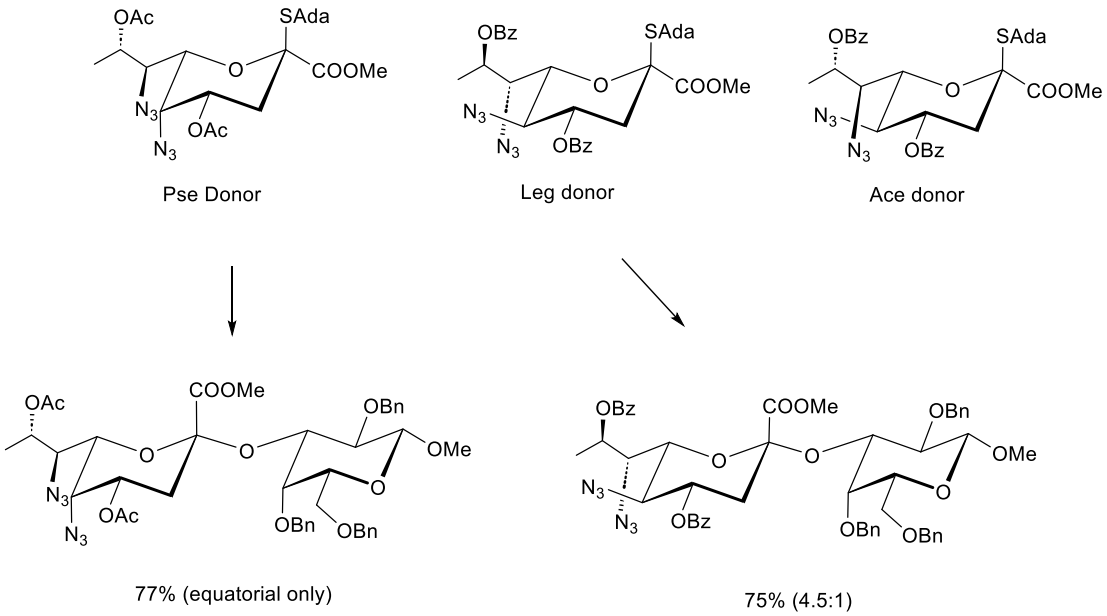


Figure 1.8 *– P. aeruginosa 1244 pilin trisacharride* ***108*** *synthesized by the Li group with the Pse component in blue,*[72] *and the chemically synthesized Pse-CRM*$_{197}$ *conjugate* ***109*** *with an ortho-phthalaldehyde linker synthesized by the Li group.*[71]

In nature, Pse has been found to be linked in both the α- and β-anomeric configurations in bacterial cell surface components and glycosylated flagella and pili, which is unusual for NonAs.[76] Unlike the typical terminal position occupied by human sialic acids on glycans, Pse is often found in the middle of the oligosaccharides decorating LPS and CPS structures on bacteria, acting as a glycosyl acceptor through derivatives of the amido functionalities on C-5 and C-7, in addition to being a donor.[77] As with the Li group, Pse donor syntheses usually produced selectivity for axial glycosylation.[72,78,79] The Crich group worked on a method for the stereoselective equatorial glycosylation, and applied it successfully for their synthesized Pse[80] and Leg[81], and more recently Ace[82] precursors (**Scheme 1.1**), which was an important step in producing

glycosylation donors that provide access to different linkages in the required saccharides, oligosaccharides and/or conjugates that must be used for therapeutic application research. Their strategy consisted of synthesizing adamantanyl (Ada) glycoside donor, which they found selectively formed equatorial linkages following glycosylation under standard conditions (**Scheme 1.1**).



Reagents and Conditions: ROH, NIS, TfOH, 4Å MS, CH_2Cl_2, MeCN

***Scheme 1.1** – Pse, Leg and Ace glycoside donors synthesized by the Crich group[80–82] and the selective glycosylation that gave the desired equatorial anomers as the only product for Pse, and the major one for Leg.*

Investigations into the biosynthetic pathways of NonAs have lately gained traction in the scientific community. These are important because not only could they provide a "greener," more selective, and even more efficient chemoenzymatic synthesis of NonAs, they could also help with discovering and characterizing the different enzymes involved in producing and glycosylating the variety of NonAs found in nature, and more importantly studying the

mechanisms of action involved. A potential therapeutic benefit of gaining information about NonA biosynthetic pathways could be the ability to discover and develop enzyme inhibitors as a novel antibacterial strategy.[83] Following the experimental evidence related to the role of Pse and other NonAs in virulence, a therapeutic treatment that interrupts the biosynthesis of Pse and others could help manage some of the symptoms associated with infection. The Fascione group recently produced an optimized chemoenzymatic syntheses of Pse, using genes from *C. jejuni* that encode the six biosynthetic enzymes involved in the process,[84] as well as demonstrated for the first time an enzymatic glycosylation of a Pse donor using a sialyltransferase to afford β-Pse-terminated glycosides.[85] Although their results were limited to Pse with a 5,7-di-N-acetylation pattern, and only produced their target in milligram quantities, their success indicates that research in this direction is ripe with opportunities.

The above overview of the recent applications of synthesized NonAs, especially Pse, demonstrates their importance towards the development of novel antibiotics against pathogens containing these unique glycan structures, many of which are multi-drug resistant. Having access to gram-quantities of the pure compounds is quite important to assure this type of research continues: the different published and developing chemical syntheses of Pse and Leg will be the focus of the following thesis. Each chapter will provide details on the various strategies used in the last decades to address the challenges of synthesizing Pse and other NonAs, with a focus on the important contributions our group has brought to the overall goal of developing expedient, versatile and efficient schemes for their synthesis, including two publications (Chapters 3 and 4). Please note that due to the extensive nature of the work presented in thesis, the numbering

format for figures, tables, schemes will be associated to the chapter number as decimals, and compounds will be numbered as integers associated to the chapters as well (ex. compound **101** is the 1st compound in Chapter 1, and compound **313** is the 13th compound in chapter 3), and some compounds will appear in more than one chapter with a different number to allow for easier referencing (ex. Pse appears in this Chapter as compound **101** in **Figure 1.7**, and will reappear in Chapter 2 as compound **206** in **Scheme 2.1**). The experimental information for each chapter will appear at the end in its own section (Experimental, p.141), which is organized by chapter as well.

Lastly, to help the reader better understand the configurational transformations involved in carbohydrate chemistry, **Figure 1.9** and **Figure 1.10** on the nomenclatures of aldopentoses and aldohexoses of both L- and D-families are provided below for reference.

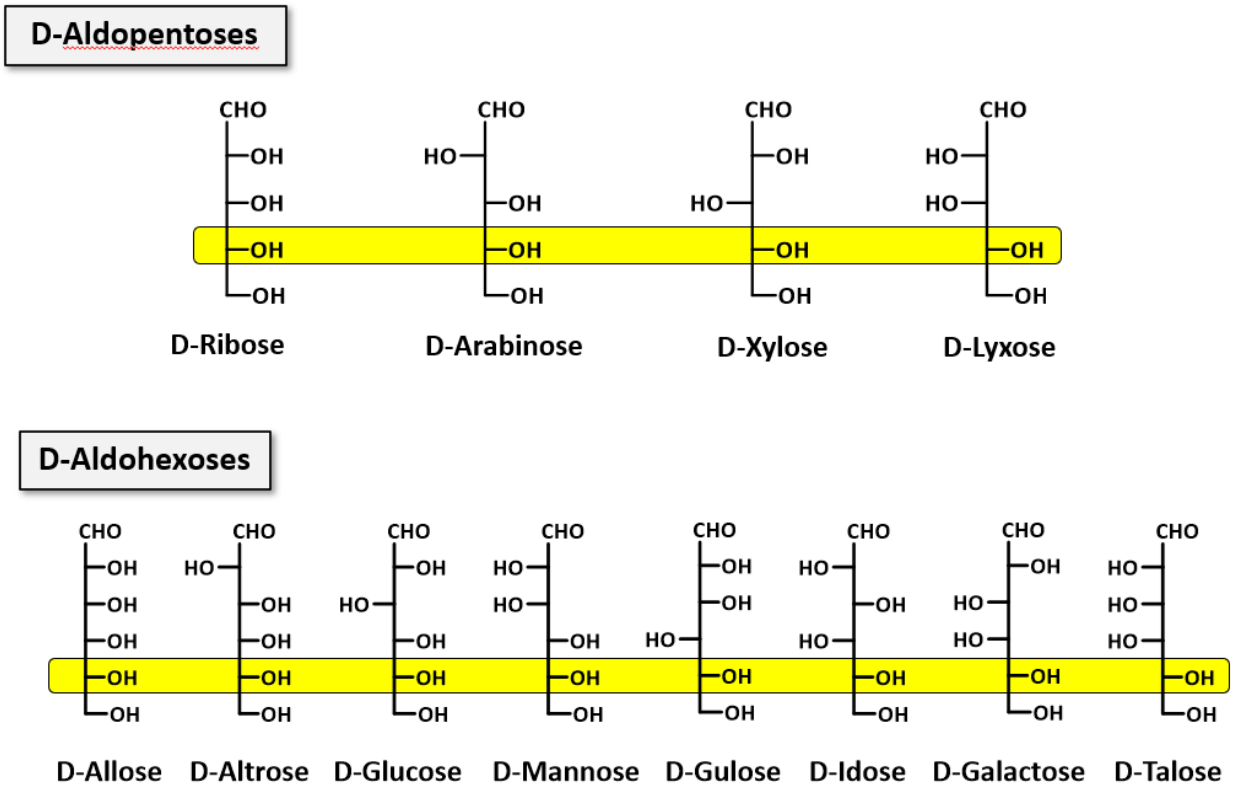


Figure 1.9 *– Overview of the nomenclature for D-conformers of aldopentoses and aldohexoses*

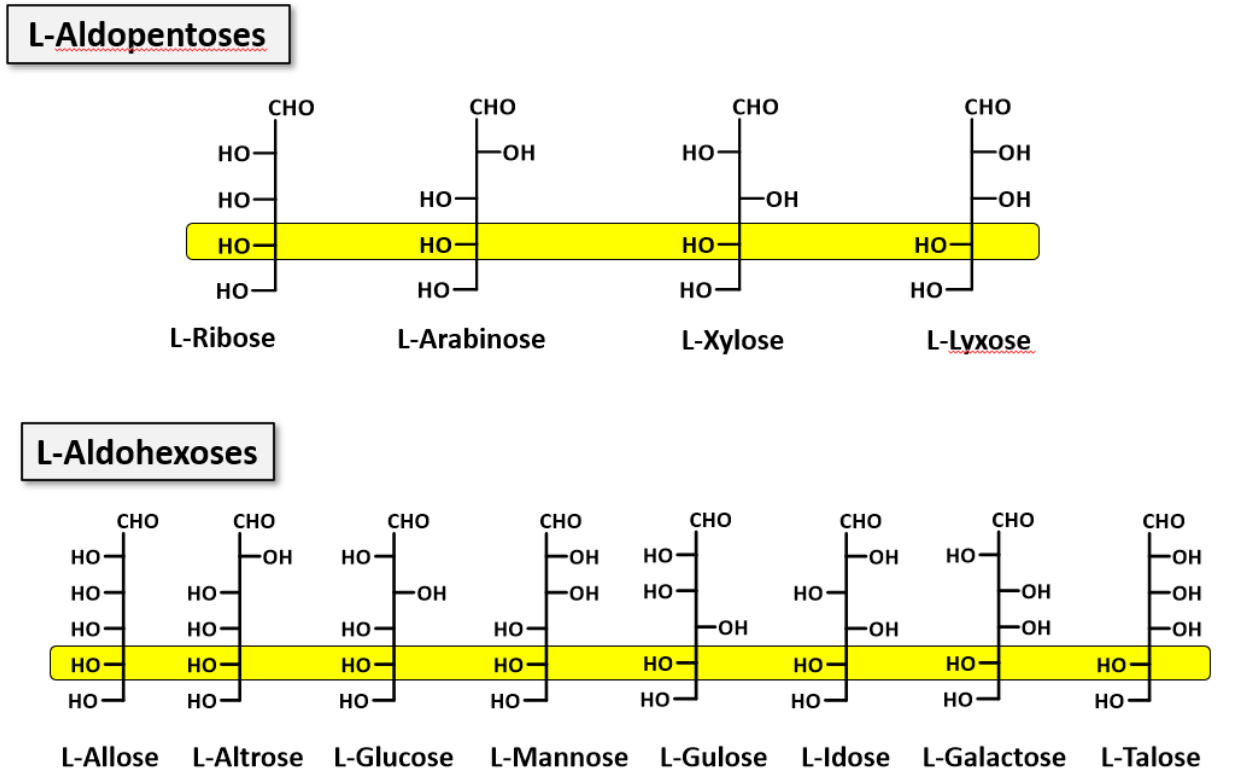


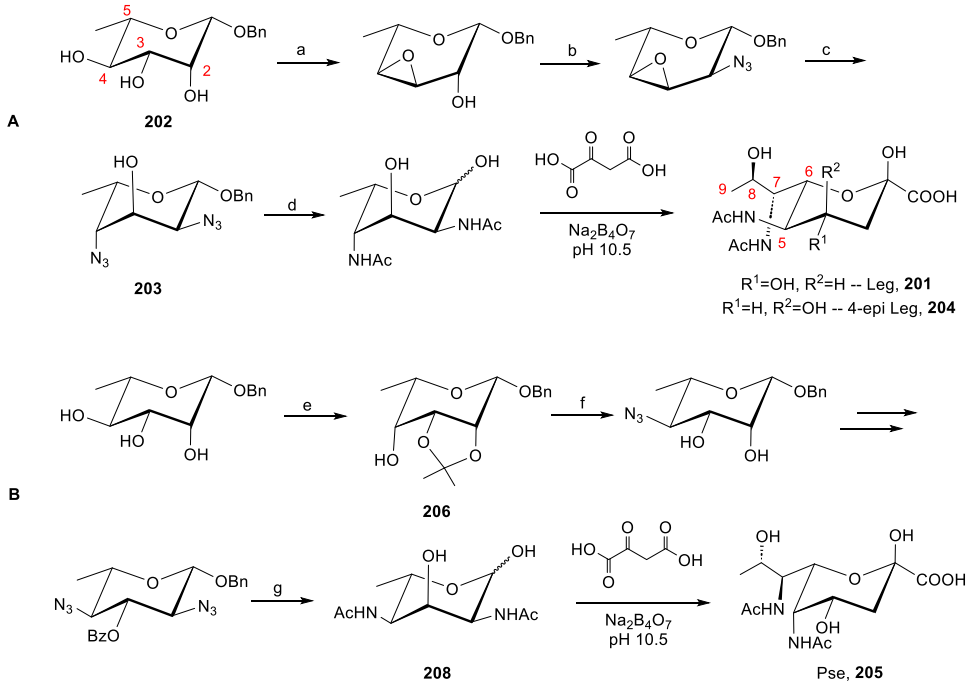
Figure 1.10 *- Overview of the nomenclature for L-conformers of aldopentoses and aldohexoses*

Chapter 2 Synthesis of Nonulosonic Acids from L-Arabinose

2.1 Previous Synthetic Strategies Towards Select Nonulosonic Acids

The distinctive structural features of Pse and Leg have long been recognized by the scientific community and published work on their chemical synthesis is abundant. Tsvetkov *et al.* were the first to synthesize bacterial NonAs by condensation of 2,4-diacetamido-2,4,6-trideoxy hexoses with oxaloacetic acid under basic conditions to obtain the 9-carbon backbone – they used commercially available sugar starting materials which could be transformed to have their C-2,3,4,5 centers correspond to C-5,6,7,8 in the desired product (**Scheme 1.1**).[86] Leg (**201**) was thus synthesized from L-rhamnose, where a prepared benzyl β-L-rhamnopyranoside (**202**) was modified by installing an epoxide at C-3 and C-4, giving the opportunity to first invert C-3, followed by a triflation and inversion with an azide at C-2, and with a final epoxide opening with azide again to invert C-4 and obtain **203** with the desired configuration. A reduction of the two azides followed by N-acetylation installed the required acetamido groups, while the final removal of the anomeric O-benzyl group provided the necessary precursor for condensation with oxaloacetic acid to give Leg along with its 4-epimer **(204)** (**Scheme 2.1**, A). The same starting material **202** was used to synthesize Pse (**205**), with extra steps as both C-2 and C-4 centers required a double inversion. After a protection of the cis-hydroxyls with an isopropylidene group, the C-4 centre was first inverted *via* an oxidation-reduction process to give **206**, followed by an inversion and activation as before, but assisted by a crown ether, to give the C-4 azide. After removing the 2,3-O-isopropylidene group, the protection of O3 with a benzoyl group allowed for a reiterated activation and inversion of C-2. The C-3 center was then simply inverted by a

deprotection, mesylation, then substitution after the reduction and selective N-acetylation of both installed azides: compound 2.8 was thus cleanly obtained in 12 steps. A simultaneous double-inversion at C-2 and C-4 by reacting a prepared 2,4-di-O-triflate with tetrabutylammonium azide resulted in a complex mixture of products, including the desired **208**: its condensation with oxaloacetic acid proved to be a mixture of isomers as well, including Pse isolated in 3% yield (**Scheme 2.1**, B).

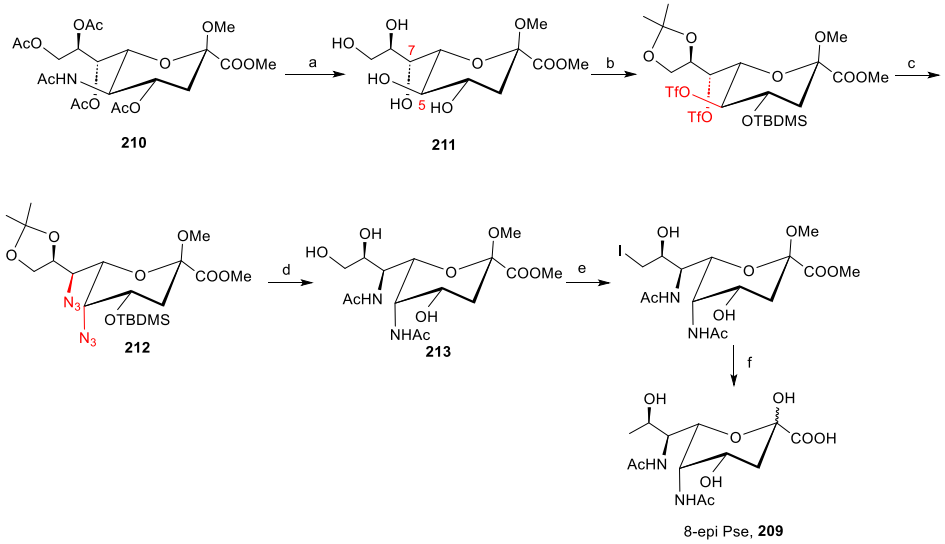


Reagents and Conditions: (**a**) i. trimethylorthoacetate, TsOH, MeCN, ii. Ac_2O,Pyr., iii. 80% aq. AcOH, iv. Tf_2O, Pyr., v. MeONa, MeOH; (**b**) i. Tf_2O, Pyr., ii. NaN_3, DMF; (**c**) NaN_3, NH_4Cl, aq. EtOH; (**d**) i. H_2, $Pd(OH)_2$/C, MeOH, ii. Ac_2O, MeOH; (**e**)i. 2,2-dimethoxypropane, TsOH, ace., ii. Oxalyl chloride, DMSO, i-Pr_2NEt, DCM, iii. $NaBH_4$. Aq. EtOH; (**f**) Tf_2O, pyr., ii. NaN_3, DMF, dibenzo-18-crown-6, iii. 80% aq. AcOH; (**g**)i. $LiAlH_4$, THF; ii. Ac_2O, MeOH; iii. MsCl, Pyr., DCM; iv. AcONa, aq 2-methoxyethanol; v. H_2, $Pd(OH)_2$/C, aq. MeOH

***Scheme 2.1** - Preparation of Leg and epimers (A) and Pse (B) by Tsvetkov et al.*[86]

A synthesis of NonAs requiring a three-carbon extension of hexoses is often exemplified in the literature (see Chapter 3 for more examples), however some groups choose to build their strategies from commercially available 9-carbon chain starting materials. As such, the Kiefel

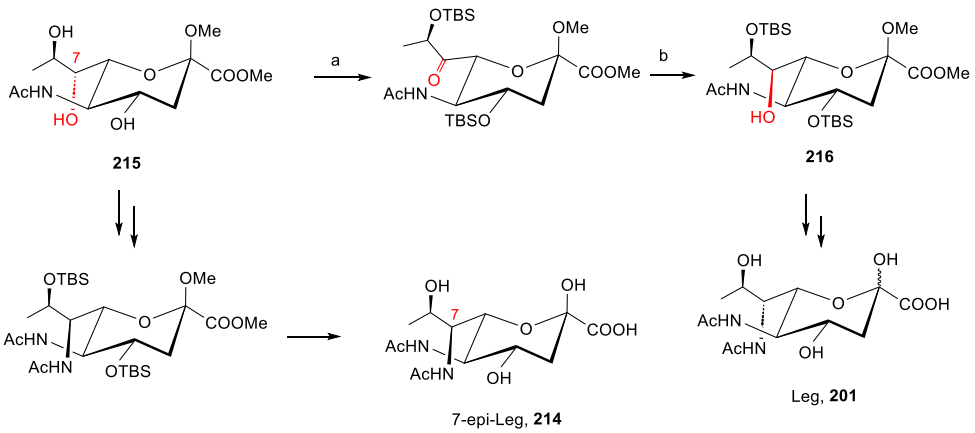
group achieved the total synthesis of 8-*epi*-Pse (**209**) by first converting per-acetylated Neu5Ac (**210**) to the C5-deaminated KDN (**211**), which possesses the right configuration at C-5 and C-7 for a double inversion (**Scheme 2.2**).[87] They achieved the first transformation via exposure of compound **210** to a large excess of sodium nitrite in a mixture of acetic anhydride and acetic acid, which gave a 5-nitroso intermediate that decomposed by heating to **211** in 57% yield. From there, they performed a regioselective 8,9-O-isopropylidenation and 4-O-silylation with tert-butyldimethylsilyl chloride; the O5,O7-diol was then activated by triflation, and substituted with sodium azide in DMF, giving C-5,C-7-diazido compound **212**. *Bis*-acetamide **213** was obtained by hydrogenation, followed by *N*-acetylation in pyridine and hydrolysis of the 8,9-O-acetal protecting group. To achieve the final 9-deoxygenation, the primary alcohol at C9 was first displaced with iodination, then a reduction of the 9-iodide by catalytic hydrogenolysis produced target **209** in a 15% total yield from Neu5Ac.



Reagents and Conditions: (**a**)i. $NaNO_2$, Ac_2O, AcOH, ii. CH_3ONa, MeOH; (**b**)i. 2,2-dimethoxypropane, TsOH-H_2O, ace., ii. TBDMSCl, imid., DMF, iii. Tf_2O, pyr.; (**c**) NaN_3, DMF; (**d**)i. H_2, $Pd(OH)_2$/C, TsOH-H_2O, MeOH, ii. Ac_2O, pyr., iii. aq. TFA; (**e**) I_2, Ph_3P, imid.; (**f**) i.$Pd(OH)_2$/C, $EtNiPr_2$, H_2, MeOH, ii. Et_3N–H_2O

***Scheme 2.2** - Synthesis of Pse from Neu5Ac by Kiefel et al. [87]*

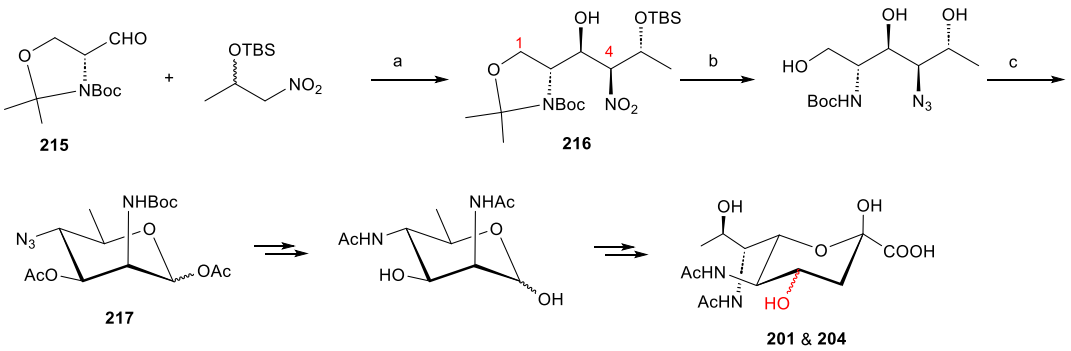
The same group later achieved the synthesis of Leg and its 7-epimer (**214**) from Neu5Ac as well, but in lower overall yields (7% and 11%, respectively) (**Scheme 2.3**).[88] After C-9 deoxygenation produced derivative **215**, oxidation-reduction using Dess-Martin periodinane was employed for the inversion of C-7 necessary to make Leg (**201**): reduction of the ketone was achieved with sodium borohydride, after mixing the compound with cerium(III) chloride in cold dichloromethane to form the Luche reagent, and the single inverted (*R*)-stereoisomer (**216**) was obtained in 88% yield. Despite the good yields, removal of the iodinane by-product proved troublesome, requiring extensive purification by column chromatography. Installing the N-acetyl at C-7 and final deprotection of both the compounds was achieved in four subsequent steps, via the well-established process of triflic anhydride treatment, followed by sodium azide, palladium catalyzed hydrogenation of the azido group followed by an N-acetylation, ultimately obtaining **201** and **214** after a global deprotection.



Reagents and conditions: (**a**) DMP, DCM; (**b**) i.$CeCl_3$, MeOH/DCM, ii. $NaBH_4$.

***Scheme 2.3**- Synthesis of Leg and epimer from Neu5Ac by the Kiefel group.*[88]

Although both syntheses by the Kiefel and co-workers are attractive due to the presence of the required 9-carbon backbone in the starting material, which circumvents the need for a C-C bond formation reaction, their low yields and high number of steps attest to the difficulty in targeting specific positions and obtaining correct stereochemistries when using complex carbohydrates as starting materials. With that in mind, the Gintner group recently published a *de novo* synthetic pathway for Leg and 4-epi-Leg, where they extended a D-serine derivative called Garner's aldehyde (**215**) with 1-nitro-2-propanol to assemble the 6-carbon backbone of **216** with the desired D-rhamno (6-deoxy-D-manno) configuration in 28% yield (**Scheme 2.4**).[89] Attempts to oxidize C-1 in the presence of the nitro group on C-4 of **216** using conventional methods did not occur in good yields, due to the electron-withdrawing properties of NO_2, and so the culprit at C-4 had to be exchanged for an azide before the required aldehyde could be formed via TEMPO-oxidation with trichloroisocyanuric acid (TCCA); a per-acetylated compound **217** with a D-rhamno configuration was obtained in the next step in a moderate yield. After converting both nitrogen functionalities to N-acetyl groups, the hexose was reacted with ethyl 2-(bromomethyl)acrylate in the presence of indium and an acid catalyst, giving the 2 diastereomers destined to become the desired compounds Leg (**201**) and 4-epi-Leg (**204)** in 68% yield, with 5% *de*. Despite the poor stereoselectivity, the versatility of this scheme in providing two analogs of Leg from one synthetic pathway is of high interest for this type of work - additionally, it provides the opportunity to chemically differentiate the two nitrogen functionalities by using different N-functionalizations on the building blocks.

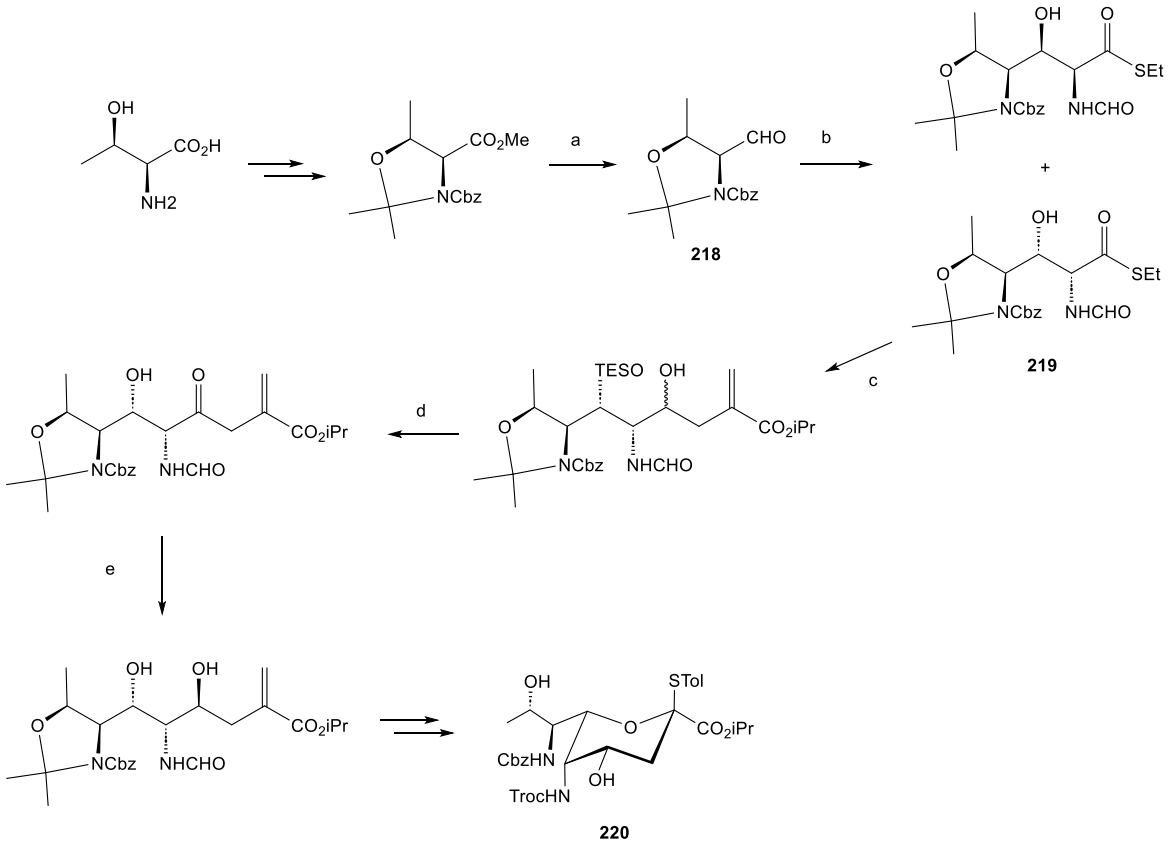


Reagents and conditions: (**a**) TBAF,THF; (**b**) i. H_2, Raney nickel, DCM, EtOH; ii. imidazole-1-sulfonyl azide hydrogen sulfate,K_2CO_3,$CuSO_4$-$5H_2O$, MeOH; iii. $CeCl_3$-$7H_2O$, $(COOH)_2$-$2H_2O$, MeCN, MeOH; (**c**) i. TEMPO, TCCA, $NaHCO_3$, DMF; ii. Ac_2O, pyr., 4-DMAP.

Scheme 2.4 *- De novo synthesis of Leg and epimer by the Gintner group.*[89]

The Li group also achieved an interesting *de novo* synthesis of a Pse thioglycoside analogue (**Scheme 2.5**)[72]: their scheme included a multistep conversion of L-threonine to the 2-amino-2,4-dideoxy-L-erythrose **218**, via inversion at the C-3 center, protection of the alcohol and amine functionalities and reduction of the C-1 acid to an aldehyde. The intermediate then underwent a key *syn* aldol-type addition step with a glycine thioester isonitrile to afford the desired L-altro-configuration thioester **219** as the major product in 67% *de*: optimization of the stereoselectivity here included choosing the right protecting group pattern on the precursor, where it was found that the presence of a bulky O-silyl group on C-3 resulted in an inverse diastereoselectivity. The three-carbon extension was also achieved via indium-mediated allylation, but gave the wrong diastereomer as the major product – the issue was successfully resolved by proceeding through a Dess-Martin oxidation, followed by the desilylation of O6 and finally a 1,3-induced diastereoselective reduction with sodium triacetoxyborohydride to give the desired (*S*)-configuration at C-4 in good yields (90% in three steps). The final Pse glycoside

structure **220** was achieved through a series of additional manipulations, including ozonolysis to cleave the alkene and boron trifluoride etherate-mediated thioglycosylation.



Reagents and conditions: (a) i. $NaBH_4$, $CaCl_2$, EtOH−THF; ii. BAIB, TEMPO, DCM; **(b)** i. $CNCH_2COSEt$, LiOTf, iPr_2NEt, DCE−DMF; ii. THF−H_2O, reflux; **(c)** i. Et_3SiH, Pd/C, THF; ii. Isopropyl bromomethacrylate, indium powder, NH_4Cl, EtOH; **(d)** i. Dess− Martin reagent, DCM; ii. TBAF, HOAc, THF; **(e)** $NaBH(OAc)_3$, HOAc, MeCN.

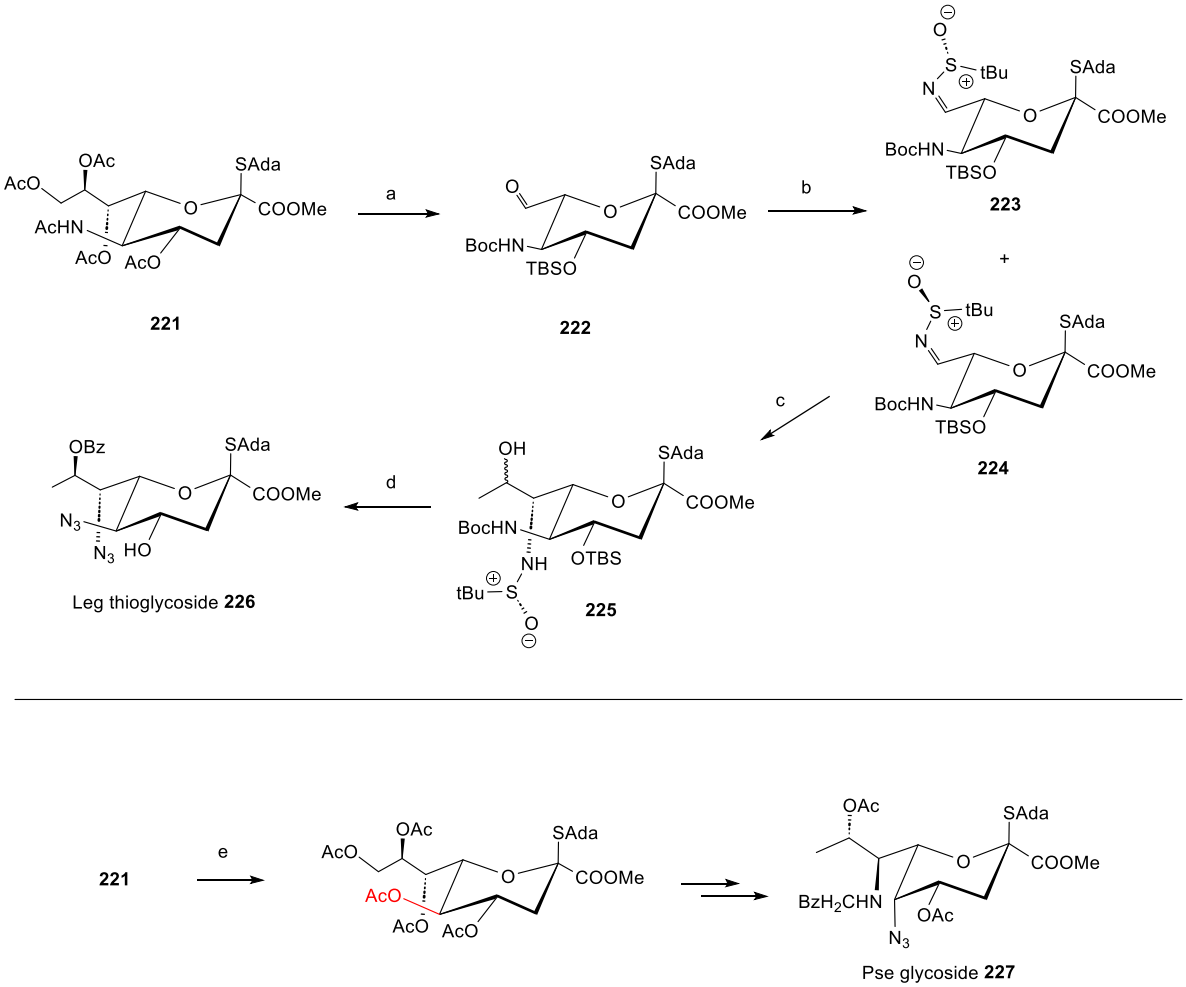
Scheme 2.5 *- Synthesis of L-Alt-diNAc and Pse from L-threonine by the Li group.*[72]

In one of the latest reported schemes towards NonAs, the Crich group provided an interesting and versatile method to obtain Leg and its 8-epimer, in addition to Ace and its 8-epimer from Neu5Ac via diastereomeric N-sulfinylimines that were manipulated to obtain the desired compounds (**Scheme 2.6**).[82] Their scheme also provided a more extensive variation that resulted in two Pse analogues. Dubbed as their second-generation synthesis based on previous

work,[80,81] the targets were obtained from Neu5Ac, where the protected equatorial thioglycoside derivative **221** was first made in 3 steps, after which an N-acylation with a tert-butyloxycarbonyl group followed by a one-pot deacetylation and oxidative cleavage gave precursor **222** with an aldehyde at C-6: condensation with either (*R*)- or (*S*)-enantiomer of the Ellman sulfanamide created the path towards the desired NonAs by way of chiral sulfoximines **223** and **224**. Leg synthesis was continued from the (*S*)-imine **224** via samarium-iodide mediated aza-pinacol condensation, giving two diastereomers in a 3:1 ratio favoring the desired (*R*)-isomer (**225**), which was then subject to prolonged treatment with hydrochloric acid in methanol, followed by a diazo-transfer with imidazole-1-sufonyl azide hydrogen chloride to give **226** in an overall 22% yield from the sulfoximine. The minor diastereomer **223** was similarly treated to give an 8-epi-Leg thioglycoside. The synthesis of Pse thioglycoside **227** and its 8-epimer using this scheme required several more steps in order to invert the chirality at C-5: in short, a Zbiral oxidative deamination of **225** gave a O-functionality with preserved configuration at C-5, allowing for the S_N2 reaction with an azide nucleophile after the 2-carbon extension via sulfoximine chemistry as described above.

While several groups have reported interesting synthetic routes towards the challenging synthesis of Pse, Leg and their analogues, there is still much room for improvements with regards to enhanced overall yields, better efficiency and more versatility. One common drawback in the above schemes was the low diastereoselectivity upon formation of new C-C bonds for the three-carbon backbone extension step. In addition, most schemes, with the exception of the Crich group's work, required different starting materials to synthesize the different NonAs, ensuing very distinct synthetic routes for each of them: this removes opportunities to share key

intermediates, lowering the overall efficiencies of the syntheses and leading to an increase of research costs.

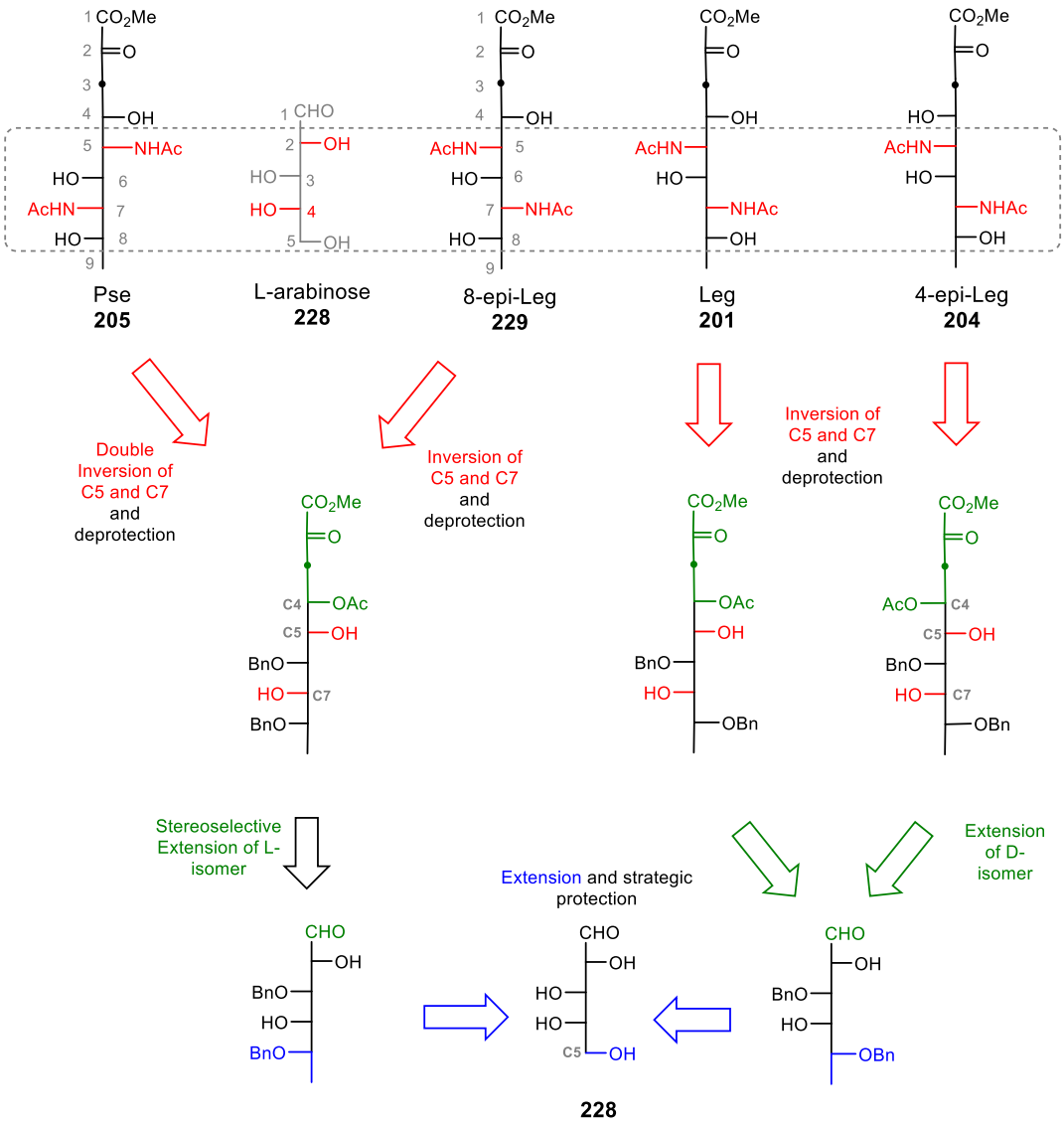


Reagents and conditions: (**a**) i. Boc_2O, DMAP, ii. NaOMe, iii. $NaIO_4$, Me_2CO, H_2O, ii. TBSCl, imidazole; (**b**) Ellman sulfanamide, PPTS, Na_2SO_4, DCM; (**c**) MeCHO, SmI_2, tBuOH, THF; **(d)** i. HCl, MeOH, ii. $imidSO_2N_3$-$CuSO_4$, iii. BzCl, pyr; **(e)** i. $NOBF_4$, pyr, DCM, ii. AcOH, TFE, iPrONa

Scheme 2.6 – *Synthesis of Pse and Leg glycosides by the Crich group.*[82]

2.2 Investigations into a New Synthetic Strategy

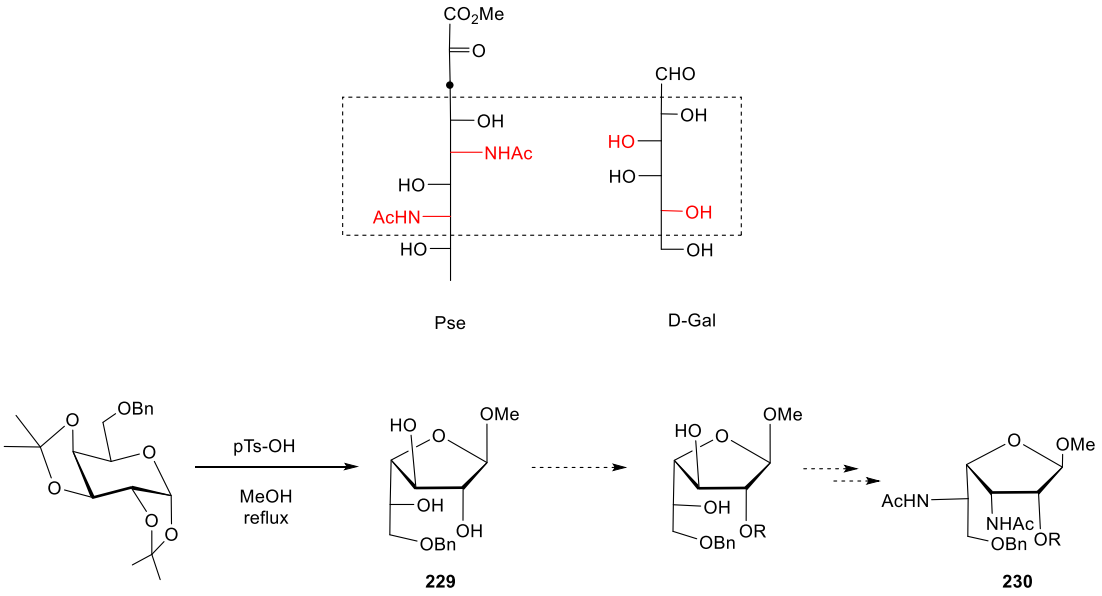
A synthetic scheme was originally developed in my work where the inexpensive L-arabinose (**228**) was envisioned as a common starting material for the synthesis of both Pse (**205**) and Leg (**201**), as well as 4-epi-Leg (**204**) and 8-epi-Leg (**229**). The proposal relied on the key stereogenic centers C-2 and C-4 on the starting material that could be manipulated to match the acetamido-substituted C-5 and C-7 centers on the targeted NonAs: this is demonstrated in **Scheme 2.7** at the top when the C-1 position of L-arabinose is aligned with C-4 of the NonAs in their Fischer projection forms. Whereas the chirality at C-3 of **228** matches the C-6 one of all the four target NonAs, its C-2 and C-4 positions match C-5 and C-7 on Pse but are exactly opposite to those on the Leg analogs. A simple strategy for the synthesis of Leg analogues from L-arabinose would then only require an inversion of both centers by a nitrogen-based nucleophiles, while the synthesis of Pse would require a less straightforward double inversion. Additionally, since the C-5 position on L-arabinose was designed to become the C-8 position of all NonA targets, we envisioned a 1-carbon chain extension from C-5 (**Scheme 2.7**, in blue) using a methyl nucleophile to complete the required terminal deoxy functionality at C-9 of all the targets. Finally, a three-carbon elongation to the anomeric position of the hexose precursors (**Scheme 2.7**, in green) would produce the required 9-carbon backbone, with the ability to generate both epimers of Leg at C-4. **Scheme 2.7** depicts a generally planned coordinated inversion of chirality and installation of acetamido substituents at the corresponding positions of the precursor hexoses for each target compound.



Scheme 2.7 *- Retrosynthetic scheme for proposed synthesis, with a comparison of corresponding stereogenic centers between starting material and target compounds.*

A preliminary inquiry into the above strategy was carried out during the first two years of my thesis work, with the much-appreciated assistance of four undergraduate students: Logan Draycott, Quyen Pham, Afsah Ali and Cole Frantz. Our first objective was to lock **228** into its L-arabinofuranose form, providing selective access to C-5 for the required one-carbon extension. As a sidenote, a similar synthetic scheme was also attempted with D-galactose as the starting material, which has an equally useful configuration towards the synthesis of Pse, with chiralities

at C-2 and C-4 matching those on C-4 and C-6 on Pse, and the opposite ones requiring inversion and functionalization at C-3 and C-5 (**Scheme 2.8**). Numerous attempts were carried out to optimize the formation of the methyl 6-O-benzyl-β-D-galactofuranoside **229** from the 6-O-benzyl-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose according to published procedures,[90–92] however, the desired furanoside product was only obtained in a maximum 50% yield. Furthermore, no strategy at the time was found to differentiate between the three secondary hydroxyls at positions 2, 3 and 5 of **229**, necessary to selectively target O2 for protection, which would have given access to C-3 and C-5 for the required inversion with a nitrogen-based nucleophile to give **230**. The scheme was therefore abandoned early on.



***Scheme 2.8** – Potential scheme towards Pse from D-galactopyranose that was abandoned.*

The free L-arabinose **228**, on the other hand, was cyclized into the furanose form smoothly using previously reported procedures, by first selectively protecting the primary O5 with the bulky tert-butyldiphenylsilyl (TBDPS) group (→**231**), followed by the installation of an isopropylidene group at the O1 and O2 centres, producing the desired compound **232** in an

overall 59% yield.[93] The product from the first step was initially isolated by silica gel column chromatography yielding the expected mixture of free-furanose anomers that were confirmed by ^{1}H NMR (**Figure 2.1**): the combined integration of the anomeric proton group at 5.23-5.45 ppm presented a 1:10 ratio to the combined integration of the aromatic group protons at 7.39-7.72 ppm, corresponding to the two phenyls on the protecting group, in addition to the 1:9 ratio to the combined tert-butyl group signals at 1.07-1.10 ppm also corresponding to the new protecting group. These ratios, combined with the presence of one major product spot when monitoring the reaction by thin layer chromatography (TLC) clearly showed that the mixture of anomers contained only one O-TBDPS group, and so the crude product was used directly for the cyclization in future iterations of this procedure.

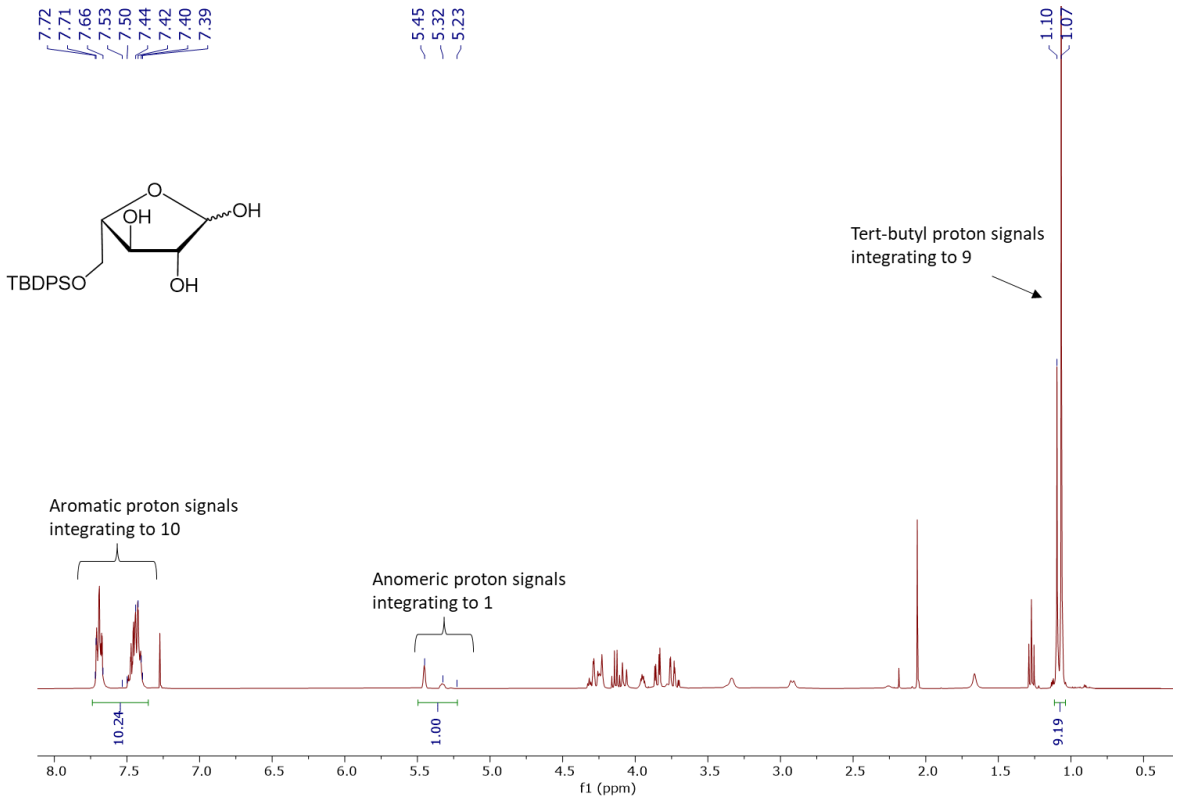
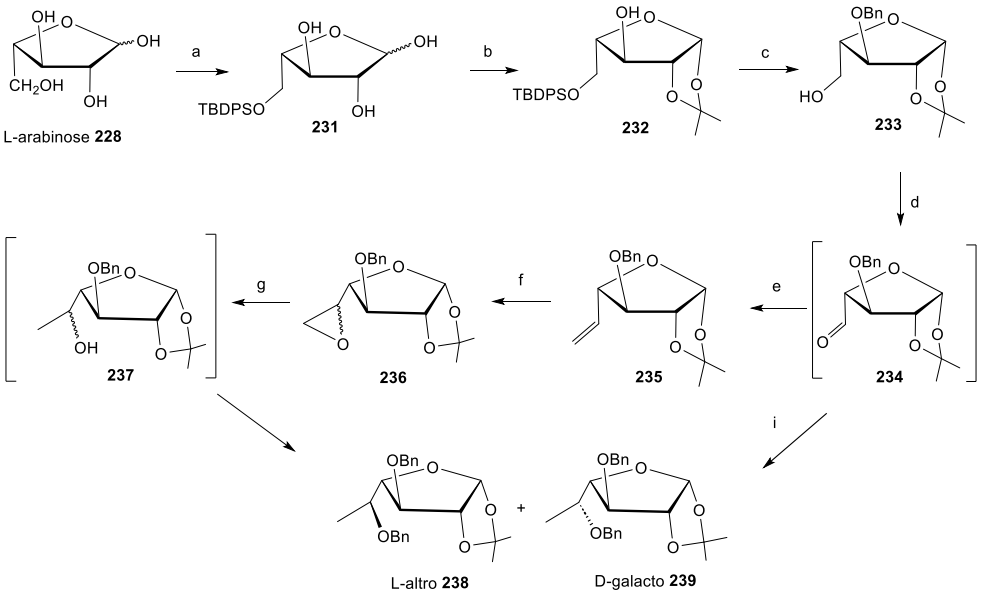


***Figure 2.1** – ^{1}H NMR spectra of compound **231**.*

The next series of steps required the selective activation of C-5 on **232** for the one-carbon extension: O3 was easily protected with a benzyl ether, however it was then noticed by TLC that the TBDPS protecting group was removed for some of the product when the benzylation reaction was quenched with methanol. It led us to the interesting discovery that this protecting group, which is usually stable in a basic media, could be completely dislodged upon treatment with sodium methoxide *in situ* in dimethylformamide, which advantageously prevented reliance on the usual route to remove TBDPS group using TBAF. Compound **233** was therefore obtained in one-pot from **232** in a 58% yield.

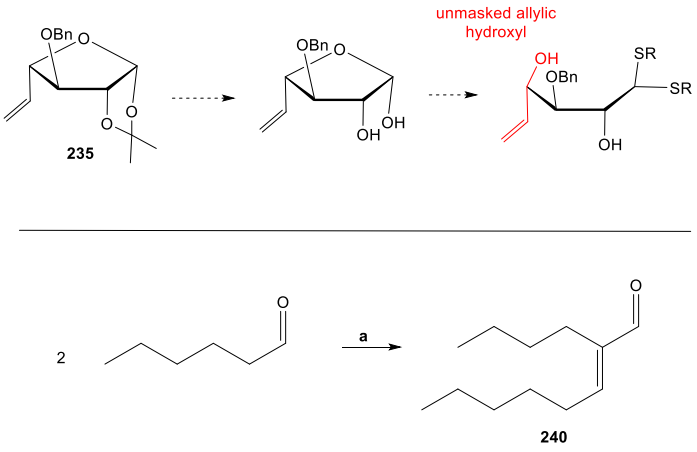


Reagents and conditions: (a) TBDPS-Cl, imidazole, DMF, 100-55°C; **(b)** $CuSO_4$, CSA, acetone; **(c)** i. BnBr, NaH, DMF; ii. NaOMe; **(d)** Oxalyl chloride, DMSO, Et_3N, DCM, -78°C; **(e)** $Ph_3P{=}CH_2$, THF; **(f)** mCPBA, DCM; **(g)** i. DIBAL-H, toluene, -78°C, ii. BnBr, NaH, DMF; **(h)** i. MeMgBr, THF, -78°C; ii. ii. BnBr, NaH, DMF

Scheme 2.9 *– Synthetic scheme towards 3,5-di-O-benzyl-6-deoxy-1,2-O-isopropylidene-α-D-galactofuranose (**239**) and 3,5-di-O-benzyl-6-deoxy-1,2-O-isopropylidene-β-L-altrofuranose (**238**).*

The single-carbon extension at C-5 step was the first expected challenge in this synthesis, as it required tuneable diastereoselectivity to achieve our versatility goals: while the target compound Pse necessitated an extension strategy favoring the L-isomer, the synthesis of Leg required the D-isomer to form. Our preliminary explorations were executed through an epoxide intermediate, made by first oxidizing **233** via Swern reaction, then adding the extra carbon by means of a Wittig reaction. We chose the Swern oxidation technique over using Dess-Martin periodinane due to cost considerations, even though the latter provided a much simpler methodology than the former, which required cryogenic cooling and constant monitoring. The reaction was also attempted under milder conditions, using a combination of dimethyl sulfoxide and acetic acid as the oxidant, but did not go to completion after 24 hours despite a large excess of both reagents. The aldehyde product **234** was found to be quite unstable at room temperature and consequently had to be subjected to the Witting reaction within a few hours of being formed: purification by silica gel column chromatography was therefore not attempted to maximize yields. Formation of compound **234** was determined by crude ^{1}H NMR: the new aldehyde singlet at 9.79 ppm was integrated, and its ratio was determined to be 1:1 with the distinctive anomeric H-1 signal (doublet present at 6.09 ppm). Additionally, it was determined to have a 1:3 integration ratio with the singlets at 1.45 and 1.31 ppm, corresponding to the methyl groups on the isopropylidene functionality. Epoxidation of the Wittig product **235** was achieved with meta-chloroperbenzoic acid (mCPBA), giving a crude equal mixture of both stereoisomers **236a** and **236b** which were carried through to the next reaction without purification: the completion of the reaction was determined by TLC monitoring, and the isomer ratio was determined later in the synthesis when the epoxide-opening products **238** and **239** were collected.

The free-rotation at C-4—C-5 of the extra-cyclic olefin on **235** makes the observed lack of selectivity foreseeable, and the use of a chiral additive, such as with the Sharpless epoxidation, was not possible at this stage due to a lack of an allylic hydroxyl group. One interesting alternative would have been to linearize the carbohydrate backbone by first deprotecting O1 and O2 on **235** then selectively reacting the anomeric carbon with a disulfide, or a similar free-sugar linearizing strategy, to unmask the allylic hydroxyl functionality at C-4 as seen in **Scheme 2.10**, allowing for the use of the (+) or (-) DET chiral catalysts: this was however not attempted. The Corey-Chaykovsky reaction was also briefly considered, where the epoxide could be formed directly from the C-5 aldehyde with a sulfonium ylide, potentially producing diastereoselectivity with the right chiral reagent or additive[94]: it was however found that the desired epoxide did not form. Presumably the strong basic sulfonium ylide intermediate was quenched by the alpha proton at C-4 of the aldehyde **234** as the reaction was not detected to progress. The enolate formation was surmised from the result of a separate "test" reaction with hexanal - the isolated compound turned out to be the aldol adduct 2-butyl-2-octenal (**240**, **Scheme 2.10**).



Reagents and conditions: (a) TMSI, DMSO, THF.

Scheme 2.10 *– Top: Possible strategy allowing for asymmetric Sharpless epoxidation. Bottom: "Test" to establish the viability of the Corey-Chaykovsky reaction, which resulted in the undesired Aldol condensation.*

Since separation of the oxirane products **236** was predicted to be difficult due to their similar R_f values, the crude product residue was subjected to a reduction using diisobutylammonium hydride (DIBAL-H) mediated epoxide opening. A 1:1 ratio of isomeric products was detected by TLC, and following the workup, the new alcohol functionality at C-5 on **237** was O-benzylated to produce the more easily isolable **238** and **239**. Indeed, purification by silica gel column chromatography produced the desired hexoses, the L-altro-configuration compound **238** and the D-galacto-configuration compound **239**, albeit in low overall yields from the olefin **235** (27% and 26% respectively). The new compounds were characterized by ^{1}H NMR (**Figure 2.2**), and identified by comparing the coupling constants between the proton on the new chiral center at C-5 and the ring proton at C4 to those of a similar compound reported in the literature, 3-O-benzyl-5-O-benzoyl-1,2-O-isopropylidene-6-deoxy-α-D-galactofuranose.[95] Despite the different protecting group at C-5, it was expected that the similar sizes and shapes of the benzyl vs the benzoyl functionalities would result in comparable conformations for the molecules, both of which were similarly distorted by the bicyclic configuration: the $J_{4,5}$ reported for the 5-O-benzoyl product was 4.0 Hz, which is very different to the much larger one of 8.6 Hz found for L-altro-configuration product **238**. The D-galacto-configuration product **239** displayed a high order coupling pattern for H-5 at 3.73-3.69 ppm because the signals for H-3 and H-4 overlapped at 3.93 ppm, making it difficult to determine the coupling constant; however, 3-O-benzyl-5-O-benzoyl-1,2-O-isopropylidene-6-deoxy-α-D-galactofuranose[95] also saw H-3 and H-4 signals that were very close to each other (3.92 and 4.05 respectively), which again matched the D-galacto-configuration compound more closely than the other. Additionally, the L-altro-configuration compound **238** experienced a stronger shielding effect (1.21 ppm) for the terminal

methyl group than that of the D-galacto configuration one (1.31 ppm). Further characterization by X-ray crystallography was attempted, but neither crystal structure was successfully obtained during the time that this project was pursued.

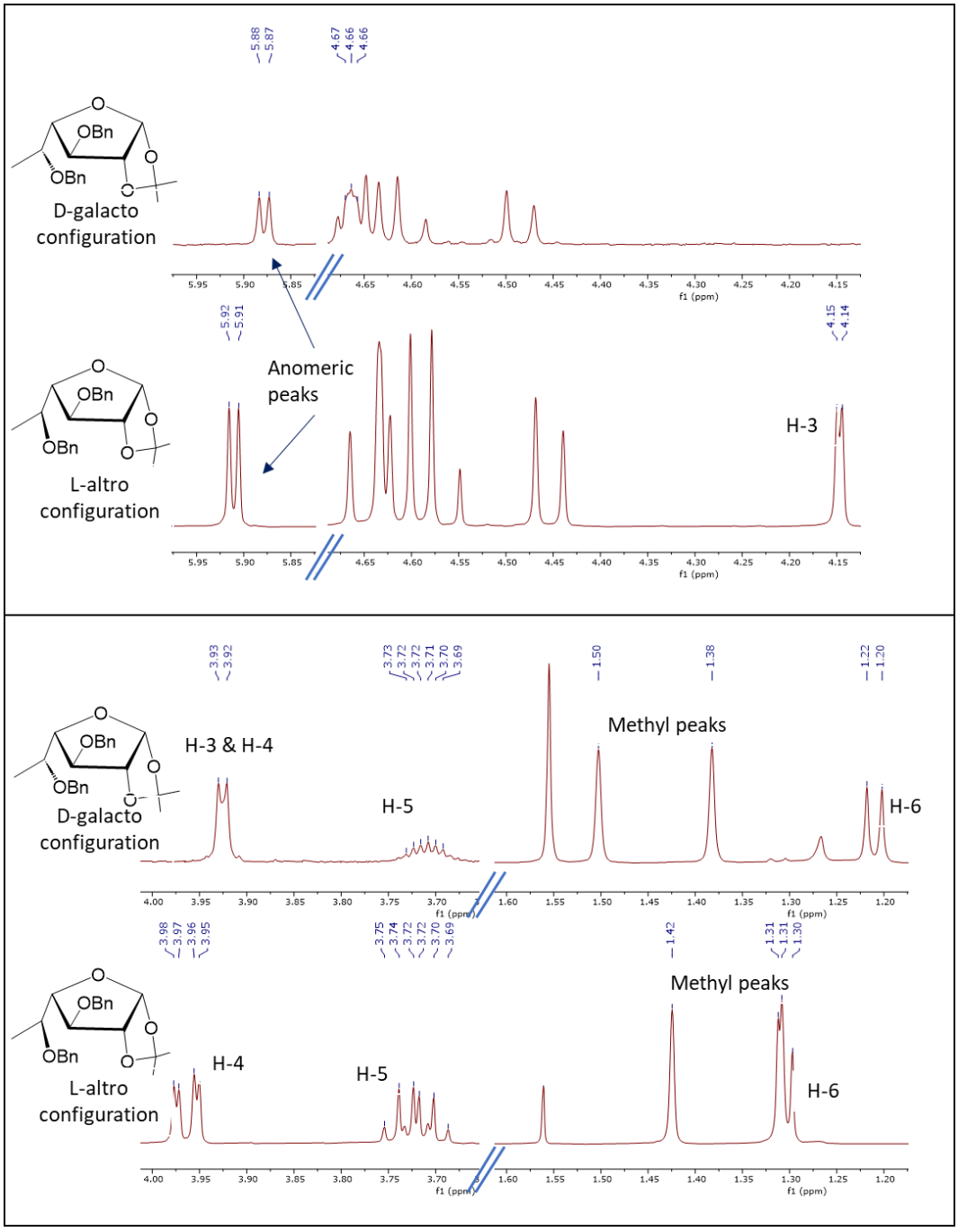
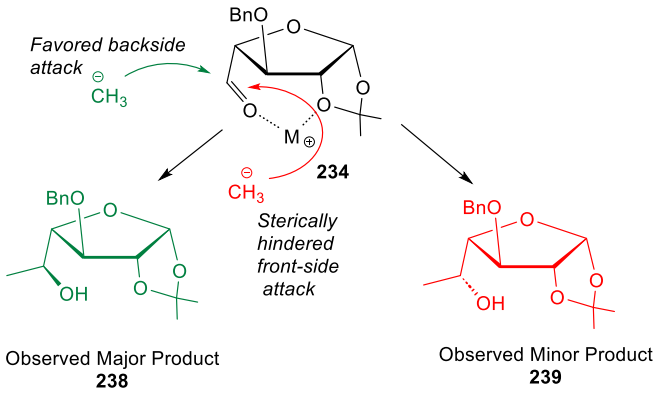


Figure 2.2 *– Comparison of four sections of ^{1}H NMR spectra of compound* ***239*** *(D-galacto configuration) and compound* ***238*** *(L-altro configuration). The top half contains sections between 6 and 4 ppm, and the bottom half contain sections between 4 and 1.1 ppm.*

The one-carbon extension of **234** was alternatively attempted via the direct nucleophilic addition to the aldehyde using either methyl Grignard or methyllithium (**Scheme 2.9**). This approach is more direct and likely more efficient, opening the potential for improving diastereoselectivity with cation-mediated chelation. For example, the oxygen at C-2 and the carbonyl of the aldehyde **234** could both chelate to an appropriate metal cation during the reaction, given their localization on the same face of the furanose ring, rigidifying the conformation of the molecule and potentially favouring the nucleophilic attack from a less sterically hindered side (**Scheme 2.11**). This selectivity was indeed observed: the reaction of methylmagnesium bromide and aldehyde **234** in anhydrous THF at -78 °C produced the L-altro isomer **238** as the major compound as per TLC monitoring after the subsequent O-benzylation. Some preliminary optimizations were attempted by varying the counter-cation (methyl magnesium bromide vs methyl lithium) and by varying the solvent[96] (diethyl ether vs tetrahydrofuran), but according to TLC, neither variation showed any change in diastereoselectivity.



***Scheme 2.11** – Nucleophilic attack on a chelated conformation of aldehyde **234** by a methyl anion favors the formation of L-altro isomer **238**.*

The one-carbon extension work that was completed with L-arabinose as a starting material towards potentially achieving a versatile synthesis of 4 different NonAs was useful in establishing preliminary results that warranted future research in this direction. A good selectivity during the one-carbon extension was demonstrated towards the synthesis of L-altro-configuration precursor **238** of Pse and 8-epi-Leg, while more work would need to be done to find a way to favor the D-galacto isomer **239** towards the synthesis of Leg and its 4-epimer.

2.3 Investigations into the Double Inversion at C-2 and C-6 of Hexoses

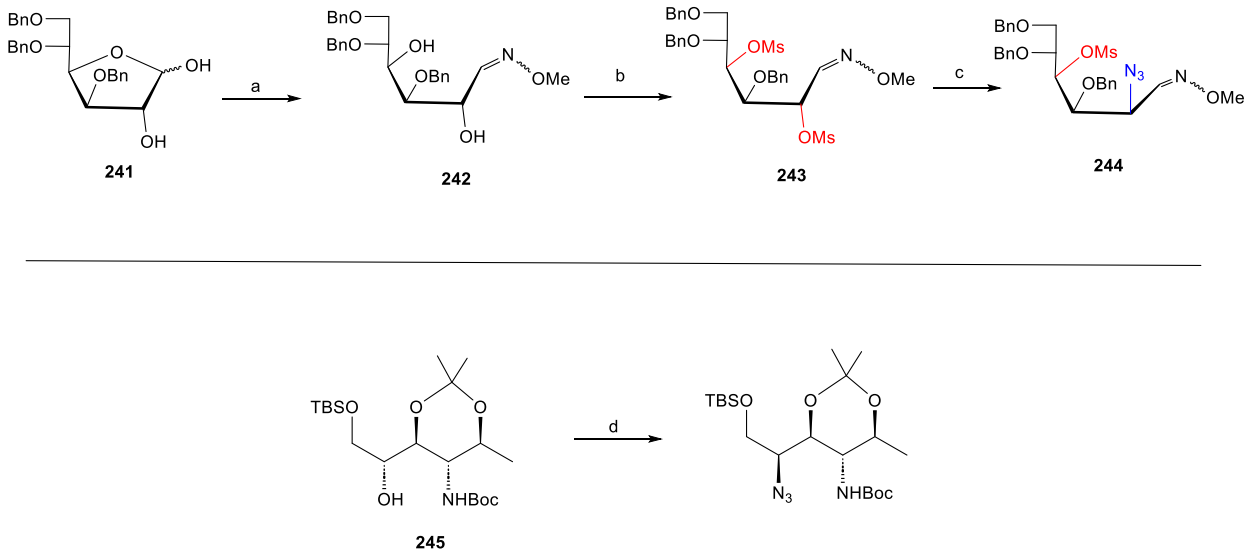
Before this project was put aside to allow me to focus on the one that will be presented in the remaining chapters of this thesis, some work was done to investigate methodologies to introduce acetamido groups at both C-2 and C-4 of products **238** and **239**. This required locking either hexose in an open chain form after the removal the 1,2-O-isopropylidene group so that both the hydroxyl groups at C-2 and C-4 could be accessed for activation and subsequent inversion. While carbohydrate linearization is traditionally achieved using dithioacetal chemistry, a less noxious approach was chosen, where the hemiacetal could be converted to an oxime functionality by a treatment of the free sugar with O- methylhydroxylamine.[97]

A protected D-glucofuranose hemiacetal, previously synthesized by our group, was used as a model compound in the preliminary investigations towards forming and manipulating the oxime substrate (**Scheme 2.12**, top). To start, an O-methylhydroxylamine group was installed at C-1 of compound **241**, leading to the desired open chain compound **242**, as a mixture of (*E,Z*)-isomers at the oxime.[97] Compatibility of the oxime functionality with the required O-activation

through mesylation was confirmed by crude ^{1}H NMR, which revealed the presence of 2 new O-mesyl groups on each of the isomers with their corresponding singlets (with some overlap) between 3.01 and 2.98 ppm; the O-methyl oxime group signals were also detected at 3.85 ppm for the major isomer, and 3.75 ppm for the minor isomer. A doublet at 6.89 ppm belonged to H-1 on the minor isomer, while the one for the major isomer overlapped the aromatic signals at 7.38-6.89 ppm. The integration ratios were in agreement with these assignments, and so compound **243** was thus shown to have formed with no side-products. Ensuing exposure of the O2,O4-dimesylate to sodium azide in DMF showed selective reactivity at the C-2 position, which was inverted, while C-4 remained unsubstituted to give compound **244**: analysis of the crude ^{1}HNMR showed the successful inversion of the C-2 position (verified by a gCOSY experiement), where the H-2 signal at 4.30 ppm matched the expected chemical shift of an azido functionality, while H-4 remained downfield with a chemical shift of 5.01 ppm, corresponding to an O-mesyl functionality.

While this finding could potentially provide a way to differentiate between the two amido functionalities, necessary for the synthesis of analogues of the NonAs that exist in nature with various N-functionalities, the issue of the low reactivity of the activated C-4 to substitution remained to be addressed. In their synthesis towards Pse derivatives, Werz *et al*[98] reported a similar difficulty in reacting the hydroxyl at C-4 on their intermediate **245** to a Mitsunobu reaction with azide as the nucleophile: low yields (<20%) were obtained when the reaction was carried out with diphenyl phosphoryl azide (DPPA), diisopropyl azodicarboxylate (DIAD) and triphenyl phosphine, but was optimized to 80% yields when diethyl azodicarboxylate (DEAD) was used instead (**Scheme 2.12**, bottom). In our case, optimizations of the inversion were attempted, with

higher reaction temperatures, a different solvent (dimethyl sulfoxide instead of dimethylformamide) and over long periods of time, with no improvements.

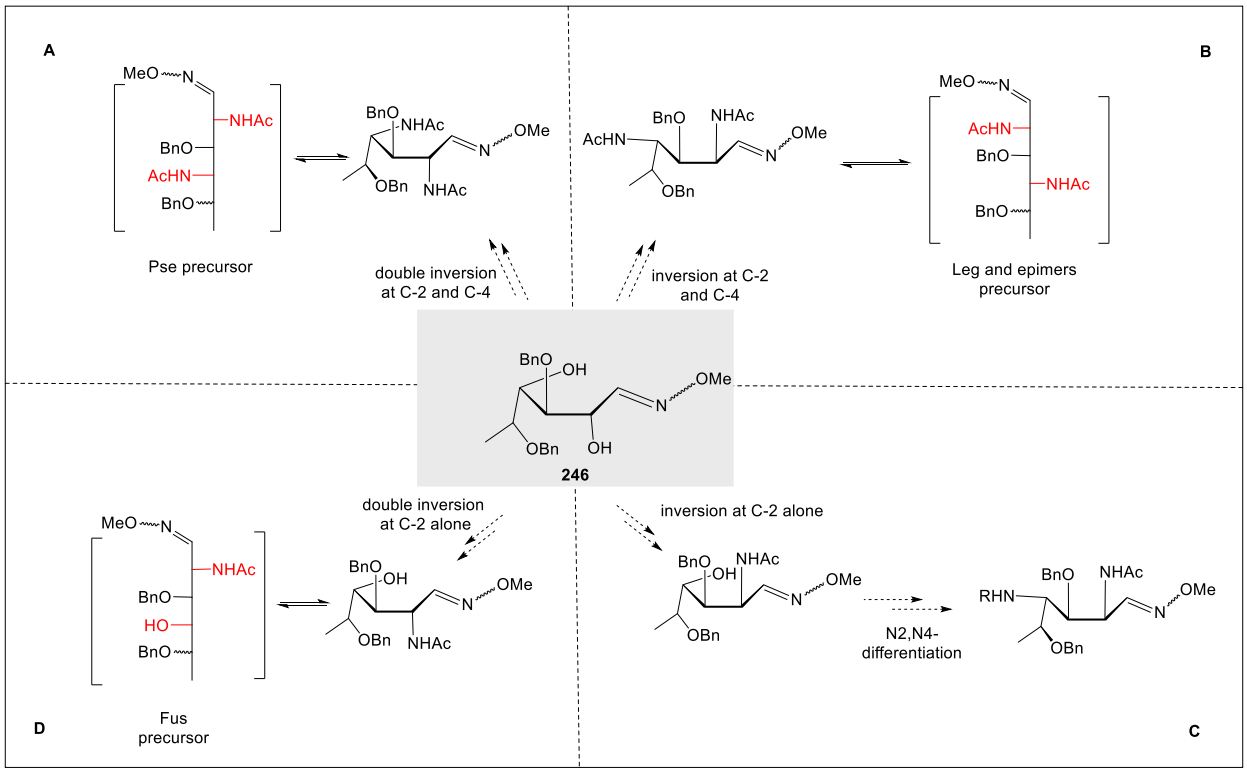


Reagents and conditions: (a) NH_2-OMe hydrochloride, Pyr; **(b)** MsCl, Pyr; **(c)** Sodium azide, DMF; **(d)** DEAD, DPPA, PPh3,THF

Scheme 2.12 *– Top: Explorations into the compatibility of linearized model substrates. Bottom: Inversion reaction in the Werz et al synthetic scheme towards Pse that also required optimizations.*

It was expected that a similar trend would be seen if the same scheme was carried out with the L-altro- and D-galacto-configuration substrates **238** and **239** due to the corresponding flanking of C-4 with O3 and O5 benzyl ethers, albeit with different configurations. Continued work towards finding the optimal conditions under which the C-2 and C-4 sulfonates on hypothetical compound **246** could be displaced simultaneously would potentially lead to accessing the configurations necessary for the synthesis of 8-epi Leg (from L-altro-configuration) and Leg and 4-epi-Leg (from D-galacto-configuration) (**Scheme 2.13**, quadrant **A**), while a double inversion of both positions on the linearized L-altro-configuration precursor would lead to the Pse backbone (**Scheme 2.13**, quadrant **B**). The ability to selectively invert C-2 with an azide nucleophile gives

rise to the opportunity to differentiate between the amido functionalities, by for example choosing a different nitrogen-based nucleophile for the subsequent inversion at C-4, like potassium phthalimide, or performing an altogether different reaction at C-4, like the Mitsunobu one, after the necessary deprotection of O4 and reduction/acetylation at C-2 (**Scheme 2.13**, quadrant **C**). Finally, a new potential route towards the synthesis of fusaminic acid (Fus, see **Figure 1.7**) has resulted from the unexpected substitution selectivity, where a double inversion at C-2 of the L-altro-configuration precursor, followed by an S-O bond cleavage at the remaining sulfonate at C-4 with a base would give the required hexose precursor for Fus (**Scheme 2.13**, quadrant **D**).



Scheme 2.13 *– Potential strategies to linearize L-altro isomer and subsequently introduce an NHAc group at both C2 and C4 via single and double inversions..*

The work presented in this chapter has produced preliminary successes towards the synthesis of several important NonAs from a single, readily available starting material, while more clearly defining some of the anticipated challenges with the planned scheme. First, it was determined that the one-carbon extension of aldehyde intermediate **234** is easily selective for the L-altro-configuration necessary for the attainment of Pse and 8-epi-Leg targets, and that selectively obtaining the D-galacto configuration for the synthesis of Leg and 4-epi-Leg requires optimizations or a brand new tactic. More work is equally required to successfully achieve an inversion at C-4, although the observed selective substitution at C-2 has created interesting new synthetic opportunities, including one for differentiating between the two N-acyl groups found in most bacterial NonAs, and introducing a potential route towards another important unique bacterial component, Fus. The work above was however put aside when a different route, which was initially investigated by a former MSc graduate student Carita Sequeira, required my attention to be carried through for publication, and ended up producing some new and exciting synthetic opportunities as well: these will be outlined and discussed in the remaining chapters of this thesis.

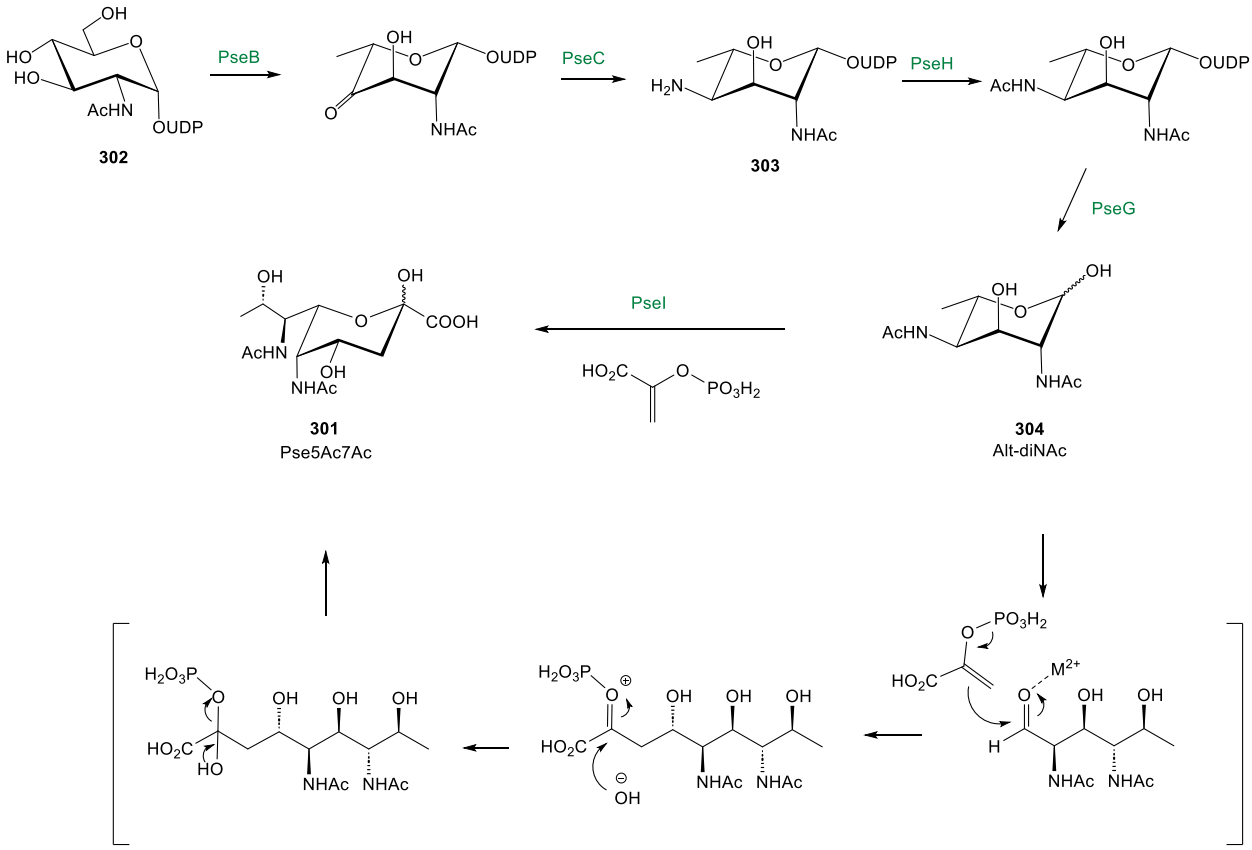
Chapter 3 Synthesis of 2,4-Di-N-Acetyl-2,4,6-Trideoxy-L-Altrose

The following chapter is based on my published work: Niedzwiecka, A.; Sequeira, C.; Zhang, P.; Ling, C.-C. An Efficient and Scalable Synthesis of 2,4-Di-N-Acetyl-L-Altrose (L-2,4-Alt-DiNAc). RSC Adv. ***2021****, 11 (19), 11583–11594. https://doi.org/10.1039/D1RA01070K.*

3.1 Biosynthesis of Pseudaminic Acid

The biosynthesis of pseudaminic acid (Pse) with di-N-acetyl functionalities at C-5 and C-7 (**301**, Pse5Ac7Ac), found in the flagella of *Campylobacter jejuni* and *Helicobacter pylori,* has been extensively studied and characterized: uridine diphosphate-N-acetyl-D-glucosamine (**302**, UDP-GlcNAc) is transformed in nature by the way of 5 essential enzymes (**Scheme 3.1**).[76] First, PseB oxidizes the C-4 hydroxyl to a ketone, and dehydrates the C-6 position via a β-elimination to form an alkene which is then reduced, resulting in an inversion of chirality at C-5[99]; PseC is an aminotransferase that adds the free amino functionality at C-4 through an enzymatic condensation with a pyridoxamine phosphate (PMP) co-factor, followed by a lysine-catalysed reduction[100]; the acetamido at C-4 is formed by the action of acyl transferase PseH[101], then PseG, a hydrolase, takes care of removing the UDP group, which frees the hexose 2,4-di-N-acetyl-2,4,6-trideoxy-L-altrose (**304**, Alt-diNAc), a precursor for the three-carbon extension by the PseI synthase.[76] This last enzyme is quite homologous to the well-characterized sialic acid producing Neu5Ac synthase, and was first identified in *C. jejuni* as NeuB3[102,103]: the extension mechanism involves a condensation with phosphoenolpyruvate (PEP), where a metal ion holds the Alt-diNAc substrate in its open form, allowing for the nucleophilic attack of the PEP enolate at the C-1

aldehyde of the open chain Alt-diNAc, followed by the water-catalyzed release of phosphate to give Pse[102] (**Scheme 3.1**).



Scheme 3.1 *– Biosynthesis of Pse5Ac7Ac detailing enzymes and intermediates identified in C. jejuni and H. pylori, including a mechanistic overview of the PseI synthase mode of action.*[76]

While the biosynthesis of Pse in *C. jejuni* and *H. pylori* is well-characterized, it is important to consider that the dozens of other bacterial strains that produce Pse exhibit deviations from the above pathway: this fact has already been seen with investigations involving *Aeromonas caviae* and *Bacillus thuringiensis*, among others.[76] Another important consideration is the occurrence of Pse derivatives in nature that have different N5,N7 acylation patterns from the di-N-acetyl one seen above, including ones that allow Pse to act as a glycosyl acceptor, like in the case of *Sinorhizobium fredii*, where the capsular polysaccharide (CPS) contains a Pse with N-

acetylation at C5 and N-(3-hydroxybutyryl) at C7, which is in turn linked to another Pse through the hydroxyl functionality on the N7 acyl group (**Figure 3.1**).[77] The specific biosynthetic pathway of these varying N-acyl transfers to the substrates has not been studied, but definitely warrants further investigations, which in turn necessitates a scalable, short and versatile synthesis of the biosynthetic precursors involved, including Alt-diNAc and its N-acyl derivatives.

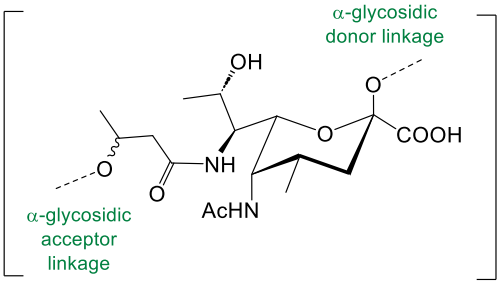


Figure 3.1 *– Example of the glycosidic linkages for Pse contained in the CPS of Sinorhizobium fredii*[77]*.*

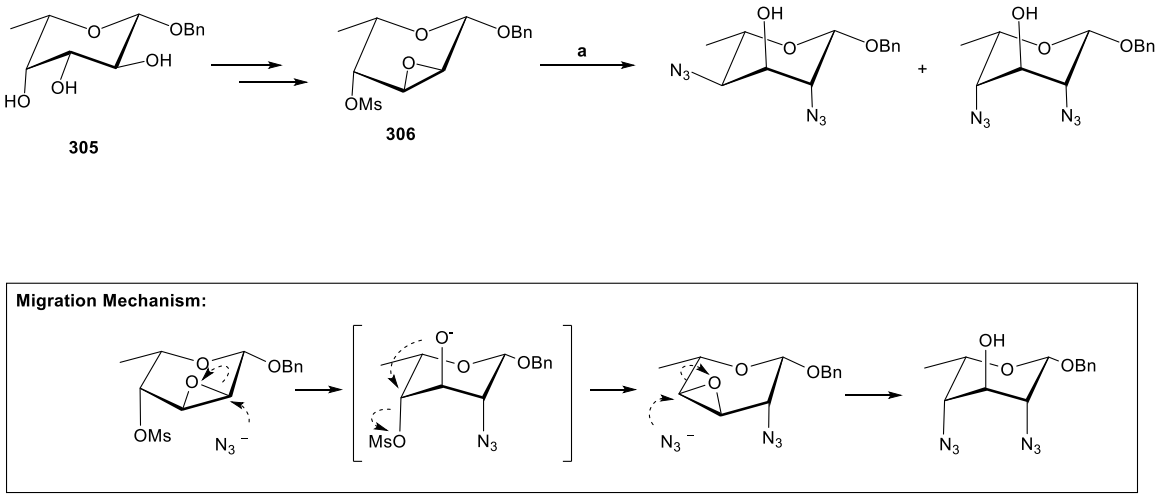
A better understanding of the biosynthesis of Pse can additionally lead to a better idea of how to include enzymes into the chemical synthesis of Pse itself. Chemoenzymatic assistance has often been used in syntheses requiring precise controls of stereochemistries for selected transformations, including glycosylations in carbohydrate chemistry.[104,105] There have been many advances towards using pyruvate-dependent aldolases and their mutants to form sialic acids from hexoses asymmetrically,[106,107] and in some cases at very large scales, starting from the early enzymatic conversion of glucosamine (GlcNAc) to N-acetylneuraminic acid (Neu5Ac) in kilogram scales, developed by Maru *et al.*[108] The *in vitro* chemoenzymatic synthesis of Pse from UDP-GlcNAc was recently reconstituted and optimized by the Fascione group, where a maximum of 39 mg of cytidine monophosphate(CMP)-Pse5Ac7Ac was collected from a one-pot multienzyme synthetic procedure involving all the enzymes from the *C. jejuni* biosynthetic pathway.[84] The

Imperiali group[109] recently reported the chemoenzymatic synthesis of the biosynthetic precursor to Alt-diNAc, with UDP at the anomeric position, through a tandem reaction of UDP-GlcNAc with *C. jejuni* PseB & C in one-pot, giving a 58% yield of UPD-6-dexoy-4-amino-2-NAc-L-Alt **303** in multi-milligram quantities. Otherwise, there are a few examples in the literature where PseI is used to extend either Alt-diNAc,[102] or a modified version of it, where the amido at C-4 beared an extra azide functionality[110]: the procedure was reported to produce the desired compounds, but yields were not given. With these limited reports of chemoenzymatic advances towards Pse, it seems clear a better-yielding and more stereochemically controlled synthesis of Pse via enzymatic catalysis is at its early stages of development, and has much room for improvement.

3.2 Previous Syntheses of Alt-diNAc

The reported literature methods for the chemical synthesis of the biosynthetic precursor to Pse, Alt-diNAc either suffer from low overall yields or long reaction sequences, caused by the difficulties in attaining the C-6 deoxy functionality from common hexose starting materials, as well as the correct stereochemistries of the two acetamido functionalities. Additionally, many of the syntheses do not present the opportunity for differentiation of the two amide groups in the final products, limiting the scope of the libraries created for Pse analogs. For example, as briefly mentioned in section 2.1, Tsvetkov *et al*[86] reported a synthesis from L-rhamnose which took advantage of its 6-deoxygenation and L-configuration. Although the use of L-sugar[111] represented an advantage because of a configurational match at the C-5 position and a deoxygenation at C-6, the conversion to a 2-epimer L-Alt-diNAc was achieved in a long and rather tedious reaction

sequence (16 steps), which gave Pse in the end as one of many isomers following the three-carbon extension, with the required isomerization at N2. (**Scheme 2.1**). In the 1970's, Liav and Sharon synthesized Alt-diNAc from benzyl-β-L-fucose **305**, but in very poor yields.[112] Despite the fact that their starting material also included the 6-deoxy functionality, their scheme to invert and functionalize the hydroxyl groups on C-2, C-3 and C-4 through the 2,3-anhydro-4-O-mesyl-β-talopyranoside intermediate **306** markedly lacked regio- and stereoselectivity (**Scheme 3.2**). Among their setbacks, despite containing both electrophilic 2,3-anhydro and 4-O-mesyl groups, these did not undergo the simultaneous S_N2 substitution and epoxide opening with an azide nucleophile as planned, producing instead a mixture of isomers through a migration side-reaction (**Scheme 3.2**).

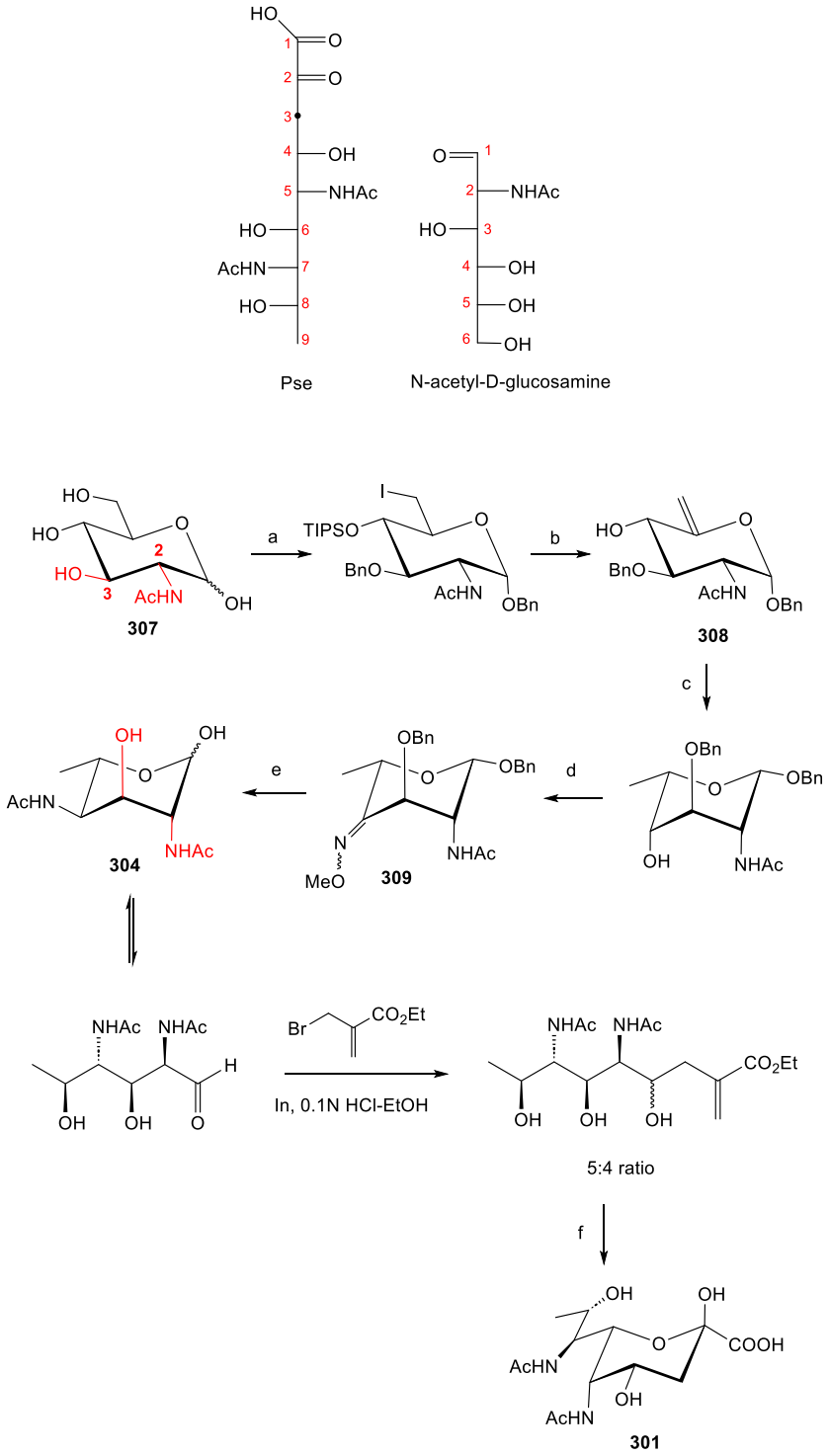


Reagents and conditions: (a) NaN_3, DMF

Scheme 3.2 *– Synthesis towards Alt-diNAc by Liav and Sharon,[112] and mechanism of migration that caused isomerization at C-4.*

The Ito group[78] recently reported their synthesis of Pse from N-acetyl-D-glucosamine (GlcNAc, **307**) via an Alt-diNAc intermediate (**Scheme 3.3**).[78] Although the starting material

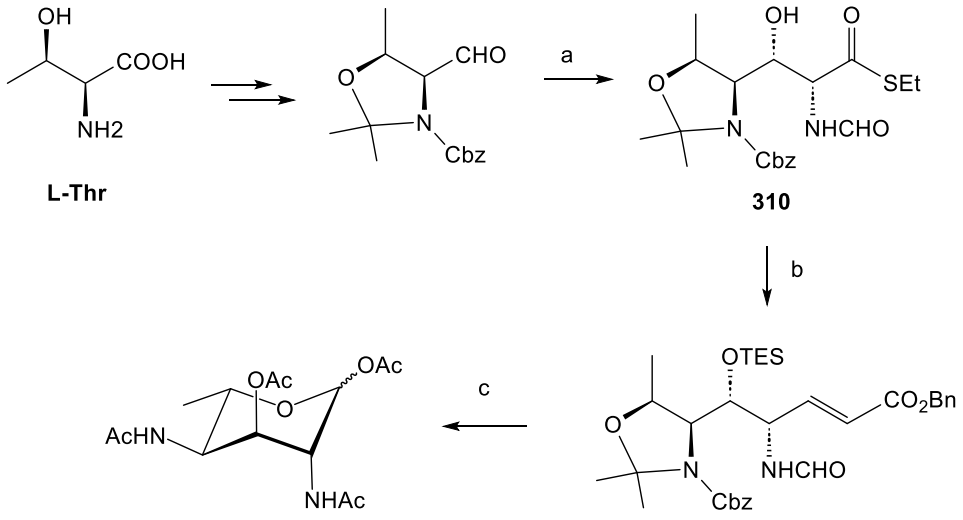
possessed the desired chirality and substitution at the C-2 (acetamido) and the C-3 (OH) positions (in red), their scheme required alterations at all of the other carbons centers, which ensued many challenges. For one, the direct deoxygenation of GlcNAc glycoside **307** using β-elimination from the C-6-iodide gave low yields, so more steps were added to first protect O4, allowing for harsher and more efficient elimination conditions using potassium *tert*-butoxyl, followed by treatment with tetra-n-butylammonium fluoride (TBAF) to remove the silyl ether, giving compound **308**. Secondly, a simple S_N2 reaction at the C-4 center via sulfonate ester was not successful, and instead required a reductive amination with samarium (II) iodide to produce oxime derivative **309**, followed by N-acetylation then hydrogenolysis, upon which Alt-diNAc **304** was obtained in a total of 14 steps. The carbon-chain elongation was executed through an indium-mediated allylation, allowing for the presence of free hydroxyl groups in the substrate, producing relatively good yields in one step (77%), but with low diastereoselectivity. Pse **301** and its 4-epimer were obtained after ozonolysis and saponification, with a total of 14 steps from **307**.



Reagents and conditions: (**a**) i. I_2, PPh_3,imidazole, THF, ii. TIPSOTf, 2,6-lutidine, DCM; (**b**) i. t-BuOK, THF,ii. TBAF, THF; (**c**) H2, $RhCl(PPh_3)_3$, benzene, EtOH; (**d**) i. Dess–Martin periodinane, $NaHCO_3$, DCM, ii. $MeONH_2$-HCl, $NaHCO_3$, MeOH; (**e**) i. SmI_2, MeOH, THF, ii. Ac_2O, pyr., iii. H_2, $Pd(OH)_2$, EtOH; (**f**) i. O_3, MeOH, ii. 30% H_2O_2, H_2O, HCO_2H, iii. TEA–H_2O (1:3).

Scheme 3.3 *- Synthesis of Pse via Alt-diNAc by the Ito group.*[78]

Of note, the *de novo* synthesis of Pse by the Li group[72] mentioned in section 2.1 included a derivatization of one of their intermediates to Alt-diNAc for structure elucidation purposes. The per-acetylated product was obtained in several low-yielding steps from the L-altro-configuration thioester **310** (**Scheme 3.4**): interestingly, they opted to "protect" the aldose functionality via Wittig reaction to allow for the manipulation of the remaining functionalities without cyclization of the sugar; deprotection was then achieved *via* ozonolysis.



Reagents and conditions: (a) i. $CNCH_2COSEt$, $LiOTf$, iPr_2NEt, DCE−DMF, r.t., 3 h; ii. THF−H_2O, reflux, 10 h, 67%, dr 5.1:1, over 2 steps; **(b)** i. Pd/C, Et_3SiH, THF, 2 h, then 1 M HCl(aq), DCM, 45 min; ii.$Ph_3P{=}CHCO_2Bn$, DCM, 45 min, 84% over 2 steps; **(c)** i. TFA, H_2O, 0 °C, 0.5 h; ii. 3% HCl(aq) in MeOH, 0 °C to r.t., 8 h; iii. Ac_2O, Na_2CO_3(aq), MeCN, 2 h, 59% over 3 steps; iv. O_3, DCM, −78 °C, 0.5 h; v. Ac_2O, N-methylmorpholine, DMAP, DCM, 93% over 2 steps; vi. $Pd(OH)_2/C$, H_2, MeOH, 2 h; vii. Ac_2O, Py, DMAP, 69%

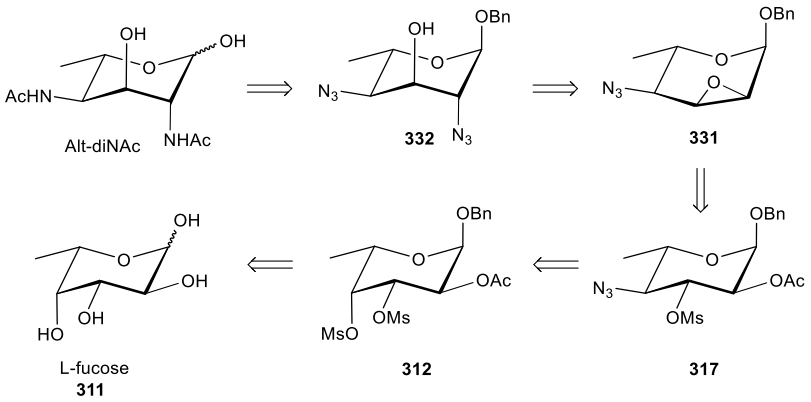
***Scheme 3.4** – Liu et al[72] derivitization of an intermediate from their de novo Pse synthesis to obtain a per-acetylated Alt-diNAc for structure elucidation.*

3.3 New Synthetic Strategy Toward Alt-diNAc[1]

We were interested in developing an efficient synthesis of Alt-diNAc from L-fucose (**311)** that could easily be scaled up to gram quantities in a better stereo control than previously reported. Like Liav and Sharon,[112] we chose L-fucose as a starting material because of the L-configuration at C-5 and 6-deoxy functionality, corresponding to the target compound, with the potential to selectively invert and functionalize the remain centers at C-2, C-3 and C-4 (**Scheme 3.5**). Our synthetic scheme was designed to produce good regio- and stereoselectivity for the required amino functionalizations by taking advantage of the significant differences in reactivity between C-3 and C-4 seen in certain polysulfonylated pyranosides.[113] With the information that nucleophiles such as benzoate or azide could be used to regioselectively displace the 4-tosylate from 2,3,4-tri-tosylated pyranosides of L-arabinose, D-ribose, D-lyxose and D-xylose, our plan avoided the previously reported need for the problematic β-L-talopyranoside intermediate **306** (**Scheme 3.2**). As shown in **Scheme 3.5**, our strategy instead involved using the di-O-mesylated substrate benzyl 2-O-acetyl-3,4-di-O-mesyl-a-L-fucopyranoside for the initial regioselective reaction with sodium azide at C-4 (**312**→**317**). Next, a 2,3-anhydro-4-azido-a-L-allopyranoside (**331**) could be formed to invert the configuration at C-3 center, while concurrently setting up a ring opening on the 2,3-epoxide by an azide nucleophile, with an expected regioselectivity following the Fürst–Plattner rules to yield the desired 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (**332**). Obtaining the final compound (**304**) would then require only a few extra

[1] Preliminary work presented in this section was performed by Carita Sequeira towards her Master's thesis.

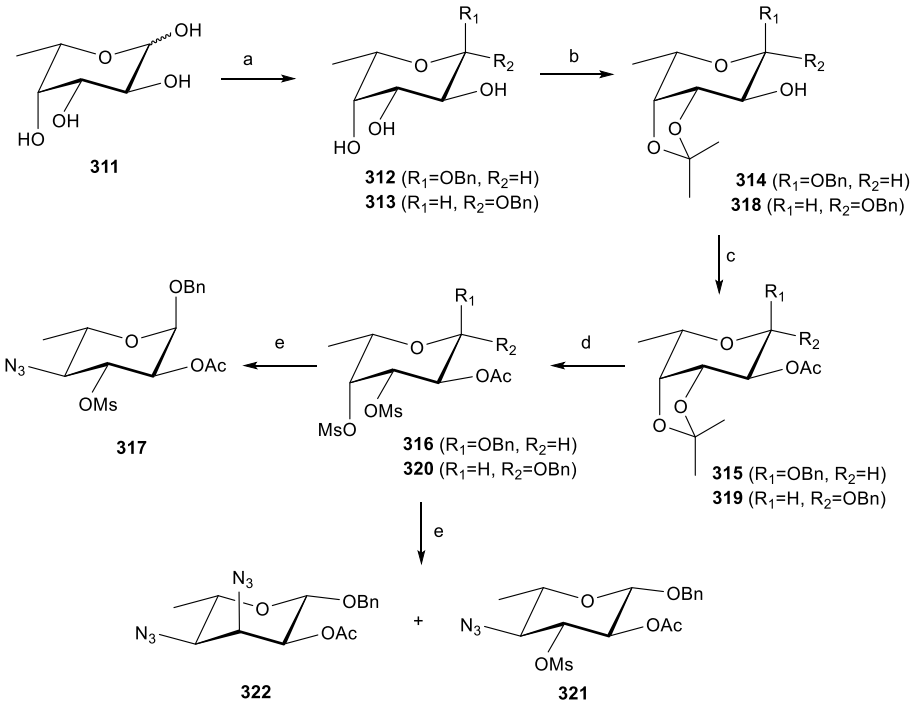
steps: the reduction of both azides followed by an *in situ* N-acetylation and a final deprotection of the anomeric benzyl group.



Scheme 3.5 *– Retrosynthesis of Alt-diNAc.*

As a first step, we developed a concise synthesis for epoxide precursors **316** and **320** that could be performed at a multigram scale (**Scheme 3.6**), improving on the previously reported synthesis of the 2-O-acetyl-3,4-O-isopropylidenated intermediate **314**[114] and its β-anomeric glycoside **318**.[112] While Liav and Sharon chose to use the β-anomer product **313** for their synthetic strategy, we opted for the α-anomer **312** as a better candidate for our scheme. In their case, a migration caused by the presence of the reactive sulfonate leaving group at C-4 (**Scheme 3.2**) gave a mixture of isomers: the issue was avoided in our scheme by an earlier substitution at C-4 with azide. Starting with L-fucose (30 g), an overnight Fischer glycosylation at 90 °C with benzyl alcohol in the presence of hydrochloric acid, generated *in-situ* from the addition of acetyl chloride to the alcohol solvent, gave the desired α-anomer **312** as the major product due the anomeric effect. A simple precipitation in hexanes was used to isolate the desired product in almost pure form (65% yield, contaminated with <5% of β-anomer **313**). A thorough rinsing of the product during vacuum filtration at this stage ensured that no remaining benzyl alcohol would complicate

purification and NMR characterizations later on. The pure α-isomer was obtained via recrystallization in ethyl acetate and hexanes, then a regioselective protection of its 3,4-*cis*-diol with isopropylidene followed by a simple acetylation gave compound **315** in a 34% overall yield from L-fucose. An eventual optimization of these conditions led to improved yields and reduced reaction times, when the above three steps were combined into a one-pot synthesis, affording **315** and its β-anomer in 48% and 20% yields from L-fucose, respectively. The higher yields can be attributed to the elimination of the precipitation and crystallization steps after making **312**, an inefficient purification method that moreover caused the loss of the more soluble β-anomer **313**. By reducing the amount of benzyl alcohol used in the first step from 5 mL per gram of L-fucose to 2 ml per gram, using a trace amount of p-toluenesulfonic acid as catalyst and heating to 130–140 °C with azeotropic removal of water, the product mixture could be subjected to the 3,4-O-isopropylidenation *in situ* with an excess amount of 2,2-dimethoxypropane, and the ultimate isolation of both anomers was achieved by silica gel column chromatography.



Reagents and conditions: (**a)** BnOH, AcCl, 130 °C; **(b)** 2,2-dimethoxypropane, CSA, acetone; **(c)** Ac_2O, pyridine; **(d)** i. 70% AcOH-H_2O, 90 °C; ii. MsCl/pyridine; **(e)** NaN_3 /DMF, 80 °C..

Scheme 3.6 *- Synthesis of Benzyl 3,4-di-O-mesylated precursors **316** and **320** and their subsequent substitutions with azide nucleophile.*

Deprotection of the 3,4-O-isopropylidene from the α-fucopyranoside **315** was achieved in 85% aqueous acetic acid at 80 °C, exposing the two hydroxyl groups at C-3 and C-4 (**Scheme 3.6**). Care had to be taken to avoid possible deacetylation at C-2 at these low pH levels, therefore the reaction was carefully monitored and stopped once the full conversion was achieved after about 2 hours. The crude 3,4-O-diol intermediate was then di-O-mesylated without difficulty using an excess amount of methanesulfonyl chloride (2.5 equivalents per OH group) in pyridine to obtain **316** in good yields. The structure of 3,4-O-dimesylate **316** was confirmed with the appearance of two new distinct singlets at 3.12 and 3.19 ppm, integrated to three protons each when compared to the anomeric doublet at 5.04 ppm (**Figure 3.2**). No mono-mesylated product was detected from analysis of the crude ^{1}H NMR, and so it was clear that by using an excess of

reactive methanesulfonyl chloride, both the axial and equatorial hydroxyl groups can be activated at the same time. The β-glycoside **319** was likewise hydrolyzed, and di-O-mesylated afterwards as above, to yield the corresponding 3,4-di-O-mesylated β-fucopyranoside **320**.

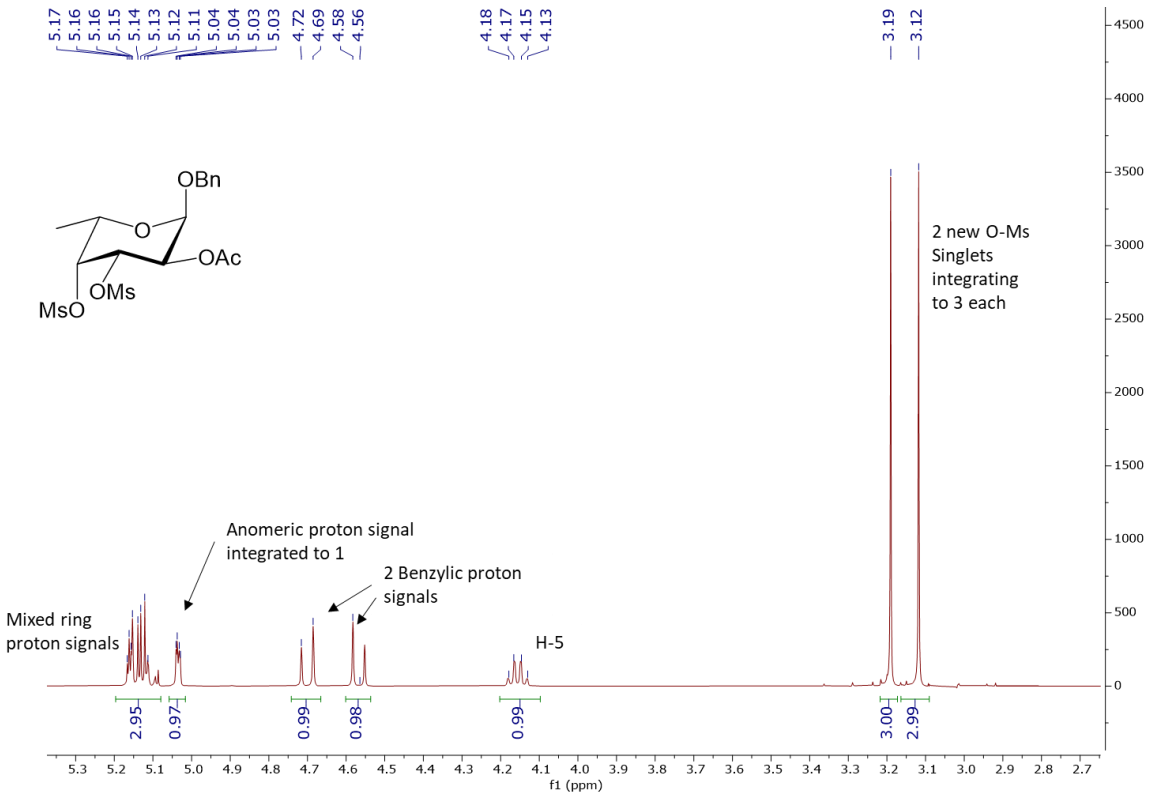


Figure 3.2 *– ^{1}H NMR spectrum of O-benzyl di-mesyl compound **316**.*

3.4 S_N2 Displacement Selectivity Investigation

When **316** was heated in DMF the presence of an excess amount of sodium azide (>5eq.), **317** was obtained exclusively, and in almost quantitative yields: despite the use of a large excess of nucleophile, high temperatures (90-100 °C) and long reaction times (up to 40 hours), no di-substituted product was isolated. The 4-O-mesylate of compound **316** was confirmed to have

been selectively displaced by ^{1}H NMR spectrum, because according to ^{1}H–^{1}H GCOSY spectrum (**Figure 3.3**), H-4 was found to shifted upfield from 5.04 ppm (compound **316**) to 3.37 ppm (compound **317**); furthermore, the coupling pattern of H-4 of formed product **317** was observed to be a doublet of doublets with two large coupling constants (9.7, 9.9 Hz), confirming that the C-4 center had an axial hydrogen, therefore an equatorial azide substituent.

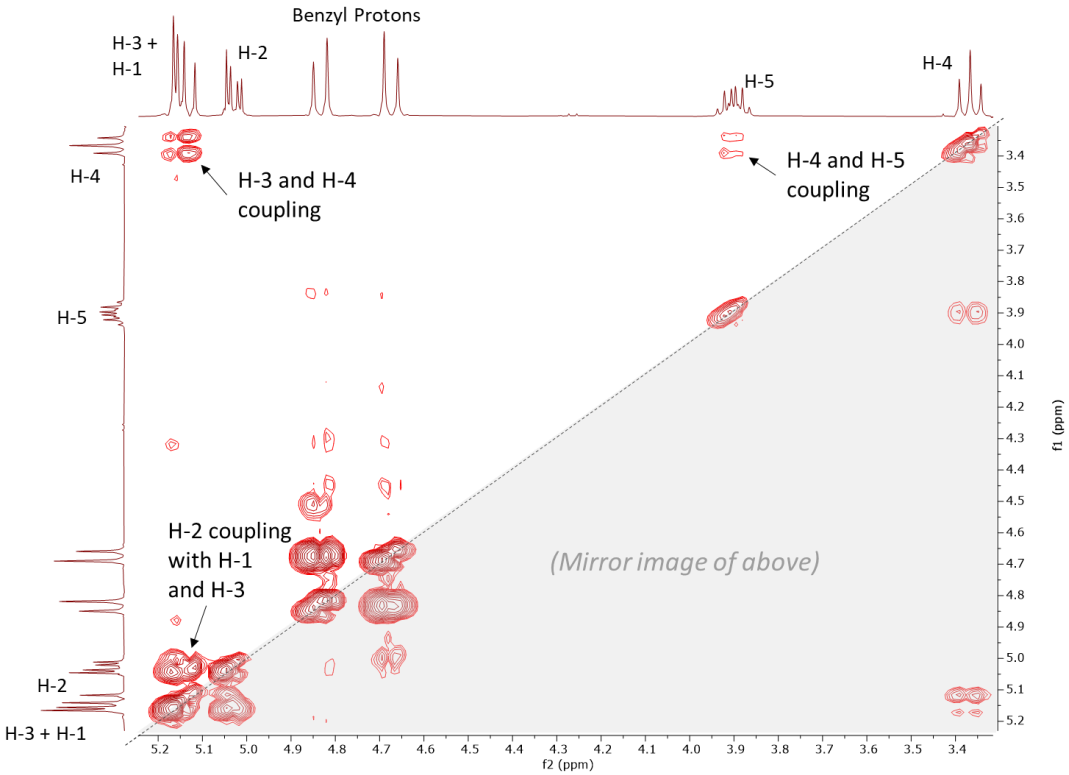


Figure 3.3 *- ^{1}H–^{1}H gCOSY spectrum of compound **317** that shows correlations between the ring protons that helped identify H-4 at 3.37 ppm due to its coupling with both H-5 at 3.91 ppm and H-3 at 5.17 ppm.*

This type of regioselectivity was also exploited, among others, by Sanapala and Kulkarni[115] in their excellent synthesis of amino L-sugar building blocks. According to the Richardson-Hough rules[113], recently updated by Hale *et al*,[116] the S_N2 displacement of sulfonates on pyranosides can be predicted based on the configuration of substrates, specifically with regards to the alignment of their dipole moments in the developing transition states (TS). As depicted in **Figure 3.4**, for α-

fucopyranoside **316**, the S_N2 displacement of mesylates on pyranosides by azide undergoes two possible TS: the attack on the 3-O-sulfonate (structure **A**) causes a repulsive alignment between the dipole of the partially bonded departing C-3---OMs and the permanent dipole of axial C-4-OMs, in addition to a 1,3-diaxial dipole clash between the C-1-OBn dipole and the partially bonded incoming C-3---N3 dipole, making the substitution of C-3-mesylate difficult. On the other hand, the S_N2 substitution of 4-O-mesylate can be illustrated by a TS (structure **B**) with misaligned dipoles between the C-3 and C-4 mesylates, where no hindering repulsion effect is present, allowing for the clean S_N2 displacement of 4-mesylate. The reaction therefore not only proceeded smoothly, but was successfully scaled up to a ~20 gram scale in 98% yield, further demonstrating the excellent regioselectivity in this scheme.

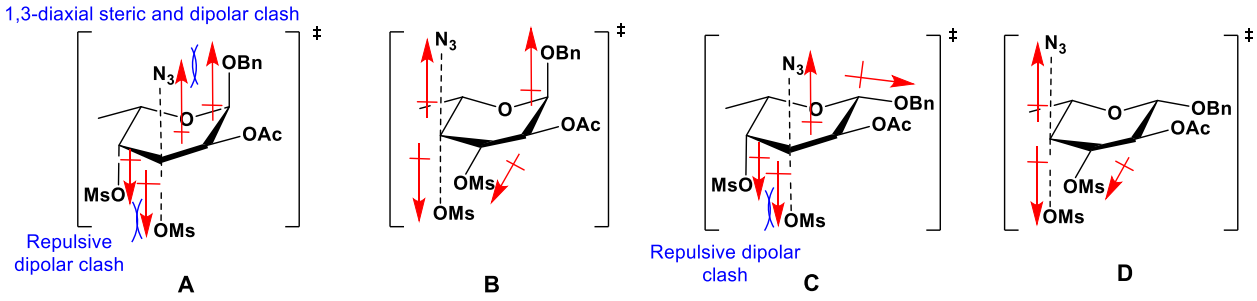


***Figure 3.4** - Transition states formed from the S_N2 displacement of 3- and 4-mesylates of α-fucopyranoside **316** (structures A and B) and β-fucopyranoside **320** (structures **C** and **D**), by an azide.*

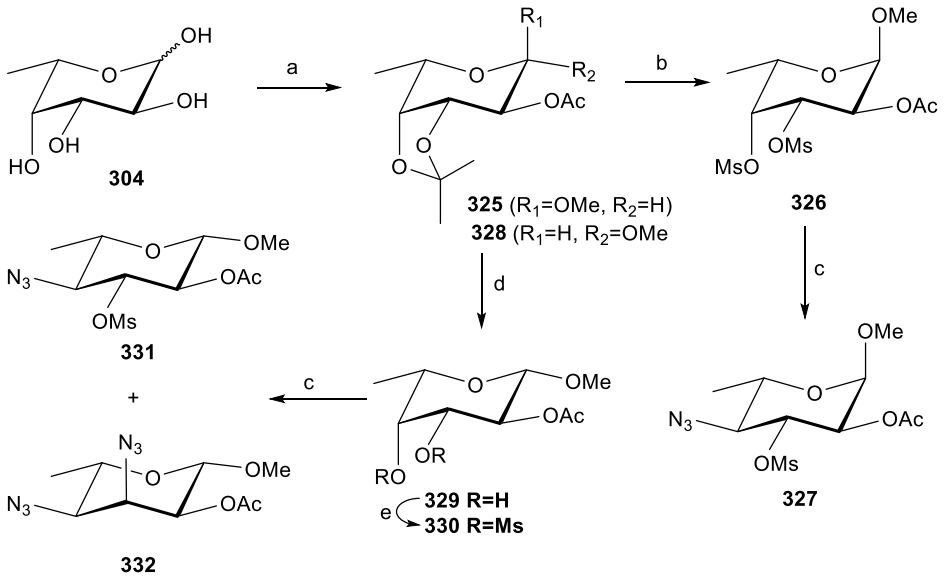
The mechanism of this selectivity was confirmed when the azide substitution reaction results were compared to ones with the β-anomer of the di-mesylated fucopyranoside **320** (**Scheme 3.6**). While the substitution of the 3-O-mesylate was completely inhibited for the axial O-benzyl anomer, a reaction under the same conditions with the equatorial O-benzyl fucopyranoside **320** resulted in the partial exchange of the 3-O-mesylate, giving **322** as a minor

product in a 29% yield, in addition to the major mono-substituted **321** in 60% yield. The H-4 signal at 3.35 ppm in **321** and 3.23 ppm in **322** indicated a shielding functional group on carbon 4, consistent with the presence of a less electron-withdrawing azide functionality. The spectrum for **321** contained an O-mesyl peak at 3.13 ppm, which confirmed a single substitution with azide, whereas in the ^{1}H spectrum for **322** a complete lack of O-mesyl peak corroborated a double substitution at both C-3 and C-4 centers. The latter was further confirmed by the upfield shift of the H-3 signal in **322**, and the significant upfield shift of the C-3 signal in the ^{13}C NMR shift, consistent with a C-N bond at 62 ppm. The small coupling constants of the H-3 proton signal in **322** ^{1}H NMR spectrum correlated with an equatorial proton at position 3, also giving evidence of a doubly substituted product with an axial shielding substituent (azide). The O-acetyl functional group was confirmed to still be present in both compounds, as the chemical shift of the H-2 protons remained downfield at around 5 ppm, suggesting the presence of an electron-withdrawing acetyl group on the O2 atom. The assignments were confirmed by ^{1}H-^{1}H GCOSY spectra.

Turning once again to the TS for explanations in the case of S_N2 attack on the 3-O- and 4-O-mesylates of β-fucopyranoside, the unfavorable dipole interactions for the substitutions at both positions are markedly reduced (**Figure 3.4**). The attack on 3-O-mesylate remains partially blocked by the bottom-face dipole repulsions as seen in structure **C**, but becomes more likely because of the of missing 1,3-diaxial clash with the incoming C-3---N3 dipole, while there are no dipole alignments whatsoever in the TS of structure **D**: this explains why both compounds **321** and **322** were formed in different yields. In an attempt to minimize substitution at C3 to obtain **321** exclusively, the reaction was performed at a lower temperature (70 °C instead of 90 °C), with

only two equivalents of sodium azide: although according to TLC a single product seemed to be forming in the first 24 hours, the second substitution did start occurring before the starting material was fully consumed, meaning that a complete selectivity in the case of the β-anomer would therefore be hard to achieve using the chosen conditions. The reaction was not observed to occur at any lower temperatures than that.

To investigate the influence of size of aglycone on the nucleophilic substitutions at C-3 and C-4 centers, we also prepared the 3,4-dimesylates of both α/β-anomers of methyl L-fucopyranosides **326** and **330** from L-fucose (**Scheme 3.7**). When the substitution reaction was performed under the same conditions as before on the methyl 2-O-acetyl-3,4-di-O-mesyl α-L-fucopyranoside **326**, the monosubstituted product **327** was isolated with no encounter of the 3,4-disubstituted product. As before, the same reaction was carried out with the corresponding β-L-fucopyranoside **330**, but this time gave the monosubstituted product **331** as the minor product in 24% yield and the 3,4-disubstituted product **332** in majority (52% yield): apparently the nature of the anomeric substituent could also entail some electronic effects that influence reactivity. Nevertheless, these results confirmed that both the steric hindrance and strong dipole/dipole repulsions in the TS play a significant role in this type of substitution.



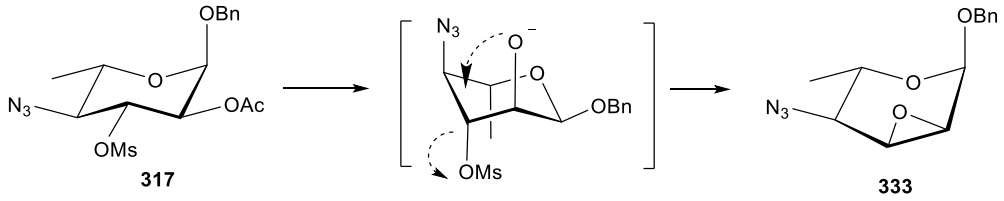
Reagents and conditions: (a) i. AcCl, MeOH; ii. 2,2-dimethoxyporpane, CSA, acetone; iii. Ac_2O, Pyr.; **(b)** i. 70% AcOH-H_2O, 90 °C; ii. MsCl/pyridine; **(c)** NaN_3 /DMF, 80 °C; **(d)** 70% AcOH-H_2O, 90 °C; **(e)** MsCl/pyridine.

Scheme 3.7 *- Synthesis of Methyl 3,4-di-O-mesylated precursors* ***326*** *and* ***330*** *and their subsequent substitutions with azide nucleophile.*

3.5 Optimizations for the Epoxide Intermediate Strategy

With large amounts of easily accessible benzyl 2-O-acetyl-4-azido-4,6-dideoxy-3-O-mesyl-α-L-fucopyranoside **317** at our disposal, an epoxidation was performed in excellent yield (89%) via transesterification in anhydrous methanol in the presence of sodium methoxide to first remove the O2-acetyl group, causing a displacement of the 3-O-mesylate through *in-situ* intramolecular ring closure (**Scheme 3.8**).[117] The oxirane formation was quite slow at room temperature, requiring overnight stirring (~20 hours) to complete: heating to 55 °C on the other hand gave the desired L-allo-epoxide **333** within a few hours in large amounts (>10 g). The reaction could easily be monitored using TLC, and the epoxide appeared to be the sole product

formed: no intermediate was detected on the TLC plate. This process successfully inverted the chirality of the C3 centre, a requirement in our designed strategy. The inversion that gave compound **333** was confirmed by ^{1}H NMR spectrum through the change in coupling constants of the H-3 signal (doublet of doublets), from two large *J* coupling constants to two small *J* coupling constants (*J* = 1.6, 4.1 Hz, H-3), indicative of a dihedral angle reduction from the previous 180 degrees between H-3 and its neighbors. Furthermore, both H-2 and H-3 signals were observed to shift upfield to 3.49 ppm and 3.55 ppm respectively, and along with the parallel C-2 and C-3 signal shifts in the ^{13}C NMR, indicated the presence of the epoxide functionality linking the two carbons.

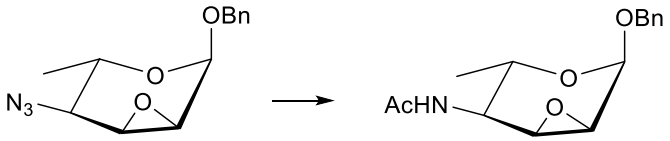


Reagents and conditions: NaOMe, MeOH.

Scheme 3.8** - Base-mediated O-deacetylation and epoxidation of compound **317.

The key to the exclusive diastereoselective formation of the desired L-allo-epoxide **333** in high yield at this point lied in the choice we made to install the azido functionality at C-4 before the epoxidation reaction. The same reaction was performed by Liav and Sharon[112] on a β-O-benzyl-2-acetyl-3,4-di-O-mesyl precursor **320**, giving them a much lower yield (49%) for the epoxide (**306**, **Scheme 3.2**), possibly due to a displacement of the C-4 sulfonate: the lack of any dipole interactions in TS of the S_N2 reaction at C-4 center makes the displacement of the sulfonate with a nucleophile, even a weak one like acetate, quite favorable. Another big advantage of our scheme is the opportunity to differentiate between the amino groups at C-2 and C-4 at this stage in the synthesis. An initial investigation was performed, where the epoxide was treated with

palladium hydroxide then acetic anhydride to produce compound **334** in 63% yield in two steps (**Scheme 3.9**). This demonstrated the adequate stability of the epoxide to reducing conditions, and paved the way towards future work for the differentiation of the two amino functionalities, which will be covered in more depth in Chapter 4.

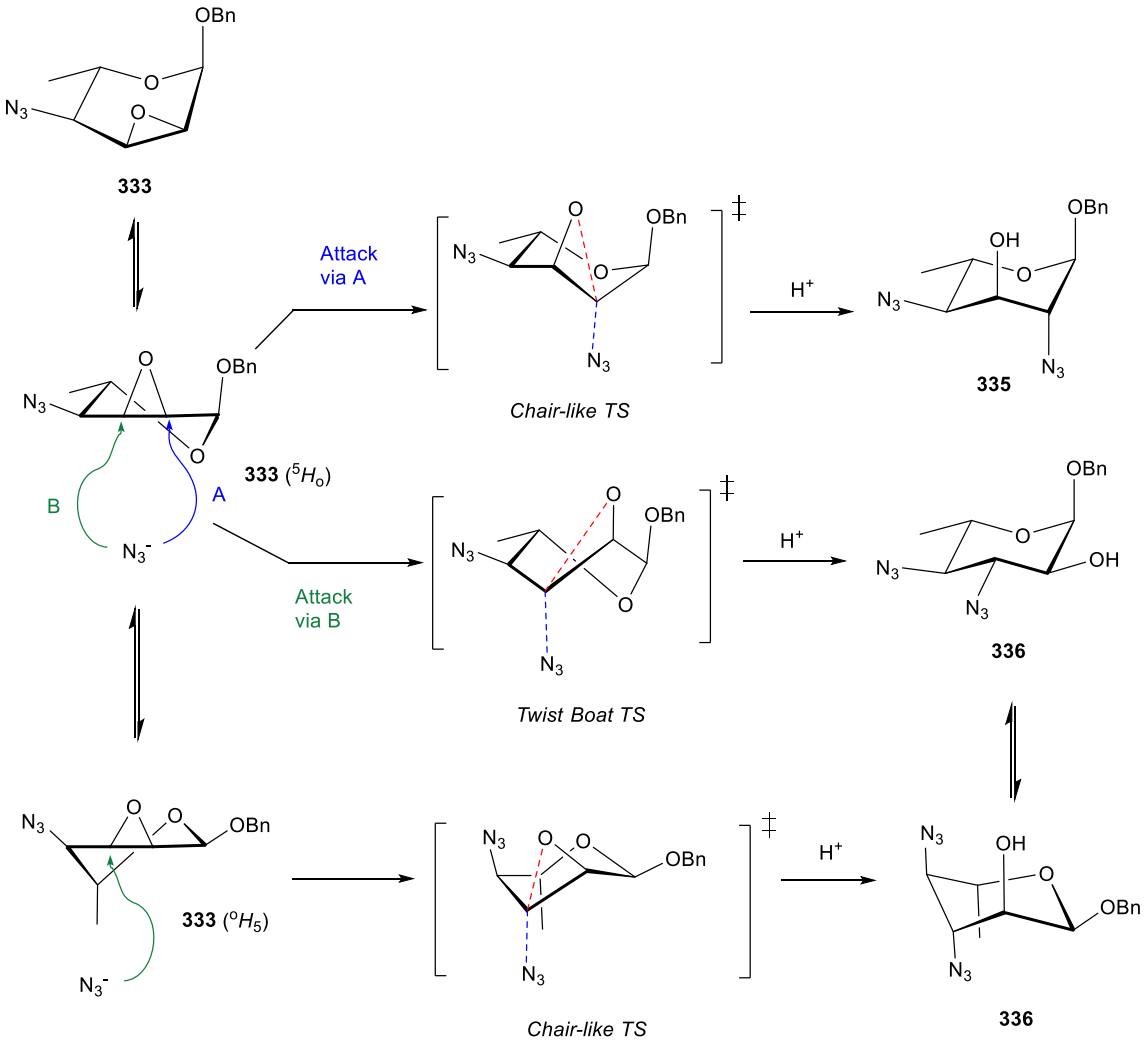


333 **334**

Reagents and conditions: i. H_2, Pd(OH)2/C, NH_4OH, MeOH, CH_2Cl_2; ii. Ac_2O / MeOH

Scheme 3.9 *– Preliminary investigation into a N2,N4 differentiation strategy.*

Following the successful synthesis of 4-azido-L-allo-epoxide **333**, we proceeded to investigate the oxirane ring opening using sodium azide as the nucleophile. A carefully optimized reaction was thought to have good potential for regioselectivity by taking advantage of the Fürst-Plattner rules,[117] where *trans*-diaxial ring opening to give the desired 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **335** was expected to be favored over the formation of 3,4-diazido-α-L-glucopyranoside configuration **336** due to its unfavorable boat-like TS (**Scheme 3.10**). However, a low-yielding mixture of the two isomers **335** and **336** was obtained in almost equal ratios (Entry 1 in **Table 3.1**) when **333** was reacted under standard conditions,[118] with five equivalents of sodium azide in anhydrous DMF at 90 °C. A change of solvent to ethanol did not increase productivity nor selectivity, despite an anticipated activation by the media engaging in hydrogen-bonding with the oxirane ring (Entry 2).



Scheme 3.10 *– Oxirane ring opening reaction with TS schematic: enhanced flexibility between the* $^{5}H_{o}$ *and* $^{o}H_{5}$ *conformations of compound* ***333*** *results in poor regio- and stereo-selectivity during azide-mediated opening of the epoxide.*

The above results were thought to be a consequence of the rapid conformational exchanges between the $^{O}H_{5}$ and $^{5}H_{O}$ conformations that occur in non-rigid ring systems such as ours, especially at higher temperatures. Since the Fürst-Plattner rules favors a product formed via the more stable chair-like TS to avoid the energetically expensive twist boat TS conformation, as seen in **Scheme 3.10**, it is likely that the formation of the undesired product **336** resulted from

a ring flipping that gave the large anomeric substituent a favored pseudo-equatorial orientation, likewise allowing for the required chair-like TS for the epoxide opening.

A fused 4,6-O-benzylidene ring is commonly appended to 2,3-epoxy-hexoses with a 6-hydroxyl group to restrict conformational exchanges in ring systems undergoing this type of reaction, producing 2,3-trans-diaxial products in excellent selectivity.[119,120] This was nicely demonstrated by a series of publications by Hevey *et al*, where a di-inversion of the rigid 4,6-O-benzylidene-2,3-di-O-sulfonyl-β-D-galactopyranosides via epoxide intermediate gave the desired C-2 and C-3 trans-diaxal products (C-3-alkoxy C-2 alcohol) in complete regio and stereo control and excellent yields.[121–125] In our case, the lack of a C-6 handle did not allow for the formation of a rigid bicyclic system, prompting us to investigate other strategies. It has been reported that using either a Brønsted[126,127] or a Lewis acid[128–133] to coordinate with the ring oxygen of the epoxide could improve reactivity and selectivity in the desired ring opening reaction: for example, the addition of ammonium chloride to the reaction mixture catalyzes the reaction and provides some regioselectivity for non-rigid ring systems.[127,134] Other literatures have successfully used additives such as lithium perchlorate[131,132], zinc (II) perchlorate[128], cerium (III) chloride[129], ferric perchlorate[130] and Amberlyst 15[135] for the regiochemical control of oxirane openings with amino- or azido-groups. We believed that a strong coordination with the oxirane would slow down ring flipping, while the added polarization would likely make the molecule preferentially adopt the $^5H_0$ conformation with a cancelled dipole moment, as illustrated in **Figure 3.5**.

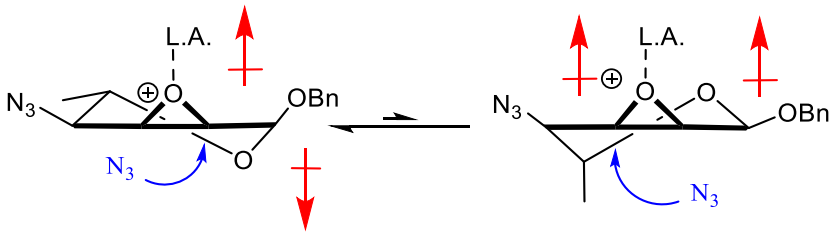


Figure 3.5 *- Coordination of the oxirane could lead to reduced conformational exchange, favoring the* $^{5}H_{O}$ *conformer due to cancellation of dipole moments.*

When adding a coordinating cation to the reaction, the fine balance between improved reactivity of the epoxide and the reduced nucleophilicity of the azide had to be carefully considered. We first investigated regioselectivity by simple protonation of the oxirane with the addition of ammonium chloride, which resulted in a slight increase in selectivity, and a large improvement in reactivity over catalyst-free conditions (Entry 3, **Table 3.1**). Cerium chloride had previously[129] been used to assist in regioselective epoxide opening in a 9:1 acetonitrile and water mixture, and so its effect was investigated for this reaction: it was expected that protons released in aqueous conditions by Lewis acid coordination would have a similar effect to ammonium chloride. However in both cases when aqueous ethanol or acetonitrile were used as solvents, the reaction did not go to completion within the allotted 48 hours, and the selectivity was not significantly improved (Entries 3 & 4).

Table 3.1 *– Epoxide Opening Optimizations*

Entry	Mass of 333	Solvent	Nucleophile	Catalyst	Temperature / time	Ratio N2,N4:N3,N4 products[a]	Yield of N2,N4 product[a]
1	2.30 g	DMF (anh.)	NaN_3 (5 eq.)	-	90 °C / 24 hrs	4:3	<50%[b]
2	20 mg	EtOH (95%)	NaN_3 (4 eq.)	-	90 °C / 2 days	1:1	50%
3	20 mg	DMF (anh.)	NaN_3 (3.5 eq.)	NH_4Cl (3.5 eq.)	90 °C / 2 days	3:2	55%
4	20 mg	EtOH (95%)	NaN_3 (8 eq.)	$CeCl_3$ (2 eq.)	85 °C / 2 days	5:4	30%
5	20 mg	MeCN / H_2O (9:1 ratio)	NaN_3 (2 eq.)	$CeCl_3$ (2 eq.)	85 °C / 2 days	5:4	10%
6	20 mg	MeCN (anh.)	NaN_3 (4 eq.)	$LiClO_4$ (2 eq.)	90 °C / 2 days	3:1	70%
7	100 mg	MeCN (anh.)	NaN_3 (4 eq.)	$LiClO_4$ (2 eq.)	85 °C / 2 days	5:1	80%
8	1.0 g	MeCN (anh.)	NaN_3 (4 eq.)	$LiClO_4$ (3 eq.)	85 °C / 2 days	5:1	62%[b]
9	1.00 g	MeCN (anh.)	NaN_3 (4 eq.)	$LiClO_4$ (4 eq.)	85 °C / 2 days	5:1	70%
10	100 mg	MeCN (anh.)	NaN_3 (4 eq.)	$LiCF_3SO_3$ (2 eq.)	85 °C / 2 days	5:1	70%
11	97 mg	MeCN (anh.)	NaN_3 (5 eq.)	$LiCF_3SO_3$ (6 eq.)	80 °C / 2 days	5:1	77%[b]
12	20 mg	MeCN (anh.)	NaN_3 (4 eq.)	12-crown-4 / $LiClO_4$ (2 eq.)	85 °C / 2 days	4:1	75%
13	100 mg	MeCN (anh.)	NaN_3 (4 eq.)	$NaClO_4$ (2 eq.)	85 °C / 2 days	-	-
14	20 mg	EtOH (anhydrous)	NaN_3 (8 eq.)	$CeCl_3$(2 eq.)	85 °C / 2 days	4:3	25%
15	20 mg	MeCN (anh.)	NaN_3 (2 eq.)	$CeCl_3$ (2 eq.)	85 °C / 2 days	5:3	32%
16	20 mg	MeCN (anh.)	NaN_3 (8 eq.)	$Mg(ClO_4)_2$ (2 eq.)	85 °C / 2 days	-	-
17	20 mg	MeCN / H_2O (9:1 ratio)	NaN_3 (8 eq.)	$Fe(ClO_4)_3$-H_2O (2 eq.)	85 °C / 2 days	-	-
18	20 mg	MeCN (anh.)	Morpholine (4 eq.)	$LiClO_4$ (2 eq.)	70 °C / 24 hrs	5:1	80%
19	100 mg	MeCN (anh.)	Morpholine (4 eq.)	$LiClO_4$ (2 eq.)	60 °C / 24 hrs	-	63%[b]
20	20 mg	EtCN (anh.)	NaN_3 (4 eq.)	$LiClO_4$ (4 eq.)	100 °C / 1 day	4:1	60%
21	21 mg	MeCN (anh.)	NaN_3 (4 eq.)	15-crown-5 / $LiClO_4$ (2 eq.)	90 °C / 2 days	3:1	60%
22	21 mg	MeCN (anh.)	NaN_3 (4 eq.)	TBACl (10 mol%) / $LiClO_4$ (2 eq.)	90 °C / 2 days	5:1	80%
23	5.53 g	MeCN (anh.)	NaN_3 (4 eq.)	$LiClO_4$ (4 eq.)	85 °C / 5 days	-	62%[b]

[a]Estimated by integration in crude ^1H NMRs, comparing integration of anomeric peaks, unless specified otherwise; [b] Actual yield % of desired regioisomer from products purified by silica gel column chromatography.

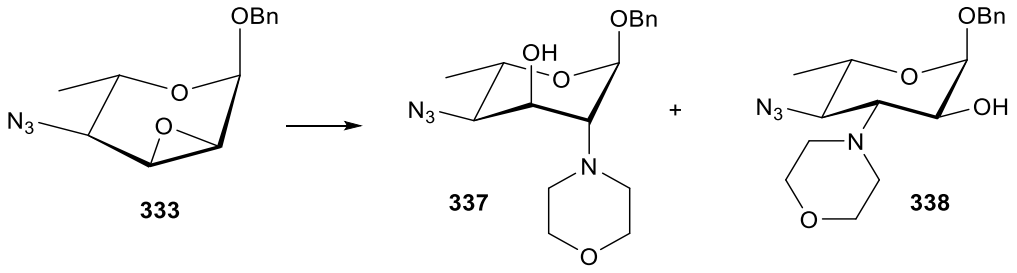
The focus was then shifted to monovalent cations for epoxide activation, by first running trials with the commonly used lithium perchlorate additive in an aprotic solvent. A much-improved reaction time and selectivity were observed at a milligram scale in numerous trials, and

the selectivity remained good as the scale was increased to 100 mg, then 1.0 g (Entries 6-9, **Table 3.1**). The effect of the counter-anion was investigated further by switching to a different reagent, lithium triflate, which produced similar results albeit in slightly lower conversion rates at larger scales (Entries 10 & 11). When lithium was made less available in the reaction procedure by trapping it inside of 12-crown-4, a slight decrease in selectivity ensued, confirming its role in regioselectivity (Entry 12).

An investigation into the scope of coordinating cations that could be used to selectively activate the epoxide was also performed for reaction optimization. It was thought that perhaps larger or multivalent cations could further rigidify the hexose ring by coordination with the multiple oxygens present in the structure. It was found however that the reaction was significantly slowed down or even halted when either sodium, cerium, magnesium(II) or iron(III) ions were used as additives (entries 13-17, **Table 3.1**). The monovalent lithium turned out to be the best choice for this reaction, with its apparent preferred affinity towards oxygen atoms over nitrogen, allowing for the azide nucleophile to remain appropriately reactive.

Despite its high nucleophilicity, reactions with sodium azide in aprotic solvents are usually slow and require high heat due to solubility issues: in our case, a minimum of 80 °C was necessary for the reaction to even begin. When, on the other hand, morpholine was used for the oxirane opening, the reaction could be performed as low as 60 °C, and went to completion in a reasonable amount of time (**Scheme 3.11**, entries 18 & 19, **Table 3.1**). We expected to see an increase in selectivity as the heat was reduced, due to possibly reduced speed of conformational exchange, but this was not evident in our preliminary results: the obtained ratios for the morpholine

products **337** and **338** were similar to the ones obtained with azide (5:1, as per crude ^{1}H NMR ratios).



Reagents and conditions: Morpholine, CH_3CN, $LiClO_4$, 60 °C.

Scheme 3.11 *- Ring opening of oxirane **333** with more soluble morpholine nucleophile.*

Going back to the azide nucleophile, a 10 degree increase in temperature did not affect selectivity either, as seen by the product ratios following the reaction in refluxing propionitrile, which has very similar properties to acetonitrile, but a higher boiling point: it did however almost double the speed of the reaction (Entry 20). Other strategies that were tried to improve the reactivity of the azide nucleophile included adding 15-crown-5 ether to the reaction with the goal of chelating the sodium counter-ion to further activate the azide anion, as well as the addition of 10 mol% of the phase transfer catalyst tetra-butyl ammonium chloride (TBACl) to increase azide solubility (Entries 21 and 22). In both cases, selectivity was not markedly improved, therefore lithium perchlorate remained the choice additive, and was used for the reaction scale-up. The large-scale reaction (5.53 g) was allowed to proceed for 5 days, which assured a near quantitative conversion rate, and a good yield was obtained (Entry 23).

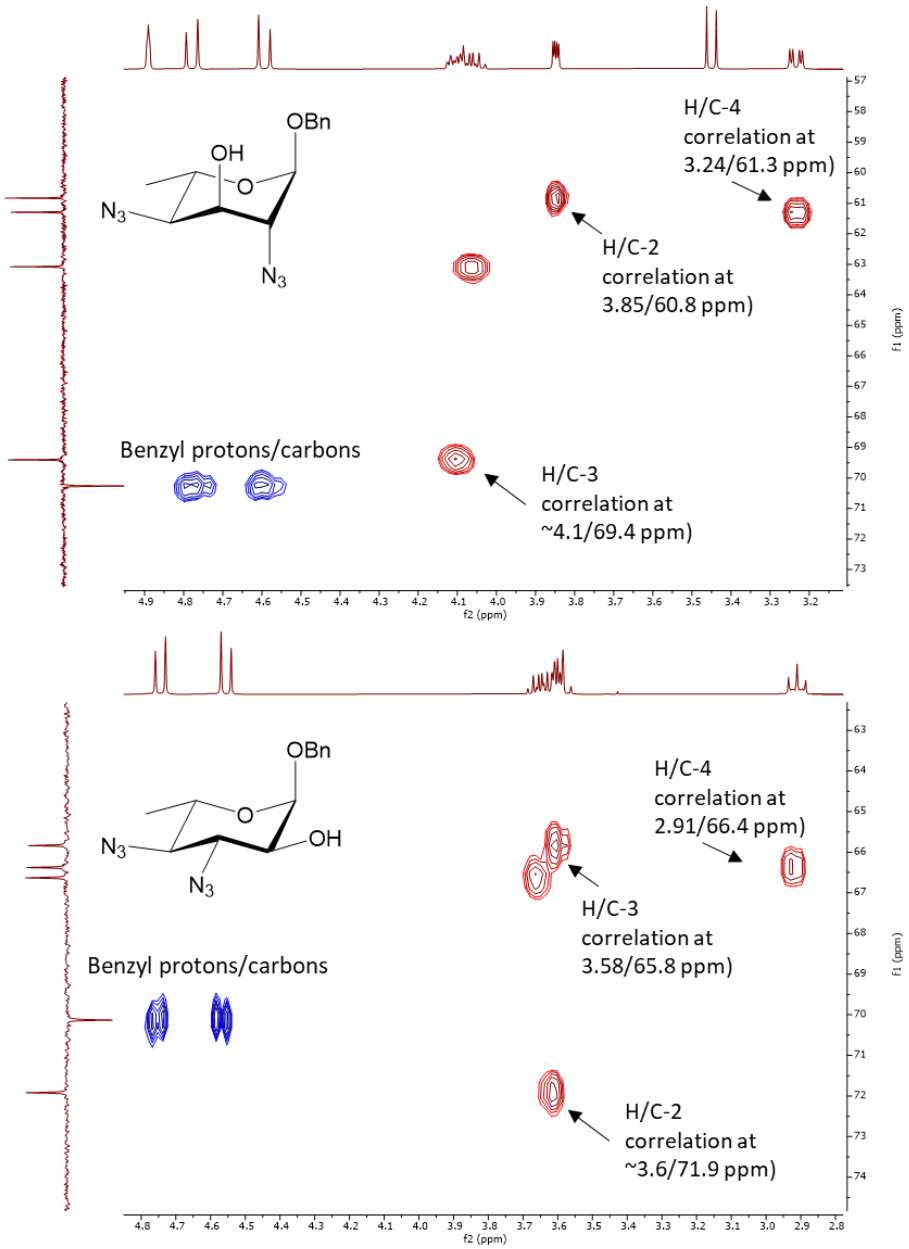


Figure 3.6 *- ^{1}H-^{13}C GHSQC spectrum of compounds **335** and **336** that shows the correlating signals for the azide-substituted carbons and associated protons. The upfield signal shift for carbons substituted with a hydroxyl to ones substituted with an azide, and vice-versa, is quite evident in these spectra.*

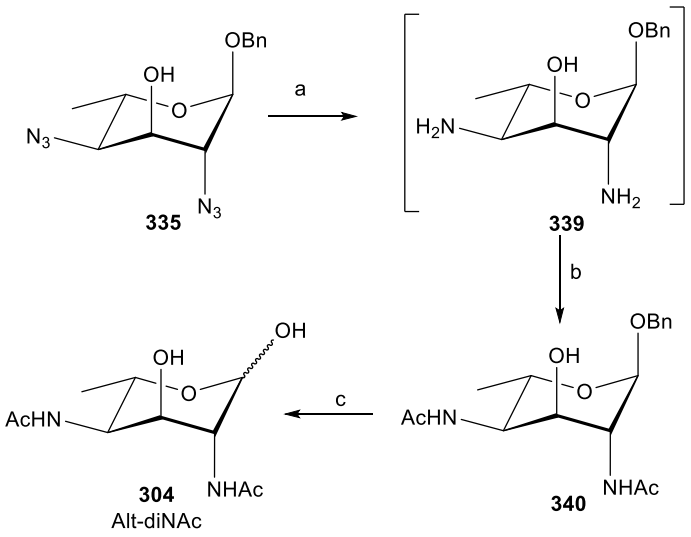
The structures of the two di-azido products **335** and **336** were confirmed through ^{1}H-^{1}H GCOSY and ^{1}H-^{13}C GHSQC correlation experiments. For compound **335**, the ^{13}C signals for the two carbons attached to an azide were observed at 60.8 ppm (C-2) and 61.3 ppm (C-4), while the

other carbon with a hydroxyl group attached to it was seen at 69.4 ppm (C-3). The shielding effect was also corroborated in the ^{1}H NMR, where signals for H-2 and H-4 moved significantly upfield (3.85 ppm and 3.24 ppm respectively). Similarly, for compound **336**, the ^{13}C signals for the two carbons attached to an azide were observed at 65.8 ppm (C-3) and 66.4 ppm (C-4), while the other carbon with a hydroxyl group attached to it was observed at 71.9 ppm (C-2). Again, the shielding effect of the azide groups was seen in the ^{1}H NMR with H-3 and H-4 upfield (3.58 ppm and 2.91 ppm respectively).

3.6 Final Derivatizations

Once the oxirane ring was successfully opened with an azide nucleophile with optimized selectivity, synthesis towards to L-Alt-2,4-diNAc **304** was continued with the correct 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside regio-isomer **335** by first chemoselectively reducing the 2,4-azido functionality through the very mild Staudinger azide reduction conditions using triphenylphosphine as a reagent in a mixture of pyridine – water (10:1), followed by an in-situ N-acetylation in methanol to give the di-N-acetyl compound **340** (**Scheme 3.12**). Previously, the same transformation had been achieved with lithium aluminum hydride as the reducing agent, and the benzyl diamino intermediate had to be worked-up before performing the acetylation, giving poor yields in the end (38%)[112] In our case, we were able to perform both reactions in one pot, giving the 2,4-di-N-acetylated compound **340** in 59% yield over two steps. A second method involving a controlled hydrogenolysis was also performed, where a few drops of ammonia were added as a mild poison against the palladium on charcoal catalyst to reduce its reactivity towards

the O-benzyl group, thus selectively reducing the two azide functionalities.[136] To be sure, when compound **335** was subjected to the latter conditions in a mixture of methanol and dichloromethane, the desired selectivity was achieved, and the crude diamine **339** was further N-acetylated as above, producing much better yield (81%). The formation of **340** was confirmed with the obvious appearance of the two amido proton doublets at 6.01 and 6.13 ppm, as well as the presence of two overlapping singlets at 2.02 ppm, attributed to the two N-acetyl groups. The increased reactivity of the amine functionality to acetylation over the hydroxyl group was exemplified in this reaction, with the conservation of the -OH signal at approximately 3.7 ppm, and no evidence of O-acetylation. Lastly, the anomeric benzyl group was removed using standard catalytic hydrogenolysis conditions to afford the targeted L-Alt-2,4-diNAc **304** in quantitative yield. ^{1}H NMR spectrum of compound **304** showed the presence of both α- and β-pyranosyl forms confirmed by electrospray high resolution mass spectrometry (calc'd m/z for $C_{12}H_{21}N_2O_6$ [M + H]+: 289.1396; found 289.1394).



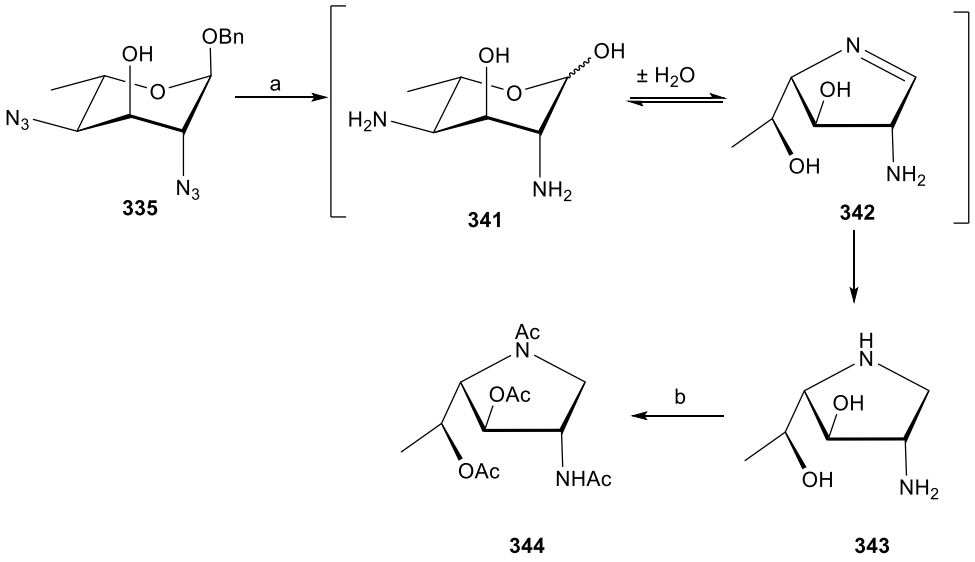
Reagents and conditions: (a) Ph_3P, Pyridine-H_2O (9:1) or H_2, MeOH-CH_2Cl_2-$NH_3.H_2O$; **(b)** Ac_2O, MeOH; **(c)** H_2, $Pd(OH)_2$-C, MeOH.

Scheme 3.12 *-Final conversion of 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **335** to L-Alt-DiNAc **304**.*

The successful selective reduction of compound **335** encouraged us to study its complete catalytic hydrogenation in one step (**Scheme 3.13**): we predicted that such an uncontrolled reduction would produce the reactive intermediate **341** that would then self-condense into a 1,4-iminofuranosyl ring (→**342**), further undergoing a reduction to afford the stable iminofuranoside **343**. This was found to be the case when compound **335** was subjected to a palladium-catalyzed hydrogenolysis in methanol in the presence of a few drops of acetic acid. A new polar product was isolated in 60% yield after a full acetylation, presumed to be the iminofuranoside compound **344**. In the ^{1}H NMR spectrum of the isolated compound, a highly deshielded proton was observed at 6.70 ppm as a doublet, which was assigned to be the single amide proton. 4 singlets were observed at 2.12, 2.11, 2.08 and 2.03 ppm, corresponding to the 4 acetyl groups present in the molecule. Two highly deshielded protons were observed at 5.45 and 5.35 ppm, attributed to the H-5 and H-3 respectively and confirmed by ^{1}H–^{1}H GCOSY experiment: their large chemical shifts were consistent with an O-acetylation on O5 and O3, further supporting that O5 is not involved in cyclization. Two mutually coupled protons (high orders) were observed at 4.24 ppm and 3.19 ppm, attributed to the two diastereotopic protons attached to C-1. These assignments were also confirmed by a ^{1}H–^{13}C 2D GHSQC correlation spectrum. The C-1 carbon was observed at a highly shielded region (52.9 ppm), confirming its attachment to a less electronegative nitrogen.

Iminosugars are known glycosidase and glycosyltransferase inhibitors due to their high analogy with enzyme carbohydrates substrates and because they contain a more basic trivalent ring heteroatom, nitrogen[137]: there is much interest in using them towards different therapeutic application,[138–140] and so new ways of synthesizing them are always welcome. The transformation of our Alt-diNAc precursor to the iminosugar **342** in moderate yield has the

potential to advance research into these types of enzyme inhibitors, especially ones necessitating amido functionalities.



Reagents and conditions: (a) H_2, $Pd(OH)_2$/C /C, MeOH, AcOH; **(b)** Ac_2O, Pyr

Scheme 3.13 *- Conversion of compound* ***335*** *to the iminofuranosides* ***343*** *and* ***344*** *via a complete hydrogenation.*

A convenient and readily scalable synthesis toward the challenging 2,4-Alt-diNAc (**304**) from L-fucose in 10 steps (23% overall yields) was hereby developed. The above scheme has overcome some major bottlenecks in previously reported syntheses, while providing milder, somewhat safer and improved regio- and stereo-controlled methodologies to achieve the target in gram-scales.[112] As such, a Staudinger reduction was used in lieu of a lithium-aluminum hydride for a di-azide reduction step, and an epoxide opening was performed under acid-free conditions with the more stable sodium azide reagent, instead of the reactive TMS-N_3 that is commonly used.[141] We also avoided using tin-based catalysts to achieve selectivity for functionalizing the hydroxyl groups at C2, C3 and C4, opting instead to use epoxide chemistry to differentiate between these positions.[115]

From this short and high-yielding synthesis of Alt-2,4-DiNAc, access to the substrate needed to study the three-carbon extension to obtain Pse and its 7-epimer from hexose has also been made easier. The extension itself has not yet shown to be achievable with a good stereoselectivity, and the most high-yielding method so far, through indium-mediated allylation, gives very slight diastereomeric excesses and is complicated by the hardship in separating the resulting mixture of isomers (See Chapter 6).

Finally, the schemes outlined above give a new possibility to manipulate each amine functionality separately, allowing for the regioselective introduction of different amino, amido or even halide groups to each amine functionality at C-2 and C-4 (see Chapter 4 for more). Further studies of the biosynthetic pathways that result in the differentiated Pse in nature, as well as potentially more efficient targeting strategies for vaccine and enzyme inhibitor-based therapies require access to these types of derivatives. Because of the shorter reaction sequence and much improved transformations in some key steps when compared to literatures, our synthesis of Alt-diNAc allows the preparation of derivatives in gram quantities that can be used to further crucial biochemical studies.

Chapter 4 Methodology to Access 2,4,6-Trideoxy-L-Altropyranosides with an N2,N4-Differentiation

The following chapter is based on my published work: Niedzwiecka, A.; Pham, Q.; Ling, C.-C. Regiospecific O → N Acyl Migration as a Methodology to Access L-Altropyranosides with an N2,N4-Differentiation. Org. Lett. ***2022****, 24 (47), 8667–8671. https://doi.org/10.1021/acs.orglett.2c03508.*

4.1 The Different Acylation Patterns of Pse in Nature

Following the successful publication of our synthetic scheme for the biological precursor to pseudaminic acid (Pse, **401**), 2,4-diacetamido-2,4,6-trideoxy-L-altrose (Alt-diNAc) in 10 steps and 23% yield,[142] we continued working on a methodology to achieve important derivatization of Alt-di**NAcy** (**402**), which can contain different acyl groups at N2 and N4. Due to its distinctive structure, Alt-diNAcy has potential to be used as a small molecule inhibitor of the Pse biosynthetic pathway, more specifically against Pse synthase, which catalyses the condensation between phosphoenolpyruvate (PEP) and Alt-diNAcy (**Figure 4.1**).

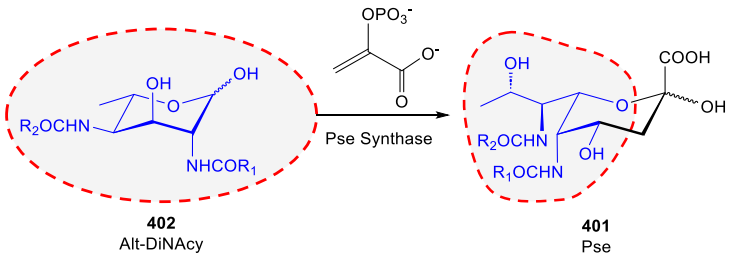
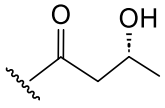
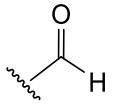
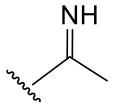
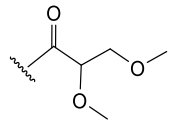
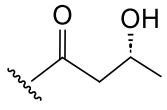
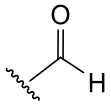
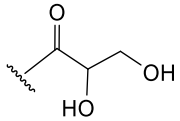


Figure 4.1 *– Biosynthesis of Pse from Altdi-NAcy* ***402****.*

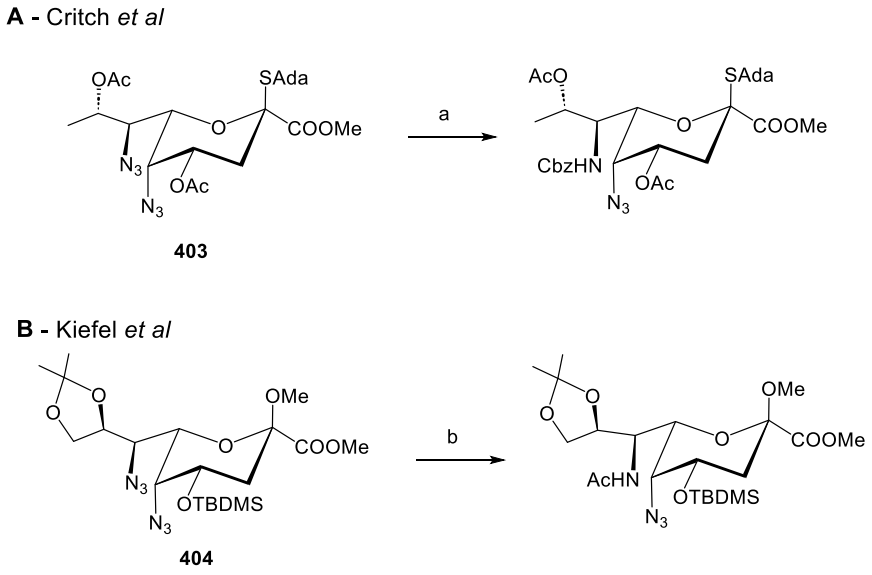
Most existing chemical and chemoenzymatic syntheses for Pse and its L-altro-configuration precursor have focused on producing their N5,N7-diacetylated (Pse5Ac7Ac) or

N2,N4- analogs: the chemical structures of the N5,N7-acyl groups on these monosaccharides are quite diverse in bacterial Pse found in nature, and usually differ intramolecularly - **Table 4.1** shows a selected list of known examples.[63] This difference in N-functionalizations is important to consider for immunochemistry as the ability to reproduce it advances the development of targeted immune responses and antimicrobial treatments. Additionally, achieving the differentiation on different Pse biosynthetic precursors could help with studies of when and how in their synthesis are the N-acyl variations introduced.

Table 4.1 *– Selected examples of N-acyl groups attached to C-5 and C-7 of Pse*

Bacteria containing Pse variant	**Acylation Pattern for Pse**
Pseudomonas aeruginosa O10a Shigella boydii type 7 Pseudomonas aeruginosa O9a, 9b Sinorhizobium fredii HH103 Kribbella spp. VKM Actinoplanes utahensis VKM Ac-674	R_1 = Ac; R_2 = 
Pseudomonas aeruginosa O7a, 7b, 7d and O7a, 7d (immunotype 6) Pseudoalteromonas distincta KMM 638	R_1 = Ac; R_2 = 
Vibrio cholera O:2 Campylobacter jejuni 81-176 Campylobacter coli VC167	R_1 =  ; R_2 = Ac
Campylobacter jejuni 11168	R_1 =  ; R_2 = Ac
Vibrio vulnificus 27562	R_1 =  ; R_2 = 
Pseudomonas aeruginosa O7a, 7b, 7c	R_1 =  ; R_2 = Ac

There exist a limited number of reports on the successful installation of differentiated N-acyl groups where specific N-substitution patterns demonstrated, but most require a long reaction sequence, or the yields have not been optimized.[72,80,82,98] The method reported by the Crich group has the potential to be more general, as it takes advantage of a difference in reactivity between the two azido groups in 5,7-diazido-substituted Pse thioglycoside **403** during reduction (**Scheme 4.1**,**A**; see **Scheme 2.6** for their more detailed synthesis).[80,82] Kiefel and co-workers first observed this phenomenon, revealing that the C-7 azido group in compound **404** could selectively be reduced over the ring C-5 azide under Staudinger conditions (**Scheme 4.1**, **B**, see **Scheme 2.2** for their more detailed synthesis).[87] The yield of this type of reaction has not been optimized beyond those obtained by both groups (55-60%).



Reagents and conditions: (a) i. $HS(CH_2)_3SH$, Pyr, Et_3N, H_2O, 0°C; ii. CbzCl, Pyr, CH_2Cl_2, 55%; **(b)** i. Ph_3P, Ac_2O, THF; ii. H_2O, 60%

Scheme 4.1 *– Demonstration of the difference in reactivities between azides at C-5 and C-7 of nonulosonic acid structures to reduction reactions by Crich et al[80,82] and Kiefel at al.[87]*

4.2 Metabolic Labelling

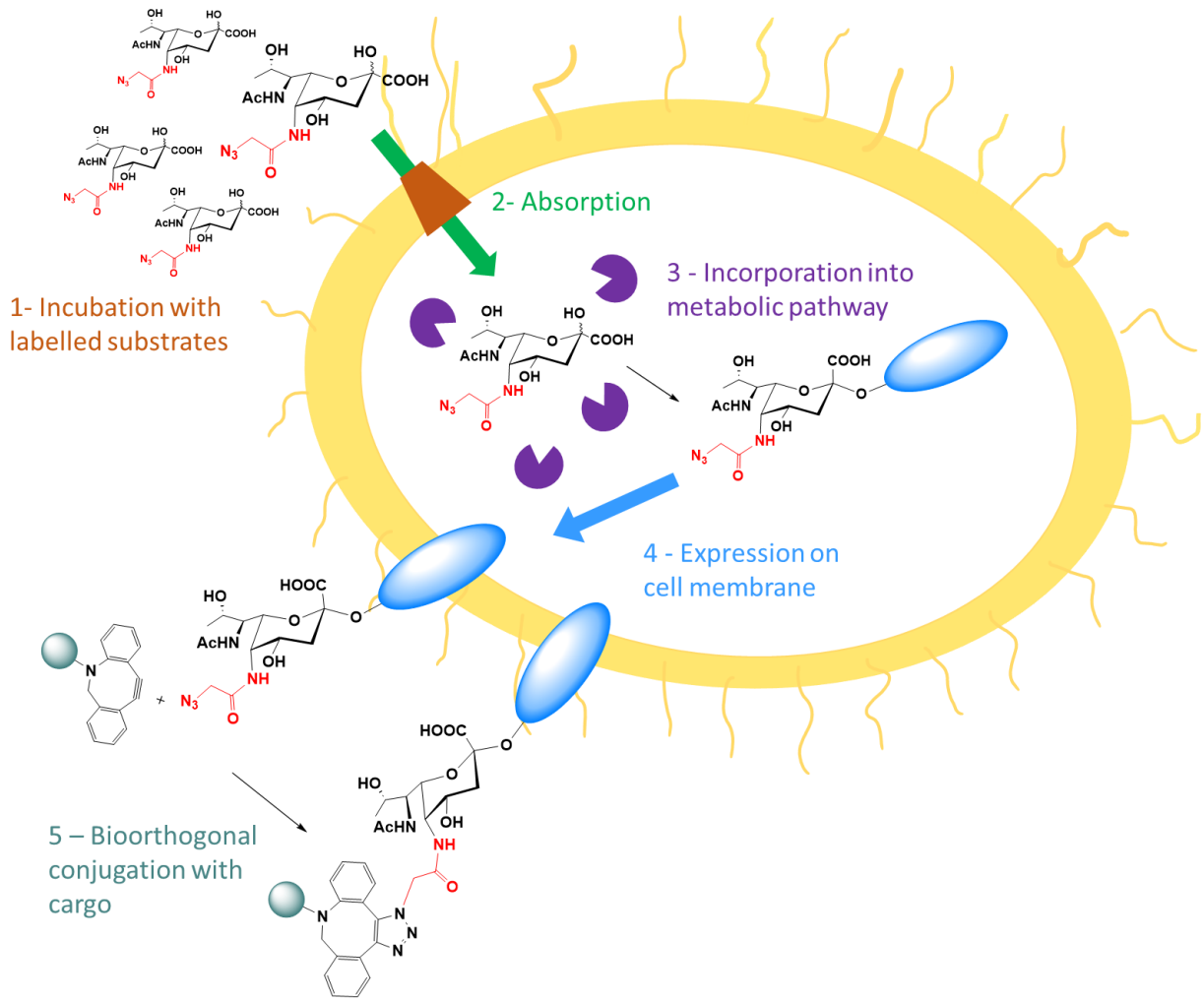


Figure 4.2 *– Schematic of general metabolic labeling process, with a C-5-azidoacetamido functionalized Pse, and a therapeutic cargo attached to DBCO, allowing for bioorthogonal "Click" chemistry once the molecule is displayed on the host cell wall.*

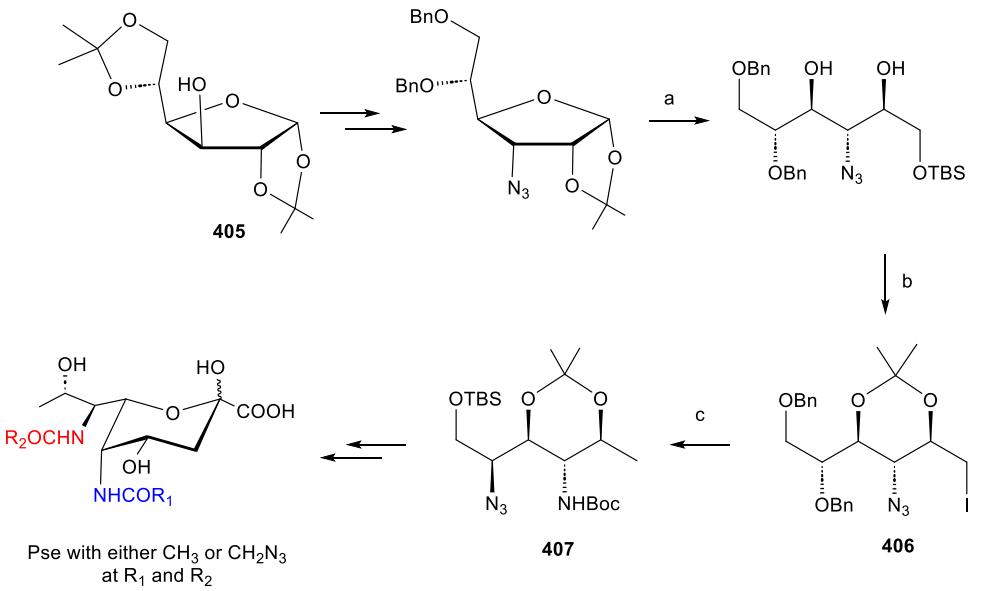
The ability to easily control the amido-functionalities during synthesis of Alt-diNAcy and Pse has introduced the idea of identifying Pse-expressing bacteria through chemical probes, which in-turn gives the potential to perform more complete biochemical investigations into the role of Pse in bacterial pathogenesis, diagnose infection more rapidly, and even specifically target

the pathogens with novel anti-microbial therapies.[98,143,144] The remodelling of cells surfaces in cancers or pathogenic bacteria with metabolic labelling has the potential to directly affect cell proliferation and virulence and/or sensitize the cells to different therapies by enabling the targeted conjugation of chemotherapeutic and other reagents (**Figure 4.2**). Metabolic labeling is being explored towards the treatment of cancers, where it is known that monosaccharides such as sialic acids (Neu5Ac) and N-acetyl-D-mannosamines (ManNAc) are displayed at the non-reducing termini of glycan chains much more than in healthy cells: modifying these sugars with chemical tags can therefore be used for the targeted delivery of therapeutic molecules to cells displaying them.[144]

Bacterial peptidoglycans or proteins that are metabolically labelled have recently provided much insight into the activity status of pathogens that survive inside hosts by immune evasion,[145,146] in addition to providing an accurate infection diagnosis tool through fluorescent imaging.[147] The modified sugars being used for these strategies must be designed in a way that still allows them to be incorporated into the metabolic pathways of cells efficiently to ensure their expression on cell membranes as glycoproteins (**Figure 4.2**). The chemical modification should be small, biocompatible and allow for conjugation under benign conditions with therapeutics that are bioorthogonal. Some great potential labelling and conjugation strategies for *in vivo* applications include bioorthogonal "Click" chemistry between azides and dibenzocyclooctynes (DBCO) (attributed to Sharpless, Meldal and Bertozzi, who received the Nobel prize in Chemistry in 2022[148]) as well as inverse Diels-Alder cycloaddition between cyclopropane/norbonene and tetrazine, and finally "Click" chemistry between isonitrile and tetrazine.[149] Monosaccharides labelled with azides have shown much promise towards metabolic

labelling in recent years with several advantages: for one, these small molecules can be presented at high densities on target cells; secondly, the complementary functional groups like DBCO that "Click" with the tags have low immunogenicity; lastly the small-sized functional groups complementary to azide can be introduced into drugs or nanomedicines without significantly affecting their binding efficiency towards receptors.[144]

Towards the goal of developing bacterial metabolic labeling, the Werz group arrived at Pse derivatives with 2-azidoacetamido functionalities at either the C-5 or C-7 positions to test which derivative, if any, could be incorporated in to the metabolic pathways of Pse-expressing bacterial strains.[98] Their synthetic strategy involved an azide substitution at C-3 of 1,2:5,6-di-isopropylidene-α-D-glucofuranose **405**, followed by a long series of iterative protection/deprotection steps to allow deoxygenation through the reductive removal of iodide on **406**, in addition to the inversion at C-5 through a Mitsunobu reaction with azide to give **407** (**Scheme 4.2**). Since the azido group at C-3 had been reduced earlier and protected with a selectively labile carbamate group, the new azido functionality could now be reduced and each N-position could be functionalized with either an acetyl or the azidoacetyl group at this point. Their synthesis continued from this point on with an indium-mediated three-carbon extension that eventually led to their desired Pse derivatives in low overall yields (<5%). Metabolic labeling experiments were carried out with two Gram positive (GP) and two Gram negative (GN) bacteria, one Pse-expressing and one negative control for each group. After incubation of the bacteria with either azide-labelled Pse derivative, the cultures were washed and conjugated to a fluorescent dye functionalized with DBCO: they found that only the C-5 azidoacetamido Pse clearly labelled both the GP and GN Pse-expressing bacteria.

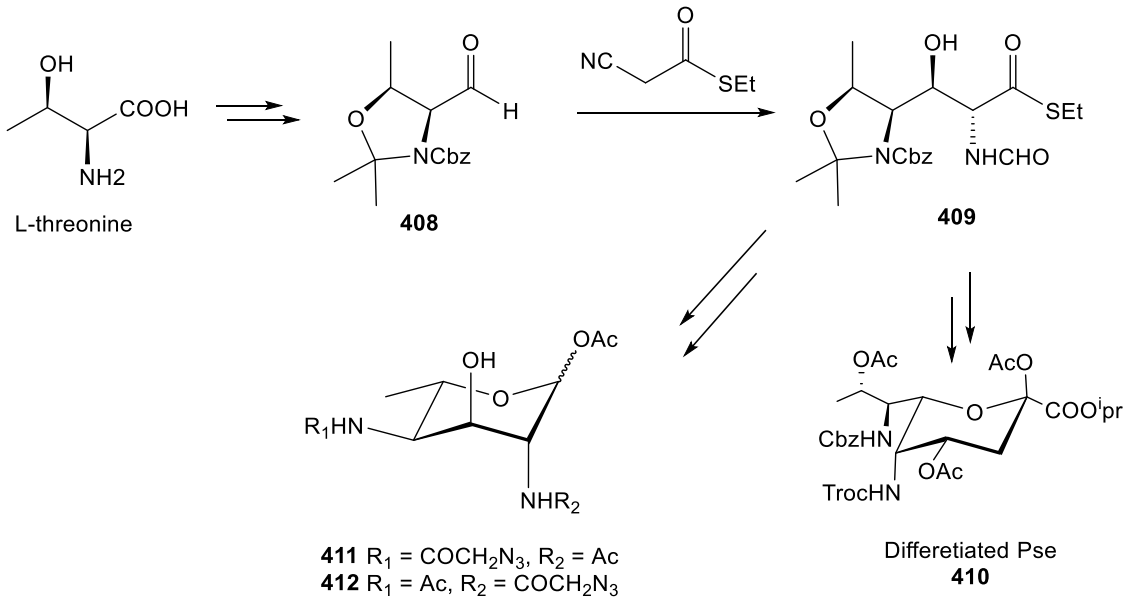


Reagents and conditions: (a) i. AcOH (60% aq),; ii. $NaBH_4$, EtOH; iii. TBSCl, Et_3N, DMAP, DCM**; (b)** i. 2,2-dimethoxypropane, p-TSA, MeCN; ii. TBAF; iii.PPh_3, imidazole, I_2,MeCN; **(c**) i. n-Bu_3SnH, AIBN, Boc_2O, toluene; ii. $Pd(OH)_2/C$, H_2, EtOH; iii. TBSCl, Et_3N, DMAP, DCM; iv. DEAD, DPPA, PPh_3

Scheme 4.2 *– Synthesis of azidoacetamido functionalized Pse derivatives by the Werz group.*[98]

In their *de novo* synthetic approach from L-threonine towards a glycosyl donor, the Li group synthesized the Pse with a benzyloxycarbonyl group at N-5, and a 2,2,2-trichloroethoxycarbonyl group at N-7 (see **Scheme 2.5** for their detailed synthesis).[72] Differentiation of the N-acyl groups was achieved through the protection of the amine functionality with a benzyloxycarbonyl (Cbz) group on their threonine-based starting material **408** before the aldol-type two-carbon extension with 4,5-trans-oxazoline, giving a differentiated L-altro-configuration intermediate **409** in 8 steps (**Scheme 4.3**). This compound was then derivatized to the N5,N7differentiated Pse **410** in 13 additional steps, with an approximate overall 5% yield. This technique later allowed the group to achieve the metabolic labelling of *P. aeruginosa, A. baumannii and V. vulnificus* with L-altro isomers **411** and **412** modified at either C-2 or C-4 with an azidoacetamido group (see **Scheme 3.4** for their detailed synthetic scheme

towards Alt-diNAc), showing that PseI in the biosynthetic pathway of some bacteria at least is tolerant to certain modifications on the N-acyl substituents.



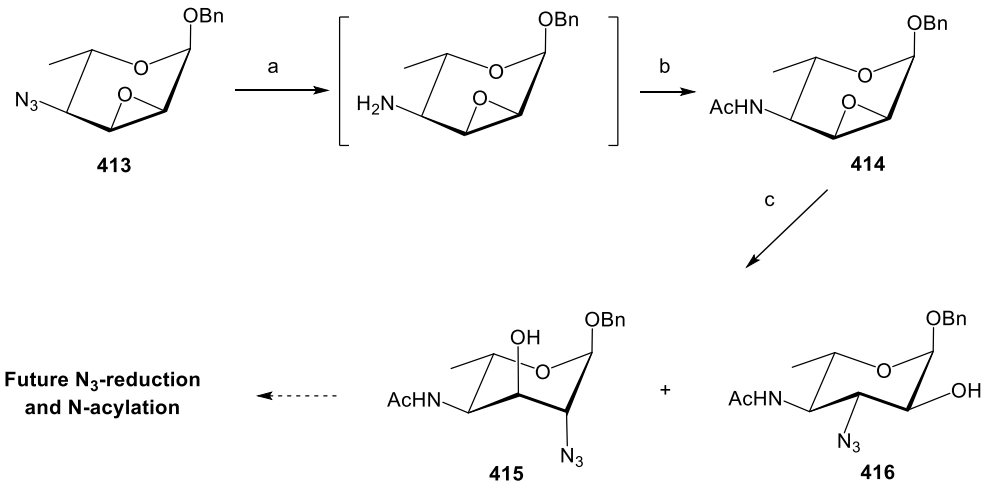
Reagents and conditions: i. $CNCH_2COSEt$, LiOTf, iPr_2NEt, DCE−DMF; ii. THF−H_2O, reflux.

Scheme 4.3 *– Synthesis of 2 azidoacetamido functionalized Alt-diNAc derivatives by the Li group.*[143]

While the above synthetic schemes allowed for the production of different N-acylation patterns on Pse and Alt-diNAcy, it was not the main goal of their schemes and were therefore not optimized for efficiency and versatility. Methodologies that allow for a broader scope in the production of Pse and Alt-diNAcy with differentiated amino functionalizations are needed for more extensive studies of the biosynthesis of Pse in pathogenetic bacteria, which in turn has the potential to yield more specific and effective antibacterial strategies: this constituted the next part of my work.

4.3 Preliminary Differentiation Strategy

The benzyl 2,3-anhydro-4-azido-4,6-dideoxy-L-allopyranoside **413** (**Scheme 4.4**), and intermediate in our scheme towards Alt-diNAc (presented in Chapter 3), was initially considered to be a good starting point for obtaining the desired N2,N4-differentiated Alt-diNAcy analogs via a sequential approach, beginning with the reduction of the C4-azide of **413** by Staudinger reduction using trimethylphosphine, followed by an *in-situ* N-acetylation (→**414**).[150–152] An epoxide opening activated by lithium perchlorate was then performed with a second azide, affording the desired 4-acetamido-2-azido-2,4,6-trideoxy-L-altropyranoside intermediate **415**, along with the undesired 4-acetamido-3-azido-L-glucopyranoside **416** in a 2:1 ratio.[142] With compound **415** in-hand, a second Staudinger reduction of the 2-azido functionality followed by an N-acylation with another type of acylating reagent would afford the N2,N4-differentiated Alt-diNAcy target.



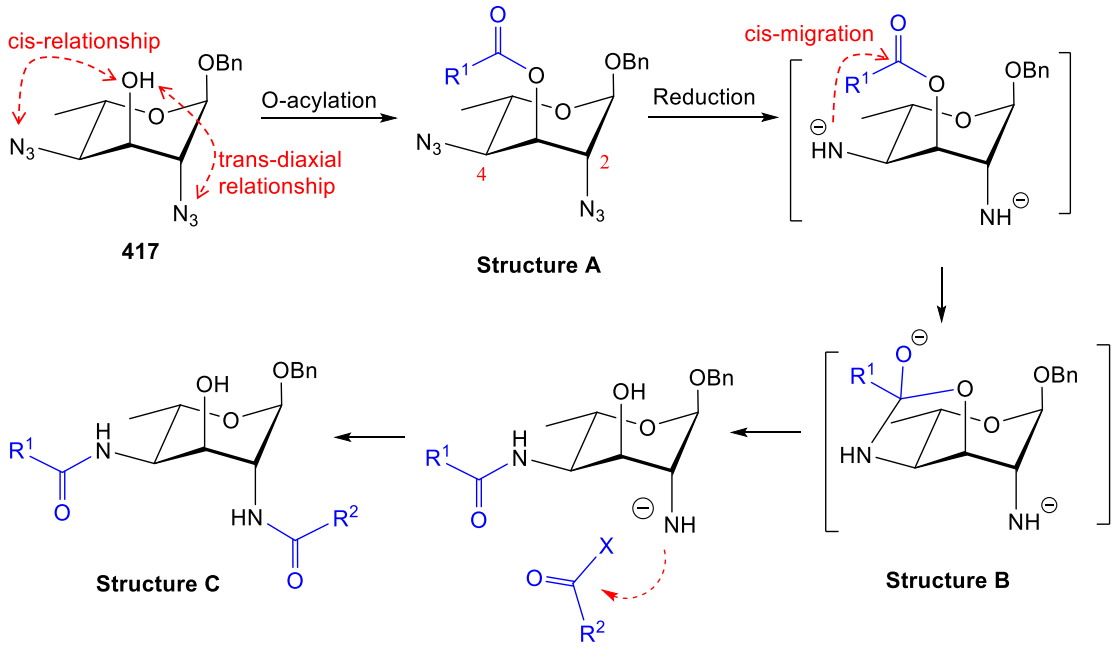
Reagents and conditions: (a) TMP, THF, H_2O; **(b)** Ac_2O, Pyr; **(c)** NaN_3, $LiClO_4$, 85-100 °C

Scheme 4.4 *– Original N2,N4 differentiation strategy via sequential Staudinger reductions and N-acylations.*

Despite the simplicity and straightforwardness of the above N-acyl differentiation strategy, many shortcomings were noticed, including the long reaction sequence and reduced efficiency and regioselectivity during the opening of epoxide functionality when compared to our previous report using **413** directly as a substrate.[142] This decreased performance could be explained by a competitive coordination of the lithium ion to the neighboring electron-rich N-acetyl group in compound **414**, in addition to a possible preferential build-up of positive charge on C-3 during the ring opening of the activated epoxide, with C-2 being adjacent to the more electron-withdrawing anomeric substituent.[153]

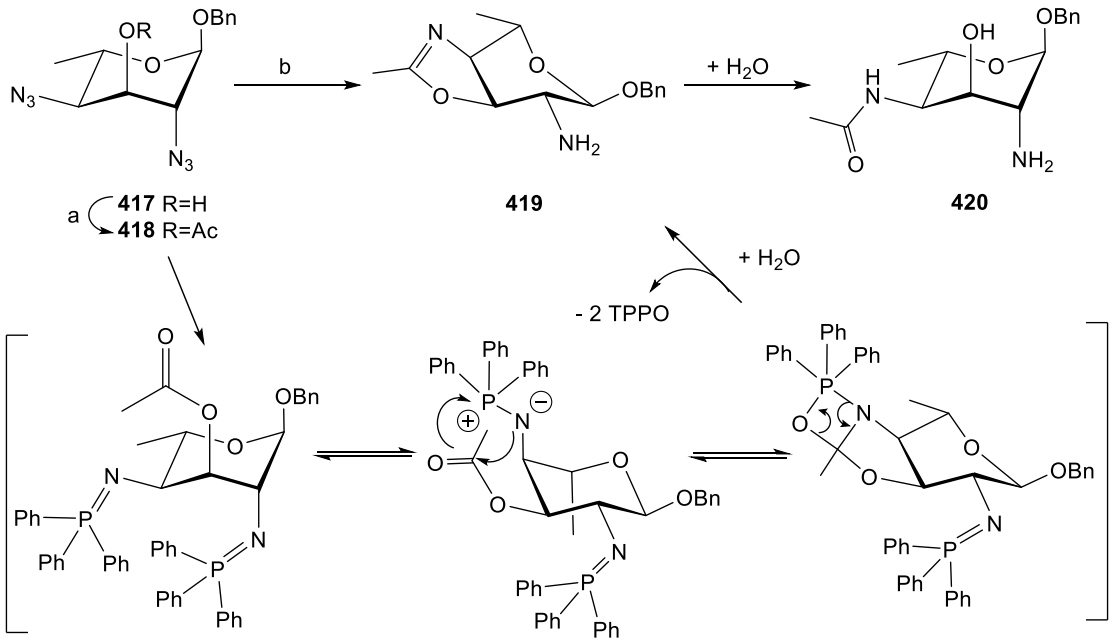
4.4 New Methodology for the Differentiation

While there are no general methods to regioselectively activate one azido group over the other in carbohydrate chemistry to our knowledge, the specific functional group relationship in compound **417** presented us with an opportunity to design an amido-group differentiation strategy which exploited the *cis*-relationship between the axial 3-OH and the equatorial 4-azido group on the pyranosyl ring, as opposed to the *trans diaxial*-relationship between the same 3-OH and the axial 2-azido group (**Scheme 4.5**). We reasoned that with an acyl group preinstalled at the 3-OH (structure **A**), a reduction of both azido groups could entail a regiospecific O3→N4 acyl migration via a favorable 5-membered ring intermediate (structure **B**). The exclusive installation of the first N-acyl group on N4 would then allow a different subsequent N2-acylation, achieving the desired N2/N4-differentiation (structure **C**).



Scheme 4.5 *– New N2,N4 differentiation strategy involving an O→N4 migration following azide reduction, favored by their exclusive cis-relationship.*

To test our hypothesis, the free hydroxyl group at C-3 of compound **417** was acetylated quantitatively to afford precursor **418** (**Scheme 4.6**). A Staudinger reduction was then performed by reacting compound **418** with triphenylphosphine (5 equiv.) in a 9:1 pyridine:water mixture at 80 °C overnight. Monitoring the reaction by thin layer chromatography revealed the formation of two new compounds, the major one being the desired product **420**, isolated in 56% yield. Analysis of the ^{1}H and 2D ^{1}H-^{1}H GCOSY NMR spectra of compound **420** confirmed the presence of a single acetamido group at C-4, where the amide proton (found at 6.06 ppm) correlated to H-4 with a large coupling constant (J_{NH} = 9.3 Hz) while the amino group at C-2 remained free of acylation. Most other coupling constants ($J_{1,2}$, $J_{2,3}$, $J_{3,4}$) were small, confirming the expected $^{1}C_{4}$ chair conformation.



Reagents and conditions : (a) Ac_2O/Pyridine; **(b)** Ph_3P/Pyridine, H_2O

Scheme 4.6 *– Exemplary regiospecific O→N-acetyl migration of compound **418** and proposed mechanism for the triphenylphosphine-promoted intramolecular O3→N4 acyl migration resulting in the oxazoline intermediate **419**.*

On the other hand, NMR analysis of the minor product, isolated in 8% yield revealed an unusually large $J_{1,2}$ coupling constant (6.7 Hz), suggesting a near trans-diaxial relationship between the anomeric H1 and H2; the remaining coupling constants of the neighboring pyranosyl protons were also found to be large ($J_{2,3}$ = 9.9 Hz), $J_{3,4}$ = 9.8 Hz, $J_{4,5}$ = 9.8 Hz), suggesting that the minor compound had adopted a different conformation (**Figure 4.3**). There was an absence of amide proton signals in the ^{1}H NMR spectrum, and a methyl group was observed at 2.02 ppm, split into a doublet with a long range coupling to H4 (~1.3 Hz, similar to a homoallylic system), confirmed by a GCOSY spectrum. Additionally, H3 was also observed as unusually deshielded at 4.39 ppm. Compound **419** was accordingly suggested as the structure which bears a fused oxazoline functionality along the C3-C4 bond. The pyranosyl ring of compound **419** would likely

exist in a $^{3}S_{O}$ twisted boat conformation (**Figure 4.4**), where the measured dihedral angles would all correlate to the observed large coupling constants, based on the Karplus curve.[154,155]

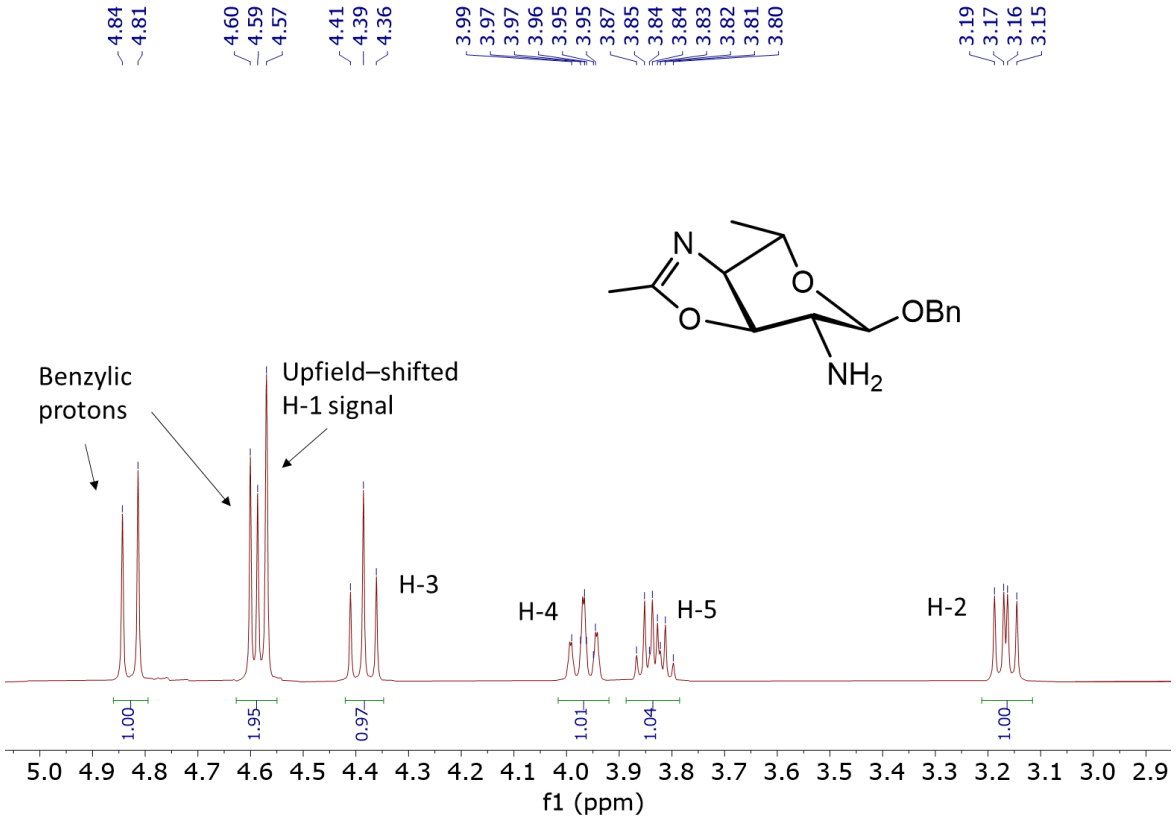
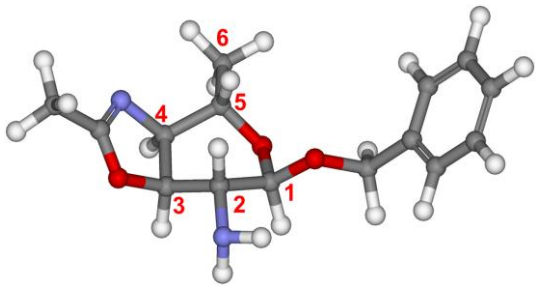


Figure 4.3 *– ^{1}H NMR of migration side-product **419**.*



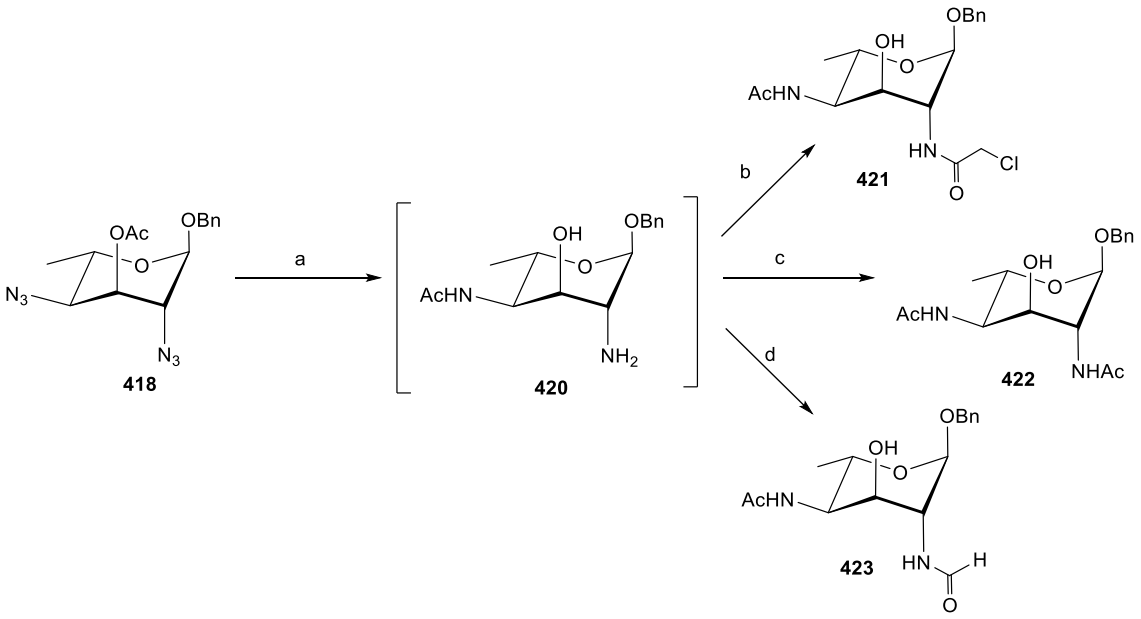
$H_1\_C_1\_C_2\_H_2$: 159.91°
$H_2\_C_2\_C_3\_H_3$: -168.00°
$H_3\_C_3\_C_4\_H_4$: 13.61°
$H_4\_C_4\_C_5\_H_5$: 161.79°

Figure 4.4 *- The measured dihedral angles of ring protons in the MM2 minimized $^{3}S_{o}$ twisted boat conformation of α-L-pyranoside **419**. All dihedral angles correspond to a large coupling constant in Karplus curve.*

No N2-acetylated product was isolated from the experiments, confirming the O→N acyl migration was regiospecific. Combined with the isolation of side-product **419**, this fact gave rise the mechanism postulated in **Scheme 4.6**. The O→N acetyl transfer likely occurred at the iminophosphorane intermediate stage, initiating the formation of an oxaphosphetidine intermediate via a Wittig-like [2+2] cycloaddition, after which the expulsion of triphenylphosphine oxide led to the formation of the oxazoline functionality. This type of O→N migration mechanism is analogous to traceless Staudinger ligation reactions, where a stabilized phosphine equipped with a neighboring cleavable ester is used to reduce and functionalize an azide via nucleophilic attack.[152] In our case, compound **419** was seemingly formed as an intermediate that underwent a partial hydrolysis under the original reaction conditions: the yield of **420** was later improved by decreasing the pH of the reaction, which promoted the complete hydrolysis of **419** with heating.

The successful regiospecific O3→N4 acetyl migration gave us access to the key N4-monoacetylated intermediate **420** where the reactive amino group at C-2 remained available to accept another type of N-acyl group. It was found that this last step could be combined with the migration reaction in one-pot: after treating compound **418** with triphenylphosphine (2.2 equiv.) in a mixture of THF-H_2O (5:1) at 65 °C for 12 hours, the solvents were evaporated and the crude mixture then subjected to a second N-acylation using the same or different acylating reagent. In the latter case, the N2,N4-differentiated product was formed exclusively (**Scheme 4.7**). For example, when chloroacetic anhydride was used in the second step, the N2-chloroacetyl-N4-acetyl analog **421** was isolated in 82% yield. The previously reported N2,N4-diacetyl-analog **422** was also obtained in 87% yield using our one-pot procedure. Compound **423**, bearing a N2-formyl

group, was prepared via Steglich esterification with formic acid and DCC, where a purification step was needed after the migration to form compound **422** as a mixture of inseparable rotamers in 82% yield, consistent with reported literature.[156]



Reagents and conditions: (a) Ph_3P, THF, H_2O; **(b)** $(ClCH_2CO)_2O$, MeOH; (**c)** $(CH_3CO)_2O$, MeOH; (**d)** HCO_2H, DCC, DMAP, MeOH

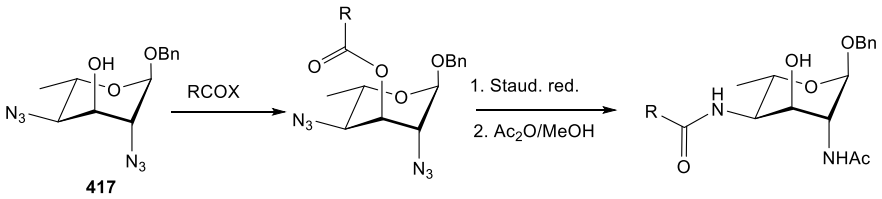
Scheme 4.7 *– Examples of one-pot or stepwise syntheses of N2,N4-diacylated O-benzyl Alt-2,4-DiNAc analogs with or without differentiation of N-acyl groups.*

The realization of the O3→N4 acetyl migration prompted an investigation into the scope of migration with different acyl groups. **Table 1** shows a list of 3-O-acylated esters of benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranosides **424**-**431** prepared in our studies. After treating each of them with a phosphine in a mixture of THF-H_2O (10:1) for 12-24 hours, the formed intermediates were concentrated and subjected to an N-acetylation in anhydrous methanol with acetic anhydride to afford the corresponding N2-acetyl-N4-acylated products (**432**-**439**) in mostly good to excellent yields (70-92% yields), with some exceptions. The Staudinger reduction was

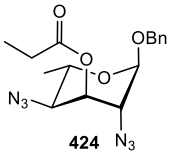
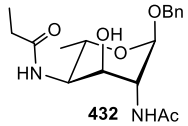
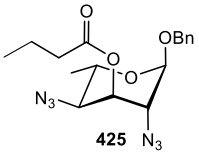
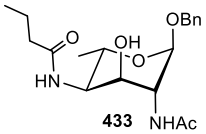
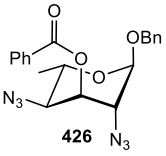
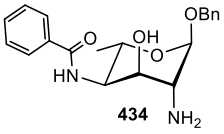
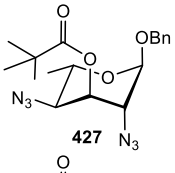
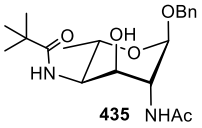
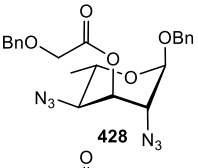
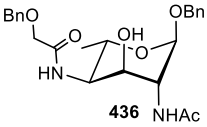
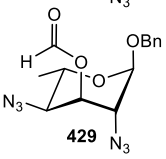
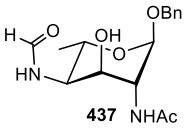
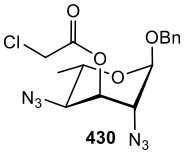
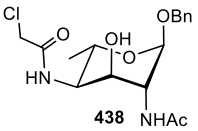
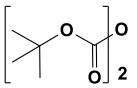
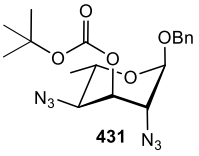
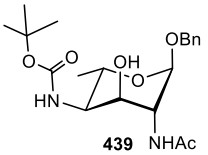
performed with either triphenyl- or trimethylphosphine, the latter being more reactive but requiring the reaction to be performed first at room temperature due to its volatility, then under heating to hydrolyse the aza-ylides: it did however allow for easier separation from the oxide side-product at the end through extraction. It is worth noting that no products with scrambled acylation patterns were detected throughout the experiments, even when a free N2-amine remained, as with **420** and **434**, as well as when the procedure was done at the molar scale to produce compound **439**. This once again demonstrated the regiospecificity of the migration. The low yield seen with the O-chloroacetyl functionalized compound **438** was attributed to the presence of a leaving group (Cl) in the acyl group that complicated the O→N migration step. Like compound **422**, the 4-formamido compound **437** was found to exist as a mixture of rotamers when characterized by NMR.

The one-pot differentiation process worked equally well with bulky acyl groups such as pivaloyl, 2-benzyloxyacetyl and aromatic group (benzoyl), revealing the versatility of the method. Furthermore, despite the possibility of cleaving the *tert*-butyl carbonate ester at two sites during the migration, we were pleased to find that the desired N4-Boc functionalized compound **439** was formed exclusively, in an overall 91% yield.

Table 4.2 *- Scope of O3→N4 acyl migration reaction on an 3-O-acyl-2,4-diazido-2,4,6-trideoxy-L-altropyranoside systems (**427**-**431**) to form N2,N4-diacylated O-benzyl Alt-2,4-DiNAc analogs (**432**-**439**) with a differentiation of N-acyl groups.*

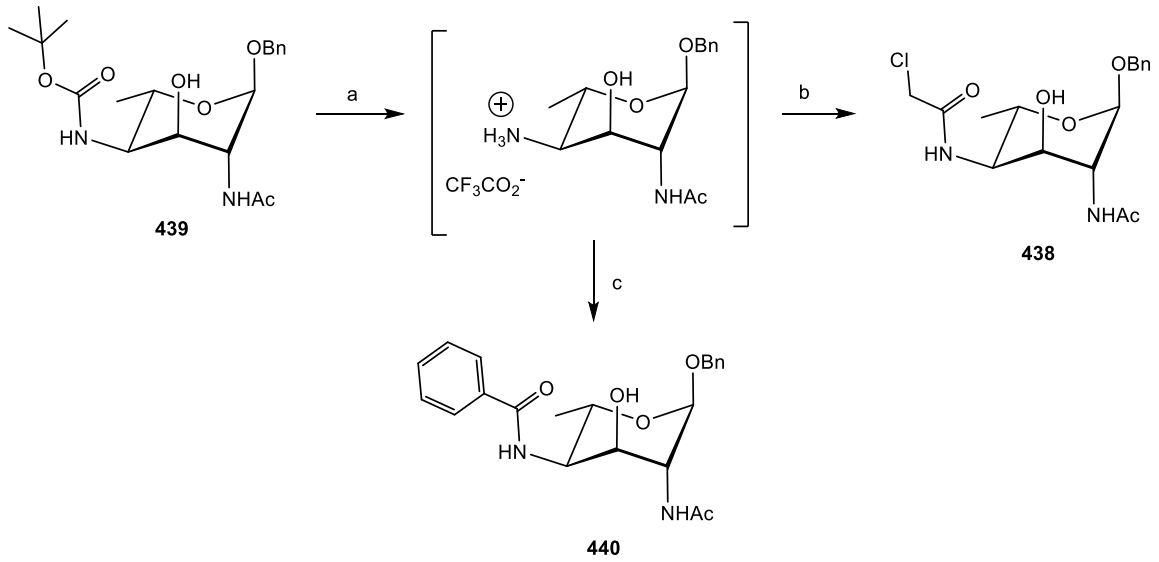


417

RCOX	**O3-acylated Ester** (yield%)	**N2-acetyl-N4-acyl-Alt** (yield%)
$(EtCO)_2O$	 **424** (99%)	 **432** (71%)
$(n\text{-}PrCO)_2O$	 **425** (96%)	 **433** (82%)
PhCOCl	 **426** (100%)	 **434** (92%)[a]
$(CH_3)_3CCOCl$	 **427** (100%)	 **435** (74%)
$BnOCH_2CO_2H$	 **428** (62%)	 **436** (100%)
HCOOH	 **429** (81%)	 **437** (76%)
$ClCH_2COCl$	 **430** (85%)	 **438** (27%)
	 **431** (97%)	 **439** (94%)

[a]The product was isolated before the second acetylation step

The ability to prepare compound **439** from precursor **431** proved to be advantageous as an alternative way to access a larger scope of N2,N4-diacylated Alt-diNAc analogs. For example, compound **438** was synthesized in a much-improved yield when **439** was stirred in a 50:50 mixture of trifluoroacetic acid (TFA)-dichloromethane for two hours, after which the N4-deprotected intermediate was directly subjected to a reaction with chloroacetic anhydride in methanol (**Scheme 4.8**). For comparison, this alternative scheme was used to produce the N4-benzoyl-N2-acetylated product **440** in a yield similar to that from the direct method that gave **434**.



Reagents and conditions: (a) TFA, DCM; **(b)** $(ClCH_2CO)_2O$, MeOH; **(c)** Bz_2O, MeOH HCO_2H, DCC, DMAP, MeOH

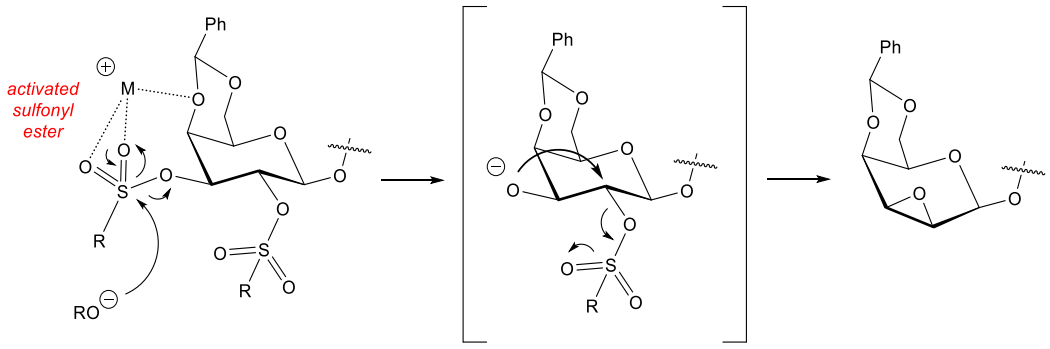
Scheme 4.8 *- Examples of one-pot or stepwise syntheses of N2,N4-diacylated O-benzyl Alt-2,4-DiNAc analogs with or without differentiation of N-acyl groups.*

In conclusion, the work outlined in this chapter led to an elegant new methodology that allows access to a wide range of Alt-diNAcy analogs, with or without N2,N4-acyl group differentiation. By taking advantage of their differences in configuration relative to a nearby OH-

3 group, it was demonstrated that it is possible to differentiate between two azido functionalities in a pyransoyl ring via the Staudinger reduction-mediated O→N acyl migration. This process was found to be high-yielding and regiospecific, and has the potential to be applied to other types of carbohydrates (such as furanosides) or molecules. Moreover, it was also found that the O→N acyl migrations occurred equally well with carbonate esters, resulting in an unprecedented approach to selectively form the labile carbamate functionality, further expanding the scope of the important Staudinger ligation methodology. The success of this new methodology should facilitate the study of differentiated Pse biosynthesis, different metabolic labelling strategies as well as the synthesis of inhibitors of Pse synthases. Additionally, when combined with the established indium-mediated Barbier allylation,[72,78] many important bacterial Pse derivatives could hence be synthesized for immunological studies. The methodology also gives rise to the potential development of a new stereoselective three-carbon extension methodology, which will be further discussed in Chapter 6.

Chapter 5 A New Methodology for Stereoselective Epoxide Formation

Following the successful development of a versatile methodology to arrive at Alt-diNAcy analogues with differentiation at N2 and N4 in an efficient and relatively short synthesis, we decided to apply one last round of optimizations to our original scheme before moving on to working on a three-carbon elongation scheme necessary for the production of Pse and derivatives (covered in Chapter 6). We were inspired by previous work in our group by Dr Rachel Hevey, who observed stereoselectivity during the epoxidation step required for their synthesis of β-D-idopyranosides from 2,3-di-O-sulfonates of 4,6-O-benzylidene-β-D-galactopyranosides.[121] The conversion of vicinal di-O-sulfonyl esters to epoxides in the presence of an alkoxide is a commonly used methodology on select hexopyranosides: Hevey's work provided evidence for the role of an alkali counter-cation in selectively activating one of the two sulfonyl esters, leading to a regioselective S-O scission, where the resulting alkoxide intermediate undergoes an intramolecular S_N2 attack to displace the neighboring O-sulfonate, furnishing the corresponding epoxide (**Scheme 5.1**). Regioselectivity was shown to occur in the presence of an oxygen on a neighboring substituent with a *cis*-relationship to the sulfonate, giving the activating cation additional coordination, and partiality, at the desired S-O scission site. This methodology and potential for selectivity was interesting to us as it could provide an optimized synthesis of Alt-diNAc from L-fucose, even shorter and more atom-economical than our published schemes.[142,157]



Scheme 5.1 *- The previously reported regioselective activation of 3-mesylate by alkali ion-mediated coordination with the assistance of a neighboring cis-oxygen O4 on a 4,6-O-benzylidene-2,3-di-O-methanesulfonyl-β-D-galactopyranoside substrate.*[121]

5.1 Optimized Synthesis of Epoxide Precursor

To start with, it was necessary to synthesize the required di-sulfonyl precursor for the epoxidation: we were encouraged by our results in Chapter 3, where we demonstrated regiospecificity in the azide substitution of precursor **316** (see **Figure 3.4**). The displacement of the 3-O-mesylate substituent in that case was inhibited due to transition-state geometries first described by Richardson and Hough, and later updated by Hale *et al.*[113,116] We hypothesized that a similar trend would be observed when performing an azide substitution reaction with the tri-mesyl compound **503** (**Scheme 5.2**), where the displacement of 3-O-mesylate would prove to be inhibited as before, with the possibility that the 2-O-mesylate would also experience steric and dipolar clashes with the α-anomeric O-benzyl group in the substitution transition state (TS) (**Figure 5.1**). In other words, we believed that during the reaction of the tri-mesylated O-benzyl-L-fucohexose **503** with a nucleophile, displacement of neither O3 nor O2-mesylates would occur, providing us with an opportunity to easily synthesize the desired di-mesyl **504** from L-fucose in a few simple steps.

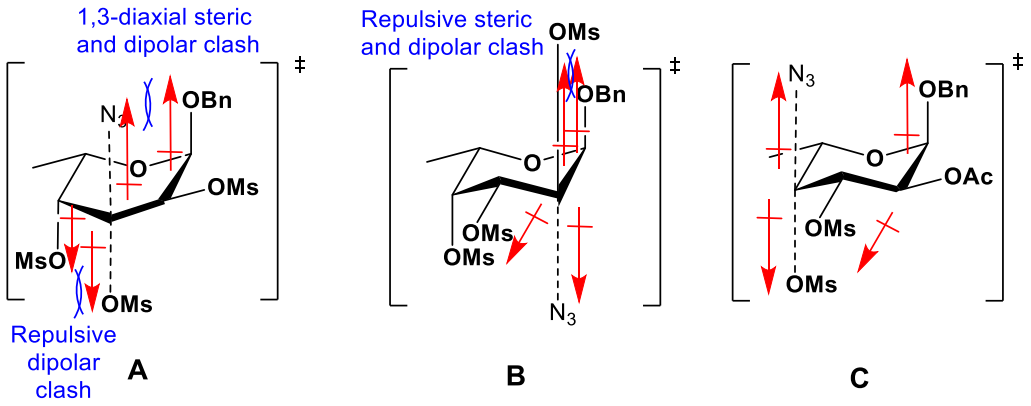
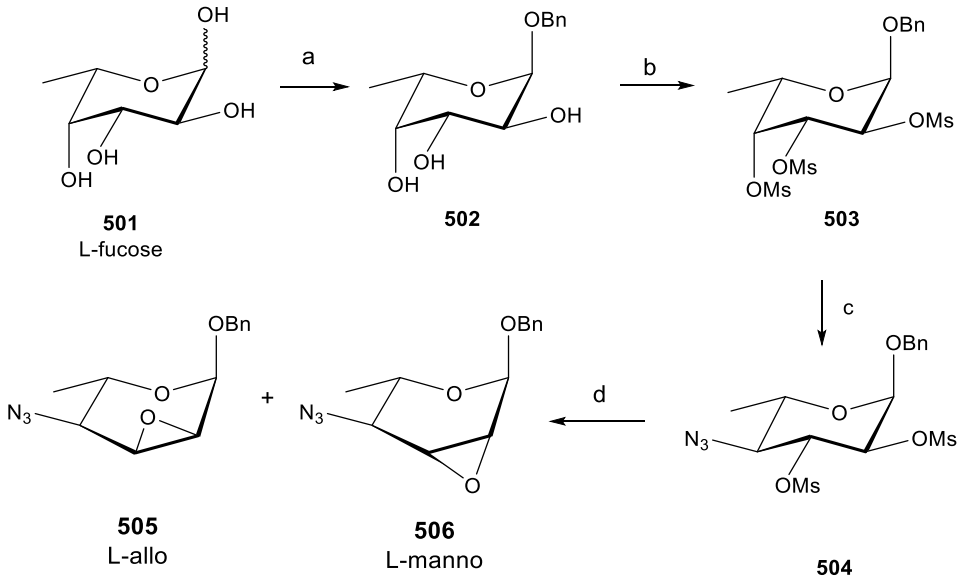


Figure 5.1 *-Transition states formed from the S_N2 displacement of 2-, 3- and 4-mesylates of α-fucopyranoside by an azide.*

As before, a Fisher glycosylation with benzyl alcohol was carried out first to obtain the desired α-O-benzyl anomer from L-fucose (**501**), isolated in 60% yield by precipitation in a large amount of hexanes, while the β-anomer remained in solution. The tri-mesylate **503** was then obtained in one additional step, bypassing the need for tedious protection and deprotection of the O3 and O4 hydroxyl functionalities for the selective O2-acetylaton that were executed in our previous approach (**Scheme 5.2**). To our delight, heating the tri-mesylate **503** in DMF in the presence of an excess of sodium azide (5 eq.) over 24 hours produced the 4-azido-2,3-dimesylate product **504** in a 91% yield, demonstrating an excellent regioselectivity in the mesylate displacement at C-4. Furthermore, when compared to the series of steps that led to the epoxide precursor in the previous scheme (6 total steps, 20% yield overall), the 52% overall yield from L-fucose in 3 steps here was much more attractive.



Reagents and conditions: (a) BnOH, CSA; **(b)** MsCl, DCM, Pyr; **(c)** NaN_3, DMF; **(d)** KOtBu, MeOH.

Scheme 5.2 *- Improved synthesis of α-L-manno and α-L-allo-epoxides **505** and **506** from L-fucose.*

5.2 Epoxidation from a Vicinal di-O-Mesylate Precursor[2]

With the C-2 and C-3 dimesylate **504** in hand, a first attempt at epoxidation was performed with an excess amount of sodium methoxide (5 equivalents) in methanol, producing the two diastereomer epoxides **505** and **506** in a 7:5 ratio (α-L-allo and α-L-manno configurations respectively) when the reaction was heated to 40 °C. When the reaction was carried out at room temperature, it proved to take too long to go to completion and gave no significant improvement in selectivity (**Table 5.1**, entries 1 & 2). Isolation of the isomers was achieved via silica gel column chromatography, where α-L-manno-epoxide **506** was eluted first, followed by desired L-allo-epoxide **505** as the majority. Some interesting variations in the NMR chemical shifts were observed when the two isomers were compared, with a marked deshielding of H-3 when

[2] Preliminary work presented in this section was performed by Carita Sequeira towards her Master's thesis.

compared to H-4 for the α-L-allo-epoxide **505**, whereas the opposite was observed for the α-L-manno-epoxide **506**.

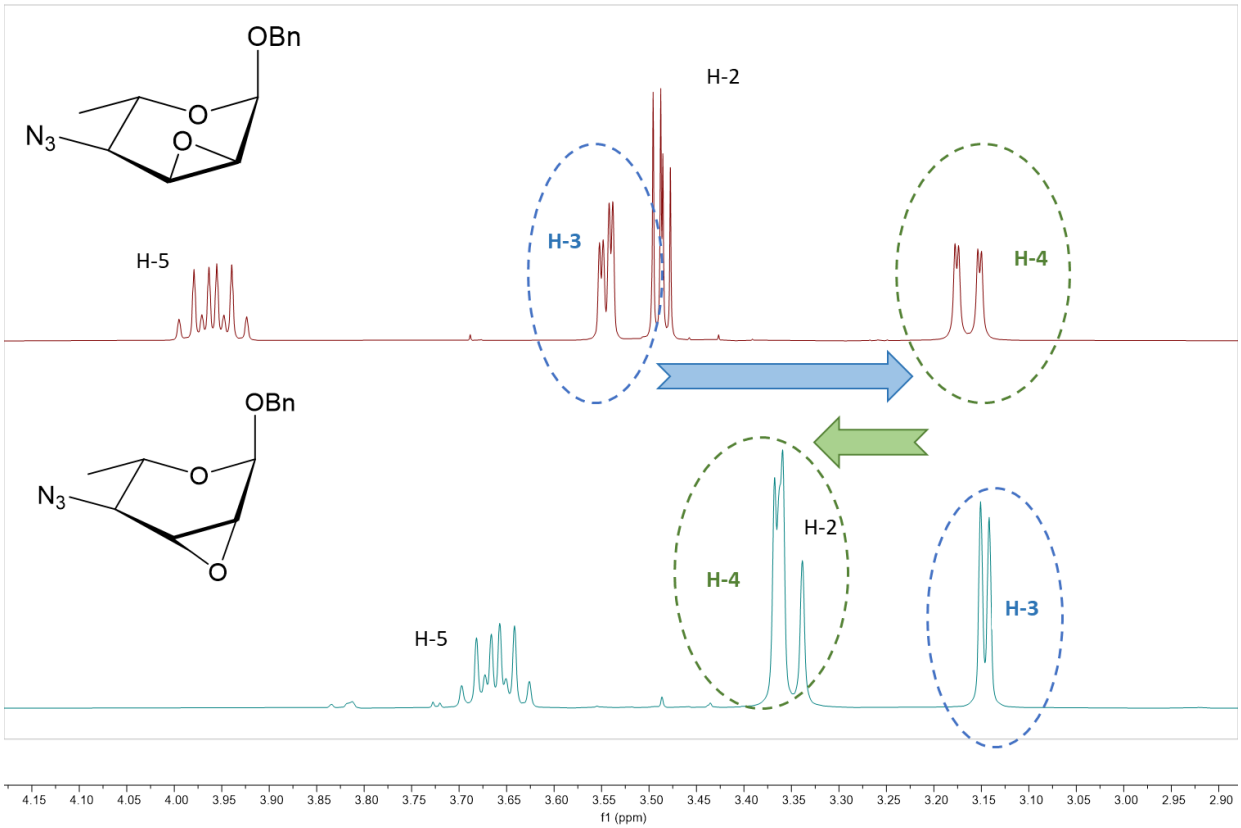
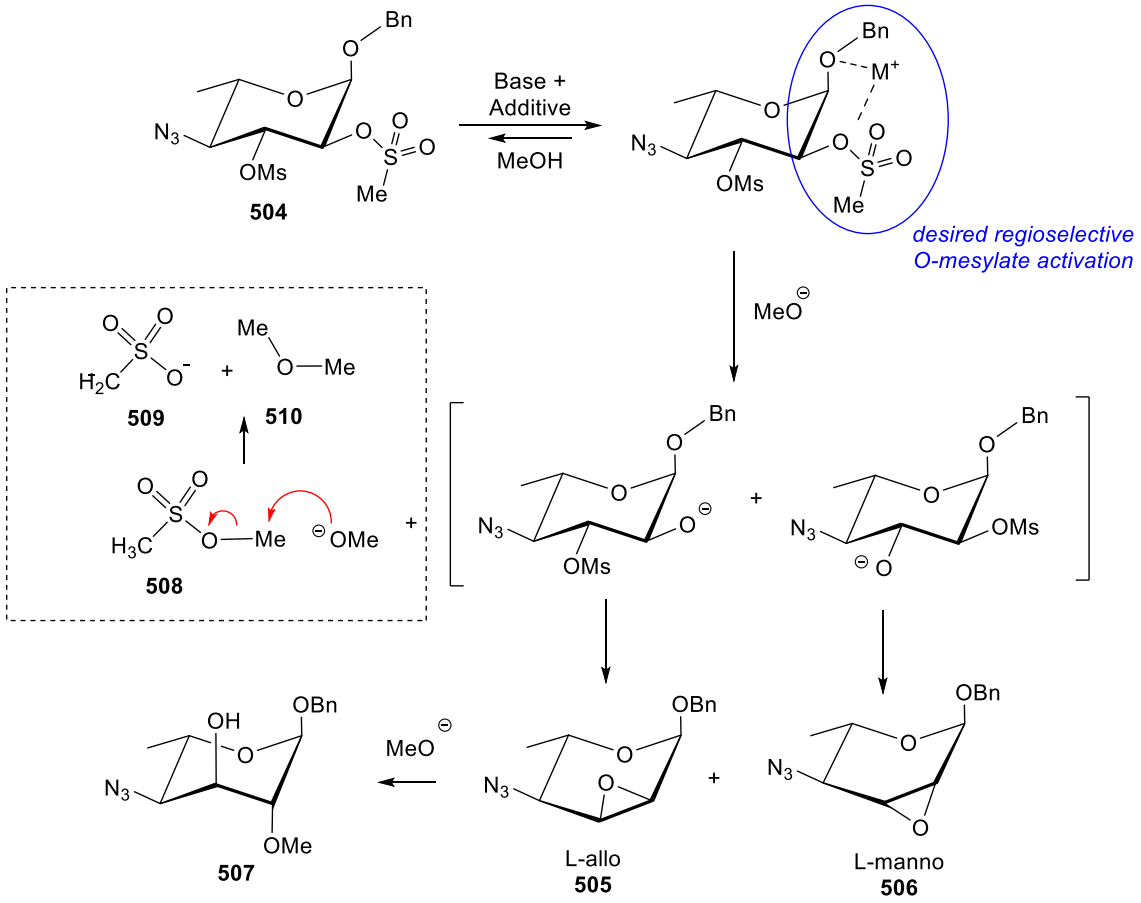


Figure 5.2 *- 1H NMR of allo-epoxide **505** (top) and manno-epoxide **506** (bottom) showing the inversion of chemical shifts for H-3 and H-4 when compared between the two compounds.*

In our previous approach (see Chapter 3), the α-L-allo-configuration epoxidation step was stereospecific from the 2-O-acetyl-3-O-mesylate compound **317** because it only had one way to form the oxirane ring, through the base-catalysed deprotection of the O-acetate at C-2, followed by intramolecular O3 sulfonyl displacement (**Scheme 3.8**): the low selectivity obtained here did not however discourage us, and many different conditions were tried, based on Hevey's previous work, and with the help of a student from our group, Jayar Espejo. Since the formation of the desired α-L-allo-epoxide **505** required the regioselective activation of the 2-O-mesylate for a

transesterification (**Scheme 5.3**), our plan involved taking advantage of the presence of the neighboring anomeric oxygen by chelation with the appropriate counter-cation.



Scheme 5.3 – *Alkali metal ion assisted activation helped improving the selectivity of α-L-allo (**505**) vs α-L-manno-(**506**) epoxides. Under heated prolonged conditions, some α-L-allo-epoxide (**505**) can further undergo an attack by methoxide to form the corresponding benzyl 4-azido-6-deoxy-2-O-methyl-α-L-altropyranoside (**507**).*

Several reaction conditions were investigated towards this goal (). First, a potential improvement of selectivity was observed when the counter-alkali cation was switched from sodium to potassium (5 equiv., entry 3), which allowed us to obtained the desired α-L-allo-epoxide **505** with an improved selectivity (L-allo : L-manno configurations, 12 : 5, entry 3) and the reaction completed after stirring for 2 days at room temperature. The potassium cation appeared

right away to have a better activation effect than sodium, and heating an analogous reaction to 40 °C shortened the reaction time to 1 day, but reduced the selectivity (L-allo : L-manno configurations, 1 : 1, entry 4). Using less potassium cation (2 equiv.) and heating the reaction to 50 °C turned out to be the optimal conditions that ensure completion of the reaction within 1 day and improved selectivity (L-allo : L-manno configurations, 4 : 1, entry 5), as carrying out the reaction at room temperature significantly prolonged the reaction time to 5 days, although a slightly better selectivity observed (L-allo : L-manno configurations, 5 : 1, entry 6). It is important to note that when the reaction was carried out at elevated temperatures combined with an excess of base, some of the formed epoxide was opened by the methoxide nucleophile present in the solution (formed by proton exchange between methanol and *t*-butoxide, **Scheme 5.3**) when the reaction was left to stir for more than 24 hours, yielding compound **507** in small amounts. A minimum of two equivalents of base were required for this specific reaction as the methoxide-derived sulfonyl ester by-product **508** likely undergoes a rapid nucleophilic attack by another methoxide to form a volatile dimethyl ether (**510**) and methanesulfonate by-product **509** (**Scheme 5.3**). The best selectivity was found when the base was made in-situ by carefully mixing 2.2 equivalents of potassium metal in anhydrous methanol at 0 °C for 10 minutes, then adding it to a solution of 2,3-di-O-mesylate **505** mixing in anhydrous methanol at 0 °C as well, and letting it warm to RT, then heating the reaction mixture at 50 °C for 12 hours (L-allo : L-manno configurations, 8 : 1, entry 7).

Table 5.1 *- List of attempts to optimize the selectivity during the formation of α-L-allo- (**505**) and α-L-manno- (**506**) epoxides.*

	Base	Equiv. of Base	Additive	Temp (˚C)	Time (days)	L-allo:L-manno Configuration Ratio
1	NaOMe	5	none	20	19	1.5:1
2	NaOMe	5	none	40	1	1.4:1
3	KOtBu	5	none	20	2	2.4:1
4	KOtBu	5	none	40	1	1:1
5	KOtBu	2	none	50	1	4:1
6	KOtBu	2	none	20	5	5:1
7	K	2.2	none	50	1	8:1
8	KOtBu	2	CsF (2 eq)	20	5	5:1
9	KOtBu	2	$Mg(ClO4)_2$ (0.1 eq)	20	5	5:1
10	NaOMe	2	none	50	3	1:1
11	LiOMe	2	none	50	5	1:2
12	KOtBu	2	18-crown-6 (2.4 eq)	50	3	4:1

Other optimization attempts revolved around varying counter-cation sizes, with bigger one (Cs^+, entry 8), so as to accommodate more coordination sites with the oxygens or with alkali earth metal ion (Mg^{2+}, entry 9) to engage a tighter binding with oxygens at the two sites: selectivity was however not improved, and the reaction was slowed down likely due to an inefficient activation. Smaller counter-cation sizes were also explored by performing the reaction with 2 equivalents of both lithium methoxide (entry 10) and sodium methoxide (entry 11) in methanol at 50 °C, producing once again slower reactions and worsened selectivity in the case of sodium cation, and a surprising reverse selectivity with lithium cation.

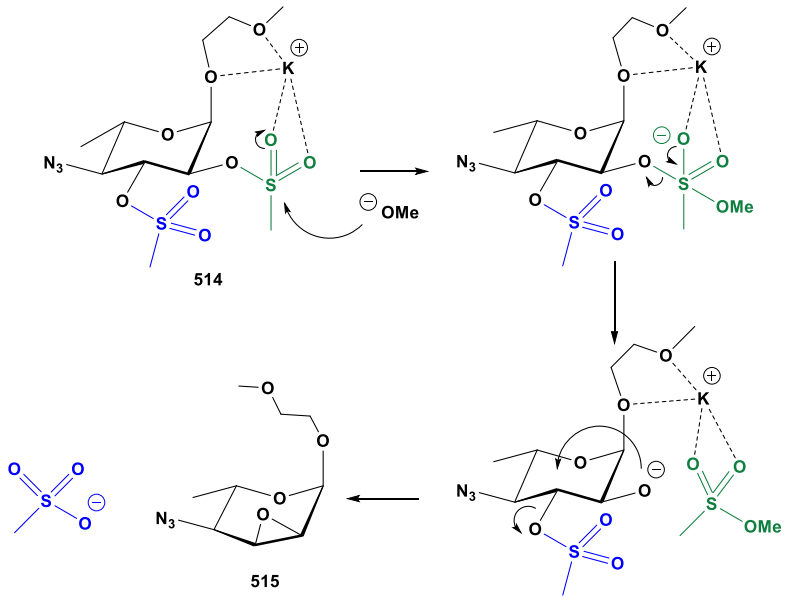
The observed variation in reaction rates can be intuitively explained by the covalent/ionic nature of the bond between the alkoxide and the metals, where potassium salt possesses the right balance of alkoxide nucleophilicity and metal cation electrophilicity, allowing it to act as a

weak Lewis acid that can accommodate electron-pair donation from several oxygen donors on the molecule, but not strong enough to inhibit alkoxide reactivity. The reversal in selectivity seen with lithium methoxide hints at a higher reactivity of the sulfonyl at C-3 towards S-O scission, likely caused by sterics, as it is the only substituent on the bottom face of the sugar. Adding 18-crown-6 ether to the reaction to attempt to take potassium out of the equation did not produce the desired results, as the equilibrating nature of the metal encapsulation by crown ether still allowed for the limited availably of potassium cations for sulfonate activation – the only effect was a slowdown of the reaction progress (entry 12). Lastly, the sulfonyl protecting group was switched to O-tosylate in an attempt to slow down the reaction with a more stable protecting group, but no increase in selectivity was observed (not shown).

5.3 More selective Epoxide formation with increased chelation sites

The previous work by Hevey[121] was carried out on a rigid pyranoside which has a fused ring system, far less flexible than the 2,3-dimesylate substate in the present studies, and so achieving the selectivity observed above was quite interesting and warranted some additional probing. While variations in the nature of the counter-cation proved to be limited due to reactivity and availability considerations, increasing the number of binding sites at the anomeric region was hypothesized to potentially help localize the alkali metal ions there, to then preferentially activate the neighboring O2 sulfonyl (**Scheme 5.4**). Compound **514**, with an α-2-methoxyethoxy anomeric substituent instead of the benzyl ether, was chosen as a good

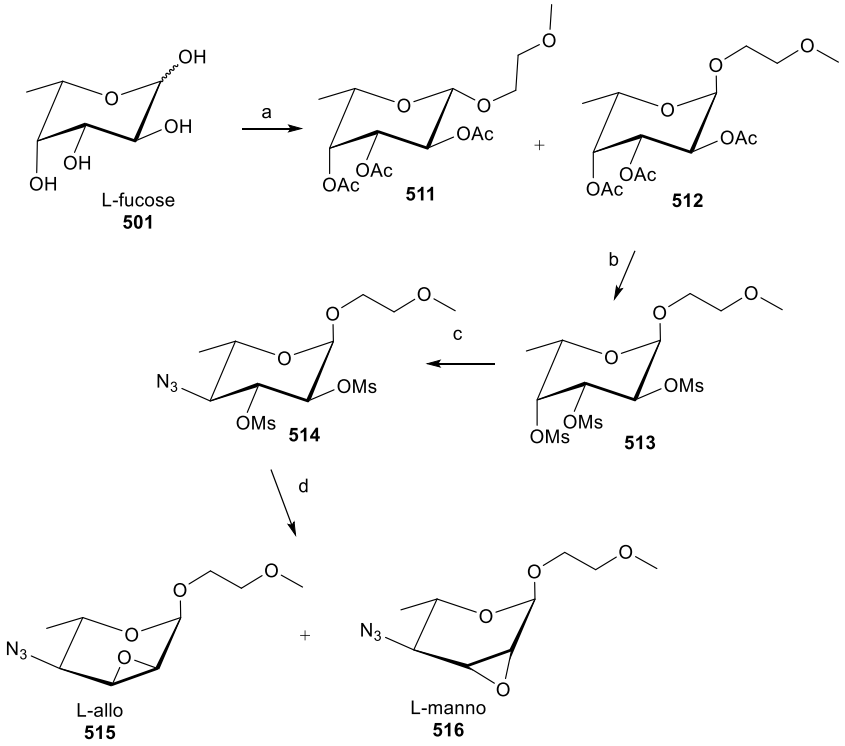
candidate for such investigations, effectively doubling the amount of oxygens available for chelation.



Scheme 5.4 – *The proposed formation of α-L-allo-epoxide (**515**) via 2-methoxyethyl promoted stronger chelation of potassium cation at the anomeric region that leads to improved regioselective activation of the 2-O-mesylate.*

Compound **514** was synthesized in 5 steps from L-fucose **501** (**Scheme 5.5**). The Fischer glycosylation of L-fucose with 2-methoxyethanol (MOE) produced the desired glycoside with a maximum yield of 37%, which was achieved in a 5-hour reaction time using a catalytic amount of sulfuric acid immobilized on silica with heating to 70 °C.[158] Additional reaction time did not improve the yield, which is thought to be so low due to MOE's ability to strongly coordinate to water – azeotropic removal of water was attempted by adding cyclohexanes to the reaction vessel and collecting the distillate in a Dean Stark apparatus under vacuum, but the yield was not improved. Many optimization attempts of this reaction produced the best results when the solvent evaporation during work-up was done at room temperature, as concentrating the acid with heating lowered the yield, likely due to an anomeric deprotection. The reaction did form the

desired α-isomer as a major (α : β = ~2:1 ratio), and separating the anomeric mixture was performed most efficiently through silica gel column chromatography after a per-acetylation of the hydroxide functionalities to form **511** and **512**. A sodium methoxide-catalyzed transesterification to remove all O-acetyl deprotection groups was then carried out in methanol before the subsequent 2,3,4-O-trimesylation; this yielded 90% of desired 2,3,4-trimesylate **513**. The mono-substitution of C-4 mesylate with sodium azide demonstrated good regioselectivity as well, where the desired C-4-azide **514** was obtained in a 68% yield.

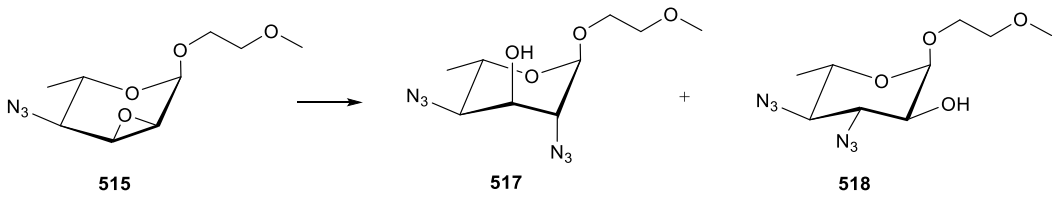


Reagents and conditions: (a) i. MOE, H_2SO_4-silica; ii. Ac_2O, Pyr; **(b)** i. NaOMe, MeOH; ii. MsCl, Pyr; **(c)** NaN_3, DMF; **(d)** KOMe

Scheme 5.5 – *Synthesis of 2-methoxyethyl α-L-fucopyranoside for better chelation of alkali metal ion at the anomeric center region to activate the 2-O-mesylate with improved selectivity.*

The epoxidation reaction was performed with compound **514** as before, with 2.2 equivalents of potassium methoxide, heated to 50 °C over 12 hours (**Scheme 5.5**). A crude ^1H

NMR after solvents were evaporated revealed that the selectivity for the desired allo-epoxide **515** was increased to 10:1. Both epoxides were isolated via silica gel column chromatography, and characterized. The allo-epoxide **515** (66% yield) was assigned based on its chemical shifts and patterns, which were very similar to the ones seen in the corresponding O-benzyl epoxide (see **Scheme 3.8**). For example, the H-4, shown as a dd, was similarly found at 3.15 ppm, while the H-2 and H-3 signals were collapsed on each other at around 3.5 ppm - this is similar to the corresponding signals for compound **331**, which had both H-2 and H-3 protons slightly separated, with a very pronounced roofing effect. The manno-epoxide **516** (7% yield), on the other hand, was characterized by an H-3 proton signal resonating at 3.16 ppm, and overlapping signals for H-2 an H-4 were observed at around 3.2 ppm, which is very similar to the chemical shifts of the corresponding protons on the O-benzyl analog **506**. Even H-5 has a similar up-field shift from the allo to the manno structure in both cases, from around 3.9 ppm to 3.7 ppm.



Reagents and conditions: NaN_3, $LiClO_4$, MeCN.

Scheme 5.6 *– Epoxide opening reaction performed on substrate **515**, giving the desired isomer **517** as a major product.*

Interestingly, when the O-MOE allo-epoxide **515** was subjected to the ring-opening reaction under the previously optimized conditions with sodium azide using lithium perchlorate as an additive in acetonitrile, an increase in regioselectivity was also observed, producing the

desired 2,4-diazido **517** in much improved regioselectivity, as seen by the crude ^{1}H NMR spectra (2,4-diazido isomer **517** : 3,4-diazido isomer **518**: 20 : 3 ratio , **Figure 5.3**).

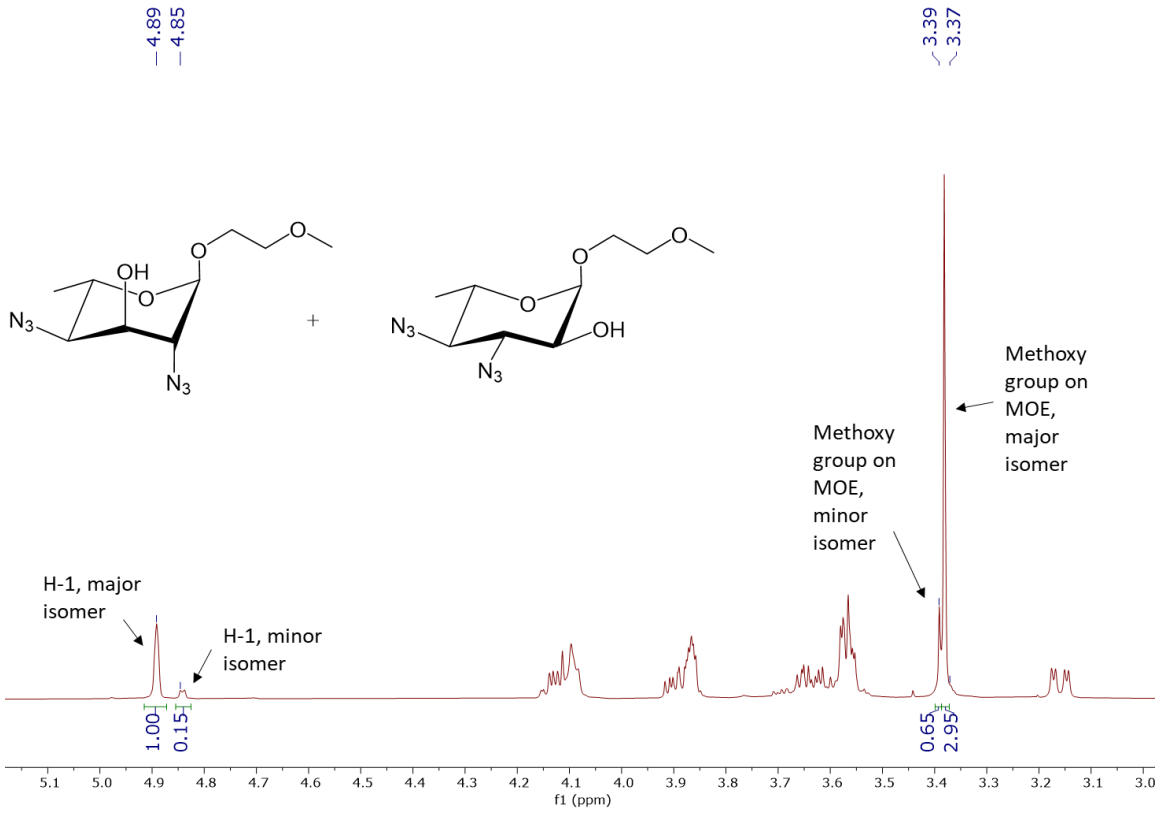


Figure 5.3 *– Crude ^{1}H NMR of the epoxide opening reaction showing a 20:3 ratio of 2,4- di-azide (**517**) vs the 3,4-di-azide (**518**).*

Although the reaction went to completion, the desired 2-methoxyethyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **517** was isolated only in a very low yield following extraction and silica gel column chromatography. We attributed the cause to be the enhanced water solubility of this series of MOE compounds due to the presence of the more hydrophilic 2-methoxyethyl aglycone. As such, an excessive loss of desired product likely occurred during liquid-liquid extraction step in the workup, warranting a switch to liquid-solid extractions in the future, among other necessary optimizations in the work-up and purification steps. Nevertheless, an explanation for the regioselectivity seen in the crude ^{1}H NMR likely follows the same logic as

previously reported in Chapter 3, where the added sites for lithium coordination with oxygens has an additive effect on the dipole moment, and so the epoxide is even more likely to adopt the preferred conformation for the desired ring-opening following Fürst-Plattner rules (see **Figure 3.5** in Chapter 3). The major product from the last reaction was isolated following silica gel column chromatography: the structures of product was confirmed through ^{1}H-^{1}H GCOSY and ^{1}H-^{13}C GHSQC correlation experiments, where it was found that C-2 and C-4 have a chemical shift at 60.6 and 61.0 ppm respectively, corresponding to their association with an azido group, while C-3, attached to a hydroxyl, was subject to deshielding effect with its signal observed at 69.6 ppm (**Figure 5.4**).

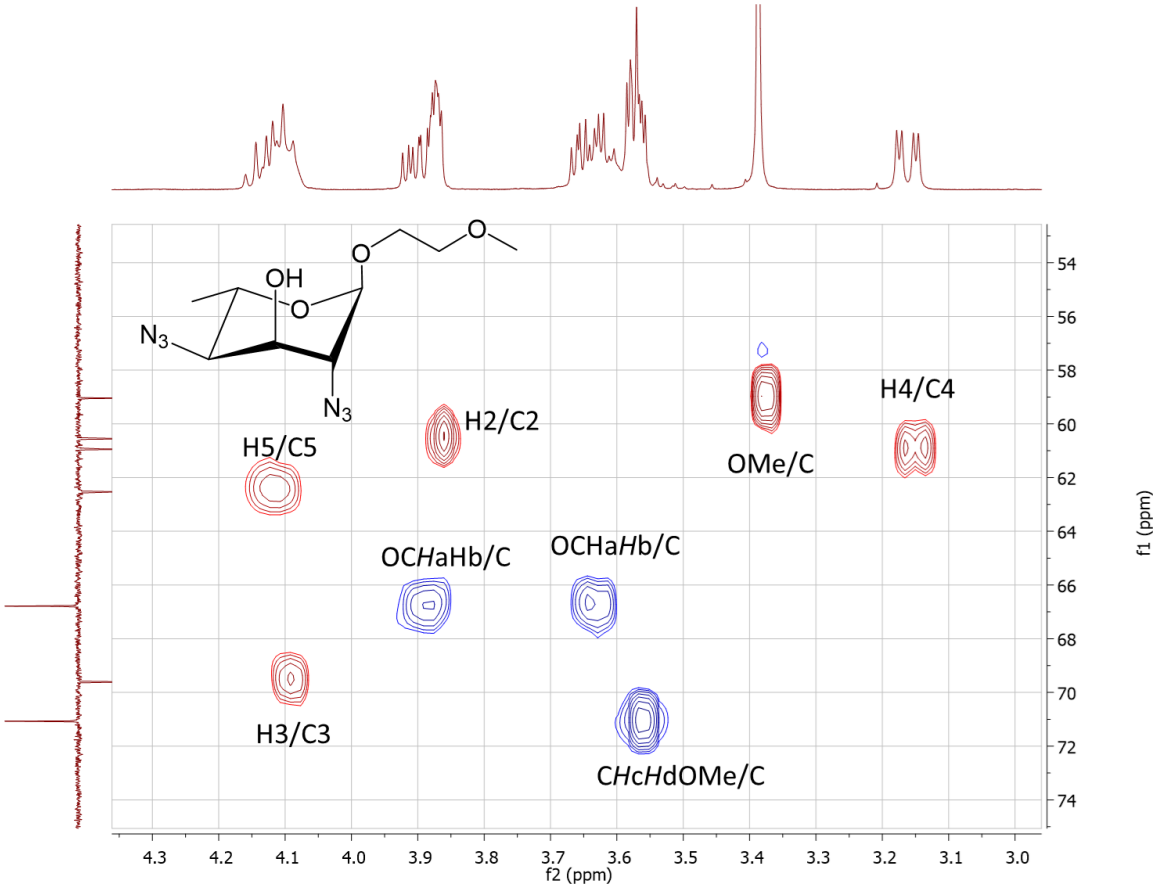


Figure 5.4 *- ^{1}H-^{13}C GHSQC spectrum of compounds **517** that shows the correlating signals for the azide-substituted C-2 and C-4 and associated protons. The upfield signal shift for C-3 matches the pattern related to substitution with a hydroxyl.*

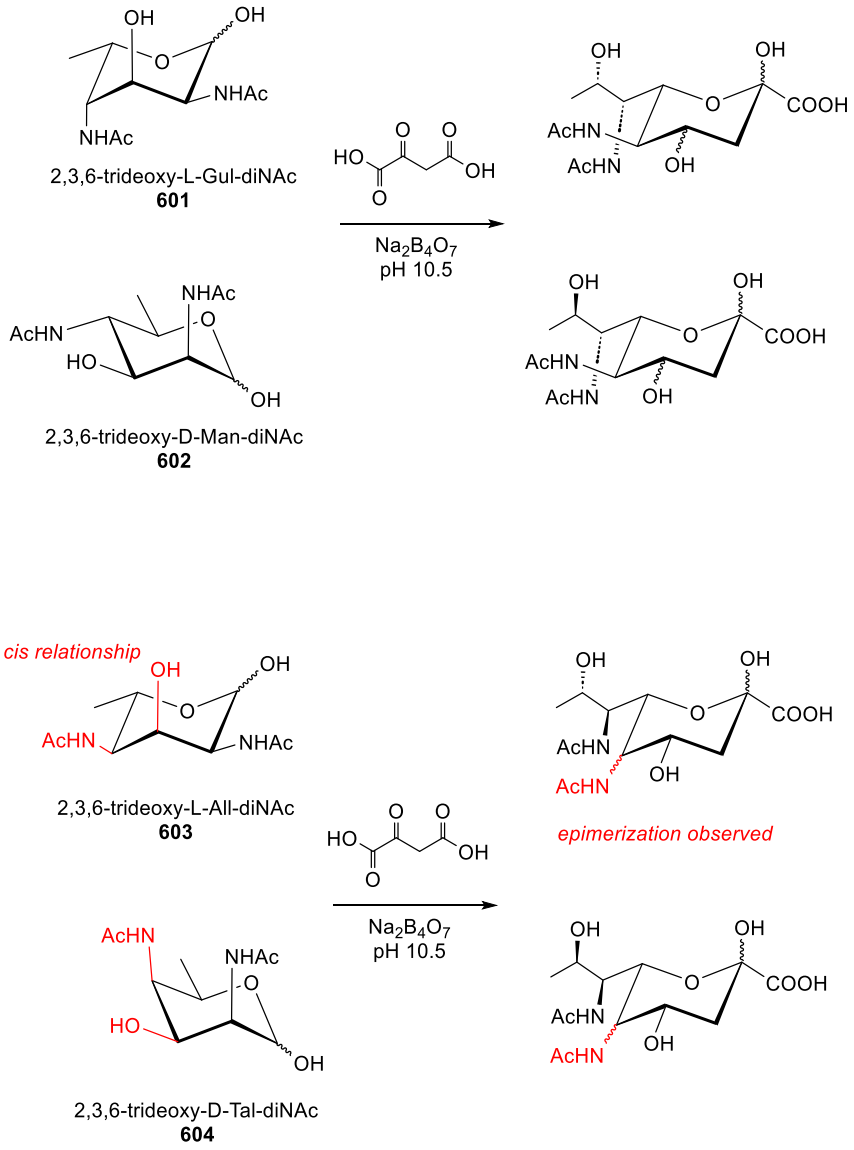
The successful work reported above using an anomeric 2-methoxyethyl glycoside further established the role of the alkali counter-cation assisted activation of 2-O-mesylate, evidenced by an increase of formation of the desired α-L-allo epoxide **515**. The much-improved selectivity in both the epoxide-forming and the epoxide-opening reactions warrants further optimizations within this scheme: first, the initial glycosylation needs upgrading to produce more of the desired α-L-fucopyranoside product, as the best yield obtained was only about 2/3 of the one obtained with the benzyl-ester protected substrate. Secondly, the work-up of reactions within this series needs to be optimized as well to prevent the loss of the more water-soluble, and possibly acid-labile products MOE anomers.

The work outlined in this chapter provides an even more expedient synthesis of Alt-diNAc, requiring only 4 steps to achieve the epoxide intermediate **505** in good yield similar to the previously reported scheme (40% vs 38%), but in less steps (4 vs 7). The challenging issue of epoxide formation selectivity from the configurationally flexible di-O-mesylate **504** was elegantly addressed with an optimized reaction with potassium methoxide, generated *in situ*, giving up to 8:1 ratios of the desired allo-epoxide **505**. Investigations into the nature of the observed selectivity with MOE-ether instead of benzyl-ether at the anomeric postion produced great results, with an unexpected additional epoxide-opening improvement in regioselectivity as well. Despite these marked selectivity improvements with the MOE-product series, work still needs to be done to optimize yields, and to find a methodology to cleave the MOE protecting group at the end, compatible with the different acylation patterns on the amido functionalities: this could potentially allow an even better-yielding route towards Alt-diNAcy. Nevertheless, with the results

and the findings outlined in the last chapters, our synthesis was finally ready for the next step towards the production of Pse: the three-carbon elongation.

Chapter 6 Preliminary three-carbon Extension Work

6.1 Literature Work on three-carbon Extension from Hexose



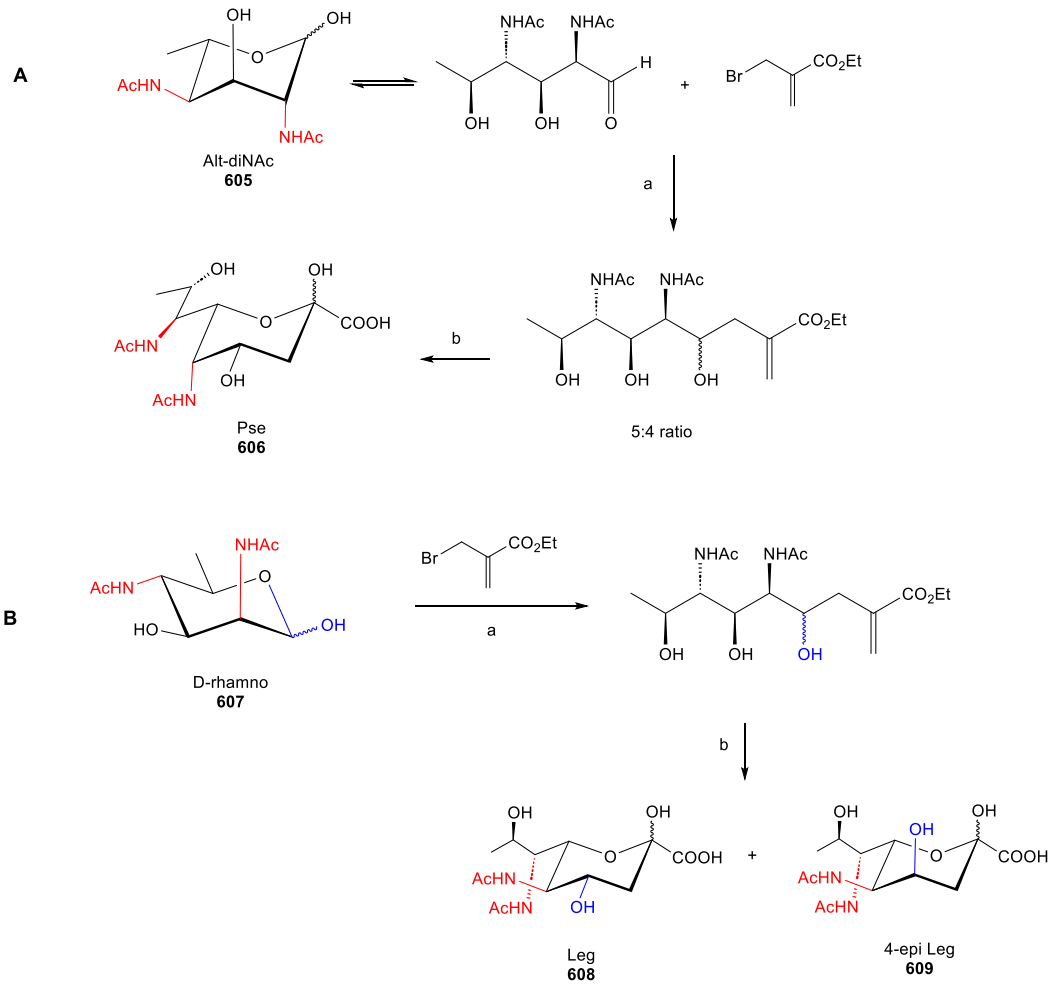
Scheme 6.1 *- Preparation of different 5,7-diacetamido-3,5,7,9-tetradeoxynono-2-ulosonic acids from the condensation of 2,3,6-trideoxy-L-hexoses-2,4-diNAc with oxalacetic acid followed by a decarboxylation.*

The three-carbon extension necessary for the synthesis of Pse from a hexose has been shown to work by several groups, using different methodologies. As seen in section 2.1, Tsvetkov

et al[86] used a condensation with oxaloacetic acid followed by a decarboxylation to extend different hexose precursors towards their synthesis of a variety of 5,7-diacetamido-3,5,7,9-tetradeoxynono-2-ulosonic acids (**Scheme 6.1**): the reaction was performed in the presence of sodium tetraborate at basic pH. When 2,3,6-trideoxy-L-Gul-diNAc **601** and 2,3,6-trideoxy-D-Man-diNAc **602** precursors were subject to these conditions, the products were isolated as racemates in low yields (18% & 20%). Interestingly, 2,3,6-trideoxy-L-All-diNAc **603** and 2,3,6-trideoxy-D-Tal-diNAc **604** precursors, where the substituents and C3 and C4 are *cis* and on the top-face, produced only one isomer each at the new C-4 center, although epimerization was detected with their respective NHAc groups at C5 of the 9-carbon product. No explanation was provided for this phenomenon.

Ito et al[78] applied the indium-mediated allylation, originally developed by Gordon and Whitesides[159] for unprotected carbohydrates, to synthesize Pse from L-Alt-diNAc with high yield. They first reacted the Alt-2,4-diNAc **605** with methyl 2-(bromomethyl)acrylate in the presence of indium to afford a mixture of diastereomers in 77% yield, with the desired *erythro* product formed in a slight majority (5:4) over the undesired *threo* isomer (**Scheme 6.2**, **A**: see **Scheme 3.3** for their detailed synthesis). The authors reported other attempted approaches to improve the diastereoselectivity, including using Lewis acid additives and using (S,S)-2,6-bis(4-isopropyl-2-oxazolin-2-yl)pyridine as a chiral ligand, which previously demonstrated enantioselectivity for allylation reactions, to no avail in this case.[160] Pse (**606**) was ultimately obtained with an ozonolysis step followed by oxidative work-up, and saponification in an aqueous triethylamine solution. The Gintner group also utilized the indium-mediated allylation to produce their Leg

(**608**) and 4-epi Leg (**609**) precursors from D-rhamno configuration intermediate **607**, obtaining poor diastereoselectivity (5% de) (**Scheme 6.2**, **B**; see **Scheme 2.4** for their detailed synthesis).[89]



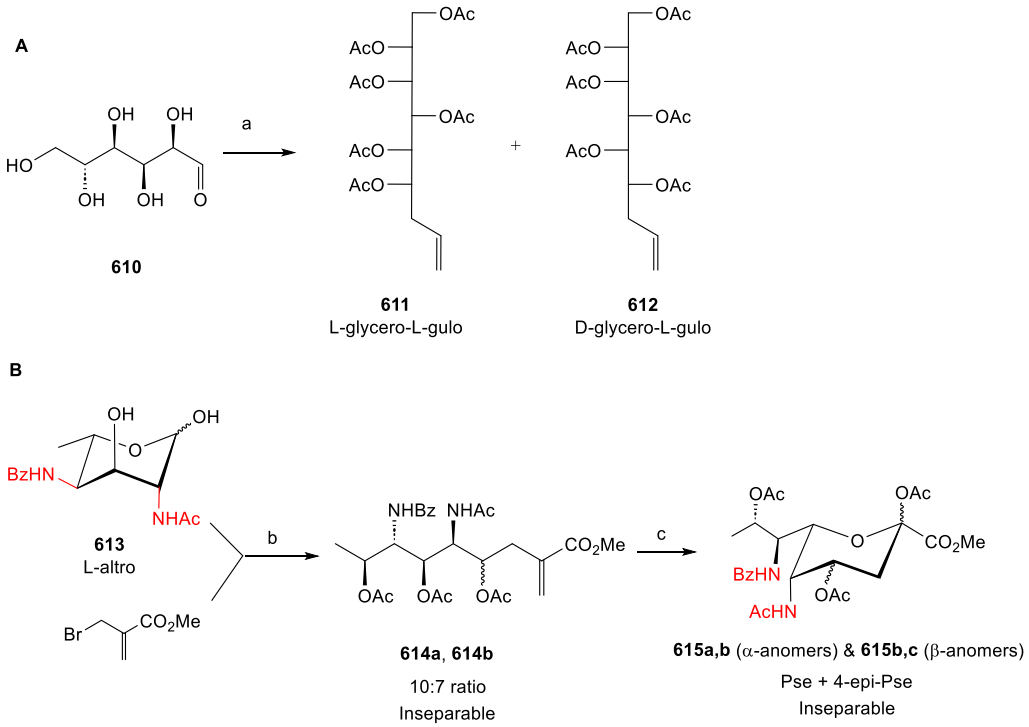
Reagents and conditions: (a) indium powder, EtOH, 01 aq. M HCl; **(b)** i. O_3, MeOH; ii. (for **A**) DMS; (for **B**) H_2O_2, H_2O, HCO_2H.

Scheme 6.2 *- Previously reported synthesis of Pse [78], Leg and its 4-epimer[89] (**B**) using indium-mediated allylation.*

6.2 Initial Attempts for Indium-Mediated Allylation

The indium-mediated intermolecular allylation was attempted in my work, with the help of undergraduate student Afsah Ali, to determine the appropriate reaction conditions. The acid-activated coupling was carried out between D-glucose **610** and allyl bromide as a model reaction

(**Scheme 6.3**) in a 1:1 ratio of ethanol and water using 4 equivalents of indium and allyl bromide, and the reaction was set to under sonication at room temperature. It was found that the reaction proceeded well, and after a full O-acetylation, the D-glycero-L-gulo- and L-glycero-L-gulo-isomers **611** and **612** were isolated in 66% and 33% yield respectively. Acidifying the reaction mixture was previously found to be conducive for metal dissolution, therefore aqueous HCl was added in all of these reactions.[160]



Reagents and conditions: (a) i. Allyl bromide, In powder, EtOH-0.1N HCl, sonication; ii. Ac_2O, Pyr; **(b)** i. indium powder, EtOH-0.1N HCl, sonication; ii. Ac_2O, Pyr.; **(c)** i. O_3, CH_2Cl_2; ii. Zn, AcOH iii. Ac_2O, Pyr.

Scheme 6.3 - A. *A model study of indium-mediated allylation using D-glucose **610** and allyl bromide.* ***B.*** *Synthesis of α-Pse derivatives **615a,c** and β-Pse **615b,d** from Alt-diNAcy **613**.*

For the reaction with methyl 2-bromomethylacrylate and the L-altro-configuration precursor **613**, preliminary studies indicated that it was indeed necessary to use a much larger excess of indium (10 equiv.) and bromo methyl acrylate (15 equiv.) in a 4:1 solution of ethanol

and 0.1 N HCl, with additional heating (40 °C, 6 hours) during sonication: Lee *et al*[78] also noticed the lower reactivity to this reaction with sugar substrates carrying an N2-acetyl group, first reported by Loh and Zhou.[160] The reaction with differentiated substrate **613** yielded the desired product **614** as a mixture of isomers in 60% yield and a 10:7 ratio, which were not able to be separated other via column chromatography on silica gel (**Scheme 6.3**). In the literature, it was noticed that specialized porous silica spheres called "Iatrobeads"[78,161] were used for separation by column chromatography instead of silica gel in one report, while the Schmid group[89] reported their products as an inseparable mixture of diastereomers, which attests to the difficulty in isolating these compounds.

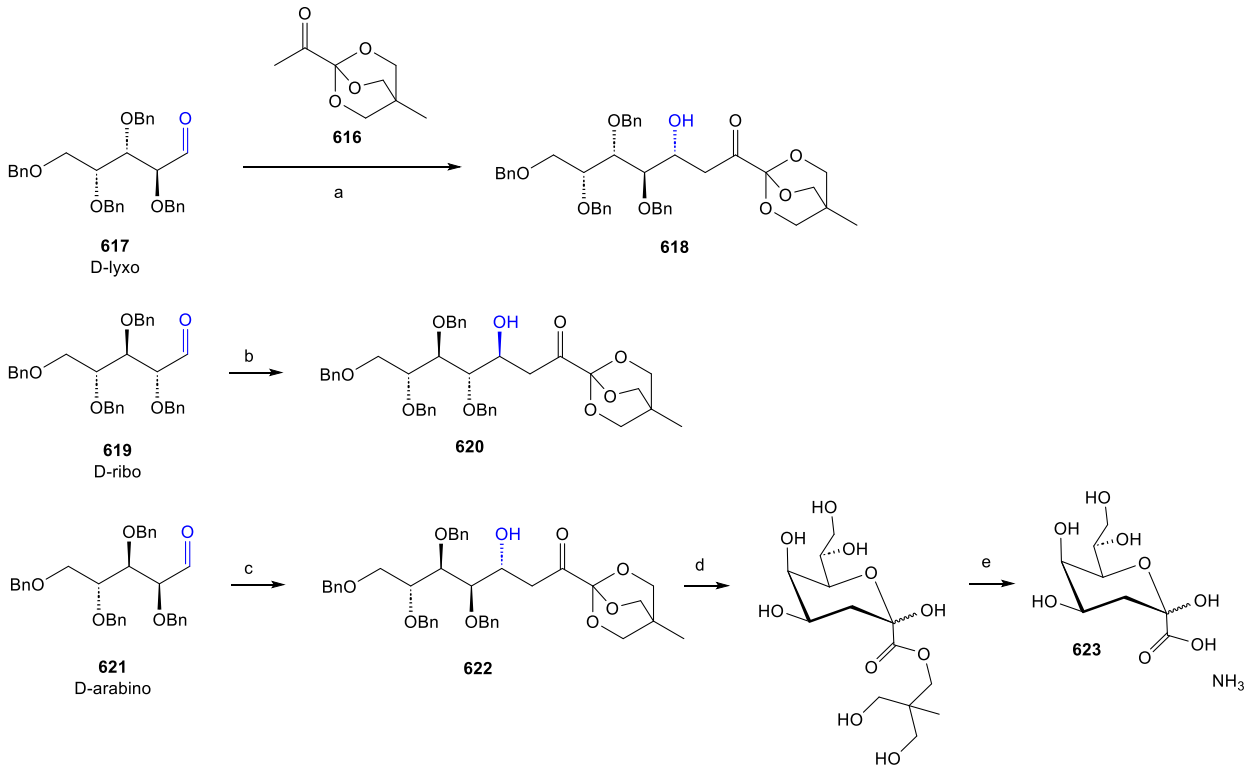
Ozonolysis was then performed with the mixture of isomers containing **614a** and **614b** (**Scheme 6.3**), which worked well and very quickly, the success of which was established by noticing the disappearance of the vinyllic hydrogen signals in the crude ^{1}H NMR spectrum of the product, seen at ~6.2 and ~5.6 ppm in the precursor. After a per-O-acetylation of the crude compound, a mixture of 4 isomers was detected by NMR, as seen by the four doublets corresponding to each of the two amide protons: apparently, the reductive step with zinc acetate caused the deprotection of all O-acetyl group, allowing for the cyclization of the 9-carbon product before the next step.[162] Isolation of the compounds **615a** and **615b**, each in their additional α- and β-anomeric configurations (4 isomers in total) was not attempted at this stage either, as TLCs did not reveal the possibility for separation in this way. Despite the moderate yield and the difficulty in isolating the products , the synthesis of a differentiated Pse, with an acetamido group at C-4 and a benzamido group at C-2, was achieved in my work, confirmed by electrospray high resolution mass spectrometry of the mixture of isomers (calc'd *m/z* for $C_{25}H_{30}D_2N_2O_{11}$ $[M+H]^+$:

539.2204; found: 539.225). A previously attempted ^{1}HNMR characterization of the sample in deuterated solvent produced a compound which was thought to have both amide protons exchanged with deuterium, which explains the observed mass. These results spurred the work on towards finding a better way to perform the three-carbon extension in a stereoselective way, which could not only improve the synthetic efficiency, but could also provide an easier way to obtain the Pse in pure form.

6.3 Intramolecular Aldol Addition as a New Strategy

As discussed before in section 2.1, several groups began their syntheses from the 9-carbon Neu5Ac, eliminating the need to create new C-C bonds in their syntheses: additionally, the C-4 position of Neu5Ac possesses the same configuration as most bacterial NonAs (except 4-epi-Leg).[80–82,87,88] While these strategies seem to have the advantage of avoiding the diastereoselectivity issue with carbon-backbone extension, most suffer from long reaction sequences and give low yields. On the other hand, carbohydrate synthesis and extensions via asymmetric aldol additions have been investigated in the past, but never for the synthesis of Pse.[163,164] As such, the Norimura group designed a three-carbon pyruvate equivalent, oxabicyclo[2.2.2]octyl orthoester (**616**), which exhibited good diastereoselectivity when it was reacted with certain open-chain hexoses protected with benzyl-ethers, with yields between 50 and 60% (**Scheme 6.4**).[97] Among their results, reactions with the D-lyxose **617** and the D-ribose **619** protected aldehydes gave extended products **618** and **620** in up to 20:1 diastereomeric product ratios when zinc chloride was used as an additive. The synthesis of 3-deoxy-D-manno-

2-octulosonic acid (KDO, **623**), a natural 3-deoxy-2-ketoacid, was demonstrated via their methodology from tetra-2,3,4,5-O-benzyl-L-*arabino* aldehyde **621**, giving a 11:1 diastereomeric ratio for the desired aldol product **622** in a 60% yield. The transformation of **622** to KDO was then achieved in three steps, with a 78% yield (**Scheme 6.4**).

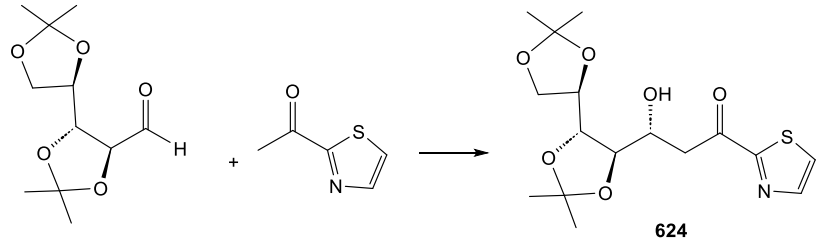


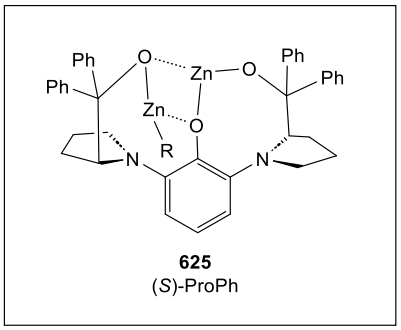
Reagents and conditions: (a) 613, LiHMDS, $ZnCl_2$, THF; **(b)** H_2, Pd/C; **(c)** i. Et_3N, H_2O; ii. aq. NH_3

Scheme 6.4 *– Norimura et al strategy towards asymmetric 3-carbon extension using orthoester **616** as the aldol adduct, giving 4,5-anti configuration preferentially in the products.[97] The synthesis of KDO is shown as well.*

The Mlynarski group has shown success in the aldol addition of pyruvic derivatives to synthesize ulosonic acids by using metal complexes acting as type II aldolases, the enzyme that performs this kind of reaction in biosynthesis.[165] Proline-based ligands coupled with metal-based catalysis produced good results, with some diastereoselectivity (**Scheme 6.5**): the synthesis of KDO precursor **624** was successful with (*S*)-ProPh (**625**) as the catalyst, first presented by Trost

and Ito,[166] giving a 77% yield with an 8:2 anti/syn selectivity. This is one example of numerous other proline-catalysed biomimetic synthetic approaches for the incorporation of PEP into molecules that have been demonstrated in the last decade[164,167].





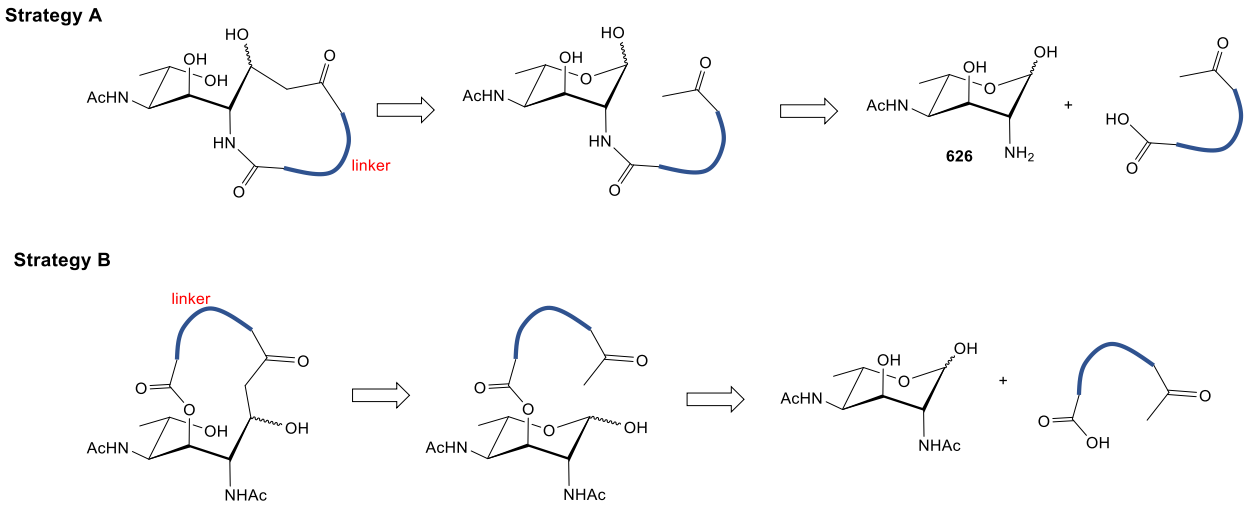
Reagents and conditions: (S)-ProPh (5 mol-%), THF

Scheme 6.5 *– Three-carbon extension of D-arabino precursor by Mlynarski et al[165] via aldol addition with organocatalysis by Trost's catalyst* ***625****.*

Considering the literature outlined above, an asymmetric three-carbon extension from hexose precursors to form 9-carbon-nonulosonic acids presents much potential, and warrants more exploration. Having successfully found a short, scalable and synthetically simple way to make an appropriate L-altro-configuration precursor to many different natural derivatives of Pse, preliminary studies to explore a new strategy to achieve the desired 3-carbon extension were carried out in my work. Our proposed methodology aims at extending the 6-carbon Alt-diNAcy backbone through a potentially stereoselective intramolecular chain extension via aldol addition, directed by a suitably positioned cleavable linker (Scheme 6.6). This would be the first time a

linker-induced stereoselective aldol addition was performed towards carbohydrate backbone extension to our knowledge, and its success could advance the synthesis of N2,N4 differentiated pseudaminic acid analogs in a meaningful way.

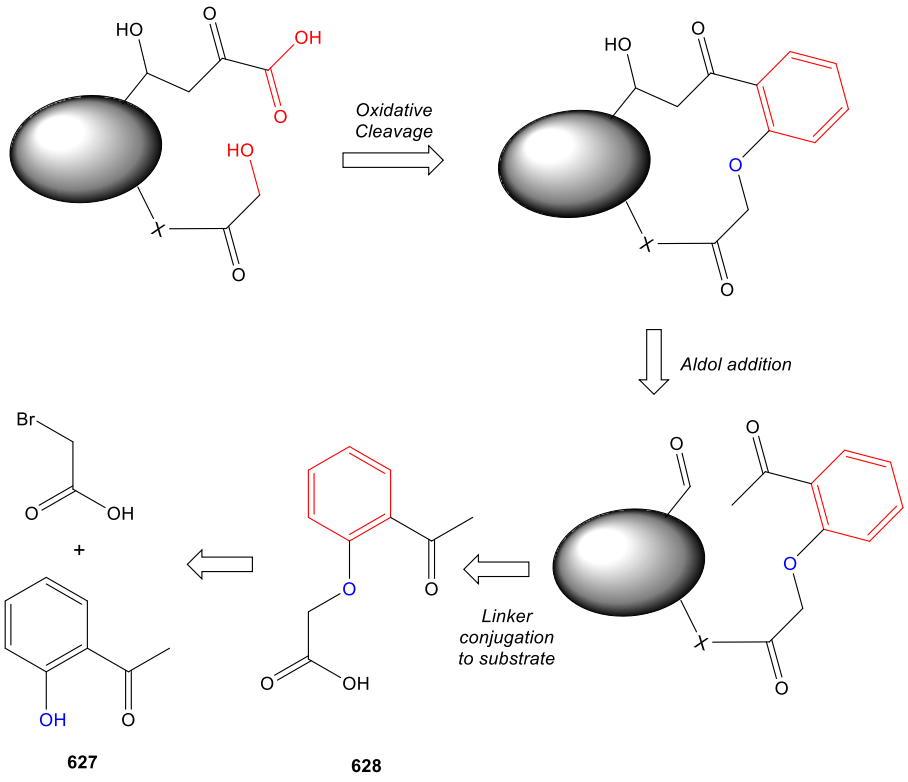
Our linker design included an enolizable methyl ketone group at the reactive end, and a carboxylate functionality that could allow for easy and versatile assembly and ultimate cleavage from the directing "arm". By choosing a carboxylic acid group we envisaged a scheme to attach the linker to either N2 or O3 of an Alt-diNAcy precursor **626** (**Strategies A** and **B Scheme 6.6**, respectively): the existing configurations at these centers are opposite to each other, meaning that an enolized methyl ketone could potentially approach the anomeric carbon of the L-altro-configuration pyranose in a diastereoselective manner under optimized conditions.



Scheme 6.6 *- Proposed retrosynthetic strategy for the 3-carbon extension from the Alt-2,4-diNAcyl via intramolecular aldol condensation between the anomeric center of the L-altro-configuration pyranose and a nucleophilic methyl ketone group preassembled with the cleavable linker.*

With this new strategy in mind, the optimal composition of the linker group was deliberated. The aldol addition strategy with PEP or equivalents is usually complicated by the alpha-keto ester structure on the pyruvate, which favors the retro-aldol product due to its

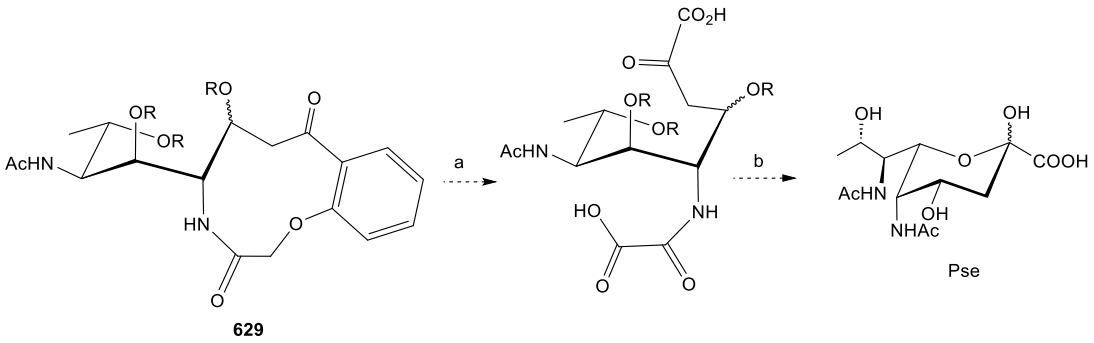
electron-withdrawing nature.[97] Masking the problematic functionality by using pyruvaldehyde dimethyl acetal is an option that has been explored, but usually produces low-yields with self-aldol side products.[97,165] We therefore opted for a terminal acetophenone functionality on the linker (**628**) that can be cleaved through oxidation to become the required carboxylic acid after the extension (**Scheme 6.7**). The phenoxy functionality on the commercially available *ortho*-hydroxyacetophenone **627**, our chosen starting material, comes with a reactive oxygen that can easily be linked to the required carboxylic acid through formation of an ether.



Scheme 6.7 *- Retrosynthetic considerations for linker design that incorporates a carboxylic acid and methyl ketone group, and allow for a cleavage through oxidation at the end.*

The proposed orthoether-functionalized acetophenone functionality has several advantages for the linker strategy. First, it leaves only one enolizable position on the methyl ketone, which decreases potential complications. Secondly, the aromatic ring is electron-

donating towards the carbonyl, potentially favoring the aldol product over the retro-aldol one: the reaction could go forward by simply adding a strong base, such as LDA in THF at -78 °C and monitoring the reaction progress by TLC. Once the cyclized product is isolated and confirmed, the proposed cleavage step is based on optimizations of the known ruthenium tetroxide catalyzed oxidation originally reported by the Sharpless group,[168] and applied in several synthetic schemes since then,[169,170] including one by our group in 2012 towards the synthesis of KDO glycosides.[171] In our design, the aromatic ring to be oxidized on hypothetical compound **629** is relatively electron-rich because of the ortho-substituted electron-donating ether functionality, which should facilitate the initial coordination with ruthenium. After the oxidation, the protecting groups can be removed and the ring might naturally cyclize to the desired ketose with the most favorable O6, as in NonAs (**Scheme 6.8**).

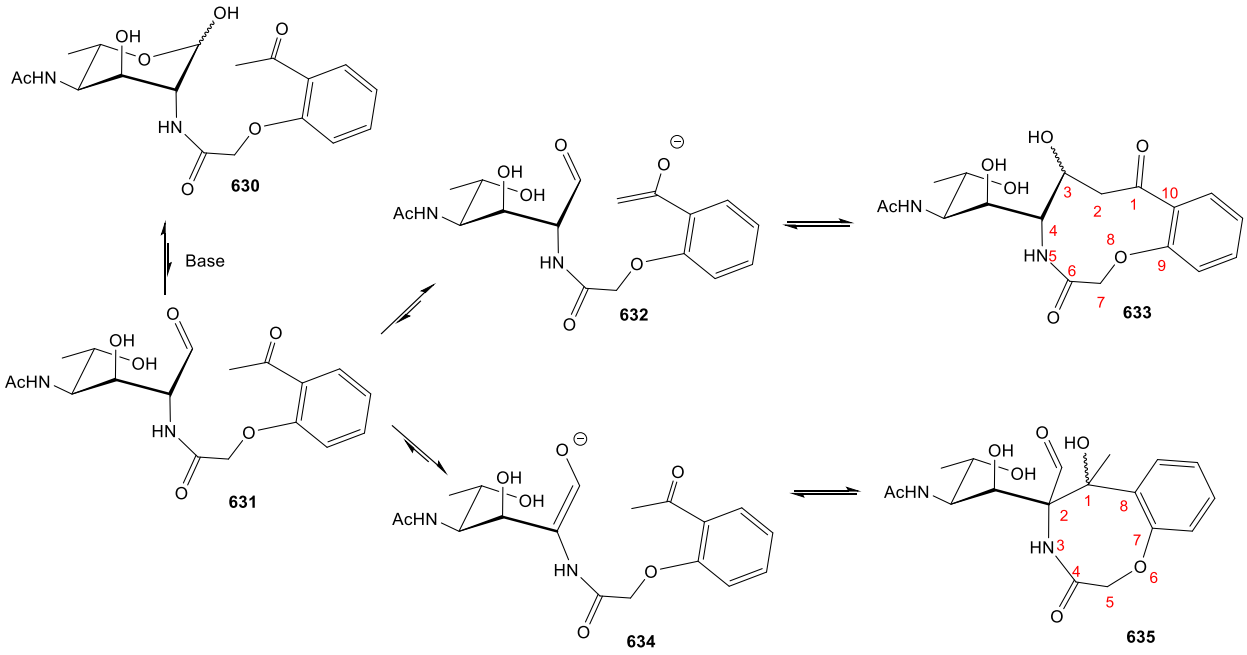


Reagents and conditions: (a) RuO_4; (b) global O-deprotection

Scheme 6.8 *- Proposed Pse synthesis following intramolecular cyclization via base-catalyzed aldol condensation with linker attached to N2.*

It should be noted that the intended intramolecular aldol addition is to proceed via nucleophilic attack by the enolized methyl ketone to the C-1 aldehyde on **630**, which is in equilibrium with hemiacetal form of the free-sugar **631** (**Scheme 6.9**). In this case, a flexible 10-membered ring on **633** would form if the addition proceeded in the desired way, but, if instead,

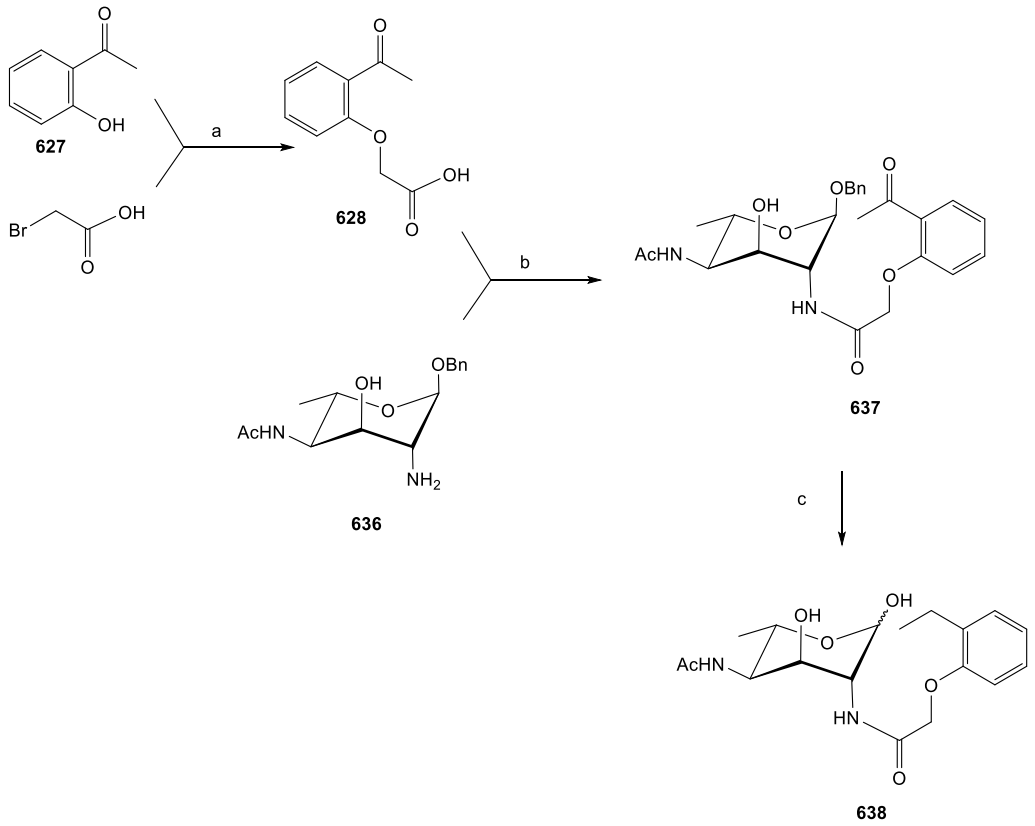
the anomeric carbon center enolized as in **634**, the methyl ketone could then be attacked itself to form an undesired aldol product **635** which has a more strained 8-membered ring (**Scheme 6.9**). Another consideration is the preferential cyclized form a free-sugar usually adopts, which could slow down the desired reaction with aldehyde functionality, which requires the open-chain form. However, considering the increased steric hindrance of the second pathway, the higher reactivity of the aldehyde vs the ketone, and the yields presented by the Norimura *et al*[97] with their orthoester aldol adduct (**Scheme 6.4**) it seems reasonable to assume that the cyclization would likely proceed through the desired pathway.



Scheme 6.9 *- Two possible intramolecular cyclization paths via base-catalyzed aldol condensation.*

6.4 Preparation and Conjugation of Linker

The synthesis of reagent **628** was carried out (**Scheme 6.10**): using sodium hydroxide as a base, *o*-hydroxyacetophenone **627** was reacted with bromoacetic acid in water,[172] to afford the desired product **628**, isolated as a precipitate after an acidification of the reaction mixture (83% yield). The structure of compound **628** was confirmed by proton NMR with the appearance of the methylene peak at 4.78 ppm, and further with the appearance of the quaternary and secondary carbon signals in the ^{13}C NMR, with chemical shifts of 200.5 and 66.9 ppm, respectively. The obtained acid was then conjugated to the 2-amino group of benzyl 4-acetamido-2-amino-2,4,6-trideoxy-α-L-altropyranoside **636** using EDC hydrochloride as a coupling reagent in the presence of 1-hydroxybenzotriazole (HOBt) in anhydrous DMF (**Scheme 6.10**), producing the desired conjugate **637** in 72% yield after a column chromatography on silica gel. In an attempt to expose the anomeric hemiacetal for the subsequent aldol addition, **637** was subjected to a catalytic hydrogenolysis in the presence of palladium hydroxide in methanol for 3 hours; unfortunately, the sole detected product was **638**, which failed to preserve the ketone functionality.



Reagents and conditions: (a) i.aq. NaOH; ii. Aq HCl; **(b)** i. EDC-HCl, HoBt, Et_3N, DMF; **(c)** $Pd(OH)_2/C$, H_2, MeOH

Scheme 6.10 *- Synthesis of O-acetic acid functionalized acetophenone – Alt-4-NAc conjugate* ***637*** *and subsequent unsuccessful deprotection of anomeric O-benzyl group.*

The unsolicited reduction was evidenced by the disappearance of the singlet at 2.67 ppm (methyl group of the acetophenone) in the proton NMR spectrum of the crude product **638** (**Figure 6.1**), with instead the appearance of quartets between 2.77 – 2.70 ppm region, consistent with the presence of an ethyl group attached to an aromatic ring that belong to both anomers of the deprotected sugar, along with some in open-chain form. The deprotection of the anomeric position was detected with the disappearance of the benzylic doublets at 4.75 and 4.58 ppm observed in the precursor **637**.

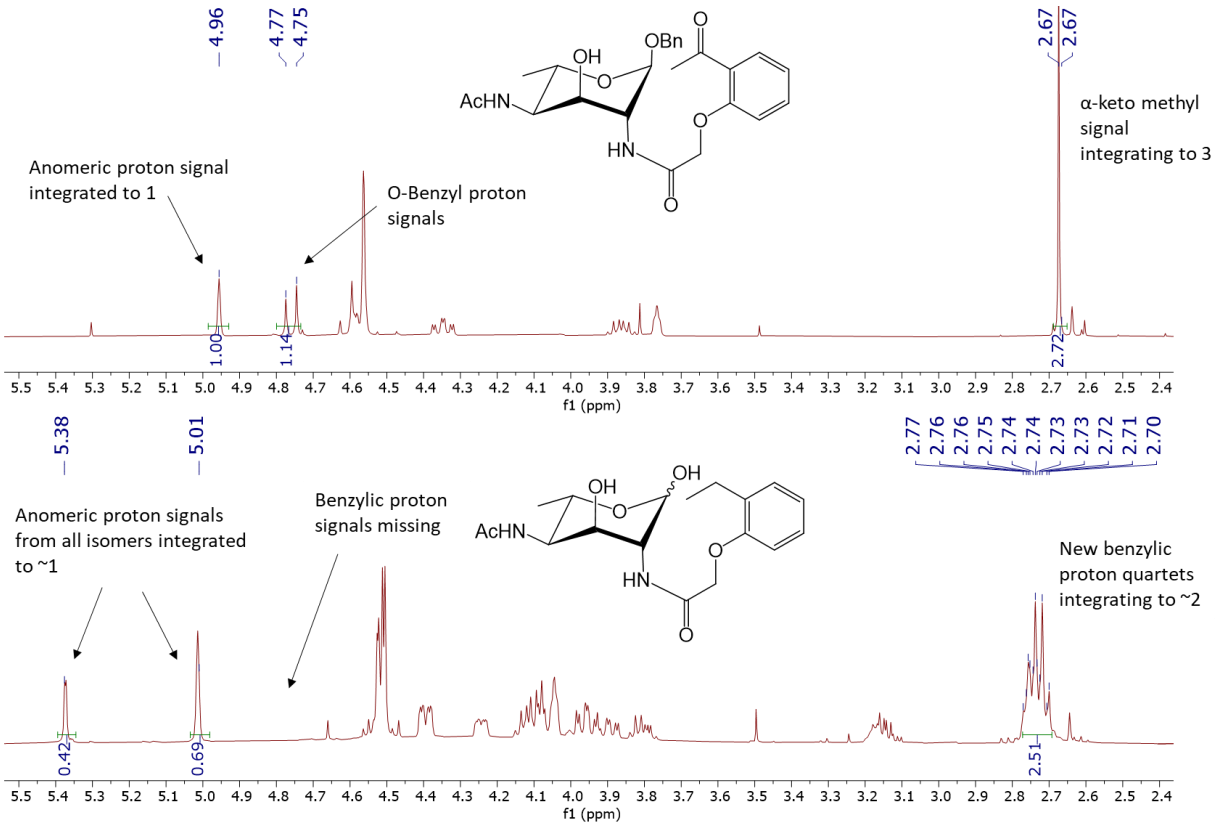
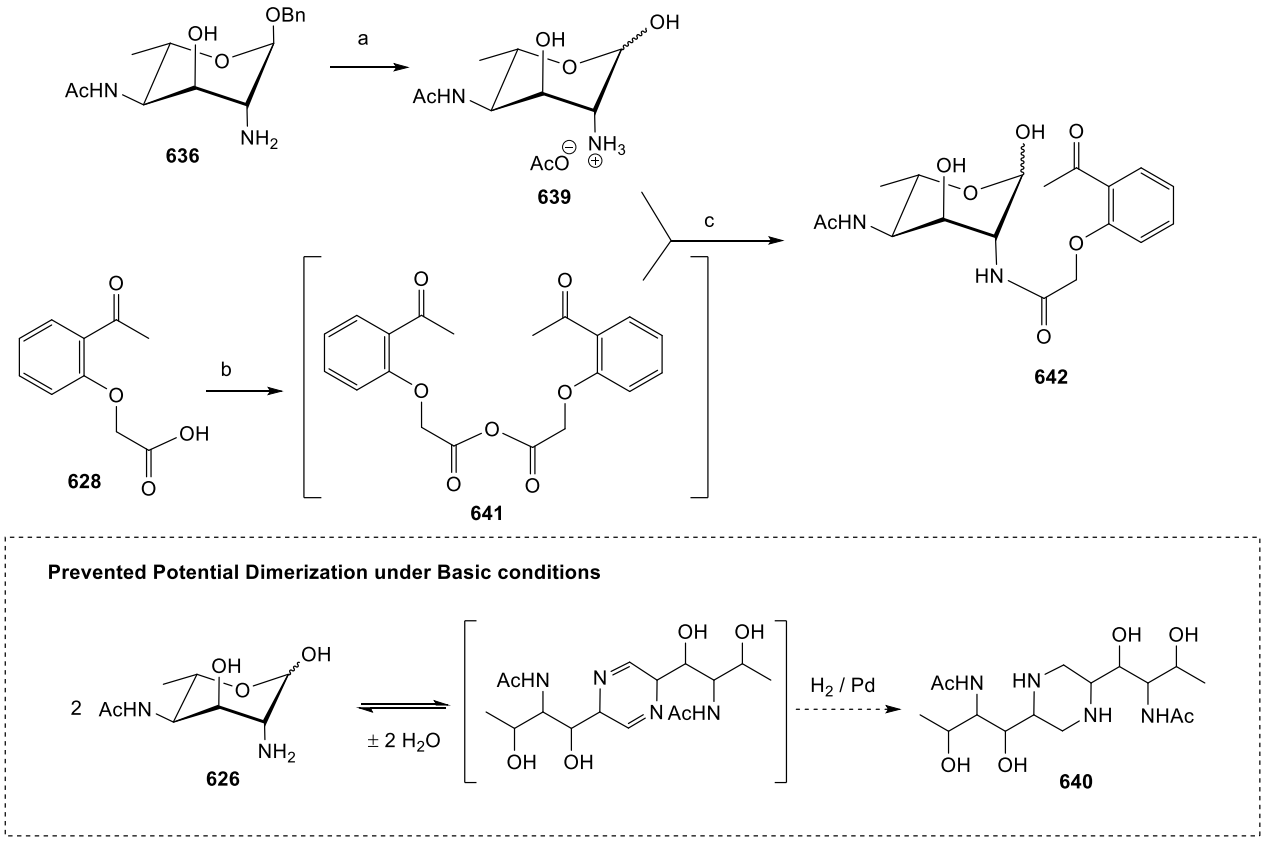


Figure 6.1 – *^{1}H NMR spectra of o-acetic acid functionalized acetophenone – Alt-4-NAc conjugate* ***637*** *(top) and of crude product* ***638*** *obtained from a catalytic hydrogenolysis.*

Alternatively, the anomeric center has been shown to undergo acidolysis with certain ethers, but the reaction usually requires strong acidic conditions, which runs the risk of hydrolyzing esters and acetamido groups and cause other complications in our scheme.[173] A modification of the reaction sequence was therefore developed, based on the well-known and high-yielding N-acetylation of glucosamine hydrochloride[174,175]: it was conceived that the conjugation necessary to append the N-linker could be performed after the hydrogenolysis of the O-benzyl group. Advantageously, a deprotection performed with hydrogen in the presence of palladium hydroxide on charcoal goes to completion and produces a pure product after a filtration, meaning that it can be used as-is for the subsequent step: a selective coupling between the carboxylic acid functionalized linker and the free amine at C-2 could then be performed, as

above. Removal of the anomeric O-benzyl group by hydrogenolysis of L-altro-configuration analogue **636** was therefore carried out in methanol with some acetic acid added as an additive to protonate the free amine, preventing the basic amine functionality from poisoning the catalyst to give **639** as a salt (**Scheme 6.11**). Furthermore, the added acid suppressed the formation of dimer **640** that could result from an inter- then intra-molecular condensation between the free amine and the aldehyde functionality at C-1 (**Scheme 6.11**). This dimer formation in known to occur under basic conditions, usually resulting in aromatized pyrazine derivatives.[176] Formation of the dimer would be detected by a shift of the anomeric proton doublets at 5.05-5.35 ppm to a more shielded position at 2-3 ppm, while splitting the signal into a doublet of doublets, which was not seen in the crude ^{1}H NMR.

The crude reaction mixture was directly subjected to an acylation in methanol with the crude anhydride **641** (1.25 equiv. per amine), formed by mixing 2-(2-acetylphenoxy)acetic acid **628** with DCC in dichloromethane (**Scheme 6.11**). It was expected that a selective functionalization of the 2-amine group on **639** should be achieved in the presence of free hydroxyl groups at C-1 and C-3 because of the enhanced nucleophilicity of amine functionality. The reaction did not go to completion according to TLC monitoring, likely due to some partial loss of the anhydride as the result of esterification with the methanol solvent. After a purification by column chromatography on silica gel, the desired product **642** was isolated in 45% yield. Despite the low yield, this preliminary exploration into the required linker-appendage chemistry exemplified the viability of our new scheme.



Reagents and conditions: (a) DCC, CH_2Cl_2; **(b)** H_2, $Pd(OH)_2/C$, AcOH, MeOH; **(c)** MeOH

Scheme 6.11 *- Altered reaction sequence to synthesize the o-acetic acid functionalized acetophenone – Alt-4-NAc conjugate* ***642****.*

The desired free-monosaccharide product **642** was isolated as an expected mixture of anomers. An analysis of the proton and ^1H-^1H COSY NMRs provided the necessary proof (**Figure 6.2**): for example, a new doublet at 8.46 ppm for the major anomer, and 8.23 ppm for the minor corresponding to the new amide on N2 appeared, in addition to the signals related to the linker in the right integration ratios, including 2 new methylene protons at 4.63-4.55, 3 new methyl protons at 2.74-2.59 and 4 new protons in the aromatic region. To improve the yield of the conjugation, in the future, a higher concentration and excess of the anhydride should be used to push the *N*-acylation to a completion.

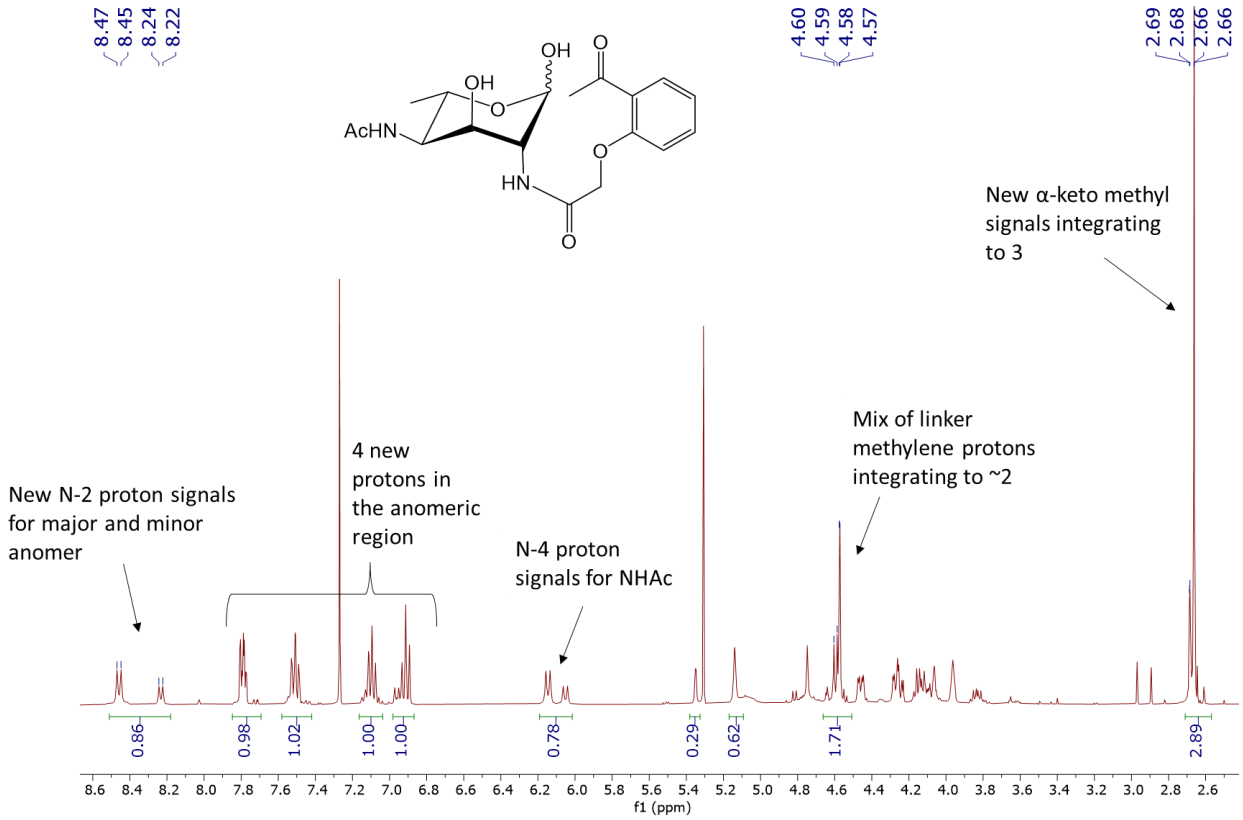


Figure 6.2 *– ^{1}H NMR of the isolated mixture of anomers resulting from product **636** synthesis.*

The preliminary success of our linker conjugation strategy is exciting, as it allows for the covalent attachment and preservation of an *ortho*-substituted acetophenone moiety to L-altro-configuration substrates that would otherwise be impossible due to functional group incompatibilities with the usual conditions required for the removal of the anomeric benzyl-ether. This route should moreover allow for the use of different L-altro-configuration precursors that bear a different acyl group at N4 without needing to revise the synthetic route further, as reagent **628** can chemo selectively be added to 2-NH_2 under mild conditions. Additionally, the work achieved earlier towards the N2,N4 differentiation advantageously allows for atom economy when coupled to this strategy, as the linker can selectively be added to one amine only: this also prevents any potential side-reactions during the three-carbon extension, had the linkers

been attached to both amines. The strategy outlined above was therefore chosen as the best one to take forward for the future synthesis of the intra-molecular three-carbon extension precursors. Unfortunately, due to time constraints, investigations toward the linker-induced asymmetric aldol extension were not achieved. With the above preliminary work, my thesis project concluded by opening the door to potentially interesting stereoselectivity studies for the problematic three-carbon extension reaction needed to produce NonAs chemically.

Chapter 7 Conclusions and Future Work

The work outlined in this thesis has provided significant contributions to the scientific community's important task to advance research into new antimicrobial strategies. Traditional antibiotics such as β-lactams, aminoglycosides and sulfonamides have been compromised by rapidly spreading antimicrobial resistance (AMR) mechanisms, and the major pharmaceutical companies have slowed down their investment into this field due to the recent gap in innovation when it comes to fighting bacterial infection in healthcare. Fortunately, the research continues in smaller settings, including academia, providing some hope for halting an almost certain imminent pandemic, if the current trend in AMR continues as is.

Complementing the widespread interest in developing novel antibacterial therapeutics is the chemical synthesis of monodisperse carbohydrate antigens, which can be applied towards investigating vaccines as a preventative strategy. Bacterial NonAs such as Pse and Leg are unique to certain pathogens, in addition to being important structural components that contribute to these pathogens' motility, immune system evasion and other virulence factors, and are perfect candidates for use as antigens in design of polysaccharide vaccines. Research has already produced some promising results with regards to immune responses to these *in vivo*. A further interesting and encouraging application of chemically synthesized NonAs comprises the studies of their biosynthetic pathways, which has already produced possibilities for metabolic labeling, and could even supply enzyme inhibitory strategies in the future.

The first successful part of my work provided a short, mild and scalable synthetic scheme towards 2,4-di-acetamido-2,4,6-trideoxy-L-altrose, the biosynthetic and often-used

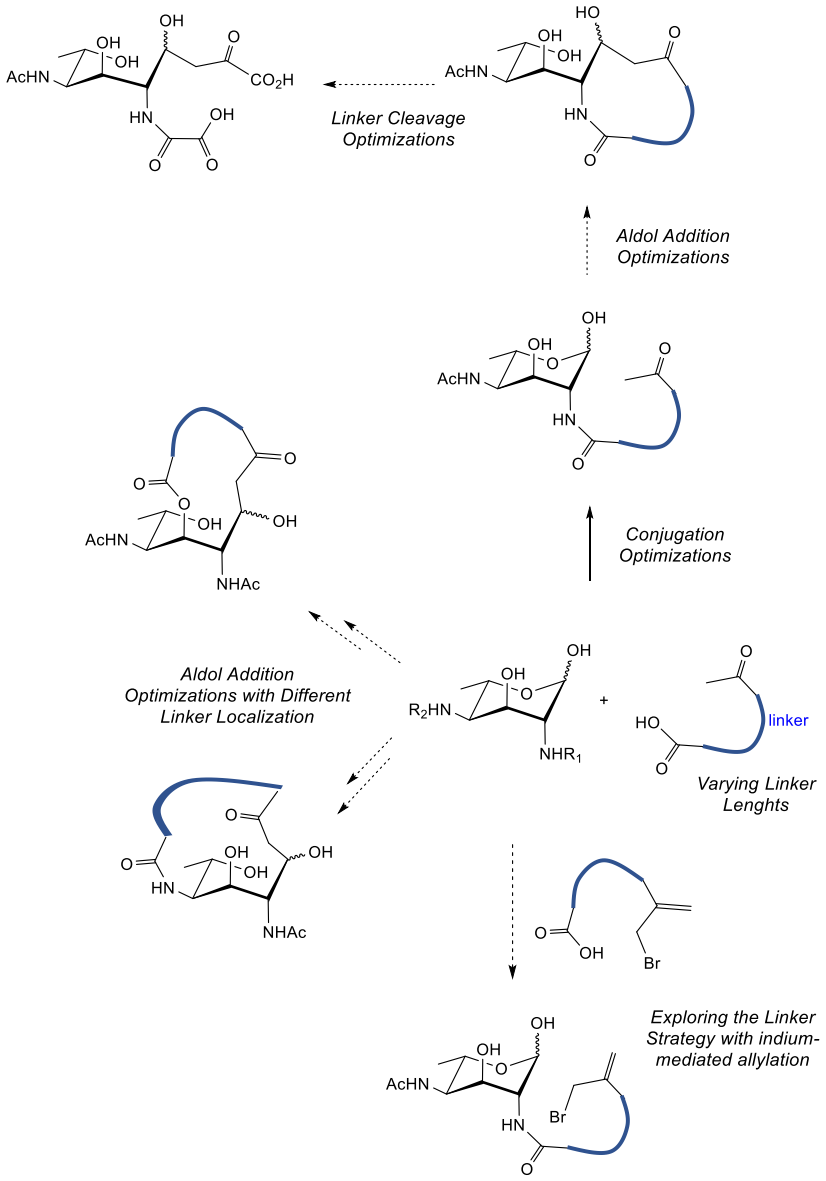
chemosynthetic precursor to Pse. The commercially available starting material for the synthesis, L-fucose, can accordingly be transformed into the desired product in 10 steps and 23% overall yield, making it the most efficient published synthesis of Alt-diNAc so far. The work was advanced later on by realizing an even shorter synthesis of the hexose, which built on our group's previous achievements with epoxide formation through regioselective sulfonyl activation: our latest synthesis of Alt-diNAc can be done in 7 steps, giving a 27% overall yield.

The second successful part of my research saw the development of a new systematic methodology for the differentiable functionalization of the N2/N4 amide groups of Alt-diNAc, which had not been achieved before with the versatility and efficiency that we provided. Our method relies on traceless Staudinger ligation-type chemistry that takes advantage of the *cis*-relationship between a hydroxy and an amino group on the hexose ring, allowing for a regiospecific O→N migration with a wide scope of acyl groups, unprecedently including one with a *tert*-butyl carbamate. The publication of this methodology, according to one of our reviewers for Organic Letters, provided "an important piece of chemistry that potentially overcomes one [of] the limitations in current approaches towards nonulosonic acids."

In addition to the published work, my research explored the possibility of obtaining up to 5 different NonA structures from L-arabinose, including Pse, Leg and Fus. A synthetic scheme was achieved that produced two C-5-(*R*)/(*S*) hexose diastereomers with the required deoxygenation at C-6, and the required configuration and protection pattern to allow for an inversion and installation of nitrogen functionalities on C-2 and C-4, before the final three-carbon extension with a PEP equivalent. In preliminary investigations, the diastereomers were produced either as racemates or with selectivity for the L-altro-configuration (the latter required for Pse synthesis),

depending on whether the addition of a methyl group to C-5 of an aldehyde intermediate was done through a Wittig addition/epoxidation/reduction series, or through a direct addition to the aldehyde with organometallic chemistry, respectively. Future work with this synthetic scheme is warranted because of the potential versatility with which select NonAs can be obtained from one starting material: it would include optimizing the selectivity for the D-galacto isomer (required for Leg) in the one-carbon extension to C-5 and optimization of the C-2 and C-4 inversion reaction with a nitrogen-based nucleophile.

Finally, it is quite evident that the whole of my work has nicely set-up the opportunity to study a new chemical methodology for a stereoselective three-carbon extension of hexose precursors to NonAs, including Alt-diNAcy and its derivatives differentiated at N2,N4. The most commonly used tactic in the literature to achieve this for Pse is through indium-mediated allylation, followed by ozonolysis, which usually produces very low diastereoselectivity. I have presented some preliminary explorations into a novel strategy using an intramolecular aldol addition that could potentially be directed by the configuration of the carbon center the aldol-adduct-containing linker is attached to. As such, a cleavable linker for the aldol reaction has been designed and synthesized, and a successful selective coupling of it to N4 of Alt-diNAcy was performed, paving the way to explore the linker strategy with high functional-group tolerance and compatibility. Future work in this interesting direction can involve optimizing reaction conditions for efficiency and stereoselectivity in the subsequent aldol addition, with variations to the linker position on the molecule (N2, O3 or even N4), addition of metal chelators to rigidify the linearized sugar undergoing the reaction, and varying linker lengths (**Scheme 7.1**).



Scheme 7.1 *– Possible future work towards stereoselective three-carbons extension with the linker strategy*

Once the addition is achieved, the linker cleavage step will also need optimizations, with the current plan involving a ruthenium-catalyzed oxidation of the phenol connector, which can be varied if needed. Furthermore, a linker design could also include the allyl bromide functionality required for indium-mediated allylation, which has the potential to proceed more diastereoselectively with an intra-molecular strategy.

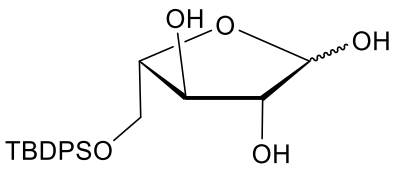
Experimental

General methods

All commercial reagents were used as supplied unless otherwise stated. Heating of the reaction vessels was performed using a paraffin oil bath. Thin layer chromatography was performed on Silica Gel 60-F254 (E. Merck, Darmstadt) with detection by fluorescence, charring with 5% H_2SO_4 (aq), or a ceric ammonium molybdate solution. Column chromatography was performed on Silica Gel 60 (Silicycle, Ontario) and solvent gradients given refer to stepped gradients and concentrations are reported as % v/v. Organic solutions were concentrated and/or evaporated to dry under vacuum in a water bath (<60 °C). Optical rotations were determined in a 5 cm cell at 20 ± 2 °C; $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg•cm^2/g. NMR spectra were recorded on Bruker spectrometers at 400 MHz, and the first-order chemical shifts of ^{1}H and ^{13}C (DEPT-Q) are reported in δ (ppm) and referenced to residual $CHCl_3$ (δ_H 7.24, δ_C 77.23, $CDCl_3$), residual CD_2HOD (δ_H 3.31, δ_C 49.1, CD_3OD), ^{1}H and ^{13}C NMR spectra were assigned with the assistance of 2D gCOSY and 2D gHSQC experiments. High-resolution ESI-QTOF mass spectra were recorded on an Agilent 6520 Accurate Mass Quadrupole Time-of-Flight LC/MS spectrometer. All the data were obtained with the assistance of the analytical services of the Department of Chemistry, University of Calgary.

Chapter 2 Procedures and Data

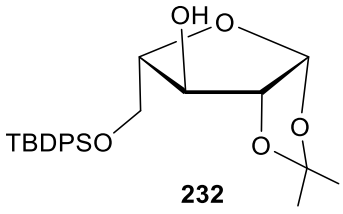
5-O-*tert*-Butyldiphenylsilyl-α/β-L-arabinofuranose (231)



231

L-arabinose **228** (8.00 g, 53.3 mmol, 1 equiv) was suspended in anhydrous dimethylformamide (120 mL) while heating to 100 °C, and once it was dissolved the solution was cooled to 55 °C. Imidazole (7.26 g, 107 mmol) and *tert*-butylchlorodiphenylsilane (14 mL, 53.3 mmol) were then added and the reaction was stirred at 55 °C for 24 hours. The solution was concentrated under reduced pressure to about half its volume, EtOAc (80 mL) was added and the organic solution was washed with water (3 x 50 mL) and the organic phase was then dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude solid was purified by column chromatography on silica gel using 70% EtOAc in hexanes as the eluent to afford the desired compound **231** (13.96 g, 72%) as an anomeric mixture. R_f = 0.16 (40% acetone in hexanes). The ^{1}H-NMR spectrum matched literature.[93]

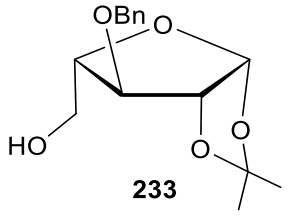
5-O-*tert*-Butyldiphenylsilyl-1,2-O-isopropylidene-β-L-arabinofuranose (232)



232

5-O-tert-Butyldiphenylsilyl-α/β-L-arabinofuranose **231** (4.80 g, 12.4 mmol) was dissolved in acetone (48.5 mL), then copper sulfate (9.25 g, 58.0 mmol) and 10-camphorsulfonic acid (0.292 g, 1.26 mmol) were added and the reaction mixture was stirred overnight at room temperature. Solid $NaHCO_3$ (2.12 g, 25.2 mmol) was added to neutralize the mixture. After stirring for 20 minutes, the mixture was filtered off and the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using a gradient of EtOAc - hexanes (2.5%→ 5% →10%) to obtain the desired compound **232** in pure form (4.04 g, 82% yield). R_f = 0.58 (40% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.10 – 7.54 (m, 4H, Ar-H), 7.52 – 7.35 (m, 6H, Ar-H), 5.92 (d, *J* = 4.0 Hz, 1H, H-1), 4.56 (d, *J* = 4.1 Hz, 1H, H-2), 4.45 (br s, 1H, H-3), 4.19 – 4.07 (m, 1H, H-4), 3.96 – 3.80 (m, 2H, H-5a, H-5b), 1.36 (s, 1H, CH_3), 1.31 (s, 3H, CH_3), 1.12 (s, 9H, t-But). The ^{1}H-NMR spectra matched the literature.[93]

3-O-Benzyl-1,2-O-isopropylidene-β-L-arabinofuranose (233)

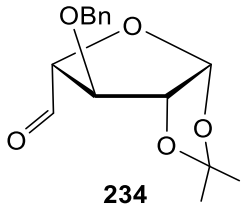


233

5-O-tert-Butyldiphenylsilyl-1,2-O-isopropylidene-β-L-arabinofuranose **232** (45.9 g, 0.107 mol) was dissolved in anhydrous dimethylformamide (500 mL), then NaH (8.56 g, 0.214 mol) was added and the mixture was cooled to 0 °C. Benzyl bromide (19.1 mL, 0.161 mol) was carefully added, and the mixture was left to stir to room temperature overnight. NaOMe (11.6 g, 0.215

mol) was added and the mixture was stirred for 5 hours. The solution was concentrated under reduced pressure, and the mixture was extracted with EtOAc (500 mL), and washed with water (200 mL) and brine (2 x 200 mL). The organic solution was dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using 20% EtOAc - hexanes as the eluent to compound **233** as an oil (16.5 g, 0.0621 mol, 58% yield). R_f = 0.38 (40% EtOAc - hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 – 7.27 (m, 5H, ArH), 5.93 (d, *J* = 4.1 Hz, 1H, H-1), 4.70 (dd, *J* = 4.2, 1.1 Hz, 1H, H-2), 4.66 (d, *J* = 11.7 Hz, 1H, CH_aH$_b$Ph), 4.58 (d, *J* = 11.7 Hz, 1H, CH$_a$$H_b$Ph), 4.21 (m, 1H, H-4), 3.99 (dd, *J* = 3.4, 1.1 Hz, 1H, H-3), 3.75 (m, 2H, H-5a, H-5b), 2.07 (t, *J* = 6.4 Hz, 1H, O-H), 1.54 (s, 3H, CH_3), 1.36 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 128.6 (ArC), 128.1 (ArC), 127.8 (ArC), 105.6 (C-1), 100.0 (*C*$(CH_3)_2$), 85.6 (C-4), 85.3 (C-2), 82.8 (C-3), 71.9 (*C*H_2Ph), 62.7 (C-5), 27.2 (CH_3), 26.4 (CH_3). The recorded ^{11}H NMR and ^{13}C NMR spectra matched the literature.[177]

3-O-Benzyl-1,2-O-isopropylidene-β-L-arabino-dialdo-1,4-furanose (234)

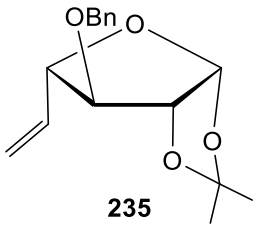


234

Oxalyl chloride (0.97 mL, 3.8 mmol) was added to anhydrous CH_2Cl_2 (14.0 mL) and the solution was cooled to -78 °C while stirring; dimethyl sulfoxide (16.0 mL, 226 mmol) was added, which resulted in the release of gas. After stirring the solution at -78°C for 30 minutes, a solution of starting material **233** (1.00 g, 3.76 mmol) in dry CH_2Cl_2 (9.0 mL) was added, and the reaction

was stirred for another 30 minutes at the same temperature. Triethyl amine (4.7 mL, 34 mmol) was added, and the reaction turned cloudy. The reaction was allowed to warm up to room temperature over 4 hours. Thin layer chromatography (20% EtOAc in hexanes) revealed the consumption of starting material. The reaction was quenched with water, and the mixture was concentrated under reduced pressure. The mixture was then extracted with 90% EtOAc in hexanes (20 mL), washed with aqueous HCl (2.0 M, 10 mL), saturated aqueous $NaHCO_3$ (10 mL) and finally brine (2 x 10 mL), dried over Na_2SO_4 and evaporated followed by several co-evaporations with with toluene (3 x 10 mL). The crude yellow oil was used directly without further purification. ^{1}H NMR spectrum showed the crude product **234** has >95% purity. R_f = 0.40 (long smear, 40% EtOAc in hexanes). ^{1}H NMR spectrum is in agreement with the literature.[178]

3-*O*-Benzyl-5,6-dideoxy-1,2-O-isopropylidene-α-L-arabino-hex-5-enofuranose (235)

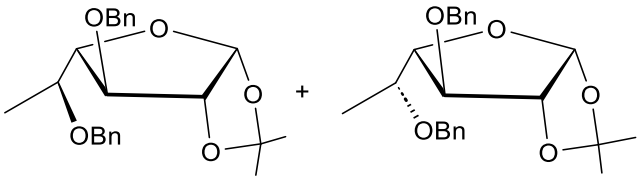


235

Methyltriphenylphosphonium bromide (7.69 g, 21.5 mmol) was suspended in anhydrous THF (30.0 mL) at 0 °C; KOBu-*t* (2.42 g, 21.5 mmol) was added to afford a bright yellow mixture. After stirring for 45 minutes, a solution of the crude aldehyde **234** (1.71 g, 6.15 mmol) in anhydrous THF (6.0 mL) was added and the reaction mixture was left stirring at room temperature overnight. A solution of saturated NH_4Cl (20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (100 mL). The organic solution was washed with aqueous

HCl (2 M, 10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and evaporated. The crude mixture was purified by column chromatography on silica gel using 5% EtOAc in hexanes as the eluent. Compound **235** (0.787 g, 46% yield) was isolated with a small amount of impurity. R_f = 0.76 (20% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 – 7.30 (m, 5H, ArH), 7.29 – 7.20 (m, impurity), 6.95 (m, impurity), 6.92 – 6.81 (m, impurity), 6.03 (ddd, *J* = 17.2, 10.4, 6.8 Hz, 1H, H-5), 5.95 (d, *J* = 4.0 Hz, 1H, H-1), 5.35 (ddd, *J* = 17.2, 1.4, <1 Hz, 1H, H-6a), 5.20 (ddd, *J* = 10.4, 1.4, <1 Hz, 1H, H-6b), 4.74 – 4.65 (m, 2H, CH_aH$_b$Ph, H-2), 4.61 (d, *J* = 11.8 Hz, 1H, CH$_a$$H_b$Ph), 4.55 (dddd, *J* = 6.9, 3.0, 1.4, <1 Hz, 1H, H-4), 3.95 (dd, *J* = 3.3, 1.1 Hz, 1H, H-3), 1.55 (s, 3H, CH_3), 1.37 (s, 3H, CH_3).

3,5-Di-O-benzyl-6-deoxy-1,2-O-isopropylidene-β-L-altrofuranose (238) and 3,5-di-O-benzyl-6-deoxy-1,2-O-isopropylidene-α-D-galactofuranose (239)



L-altro **238** D-galacto **239**

Method 1

A solution of 3-O-benzyl-5,6-dideoxy-1,2-O-isopropylidene-α-L-arabino-hex-5-enofuranose (**235**, 787 mg, 2.85 mmol) in anhydrous CH_2Cl_2 (30.0 mL) was cooled to 0 °C under an atmosphere of argon; MCPBA (2.11 g, 8.55 mmol) was added, and the reaction was stirred overnight at room temperature. Monitoring by TLC revealed the disappearance of the starting material, and the appearance of two new compounds (R_f = 0.38 and 0.44, 20% ethanol in

hexanes). The reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL), and the organic phase was washed with brine (20 mL) and water (20 mL), dried over anhydrous Na_2SO_4, and evaporated to afford the crude mixture **236** (1.22 g, 4.17 mmol) which was used directly for the next reaction without separating the two isomers.

The above crude mixture (ca ~ 2.85 mmol) was dissolved in anhydrous toluene (10.0 mL) and the solution was cooled to -78°C. a solution of DIBAL-H in toluene (1.5 M, 1.9 mL, 4.3 mmol) was added; the reaction was slowly warmed to room temperature over 2 hours. TLC revealed the formation of two isomer products **237a** and **237b** (R_f = 0.18 and 0.22, 20% EtOAc in hexanes). After stirring overnight, more DIBAL-H in toluene (1.5 M solution, 1.9 mL, 4.3 mmol) was added. After two hours, the reaction was quenched with methanol (10 mL). The solution was concentrated and the residue was suspended in EtOAc (25 mL), washed with aqueous HCl (2 M, 25 mL), saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), and dried over sodium sulfate and evaporated.

The crude product **237** (ca ~2.85 mmol) was dissolved in anhydrous DMF under argon atmosphere; NaH (60% in mineral oil, 342 mg, 8.55 mmol) was added, and the solution was cooled to 0°C followed by the addition of benzyl bromide (0.51 mL, 4.28 mmol) dropwise. After stirring the reaction for 48 hours, more sodium hydride (60% in mineral oil, 100 mg, 2.50 mmol) and benzyl bromine (0.15 mL, 1.3 mmol) were added to push the reaction to completion. After an additional 48 hours, the reaction was quenched with methanol (10 mL). The mixture was evaporated under reduced pressure and the residue was dissolved in EtOAc (20 mL); the organic solution was washed with brine (2 x 20 mL), dried over anhydrous Na_2SO_4, and evaporated. The mixture, containing two products by TLC (R_f = 0.31 and 0.42, 10% EtOAc in hexanes), was carefully

purified by column chromatography on silica gel using 2% EtOAc in hexanes to afford first compound **238** (290 mg, 0.754 mmol, 27% yield for 3 steps) and compound **239** (283 mg, 0.740 mmol, 26% yield for 3 steps).

Method 2

Crude 3-O-benzyl-1,2-O-isopropylidene-β-L-arabino-dialdo-1,4-furanose **234** (ca ~58 mg, 0.21 mmol) was dissolved in anhydrous diethyl ether (1.0 mL) under argon atmosphere, and the solution was cooled to -78°C. A solution of MeMgBr in diethyl ether (3.0 M solution, 0.14 mL, 0.42 mmol) was added, and the reaction mixture was stirred for 2 hours. Monitoring by TLC showed the appearance of two new spots. The reaction was quenched with a sat. solution of NH_4Cl (1.0 mL). EtOAc (5 mL) was added to extract the product and the organic solution was washed with brine (2 x 5 mL), dried over Na_2SO_4 and evaporated. The crude residue was redissolved in anhydrous DMF (2.0 mL) under argon atmosphere at 0 °C; to the solution, was added NaH (60% in mineral oil, 21 mg, 0.52 mmol) and benzyl bromide (65 µl, 0.52 mmol). Stirring was continued for 24 hours at room temperature. TLC revealed the formation of two products as above with a 9:1 ratio (**238** : **239**). No further purification steps were taken.

A similar reaction was repeated with the same amounts in anhydrous THF (1.0 mL) without the O-benzylation step. TLC revealed similar results.

Method 3

Crude 3-O-benzyl-1,2-O-isopropylidene-β-L-arabino-dialdo-1,4-furanose **234** (100 mg, 0.359 mmol) was dissolved in anhydrous diethyl ether (1.0 mL) under an atmosphere of argon at -78 °C; a solution of MeLi-LiBr complex in diethyl ether (1.5 M solution, 0.48 mL, 0.72 mmol) was

added. After stirring for 2 hours, TLC shows the reaction is complete. The reaction was warmed up and quenched with saturated solution of NH_4Cl (1 mL), and the solution was extracted with EtOAc (5 mL). The organic phase was washed with brine (2 x 5 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. TLC showed the formation of two products with a similar ratio as above.

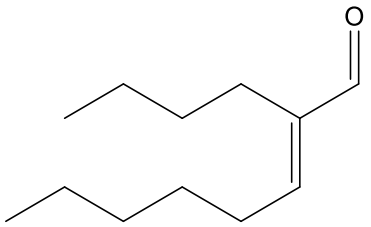
Another reaction was repeated with the same amounts of aldehyde and MeLi-LiBr complex in diethyl ether in tetrahydrofuran (1.0 mL) as the solvent to afford similar results.

Data for Compound **238**: R_f = 0.42 (10% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38 – 7.24 (m, 10H, ArH), 5.91 (d, *J* = 4.0 Hz, 1H, H-1), 4.72 – 4.59 (m, 3H, H-2, C3-OCH_aH$_b$Ph, C5-OCH_aH$_b$Ph), 4.56 (d, *J* = 12.0 Hz, 1H, C3-OCH$_a$$H_b$Ph), 4.45 (d, *J* = 11.6 Hz, 1H, C5-OCH$_a$$H_b$Ph), 4.15 (d, *J* = 2.1 Hz, 1H, H-3), 3.96 (dd, *J* = 8.6, 2.1 Hz, 1H, H-4), 3.72 (dq, *J* = 8.5, 6.2 Hz, 1H, H-5), 1.42 (s, 3H, CH_3), 1.33 – 1.27 (m, 6H, H-6, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 137.5 (ArC), 128.4 (ArC), 128.3 (ArC), 127.82 (ArC), 127.76 (ArC), 127.6 (ArC), 105.9 (C-1), 88.2 (C-4), 85.0 (C-2), 82.7 (C-3), 74.2 (C-5), 71.3 (C3-O*C*H$_2$Ph), 70.8 (C5-O*C*H$_2$Ph), 26.9 (CH_3), 26.1 (CH_3), 16.2 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{23}H_{32}NO_5$ $[M+NH_4]^+$: 402.2275; found: 402.2278.

Data for compound **239**: R_f = 0.31 (10% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.39 – 7.24 (m, 10H, ArH), 5.88 (d, *J* = 4.2 Hz, 1H, H-1), 4.71 – 4.62 (m, 3H, H-2 H-2, C3-OCH_aH$_b$Ph, C5- OCH_aH$_b$Ph), 4.60 (d, *J* = 12.0 Hz, 1H, C3-OCH$_a$$H_b$Ph), 4.48 (d, *J* = 11.6 Hz, 1H, C5-OCH$_a$$H_b$Ph), 3.93 (m, 2H, H-3, H-4), 3.71 (high order dq, 1H, H-5), 1.50 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.21 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 138.7 (ArC), 137.0 (ArC), 128.5 (ArC), 128.2 (ArC), 128.0 (ArC), 127.9 (ArC), 127.2 (ArC), 105.0 (C-1), 87.0 (C-3 or C-4), 85.7 (C-2), 83.0 (C-3 or C-4),

74.1 (C-5), 71.9 (C3-O*C*H_2Ph), 71.5 (C5-O*C*H_2Ph), 27.4 (CH_3), 26.9 (CH_3), 16.5 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{23}H_{32}NO_5$ $[M+NH_4]^+$: 402.2275; found: 402.2283.

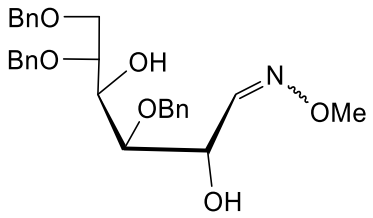
(*E*)-2-Butyl-2-octenal (240)



240

DMSO (3.0 mL) was combined with toluene (10.0 mL) and the mixture was concentrated under reduced pressure to approximately half the DMSO remained. Anhydrous THF (2.0 mL) was then added, and the solution was cooled to 0 °C. Purified TMSI (153 mg, 0.750 mmol, recrystallized from water and thoroughly dried in a desiccator overnight) was added and the mixture continued to be stirred for 30 minutes while warming to room temperature. Hexanal (0.60 mL, 0.50 mmol) was added to the dark orange mixture, and stirring was continued until the hexanal spot disappeared by TLC. After 2.5 hours, the mixture was concentrated under reduced pressure. The crude mixture was extracted with EtOAc (10 mL), washed with brine (2 x 10 mL) and water (10 mL), dried over anhydrous Na_2SO_4, and evaporated. The obtained syrup was purified by column chromatography on silica gel using 1% EtOAc in hexanes as the eluent to afford a yellow oil (39 mg, 0.19 mmol, 76% yield). The 1H NMR spectrum of isolated product **240** is in agreement with previously reported data for the (*E*)-isomer.[179] R_f = 0.69 (10% EtOAc in hexanes).

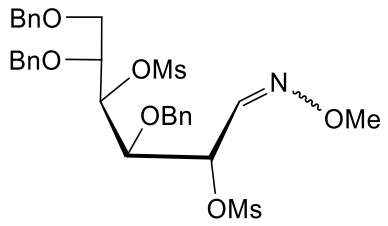
3,5,6-Tri-O-benzyl-D-glucose methyloxime (242)



242

A solution of 3,4,6-tri-O-benzyl-α/β-D-glucofuranose **241** (3.01 g, 6.08 mmol) in anhydrous pyridine (30.0 mL) was heated to 70 °C under an atmosphere of argon; $NH_2OMe•HCl$ (0.67 g, 8.0 mmol) was added. The golden yellow-colored mixture was stirred overnight to afford a deep brown coloured solution. Reaction progress was monitored via TLC using 20% EtOAc in hexanes: two new product spots had formed (R_f = 0.25 and 0.33) along with some starting material (R_f = 0.05). The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using 5% EtOAc in hexanes as the eluent to afford compound **242** as a mixture of cis and trans isomers (2.42 g, 5.05 mmol, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 4.6 Hz, 1H, H-1 major isomer), 7.42 – 7.21 (m, 18H, ArH both isomers), 6.89 (d, *J* = 5.0 Hz, 0.3H, H-1 minor isomer), 4.96 (dd, *J* = 5.0, 3.3 Hz, 0.3H), 4.77 – 4.61 (m, 3H, benzylic protons both isomers), 4.59 (d, *J* = 2.0 Hz, 3H), 4.55 – 4.37 (m, 4H, benzylic protons), 4.13 (dd, *J* = 3.3, 2.1 Hz, 0.3H), 4.01 – 3.90 (m, 2H), 3.89 (s, 1H, OMe minor isomer), 3.87 (s, 3H, OMe major isomer), 3.79 – 3.64 (m, 2H).

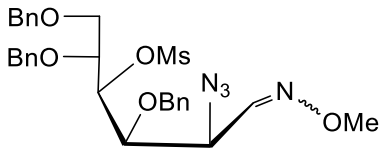
3,5,6-Tri-O-benzyl-2,4-di-O-methanesulfonyl-D-glucose methyloxime (243)



243

A solution of 3,5,6-tri-O-benzyl-D-glucose methyloxime **242** (1.37 g, 2.86 mmol) in anhydrous pyridine (15.0 mL) was purged with an atmosphere of argon, then mesyl chloride (1.32 mL, 17.1 mmol) was added, and the reaction was stirred at room temperature overnight. TLC monitoring revealed a product spot (R_f = 0.51, 40% EtOAc in hexanes) was formed. The reaction was quenched with methanol, and the reaction mixture was concentrated under reduced pressure. The residue was redissolved in EtOAc, and the organic solution was washed with aqueous HCl (2.0 M, 10 mL), saturated aq. $NaHCO_3$ (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and evaporated to afford the crude product **243** without further purification (1.87 g, quant.). ^{1}H NMR revealed the presence of 2 new O-mesyl groups at 3.01 and 2.98 ppm; the OMe groups were also observed at 3.85 ppm (major isomer) and 3.75 ppm (minor isomer).

2-Azido-3,5,6-tri-O-benzyl-4-O-methanesulfonyl-D-mannose methyloxime (**244**)

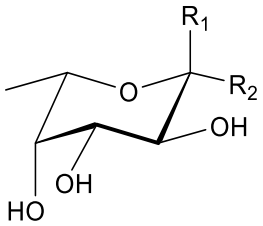


244

A solution of crude 3,5,6-tri-O-benzyl-2,4-O-methanesulfonyl-D-glucose methyloxime **243** (0.145 mg, 0.267 mmol) and NaN_3 (87 mg, 1.34 mmol) in anyhydrous DMF (4.0 mL) was stirred under un atmosphere of argon overnight. TLC monitoring revealed the formation of a new product spot (R_f =0.47, 40% EtOAc in hexanes). The reaction was quenched with water, and the product was extracted with EtOAc (20 mL); the organic solution was washed with HCl (2 M, 5 mL), sat. $NaHCO_3$ (5 mL) and brine (5 mL), dried over with Na_2SO_4 and concentrated. Select ^{1}H NMR data for the major isomer (**244**): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 (d, *J* = 7.8 Hz, 1H, H-1), 5.02 (dd, *J* = 6.8, 3.4 Hz, 1H, H-4), 4.30 (dd, *J* = 7.9, 4.3 Hz, 1H, H-2), 3.98 (dd, *J* = 6.8, 4.3 Hz, 1H, H-3), 3.86 (s, 3H, OMe), 2.96 (s, 3H, OMs).

Chapter 3 Procedures and Data

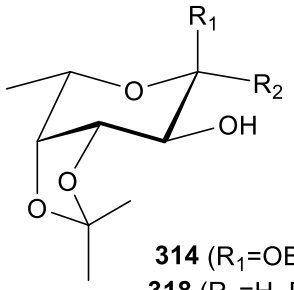
Benzyl α-L-fucopyranoside (312) and benzyl β-L-fucopyranoside (313)



312 (R_1=OBn, R_2=H)
313 (R_1=H, R_2=OBn)

Acetyl chloride (~5.0 ml) was added to benzyl alcohol (150 ml) at 0 °C and the solution was stirred for 10 minutes. L-fucose **304** (30.0 g, 183 mmol) was then added into the solution, and the mixture was heated to 90 °C for 24 hours. Cyclohexanes (100 ml) were added to the solution, and the solution was distilled off to remove the water azeotrope under reduced pressure at 90°C. The solution was then poured into hexanes (3.5 L) with vigorous stirring to afford precipitate. Vacuum filtration afforded an off-white powder which consisted of a mixture of α/β-anomers (**312**/**313**: 95/5; 30.21 g, 65% yield). Some pure α-anomer was isolated by recrystallization of the crude product mixture (~20 g) in 5% ethyl acetate in hexanes (400 ml) to afford a white powder. Characterization of major anomer **312**: R_f = 0.47 (10% MeOH - CH_2Cl_2). ^{1}H NMR (400 MHz, CD_3OD): δ_H 7.44 – 7.26 (m, 5H, ArH), 4.87 (d, 1H, *J* = 3.3 Hz, H-1), 4.71 (d, 1H, *J* = 12.0 Hz, C*H*$_a$H$_b$Ph), 4.58 (d, 1H, *J* = 12.0 Hz, CH$_a$*H*$_b$Ph), 3.97 (dq, 1H, *J* = 6.6, 0.7 Hz, H-5), 3.80 (dd, 1H, *J* = 10.1, 3.0 Hz, H-3), 3.76 (dd, 1H, *J* = 10.1, 3.4 Hz, H-2), 3.68 (m, 1H, H-4), 1.20 (d, 3H, *J* = 6.6 Hz, H-6). ^{13}C NMR data are in agreement with previously reported.

Benzyl 3,4-O-isopropylidene-α-L-fucopyranoside (**314**) and benzyl 3,4-O-isopropylidene-β-L-fucopyranoside (**318**).



314 (R_1=OBn, R_2=H)
318 (R_1=H, R_2=OBn)

Method 1

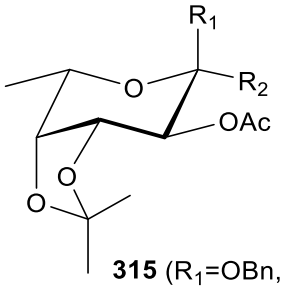
To a mixture of benzyl α-L-fucopyranoside **312** (5.27 g, 20.7 mmol) in acetone (30 mL), was added 2,2-dimethoxypropane (10.0 mL), and 10-camphorsulfonic acid (CSA, 120 mg), and the mixture was heated to reflux for 0.5 h. The mixture was then neutralized with triethylamine (~2 mL) and concentrated under reduced pressure to afford a yellow oil. The mixture was dissolved in a 10:1 mixture of methanol and water (220 ml) and heated to reflux for one hour to hydrolyze any potentially formed hemi-acetal at O2, then concentrated under reduced pressure. The mixture was dissolved in ethyl acetate (50 mL), and the obtained organic solution was washed successively with brine (50 ml), 2 N HCl (50 ml) and saturated aqueous $NaHCO_3$ (50 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel using a gradient of 5 → 10% ethyl acetate in hexanes as an eluent, to yield the desired compound (**314**) as a colourless syrup (5.70 g, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.41 – 7.30 (m, 5H, ArH), 4.95 (d, 1H, *J* = 3.9 Hz, H-1), 4.80 (d, 1H, *J* = 11.8 Hz, CH_aH_bPh), 4.59 (d, 1H, *J* = 11.8 Hz, CH_aH_bPh), 4.24 (t, 1H, *J* = 6.2 Hz, H-3), 4.17 (dq, 1H, *J* = 6.6 Hz, *J* = 2.3 Hz, H-5), 4.07 (dd, 1H, *J* = 6.1 Hz, *J* = 2.3 Hz, H-4), 3.84 (dt, 1H, *J* = 6.7 Hz, *J*

= 4.0 Hz, H-2), 2.28 (d, 1H, *J* = 6.9 Hz, OH), 1.53 (s, 3H, Me_ISP), 1.37 (s, 3H, Me_ISP), 1.32 (d, 3H, *J* = 6.6 Hz, H-6). ^{13}C NMR data are in agreement with previously reported.[114]

Method 2

To a mixture of the crude precipitate that contained the benzyl α/β-L-fucopyranosides (**312/313**, 18.2 g, 62.6 mmol) in acetone (50.0 ml) and 2,2-dimethoxypropane (50 ml), was added CSA (200 mg), and the solution was heated to reflux for 24 hours. The mixture was then neutralized with triethylamine, and solution was concentrated under reduced pressure. The yellow syrup was purified by column chromatography on silica gel using a gradient of 5→10% ethyl acetate - hexanes as an eluent to yield the pure β-fucopyranoside **318** (3.89 g, 20% yield) and also the α-fucopyranoside **314** (7.17 g, 37% yield), as a colourless syrups. Data for compound **318**: R_f = 0.39 (40% ethyl acetate - hexanes). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.41 – 7.23 (m, 5H, ArH), 4.93 (d, *J* = 11.6 Hz, 1H, C*H*$_a$H$_b$Ph), 4.58 (d, *J* = 11.7 Hz, 1H, CH$_a$*H*$_b$Ph), 4.22 (d, *J* = 8.3 Hz, 1H, H-1), 4.01 (dd, *J* = 7.2, 5.5 Hz, 1H, H-3), 3.97 (dd, *J* = 5.5, 2.2 Hz, 1H, H-4), 3.82 (qd, *J* = 6.6, 2.2 Hz, 1H, H-5), 3.58 (ddd, *J* = 8.2, 7.2, 2.5 Hz, 1H, H-2), 2.80 (d, *J* = 2.6 Hz, 1H, OH-2), 1.53 (s, 3H, Me_ISP), 1.44 (d, *J* = 6.6 Hz, 3H, H-6), 1.35 (s, 3H, Me_ISP). ^{13}C NMR data are in agreement with previously reported.

Benzyl 2-O-acetyl-3,4-O-isopropylidene-α-L-fucopyranoside (315) and benzyl 2-O-acetyl-3,4-O-isopropylidene-β-L-fucopyranoside (319).

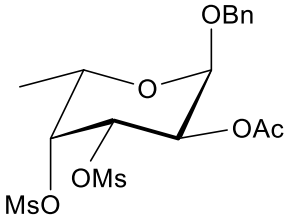


315 (R_1=OBn, R_2=H)
319 (R_1=H, R_2=OBn)

To a solution of L-fucose **304** (11.0 g, 67.0 mmol) in benzyl alcohol (22 mL), was added p-toluenesulfonic acid (0.5 g), and the mixture was stirred and heated to 130 °C for 30 min. Toluene (10 mL) was added and the reaction flask was connected to a Dean-stark. The temperature was raised to 140 °C to allow azeotropic removal of toluene – water mixture. More toluene (3 × 10 mL) was repeatedly added to reaction mixture, and each time the toluene – water azeotrope was removed. The reaction was cooled to room temperature. To the above reaction mixture, was added 2,2-dimethoxypropane (25 mL, 204 mmol), and the reaction was continued overnight. The solution was concentrated under reduced pressure. Anhydrous pyridine (80 mL) was added to the reaction followed by acetic anhydride (80 mL), and the mixture was heated to 70 °C for 4 hours. The reaction solution was then concentrated under reduced pressure, and co-evaporated with toluene (2 × 100 mL). The mixture was diluted with ethyl acetate (~300 mL), and the organic solution was washed successively with a solution of aqueous 2 N HCl (1 × 100 mL), saturated aqueous $NaHCO_3$ (1 × 100 mL), 10% brine (1 × 100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The mixture was purified by column chromatography on silica gel using a gradient of ethyl acetate – hexanes (0 → 12.5%) to afford the desired α-anomer

315 (10.92 g, 48% over 3 steps), and β-anomer **319** (4.54 g, 20% over 3 steps). Both products can also be recrystallized from hexanes. Data for compound **315**: R_f = 0.40 (20% EtOAc in hexanes). $[\alpha]_D^{25}$ -155.5 (*c* 1.2, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.41 – 7.25 (m, 5H, ArH), 5.00 (d, *J* = 3.6 Hz, 1H, H-1), 4.92 (dd, *J* = 8.2, 3.6 Hz, 1H, H-2), 4.72 (d, *J* = 12.3 Hz, 1H, C*H*$_a$H$_b$Ph), 4.53 (d, *J* = 12.3 Hz, 1H, CH$_a$*H*$_b$Ph), 4.37 (dd, *J* = 8.3, 5.3 Hz, 1H, H-3), 4.18 (dq, *J* = 6.7, 2.6 Hz, 1H, H-5), 4.10 (dd, *J* = 5.4, 2.6 Hz, 1H, H-4), 2.10 (s, 3H, Ac), 1.54 (s, 3H, Me_ISP), 1.37 (d, *J* = 6.7 Hz, 3H, H-6), 1.36 (s, 3H, Me_ISP). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.5 (Ac), 137.3 (ArC), 128.4 (ArC) , 127.9 (ArC), 127.6 (ArC), 109.3 (*C*(CH$_3$)$_2$), 95.4 (C-1), 76.13 (C-4), 73.38 (C-3), 71.94 (C-2), 69.6 (O*C*H$_a$H$_b$Ph), 63.4 (C-5), 26.39 (CH$_3$–ISP), 20.97 (Ac), 16.25 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{18}H_{24}O_6$ [M+Na]$^+$: 359.1465; found: 359.1452. Data for compound **319**[112]**:** R_f = 0.20 (20% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.42 – 7.24 (m, 5H, ArH), 5.06 (dd, *J* = 8.3, 7.6 Hz, 1H, H-2), 4.91 (d, *J* = 12.5 Hz, 1H, C*H*$_a$H$_b$Ph), 4.62 (d, *J* = 12.5 Hz, 1H, CH$_a$*H*$_b$Ph), 4.37 (d, *J* = 8.4 Hz, 1H, H-1), 4.13 (dd, *J* = 7.6, 5.3 Hz, 1H, H-3), 4.03 (dd, *J* = 5.3, 2.1 Hz, 1H, H-4), 3.86 (dq, *J* = 6.6, 2.2 Hz, 1H, H-5), 2.09 (s, 3H, Ac), 1.60 (s, 3H, Me_ISP), 1.47 (d, *J* = 6.6 Hz, 3H, H-6), 1.37 (s, 3H, Me_ISP).

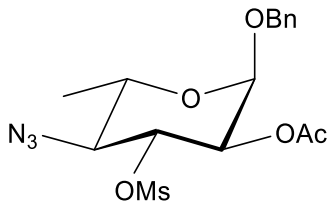
Benzyl 2-O-acetyl-3,4-di-O-methanesulfonyl-α-L-fucopyranoside (316).



316

A solution of compound **315** (17.2 g, 51.2 mmol) in 85% acetic acid - water (330 mL) was heated to 80 °C. After 2 hours, the solution was concentrated and co-evaporated with toluene (3 × 150 mL) to give an off-white solid (15.1 g, ~100% yield). The hydrolyzed diol was dissolved in anhydrous dichloromethane (100 mL) and pyridine (50 mL) and was cooled to 0 °C; mesyl chloride (20.0 mL, 0.26 mol) was added, and the reaction was allowed to warm up to room temperature. After stirring for 20 hours, water (20 mL) was added to quench the reaction, and the mixture was diluted with ethyl acetate (200 mL), and washed with 2 N HCl (2 × 100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using 25% ethyl acetate - hexanes as an eluent to afford compound **316** as a white solid (20.7 g, 90% yield). R_f = 0.23 (40% EtOAc - hexanes). $[\alpha]_D^{25}$ -119.3 (*c* 0.63, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.40 – 7.28 (m, 5H, ArH), 5.17 – 5.07 (m, 3H, H-1, H-2, H-3), 5.04 (dd, *J* = 1.2, 3.2 Hz, 1H, H-4), 4.70 (d, 1H, *J* = 12.2 Hz, C*H*aHbPh), 4.57 (d, 1H, *J* = 12.2 Hz, CHa*H*bPh), 4.16 (dq, 1H, *J* = 6.5, 0.8 Hz, H-5), 3.19 (s, 3H, Ms), 3.12 (s, 3H, Ms), 2.09 (s, 3H, OAc), 1.29 (d, 3H, *J* = 6.5 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 169.7 (Ac), 136.6 (ArC), 128.6 (ArC), 128.2 (ArC), 128.0 (ArC), 95.3 (C-1), 80.6 (C-4), 74.4 (C-3), 70.3 (*C*H_2Ph), 67.7 (C-2), 64.8 (C-5), 39.0 (Ms), 38.6 (Ms), 20.7 (Ac), 16.3 (C-6). HRMS (ESI-QTOF) calc'd m/z for $C_{17}H_{24}O_{10}S_2$ $[M+Na]^+$: 475.0709; found: 475.0703.

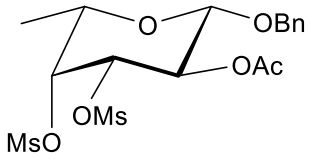
Benzyl 2-O-acetyl-4-azido-4,6-dideoxy-3-O-mesyl-α-L-glucopyranoside (317).



317

To a mixture of 3,4-di-O-mesylate **316** (20.7 g, 45.8 mmol) in anhydrous DMF (200 mL), was added NaN_3 (15.5 g, 0.240 mol), and the mixture was heated to 90 °C for 40 hours. The temperature was raised to 100 °C for additional 4 hours. The mixture was concentrated under reduced pressure, and the residue was partitioned between 10% brine (400 mL) and ethyl acetate (300 mL); the aqueous layer was separated and re-extracted with more EtOAc (200 mL). The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using 5% ethyl acetate – hexanes as an eluent to afford compound **317** as a white solid (17.9 g, 98% yield). R_f = 0.53 (40% EtOAc - hexanes). $[\alpha]_D^{25}$ -172.0 (*c* 4.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.61 – 7.37 (m, 5H, ArH), 5.16 (d, *J* = 3.6 Hz, 1H, H-1), 5.14 (dd, *J* = 9.8, 9.8 Hz, 1H, H-3), 5.03 (dd, *J* = 10.0, 3.7 Hz, 1H, H-2), 4.83 (d, *J* = 12.4 Hz, 1H, C*H*aHbPh), 4.67 (d, *J* = 12.4 Hz, 1H, CHa*H*bPh), 3.90 (dq, *J* = 9.9, 6.2 Hz, 1H, H-5), 3.37 (dd, *J* = 9.8, 9.8 Hz, 1H, H-4), 3.26 (s, 3H, Ms), 2.24 (s, 3H, Ac), 1.48 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.2 (Ac), 136.8 (ArC), 128.6 (ArC), 128.2 (ArC), 128.0 (ArC), 95.3 (C-1), 78.5 (C-3), 70.6 (C-2), 70.1 (*C*HaHbPh), 66.8 (C-4), 66.5 (C-5), 39.1 (Ms), 20.8 (Ac), 18.3 (C-6). HRMS (ESI-QTOF) calc'd m/z for $C_{18}H_{28}O_8N$ $[M+NH_4]^+$: 417.1438; found: 417.1455.

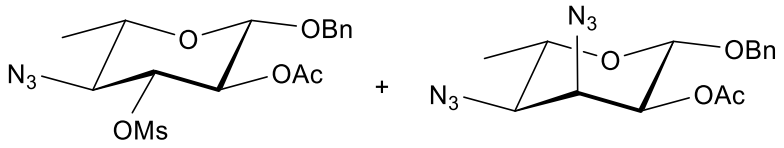
Benzyl 2-O-acetyl-3,4-di-O-mesyl-β-L-fucopyranoside (320).



320

Benzyl 3,4-O-isopropylidene-β-L-fucopyranoside **318** (3.39 g, 11.5 mmol) was acetylated in a mixture of acetic anhydride (4.4 mL, 47 mmol) and pyridine (30 mL) in a similar manner as compound **314**. The crude product **319** was then hydrolysed in 80% acetic acid - water (100 mL) to 90˚C for 4 hours. The reaction mixture was concentrated and co-evaporated with toluene (50 mL) to provide crude diol intermediate which was re-dissolved in a mixture of anhydrous dichloromethane (50 mL) and anhydrous pyridine (25 mL). To this solution, mesyl chloride (5.0 mL, 65 mmol) was added, and the mixture was stirred at room temperature for 20 hours. Methanol (~2 mL) was added to quench the reaction, and the reaction mixture was concentrated and co-evaporated with toluene (100 mL). The residue was partitioned between EtOAc (100 mL) and 1 N HCl (100 mL), and the aqueous phase was extracted with more EtOAc (2 × 100 mL). The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by precipitation in 30% ethyl acetate in hexanes to afford the desired compound **320**[112] (2.19 g, 42% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.40 – 7.26 (m, 5H, ArH), 5.27 (dd, 1H, *J* = 10.3, 7.9 Hz, H-2), 4.98 (dd, 1H, *J* = 3.4, 0.7 Hz, H-4), 4.92 (d, 1H, *J* = 12.3 Hz, C*H*$_a$H$_b$Ph), 4.78 (dd, 1H, *J* = 10.3, 3.4 Hz, H-3), 4.63 (d, 1H, *J* = 12.4 Hz, CH$_a$*H*$_b$Ph), 4.51 (d, 1H, *J* = 7.9 Hz, H-1), 3.78 (dq, 1H, *J* = 6.4, 0.7 Hz, H-5), 3.21 (s, 3H, Ms), 3.10 (s, 3H, Ms), 2.08 (s, 3H, Ac), 1.43 (d, 3H, *J* = 6.4 Hz, H-6).

Benzyl 2-O-acetyl-4-azido-4,6-dideoxy-3-O-mesyl-β-L-glucopyranoside (321) and Benzyl 2-O-acetyl-3,4-diazido-3,4,6-trideoxy-β-L-allopyranoside (322).

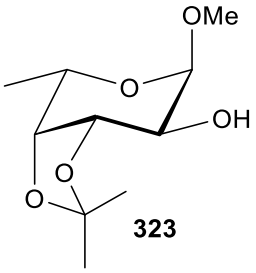


321 **322**

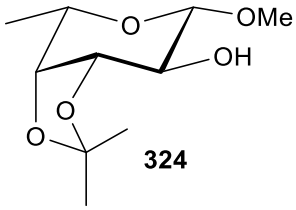
A mixture of 3,4-dimesylate **320** (1.77 g, 3.90 mmol) and NaN_3 (1.27 g, 20.0 mmol) in anhydrous DMF (15 mL) was heated to 90 °C for 40 h. The reaction mixture was poured into water (50 mL) and the product extracted into EtOAc (3 × 20 mL). The combined organic solutions were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography using a mixture of 10% ethyl acetate - hexanes as the eluent to afford compound **321** (0.804 g, 60% yield) as a syrup, and compound **322** (0.447 g, 29% yield) as a white powder. Data for compound **321**: R_f = 0.58 (40% ethyl acetate - hexanes). $[\alpha]_D^{25}$ +27.8 (*c* 0.58, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.38 – 7.27 (m, 5H, ArH), 5.08 (dd, 1H, *J* = 9.6 Hz, 8.0 Hz, H-2), 4.89 (d, 1H, *J* = 12.4 Hz, C*H*$_a$H$_b$Ph), 4.63 (dd, 1H, *J* = 9.5, 9.5 Hz, H-3), 4.61 (d, 1H, *J* = 12.3 Hz, CH$_a$*H*$_b$Ph), 4.46 (d, 1H, *J* = 8.0 Hz, H-1), 3.39 – 3.28 (m, 2H, H-4 and H-5), 3.11 (s, 3H, Ms), 2.09 (s, 3H, Ac), 1.46 (d, 3H, *J* = 5.8 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 169.5 (Ac), 136.7 (ArC), 128.5 (ArC), 128.0 (ArC), 127.7 (ArC), 98.9 (C-1), 80.1 (C-3), 70.8 (C-2), 70.8 (*C*H$_2$Ph), 70.6 (C-5), 66.2 (C-4), 38.9 (Ms), 20.8 (Ac), 18.3 (C-6). HRMS (ESI) calc'd *m/z* for $C_{16}H_{25}N_4O_7S$ $[M+NH_4]^+$: 417.1438; found: 417.1441. Data for compound **322**: R_f = 0.20 (40% ethyl acetate - hexanes). $[\alpha]_D^{25}$ +12.19 (*c* 2.1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.38 – 7.27 (m, 5H, ArH), 4.89 (d, 1H, *J* = 12.1 Hz, C*H*$_a$H$_b$Ph), 4.84 (m, 2H, H-1 and H-2), 4.62 (d, 1H, *J* = 12.1 Hz, CH$_a$*H*$_b$Ph), 4.34 (dd, 1H, H-3), 3.81 (dq, 1H, *J* = 9.7, 6.2 Hz, H-5), 3.23 (dd, *J* = 9.7, 3.1 Hz, 1H, H-4), 2.14 (s, 3H, Ac), 1.37 (d,

J = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 169.7 (Ac), 137.1 (ArC), 128.4 (ArC), 127.9 (ArC), 127.6 (ArC), 97.1 (C-1), 71.5 (C-2), 70.9 (CH_aH_bPh), 68.8 (C-5), 63.1 (C-4), 62.3 (C-3), 20.6 (Ac), 18.1 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{15}H_{22}N_7O_4$ $[M+NH_4]^+$: 364.1728; found: 364.1722.

Methyl 3,4-O-isopropylidene-α-L-fucopyranoside (323) & methyl 3,4-O-isopropylidene-β-L-fucopyranoside (324)



323

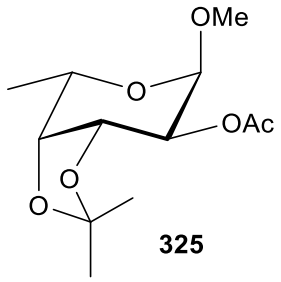


324

L-Fucose **304** (20.0 g, 122 mmol) was suspended in a solution of anhydrous methanol (200 mL) and acetyl chloride (2.0 mL, 28 mmol) at 0˚C. The mixture was then heated to reflux for 2 h. Ammonium hydroxide (1.0 mL) was added to neutralize the mixture. The mixture was then concentrated under reduced pressure to obtain a sticky white solid (11.0 g), which was used without further purification. Crude methyl-L-fucopyranoside (4.87 g, 27.3 mmol) was suspended in a mixture of acetone (35 mL) and 2,2-dimethoxypropane (5 mL), and treated with 10-camphorsulfonic acid (CSA) (153 mg). The mixture was heated to 40˚C for 20 h. The mixture was neutralized with ammonium hydroxide (1.0 mL), then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (20% ethyl acetate in hexanes) to obtain the α-anomer **323** (3.34 g, 56% yield), and the β-anomer **324** (1.32 g, 22% yield). Data for **323**: ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.72 (d, 1H, *J* = 3.9 Hz), 4.19 (t, 1H, *J* = 6.3 Hz), 4.10 (dq, 1H, *J*

= 6.6 Hz, *J* = 2.0 Hz), 4.05 (dd, 1H, *J* = 6.0 Hz, *J* = 2.3 Hz), 3.79 (dt, 1H, *J* = 6.6 Hz, *J* = 3.9 Hz), 3.45 (s, 3H), 2.35 (d, 1H, *J* = 6.6 Hz), 1.53 (s, 3H), 1.35 (s, 3H), 1.33 (d, 3H, *J* = 6.7 Hz). Data for **324**: ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.97 (d, 1H, *J* = 8.2 Hz), 3.95 (dd, 1H, *J* = 7.1 Hz, *J* = 5.6 Hz), 3.90 (dd, 1H, *J* = 5.5 Hz, *J* = 2.15 Hz), 3.77 (dq, 1H, *J* = 6.6 Hz, *J* = 2.1 Hz), 3.43 (s, 3H), 3.40 (dt, 1H, *J* = 7.3 Hz, *J* = 2.7 Hz), 3.15 (d, 1H, *J* = 2.5 Hz), 1.42 (s, 3H), 1.31 (d, 3H, *J* = 6.6 Hz), 1.25 (s, 3H). ^{1}H NMR matches literature.[181]

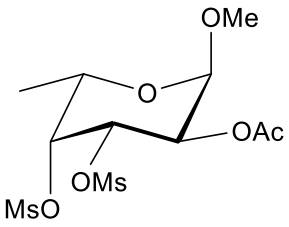
Methyl 2-O-acetyl-3,4-O-isopropylidene-α-L-fucopyranoside (325)



325

A mixture of methyl 3,4-O-isopropylidene-α-L-fucopyranoside **323** (0.43 g, 2.0 mmol), anhydrous pyridine (2 mL), and acetic anhydride (0.75 mL, 7.9 mmol) was stirred at room temperature for 4 h. The reaction mixture was quenched with water (1 mL), poured into aqueous sodium bicarbonate (10 mL) and extracted into $CHCl_3$ (3 × 10 mL). The organic fractions were combined, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a pale yellow oil (0.44 g). The crude product **325** was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.91 (dd, 1H, *J* = 8.3 Hz, *J* = 3.6 Hz), 4.79 (d, 1H, *J* = 3.6 Hz), 4.29 (dd, 1H, *J* = 8.3 Hz, *J* = 5.2 Hz), 4.13 – 4.06 (m, 2H), 3.37 (s, 3H), 2.13 (s, 3H), 1.53 (s, 3H), 1.37 (d, 3H, *J* = 6.5 Hz), 1.35 (s, 3H). ^{1}H NMR matches literature.[182]

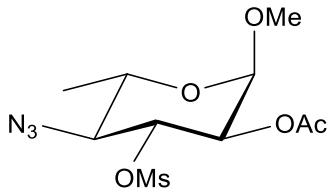
Methyl 2-O-acetyl-3,4-di-O-mesyl-α-L-fucopyranoside (326).



326

Crude methyl 2-O-acetyl-3,4-O-isopropylidene-α-L-fucopyranoside **325** (0.36 g, 1.4 mmol) was suspended in 80% acetic acid in water (5 mL) and heated to 80˚C for 1 h. The mixture was cooled back to room temperature and the solvent co-evaporated with toluene (30mL) to give a pale yellow oil (0.35 g). A mixture of crude methyl 2-O-acetyl-α-L-fucopyranoside (0.18 g, 0.81 mmol), dichloromethane (1 mL), anhydrous pyridine (0.5 mL), was cooled to 0˚C then mesyl chloride (0.31 mL, 4.0 mmol) was added and the reaction mixture allowed to warm to room temperature over a period 4.5 h. The reaction mixture was quenched with methanol (1 mL) and the solvent co-evaporated with toluene (30 mL). The residue was then purified by column chromatography (30% ethyl acetate in hexanes) to afford **326** (0.23 g, 74%) yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.13 (dd, 1H, *J* = 10.6 Hz, *J* = 3.1 Hz), 5.09 (dd, 1H, *J* = 10.6 Hz, *J* = 2.8 Hz), 5.04 (dd, 1H, *J* = 3.0 Hz, *J* = <1 Hz), 4.97 (d, 1H, *J* = 3.1 Hz), 4.12 (dq, 1H, *J* = 6.5 Hz, *J* = <1 Hz), 3.40 (s, 3H), 3.21 (s, 3H), 3.12 (s, 3H), 2.15 (s, 3H), 1.34 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR matches literature.[183]

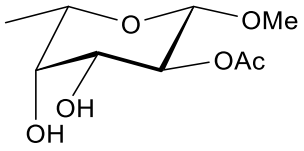
Methyl 2-O-acetyl-4-azido-4,6-dideoxy-3-O-mesyl-α-L-glucopyranoside (327).



327

A mixture of methyl 2-O-acetyl-3,4-di-O-mesyl-α-L-fucopyranoside **326** (97.8 mg, 0.260 mmol), DMF (2 mL), and sodium azide (94 mg, 1.4 mmol) was heated to 100˚C for 16 h. The reaction mixture was poured into brine (10 mL) and the product extracted into ethyl acetate (3 × 10 mL). The organic fractions were combined, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography (15% ethyl acetate in hexanes) on silica gel to obtain **327** as a yellow oil (57.3 mg, 68% yield). R_f = 0.51 (40% ethyl acetate in hexanes). $[\alpha]_D^{25}$ -139.4 (*c* 1.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.99 – 4.88 (m, 2H, H-2 and H-3), 4.84 (d, 1H, *J* = 3.5 Hz, H-1), 3.70 (dq, 1H, *J* = 10.0 Hz, *J* = 6.2 Hz), 3.38 (s, 3H, OMe), 3.23 (dd, 1H, *J* = 9.7 Hz, *J* = <1 Hz), 3.12 (s, 3H, OMs), 2.15 (s, 3H, OAc), 1.39 (d, 3H, *J* = 6.2 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.2 (OAc), 96.9 (C-1), 78.2 (C-2), 70.5 (C-3), 66.6 (C-4), 66.0 (C-5), 55.4 (OMe), 38.9 (SO_2CH_3), 20.8 ($COCH_3$), 18.2 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{10}H_{17}N_3O_7S$ $[M+Na]^+$: 346.0679; found: 346.0666.

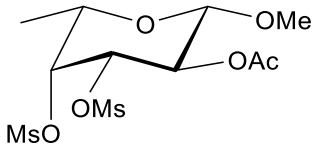
Methyl 2-O-acetyl-β-L-fucopyranoside (329).



329

A mixture of methyl 3,4-O-isopropylidene-β-L-fucopyranoside **324** (1.51 g, 6.90 mmol), acetic anhydride (2.6 mL, 28 mmol) in anhydrous pyridine (15 mL) was stirred at room temperature for 20 h. The reaction was quenched with water (5 mL), poured into 10% aqueous $NaHCO_3$ (50 mL) and extracted into $CHCl_3$ (3 × 30 mL). The combined organic solutions were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude solid **328** (1.62 g). The crude mixture was dissolved in 80% acetic acid - water (20 mL), and the solution was heated to 75 °C for 3 hours. After cooled back to room temperature, the reaction solution was concentrated under reduced pressure and co-evaporated with toluene (100 mL). The crude mixture was purified by column chromatography on silica gel using a mixture of 60% ethyl acetate - hexanes to afford the desired compound **329** (1.27 g, 83% yield). R_f = 0.66 (10% MeOH in CH_2Cl_2). $[\alpha]_D^{25}$ +3.6 (*c* 1.5, MeOH). ^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 4.97 (dd, *J* = 9.6, 8.0 Hz, 1H, H-2), 4.29 (d, *J* = 8.0 Hz, 1H, H-1), 3.75 – 3.61 (m, 3H, H-3, H-4, H-5), 3.39 (s, 3H, -OMe), 2.01 (s, 3H, Ac), 1.28 (d, *J* = 6.5 Hz, 3H, H-6). ^{13}C NMR (101 MHz, CD_3COCD_3): δ_C 101.7 (C-1), 72.2, 72.1, 71.7, 70.4 (C-2,3,4,5), 55.2 (OMe), 20.1 (Ac), 15.8 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_9H_{16}O_6$ $[M+Na]^+$: 243.0839; found 243.0831.

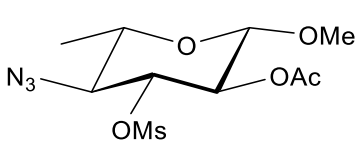
Methyl 2-O-acetyl-3,4-O-mesyl-β-L-fucopyranoside (330).



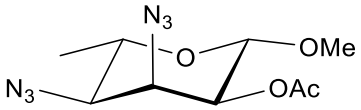
330

To a solution of compound **329** (0.12 g, 0.54 mmol) in a mixture of anhydrous dichloromethane (1.0 mL) and pyridine (0.5 mL), was added mesyl chloride (0.21 mL, 2.7 mmol) at 0˚C. The temperature was allowed to warm up to room temperature. After stirring for 6 hours, the reaction mixture was quenched with methanol (~1 mL), and the solvent was removed under reduced pressure, and the residue was co-evaporated with toluene (30 mL). The crude mixture was purified by column chromatography on silica gel using 30% ethyl acetate - hexanes as an eluent to afford compound **330** in pure form (0.20 g, 99% yield). R_f = 0.14 (40% EtOAc in hexanes). $[\alpha]_D^{25}$ -17.93 (*c* 0.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.18 (dd, *J* = 10.3, 7.9 Hz, 1H, H-2), 4.99 (dd, *J* = 3.4, 0.9 Hz, 1H, H-4), 4.82 (dd, *J* = 10.3, 3.4 Hz, 1H, H-3), 4.40 (d, *J* = 7.9 Hz, 1H, H-1), 3.80 (dd, *J* = 6.4, 0.9 Hz, 1H, H-5), 3.52 (s, 3H, OMe), 3.20 (s, 3H, Ms), 3.12 (s, 3H, Ms), 2.13 (s, 3H, Ac), 1.41 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 101.5 (C-1), 78.19 (C-4), 75.87 (C-3), 68.92 (C-5), 68.14 (C-2), 56.93 (OMe), 38.91 (Ms), 38.71 (Ms), 20.83 (Ac), 16.57 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{11}H_{20}O_{10}S_2$ $[M+Na]^+$: 399.0396; found: 399.0411.

Methyl 2-O-acetyl-4-azido-4,6-dideoxy-3-O-mesyl-β-L-glucopyranoside (331) and methyl 2-O-acetyl-3,4-diazido-3,4,6-trideoxy-β-L-glucopyranoside (332).



331

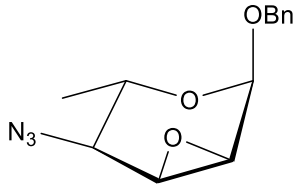


332

A mixture of 3,4-dimesylate **330** (240 mg, 0.540 mmol) and sodium azide (0.16 g, 2.5 mmol) in DMF (2.5 mL) was heated to 90 °C for 40 h. The solvent was then removed by evaporatation *in vacuo*. The residue was extracted into ethyl acetate (20 mL) and washed with brine (3 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 5% ethyl acetate - hexanes as the eluent to afford compound **332** as a white solid (76 mg, 0.28 mmol, 52% yield) and compound **331** (4-azido-3-mesyl) as another white solid (34 mg, 20% yield). Data for compound **331**: R_f = 0.58 (40% EtOAc - hexanes). $[\alpha]_D^{25}$ -5.14 (*c* 2.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.01 (dd, *J* = 9.7, 7.9 Hz, 1H, H-2), 4.68 (dd, *J* = 9.6, 9.6 Hz, 1H, H-3), 4.35 (d, *J* = 8.0 Hz, 1H, H-1), 3.51 (s, 3H, OMe), 3.39 (dq, *J* = 9.8, 6.0 Hz, 1H, H-5), 3.30 (d, *J* = 9.6, 9.6 Hz, 1H, H-4), 3.14 (s, 3H, Ms), 2.15 (s, 3H, Ac), 1.47 (d, *J* = 6.0 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 101.2 (C-1) , 80.2 (C-3), 70.8 (C-2) , 70.6 (C-5) , 66.3 (C-4), 57.0 (OMe) , 38.94 (Ms), 20.89 (Ac), 18.29 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_9H_{17}O_7N_3S$ $[M+Na]^+$: 346.0679; found: 346.0683. Data for compound **332**: R_f = 0.15 (10% Ethyl acetate - hexanes). $[\alpha]_D^{25}$ +25.5 (*c* 0.80, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.76 (dd, *J* = 8.1, 3.4 Hz, 1H, H-2), 4.67 (d, *J* = 8.1, 1H, H-1), 4.34 (dd, *J* = 3.4, 3.2 Hz, 1H, H-3), 3.82 (dq, *J* = 9.7, 6.2 Hz, 1H, H-5), 3.52 (s, 3H, OMe), 3.22 (dd, *J* = 9.7, 3.2 Hz, 1H, H-4), 2.18 (s, 3H, Ac), 1.37 (d, *J* = 6.3, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 169.77 (Ac),

98.78 (C-1), 71.49 (C-2), 68.72 (C-5), 63.16 (C-4), 62.27 (C-3), 56.93 (OMe), 20.64 (Ac), 18.08 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_9H_{24}O_4N_6$ $[M+Na]^+$: 293.0969; found: 293.0971.

Benzyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-allopyranoside (333).

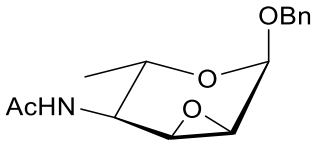


333

To a solution of benzyl 2-O-acetyl-4-azido-4,6-dideoxy-3-O-mesyl-α-L-glucopyranoside **317** (17.85 g, 44.69 mmol) in anhydrous methanol (200 mL) was added sodium methoxide (2.68 g, 47.0 mmol), and the mixture was stirred at room temperature for 20 hours under an atmosphere of argon. The reaction solution was concentrated under reduced pressure and the residue was resuspended in ethyl acetate (100 mL). The organic solution was washed with distilled water (3 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The syrupy mixture was purified by precipitation in a mixture of 5% ethyl acetate – hexanes (~20 mL) to afford the desired compound **333** as an off-white solid (10.36 g, 89% yield). R_f = 0.65 (40% ethyl acetate - hexanes). $[\alpha]_D^{25}$ -197.5 (*c* 0.76, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.42 – 7.28 (m, 5H, ArH), 5.01 (dd, *J* = 3.2 Hz, <1 Hz, 1H, H-1), 4.78 (d, *J* = 12.0 Hz, 1H, C*H*$_a$H$_b$Ph), 4.65 (d, *J* = 12.3 Hz, 1H, CH$_a$*H*$_b$Ph), 3.96 (dq, *J* = 9.6, 6.3 Hz, 1H, H-5), 3.55 (dd, *J* = 4.1 Hz, 1.6 Hz, 1H, H-3), 3.49 (dd, *J* = 4.1, 3.2 Hz, 1H, H-2), 3.16 (dd, *J* = 9.6 Hz, 1.5 Hz, 1H, H-4), 1.23, (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 137.4 (ArC), 128.5 (ArC), 128.1 (ArC), 127.9 (ArC), 92.0

(C-1), 69.5 (*C*H_2Ph), 63.2 (C-5), 61.5 (C-4), 53.7 (C-2), 52.3 (C-3), 17.8 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{13}H_{22}N_4O_3$ $[M+NH_4]^+$: 279.1452; found: 279.1460.

Benzyl 4-acetamido-2,3-anhydro-4,6-dideoxy-α-L-allopyranoside (334)

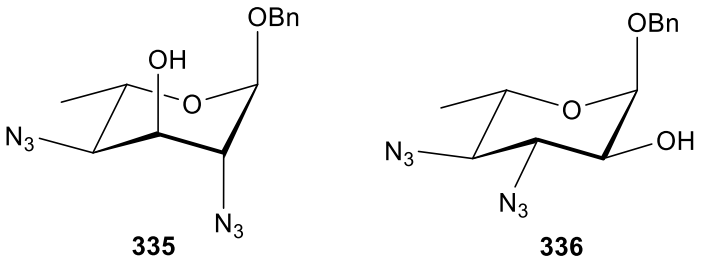


334

A mixture of benzyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-allopyranoside **333** (0.517 g, 1.98 mmol), methanol (25 mL), dichloromethane (5 mL), ammonium hydroxide (0.125 mL, 1.80 mmol) and palladium hydroxide (20% over charcoal; 163.4 mg) was stirred at room temperature for 3 h. The mixture was then filtered through a 2 μm PTFE syringe filter and the solvent removed under reduced pressure. The residue was redissolved in methanol (4 mL) and treated with acetic anhydride (0.95 mL) at room temperature for 21 h. The solvent was removed under reduced pressure and the pink residue purified by column chromatography (30% ethyl acetate in hexanes) to give **334** as a white solid (0.345 g, 63% yield). R_f = 0.40 (5% methanol in dichloromethane). $[\alpha]^{25}_D$ -152.6˚ (c 0.23 $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.42 – 7.30 (m, 5H, ArH), 5.69 (d, 1H, *J* = 9.5 Hz, N*H*Ac), 5.06 (d, 1H, *J* = 3.2 Hz, H-1), 4.77 (d, 1H, *J* = 12.2 Hz, -CH_2Ph), 4.65 (d, 1H, *J* = 12.2 Hz, -CH_2Ph), 4.20 (td, 1H, *J* = 9.7 Hz, *J* = 1.8 Hz, H-4), 3.68 (dq, 1H, *J* = 9.7 Hz, *J* = 6.3 Hz, H-5), 3.51 (dd, 1H, *J* = 4.1 Hz, *J* = 3.4 Hz, H-2), 3.37 (dd, 1H, *J* = 4.1 Hz, *J* = 1.8 Hz, H-3), 2.03 (s, 3H, NHAc), 1.17 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.0 (NHAc), 137.5 (ArC), 128.5 (ArC), 128.1 (ArC), 127.9 (ArC), 92.2 (C-1), 69.6 (-CH_2Ph), 64.0 (C-5), 54.9 (C-2), 53.4 (C-3), 50.0 (C-4),

23.4 (NHCO*C*H_3), 17.5 (C-6). HRMS (ESI) calc'd *m/z* for $C_{15}H_{20}NO_4$ $[M+H]^+$: 278.1387 found 278.1382.

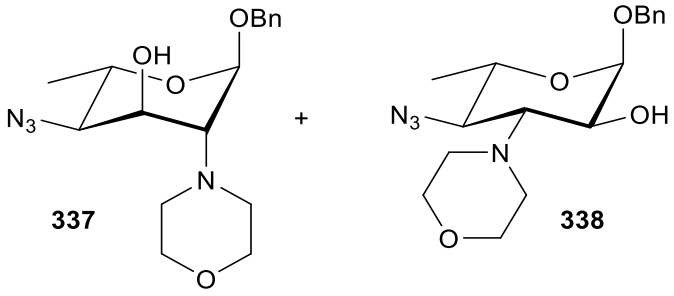
Benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (335) and benzyl 3,4-diazido-3,4,6-trideoxy-α-L-glucopyranoside (336)



General Method: A solution of benzyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-allopyranoside (**333**), sodium azide and catalyst in the specified solvent (**Table 1**) was heated to the specified temperature for the specified amount for time. The solvent was then evaporated under reduced pressure. The residue was extracted into ethyl acetate, and the organic solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. ^{1}H NMR experiment was used to directly determine the conversion rates and product ratios (**335/336**). For some experiments, the obtained residue was purified by column chromatography on silica gel using 2.5% ethyl acetate - hexanes as the eluent to yield the desired compounds **335** and **336** respectively. Data for compound **335**: R_f = 0.43 (20% ethyl acetate – hexanes, twice). $[\alpha]_D^{25}$ -116.1 (*c* 0.43, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.45 – 7.31 (m, 5H, ArH), 4.89 (s, 1H, H-1), 4.78 (d, 1H, *J* = 11.7 Hz, C*H*$_a$H$_b$Ph), 4.59 (d, 1H, *J* = 11.8 Hz, CH$_a$*H*$_b$Ph), 4.13 – 4.02 (m, 2H, H-3 and H-5), 3.85 (dd, 1H, *J* = 4.0 Hz, 1.9 Hz, H-2), 3.45 (d, 1H, *J* = 9.8 Hz, OH), 3.23 (dd, 1H, *J* = 9.8,

3.1 Hz, H-4), 1.38 (d, 3H, *J* = 6.3 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 135.8 (ArC), 128.7 (ArC), 128.5 (ArC), 128.3 (ArC), 96.9 (C-1), 70.3 (*C*H_aH_bPh), 69.4 (C-3), 63.1 (C-5), 61.3 (C-4), 60.8 (C-2), 18.1 (C-6). HRMS (ESI-TOF) calc'd *m/z* for $C_{13}H_{16}N_6O_3$ $[M+NH_4]^+$: 322.1626; found 322.1622. Data for compound **336**: R_f = 0.37 (20% ethyl acetate – hexanes, twice). $[\alpha]_D^{25}$ -330.0 (*c* 1.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.43 -7.32 (m, 5H, ArH), 4.93 (d, *J* = 3.1 Hz, 1H, H-1), 4.75 (d, *J* = 11.7 Hz,1H, C*H*$_a$H$_b$Ph), 4.55 (d, *J* = 11.7 Hz, 1H, CH$_a$*H*$_b$Ph), 3.69 – 3.57 (m, 3H, H-2, H-4, and H-5), 2.92 (*high order* t, *J* = 9.9, 9.9 Hz, 1H, H-3) 2.17 (*high order* d, *J* = 10.4 Hz, 1H, OH-2), 1.30 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 128.7 (ArC), 128.4 (ArC), 128.2 (ArC), 96.8 (C-1), 71.9 (C-2), 70.1 (*C*H_aH_bPh), 66.6 (C-5), 66.4 (C-3), 65.8 (C-4), 18.2 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{13}H_{16}N_6O_3$ $[M+NH_4]^+$: 322.1626; found 322.1617.

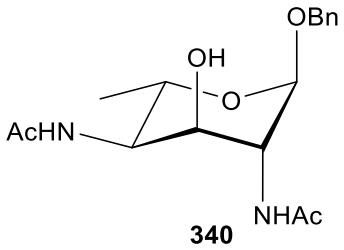
Benzyl 3-azido-2,4,6-trideoxy-2-morpholino-α-L-altropyranoside (337) and Benzyl 4-azido-3,4,6-trideoxy-3-morpholino-α-L-glucopyranoside (338).



A mixture of compound **333** (100 mg, 0.383 mmol), morpholine (0.07 ml, 0.765 mmol) and lithium perchlorate (82 mg, 0.77 mmol) in anhydrous acetonitrile (2.0 mL) was heated to 60°C for 48 hours. The solution was concentrated under reduced pressure and the residue

extracted with ethyl acetate (20 mL) and washed with brine (3 × 20 mL). The organic solution was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 10% ethyl acetate - hexanes as the eluent to yield the 2-morpholino product **337** (84 mg, 63% yield), and the 3-morpholino analog **338** (20 mg, 15% yield). Data for compound **337**: R_f = 0.41 (40% ethyl acetate - hexanes). $[\alpha]_D^{25}$ -117.2 (*c* 1.7, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.29 (m, 5H, ArH), 4.98 (d, *J* = 4.4 Hz, 1H, H-1), 4.76 (d, *J* = 11.8 Hz, 1H, C*H*$_a$H$_b$Ph), 4.56 (d, *J* = 11.8 Hz, 1H, CH$_a$*H*$_b$Ph), 4.03 (dd, *J* = 7.6, 4.3 Hz, 1H, H-3), 3.92 (dq, *J* = 6.5, 6.5 Hz 1H. H-5), 3.65 – 3.73 (m, 4H, CH_2OCH_2), 3.61 (dd, *J* = 7.6, 4.2 Hz, 1H, H-4), 2.78 (dd, *J* = 7.6, 4.4 Hz, 1H, H-2), 2.73 (m, 2H, C*H*$_a$H$_b$NC*H*$_a$H$_b$), 2.55 - 2.61 (m, 2H, CH$_a$*H*$_b$NCH$_a$*H*$_b$), 1.34 (d, *J* = 6.5 Hz, 1H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 136.8 (ArC), 128.6 (ArC), 128.2 (ArC), 128.1 (ArC), 95.7 (C-1) , 69.7 (*C*H$_a$H$_b$Ph), 67.4 (CH_2OCH_2), 66.6 (C-3) , 66.0 (C-5) , 65.26 (C-2) , 63.61 (C-4) , 50.3 (CH$_a$H$_b$NCH$_a$H$_b$), 19.02 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{17}H_{24}N_4O_4$ $[M+H]^+$: 349.1880; found: 349.1870. Data for compound **338**: R_f = 0.29 (40% ethyl acetate - hexanes). $[\alpha]_D^{25}$ -92.0 (*c* 1.9, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 – 7.31 (m, 5H, ArH), 4.97 (d, *J* = 3.8 Hz, 1H, H-1), 4.75 (d, *J* = 11.8 Hz, 1H, C*H*$_a$H$_b$Ph), 4.59 (d, *J* = 11.8 Hz, 1H, CH$_a$*H*$_b$Ph), 3.78 – 3.68 (m, 5H, H-2 + CH_2OCH_2), 3.65 (dq, *J* = 9.7, 6.2 Hz, 1H, H-5), 3.02 (dd, *J* = 10.1, 9.7 Hz, 1H, H-4), 2.94 – 2.84 (m, 5H, H-3, CH$_a$H$_b$NCH$_a$H$_b$), 1.28 (d, *J* = 6.2 Hz, 3H, H-6). δ_C ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 137.0 (Ar-C), 128.6 (Ar-C), 128.1 (Ar-C), 128.1 (Ar-C), 97.4 (C-1), 69.9 (*C*H$_a$H$_b$Ph), 68.1 (C-2), 67.5 (CH_2OCH_2), 67.3 (C-5), 66.7 (C-3), 63.3 (C-4), 50.1 (CH$_a$H$_b$NCH$_a$H$_b$), 18.6 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{17}H_{24}N_4O_4$ $[M+H]^+$: 349.1870; found 349.1863.

Benzyl 2,4-diacetamido-2,4,6-trideoxy-α-L-altropyranoside (340)



340

Method 1

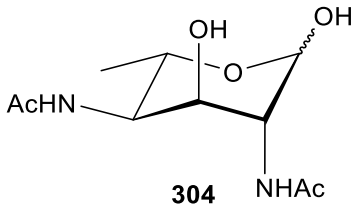
To a solution of 2,4-diazido compound **335** (80 mg, 0.26 mmol) in a 9:1 mixture of pyridine -H_2O (2.0 mL) was added triphenylphosphine (275 mg, 1.05 mmol, 4 equiv.), and the mixture was stirred at ambient temperature for 48 hours. The solution was concentrated under reduced pressure. The residue (**339**) was redissolved in methanol (2.0 mL), and acetic anhydride (250 µL) was added. After stirring for 4 hours, the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of 30 → 40% acetone - toluene as the eluent to afford the desired Alt-2,4-DiNHAc compound **340** (52 mg, 59% yield).

Method 2

To a solution of 2,4-diazido compound **335** (206 mg, 0.680 mmol) in dichloromethane (5.0 mL) and methanol (5.0 mL), was added concentrated ammonia (30%, 4 drops), and palladium on charcoal (5%, 28 mg) was added; the mixture was purged with hydrogen and stirred continuously under an atmosphere of hydrogen for 5 hours. The reaction solution was filtered over a 0.22 µM membrane disk, and the solution was concentrated under reduced pressure. The residue was redissolved in methanol (5.0 mL), and acetic anhydride (0.5 mL) was added. After stirring for 2

hours, the reaction solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel as above to afford compound **340** in pure form (184 mg, 81% yield). R_f = 0.55 (10% MeOH - CH_2Cl_2). $[\alpha]_D^{25}$ -74.8 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.41 – 7.26 (m, 5H, Ph), 6.45 (d, *J* = 8.8 Hz, 1H, N*H*Ac-C_2), 6.25 (d, *J* = 9.2 Hz, 1H, N*H*Ac-C4), 4.79 (br s, 1H, H-1), 4.69 (d, *J* = 11.8 Hz, 1H, C$H_a$$H_b$Ph), 4.52 (d, *J* = 11.8 Hz, 1H, C$H_a$$H_b$Ph), 4.42 (dd, *J* = 8.8, 2.8 Hz, 1H, H-2), 4.03 (ddd, *J* = 10.1, 10.1, 2.9 Hz, 1H, H-4), 3.89 – 3.78 (m, 2H, H-5 + OH-3), 3.73 (m, 1H, H-3), 1.98 (s, 6H, 2 × Ac), 1.24 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.0 (C=O), 169.7 (C=O), 136.1 (Ph), 128.7 (Ph), 128.3 (Ph), 128.1 (Ph), 98.3 (C-1), 69.9 (*C*HaHbPh), 69.1 (C-3), 63.9 (C-5), 50.1 (C-2), 49.7 (C-4), 23.4 (Ac), 23.2 (Ac), 17.8 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{17}H_{25}N_2O_5$ $[M+H]^+$: 337.1751; found 337.1758.

2,4-Di-N-acetamido-2,4,6-trideoxy-L-altropyranose (304).

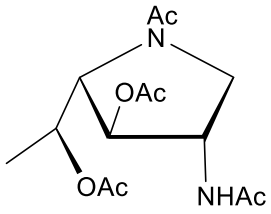


304

A mixture of benzyl 2,4-di-N-acetyl-2,4,6-trideoxy-α-L-altropyranoside **340** (106 mg, 0.280 mmol), palladium hydroxide (68 mg) in methanol (10.0 mL), dichloromethane (2.0 mL) and acetic acid (100 µL) was stirred under a hydrogen atmosphere (balloon). After stirring for 20 h, the mixture was filtered through a 0.2 µm PTFE syringe filter and the clear solution was evaporated under reduced pressure to give a white solid (77 mg, ~quantitative yield). Selected

^{1}H NMR (400 MHz, D_2O): δ_H: 5.16 (d, *J* = 1.93 Hz, H-1 (α-pyranose), 4.93 (d, *J* = 2.6 Hz, H-1 (β-pyranose), 3.99 -3.97 (m, major isomer), 3.94 – 3.92 (m, minor isomer), 3.87 -3.79 (m), 3.70 (dd, *J* = 10.4 Hz, *J* = 3.0 Hz), 1.97 (s), 1.91 (s), 1.12 (d, *J* = 6.5 Hz, H-6 (major isomer)), 1.09 (d, *J* = 6.2 Hz, (minor isomer)). HRMS (ESI-QTOF) calc'd *m/z* for $C_{12}H_{21}N_2O_6$ $[M+H]^+$: 289.1396; found 289.1394. ^{1}H NMR matches literature.[78]

2-Acetamido-5-O-acetyl-1,4-(N-acetylimino)-1,2,4,6-tetradeoxy-L-altritol (344).



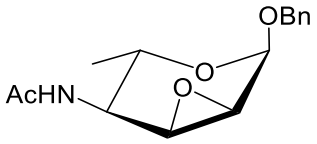
344

To a solution of 2,4-diazido compound **335** (31 mg, 0.10 mmol) in a 1:1 mixture of CH_2Cl_2-MeOH (5.0 mL) was added ten drops of water and acetic acid (12 µL, 0.20mmol) and $Pd(OH)_2$/C /C on charcoal (42 mg), and the flask was purged with an atmosphere of hydrogen, and kept under a positive hydrogen pressure with stirring for 48 hours. The solids were filtered off using a 0.22 mm membrane filter, and the solution was concentrated under reduced pressure. The crude syrup was acetylated using a mixture of acetic anhydride (1.0 mL) and pyridine (1.0 mL). After stirring overnight, the reaction solution was concentrated and co-evaporated with toluene (3 × 5 mL). The crude residue was purified by column chromatography on silica gel using 2% methanol - dichloromethane to afford a syrupy product (19 mg, 60% yield). R_f = 0.55 (10% MeOH – CH_2Cl_2).

$[\alpha]_D^{25}$ -9.85 (*c* 1.3, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.70 (d, *J* = 4.7 Hz, 1H, N*H*Ac), 5.45 (qd, *J* = 6.6, 4.1 Hz, 1H, H-5), 5.35 (dd, *J* = 4.7, 3.9 Hz, 1H, H-3), 4.30 – 4.23 (m, 2H, H-1a + H-2), 4.22 (dd, *J* = 4.1, 4.1 Hz, H-4), 3.19 (high order dd, *J* = 10.3, 14.2 Hz, 1H, H-1b), 2.12 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.28 (d, *J* = 6.6 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 171.0 (Ac), 170.5 (Ac), 170.0 (Ac), 169.7 (Ac), 75.9 (C-3), 69.4 (C-5), 63.7 (C-4), 55.0 (C-2), 52.9 (C-1), 23.1 (Ac), 22.3 (Ac), 21.4 (Ac), 20.9 (Ac), 17.0 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{14}H_{22}N_2O_6$ $[M+Na]^+$: 337.1376; found 337.1365.

Chapter 4 Procedures and Data

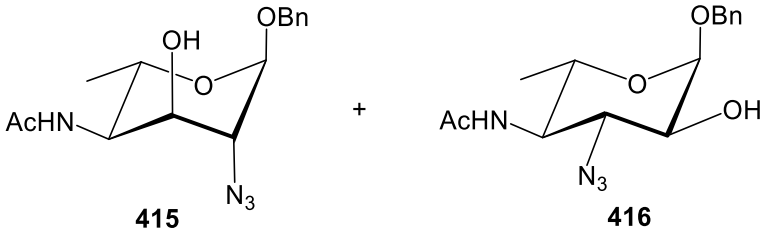
Benzyl 4-acetamido-2,3-anhydro-4,6-dideoxy-α-L-allopyranoside (404)



404

See synthesis of Benzyl 4-acetamido-2,3-anhydro-4,6-dideoxy-α-L-allopyranoside (**334**) *in Chapter 3 Procedures and Data.*

Benzyl 4-acetamido-2-azido-2,4,6-trideoxy-α-L-altropyranoside (415) and benzyl 4-acetamido-3-azido-3,4,6-trideoxy-α-L-glucopyranoside (416)

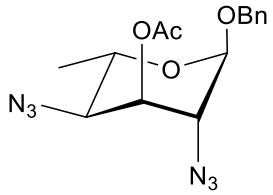


415 **416**

Benzyl 4-acetamido-2,3-anhydro-4,6-dideoxy-α-L-allopyranoside (**414**, 21 mg, 0.076 mmol) was combined with sodium azide (20 mg, 0.30 mmol) and lithium perchlorate (32 mg, 0.30 mmol) in anhydrous acetonitrile (5.0 mL), and the mixture was heated to 85 °C for 48 hours. The solvent was removed under reduced pressure. ^{1}H NMR spectrum of the crude revealed that the reaction was incomplete. The crude was then redissolved in a mixture of water (3.0 mL) and ethanol (3.0 mL), and the solution was heated to reflux for 24 hours. The solvents were removed

under reduced pressure and the crude was then extracted with ethyl acetate (5.0 mL), and the organic solution was washed with brine (2 x 5.0 mL) and water (5.0 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The mixture was purified by column chromatography on silica gel using 25% ethyl acetate in toluene as the eluent to afford compound **416** (8 mg, 0.02 mmol, 33% yield) as a white powder **415** (15 mg, 0.047 mmol, 62% yield as a white powder. Data for compound **415**: R_f = 0.13 (40% toluene - hexanes, x 2). $[\alpha]^{25}_D$ -66.6 (*c* 0.6, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 – 7.30 (m, 5H, ArH), 5.95 (d, *J* = 9.5 Hz, 1H, N*H*Ac), 4.94 (d, *J* = 1.6 Hz, 1H, H-1), 4.75 (d, *J* = 11.6 Hz, 1H, CH_aH$_b$Ph), 4.58 (d, *J* = 11.6 Hz, 1H, CH$_a$$H_b$Ph), 4.13 (ddd, *J* = 12.15, 9.5, 2.6 Hz, 1H, H-4), 3.91 – 3.72 (m, 3H, H-2, H-3, H-4), 3.61 (d, *J* = 9.7 Hz, 1H, OH-3), 2.02 (s, 3H, Ac), 1.29 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.7 (CO), 136.7 (ArC), 128.8 (ArC), 128.5 (ArC), 128.3 (ArC), 96.82 (C-1), 70.17 (Ph*C*H$_2$CO), 69.20 (C-3), 64.53 (C-5), 60.01 (C-2), 49.30 (C-4), 23.46 (Ac), 17.75 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{15}H_{21}N_4O_4$ $[M+Na]^+$: 343.1377; found 343.1374. Data for compound **416**: R_f = 0.21 (40% toluene - hexanes, x2). $[\alpha]^{25}_D$ -79.67 (*c* 0.4, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 – 7.30 (m, 5H, ArH), 5.31 (d, *J* = 8.5 Hz, 1H, N*H*Ac), 4.96 (d, *J* = 3.2 Hz, 1H, H-1), 4.75 (d, *J* = 11.7 Hz, 1H, CH_aH$_b$Ph), 4.57 (d, *J* = 11.7 Hz, 1H, CH$_a$$H_b$Ph), 3.83 (dq, *J* = 12.4, 6.3 Hz, 1H, H-5), 3.75 – 3.54 (m, 3H, H-2, H-3, H-4), 2.19 (d, *J* = 9.3 Hz, 1H, OH-2), 2.04 (s, 3H, Ac), 1.20 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.0 (CO), 136.7 (ArC), 128.7 (ArC), 128.3 (ArC), 128.2 (ArC), 97.0 (C-1), 72.1 (C-2), 70.0 (Ph*C*H$_2$CO), 66.9 (C-5), 64.5 (C-3), 55.1 (C-4), 23.4 (Ac), 17.6 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{15}H_{21}N_4O_4$ $[M+Na]^+$: 343.1377; found 343.1377.

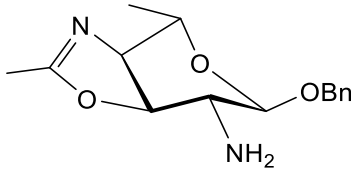
Benzyl 3-O-acetyl-2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (418)



418

Benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (**417**, 206 mg, 0.677 mmol) was combined with acetic anhydride (1.0 mL) and anhydrous pyridine (1.0 mL) and stirred at room temperature for 12 hours. Solvents were removed under reduced pressure and Compound **418** was isolated by silica gel column chromatography, eluted with 2% ethyl acetate in hexanes to give a clear oil (233 mg, 0.672 mmol, 99% yield). R_f = 0.47 (10% EtOAc - hexanes). $[\alpha]^{25}_D$ −76.5 (*c* 12.5, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 – 7.30 (m, 5H, ArH), 5.08 (dd, *J* = 6.0, 3.7 Hz, 1H, H-3), 4.87 – 4.71 (m, 2H, H-1, C*H*$_a$H$_b$Ph), 4.54 (d, *J* = 11.8 Hz, 1H, CH$_a$*H*$_b$Ph), 4.16 (dq, *J* = 8.2, 6.5 Hz, 1H, H-5), 3.93 (dd, *J* = 6.0, 3.2 Hz, 1H, H-2), 3.50 (dd, *J* = 8.2, 3.7 Hz, 1H, H-4), 2.11 (s, 1H, Ac) , 1.34 (d, *J* = 6.5 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.1 (CO), 137.0 (ArC), 128.5 (ArC), 128.4 (ArC), 128.0 (ArC), 97.1 (C-1), 69.9 (C-3), 69.9 (Ph*C*H$_2$CO), 65.4 (C-5), 60.3 (C-4), 59.6 (C-2), 20.7 (Ac), 18.0 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{15}H_{18}N_6O_4Na$ $[M+Na]^+$: 369.1282; found 369.1275.

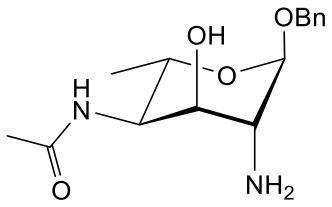
Benzyl 2,4-diamino-3-O,4-N-ethylylidene-2,4,6-trideoxy-α-L-altropyranoside (419)



419

Benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **417** (170 mg, 0.599 mmol) was acetylated in a mixture of acetic anhydride (2.0 mL) and anhydrous pyridine (2.0 mL) as in procedure A. The crude oil was re-dissolved in anhydrous pyridine (5.0 mL), and a solution of trimethylphosphine in THF (1.0 M, 4.7 mL) was added along with 4 Å molecular sieves (800 mg). After stirring for 12 hours, aqueous NaOH (10%, 1.0 mL) was added and the reaction was left at room temperature for 24 hours. The reactive mixture was concentrated under reduced pressure, and the residue was purified by chromatography on silica gel using 1% methanol in dichloromethane as the eluent to afford the oxazoline **419** as a white powder (40 mg, 0.15 mmol, 24% yield). R_f = 0.67 (10% MeOH – CH_2Cl_2). $[\alpha]^{25}_D$ -99.56 (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.26 (m, 5H, ArH), 4.83 (d, *J* = 11.9 Hz, 1H, CH_aH$_b$Ph), 4.58 (d, *J* = 7.0 Hz, 1H, H-1), 4.57 (d, *J* = 11.9 Hz, 1H, CH$_a$$H_b$Ph), 4.39 (dd, *J* = 9.8, 9.9 Hz, 1H, H-3), 3.97 (ddq, *J* = 9.8, 9.8, 1.4 Hz, 1H, H-4), 3.83 (dq, *J* = 9.8, 6.0 Hz, 1H, H-5), 3.17 (dd, *J* = 9.9, 7.0 Hz, 1H, H-2), 2.02 (d, *J* = 1.4 Hz, 1H, Ac), 1.38 (d, *J* = 6.0 Hz, 1H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 165.7 (Me*C*O), 138.0 (ArC), 128.5 (ArC), 127.9 (ArC), 127.4 (ArC), 101.0 (C-1), 81.7 (C-3), 72.4 (C-4), 69.4 (Ph*C*H$_2$CO), 67.56 (H-5), 53.51 (C-2), 19.73 (Ac), 14.07 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{15}H_{21}N_2O_3$ $[M+H]^+$: 277.1561; found 277.1547.

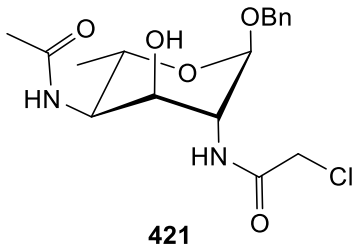
Benzyl 2-amino-4-acetamido-2,4,6-trideoxy-α-L-altropyranoside (420)



420

To a solution of benzyl 3-O-acetate-2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **418** (522 mg, 1.51 mmol) in a mixture of tetrahydrofuran (5.0 mL) and water (0.5 mL), was added triphenylphosphine (791 mg, 3.01 mmol), and the mixture was stirred under reflux for 24 hours. The solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using a gradient of 0.5 → 1.0% methanol-dichloromethane (containing 0.25% ammonium hydroxide) as the eluent to afford compound **420** as a white solid (331 mg, 1.07 mmol, 71% yield). R_f = 0.36 (10% MeOH – CH_2Cl_2). $[\alpha]^{25}_D$ -147.72 (*c* 0.95, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 – 7.32 (m, 5H, ArH), 6.06 (d, *J* = 9.4 Hz, 1H, N4*H*CO), 4.82 (d, *J* = 1.0 Hz, 1H, H-1), 4.75 (d, *J* = 11.7 Hz, 1H, C*H*$_a$H$_b$Ph), 4.56 (d, *J* = 11.7 Hz, 1H, CH$_a$*H*$_b$Ph), 4.11 (ddd, *J* = 11.8, 9.4, 1.8 Hz, 1H, H-4), 3.81 (dq, *J* = 11.8, 6.3 Hz, 1H, H-5), 3.67 (dd, *J* = 2.2, 1.8 Hz, 1H, H-3), 3.22 (dd, *J* = 2.2, 1.0 Hz, 1H, H-2), 2.03 (s, 3H, Ac), 1.30 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.0 (CO), 136.4 (ArC), 128.6 (ArC), 128.2 (ArC), 128.1 (ArC), 100.2 (C-1), 72.1 (C-3), 69.9 (Ph*C*H$_2$CO), 64.6 (C-5), 52.6 (C-2), 49.2 (C-4), 23.4 (Ac), 17.9 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{15}H_{23}N_2O_4$ $[M+H]^+$: 295.1652; found 295.1667.

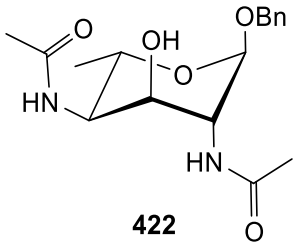
Benzyl 4-acetamido-2-chloroacetamido-2,4,6-trideoxy-α-L-altropyranoside (421)



421

To a solution of benzyl 3-O-acetate-2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **418** (47 mg, 0.14 mmol) in tetrahydrofuran (1.0 mL) and water (0.1 mL), was added triphenylphosphine (79 mg, 0.30 mmol), and the reaction mixture was stirred under reflux for 24 hours. The solvent was removed under reduced pressure. The crude mixture was redissolved in anhydrous methanol (2.0 mL), and chloroacetic anhydride (120 mg, 1.27 mmol) was then added. After stirring at room temperature for 12 hours, then the reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 30 % ethyl acetate – hexanes as the eluent to afford compound **421** as a white solid (42 mg, 0.11 mmol, 82% yield). R_f = 0.43 (60% acetone - hexanes). $[\alpha]^{25}_D$ -79.1 (*c* 2.98, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 – 7.31 (m, 5H, ArH), 6.78 (d, *J* = 8.6 Hz, 1H, N2*H*CO), 6.08 (d, *J* = 9.0 Hz, 1H, N4*H*CO), 4.84 (d, *J* = 1.4 Hz, 1H, H-1), 4.74 (d, *J* = 11.7 Hz, 1H, CH_aH$_b$Ph), 4.58 (d, *J* = 11.7 Hz, 1H, CH$_a$$H_b$Ph), 4.37 (ddd, *J* = 8.6, 3.1, 1.4 Hz, 1H, H-2), 4.08 (d, *J* = 15.3 Hz, 1H, Cl-CH_aH$_b$-CO), 4.02 (d, *J* = 15.3 Hz, 1H, Cl-CH$_a$$H_b$-CO), 3.94 (ddd, *J* = 11.8, 9.1, 2.7 Hz, 1H, H-4), 3.84 (dq, *J* = 11.8, 6.2 Hz, 1H, H-5), 3.77 (dd, *J* = 3.1, 2.7 Hz, 1H, H-3), 2.01 (s, 3H, Ac), 1.29 (d, *J* = 6.2 Hz, 3H, H-6).^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.9 (ClCH$_2$*C*O), 165.7 (CO), 135.7 (ArC), 128.8 (ArC), 128.6 (ArC), 128.2 (ArC), 97.7 (C-1), 70.2 (Ph*C*H$_2$O), 68.7 (C-3), 64.3 (C-5), 50.4 (C-2), 49.7 (C-4), 42.4 (Cl*C*H$_2$CO), 23.5 (Ac), 17.9 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{17}H_{23}ClN_2O_5Na$ $[M+Na]^+$: 393.1188; found 393.1194.

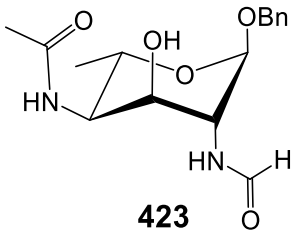
Benzyl 2,4-diacetamido-2,4,6-trideoxy-α-L-altropyranoside (422)



422

Benzyl 3-O-acetate-2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (**418**) (47 mg, 0.14 mmol) was dissolved in a mixture of tetrahydrofuran (1.0 mL) and water (0.1 mL), then triphenylphosphine (79 mg, 0.30 mmol) was added. After 24 hours of stirring under reflux, the solvent was evaporated under reduced pressure. The crude mixture was redissolved in anhydrous methanol (2.0 mL), to which was added excess acetic anhydride (1.0 mL). After stirring for 12 hours at room temperature, the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 40% ethyl acetate - hexanes to provide compound **422** as a white solid (40 mg, 0.12 mmol, 87% yield). R_f = 0.16 (60% acetone - hexanes). ^{1}H NMR and ^{13}C NMR data are in agreement with previously reported.[142]

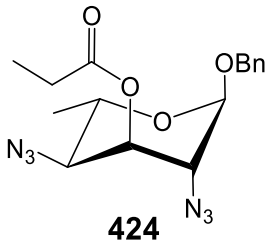
Benzyl 4-acetamido-2-formamido-2,4,6-trideoxy-α-L-altropyranoside (423)



423

To a solution of benzyl 3-O-acetate-2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **418** (53 mg, 0.15 mmol) in a mixture of tetrahydrofuran (1.0 mL) and water (0.1 mL), was added triphenylphosphine (84 mg, 0.32 mmol). After stirring under reflux for 24 hours, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using 2% methanol – dichloromethane as the eluent. The obtained compound (slightly impure according to ^{1}H NMR) was then redissolved in anhydrous dichloromethane (1.0 mL) to which were added DCC (95 mg, 0.459 mmol), 4-N,N-dimethylaminopyridine (DMAP, 6.0 mg, 0.046 mmol) and formic acid (7.0 µL, 0.18 mmol). After stirring the reaction for 12 hours at room temperature, the reaction mixture was evaporated to dry under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of 30 → 50 % acetone – hexanes as the eluent to afford compound **423** (41 mg, 0.13 mmol, 83% yield). Compound **12** was found to be a mixture of rotamer by ^{1}H NMR experiment. R_f = 0.55 (10% MeOH – CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) for the major rotamer: δ_H 8.17 (s, 1H, CHO), 7.44 – 7.30 (m, 5H, ArH), 6.30 (d, *J* = 9.0 Hz, 1H, N4*H*C0), 6.09 (d, *J* = 9.2 Hz, 1H, N2*H*CO), 4.83 (d, *J* = 0.70 Hz, 1H, H-1), 4.73 (d, *J* = 11.6 Hz, 1H, CH_aH$_b$Ph), 4.57 (d, *J* = 11.6 Hz, 1H, CH$_a$$H_b$Ph), 4.50 (ddd, *J* = 9.2, 2.9, 0.70 Hz, 1H, H-2), 4.02 (ddd, *J* = 12.1, 9.0, 2.8 Hz, 1H, H-4), 3.84 (dq, *J* = 12.1, 6.2 Hz, 1H, H-5), 3.76 (dd, *J* = 2.9, 2.8 Hz, 1H, H-3), 2.01 (s, 3H, Ac), 1.27 (d, *J* = 6.5 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 187.7 (*C*HO), 176.1 (CO), 146.6 (ArC), 138.1 (ArC), 137.5 (ArC), 137.4 (ArC), 101.1 (C-1), 67.7 (C-3), 66.3 (Ph*C*H$_2$CO), 60.5 (C-5), 43.1 (C-4), 41.8 (C-2), 23.5 (Ac), 17.8 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{16}H_{22}N_2O_5Na$ $[M+Na]^+$: 345.1421; found 345.1426.

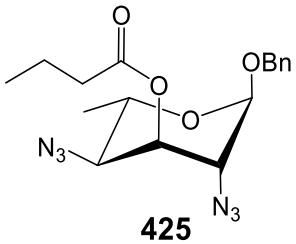
Benzyl 2,4-diazido-2,4,6-trideoxy-3-O-propionyl-α-L-altropyranoside (424)



424

Benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (**417**) (35 mg, 0.12 mmol) was dissolved in a mixture of propionic anhydride (0.2 mL) and anhydrous pyridine (1.0 mL) under argon atmosphere, and the reaction was stirred at room temperature for 24 hours. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 2% ethyl acetate - hexanes as the eluent to afford the pure compound **424** as a white solid (41 mg, 11 mmol, 99% yield). R_f = 0.43 (10% EtOAc - hexanes). $[\alpha]^{25}_D$ -68.6 (*c* 1.2, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.29 (m, 5H, ArH), 5.09 (dd, *J* = 5.8, 3.7 Hz, 1H, H-3), 4.77 (d, *J* = 11.8 Hz, 1H, CH_aH$_b$Ph), 4.76 (d, *J* = 3.1 Hz, 1H, H-1), 4.54 (d, *J* = 11.8 Hz, 1H, CH$_a$$H_b$Ph), 4.16 (dq, *J* = 8.2, 6.5 Hz, 1H, H-5), 3.93 (dd, *J* = 5.8, 3.1 Hz, 1H, H-2), 3.49 (dd, *J* = 8.1, 3.7 Hz, 1H, H-4), 2.39 (dq, *J* = 7.5, 5.0 Hz, 2H, CH_3CH_2CO), 1.34 (d, *J* = 6.5 Hz, 3H, H-6), 1.13 (t, *J* = 7.5 Hz, 3H, C$H_3$$CH_2$CO). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 173.6 (CH_3CH_2*C*O), 136.9 (ArC), 128.5 (ArC), 128.0 (ArC), 127.8 (ArC), 97.1 (C-1), 70.0 (Ph*C*H_2CO), 69.9 (C-3), 65.2 (C-5), 60.3 (C-4), 59.7 (C-2), 27.4 (CH_3*C*H_2CO), 18.07 (C-6), 8.71 (*C*H_3CH_2CO). HRMS (ESI-QTOF) calc'd *m/z* for $C_{16}H_{20}N_6O_4Na$ $[M+Na]^+$: 383.1438; found 383.1448.

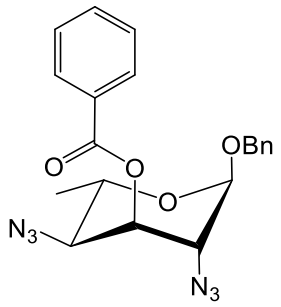
Benzyl 2,4-diazido-3-O-butyryl-2,4,6-trideoxy-α-L-altropyranoside (**425**)



425

Benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (**417**) (45 mg, 0.15 mmol) was dissolved in mixture of anhydrous pyridine (1.0 mL) and butyric anhydride (194 mg, 0.739 mmol), and the mixture was stirred at room temperature for 12 hours. The solution was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using 1% ethyl acetate – hexanes as the eluent to provide the pure compound **425** as a white foam (53 mg, 0.14 mmol, 96% yield). R_f = 0.42 (10% EtOAc - hexanes). $[\alpha]^{25}_D$ -81.8 (*c* 1.2, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.28 (m, 5H, ArH), 5.09 (dd, *J* = 5.7, 3.7 Hz, 1H, H-3), 4.78 (d, *J* = 3.0 Hz, 1H, H-1), 4.76 (d, *J* = 11.7 Hz, 1H, C*H*$_a$H$_b$Ph), 4.53 (d, *J* = 11.7 Hz, 1H, CH$_a$*H*$_b$Ph), 4.16 (dq, *J* = 8.4, 6.5 Hz, 1H, H-5), 3.92 (dd, *J* = 5.8, 3.0 Hz, 1H, H-2), 3.48 (dd, *J* = 8.2, 3.7 Hz, 1H, H-4), 2.36 (ddd, *J* = 16.2, 7.6, 7.6, 1H, $CH_3CH_2CH_aH_bCO$), 2.30 (ddd, *J* = 16.2, 7.3, 7.3, 1H, $CH_3CH_2CH_aH_bCO$), 1.63 (dt, *J* = 14.6, 7.4 Hz, 2H, $CH_3CH_2CH_2CO$), 1.34 (d, *J* = 6.5 Hz, 1H, H-6), 0.92 (t, *J* = 7.4 Hz, 3H, $CH_3CH_2CH_2CO$). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 172.8 ($CH_3CH_2CH_2CO$), 136.9 (ArC), 128.4 (ArC), 128.0 (ArC), 127.8 (ArC), 97.1 (C-1), 70.0 ($PhCH_2O$), 69.7 (C-3), 65.1 (C-5), 60.3 (C-2), 59.7 (C-4), 36.0 ($CH_3CH_2CH_2CO$), 18.1 ($CH_3CH_2CH_2CO$), 18.0 (C-6), 13.5 ($CH_3CH_2CH_2CO$). HRMS (ESI-QTOF) calc'd *m/z* for $C_{17}H_{22}N_6O_4Na$ $[M+Na]^+$: 397.1595; found 397.1601.

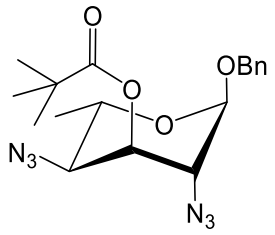
Benzyl 2,4-diazido-3-O-benzoyl-2,4,6-trideoxy-α-L-altropyranoside (426)



426

To a solution of benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **417** (47 mg, 0.15 mmol) in anhydrous dichloromethane (1.0 mL) and pyridine (1.0 mL), was added benzoyl chloride (0.10 mL, 0.86 mmol), and the reaction was stirred at room temperature overnight. Water was added to quench the reaction, and the mixture was extracted with ethyl acetate (5.0 mL) and washed with brine (5.0 mL x 3), 10% aqueous NaOH (10 mL) and dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 0.5% ethyl acetate – hexanes as the eluent to afford the pure compound **426** in quantitative yield (60 mg, 0.15 mmol). R_f = 0.21 (5% EtOAc - hexanes). $[\alpha]^{25}_D$ -64.9 (*c* 3.2, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.02 – 7.95 (m, 2H, BzH), 7.61 – 7.51 (m, 1H, BzH), 7.36 (m, 2H, BzH), 7.30 (m, 5H, BnH), 5.41 (dd, *J* = 4.9, 3.5 Hz, 1H, H-3), 4.88 (d, *J* = 2.4 Hz, 1H, H-1), 4.80 (d, *J* = 11.5 Hz, 1H, C*H*$_a$H$_b$Ph), 4.55 (d, *J* = 11.5 Hz, 1H, CH$_a$*H*$_b$Ph), 4.29 (dq, *J* = 9.0, 6.5 Hz, 1H, H-5), 4.03 (dd, *J* = 4.9, 2.4 Hz, 1H, H-2), 3.59 (dd, *J* = 8.9, 3.5 Hz, 1H, H-4), 1.40 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 165.6 (Ph*C*O), 136.8 (ArC), 133.5 (ArC), 130.0 (ArC), 129.0 (ArC), 128.4 (ArC), 127.9 (ArC), 97.2 (C-1), 70.2 (Ph*C*H$_2$CO), 69.6 (C-3), 64.5 (C-5), 60.4 (C-4), 59.9 (C-2), 18.2 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{20}H_{20}N_6O_4Na$ $[M+Na]^+$: 431.1444; found 431.1438.

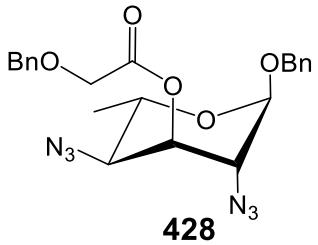
Benzyl 2,4-diazido-2,4,6-trideoxy-3-O-pivaloyl-α-L-altropyranoside (**427**)



427

Benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (**417**) (34 mg, 0.11 mmol) was dissolved in a solution of anhydrous pyridine (1.0 mL) and pivaloyl chloride (70 µL, 0.56 mmol) was added. The mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel using 1% ethyl acetate – hexanes as the eluent to yield the pure compound **427** (43 mg, 0.11 mmol, quantitative yield). R_f = 0.44 (10% EtOAc – hexanes). $[\alpha]^{25}_D$ -80.3 (*c* 5.2, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.41 – 7.27 (m, 5H, ArH), 5.05 (dd, *J* = 5.2, 3.6 Hz, 1H, H-3), 4.76 (d, *J* = 2.6 Hz, 1H, H-1), 4.72 (d, *J* = 11.6 Hz, 1H, CH_aH$_b$Ph), 4.54 (d, *J* = 11.6 Hz, 1H, CH$_a$$H_b$Ph), 4.16 (dq, *J* = 8.7, 6.5 Hz, 1H, H-5), 3.87 (dd, *J* = 5.2, 2.6 Hz, 1H, H-2), 3.48 (dd, *J* = 8.6, 3.6 Hz, 1H, H-4), 1.35 (d, *J* = 6.5 Hz, 3H, H-6), 1.17 (s, 9H, Piv). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 177.7 (tBut*C*O), 136.7 (ArC), 128.4 (ArC), 128.4 (ArC), 128.1 (ArC), 97.0 (C-1), 70.2 (Ph*C*H$_2$CO), 69.4 (C-3), 64.7 (C-5), 60.3 (C-4), 59.8 (C-2), 39.00 [(CH$_3$)$_3$*C*CO], 26.8 [(*C*H$_3$)$_3$CCO], 18.1 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{18}H_{24}N_6O_4Na$ $[M+Na]^+$: 411.1751; found 411.1753.

Benzyl 2,4-diazido-3-O-benzyloxyacetyl-2,4,6-trideoxy-α-L-altropyranoside (428)



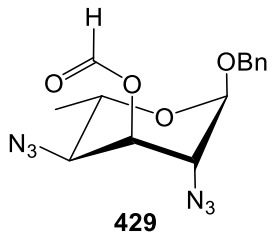
428

Sodium hydride (60% in mineral oil, 1.15 g, 28.8 mmol) was suspended in anhydrous THF (10.0 mL), and benzyl alcohol (3.13 mL, 30.2 mmol) was slowly added. The mixture was cooled to 0°C, then a solution of bromoacetic acid (2.0 g, 14.4 mmol) in anhydrous THF (8.0 mL) was slowly added. After stirring for 12 hours at room temperature, the desired benzyloxyacetic acid (1.70 g, 10.2 mmol, 71% yield) was isolated according to published literature.[184] R_f = 0.45 (40% EtOAc - hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.11 (s, 1H, COO*H*), 7.48 – 7.20 (m, 5H, ArH), 4.64 (s, 2H, PhC*H*$_2$O), 4.14 (s, 2H, BnOC*H*$_2$CO$_2$H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 175.0 (*C*OOH), 136.7 (ArC), 128.59 (ArC), 128.22 (ArC), 128.18 (ArC), 73.28 (Ph*C*H$_2$CO), 66.78 (BnO*C*H$_2$CO$_2$H).

To a solution of benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (**417**) (52 mg, 0.17 mmol) in anhydrous dichloromethane (1.0 mL), was added DCC (71 mg, 0.34 mmol), DMAP (4.0 mg, 0.034 mmol) and benzyloxyacetic acid (43 mg, 0.26 mmol). After stirring at room temperature for 24 hours, the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using 3% ethyl acetate - hexanes as the eluent to afford compound **428** in pure form (48 mg, 0.11 mmol, 62% yield). R_f = 0.39 (10% EtOAc - hexanes). $[\alpha]^{25}_D$ -69.6 (*c* 1.7, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 – 7.28 (m, 10H, ArH), 5.16 (dd, *J* = 5.5, 3.6 Hz, 1H, H-3), 4.79 (d, *J* = 2.8 Hz, 1H, H-1), 4.73

(d, *J* = 11.7 Hz, 1H, PhCH_aH$_b$), 4.59 (d, *J* = 12.3 Hz, 1H, PhCH_aH$_b$), 4.56 (d, *J* = 12.3 Hz, 1H, PhCH$_a$$H_b$), 4.51 (d, *J* = 11.7 Hz, 1H, PhCH$_a$$H_b$), 4.21 – 4.12 (m, 2H, H-5, BnOCH_aH$_b$CO), 4.06 (d, *J* = 16.8 Hz, 1H, BnOCH$_a$$H_b$CO), 3.96 (dd, *J* = 5.5, 2.8 Hz, 1H, H-2), 3.52 (dd, *J* = 8.4, 3.6 Hz, 1H, H-4), 1.34 (d, *J* = 6.5 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.7 (BnOCH$_2$*C*O), 136.8 (ArC), 128.5 (ArC), 128.5 (ArC), 127.9 (ArC), 96.9 (C-1), 73.4 (Ph*C*H$_2$O), 70.3 (C-3), 70.0 (Ph*C*H$_2$O), 66.7 (BnO*C*H$_2$CO), 65.0 (C-5), 60.2 (C-4), 59.4 (C-2), 18.0 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{22}H_{24}N_6O_5Na$ $[M+Na]^+$: 475.1700; found 475.1703.

Benzyl 2,4-diazido-3-O-formyl-2,4,6-trideoxy-α-L-altropyranoside (429)

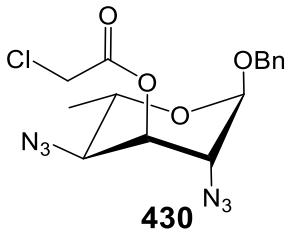


429

To a solution of benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **417** (104 mg, 0.342 mmol) in anhydrous dichloromethane (2.0 mL), was added DCC (141 mg, 0.684 mmol), DMAP (8.0 mg, 0.068 mmol) and formic acid (26 µL, 0.68 mmol), and the mixture was stirred for 24 hours at room temperature. The precipitate was filtered off and the solution was concentrated under reduced pressure. The crude was purified by column chromatography on silica gel using 2% ethyl acetate – hexanes as the eluent to afford compound **429** (92 mg, 0.28 mmol, 81% yield). R_f = 0.30 (10% EtOAc - hexanes). $[\alpha]^{25}_D$ -87.4 (*c* 2.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.15 (s, 1H, CHO), 7.36 (m, 5H, ArH), 5.21 (dd, *J* = 5.6, 3.7 Hz, 1H, H-3), 4.81 – 4.74 (m, 2H, H-1, PhCH_aH$_b$),

4.56 (d, *J* = 12.0 Hz, 1H, PhCH$_a$*H$_b$*), 4.16 (dq, *J* = 8.2, 6.5 Hz, 1H, H-5), 3.92 (dd, *J* = 5.6, 3.0 Hz, 1H, H-2), 3.55 (dd, *J* = 8.2, 3.7 Hz, 1H, H-4), 1.34 (d, *J* = 6.5 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 159.8 (*C*HO), 136.75 (ArC), 128.52 (ArC), 128.02 (ArC), 127.78 (ArC), 96.8 (C-1), 69.9 (Ph*C*H$_2$O), 69.1 (C-3), 65.2 (C-5), 60.3 (C-2), 59.6 (C-4), 18.0 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{14}H_{16}N_6O_4Na$ $[M+Na]^+$: 355.1125; found 355.1121.

Benzyl 2,4-diazido-3-O-chloroacetyl-2,4,6-trideoxy-α-L-altropyranoside (430)

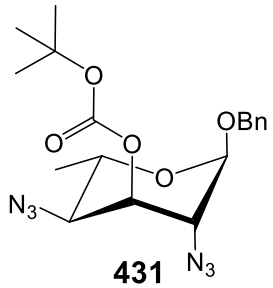


430

To a solution of benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **417** (104 mg, 0.341 mmol) in anhydrous pyridine (2.0 mL), was added chloroacetic anhydride (292 mg, 1.71 mmol), and the reaction was stirred room temperature for 3 hours. The reaction was then quenched with water, and the mixture was diluted with ethyl acetate (10 mL), washed with brine (10 mL x 3). The organic solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using 2% ethyl acetate – hexanes as the eluent to afford the pure compound **430** (111 mg, 0.291 mmol 85% yield). R_f = 0.27 (10% EtOAc - hexanes). $[\alpha]^{25}_D$ -59.6 (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 – 7.29 (m, 5H, ArH), 5.13 (dd, *J* = 5.2, 3.5 Hz, 1H, H-3), 4.81 (d, *J* = 2.7 Hz, 1H, H-1), 4.75 (d, *J* = 11.6 Hz, 1H, PhC*H$_a$*H$_b$), 4.53 (d, *J* = 11.6 Hz, 1H, PhCH$_a$*H$_b$*), 4.16 (dq, *J* = 8.7, 6.5 Hz, 1H, H-5), 4.08

(d, *J* = 15.4 Hz, 1H, ClC*H*$_a$H$_b$CO), 3.98 (d, *J* = 15.3 Hz, 1H, ClCH$_a$*H*$_b$CO), 3.96 (dd, *J* = 5.2, 2.7 Hz, 1H, H-2), 3.50 (dd, *J* = 8.6, 3.5 Hz, 1H, H-4), 1.35 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 166.6 (ClCH$_2$*C*O), 136.8 (ArC), 128.5 (ArC), 128.1 (ArC), 127.9 (ArC), 96.8 (C-1), 71.56 (C-3), 70.02 (Ph*C*H$_2$CO), 64.72 (C-5), 59.97 (C-4), 59.13 (C-2), 40.45 (Cl*C*H$_2$CO), 18.03 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{15}H_{17}ClN_6O_4Na$ $[M+Na]^+$: 403.0892; found 403.0904.

Benzyl 2,4-diazido-3-O-tert-butyloxycarbonyl-2,4,6-trideoxy-α-L-altropyranoside (431)



431

To a solution of benzyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside **417** (344 mg, 1.13 mmol) in anhydrous CH_2Cl_2 (10.0 mL), was added Boc_2O (987 mg, 4.52 mmol) and DMAP (55 mg, 0.45 mmol). After stirring at room temperature for 12 hours, the reaction mixture was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using 1% ethyl acetate - hexanes as the eluent to afford the pure compound **431** as a white foam (448 mg, 1.11 mmol, 98% yield). R_f = 0.51 (10% EtOAc - hexanes). $[\alpha]^{25}_D$ -90.5 (*c* 1.3, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.29 (m, 5H, ArH), 4.85 (dd, *J* = 6.2, 3.7 Hz,1H, H-3), 4.82 (d, *J* = 12.1 Hz, 1H, PhC*H*$_a$H$_b$), 4.78 (d, *J* = 3.4 Hz, 1H, H-1), 4.54 (d, *J* = 12.1 Hz, 1H, PhCH$_a$*H*$_b$), 4.15 (dq, *J* = 7.8, 6.6 Hz,1H, H-5), 3.99 (dd, *J* = 6.2, 3.4 Hz, 1H, H-2), 3.60 (dd, *J* = 7.8, 3.7 Hz, 1H,

H-4), 1.50 (s, 9H, Boc), 1.33 (d, *J* = 6.6 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 152.6 (tButO*C*O), 137.1 (ArC), 128.4 (ArC), 127.8 (ArC), 127.5 (ArC), 97.1 (C-1), 72.1 (C-3), 69.7 (Ph*C*H_2O), 65.8 (C-5), 60.8 (C-2), 59.8 (C-4), 27.6 (Boc), 17.9 (C6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{18}H_{24}N_6O_5Na$ $[M+Na]^+$: 427.1700; found 427.1713

General Procedure A for one-pot O3→N4 Migration and N2 acetylation

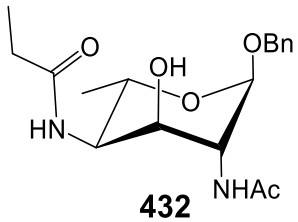
To a solution of O-acylated precursor in a 10:1 mixture of tetrahydrofuran and water was added triphenylphosphine (TPP) (2.2 eq.) and the reaction was stirred at 66 °C until monitoring by thin layer chromatography showed a complete reaction. The solution was then concentrated under reduced pressure, and the crude residue was redissolved in a 10:1 solution of anhydrous methanol and acetic anhydride. After stirring at room temperature until the reaction was completed, the solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to afford the pure desired compound.

General Procedure B for one-pot O3→N4 Migration and N2 acetylation

To a solution of O-acylated precursor in a 10:1 mixture of tetrahydrofuran and water was added trimethylphospine (TMP) (2.2 eq.) and the reaction was stirred at room temperature for 12 hours, after which it was heated to 60 °C until monitoring by thin layer chromatography showed complete hydrolysis. The solution was then concentrated under reduced pressure, and

the crude residue was redissolved in a 10:1 solution of anhydrous methanol and acetic anhydride. After stirring at room temperature until the reaction was completed, the solvents were evaporated under reduced pressure. The crude product was extracted with ethyl acetate and washed with water three times, the organic solution was dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel to afford the pure desired compound.

Benzyl 2-acetamido-2,4,6-trideoxy-4-propanamido-α-L-altropyranoside (432)

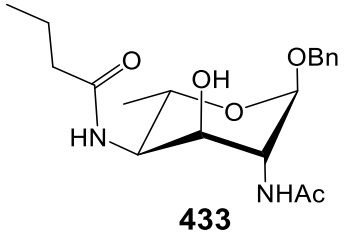


432

Compound **424** (13 mg, 0.036 mmol) was treated as per **procedure A** in 10:1 THF - water (1.1 mL) with TPP (21 mg, 0.079 mmol), followed by the acetylation in the MeOH:Ac_2O solution (1.1 ml). The residue was eluted using a gradient of 20 → 40 % acetone – hexanes to afford compound **432** (9.0 mg, 0.026 mmol, 71% yield). R_f = 0.24 (60% acetone - hexanes). $[\alpha]^{25}_D$ -76.1 (*c* 0.6, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 – 7.30 (m, 5H, ArH), 6.06 (d, *J* = 9.1 Hz, 1H, N^{4}HCO), 5.81 (d, *J* = 8.9 Hz, 1H, N2HCO), 4.80 (d, *J* = 2.0 Hz, 1H, H-1), 4.72 (d, *J* = 11.6 Hz, 1H, CH_aH$_b$Ph), 4.56 (d, *J* = 11.7 Hz, 1H, CH$_a$$H_b$Ph), 4.42 (ddd, *J* = 8.9, 2.0, 1.3 Hz, 1H, H-2), 3.95 (ddd, *J* = 12.3, 9.1, 2.1 Hz, 1H, H-4), 3.83 (dq, *J* = 12.3, 6.2 Hz, 1H, H-5), 3.73 (dd, *J* = 2.1, 1.3 Hz, 1H, H-3), 2.23 (q, *J* = 7.6 Hz, 2H, CH$_3$CH_2CO), 2.02 (s, 3H, Ac), 1.27 (d, *J* = 6.2 Hz, 3H, H-6), 1.16 (t, *J* = 7.6 Hz, 3H, CH_3CH$_2$CO). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 173.77 (CH$_3$CH$_2$*C*O), 169.41 (Me*C*O), 135.85 (ArC),

128.7 (ArC), 128.5 (ArC), 128.2 (ArC), 98.2 (C-1), 70.1 (Ph*C*H_2CO), 69.1 (C-3), 64.2 (C-5), 50.0 (C-4), 49.5 (C-2), 29.7 (*C*H_3CH_2CO), 23.3 (Ac), 17.8 (C-6), 9.8 (CH_3*C*H_2CO). HRMS (ESI-QTOF) calc'd *m/z* for $C_{18}H_{26}N_2O_5Na$ $[M+Na]^+$: 373.1734; found 373.1749

Benzyl 2-acetamido-4-butanamido-2,4,6-trideoxy-α-L-altropyranoside (433)

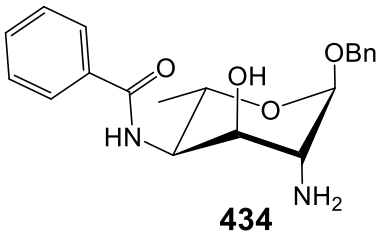


433

Compound **425** (25 mg, 0.064 mmol) was treated as per **procedure A** in 10:1 THF - water (1.1 mL) with TMP (1.0 M solution in THF, 0.14 mL, 0.14 mmol), followed by the acetylation in the MeOH:Ac_2O solution (1.1 ml). The residue was purified using a gradient of 20 → 30% acetone – hexanes as the eluent to afford compound **433** (20 mg, 0.053 mmol, 82% yield). R_f = 0.30 (60 % acetone - hexanes). $[\alpha]^{25}_D$ -11.9 (*c* 1.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.53 – 7.29 (m, 5H, ArH), 6.11 (d, *J* = 9.1 Hz, 1H, N^{4}HCO), 6.02 (d, *J* = 8.5 Hz, 1H, N2HCO), 4.80 (d, *J* = 1.3 Hz, 1H, H-1), 4.73 (d, *J* = 11.7 Hz, 1H, C*H*$_a$H$_b$Ph), 4.55 (d, *J* = 11.7 Hz, 1H, CH$_a$*H*$_b$Ph), 4.42 (ddd, *J* = 8.5, 1.9, 1.3 Hz, 1H, H-2), 3.99 (ddd, *J* = 10.6, 9.0, 2.9 Hz, 1H, H-4), 3.83 (dq, *J* = 10.6, 6.3 Hz, 1H, H-5), 3.73 (dd, *J* = 2.9, 1.9 Hz, 1H, H-3), 2.20 (ddd, *J* = 16.2, 7.6, 7.6, 1H, CH_3CH_2C*H*$_a$H$_b$CO), 2.14 (ddd, *J* = 16.2, 7.3, 7.3, 1H, CH_3CH_2CH$_a$*H*$_b$CO), 2.01 (s, 2H, Ac), 1.66 (dt, *J* = 14.6, 7.3 Hz, 2H, CH_3C*H*$_2$$CH_2$CO), 1.25 (d, *J* = 6.3 Hz, 3H, H-6), 0.94 (t, *J* = 7.4 Hz, 3H, C*H*$_3$$CH_2CH_2$CO). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 173.0 ($CH_3CH_2CH_2$*C*O), 169.6 (Me*C*O), 135.9 (ArC), 128.7 (ArC), 128.4 (ArC), 128.1 (ArC), 98.2 (C-1), 70.0 (Ph*C*H_2CO), 69.2 (C-3), 64.1 (C-5), 50.0 (C-2), 49.6 (C-4), 38.7 (CH_3CH_2*C*H_2CO), 23.3 (Ac), 19.1

($CH_3CH_2CH_2CO$), 17.9 (C-6), 13.7 ($CH_3CH_2CH_2CO$). HRMS (ESI-QTOF) calc'd *m/z* for $C_{19}H_{28}N_2O_5Na$ $[M+Na]^+$: 387.1890; found 387.1901.

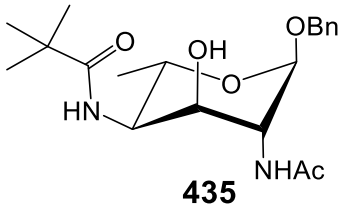
Benzyl 2-amino-4-benzamide-2,4,6-trideoxy-α-L-altropyranoside (**434**)



434

To a solution of compound **426** (63 mg, 0.105 mmol) in 10:1 THF - water (2.2 mL), was added TPP (81 mg, 0.31 mmol), and the solution was heated to reflux overnight. After 24 hours, the solution was evaporated to dry under reduced pressure. The residue was purified by column chromatography on silica using 20% acetone – toluene as the eluent to afford compound **434** (34 mg, 0.095 mmol, 92% yield). R_f = 0.33 (60% acetone - toluene). $[\alpha]^{25}_D$ -91.2 (*c* 3.3, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.87 – 7.71 (m, 2H, BzH), 7.56 – 7.31 (m, 8H, BzH & BnH), 6.79 (d, *J* = 9.1 Hz, 1H, N4HCO), 4.89 (d, *J* = 1.6 Hz, 1H, H-1), 4.76 (d, *J* = 11.7 Hz, 1H, CH_aH_bPh), 4.57 (d, *J* = 11.7 Hz, 1H, CH_aH_bPh), 4.35 (ddd, *J* = 9.7, 9.1, 3.1 Hz, 1H, H-4), 3.94 (dq, , *J* = 9.7, 6.3 Hz, 1H, H-5), 3.82 (dd, *J* = 3.4, 3.1 Hz, 1H, H-3), 3.30 (dd, *J* = 3.3, 1.6 Hz, 1H, H-2), 1.34 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 167.2 (Ph*C*O), 136.4 (ArC), 134.2 (ArC), 131.6 (ArC), 128.6 (ArC), 128.5 (ArC), 128.3 (ArC), 128.2 (ArC), 127.0 (ArC), 100.2 (C-1), 72.1 (C-3), 70.0 ($PhCH_2CO$), 64.7 (C-5), 52.6 (C-2), 49.6 (C-4), 18.1 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{20}H_{24}N_2O_4Na$ $[M+Na]^+$: 379.1628; found 379.1615.

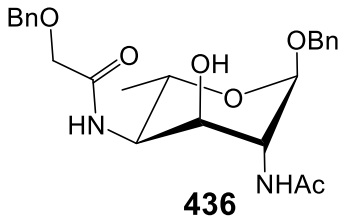
Benzyl 2-acetamido-2,4,6-trideoxy-4-(2',2'-dimethyl)propanamido-α-L-altropyranoside (435)



435

Compound **427** (34 mg, 0.88 mmol) was treated as per **procedure A** in 10 : 1 THF - water (1.65 mL) with TPP (51 mg, 0.19 mmol), followed by the acetylation in the 10: 1 MeOH:Ac_2O solution (1.1 mL). The residue was purified using 10% acetone in hexanes as the eluent to afford compound **435** in pure form (22 mg, 0.058 mmol, 74% yield). R_f = 0.47 (60% acetone - hexanes). $[\alpha]^{25}_D$ -91.0 (*c* 1.96, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 – 7.30 (m, 5H, ArH), 6.30 (d, *J* = 8.9 Hz, 1H, N^{4}HCO), 5.92 (d, *J* = 8.8 Hz, 1H, N2HCO), 4.81 (d, 1H, *J* = 1.3 Hz, H-1), 4.73 (d, *J* = 11.7 Hz, 1H, CH_aH$_b$Ph), 4.56 (d, *J* = 11.7 Hz, 1H, CH$_a$$H_b$Ph), 4.42 (dd, *J* = 8.9, 2.6, 1.3 Hz, 1H, H-2), 3.95 (dq, *J* = 10.4, 8.8, 2.9 Hz, 1H, H-4), 3.85 (dq, *J* = 10.4, 6.2 Hz, 1H, H-5), 3.71 (dd, *J* = 2.9, 2.6 Hz, 1H, H-3), 2.01 (s, 3H, Ac), 1.24 (d, *J* = 6.2 Hz, 3H, H-6), 1.20 (s, 9H, N4-Piv). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 178.6 (tBut*C*O), 169.5 (CO), 135.9 (ArC), 128.7 (ArC), 128.4 (ArC), 128.1 (ArC), 98.2 (C-1), 70.0 (Ph*C*H$_2$CO), 69.1 (C-1), 64.2 (C-5), 50.1 (C-2), 49.5 (C-4), 38.8 [(CH$_3$)$_3$*C*CO], 27.5 [(*C*H$_3$)$_3$CCO], 23.3 (Ac), 17.77 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{20}H_{30}N_2O_5Na$ $[M+Na]^+$: 401.2052; found 401.2062.

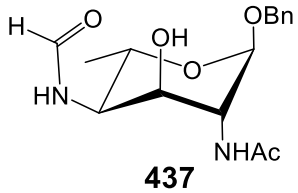
Benzyl 2-acetamido-4-benzyloxyacetamido-2,4,6-trideoxy-α-L-altropyranoside (436)



436

Compound **428** (24 mg, 0.053 mmol) was treated as per **procedure A** in 10:1 THF - water (1.1 mL) with TPP (31 mg, 0.12 mmol), followed by the acetylation in the 10 : 1 MeOH - Ac_2O solution (2.2 mL). The residue was purified using 30% acetone in hexanes as the eluent to afford compound **436** as a clear oil (23 mg, 0.053 mmol, 100% yield). R_f = 0.29 (60% acetone in hexanes). $[\alpha]^{25}_D$ -60.7 (*c* 1.7, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 – 7.30 (m, 10H, ArH), 7.20 (d, *J* = 9.3 Hz, 1H, N^{4}HCO), 5.80 (d, *J* = 8.8 Hz, 1H, N2HCO), 4.80 (d, *J* = 1.3 Hz, 1H, H-1), 4.73 (d, *J* = 11.7 Hz, 1H, PhCH_aH$_b$), 4.61 (d, *J* = 11.8 Hz, 1H, PhCH_aH$_b$), 4.56 (d, *J* = 11.7 Hz, 1H, PhCH$_a$$H_b$), 4.54 (d, *J* = 11.8 Hz, 1H, PhCH$_a$$H_b$), 4.43 (ddd, *J* = 8.9, 3.1, 1.3 Hz, 1H, H-2), 4.03 (d, *J* = 15.4 Hz, 1H, BnOCH_aH$_b$CO), 3.96 (d, *J* = 15.4 Hz, 1H, BnCH$_a$$H_b$CO), 3.94 (ddd, *J* = 10.6, 9.3, 2.8 Hz, 1H, H-4), 3.87 (dq, *J* = 10.6, 6.1 Hz, 1H, H-5), 3.75 (dd, *J* = 3.1, 2.8 Hz, 1H, H-3), 2.01 (s, 3H, Ac), 1.25 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.7 (BnOCH$_2$*C*O), 169.4 (CO), 136.6 (ArC), 135.8 (ArC), 128.7 (ArC), 128.6 (ArC), 128.5 (ArC), 128.3 (ArC), 128.2 (ArC), 128.1 (ArC), 98.2 (C-1), 73.7 (Ph*C*H$_2$OCH$_2$CO), 70.1 (C1-*C*H$_2$Ph), 69.3 (PhCH$_2$O*C*H$_2$CO), 69.0 (C-3), 63.9 (C-5), 50.0 (C-2), 49.4 (C-4), 23.3 (Ac), 17.9 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{24}H_{30}N_2O_6Na$ $[M+Na]^+$: 465.1996; found 465.2009.

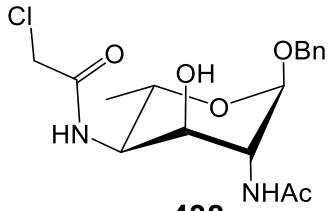
Benzyl 2-acetamido-2,4,6-trideoxy-4-formamido-α-L-altropyranoside (437)



437

Compound **429** (31 mg, 0.093 mmol) was treated as per **procedure A** in 10 : 1 THF - water (1.1 mL) with TPP (52 mg, 0.20 mmol), followed by the acetylation in the 10 : 1 MeOH - Ac_2O solution (2.2 ml). The residue was purified using a gradient of 20 → 40% acetone – hexanes as the eluent to afford compound **437** as a mixture of rotamer (23 mg, 0.071mmol, 76% yield). R_f = 0.45 (80% acetone - hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.23 (s, 1H, CHO) for the major rotamer, 7.43 – 7.28 (m, 5H, ArH), 6.25 (d, *J* = 9.4 Hz, 1H, N^4*H*CHO), 5.94 (d, *J* = 8.8 Hz, 1H, N2HCO), 4.81 (d, *J* = 0.9 Hz, 1H, H-1), 4.73 (d, *J* = 11.6 Hz, 1H, C*H*$_a$H$_b$Ph), 4.57 (d, *J* = 11.7 Hz, CH$_a$*H*$_b$Ph), 4.42 (ddd, *J* = 8.8, 2.2, 0.9 Hz, 1H, H-2), 4.07 (ddd, *J* = 10.6, 9.4, 3.6 Hz, 1H, H-4), 3.84 (dq, *J* = 10.6f, 6.2 Hz 1H, H-5), 3.75 (dd, *J* = 3.6, 2.2 Hz, 1H, H-3), 2.02 (s, 3H, Ac), 1.29 (d, *J* = 6.2 Hz, 3H. H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.49 (CO), 161.1 (*C*HO), 135.9 (ArC), 128.7 (ArC), 128.5 (ArC), 128.2 (ArC), 98.2 (C-1), 70.2 (Ph*C*H$_2$O), 68.9 (C-3), 63.8 (C-5), 49.9 (C-2), 48.5 (C-4), 23.2 (Ac), 17.8 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{16}H_{22}N_2O_5Na$ $[M+Na]^+$: 345.1421; found 345.1427.

Benzyl 2-acetamido-4-chloroacetamido-2,4,6-trideoxy-α-L-altropyranoside (438)



438

Procedure A

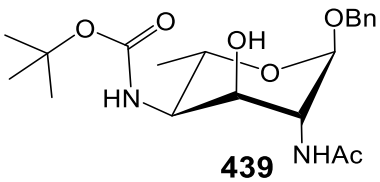
Compound **430** (23 mg 0.060 mmol) was treated as per **procedure A** in 10 : 1 THF - water (1.1 mL) with TPP (35 mg, 0.13 mmol), followed by the acetylation in the 10 : 1 MeOH - Ac_2O solution (2.2 mL). The residue was purified using a gradient of 10 → 20% acetone - hexanes as the eluent to afford compound **438** (6.0 mg, 0.016 mmol, 27% yield).

Procedure B

To a solution of benzyl 2-acetamido-4-tert-butyloxycarbonylamino-2,4,6-trideoxy-α-L-altropyranoside **439** (72 mg, 0.18 mmol) in dichloromethane (2.0 mL) was added trifluoroacetic acid (2.0 mL), and the mixture was stirred at room temperature for 2 hours. The reaction solution was evaporated to dry. The residue was redissolved in anhydrous methanol (3.0 mL), and chloroacetic anhydride (156 mg, 0.912 mmol) was added along with triethylamine (0.3 mL). After stirring the mixture for 12 hours, the reaction solution was evaporated to dry under reduced pressure. The crude product was purified by column chromatography on silica gel using a gradient of 20 → 30% acetone - hexanes as the eluent to afford the pure product **438** (56 mg, 0.15 mmol, 82% yield). R_f = 0.18 (10% MeOH – CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.46 – 7.30

(m, 5H, ArH), 7.14 (d, *J* = 8.4 Hz, 1H, N^4HCO), 5.93 (d, *J* = 8.8 Hz, 1H, N2HCO), 4.81 (d, *J* = 1.3 Hz, 1H, H-1), 4.74 (d, *J* = 11.7 Hz, 1H, PhCH_aH_b), 4.56 (d, *J* = 11.7 Hz, 1H, , PhCH_aH_b), 4.42 (ddd, *J* = 8.8, 3.1, 1.3 Hz, 1H, H-2), 4.06 (s, 2H, ClCH_2CONH), 4.01 – 3.86 (m, 2H, H-4, H-5), 3.77 (dd, *J* = 3.1, 1.8 Hz, 1H, H-3), 2.02 (s, 3H, Ac), 1.26 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.6 (CO), 166.2 ($ClCH_2CON$), 135.8 (ArC), 128.7 (ArC), 128.5 (ArC), 128.2 (ArC), 98.1 (C-1), 70.2 (PhCH_2O), 68.8 (C-3), 63.7 (C-5), 50.0 (C-2), 50.4 (C-4), 42.6 ($ClCH_2$CON), 23.3 (CH_3CO), 17.8 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{17}H_{23}ClN_2O_5Na$ $[M+Na]^+$: 393.1188; found 393.1201.

Benzyl 2-acetamido-4-(tert-butoxycabonyl)amido-2,4,6-trideoxy-α-L-altropyranoside (439)

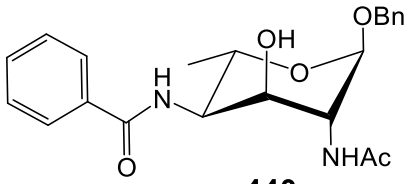


439

Compound **431** (951 mg, 2.21 mmol) was treated as per **procedure B** in 10 : 1 THF - water (5.5 mL) with TMP in THF (1.0 M, 4.7 mL, 4.7 mmol), followed by the acetylation in the 10 : 1 MeOH - Ac_2O solution (5.5 ml). The crude mixture was purified using 20% acetone - hexanes as the eluent to afford the pure product **439** as an oil (844 mg, 2.08 mmol, 94% yield). R_f = 0.32 (40% acetone in hexanes). $[\alpha]^{25}_D$ -96.9 (*c* 1.1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 – 7.28 (m, 5H, ArH), 5.93 (d, *J* = 9.0 Hz, 1H, N2HCO), 5.23 (d, *J* = 9.4 Hz, 1H, , N^4HCO), 4.79 (d, *J* = 1.3 Hz, 1H, H-1), 4.71 (d, *J* = 11.7 Hz, 1H, PhCH_aH_b), 4.54 (d, *J* = 11.6 Hz, 1H, PhCH_aH_b), 4.41 (ddd, *J* = 9.0, 3.1, 1.3 Hz, 1H, H-2), 3.81 (dq, *J* = 10.5, 6.3 Hz, 1H, H-5), 3.78 (dd, *J* = 3.1, 2.8 Hz, 1H, H-3), 3.57 (ddd, *J* = 10.5, 9.4, 2.8 Hz, 1H, H-4), 2.01 (s, 3H, Ac), 1.43 (s, 9H, Boc), 1.29 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C

NMR (101 MHz, $CDCl_3$) δ_C 169.56 (CO), 155.81 (tBuO*C*O), 135.93 (ArC), 128.69 (ArC), 128.31 (ArC), 128.16 (ArC), 98.20 (C-1), 70.05 (Ph*C*H_2O), 69.22 (C-3), 64.43 (C-5), 51.15 (C-4), 50.15 (C-2), 28.30 (tBu), 23.25 (Ac), 17.72 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{20}H_{30}N_2O_6Na$ $[M+Na]^+$: 417.1992; found 417.2008.

Benzyl 2-acetamido-4-benzamido-2,4,6-trideoxy-α-L-altropyranoside (440)



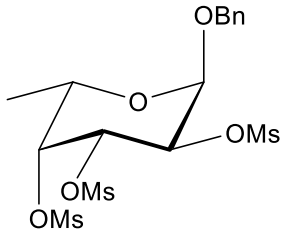
440

To a solution of benzyl 2-acetamido-4-tert-butyloxycarbonylamino-2,4,6-trideoxy-α-L-altropyranoside **439** (154 mg, 0.390 mmol) in dichloromethane (2.0 mL), was added trifluoroacetic acid (2.0 mL), and the reaction mixture was stirred at room temperature for 1.5 hours. The reaction solution was concentrated under reduced pressure. The residue was redissolved in anhydrous methanol (4 mL), and benzoic anhydride (272 mg, 1.21 mmol) was added along with triethylamine (0.4 mL). After stirring for 12 hours, reaction solution was evaporated to dry under reduced pressure. The crude product was purified by column chromatography on silica gel using a gradient of 10 → 15% acetone - hexanes as the eluent to afford the pure product **440** (148 mg, 0.371 mmol, 95% yield). R_f = 0.15. $[\alpha]^{25}_D$ -77.20 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.81 – 7.74 (m, 2H, ArH), 7.54 – 7.32 (m, 8H, ArH), 6.87 (d, *J* = 9.1 Hz, 1H, , N^4HCO), 6.37 (d, *J* = 8.8 Hz, 1H, , N2HCO), 4.87 (d, *J* = 1.3 Hz, 1H, H-1), 4.75 (d, *J* = 11.7 Hz, 1H, PhC$H_a$$H_b$), 4.59 (d, *J* = 11.7 Hz, 1H, PhC$H_a$$H_b$), 4.50 (ddd, *J* = 8.9, 3.2, 1.3 Hz, 1H, H-2),

4.29 (ddd, *J* = 10.6, 9.1, 2.8 Hz, 1H, H-4), 3.80 (dq, *J* = 10.6, 6.3 Hz, 3H, H-5), 3.86 (dd, *J* = 3.2, 2.8 Hz, 1H, H-3), 2.02 (s, 3H, Ac), 1.35 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.83 (CO), 167.44 (Bz*C*ON), 136.05 (ArC), 133.95 (ArC), 131.87 (ArC), 128.72 (ArC), 128.66 (ArC), 128.42 (ArC), 128.16 (ArC), 127.01 (ArC), 98.36 (C-1), 70.14 (Ph*C*H_2O), 69.21 (C-3), 64.19 (C-5), 50.24 (C-2), 50.14 (C-4), 23.26 (*C*H_3COO), 17.97 (C-6). HRMS (ESI-QTOF) calc'd *m/z* for $C_{22}H_{26}N_2O_5Na$ $[M+Na]^+$: 421.1734; found 421.1746.

Chapter 5 Procedures and Data

Benzyl 2,3,4-tri-O-methanesulfonyl-α-L-fucopyranoside (503)

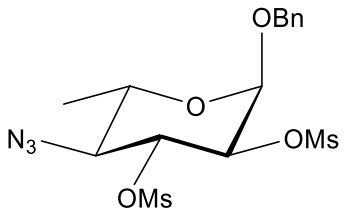


503

A mixture of benzyl α-L-fucopyranoside **502** (1.47 g, 5.78 mmol), dichloromethane (10 mL) and anhydrous pyridine (10 mL) was cooled to 0˚C under an argon atmosphere. Methanesulfonyl chloride (4.0 mL, 52 mmol) was added to the reaction mixture dropwise, and the temperature was allowed to warm up to ambient temperature while stirring for 20 hours. Methanol (5 mL) was added to quench the reaction, and the mixture was concentrated under reduced pressure. The residue was then redissolved in EtOAc (15 mL) and washed with 2 N HCl (10 mL) then brine (2 x 10 mL) and, after drying over sodium sulfate and evaporating the solvents under reduced pressure, the residue was purified by column chromatography on silica gel (10% acetone in hexanes) to yield the desired compound **503** as a sticky white solid (2.62 g, 5.36 mmol, 93% yield). R_f 0.54 (50% EtOAc in toluene). $[\alpha]^{25}_D$ -123 (*c* 0.78, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.33 (m, 5H, ArH), 5.17 (d, 1H, *J* = 3.8 Hz, H-1), 5.14 (dd, 1H, *J* = 3.4 Hz, 10.4 Hz, H-3), 5.05 (dd, 1H, *J* = 1 Hz, 3.4 Hz, H-4), 4.88 (dd, 1H, *J* = 3.8 Hz, 10.4 Hz, H-2), 4.74 (d, 1H, *J* = 12.0 Hz, OC*H*$_a$H$_b$Ph), 4.63 (d, 1H, *J* = 12.0 Hz, OCH$_a$*H*$_b$Ph), 4.17 (dq, 1H, *J* = 1 Hz, 6.6Hz, H-5), 3.22 (s, 3H, -OSO_2CH_3), 3.17 (s, 3H, -OSO_2CH_3), 2.92 (s, 3H, -OSO_2CH_3), 1.3 (d, 3H, *J* = 6.5 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 128.7 (ArC), 128.5 (ArC), 95.4 (C-1), 80.9 (C-4), 73.6 (C-3), 73.0 (C-2), 70.4 (-

CH_2Ph), 65.0 (C-5), 39.1 (-OSO_2CH_3), 39.05 (-OSO_2CH_3), 38.9 (-OSO_2CH_3), 16.2 (C-6). HRMS (ESI) calc'd *m/z* for $C_{16}H_{24}O_{11}S_3Na$ $[M+Na]^+$: 511.0373; found: 511.0369.

Benzyl 4-azido-4,6-dideoxy-2,3-di-O-methanesulfonyl-α-L-glucopyranoside (504)

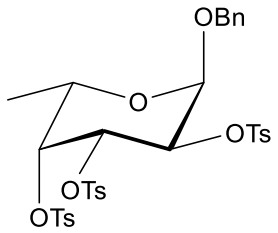


504

A mixture of benzyl 2,3,4-tri-O-methanesulfonyl-α-L-fucopyranoside **503** (5.02 g, 10.3 mmol), sodium azide (2.7 g, 41 mmol) and anhydrous DMF (50 mL) was heated to 80 ˚C under an atmosphere of argon for 48 hours. The reaction mixture was then cooled back to room temperature and concentrated under reduced pressure. EtOAc (200 mL) was added and the organic solution was washed with brine (3 × 200 mL), dried over anhydrous Na_2SO_4, and concentrated to afford a brown syrup. The crude mixture was purified by column chromatography on silica gel using a gradient of EtOAc - hexanes (10 → 20%) as the eluent to yield the desired compound **504** as a colourless syrup (4.07 g, 9.34 mmol, 91% yield). R_f = 0.75 (50% EtOAc in toluene). $[\alpha]^{25}_D$ -62 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.31 (m, 5H, ArH), 5.15 (d, 1H, *J* = 3.7 Hz, H-1), 4.96 (t, 1H, *J* = 9.7 Hz, H-3), 4.73 (d, 1H, *J* = 12.0 Hz, -CH_2Ph), 4.64 – 4.57 (m, 2H, -CH_2Ph and H-2), 3.78 (dq, 1H, *J* = 10.0 Hz, 6.2 Hz, H-5), 3.22 (t, 1H, *J* = 9.9 Hz, H-4), 3.19 (s, 3H, -OSO_2CH_3), 3.01 (s, 3H, -OSO_2CH_3), 1.36 (d, 3H, *J* = 6.2 Hz, H-6). ^{13}C NMR (101

MHz, $CDCl_3$) δ_C 136.2 (ArC), 128.6 (ArC), 128.3 (ArC), 128.2 (ArC), 95.5 (C-1), 77.5 (C-3), 75.2 (C-2), 70.4 (CH_2Ph), 66.9 (C-5), 66.5 (C-4), 39.2 (-OSO_2CH_3), 38.7 (-OSO_2CH_2), 18.0 (C-6). HRMS (ESI) calc'd *m/z* for $C_{15}H_{21}N_3O_8S_2Na$ $[M+Na]^+$: 458.0655; found: 458.0662.

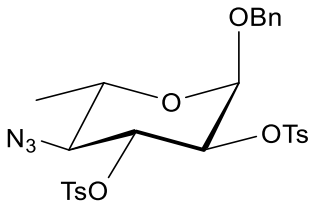
Benzyl 2,3,4-tri-O-*p*-toluenesulfonyl-α-L-fucopyranoside



Benzyl α-L-fucopyranoside **502** was prepared according to a previous procedure.[142] A solution of benzyl α-L-fucopyranoside (1.0 g, 3.93 mmol) in anhydrous pyridine (1.5 mL) and CH_2Cl_2 (10.0 mL) was cooled to 0 °C under an atmosphere of argon; p-toluenesulfonyl chloride (3.37 g, 17.7 mmol) was then added portion wise, and the temperature was allowed to warm up to ambient temperature. After stirring for 24 hours, the reaction was heated to 50 °C for 3 days. EtOAc (50 mL) was added to dilute the reaction mixture and the organic solution was washed with brine (2 x 50 mL), dried with anhydrous Na_2SO_4 and evaporated under reduced procedure. The crude mixture was purified by column chromatography on silica gel using 5% EtOAc-hexanes as an eluent to yield the desired compound as a clear syrup (2.4 g, 86% yield). R_f = 0.45 (40% EtOAc in hexanes). $[\alpha]^{25}_D$ -48.1 (*c* 10.1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.78 (d, 2H,-OSO_2ArH-), 7.66 (d, 2H, -OSO_2ArH-), 7.53 (d, 2H, -OSO_2ArH-), 7.42 – 7.33 (m, 5H, ArH), 7.33 (d, 2H, - OSO_2ArH-), 7.28 (d, *2*H, - OSO_2ArH-), 7.20 (d, *2*H, - OSO_2ArH-),5.13 (dd, *J* = 3.0 Hz, 0.7, 1H,

H-4), 4.94 (d, *J* = 3.7 Hz, 1H, H-1), 4.91 (dd, *J* = 10.6 Hz, 3.0 Hz, 1H, H-3), 4.58 (dd, *J* = 10.6 Hz, 3.70 Hz, 1H, H-2), 4.56 (d, *J* = 12.0 Hz, 1H, C*H*$_a$H$_b$Ph), 4.35 (d, *J* = 12.0 Hz, 1H, CH$_a$C*H*$_b$Ph), 4.05 (dq, *J* = 6.8 Hz, 0.7 Hz, 1H, H-5), 2.46 (s, 3H, -OSO$_2$BnC*H*$_3$), 2.45 (s, 3H, -OSO$_2$BnC*H*$_3$), 2.42 (s, 3H, -OSO$_2$BnC*H*$_3$), 1.19 (t, *J* = 6.8 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 145.1 (-OSO$_2$ArC-), 144.9 (-OSO$_2$ArC-), 144.7 (-OSO$_2$ArC-), 129.6 (ArC), 129.6 (ArC), 129.6 (ArC), 128.5 (-OSO$_2$ArC-), 128.4 (-OSO$_2$ArC-), 128.0(-OSO$_2$ArC-), 127.8 (-OSO$_2$ArC-), 127.6(-OSO$_2$ArC-), 96.1 (C-1), 81.0 (C-4), 73.0 (C-3), 72.6 (C-2), 70.4 (-CH$_2$Ph), 65.3 (C-5), 21.73 (-OSO$_2$Bn*C*H$_3$), 21.7 (-OSO$_2$Bn*C*H$_3$), 21.68 (-OSO$_2$Bn*C*H$_3$), 16.4 (C-6). HRMS (ESI) calc'd *m/z* for $C_{34}H_{40}NO_{11}S_3$ $[M+NH_4]^+$: 734.1758; found: 734.1760.

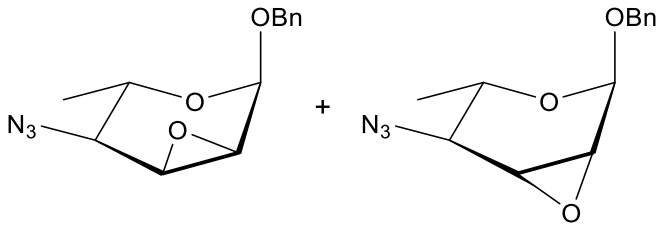
Benzyl 4-azido-4,6-dideoxy-2,3-di-O-*p*-toluenesulfonyl-α-L-glucopyranoside



To a solution of benzyl 2,3,4-tri-O-*p*-toluenesulfonyl-α-L-fucopyranoside (1.0 g, 1.7 mmol) in anhydrous DMF (10.0 mL) was added sodium azide (0.23 g, 4.3 mmol), and the mixture was heated to 90 °C under an atmosphere of argon. After stirring for 36 hours. the reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), and the solution was washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using 10% EtOAc in hexanes as a eluent to yield the

desired compound as a colorless paste (620 mg, 82% yield). R_f 0.73 (40% EtOAc in hexanes). $[\alpha]^{25}_D$ -746 (*c* 0.6, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.86 (m, 1H, -OSO_2ArH-), 7.83 (m, 1H, -OSO_2ArH-), 7.67 (m, 1H, -OSO_2ArH-), 7.65 (m, 1H, -OSO_2ArH-), 7.42 – 7.33 (m, 5H, ArH), 7.27 (m, 1H, -OSO_2ArH-), 7.24 (m, 1H, -OSO_2ArH-), 5.02 (d, *J* = 3.6 Hz, 1H, H-1), 4.94 (dd, *J* = 9.7 Hz, *J* = 9.7 Hz, 1H, H-3), 4.61 (d, *J* = 11.9 Hz, 1H, C$H_a$$H_b$Ph), 4.45 (d, *J* = 11.9 Hz, 1H, CH_aCH_bPh), 4.33 (dd, *J* = 9.7, 3.6 Hz, 1H, H-2), 3.65 (dq, *J* = 11.9 Hz, 6.2 Hz, 1H, H-5), 3.03 (dd, *J* = 9.9 Hz, 9.7 Hz, 1H, H-4), 2.45 (s, 3H, -OSO_2BnCH_3), 2.43 (s, 3H, -OSO_2BnCH_3), 1.24 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_H 145.2 (-OSO_2ArC-), 144.9 (-OSO_2ArC-), 129.8 (ArC), 129.7 (ArC), 128.5 (ArC), 128.2 (ArC), 128.1 (-OSO_2ArC-), 128.0 (-OSO_2ArC-), 95.8 (C-1), 77.3 (C-3),75.6 (C-2), 70.6 (-CH_2Ph), 67.4 (C-4), 66.5 (C-5), 21.7 (-OSO_2Bn*C*H_3), 21.68 (-OSO_2Bn*C*H_3), 17.9 (C-6). HRMS (ESI) calc'd *m/z* for $C_{27}H_{33}N_4O_8S_2$ $[M+NH_4]^+$: 605.1734; found: 605.1735.

Benzyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-allopyranoside (505) and Benzyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-mannopyranoside (506)



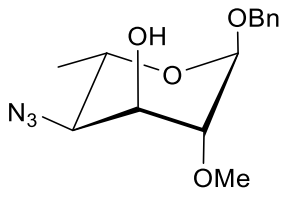
505
L-allo

506
L-manno

A solution of benzyl 4-azido-4,6-dideoxy-2,3-di-O-methanesulfonyl-α-L-glucopyranoside **504** (1.38 g, 3.17 mmol) in anhydrous methanol (10.0 mL) was cooled to 0 °C under an

atmosphere of argon, then a solution of potassium methoxide, prepared by reacting potassium (0.30 g, 6.98 mmol) with anhydrous methanol (10 mL), was added, and the mixture was heated to 50 °C. After stirring for 24 hours, the mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), and the obtained solution was washed with water (3 × 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using a gradient of EtOAc in hexanes (1→2%) as the eluent to yield the desired compound **505** (680 mg, 2.6 mmol, 82% yield) and its isomer **506** (84 mg, 0.32 mmol, 10% yield). *See Chapter 3 for **505** data: Benzyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-allopyranoside (**333**).* Data for **506**: R_f = 0.61 (40% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 – 7.28 (m, 5H, ArH), 5.01 (dd, 1H, *J* = 3.2 Hz, <1 Hz, H-1), 4.78 (d, 1H, *J* = 12.0 Hz, -CH_2Ph), 4.65 (d, 1H, *J* = 12.3 Hz, -CH_2Ph), 3.96 (dq, 1H, *J* = 9.6 Hz, 6.3 Hz, H-5), 3.55 (dd, 1H, *J* = 4.1 Hz, 1.6 Hz, H-3), 3.49 (dd, 1H, *J* = 4.1 Hz, 3.2 Hz, H-2), 3.16 (dd, 1H, *J* = 9.6 Hz, 1.5 Hz, H-4), 1.23, (d, 3H, *J* = 6.3 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 137.4 (ArC), 128.5 (ArC), 128.1 (ArC), 127.9 (ArC), 92.0 (C-1), 69.5 (-CH_2Ph), 63.2 (C-5), 61.5 (C-4), 53.7 (C-2), 52.3 (C-3), 17.8 (C-6). Both ^{1}H and ^{13}C NMR are in agreement with previously reported data.[142]

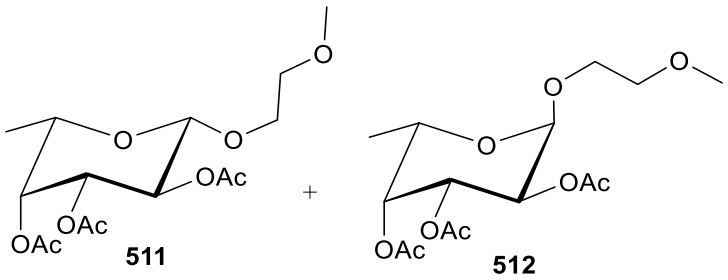
Benzyl 4-azido-4,6-dideoxy-2-O-methyl-α-L-altropyranoside (507)



507

Benzyl 4-azido-4,6-dideoxy-2,3-di-O-methanesulfonyl-α-L-glucopyranoside **504** (1.28 g, 2.94 mmol) was dissolved in anhydrous methanol (30.0 mL), and KOBu-*t* (1.5 g, 13.38 mmol) was added. The mixture was heated to 50 °C for 48 hours. The mixture was cooled and concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), and the organic solution was washed with water (10 mL) and brine (2 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by column chromatography on silica gel using a gradient of EtOAc in hexanes (1→2%) as an eluent to yield first compound **505** (294 mg, 1.13 mmol, 38% yield), and then compound **506** (236 mg, 0.903 mmol, 31% yield) and compound **507** (89 mg, 0.30 mmol, 10% yield). Data for compound **507**: R_f 0.20 (20% EtOAc in hexanes, run twice). $[\alpha]^{25}_D$ -121˚ (*c* 1.9, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 – 7.30 (m, 5H, ArH), 4.92 (d, *J* = 1.5 Hz, 1H, H-1), 4.77 (d, *J* = 11.8 Hz, 1H, CH_aH$_b$Ph), 4.57 (d, *J* = 11.8 Hz, 1H, , CH$_a$$H_b$Ph), 4.18 (br s, 1H, H-2), 4.04 (dq, *J* = 10.2, 6.2 Hz, 1H, H-5), 3.52 – 3.44 (m, 2H, H-3, OH), 3.41 (s, 3H, OCH_3), 3.21 (dd, *J* = 10.3, 3.0 Hz, 1H, H-4), 1.37 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 136.20 (ArC), 128.67 (ArC), 128.33 (ArC), 128.14 (ArC), 96.95 (C-1), 77.52 (C-3), 69.89 (CH_2Ph), 68.36 (C-2), 62.28 (C-5), 61.59 (C-4), 58.24 (OMe), 18.26 (C-6). HRMS (ESI) calc'd *m/z* for $C_{14}H_{23}N_4O_4$ $[M+NH_4]^+$: 311.1714; found: 311.1721.

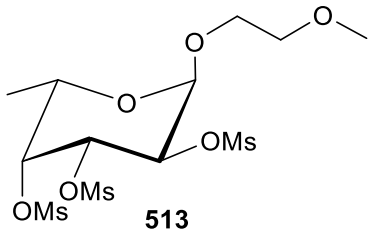
2-Methoxyethyl 2,3,4-tri-O-acetyl-α-L-fucopyranoside (511) and 2-methoxyethyl 2,3,4-tri-O-acetyl-β-L-fucopyranoside (513)



To a suspension of L-fucose (1.26 g, 7.68 mmol) in anhydrous 2-methoxyethanol (4.0 mL), was added H_2SO_4-silica (20 mg), and the mixture was heated to 70 °C for 5 hours. The obtained clear solution was evaporated under reduced pressure. The residue was redissolved in anhydrous pyridine (5.0 mL) and acetic anhydride (5.0 mL) was added. After stirring the reaction overnight at room temperature, the solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of EtOAc in hexanes (2→5%) as the eluent to afford first the α-anomer **511** (990 mg, 2.84 mmol, 37% yield) as a white solid, then the β-anomer **512** (454 mg, 1.31mmol, 17% yield) as a yellowish oil. Data for compound **511**: R_f = 0.22 (40% EtOAc in hexanes). $[\alpha]^{25}_D$ -95.4 (*c* 0.73, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.43 – 5.34 (high order m, 1H, H-3), 5.31 (dd, *J* = 3.4, 1.3 Hz, 1H, H-4), 5.17 – 5.08 (m, 2H, H-2 + H-1), 4.24 (dq, *J* = 6.6, 1.3 Hz, 1H, H-5), 3.80 (ddd, *J* = 11.2, 5.6, 3.9 Hz, 1H, OCH_aH_b), 3.64 (ddd, *J* = 11.3, 5.9, 3.5 Hz, 1H, OCH$_a$*H$_b$*), 3.55 (m, 2H, CH_cH_dOCH$_3$), 3.38 (s, 3H, OCH$_3$), 2.17 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.15 (d, *J* = 6.6 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 96.3 (C-1), 71.4 (*C*H$_c$H$_d$OCH$_3$), 71.2 (C-4), 68.2 (C-2 or C-3), 68.0 (C-2 or C-3), 67.5 (O*C*H$_a$H$_b$), 64.3 (C-5), 59.0 (OCH$_3$), 20.8 (OAc), 20.70 (OAc), 20.66 (OAc), 15.9 (C-6). HRMS (ESI) calc'd *m/z* for $C_{15}H_{24}O_9Na$ $[M+Na]^+$: 371.1324; found: 371.1313. Data for compound **512**: R_f = 0.18 (40% EtOAc in hexanes).

$[\alpha]^{25}_{D}$ -3.11 (*c* 0.60, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.27 – 5.16 (m, 2H, H-2, H-4), 5.03 (dd, *J* = 10.4, 3.5 Hz, 1H, H-3), 4.53 (d, *J* = 8.0 Hz, 1H, H-1), 3.97 (ddd, *J* = 11.1, 4.2, <1 Hz, 1H, OC*H*$_a$H$_b$), 3.81 (dq, *J* = 6.4, <1 Hz, 1H, H-5), 3.72 (ddd, *J* = 11.0, 6.3, 4.6 Hz, 1H, OCH$_a$*H$_b$*), 3.60 – 3.50 (m, 2H, C*H*$_c$*H*$_d$OCH$_3$), 3.37 (s, 3H, OCH_3), 2.18 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.23 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.6 (O*C*OCH$_3$), 101.3 (C-1), 71.7 (*C*H$_c$H$_d$OCH$_3$), 71.34 (C-3), 70.28 (C-4), 69.2 (C-5), 68.9 (C-2), 68.8 (O*C*H$_a$H$_b$), 59.1 (OCH_3), 20.8 (Ac), 20.7 (Ac), 20.6 (Ac), 16.1 (C-6). HRMS (ESI) calc'd *m/z* for $C_{15}H_{24}O_9Na$ $[M+Na]^+$: 371.1327; found: 371.1313.

2-Methoxyethyl 2,3,4-tri-O-methanesulfonyl-α-L-fucopyranoside (513)

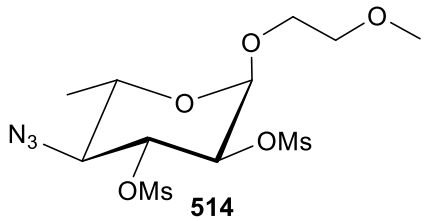


513

To a solution of 2-methoxyethyl 2,3,4-tri-O-acetyl-α-L-fucopyranoside **511** (508 mg, 1.46 mmol) in anhydrous methanol (10.0 mL) was added NaOMe (10 mg, 0.15 mmol), and the mixture was stirred at room temperature overnight. The solution was evaporated to dry. The residue was redissolved in anhydrous pyridine (10.0 mL) at 0 °C, and methanesulfonyl chloride (1.0 mL, 13 mmol) was added dropwise. After stirring the reaction at room temperature overnight, the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), and the solution was washed with aq. HCl (1.0 M, 10 mL), sat. brine (10 mL) and water (10 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel using a gradient of acetone in hexanes (10→40%) as the eluent to

afford the desired product **513** as a light yellow oil (598 mg, 1.31 mmol, 90% yield). R_f = 0.18 (40% acetone in hexanes). $[\alpha]^{25}_D$ -60.3 (*c* 7.5, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.19 (d, *J* = 3.8 Hz, 1H, H-1), 5.14 (dd, *J* = 10.4, 3.4 Hz, 1H, H-3), 5.05 (dd, *J* = 3.4, <1 Hz 1H, H-4), 4.88 (dd, *J* = 10.3, 3.7 Hz, 1H, H-2), 4.25 (dq, *J* = 6.6, <1 Hz, 1H, H-5), 3.86 (ddd, *J* = 11.6, 4.9, 3.9 Hz, 1H, OC*H*$_a$H$_b$), 3.75 (ddd, *J* = 11.6, 5.1, 3.9 Hz, 1H, OCH$_a$*H$_b$*), 3.59 (m, 2H, C*H*$_c$*H*$_d$OCH$_3$), 3.38 (s, 3H, OCH_3), 3.23 (s, 3H, OMs), 3.18 (s, 3H, OMs), 3.14 (s, 3H, OMs), 1.33 (d, *J* = 6.5 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 96.4 (c-1), 80.9 (C-4), 73.6 (C-3), 73.1 (C-2), 71.3 (*C*H$_c$H$_d$OCH$_3$), 67.7 (O*C*H$_a$H$_b$), 64.6 (OCH_3), 58.9 (OMs), 39.1 (OMs), 39.08 (OMs), 16.3 (C-6). HRMS (ESI) calc'd *m/z* for $C_{12}H_{28}NO_{12}S_3$ $[M+NH_4]^+$: 474.0768; found: 474.0774.

2-Methoxyethyl 4-azido-4-deoxy-2,3-di-O-methanesulfonyl-α-L-fucopyranoside (514)

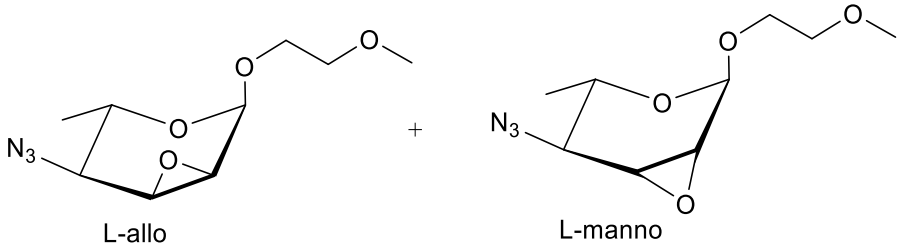


514

A solution of 2-methoxyethyl 2,3,4-tri-O-methanesulfonyl-α-L-fucopyranoside **513** (576 mg, 1.26 mmol) and sodium azide (328 mg, 5.04 mmol) in DMF (10.0 mL) was heated to 90 °C with stirring overnight. The mixture was cooled and evaporated under reduced pressure. The crude mixture was extracted with hot EtOAc (3 x 10 mL), and the combined solutions were evaporated. The obtained residue was purified by column chromatography on silica gel using a gradient of EtOAc – hexanes (10→20%) as the eluent to afford the desired product **514** in pure

form (347 mg, 0.861mmol, 68% yield). R_f = 0.41 (40% acetone in hexanes). $[\alpha]^{25}_D$ -100 (*c* 2.9, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.14 (d, *J* = 3.7 Hz, 1H, H-1), 4.91 (t, *J* = 9.7 Hz, 1H, H-3), 4.57 (dd, *J* = 9.8, 3.7 Hz, 1H, H-2), 3.96 – 3.73 (m, 2H, H-5, OC*H*$_a$H$_b$), 3.69 (ddd, *J* = 11.6, 5.1, 3.9 Hz, 1H, OCH$_a$*H$_b$*), 3.58-3.52 (m, 2H, C*H$_c$H$_d$*OCH$_3$), 3.35 (s, 3H, OCH$_3$), 3.23-3.18 (m, 2H, H-4, OMs), 3.14 (s, 3H, OMs), 1.36 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 96.2 (C-1), 77.6 (C-3), 75.4 (C-2), 71.3 (*C*H$_c$H$_d$OCH$_3$), 67.7 (O*C*H$_a$H$_b$), 66.8 (C-4), 66.1 (C-5), 58.9 (OCH$_3$), 39.3 (OMs), 38.9 (OMs), 18.1 (C-6). HRMS (ESI) calc'd *m/z* for $C_{11}H_{25}N_4O_9S_2$ $[M+NH_4]^+$: 421.1058; found: 421.1072.

2-Methoxyethyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-allopyranoside (515) and 2-methoxyethyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-mannopyranoside (516)

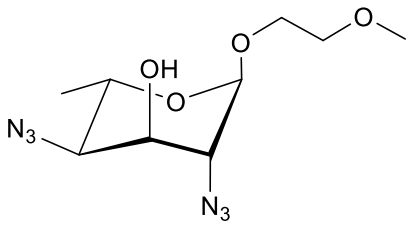


L-allo **515** + L-manno **516**

To a solution of 2-methoxyethyl 4-azido-4-deoxy-2,3-di-O-methanesulfonyl-α-L-fucopyranoside **514** (305 mg, 0.756 mmol) in anhydrous methanol (5.0 mL), was added a solution of KOMe (prepared by dissolving K (118 mg, 3.02 mmol) in anhydrous methanol (10 mL)) at 0°C, and the mixture was warmed up to ambient temperature and then heated to 50 °C. After stirring the mixture for 12 hours, the solution was evaporated to dry and the residue was then redissolved in EtOAc (10 mL), and the solution was washed with water (10 mL) and sat. brine (2 x 10 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue

was purified by column chromatography on silica gel using a gradient of EtOAc in hexanes (1→5%) as the eluent to afford first the L-manno-epoxide **516** (12 mg, 0.052 mmol, 7% yield) and then desired L-allo-epoxide **515** (114 mg, 0.499 mmol, 66% yield). Data for L-manno-epoxide **516**: R_f = 0.70 (40% acetone in hexanes). $[\alpha]^{25}_D$ -64˚ (*c* 0.33, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.00 (br-s, 1H, H-1), 3.87 (ddd, *J* = 3.9, 4.9, 11.0 Hz, 1H, OC*H*$_a$H$_b$), 3.75 – 3.56 (m, 4H, OCH$_a$*H*$_b$ + H-5 + C*H*$_c$*H*$_d$OCH$_3$), 3.41 (s, 3H, OCH$_3$), 3.37 – 3.29 (m, 2H, H-2 + H-4), 3.16 (dd, *J* = <1, 3.6 Hz, 1H, H-3), 1.28 (d, 1H, *J* = 3.9 Hz, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 94.9 (C-1), 71.6 (*C*H$_c$H$_d$OCH$_3$), 67.5 (OCH$_a$H$_b$), 63.6 (C-5), 59.3 (C-4), 59.1 (OCH$_3$), 53.5 (C-2), 49.5 (C-3), 18.8 (C-6). HRMS (ESI) calc'd *m/z* for $C_9H_{15}N_3O_4Na$ $[M+Na]^+$: 252.0955; found: 252.0966. Data for the L-allo-epoxide **515**: R_f = 0.61 (40% acetone in hexanes). $[\alpha]^{25}_D$ -139 (*c* 1.8, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.04 (br-s, 1H, H-1), 3.93 (dq, *J* = 6.4, 9.7 Hz, 1H, H-5), 3.85 (ddd, *J* = 3.5, 5.0, 11.2 Hz, 1H, OC*H*$_a$H$_b$), 3.72 (ddd, *J* = 3.7, 7.0, 11.2 Hz, 1H, OCH$_a$*H*$_b$), 3.47-3.67 (m, 4H, C*H*$_c$*H*$_d$OCH$_3$ + H-2 + H-3), 3.39 (s, 3H, OCH$_3$), 3.15 (dd, *J* = <1, 9.6 Hz, H-4), 1.26 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 93.5 (C-1), 71.8 (*C*H$_c$H$_d$OCH$_3$), 67.3 (OCH$_a$H$_b$), 63.0 (C-5), 61.5 (C-4), 59.1 (OCH$_3$), 53.5 (C-2 or C-3), 52.2 (C-3 or C-2), 17.9 (C-6). HRMS (ESI) calc'd *m/z* for $C_9H_{15}N_3O_4Na$ $[M+Na]^+$: 252.0955; found: 252.0964.

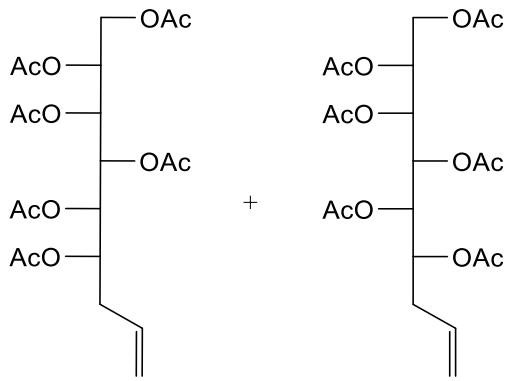
2-Methoxyethyl 2,4-diazido-2,4,6-trideoxy-α-L-altropyranoside (517)



517

To a solution of 2-methoxyethyl 2,3-anhydro-4-azido-4,6-dideoxy-α-L-allopyranoside **515** (71 mg, 0.31 mmol) in anhydrous acetonitrile (10.0 mL), was added sodium azide (80 mg, 1.2 mmol) and lithium perchlorate (132 mg, 1.24 mmol), and the reaction was heated to 85 °C for 48 hours. The solvents were removed under reduced pressure. The residue was dissolved in EtOAc (10 mL), and the solution was washed with water (10 mL) and brine (2 x 10 mL), and dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using a gradient of EtOAc – hexanes (5→10%) as the eluent to afford the pure desired product **517** (12 mg, 0.044 mmol, 14% yield). The aqueous wash layer was evaporated to dry, then more crude product was recovered by extraction using hot EtOAc (3 x 10 mL) using ^{1}H NMR, but not quantified. R_f = 0.52 (40% acetone in hexanes, run twice). $[\alpha]^{25}_D$ -18 (*c* 0.7, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.90 (d, *J* = 1.6 Hz, 1H, H-1), 4.16-4.09 (m, 2H, H-3, H-5), 3.95 – 3.83 (m, 2H, H-2, OC*H*$_a$H$_b$), 3.64 (ddd, *J* = 11.7, 4.9, 3.2 Hz, 1H, OCH$_a$*H*$_b$), 3.59-3.56 (m, 2H, C*H*$_c$*H*$_d$OCH$_3$), 3.39 (s, 3H, OCH_3), 3.16 (dd, *J* = 10.0, 3.1 Hz, 1H, H-4), 1.38 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 97.6 (C-1), 71.1 (*C*H$_c$H$_d$OCH$_3$), 69.6 (C-3), 66.8 (OCH$_a$H$_b$), 62.5 (C-5), 61.0 (C-4), 60.6 (C-2), 59.1 (OCH_3), 18.2 (C-6). HRMS (ESI) calc'd *m/z* for $C_9H_{20}N_7O_4$ $[M+NH_4]^+$: 290.1571; found: 290.1577.

1,2,3,4,5,6-Hexa-O-acetyl-7,8,9-trideoxy- L-*glycero*-D-*gulo*-8-octenitol (611) and 1,2,3,4,5,6-Hexa-*O*-acetyl-7,8,9-trideoxy- L-*glycero*-D-*gulo*-8-octenitol (612)



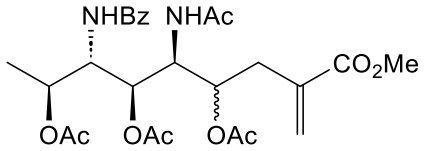
611
L-glycero-L-gulo

612
D-glycero-L-gulo

D-glucose **610** (49 mg, 0.27 mmol) was dissolved in a 1:1 (v/v) solution of ethanol and water (1.0 mL), then indium (37 mg, 0.33 mmol) and allyl bromide (28 µL, 0.33 mmol) were added, and the cloudy and bluish mixture was sonicated overnight at room temperature. Monitoring by TLC revealed that the reaction was halfway done, and so 1 drop of aq. HCl (2.0 M) was added followed by more allyl bromide (28 µL, 0.33 mmol) and indium (37 mg, 0.33 mmol). After stirring the reaction mixture for another night with sonication, the solution was concentrated under reduced pressure. The residue was then acetylated using a mixture of anhydrous pyridine (1.0 mL) and acetic anhydride (1.0 mL) along with a catalytic amount of DMAP (5 mg). After stirring the mixture at room temperature overnight, the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 20% ethyl acetate – hexanes as the eluent to afford the first isomer **612** (20 mg, 0.090 mmol, 33% yield), then the second isomer **611** (40 mg, 0.18 mmol, 66% yield). The ^{1}H NMR spectra were

found to match the literature.[185] Data for **611**: ^{1}H NMR (401 MHz, $CDCl_3$) δ_H 5.82 – 5.67 (m, 1H, H-8), 5.48 – 5.33 (m, 3H, H-3, H-4, H-6), 5.17 – 5.08 (m, 2H), 5.07 – 5.02 (m, 1H), 5.00 – 4.95 (m, 1H, H-2), 4.28 (dd, *J* = 12.2, 4.3 Hz, 1H, H-1b), 4.13 (dd, *J* = 12.2, 5.8 Hz, 1H, H-1a), 2.43 – 2.40 (m, 2H, H-7a, H7-b), 2.15 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.10 (s, 4H, OAc), 2.05 (s, 4H, OAc), 2.04 (s, 3H, OAc), 2.00 (s, 3H, OAc). Data for **612**: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.83 – 5.60 (m, 1H, H-8), 5.45 (dd, *J* = 8.3, 2.5 Hz, 1H, H-3), 5.39 – 5.28 (m, 2H, H-4, H-6), 5.19 – 5.07 (m, 3H, H-5, H-9a, H-9b), 5.01 (ddd, *J* = 8.0, 4.8, 2.7 Hz, 1H, H-2), 4.25 (dd, *J* = 12.5, 2.8 Hz, 1H, H-1b), 4.15 (dd, *J* = 12.5, 4.8 Hz, 1H, H-1a), 2.37 – 2.26 (m, 2H, H-7a, H7-b), 2.16 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc).

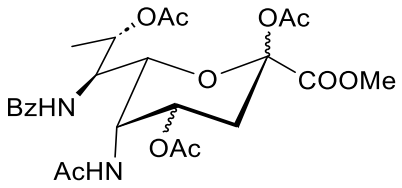
(4S/R,5S,6S,7S,8S)-Methyl 5-acetamido-7-N-benzoyl-4,6,8-trihydroxy-2-methylenenonanoate (614a/614b)



Previously synthesized[157] benzyl 2-acetamido-4-benzamido-2,4,6-trideoxy-α-L-altropyranoside (71 mg, 0.18 mmol) was hydrogenated methanol (10 mL) and dichloromethane (2 mL) in the presence of palladium hydroxide on charcoal (20%, 50 mg) as above. After stirring at room temperature overnight, the solids were then filtered off with a 0.2 µm PTFE syringe filter and the clear solution was evaporated under reduced pressure to give the free-sugar intermediate **613** (42 mg, 0.14 mmol). The crude hemiacetal was suspended in ethanol (4 mL)

and aq. HCl (0.1 N, 1.0 mL), then indium powder (150 mg, 1.36 mmol) and methyl bromomethylacrylate (0.25 mL, 2.04 mmol) were added, and the mixture was sonicated at 40 °C for 6 hours. The solution was filtered through a Celite plug and the celite washed with methanol; the solution was evaporated off under reduced pressure. The residue was acetylated in a mixture of anhydrous pyridine (5.0 mL) and acetic anhydride (5.0 mL) at room temperature overnight. The solution was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel using a gradient of acetone - hexanes (5→40%) as the eluent to afford the desired products (49 mg, 0.082 mmol, 60% yield) as an inseparable mixture of both expected diasteromers **614a** and **614b** in a 10:7 ratio, as determined by ^{1}H NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.84 – 7.68 (m, ArH), 7.56 – 7.40 (m, ArH), 6.95 (d, *J* = 10.0 Hz, amide peak on major isomer), 6.68 (d, *J* = 10.3 Hz, amide peak on minor isomer), 6.25 (d, *J* = 1.2 Hz, C=CH_aH$_b$ on major isomer), 6.18 (d, *J* = 1.3 Hz, C=CH_aH$_b$ on minor isomer), 5.95 (dd, *J* = 15.9, 8.6 Hz, amide peak on minor isomer), 5.88 (d, *J* = 10.0 Hz, amide peak on major isomer), 5.65 (br s, C=CH$_a$*H$_b$* on minor isomer), 5.62 (br s, C=CH$_a$*H$_b$* on major isomer), 5.42 – 5.36 (m), 5.31 (dd, *J* = 4.2, 2.3 Hz), 5.23 – 5.06 (m, 2H), 4.91 (td, *J* = 8.6, 3.5 Hz), 4.75 – 4.53 (m), 3.74 (2 s, OCH_3 on both isomers), 2.80 (dd, *J* = 14.1, 3.5 Hz, CH_aH$_b$C=CH$_2$ minor isomer), 2.63 (dd, *J* = 14.2, 4.7 Hz, CH_aH$_b$C=CH$_2$ major isomer), 2.49 (dd, *J* = 14.1, 8.2 Hz, CH$_a$*H$_b$*C=CH$_2$ minor isomer), 2.32 (dd, *J* = 14.2, 9.0 Hz, CH$_a$*H$_b$*C=CH$_2$ minor isomer), 2.23 – 2.03 (m, Ac both isomers), 2.00 (2 s, Ac both isomers), 1.85 (s, Ac), 1.82 (s, Ac), 1.38 (d, *J* = 6.4 Hz, H-9 both isomers).

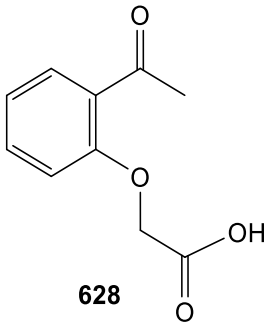
Methyl (5,7-diacetamido-2,4,8-tri-O-acetyl-3,5,7,9-tetradeoxy-L-glycero-α/β--L-manno-non-2-ulopyranosonate (615a,c) and methyl (5,7-diacetamido-2,4,8-tri-O-acetyl-3,5,7,9-tetradeoxy-L-glycero-α/β-L-gluco-non-2-ulopyranosonate (615b,d)



615a,b (α-anomers) & **615b,c** (β-anomers)

The mixture of isomers (4S/R,5S,6S,7S,8S)-methyl-5-acetamido-7-N-benzoyl-4,6,8-trihydroxy-2-methylenenonanoate (36 mg, 0.061 mmol) was dissolved in CH_2Cl_2 (2.0 mL) and the solution was cooled to -78 °C. Ozone was bubbled through the solution for 10 minutes until the mixture adopted a bluish color. The reaction flask was then purged with argon and warmed up to room temperature. Zinc powder (0.65 mg) was mixed in AcOH (1.1 mL) and the slurry was added to the reaction vessel and stirred at room temperature for 5 hours, after which the solids were filtered out and the product extracted from the solution with 20% CH_2Cl_2 in EtOAc (5 mL), washed with saturated aqueous sodium bicarbonate (5 mL), water (5 mL) and finally brine (5 mL). The organic solution was dried over Na_2SO_4, then evaporated to dry. The residue was redissolved in a mixture of anhydrous pyridine (2.0 mL) and acetic anhydride (2.0 mL). After stirring at room temperature overnight, the solution was evaporated to dry under reduced pressure. The crude product was characterized by ^{1}H NMR as a mixture that consists of 4 inseparable compounds, in the L-glycero-α/β-L-manno and L-glycero- α/β-L-gluco configurations. Due to the complexity of the ^{1}H NMR spectra, further analysis by ^{1}H NMR was not achieved. HRMS (ESI) calc'd *m/z* for $C_{25}H_{30}D_2N_2O_{11}$ $[M+H]^+$: 539.2204; found: 539.2250.

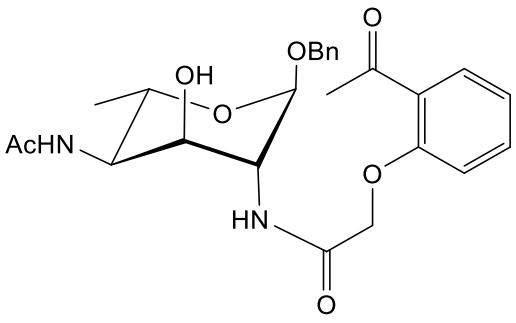
2-(2-acetylphenoxy)acetic acid (628)



628

To a stirred mixture containing *ortho*-hydroxyacetophenone **627** (5.0 mL, 42 mmol) and bromoacetic acid (8.65 g, 62.3 mmol) in water (30 mL), was added dropwise a solution of NaOH (4.98 g, 125 mmol) dissolved in 20 mL within 10 min at room temperature, and the mixture was heated to reflux for 3 hours with stirring. After cooling down the reaction, a solution of aq. HCl (2.0 M) was added to adjust the pH of the solution to ~1-2. This resulted in precipitation. The precipitate was collected by filtration to obtain compound **628** as an off-white powder which was dried under vacuum (6.67 g, 34.4mmol, 83% yield). No further purification was needed. R_f = 0.34 (40% acetone in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.80 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.54 (ddd, *J* = 8.3, 7.4, 1.8 Hz, 1H), 7.14 (td, *J* = 7.6, 1.0 Hz, 1H), 6.97 (dd, *J* = 8.4, 1.0 Hz, 1H), 4.78 (s, 2H), 2.69 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 200.5, 170.5, 156.8, 134.7, 131.3, 127.4, 122.5, 114.8, 66.9, 29.9. Both ^{1}H and ^{13}C NMR data are in agreement with previously reported.[172]

Benzyl 4-acetamido-2-(2-(2-acetylphenoxy)acetamido)-2,4,6-trideoxy-α-L-altropyranoside (637)

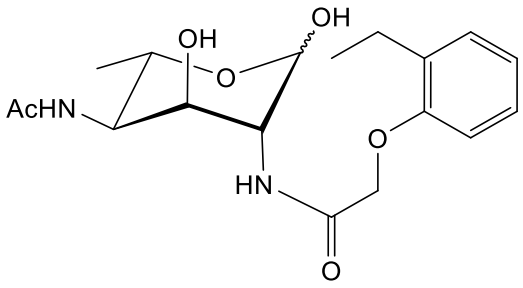


637

Benzyl 4-acetamido-2-amino-2,4,6-trideoxy-α-L-altropyranoside (48 mg, 0.16 mmol) was dissolved in anhydrous DMF (1.0 mL) at 0 °C, was added triethylamine (0.05 mL). In a separate flask, EDC-HCl (37 mg, 0.20 mmol), HOBt (33 mg, 0.24 mmol) and 2-(2-acetylphenoxy)acetic acid **628** (64 mg, 0.33 mmol) were mixed in anhydrous DMF (2.0 mL) at 0 °C, and this solution was carefully added to the first reaction vessel. The mixture was stirred overnight at room temperature. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 30% acetone - hexanes as the eluent to afford compound **637** (55 mg, 0.12 mmol, 72% yield). ^{1}H NMR showed the product is slightly impure but was used for the next step without further purification. R_f = 0.44 (5% MeOH in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.59 (d, *J* = 9.3 Hz, 1H, CONH_2), 7.80 (dd, *J* = 7.8, 1.7 Hz, 1H, ArH), 7.50 (ddd, *J* = 8.4, 7.4, 1.7 Hz, 1H, ArH), 7.41 – 7.30 (m, 5H, ArH), 7.08 (td, *J* = 7.6, 1.0 Hz, 1H, ArH), 6.89 (dd, *J* = 8.3, 1.1 Hz, 1H, ArH), 6.09 (d, *J* = 9.3 Hz, 1H, CONH_4), 4.95 (d, *J* = 1.4 Hz, 1H, H-1), 4.76 (d, *J* = 11.6 Hz, 1H, CH_aH$_b$Ph), 4.65 – 4.56 (m, 4H, COCH_2OPh, CH$_a$$H_b$Ph, H-2), 4.35 (ddd, *J* = 10.4, 9.3, 3.1

Hz, 1H, H-4), 3.86 (dq, *J* = 10.3, 6.3 Hz, 1H, H-5), 3.77 (dd, *J* = 3.2, 1.3 Hz, 1H, H-3), 2.67 (s, 3H, C*H*$_3$COPh), 1.99 (s, 3H, Ac), 1.40 (d, *J* = 6.3 Hz, 3H, H-6).

4-Acetamido-2-(2-(2-ethylphenoxy)acetamido)-2,4,6-trideoxy-α/β-L-altropyranose (638)

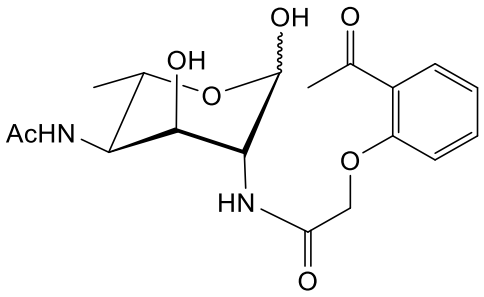


638

To as solution of benzyl 4-acetamido-2-(2-(2-acetylphenoxy)acetamido)-2,4,6-trideoxy-α-L-altropyranoside **637** (55 mg, 0.12 mmol) in methanol (10 mL) was added palladium hydroxide on charcoal (20%, 50 mg), and the mixture was purged with a hydrogen atmosphere. After stirring the mixture at room temperature for 3 hours, the mixture was filtered off using a 0.2 µm PTFE syringe filter and the clear solution was evaporated under reduced pressure to give compound **638** as a white solid (44 mg, quantitative yield). ^{1}H NMR showed that the product is a anomeric mixture. Selected ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36 (d, *J* = 7.8 Hz, CONH_2, major anomer), 7.24 – 7.13 (m, ArH), 7.10 (d, *J* = 8.6 Hz, CONH_2, minor anomer), 7.04-6.94 (m, ArH), 6.81 – 6.71 (m, ArH), 6.17 (2 doublets, CONH_4, from both anomers), 5.37 (d, *J* = 1.7 Hz, H-1 minor anomer), 5.01 (d, *J* = 1.6 Hz, H-1, major anomer), 4.59 – 4.45 (m, COC*H*$_2$OPh from both anomers), 4.39 (ddd, *J* = 8.6, 3.6, 1.5 Hz, H-2 major anomer), 4.24 (m, H-2 minor anomer), 4.15 – 3.76 (m, H-3, H-4, H-5

from both anomers), 2.85 – 2.69 (2 q, CH_3CH_2Ph from both anomers), 2.02 (2 s, Ac from both anomers), 1.35 – 1.18 (m, CH_3CH_2Ph, H-6 from both anomers).

4-Acetamido-2-(2-(2-acetylphenoxy)acetamido)-2,4,6-trideoxy-α/β-L-altropyranose (642)



642

To a solution of benzyl 4-acetamido-2-amino-2,4,6-trideoxy-α-L-altropyranoside **636** (114 mg, 0.387 mmol) in methanol (8 mL) and dichloromethane (3 mL) was added palladium hydroxide on charcoal (20%, 100 mg) and AcOH (25 drops), and the solution was purged with a hydrogen atmosphere. The hydrogenation reaction was continued at room temperature overnight. The mixture was then filtered through a 0.2 µm PTFE syringe filter and the clear solution was evaporated under reduced pressure to give compound **639** as an anomeric mixture (quantitative yield). The crude ^{1}H NMR spectrum of the product showed the disappearance of the aromatic peaks.

The crude product **639** obtained from above was redissolved in anhydrous methanol (4.0 mL). In a flask, 2-(2-acetylphenoxy)acetic acid **628** (188 mg, 0.968 mmol) was dissolved in anhydrous CH_2Cl_2 (4.0 mL); DCC (176 mg, 0.851 mmol) was added, and the mixture was stirred

for 1 hour. The formed precipitates were filtered off. The above prepared methanolic solution of compound **639** was then added to the filtrate, and the mixture was stirred overnight at room temperature. TLC monitoring revealed the formation of three new spots (R_f's are between 0.4 and 0.53, 10% methanol - CH_2Cl_2), with some starting material **639** spot still remained (R_f = 0.07, 10% methanol – CH_2Cl_2). DMAP (~50 mg) was then added to catalyze the reaction, and the reaction was continued for another 24 hours. The reaction solution was then evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel. O-methyl 2-(2-acetylphenoxy)acetate came out first (eluted with 1% methanol – CH_2Cl_2), then the desired product **642** came out as a clear syrup (eluted with 5% methanol – CH_2Cl_2) (66 mg, 0.17 mmol, 45% yield). The product was found slightly impure and identified by ^{1}H NMR and ^{1}H–^{1}H GCOSY as the expected compound containing a mixture of α- and β-anomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.46 (d, *J* = 8.8 Hz, CONH_2, major anomer), 8.23 (d, *J* = 7.9 Hz, CONH_2, minor anomer), 7.81-7.77 (m, ArH), 7.55 – 7.49 (m, ArH), 7.19 – 7.03 (m, ArH), 7.01 – 6.74 (m, ArH), 6.15 (d, *J* = 9.1 Hz, CONH_4, major anomer), 6.05 (d, *J* = 9.2 Hz, CONH_4, minor anomer), 5.35 (d, *J* = 1.8 Hz, H-1, minor anomer), 5.14 (br s, H-1, major anomer), 4.63 – 4.55 (m, COC*H*$_2$OPh from both anomers), 4.50 – 4.41 (m, H-2, major anomer), 4.29-4.23 (m, H-4, major anomer, H-2 minor anomer), 4.20 – 4.01 (m, H-5, major anomer, H-3 & H-4, minor anomer), 3.96 (br s, H-3, major anomer), 3.90 – 3.76 (m, H-5, minor anomer), 2.74 – 2.59 (2 × s, CH_3COPh, both anomers), 2.01 (2 × s, Ac, both anomers), 1.36 (d, *J* = 6.2 Hz, H-6, major anomer), 1.28 (d, *J* = 6.2 Hz, H-6, minor anomer).

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> PhD Candidate & Graduate Teaching Assistant
>
> **Ling Research Group**
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Anna Niedzwiecka

From: Quyen Pham <qtpham1@outlook.com>
Sent: June 2, 2023 2:50 PM
To: Anna Niedzwiecka
Subject: RE: Permission to Use our Paper in my Thesis

[△EXTERNAL]

Hi Anna,

Absolutely, you have my full permission!

Thank you,
Quyen

From: Anna Niedzwiecka
Sent: June 2, 2023 2:47 PM
To: qtpham1@outlook.com
Subject: Permission to Use our Paper in my Thesis

Hi Quyen!

I'm writing to you to ask for your permission to use the paper entitled: "Regiospecific O → N Acyl Migration as a Methodology to Access L-Altropyranosides with an *N*2,*N*4-Differentiation," which appears in Organic Letters (published November 16th 2022) in my thesis write-up, as you are one of the co-authors. I will be including all of the information that appears in the paper.

If you could reply to this email with you explicit permission, that would be appreciated.

Thank you!

Anna Niedzwiecka
PhD Candidate & Graduate Teaching Assistant

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