Development of an Alternative Synthesis of 2-acetamido-2-deoxy-L-altruronic acid:

An unusual sugar found in the *O*-specific polysaccharide of *Shigella sonnei*

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by

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Abstract

A new synthetic route has been explored for the preparation of derivatives of 2-acetamido-2-deoxy-L-altruronic acid (L-AltNAcA). This is a rare sugar found together with 2-acetamido-4-amino-2,4-dideoxy-D-fucose(D-FucNAc4N) in the repeating unit of *Shigella sonnei.* Derivatives are needed *inter alia* for chemical and spectroscopic calibration standards, and as building blocks for preparing oligomeric subunits of the O-polysaccharide antigen for possible incorporation into a synthetic glycoconjugate vaccine. Two synthetic routes were investigated. The first route successfully repeated a published four step sequence converting diacetone-D-glucose to 1,6-anhydro-β-L-idopyranose in a 38% yield overall, and a further selective benzylation at O-3. Attempts to discriminate between O-2, O-3 and O-4 using low temperature acylation or alkylation conditions were unsuccessful, but modest selectivity for the 4-benzoate was observed in a Bu_2SnO-mediated benzoylation, although this product could not be easily separated from other mono-benzoates. The second route started from N-acetyl-D-glucosamine which was successfully converted in the first step to 2-methyl-(1,2-dideoxyl-5,6-*O*-isopropylidene-α-D-glucofurano)-[2,1-d]-2-oxazoline. The oxazoline and dioxolane units could be selectively manipulated in a series of steps to afford 2-acetamido-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucofuranosyl acetate in a 41% yield over four steps. This is a key synthetic intermediate in which the 5-OH is available for the required inversion step. During this study, an unusual minor side-product, 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose, was isolated. While this was also a potentially useful intermediate, having only the 5-OH unprotected, it proved not possible to find conditions for optimizing this product. Inversion of configuration at C-5 in the 6-*O*-silylated glucofuranose was attempted *via* the 5-*O*-triflate and 5-*O*-mesylate: the triflate formed but was displaced *in situ* by the solvent pyridine to give an unusual 5-pyridinium derivative, while the mesylate was stable but unreactive towards subsequent S_N2 inversion. These outcomes were attributed to the steric congestion imposed by the combination of the 3,4-*cis*-disubstitution of the furanose ring and the very bulky silyl substituent at O-6. While the goal of preparing L-AltNAcA was not achieved *via* these approaches, useful insights have been contributed towards the ongoing study.

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Abbreviations

$(CD_3)_2CO$	Deuterated Acetone
BAIB	Bis(acetoxy)iodobenzene
BF_3Et_2O	Borontrifluoride Dietherate
BH_3THF	Borane Tetrahydrofuran
BnBr	Benzyl Bromide
bs	Broad Singlet
Bu_2SnO	Dibutyltin Oxide
BzCl	Benzoyl Chloride
CAS	Ceric Ammonium Sulfate
$CDCl_3$	Deuterated Chloroform
COSY	Correlation Spectroscopy
CSA	Camphorsulfonic Acid
d	Doublet
DAST	Diethylaminosulfur Trifluoride
DCM	Dichloromethane
dd	Doublet of Doublets
D-FucNAc4N	2-Acetamido-4-amino-2,4,6-trideoxy-D-galactose
DIAD	Diisopropylazodicarboxylate
DMAP	Dimethylaminopyridine
DMDO	Dimethyldioxirane
DMF	N,N-Dimethylformamide
Et_2O	Diethyl Ether
Et_3N	Triethylamine
EtOAc	Ethyl Acetate
EtOH	Ethanol
h	Hours
HMBC	Heteronuclear Multiple-Bond Correlation Spectroscopy
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
KOH	Potassium Hydroxide
L-AltNAcA	2-Acetamido-2-deoxy-L-altruronic acid
m	Multiplet
m.p.	Melting Point
MeOD	Deuterated Methanol

MeOH	Methanol
Mesyl Chloride	Methanesulfonyl Chloride
MHz	Mega Hertz
mL	Millilitres
mmol	Millimoles
MS	Molecular Sieves
NaOBz	Sodium Benzoate
NaOPiv	Sodium Pivaloate
NBS	N-Bromosuccinimide
n-BuLi	n-Butyllithium
NIS	N-Iodosuccinamide
NMR	Nuclear Magnetic Resonance
Ph	Phenyl
PhCHO	Benzaldehyde
PhCOOH	Benzoic acid
PMBCl	*p*-Methoxybenzyl Chloride
PTFACl	N-(phenyl)trifluoroacetimidoyl Chloride
py	Pyridine
RT	Room Temperature
s	Singlet
t	Triplet
TBABr	Tetrabutylammonium Bromide
TBAF	Tetrabutylammonium Fluoride
TBDMSCl	Tertiarybutyldimethylsilyl Chloride
td	Triplet of Doublets
TEMPO	(2,2,6,6-Tetramethyl-piperidin-1-yl)oxyl
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMSCl	Trimethylsilyl Chloride
TMSOTf	Trimethylsilyl Trifluoromethanesulfonate
Tosyl Chloride	4-Toluenesulfonyl Chloride
UV	Ultra-Violet

Table of Contents

CHAPTER 1

Introduction

1.1 Carbohydrates in Nature

Carbohydrates are one of the four essential building blocks of life.[1] In their simplest form they exist as monomers known as monosaccharides. D-Glucose is the most common monosaccharide and the most abundant organic molecule on earth.[2] Monosaccharides link together through glycosidic bonds to form oligosaccharides (2-10) and polysaccharides (>10). Apart from being one of our main energy sources, carbohydrates are vital structural components in nature, forming part of DNA, RNA, starch, cellulose and cotton.[3] These versatile molecules are also involved in biological signalling and recognition processes through interactions with cell surfaces. The carbohydrates expressed on the surface of pathogens enable them to evade humans' innate immune response. However, cell surface recognition binding stimulates the adaptive immune system which elicits an immune response against the pathogen. Consequently, carbohydrates form the basis of many vaccines.[4] Research into the synthesis of biologically relevant carbohydrates is therefore of great importance in the prevention of diseases.

1.2 *Shigella sonnei*

Diarrhoeal diseases account for approximately 1.5 million deaths annually predominantly affecting low-middle income countries.[5,6] Dysentery due to shigellosis is responsible for 1.1 million of those deaths. It consistently ranks in the WHO top 10 causes of death in developing countries with 60% of those deaths in children under 5 years old.[7,8] Shigellosis is a bacterial infection of the mucosal epithelial cells in the small intestine.[9] It is caused by the bacteria of the genus *Shigella*, which is divided into four serogroups; *S. dystenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*.[10] 77% of infections in industrialized countries are caused by *S. sonnei*[9,11] and this serogroup was the focus of this study.

Shigellosis is a waterborne disease and is primarily transmitted through faecal-oral contamination.[12] Poor hygiene and limited sanitation in addition to the low infectious dose make this disease highly prevalent in low income developing countries.[13] Shigellosis is one of the many opportunistic infections that occur in HIV and AIDS patients which is of great concern particularly in Africa.[8,9,11,14] Further studies have also shown that the effects of Shigellosis are not only short term but can cause physical and cognitive impairments in the long term.[15,16]

Recent reports have shown that the mortality rate due to diarrhoea has decreased from effective treatments with zinc, fluid replacement and improvement of general hygiene such as hand washing.[5,6] Antimicrobial drugs have also been very effective in reducing the spread of the infection, duration of diarrhoea and fevers.[17] However, the disease severity and antibiotic resistance are ever growing challenges especially for children under 5 years.[18,19] Increased resistance to antibiotics such as ciprofloxacin, pivmecillinam, azithromycin and cephalosporins has encouraged investigation into vaccines as the primary form of prevention.[10] Many investigations into the pathogenesis of shigellosis and elicited immune response have formed the basis of vaccine requirements.

1.3 Vaccine Development

Shigella cells contain a bacterial surface antigen, the lipopolysaccharide (LPS), which allows the invasion of numerous host cells.[10] The LPS contains an O-specific polysaccharide chain (O-SP), which is made up of repeating unit such as the disaccharide in the O-SP from *S. sonnei*, shown in Figure 1. It is this repeating unit that varies and gives rise to different serotypes in the aforementioned serogroups of *Shigella*. Robbins *et al.*[20,21] identified that this serotype-specific O-SP chain plays an essential role in eliciting an immune response and is therefore a key virulence factor which can be exploited in vaccines development. However, it does not stimulate a strong immune

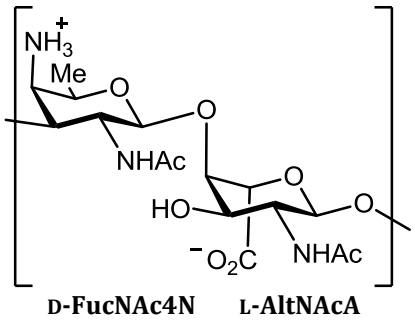


Figure 1: Repeating unit of *S. sonnei*

response[22] and requires covalent linkage to a carrier protein to become immunogenic.[23] The covalently linked carrier protein and polysaccharide are known as a conjugate vaccine, Figure 2.

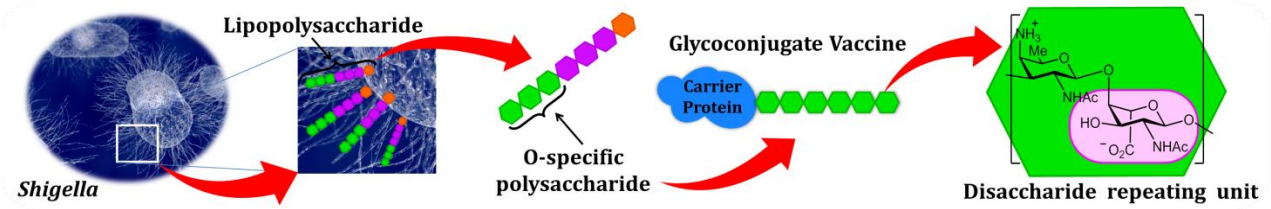


Figure 2: Showing the carbohydrate component of the potential glycoconjugate vaccine and its source in *S. sonnei*

1.4 Conjugate Vaccines

In the last 5 years major breakthroughs with conjugate vaccines have shown potential to produce a safe and effective solution to shigellosis. GlycoVaxyn, a biopharmaceutical company, is currently investigating the development of a multivalent conjugate vaccine against the five most common serotypes of *Shigella*: *S. sonnei*, *S. dysenteriae* 01, *S. flexneri* 2a, *S. flexneri* 3a and *S. flexneri* 6. A potential candidate against *S. dysenteriae* 01 has performed well in a Phase I clinical trial[24] and a candidate for *S. flexneri* 2a is currently in clinical trials.

GlycoVaxyn's technology makes use of a bioconjugation platform to produce conjugate vaccines in bacterial cells, avoiding chemical conjugation.[25,26] This exploits recombinant DNA techniques to modify *E. coli* bacteria to reproducibly manufacture specific glycoproteins.[27] The technology enables almost any bacterial polysaccharide to be conjugated to almost any bacterial protein.[26,28] This technology allows the challenges of current methods for conjugate vaccine production to be avoided through a simplified biological process which lowers production costs and improves efficiency.[26,28]

Since this method produces the vaccine in one process, the protein and carbohydrate constituents are not able to be analysed independently. Authentic samples of the monosaccharides, which are not commercially available, are therefore needed as chemical and spectroscopic calibration standards for

use in physicochemical and biological assays. This is essential for the analysis and verification of the carbohydrate component of the conjugate vaccine.

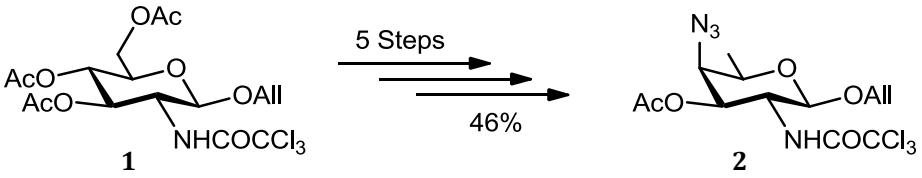
1.5 Synthesis of the Disaccharide Repeating Unit of *S. sonnei*

Synthesis of the any oligosaccharide repeating unit, such as that of *S. sonnei* (Figure 1), involves synthesising each monosaccharide in a suitably protected form, followed by formation of the glycosidic linkages in the correct sequence. It is therefore essential that each monosaccharide can be suitably activated at the anomeric position to act as a glycosyl donor, and have a selectively removable protecting group at the hydroxyl group where it will form the glycosidic bond. Accordingly, the protected forms of D-FucNAc4N and L-AltNAcA are the first target compounds towards the synthesis of the repeating unit. Sugars in the D-configuration, such as D-FucNAc4N, are generally accessible from abundant D-sugar precursors, whereas the L-sugar, L-AltNAcA, has to be prepared from much rarer and more expensive L-sugars, or *via* challenging routes from the D-sugars. There are only two successful methods reported for the total syntheses of L-AltNAcA,[29,30] both starting from sugars already in the L-configuration, thus reducing the synthetic challenges although increasing production costs. The published synthetic routes to these unusual sugars are briefly reviewed here as to provide context for the present study.

1.5.1 Synthesis of D-FucNAc4N

This monosaccharide has been successfully synthesised by a number of groups in various protected forms.[30–40] Since it is a component of a number of bacterial surface antigens including *Streptococcus pneumoniae,*[31,38] *Streptococcus mitis*[41]*, Providencia alcalifaciens,*[42] *Bacteroides fragilis*[43] and various serogroups of *Shigella*[9], its synthesis has been more widely investigated. The most recent study provides a review of synthetic approaches to D-FucNAc4N (or AAT), and presents a particularly efficient synthesis of protected D-FucNAc4N (**2**) requiring 5 steps from allyl glycoside **1** in an overall yield of 46%, shown in Scheme 1.[30]

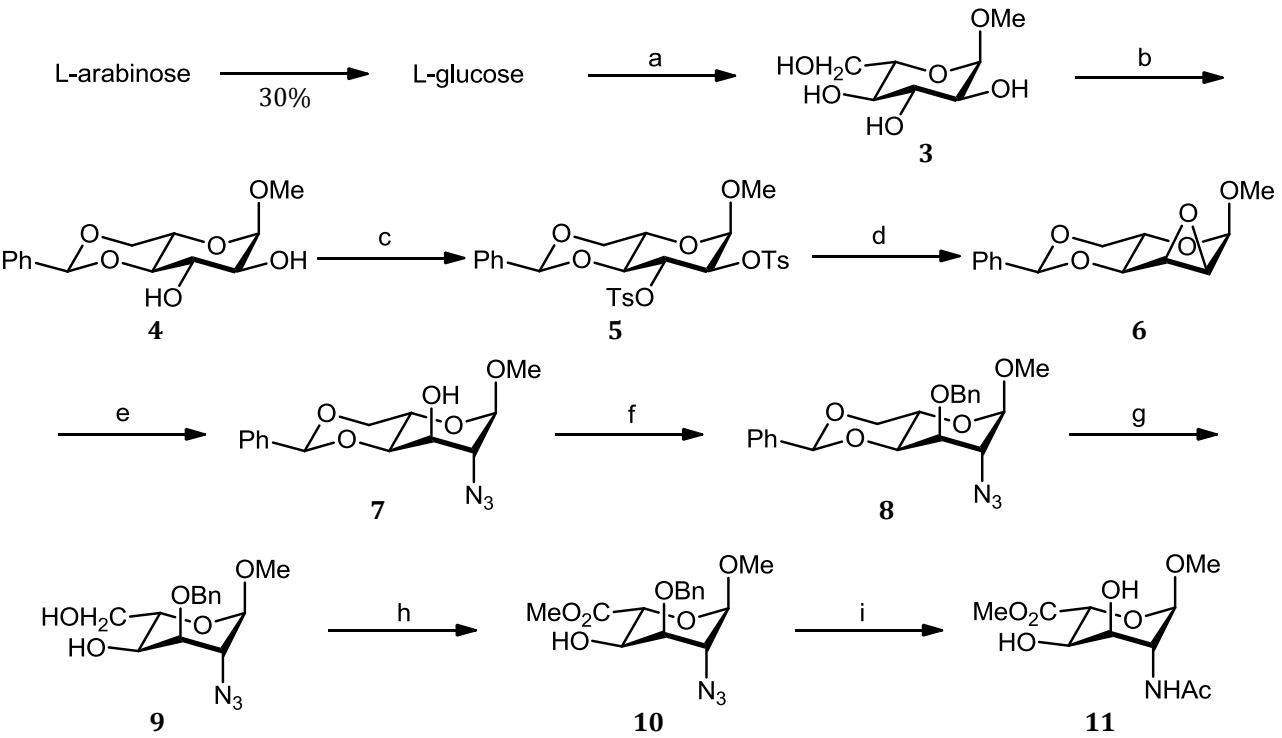
This was a novel strategy with a significant improvement in yield compared to the original reported synthesis of D-FucNAc4N by Medgyes *et al.*[32]



Scheme 1: Synthesis of D-FucNAc4N (**2**) from Glycoside **1**

1.5.2 Synthesis of L-AltNAcA

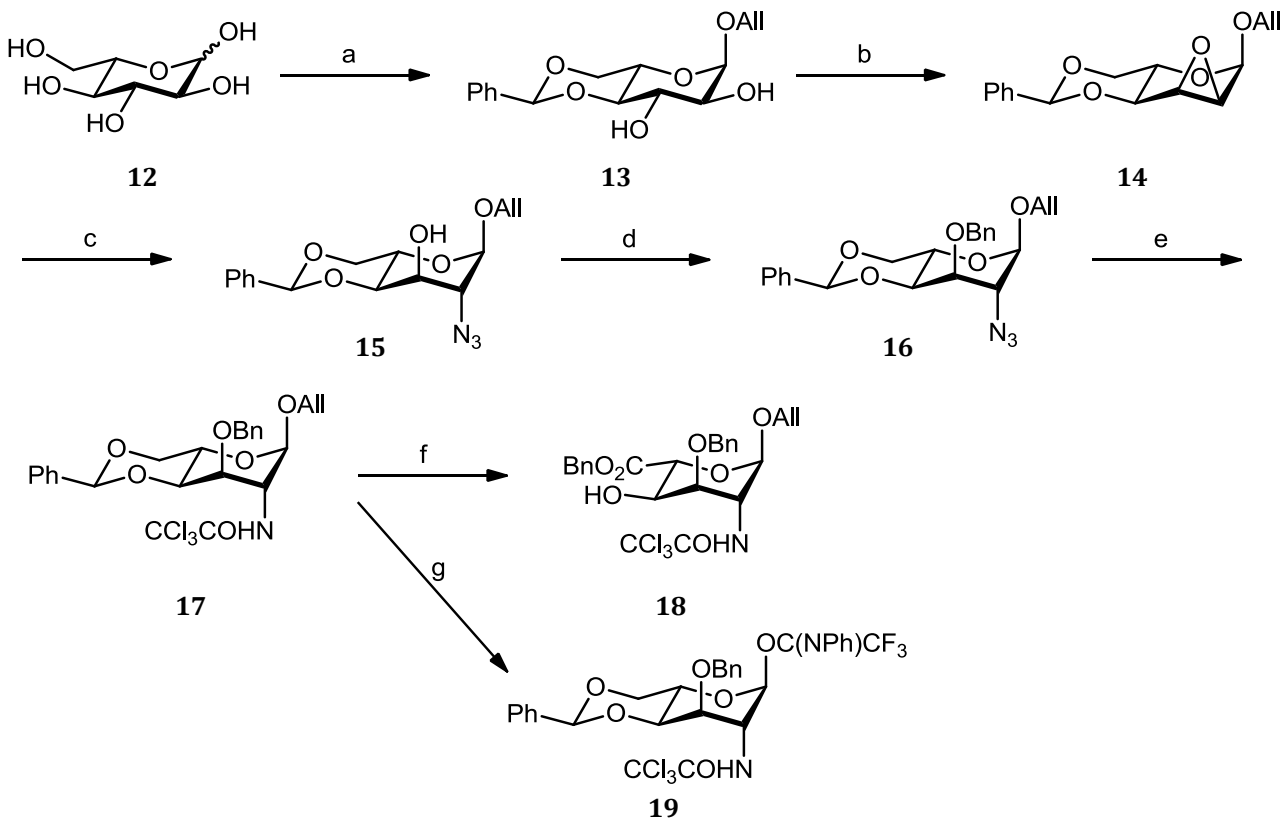
The original synthesis of L-AltNAcA was also achieved by Medgyes *et al.*[32] Starting with L-arabinose they followed a previously published nitromethane method[44] to L-glucose in a 30% yield. Focus then shifted to the challenges in selective functional group conversions.



Scheme 2: Reagents and conditions (a) HCl, MeOH, 65°C, 24 h, 61% (b) C_6H_5CHO, $ZnCl_2$, 62% (c) TsCl, NaOH, K_2CO_3, 20°C, 1 h, 76% (d) NaOMe, MeOH, 65°C, 4 h, 77% (e) NaN_3, NH_4Cl, 2-methoxylethanol.H_2O, reflux, 4 h, 66% (f) BnBr, NaH, DMF, 20°C, 73% (g) AcOH, H_2O, 60°C (h) i: NaOCl, tetramethylpiperidine-1-oxyl, KBr, aq. $NaHCO_3$, 20°C, 2 h; ii: MeI, DMF, 20°C, 2 h, 62% (i) i: H_2, 20% $Pd(OH)_2/C$, MeOH, 20°C, 24 h; ii: Ac_2O, 0°C, 1 h, 90%

Fischer glycosylation of L-glucose converted the hydroxyl group at C-1 to the methyl-α-L-glucoside **3**. Acetal protection of O-4 and O-6 followed by tosylation at O-2 and O-3 gave ditosylate **5** which was transformed to the 2,3-epoxide **6**. This key intermediate allowed for eventual inversion of configuration at both C-2 and C-3 via selective nucleophilic addition of azide at C-2, giving the desired configuration of the final product. Benzylation at O-3 and deprotection of O-4 and O-5 afforded L-altrose derivative **9**. Selective oxidation of the primary hydroxyl group at C-6 gave the desired carboxyl group in **10**. The final step included a catalytic reduction of the azide and acetylation to afford the final product, L-AltNAcA (**11**), as its methyl glycoside methyl ester.

Two significant disadvantages in this route are clear: firstly, it started with monosaccharide L-arabinose and secondly, converting this to L-glucose was very low yielding (30%). This has prompted other efforts to develop a more efficient and cost effective route.



Scheme 3: Reagents and conditions (a) i: AcCl, AllOH, 90°C, 16 h; ii: $PhCH(OMe)_2$, CSA, CH_3CN, 80°C, 2 h, 54% (b) i: MsCl, py, RT, 16 h; ii: KOH, THF/MeOH, 70°C, 16 h, 69% (c) NaN_3, NH_4Cl, DMF, 80°C, 48 h, 70% (d) BnBr, NaH, DMF, RT, 1 h, 91% (e) i: PPh_3, H_2O, THF, 60°C, 16 h; ii: $(CCl_3CO)_2O$, py, 0°C, 15 min, 91% (f) i: 80% aq. AcOH, 60°C, 1 h; ii: TEMPO, BAIB, DCM/H_2O, RT, 1 h; iii: BnBr, K_2CO_3, DMF, RT, 16 h, 74% (g) i: $[Ir(COD)(PMePh_2)_2]^+PF_6^-$, H_2, RT, 30 min; ii: I_2 THF/H_2O, RT, 30 min; iii: PTFACl, Cs_2CO_3, $(CH_3)_2CO$, RT, 2 h, 88%

More recently, Pfister *et al.*[30] proposed a new synthetic route to L-AltNAcA (Scheme 3) in a form in which it can be readily converted to both a glycosyl acceptor (**18**) and donor (**19**), for use in chain elongation towards the *S. sonnei* repeating unit.

This route was largely based on the work of Medgyes *et al*.[32] starting from L-glucose. Again starting with a Fischer-type glycosylation and regioselective protection of O-4 and O-6, the allyl α-glycoside **13** was synthesised in a 3:1 excess over the β-isomer. Following a similar sequence to invert C-2 and C-3, epoxide **14** was accessed *via* the 2,3-di-*O*-mesylate and trans-diaxial opening of **14** afforded 2-azido altroside **15** preferentially (70%) over the trans-diequatorial product (6%). Protection of O-3 and reduction at C-2 successfully produced the fully protected allyl α-L-altrosamine (**17**). Deprotection and oxidation at C-6 lead to the desired glycosyl acceptor **18**. **17** was also converted to imidate **19,** through a deallylation and subsequent reaction of the formed hemiacetal with PTFACl, for use as a glycosyl donor. Overall the protected form of L-AltNAcA was synthesised in a 14% yield from L-glucose.[30]

While this route does offer an effective synthesis of the desired target compound, the cost of the starting material (R1 429.75 per gram compared to D-glucose at R9.10 per gram) is a disadvantage.

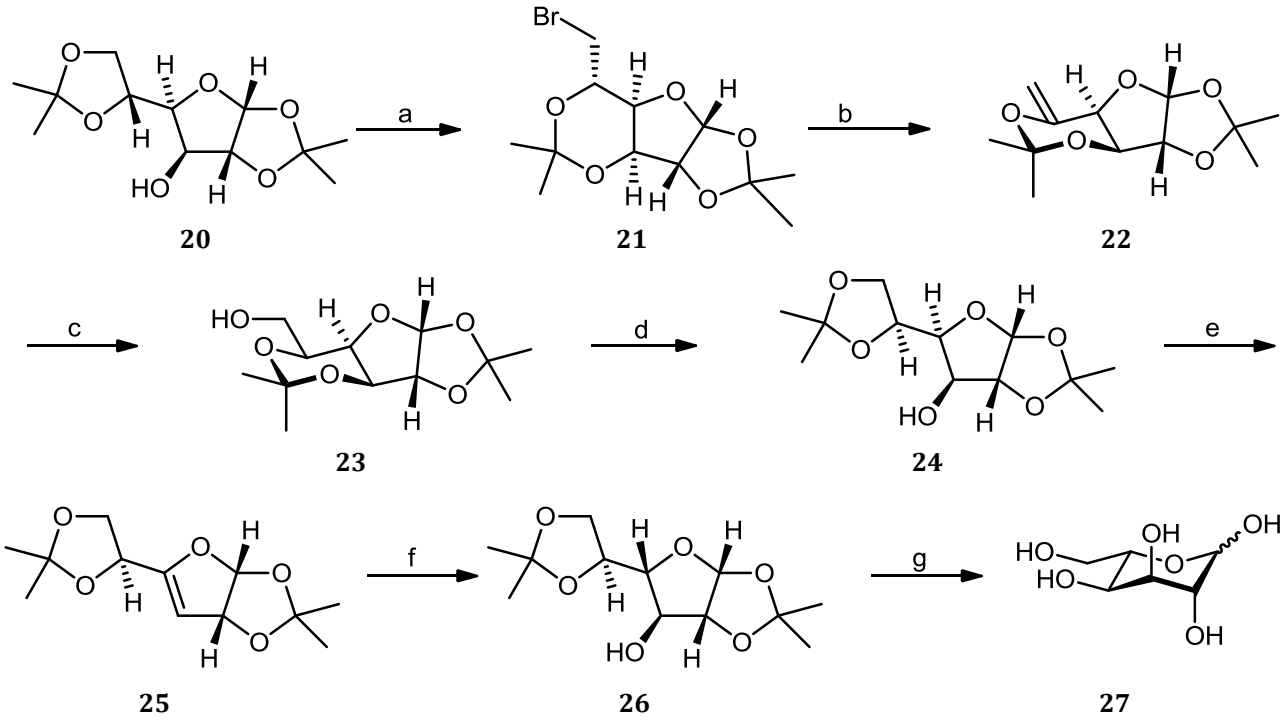
1.6 Synthesis of L-Sugars

Since these are the only published syntheses of L-AltNAcA, the synthesis of other L-sugars from D-sugar starting materials was explored, in particular L-altrose which is a potent biological synthon and would be a valuable intermediate towards the desired L-altruronic acid. Recent reviews of the synthesis of L-sugars was conducted by Zululeta *et al.*[45] and Frihed *et al*.[46] some of the key strategies are discussed below.

1.6.1 Synthetic strategies towards L-altrose

Two methods were identified which form L-altrose from D-sugar starting materials.

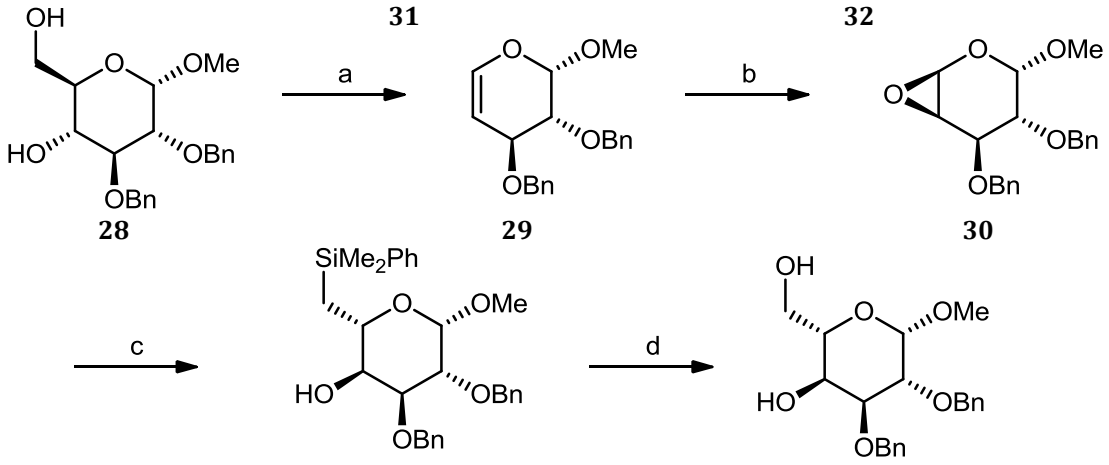
The first strategy shown in Scheme 4 effectively converts diacetone-D-glucose (**20**) to L-altrose (**27**) in its unprotected form in a 9% yield over 7 steps.[47] The initial sequence involved an interesting 5,6- to 3,5-acetal rearrangement and bromination at C-6 affording 6-bromoglucofuranoside **21**. This was ideally set up for the elimination at C-6 followed by a stereoselective hydroboration coupled with an oxidative work-up to efficiently invert the configuration at C-5. The protected L-glucofuranoside **23** now differed from the desired L-altrose only at position 4. This inversion was also achieved *via* an elimination-oxidation sequence. Firstly, the migration of the acetal protecting group to its original 5,6 position was achieved using 2,2-dimethyoxypropane and a catalytic amount of CSA leaving an OH at C-3 which underwent elimination on treatment with DAST. The hydroboration of endocyclic olefin **25** was stereoselective and the oxidative work-up led to inversion at C-4 and restoration of the hydroxyl group at C-3. Acid hydrolysis of **26** removed the isopropylidene groups affording the desired target L-altrose (**27**).[47] This route offered a viable option for the synthesis of L-AltNAcA from D-glucose.



Scheme 4: Reagents and conditions (a) PPh_3, NBS, $PhCH_3$, 110°C, 2 h, 75% (b) DBU, DMF, 110°C, 2 h, 84% (c) BH_3.THF, THF, H_2O_2, NaOH, RT, 3 h, 90% (d) CSA, $(CH_3)_2CO$, 2,2-dimethoxypropane, RT, 42% (e) DAST, py, 100°C, 0.12 mmHg, 51% (f) BH_3.THF, THF, H_2O_2, NaOH, RT, 3 h, 78% (g) amberlite-120 resin (H^+), H_2O/1,4-dioxane, 99%

The second successful method started with a selectively protected form of D-glucose (**28**) to produce a similarly protected L-altrose (**32**) in 4 steps with an overall yield of 49% (Scheme 5).[48]

4-deoxypentenoside **29** was successfully prepared in a two-step sequence of oxidation followed by decarboxylative elimination. An epoxide which could be selectively opened to attain the desired configuration at both C-4 and C-5 was then synthesised. The epoxidation was achieved using DMDO with high facial selectivity to produce **30**, with this high selectivity presumably due to the pyran system adopting a preferred $^2H_1$ half-chair, where the two benzyloxy groups are *pseudo*-equatorial and the methoxy group *pseudo*-axial.[48] This epoxide was found to undergo completely regioselective opening with a broad set of nucleophiles of which the most effective were Grignard reagents. Use of dimethylphenylsilyl methylenyl Grignard reagent provided silane **31** which was readily converted to the L-hexopyranoside (**32**) in which the hydroxyl at C-6 is reintroduced.



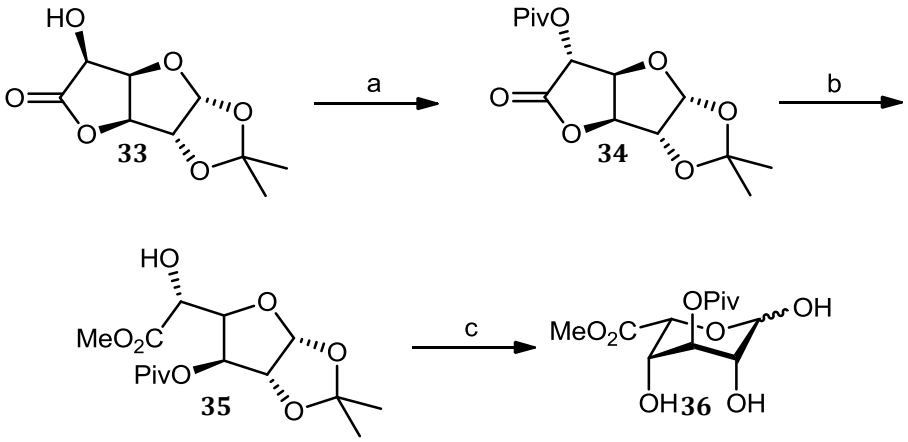
Scheme 5: Reagents and conditions (a) i: TEMPO, KBr, *n*-Bu_4NBr, NaOCl, $NaHCO_3$, DCM/H_2O, 0°C; ii: *N,N*-dimethylformamide dineopentyl acetal, $PhCH_3$, 120°C, 70% (b) DMDO, $(CH_3)_2CO$, DCM, -55°C, 99% (c) $PhMe_2SiCH_2MgCl$, Et_2O, RT, 86% (d) CH_3CO_3H, KBr, AcOH/NaOAc, 0°C to RT, 75%

1.6.2 Synthetic strategies towards general L-sugars

Synthetic routes to more general L-sugars were then reviewed. Four basic methods were identified that showed potential adaptability towards preparation of L-AltNAcA.

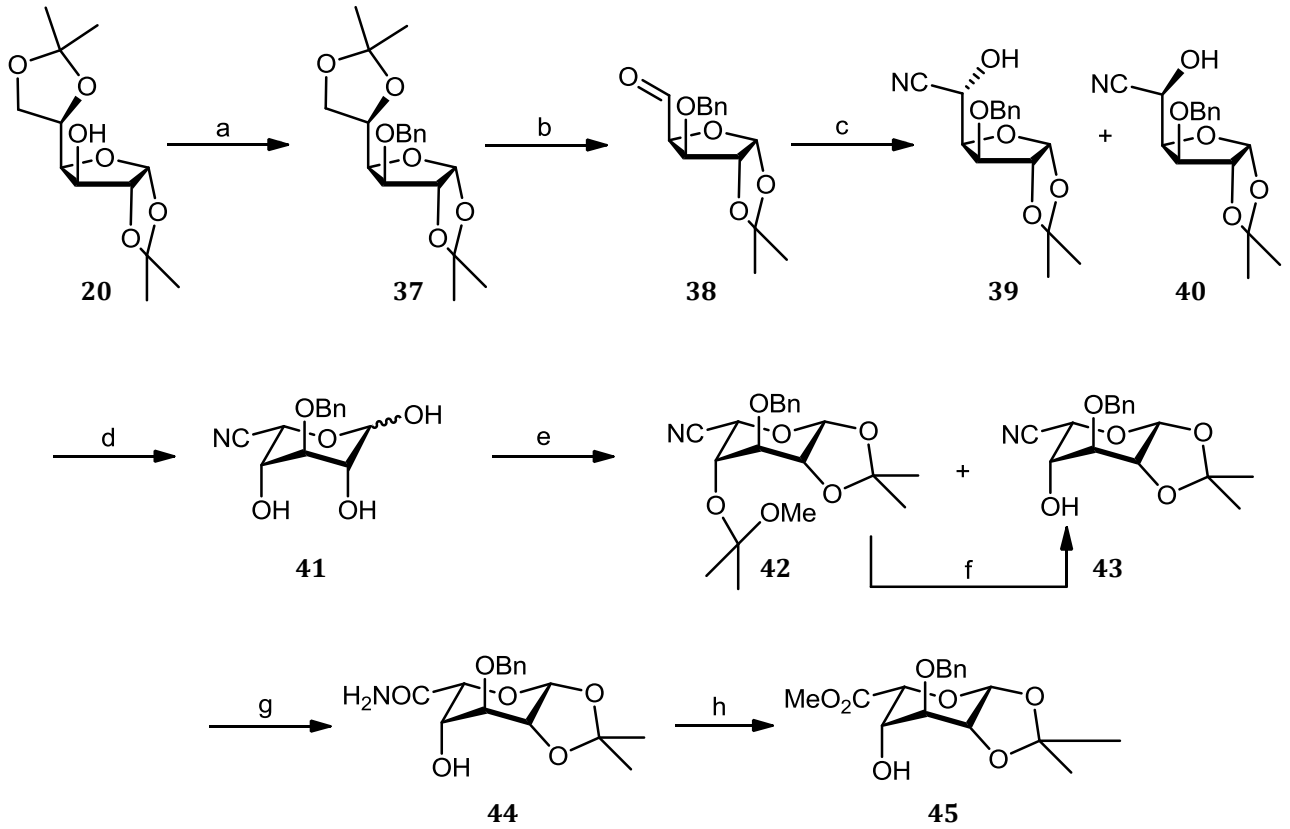
The first strategy (Scheme 6) has been published by two separate groups[49,50] and used the readily available, 1,2-*O*-isopropylidene-6,3-D-glucoronolactone (**33**) as the starting material. The inversion of configuration at C-5 was accomplished by means of O-triflation at C-5 followed by S_N2 inversion using the pivaloyl anion as a nucleophile to give **34**. When the lactone was opened, this protecting group then

migrated to O-3 to produce the 3-*O*-pivaloyl methyl ester **35**[49] which was subjected to hydrolysis of the isopropylideneacetal group to form L-iduronate **36**.



Scheme 6: Reagents and conditions (a) i: Tf_2O, pyridine; ii: NaOPiv, DMF (b) Et_3N, MeOH, 0°C, overnight (c) TFA, RT, 3 h

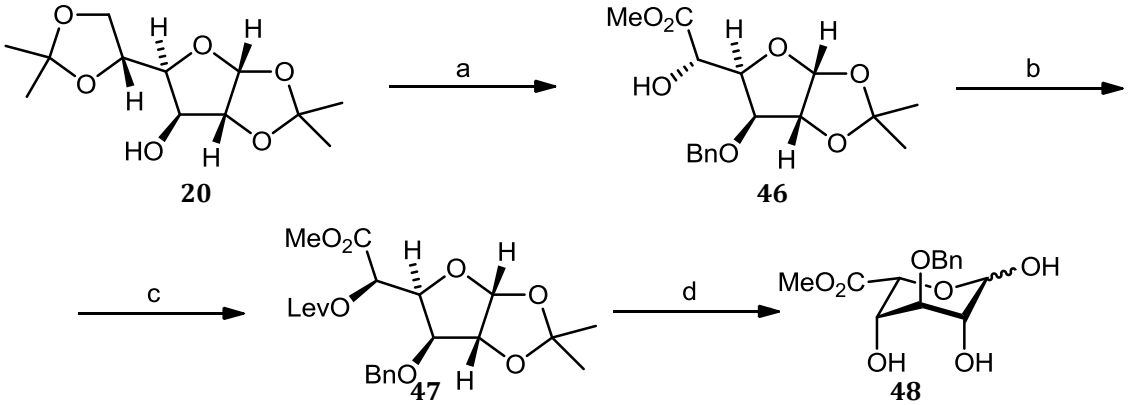
However, transformation of **36** to an L-AltNAcA derivative would require a significant number of complex challenges, including selectively introducing the acetamido group at C-2 with retention of configuration, and inversion of configuration at C-4, and seemed an impractical alternative at this stage.



Scheme 7: Reagents and conditions (a) BnO, Bu_4NHSO_3, NaOH (b) i) AcOH/H_2O ii) $NaIO_4$ (c) KCN, $MgCl_2$, H_2O, MeOH (d) TFA, H_2O (e) CSA, 2-methoxypropene, THF (f) TsOH, H_2O, THF (g) H_2O_2, K_2CO_3, dioxane, H_2O (h) DMF, DMA(i) as seen above in Scheme 2

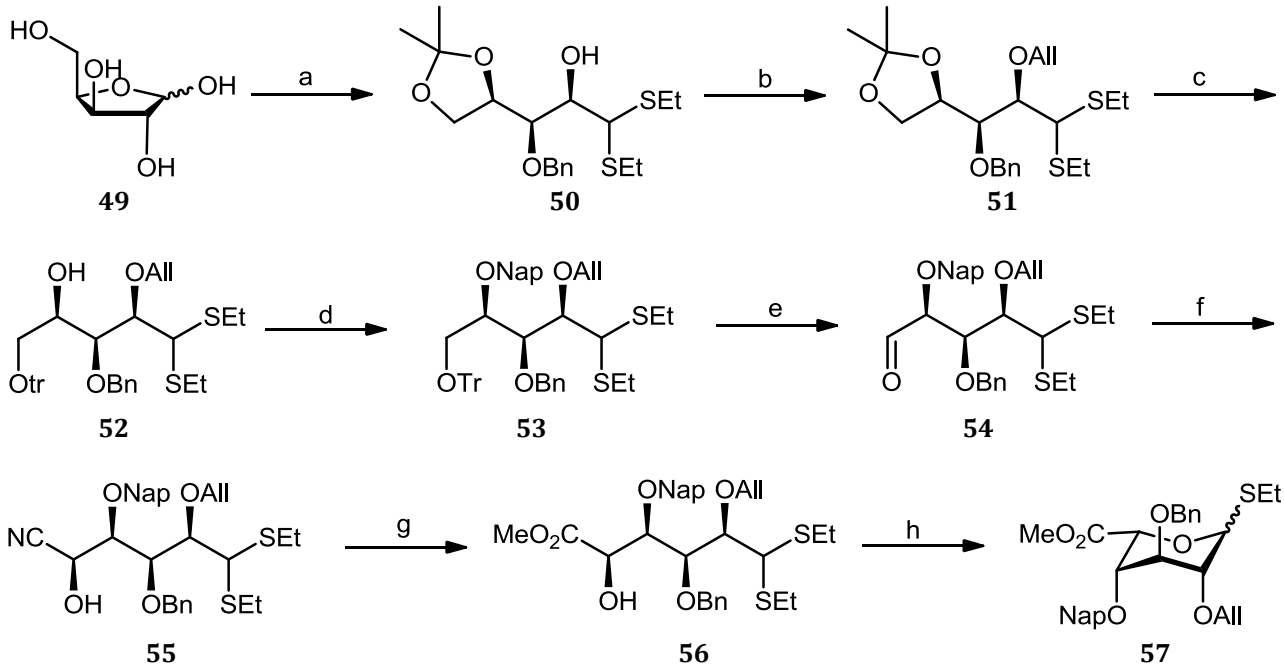
The second method (Scheme 7) started with the inexpensive dicaetone-D-glucose (**20**) which was protected at O-3 and then selectively deprotected at O-5 and O-6 before being reduced to the aldehyde **38**.[51] Following this, a stereoselective cyanohydrin reaction successfully inverted the configuration at C-5 giving the desired L-configuration. This was achieved in a 90% diastereomeric excess producing **39** and a minor amount of **40**. Carrying forward **39**, treatment with TFA afforded the cyanated pyranoside **41**. Synthesis of the desired product **43** and a side product (**42**) occurred via a tris-acetal-protected intermediate. Removal of the acetal group at C-4 conveniently facilitated the conversion of **42** to alcohol **43**. The cyano group at C-5 was then converted to the methyl ester in **45** via amide **44**.[52] The disadvantage of this route is that it is long and many of the steps require difficult separations to get the desired product. While there have been more efficient versions of this route published recently by Gardiner *et al*[53], unfortunately time did not permit exploration of this.

Another approach, *via* an S_N2 inversion at C-5, was successfully carried out by Orgueira *et al.*[54], Ojeda *et al*[50] and more recently Saito *et. al.*[55] Once again starting with the readily available diacetone-D-glucose (**20**), an 8 step procedure was carried out to form **46** in an overall yield of 65%. It is reported to be scalable to 100g and requires little purification,[54] factors which are definite benefits in a total synthesis. The inversion at C-5 is achieved on this furanose **46** through conversion of the hydroxyl in question to a better leaving group which can then be displaced by a nucleophile. In this case a levulinate anion was used which effectively formed the L-sugar **47**. Treatment with hydrazine in acetic acid yielded the L-sugar the structure is in the pyranose form (**48**) analogous to **36** in Scheme 6, and presenting similar challenges for the conversion to an L-AltNAcA derivative.



Scheme 8: Reagents and conditions (a) i: NaH, BnBr, THF, Bu_4NI; ii: AcOH, 40°C; iii: TBSCl, DMAP, DCM, py; iv: Ac_2O, DMAP, py; v: HF/py, THF; vi: TEMPO, KBr, Bu_4NBr, $NaHCO_3$, NaOCl, DCM/H_2O; vii: NaOH, MeOH; viii: MeI, $KHCO_3$, DMF, 65% (b) TFA, 99% (c) i: TF_2O, py, DCM; ii: NaOLev, DMF, 80°C, 82% (d) N_2H_4, AcOH, py, 91%

The last method involved a very interesting sequence involving the initial ring-opening of D-xylose (**49**), shown in Scheme 9.[56] **49** was initially converted in 6 steps to di-thio acetal **50** which was immediately protected to form **51** with different protecting groups at O-2 and O-3.[51] Removal of the isopropylidene acetal *via* acid hydrolysis was followed by a selective tritylation of the primary hydroxyl to produce alcohol **52**. The hydroxyl group at C-4 was then alkylated to give a fully protected product which could be selectively modified at each carbon. The pentane chain of **53** was elongated in the key step of this synthesis via a sequence of deprotection, oxidation and subsequent diastereoselective cyanation to give **55**.[56] Aldehyde **54** was a key intermediate as it allowed for the inversion of configuration at C-5 to produce L-idose after ring closure. Methyl ester **56** was synthesised using the Pinner reaction and was cyclised to produce pyranose **57** using NIS.



Scheme 9: Reagents and conditions (a) Lubineau, 6 step reaction (b) AllBr, NaH, DMF (c) i) AcOH ii) TrCl, pyridine (d) NapBr, NaH, DMF (e) TFA, Et_3SiH, CH_2Cl_2 (f) TMS-CN, $MgBr_2.OEt_2$, CH_2Cl_2 (g) i) AcCl, MeOH, toluene ii) H_2O (h) NIS, CH_2Cl[51]

This route has some very exciting chemistry however, the number of synthetic steps, the lack of crystalline intermediates thus leading to a lot of chromatography steps and the use of toxic reagents like cyanide, undesirable in terms of both human and environmental safety, make it unsuitable for the total synthesis of L-AltNAcA.

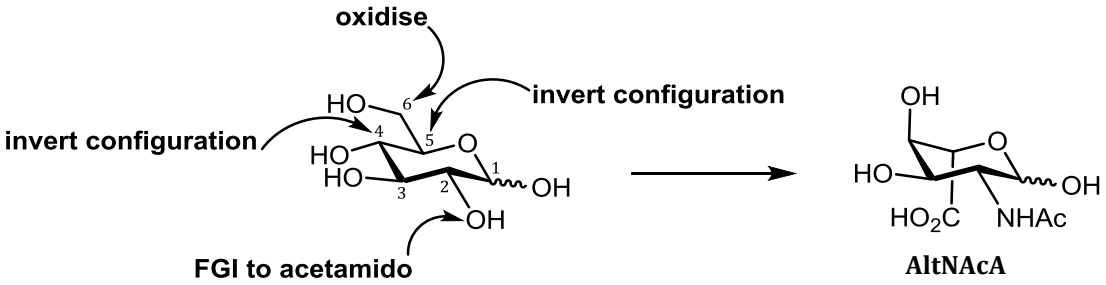
1.6.3 Synthetic strategies in the inversion of configuration in sugars

The inversion of a stereocentre in carbohydrates is of course not only confined to position 5. A common way to achieve this is by formation of a reactive triflate at the appropriate hydroxyl group followed by an S_N2 inversion by a nucleophile. This was described above for molecules in the furanose form but has also been successful on pyranose rings at a number of difference positions.[36,57,58] Other options include the Mitsunobu inversion, reactions proceeding *via* oxidations of free hydroxyl groups followed by stereoselective reduction, and eliminations followed by stereoselective oxidations, similar to the hydroboration reactions described above.

The foregoing summary provides some insight into the challenges and difficulties of synthesising L-sugars from D-sugars and justified the investigation of a new, efficient synthetic route to L-AltNAcA starting from readily available materials.

1.7 Proposed Alternative Synthetic Routes to L-AltNAcA

Starting from the most abundant monosaccharide, D-glucose, the key challenges in the synthesis of L-AltNAcA are highlighted in Scheme 10. Two inversions of configuration, at C-4 and C-5, would be

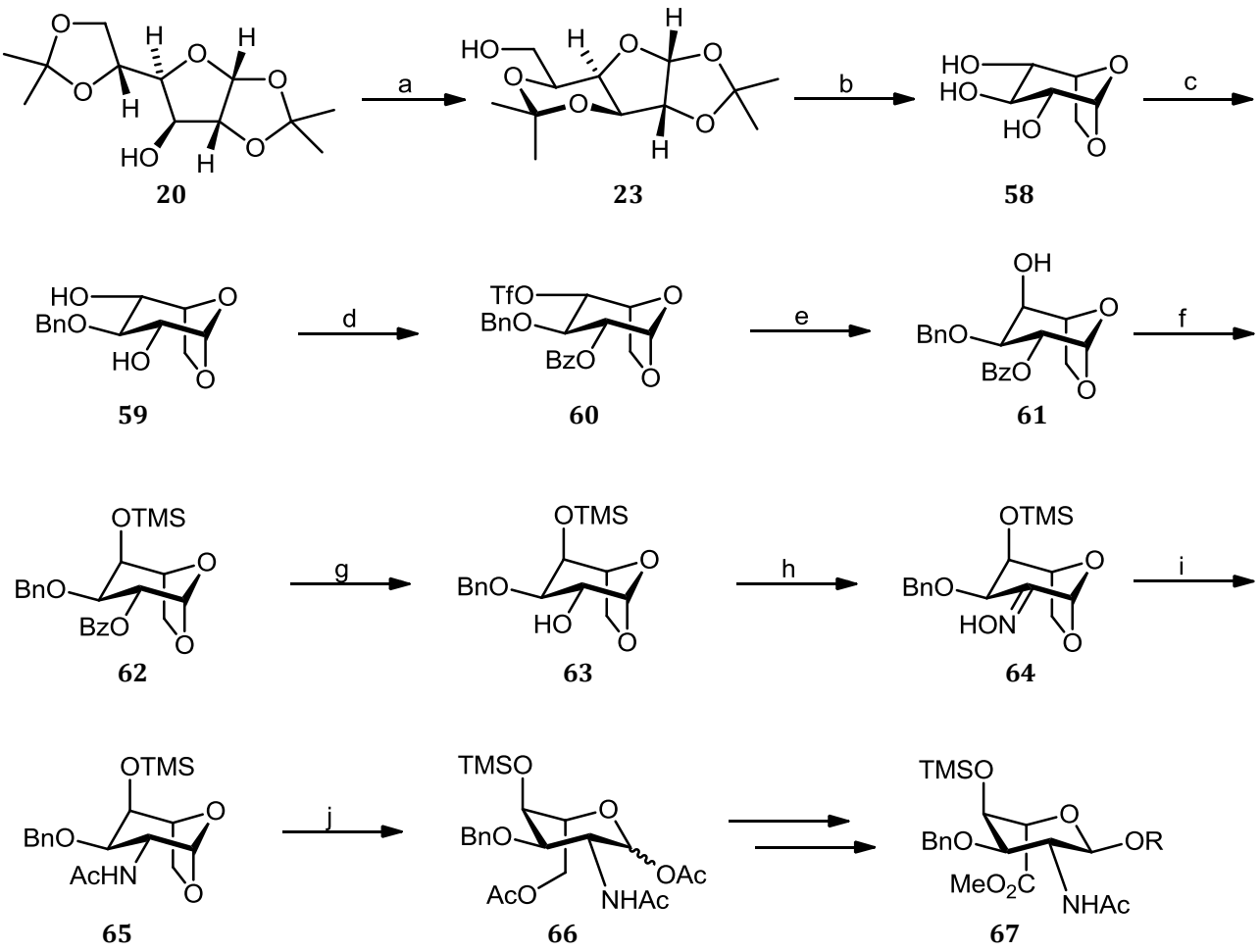


Scheme 10: Key synthetic challenges in the synthesis of AltNAcA from D-glucose

required in order to prepare L-altrose. Following this, oxidation at C-6 would provide the desired L-altruronic acid, and finally changing the functionality at C-2 to an acetamido group with retention of configuration to give the target compound, L-AltNAcA. With these challenges in mind and based on the reviewed literature, two synthetic routes were proposed for the synthesis of L-AltNAcA, the first starting from diacetone-D-glucose and the second from N-acetyl-D-glucosamine.

1.7.1 Proposed route starting from diacetone-D-glucose

The most effective synthesis reported in the literature that converted D-glucose to L-altrose was described in Scheme 4.[47] This route was adapted for the synthesis of biologically important L-hexoses by Lee *et al.*[59] The potential of this modified route to be adapted to the synthesis of L-AltNAcA was recognised and is described in Scheme 11. This route also presented the opportunity to explore interesting and novel chemistry.



Scheme 11: Reagents and conditions for proposed synthetic route from **20** to L-AltNAcA (a) 3 step route described in Scheme 4 (b) HCl, EtOH (c) i: TMSCl, Et_3N, DCM; ii: PhCHO, TMSOTf, Et_3SiH, DCM (d) i: BzCl, DCM, py; ii: Tf_2O, py (e) $NaNO_2$, H_2O (f) TMSCl, py (g) NaOMe, MeOH (h) i: oxidation; ii: oximation (i) i: LAH; ii: Ac_2O (j) TFA, Ac_2O

Following the original route carried out by Hung *et al.*[47] (Scheme 4) the sequence would start with a reaction of di-acetal **20** with PPh_3 and NBS to form **21**, in which bromination at C-6 would be achieved with rearrangement of the 5,6-acetal to the 3,5-acetal. The base induced elimination forming **22** would be carried out using either NaH or DBU. The inversion at C-5 to give L-idose derivative **23** would be

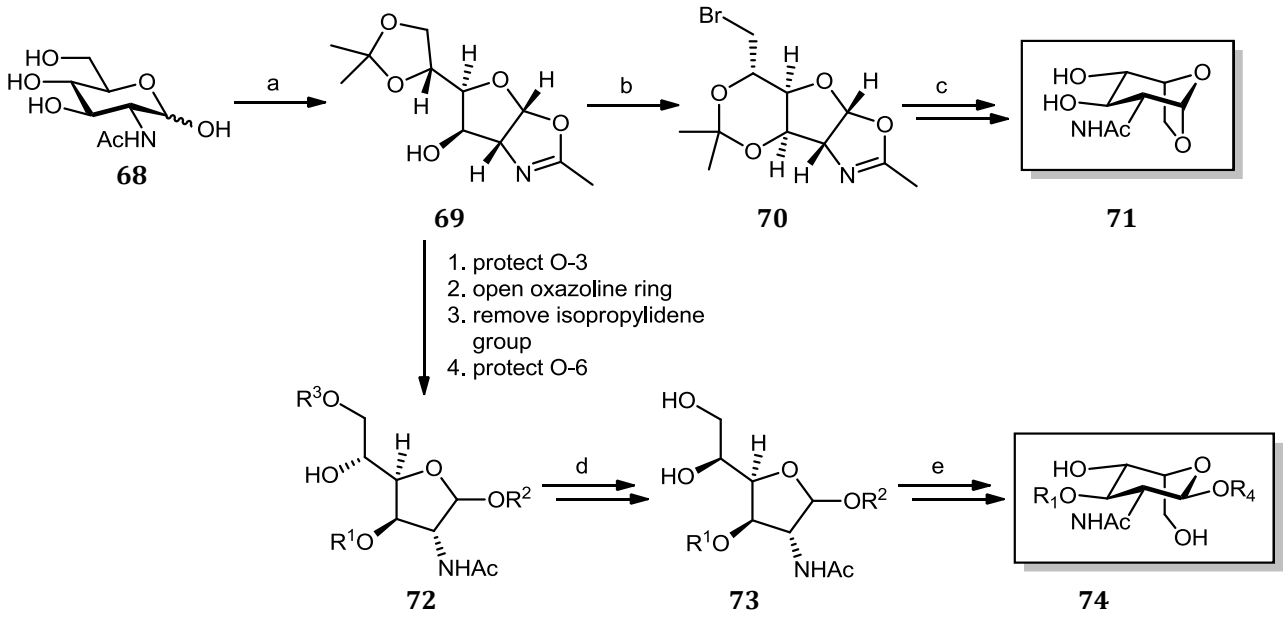
carried out by means of the stereoselective hydroboration-oxidation process with hydrolysis then providing 1,6-anhydro-L-idose. A modification of published procedures[59] would be followed to achieve the desired selective inversion at C-4 resulting in the L-altrose configuration seen in **61**. This route made use of the 1,6-anhydro bicyclic system seen in compound **58** which was then able to be selectively modified at positions, 2, 3 and 4. This method offered more selectivity than the original route for the functional group interchange that would need to be performed at C-2. The selective benzylation of O-3 would be carried out first *via* a tris-trimethylsilyl intermediate. The subtly different electronic environments at C-2 and C-4 would then be exploited to achieve the inversion *via* the triflate (**60**) to form L-altrose **61**. At this point the route would be adapted for the purpose of synthesising L-AltNAcA where the functionality at C-2 would be converted to an NHAc group and O-6 oxidised to give the desired selectively protected L-altrouronic acid **67**.

1.7.2 Proposed alternative route starting from N-acetyl-D-glucosamine

The obvious benefit of starting from N-acetyl-D-glucosamine (**68**) is that the desired NHAc is already in place at C-2. An interesting rearrangement and derivatization of compound **68** was reported by Mack *et al.*[60] (Scheme 12) in which the fused furano-oxazoline (**69**) is formed, while simultaneously protecting O-5 and O-6 as an isopropylidene acetal.

Compound **69** has a very similar structure to **20** and it was thus proposed that the sequence in Scheme 11 could potentially be carried out to synthesise compound **71** which could be further modified to form L-AltNAcA. Failing this, the sequence forming **72** would be attempted which takes advantage of the selectively unprotected O-3 and different reactivity of the oxazoline and isopropylidene groups. From

72, the key inversion at C-5 could be achieved following a similar method as Orgueira[54] and Saito[55] as described in Section 1.6.2.



Scheme 12: Overview of the proposed routes starting from N-acetyl-D-glucosamine (a) $BF_3.Et_2O$, $(CH_3)_2CO$ (b) PPh_3, NBS, CH_3CN (c) as detailed in Scheme 11 (d) i: inversion of C-5; ii: Deprotection of O-6 (e) hydrolysis

The aim of this project was therefore to contribute to the preparation of a glycoconjugate vaccine candidate for *S. sonnei* by synthesising a suitable derivative of the acidic sugar, L-AltNAcA, found in the repeating unit of *S. sonnei*. The proposed synthetic routes as discussed above were the focus of this project. In relation to the first route, specific emphasis would be placed on the investigation of alternative methods for discriminating between the three hydroxyl groups in 1,6-anhydro-β-L-idopyranose. Achievement of such selectivity would allow for the selective inversion of the configuration at C-4 and converting the hydroxyl at C-2 to the desired acetamido group. The emphasis in the second route, which already has the 2-acetamido group in place, would be placed on optimizing the chemistry required for achieving the inversion of configuration at C-5. All compounds synthesised en route to the target compound L-AltNAcA would be fully characterised by 1D ^1H and ^{13}C NMR spectroscopy and 2D NMR where necessary, together with other spectroscopic and analytical techniques.

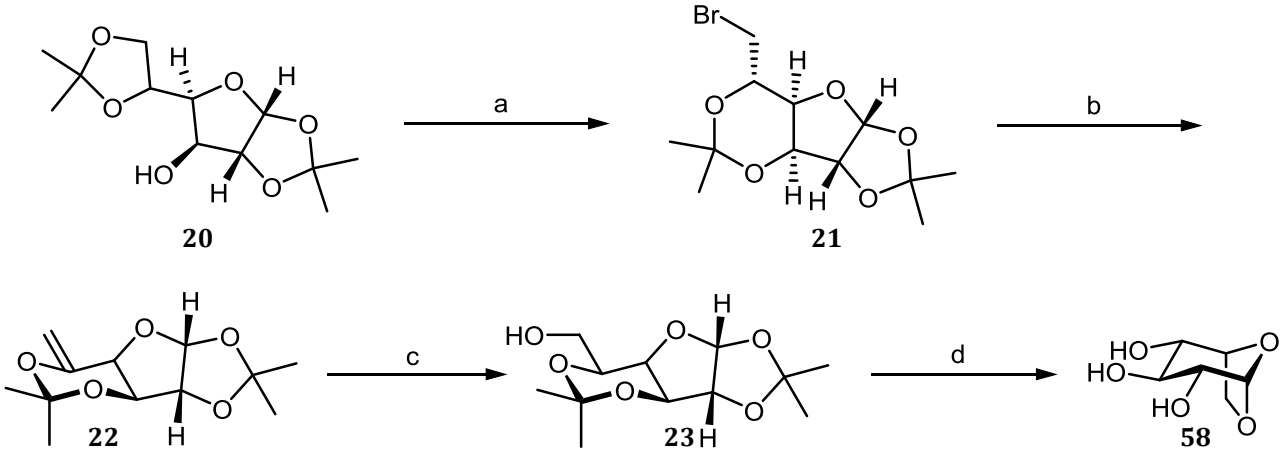
CHAPTER 2

Synthetic Route 1:

Since L-sugars are rare and proportionately expensive, a monosaccharide in the D–configuration was selected as the starting material. Therefore, it was essential to arrange the carbohydrate structure to allow selective manipulation of C-5 to achieve the key inversion of configuration at that centre. D-Glucose is the cheapest and most readily available monosaccharide however is difficult to selectively protect, particularly at positions 1 through 5 where the hydroxyl groups are in similar chemical environments. Diacetone-D-glucose which is already selectively protected was thus selected preferentially. Following literature procedures,[1–3] the key intermediate 1,6-anhydro-β-L-idopyranose was successfully synthesised via the proposed four step synthesis. A study of chemo-and regioselectivity was subsequently carried out in order to derivatise 1,6-anhydro-β-L-idopyranose into the desired L-AtlNAcA.

2.1 Preparation of 1,6-Anhydro-β-L-idopyranose

Following the procedure of Lee *et al.*[59] this four step sequence successfully achieved the inversion of configuration at C-5 and a further transformation to triol **58** which is set up for selective protections and modifications towards L-AltNAcA.



Scheme 13: Reagents and conditions (a) NBS, PPH_3, CH_3CN, MS, 80°C, 20 h, 70% (b) NaH, DMF, 0 - 20°C, 16 h, 95% (c) i: BH_3.THF, THF, RT, 3.5 h; ii: H_2O_2, NaOH, 0°C, 0.5 h, 71% (d) 0.2M HCl in EtOH, 95°C, 17 h, 80%

The first step in this sequence involved an interesting rearrangement of the isopropylidene group together with selective bromination at C-6 in order to set up the possibility of a selective elimination at C-5, C-6, while leaving all the other functional groups protected. Treatment of diacetone-D-glucose with NBS and PPh_3 in acetonitrile gave the desired product (**21**) in low yield (<40%) after the recommended aqueous workup. This was eventually improved to 70% on a three gram scale through rigorous exclusion of water by using 4 Å molecular sieves in the reaction, extending the reaction time and avoiding the aqueous workup. NMR analysis confirmed the formation of the desired product (**21**) with all data corresponding to that in the literature.[61]

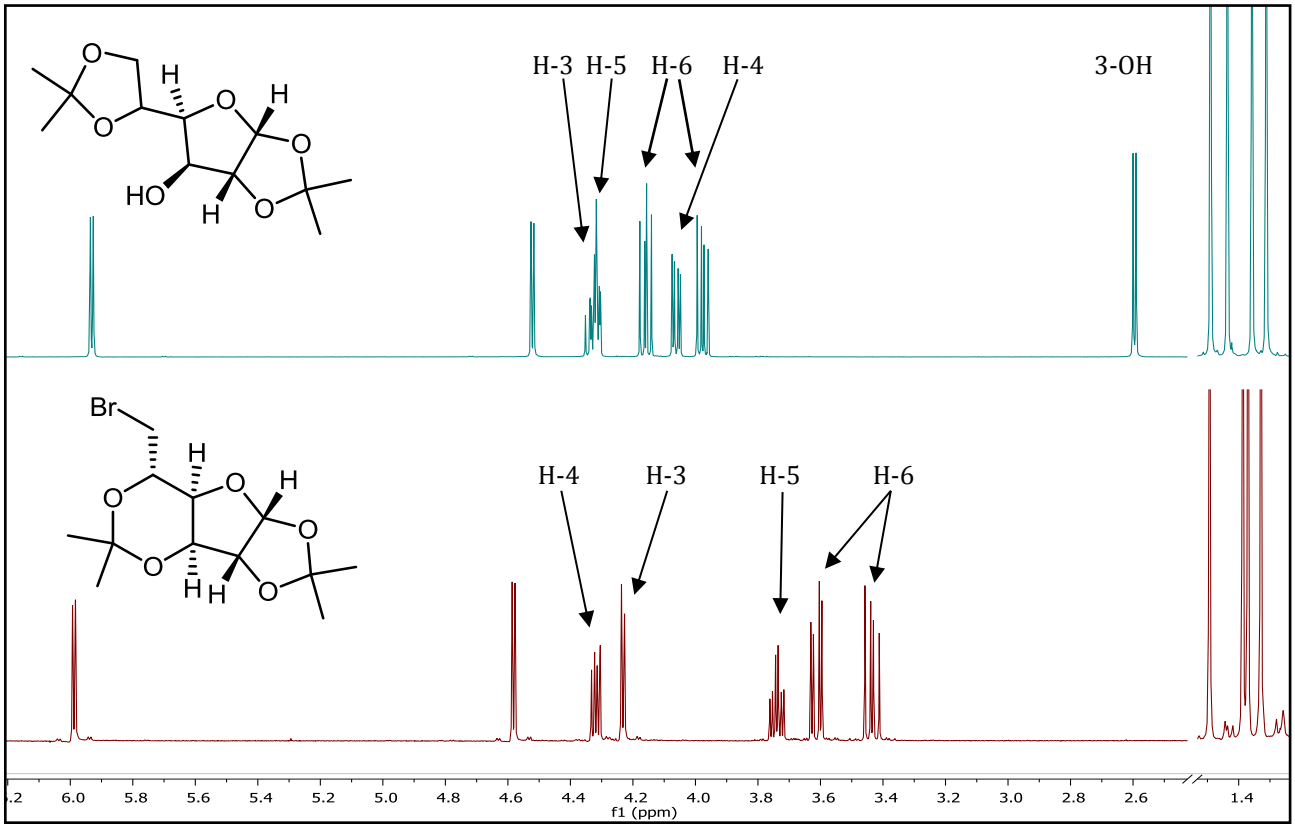


Figure 3: Diagnostic shifts in ^{1}H NMR signals from compound **20** to **21**

The diagnostic peaks shifts are seen in Figure 3, which shows in particular the significant upfield shifts of signals for H-5 and H-6 due to the electron donating bromine. H-5 couples to the two H-6 protons and H-4 which is evident from its signal at 3.74 ppm, which changed from a multiplet to an apparent triplet of doublets due to the increased rigidity of the product. This resulted in more distinct splitting patterns in the H-3, H-4 and H-6 signals and the disappearance of the doublet at 2.60 ppm for the hydroxyl at C-3.

The proposed mechanism for this reaction is shown in Figure 4. It is suggested by Hodosi *et al.*[61] that, based on their NMR experiment results, an initial reaction occurs between NBS and PPh_3 forming a phosphonium salt. The least hindered oxygen in **20** then forms an intermediate phosphonium salt, **A**, followed by opening of the dioxolane ring to form oxocarbenium intermediate, **B**. This rearranges to the 3,5-ketal while at the same time the bromide ion attacks at C-6 to release PPh_3O to give the 6-deoxy-6-bromo product **21**.

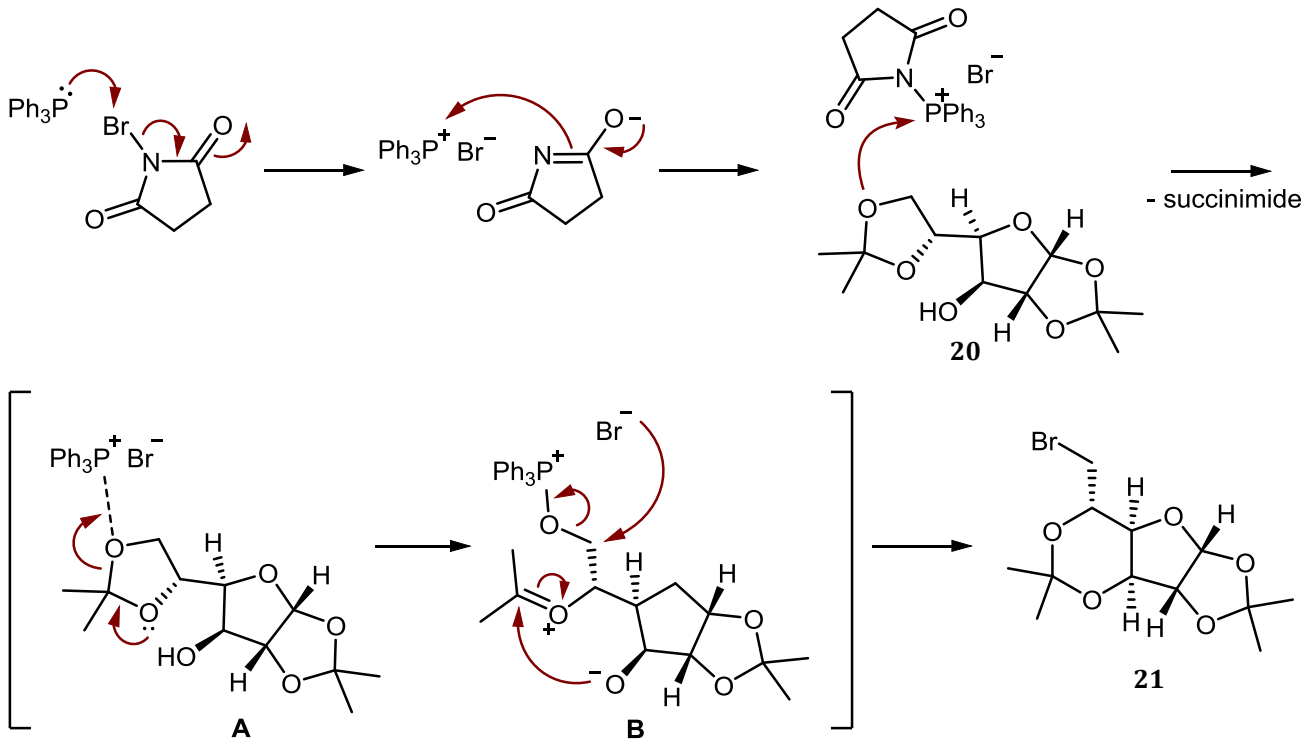


Figure 4: Proposed mechanism for rearrangement and bromination of diacetone-D-glucose

The next step required an elimination to prepare the *exo*-olefin required for the anticipated stereoselective hydroboration reaction. This was achieved using NaH in DMF[62] affording the desired enol ether **22** in 95% yield after purification by column chromatography.

Confirmation was seen in the NMR spectra which agreed with reported data.[59] Figure 5 shows, in particular, the signals for two H-6 protons which appear as two doublets at 4.76 and 4.69 ppm, considerably further downfield from their position in the spectrum of **21**, together with the expected absence of a signal for H-5.

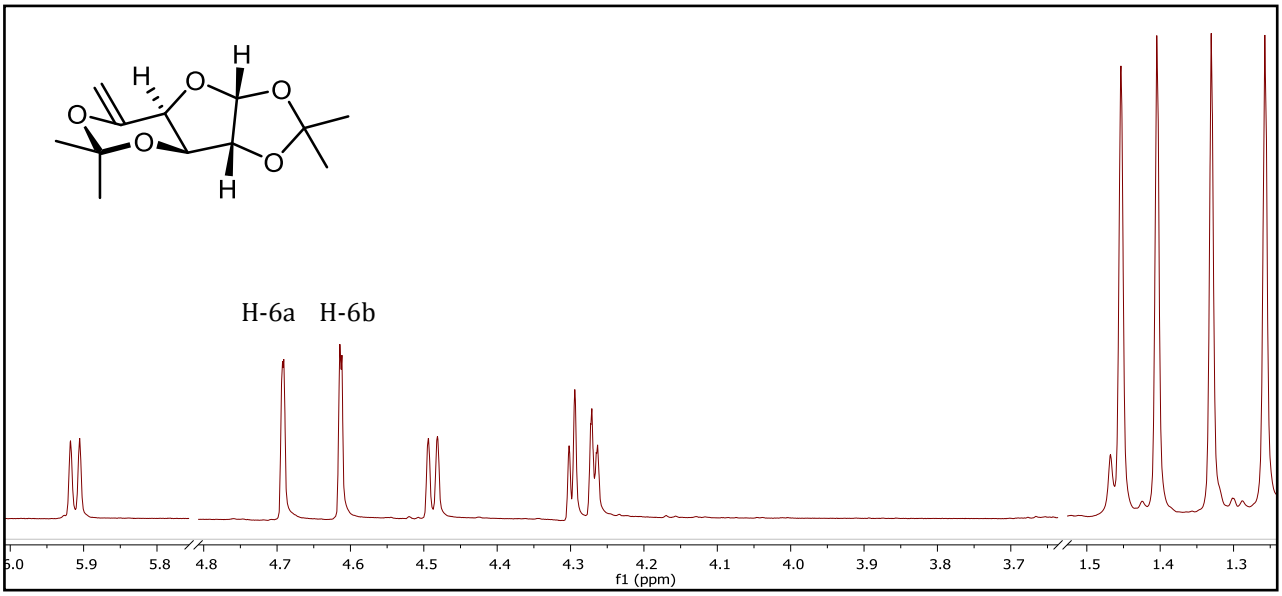


Figure 5: ^{1}H NMR spectrum of enol ether **22**

The elimination of HBr presumably occurred *via* an E2 type mechanism shown in Figure 6 .

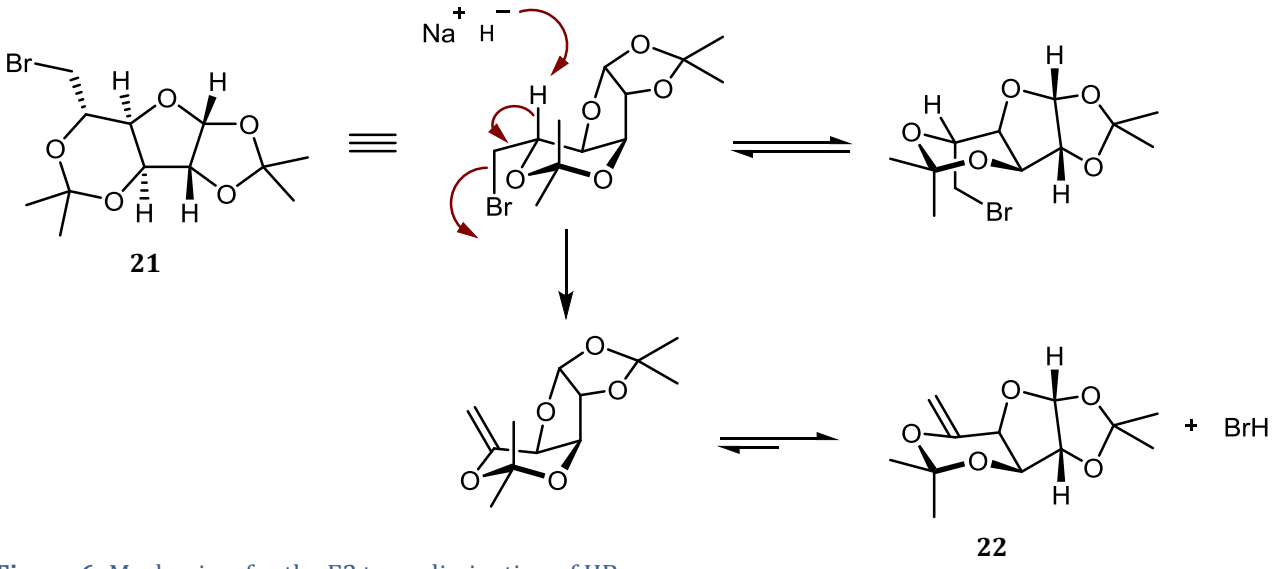


Figure 6: Mechanism for the E2 type elimination of HBr

The next reaction addressed the key challenge in this synthetic route, the inversion of the stereocentre at C-5 to give the desired L-configuration for the target molecule. This was achieved by stereoselective hydroboration and subsequent oxidation to give **23** in a 71% yield. The product was confirmed as a single diastereomer by NMR spectroscopy with data corresponding to that in the literature.[59] The most diagnostic feature in this spectrum (Figure 7) was the appearance of a multiplet for H-5 at 4.11 ppm significantly downfield compared to H-5 in the 6-bromo product. The two signals for H-6 have

correspondingly shifted further upfield, again confirming the presence of a hydroxyl at that position. The signal for H-4 appears as a triplet resonating at 4.01 ppm but is in fact an overlapping doublet of doublets with two small couplings of 2.2 Hz. The signal for H-4 in compound **21** is a doublet of doublets with one large coupling (7.2 Hz) and one smaller coupling (3.9 Hz) corresponding to the close-to anti peri-planar relationship between H-4 and H-5. The change observed in **23** corresponds to a much smaller dihedral angle between H5-C5-C4-H4, close to 0°, which is consistent with the inversion of configuration at C-5.

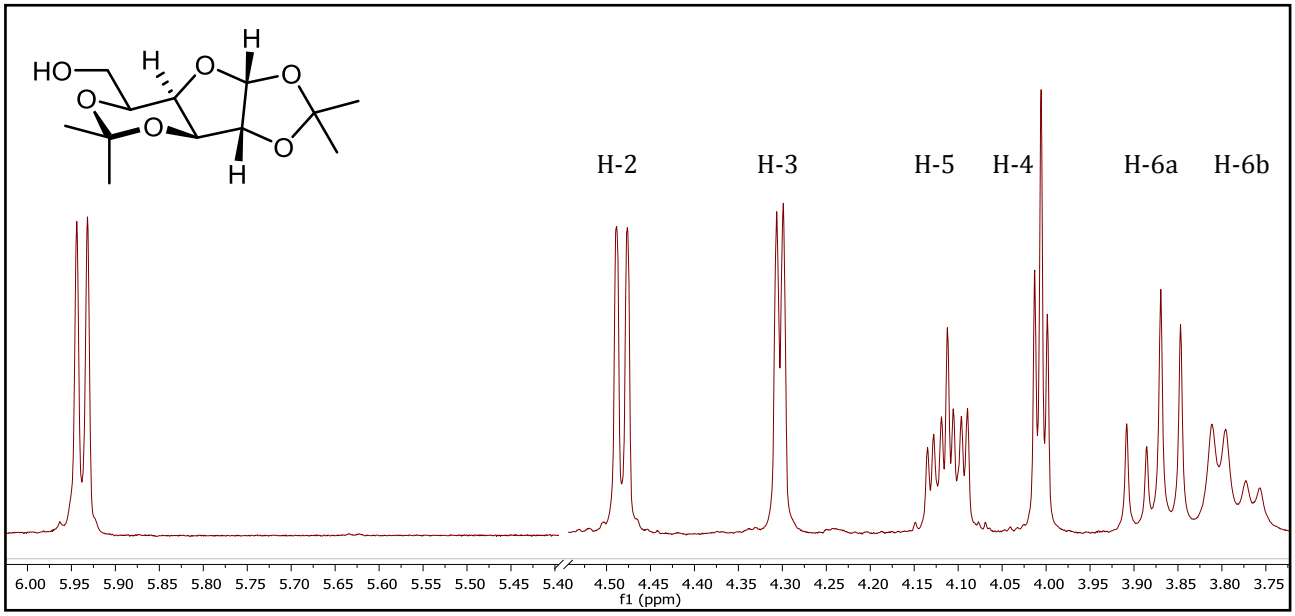


Figure 7: ^{1}NMR spectrum of compound **23**

The observed stereoselectivity in the addition of borane can be explained by the steric congestion in compound **23** (Figure 8). The β-face of transition state **C** has the C4–O4 in a *pseudo*-axial orientation eclipsing the incoming borane, which discourages this approach to give alkyl borane **C'** (or its tri-alkyl analogue) as an intermediate, which is itself high in energy due to diaxial repulsion between C-6 and the isopropylidene CH_3. In contrast, addition to the α-face in transition state **D** is less sterically hindered and thus lower in energy than **C**.

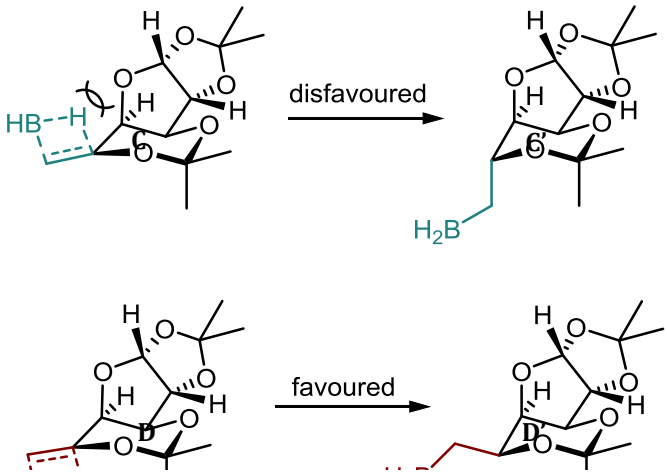


Figure 8: Facial selectivity in hydroboration step

This forms intermediate **D'** which is more stable than its counterpart **C'** since the bulky borane group is equatorially-oriented.[59] Therefore pathway **D** is both kinetically and thermodynamically favoured.

The reaction thus starts with the syn addition of borane to the α-face of the double bond (transition state **D** in Figure 8) with possible formation of the trialkyl borane, and then subsequent oxidation to give 1,2:3,5-di-*O*-isopropylidene-β-L-idofuranoside (**23**) in an overall anti-Markovnikov addition of H_2O.

After successfully inverting the stereocentre at C-5 *via* the stereoselective hydroboration of the *exo*-cyclic double bond, the final step in this initial sequence involved preparation of 1,6-anhydro-L-idose (**58**) as a potentially versatile synthetic intermediate. This involved refluxing idofuranoside **23** in acidified ethanol, effectively removing the isopropylidene groups and catalysing the rearrangement to form the 1,6-anhydro pyranose form as shown in Figure 10. A single product was isolated after column chromatography and was confirmed by NMR analysis to be the desired 1,6-anhydro-β-L-idopyranose in an 80% yield which agreed with reported literature data.[59]

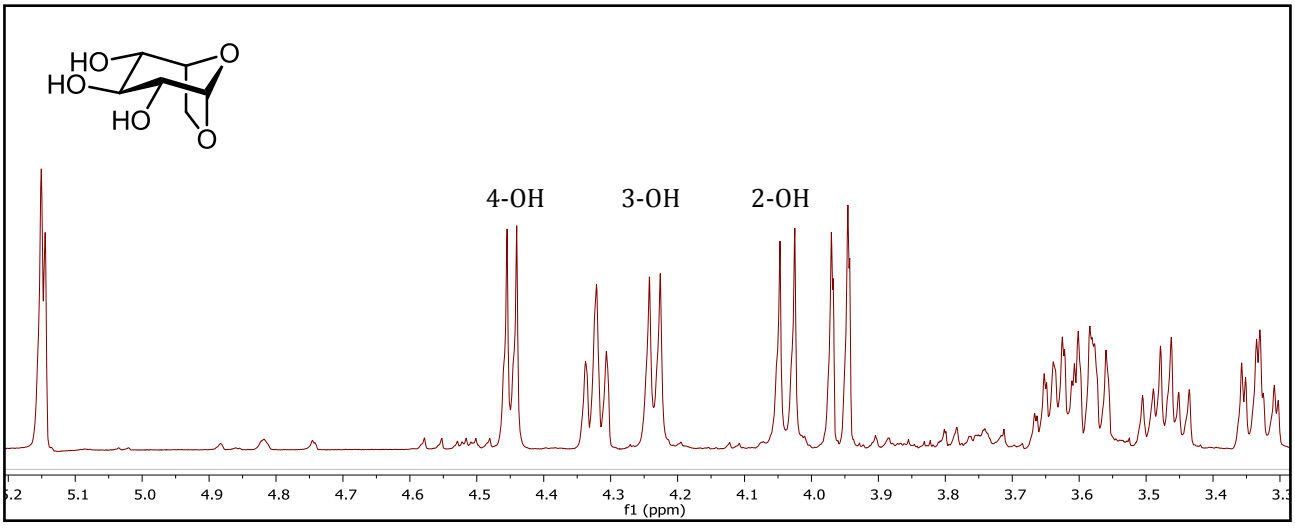


Figure 9: ^{1}H NMR spectrum of compound **58**

Due to the increased polarity of this compound the NMR were recorded in $(CD_3)_2CO$ which facilitates the appearance of OH peaks as clear doublets (Figure 9) in addition to the signals for 7 protons of the carbohydrate skeleton. These were assigned with reference to literature data[59]. The disappearance of the four methyl singlets between 1.3 and 1.5 ppm confirmed that the isopropylidene group had been removed.

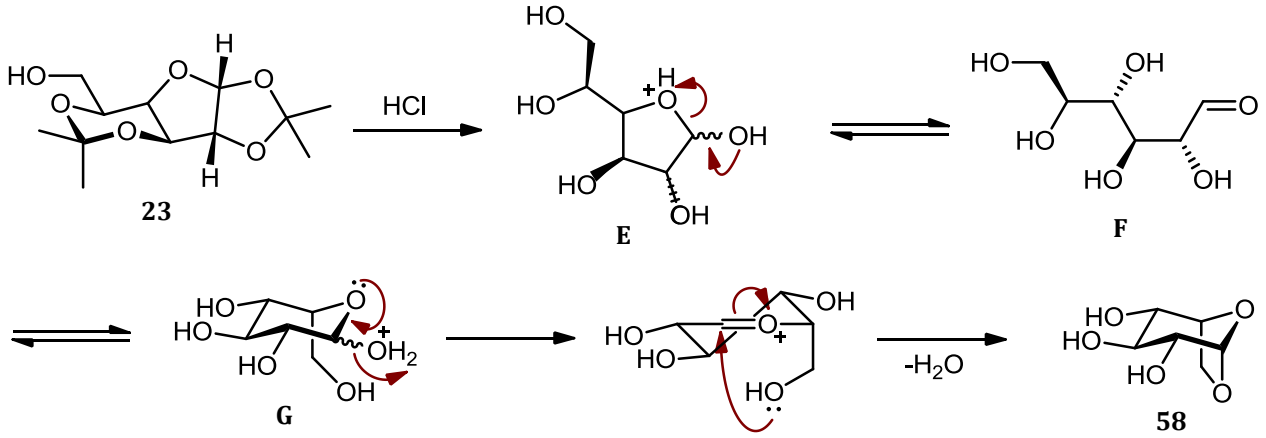


Figure 10: Proposed mechanism for deprotection and anhydro ring formation

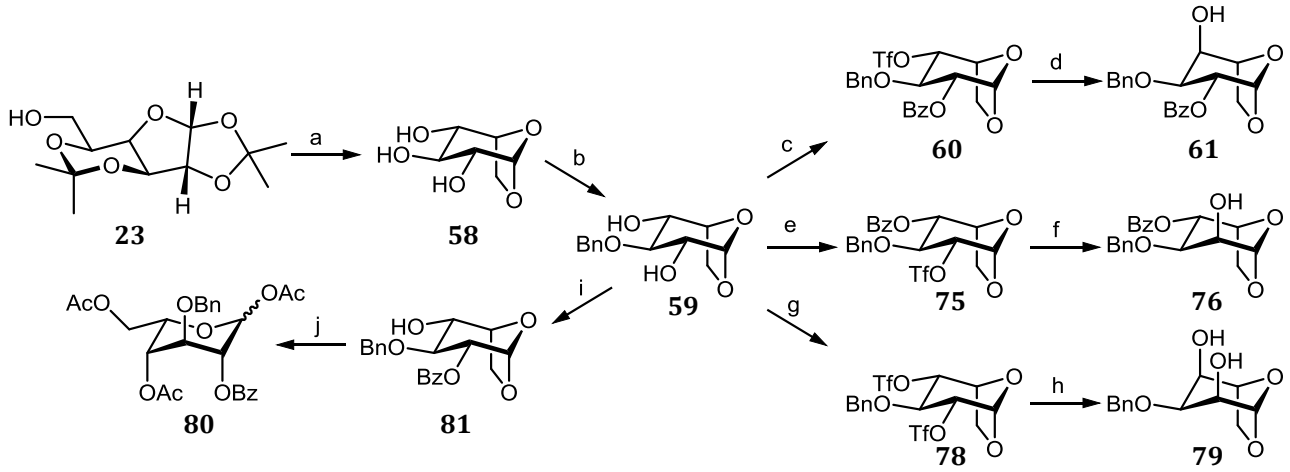
The reaction presumably proceeds as shown in Figure 10, with initial removal of the isopropylidene protecting groups to give the furanose intermediate (**E**) which is in equilibrium with the open chain aldehyde, **F**. This is in turn in equilibrium with the 6-membered pyranose ring (**G**) which under acid hydrolysis loses the anomeric hydroxyl to form an oxocarbenium ion which is trapped by the 6-OH to form 1,6-anhydropyranose, **58**.

2.2 Attempted Selective Protections of 1,6-Anhydro-β-L-idopyranose

With this key intermediate in hand an investigation was initiated into the possibility of differentiating between the three free hydroxyl groups through selective protection/deprotection sequences. The ultimate aim, as shown in Figure 2, was to achieve the inversion of configuration at C-4 and functional group conversion at C-2, while O-6 remained protected to allow for oxidation at C-6 at a later stage.

A review of the literature showed that a number of research groups had been successful in derivatising triol **58** and other monosaccharides with a similar structure.[45,59,63–68] As noted earlier, the intention of

this study was to follow the approach of Hung's group at the Academia Sinica in Taiwan[45,59], who had successfully carried out a series of regioselective protections which enabled further selectivity in the inversion of C-2 and C-4.

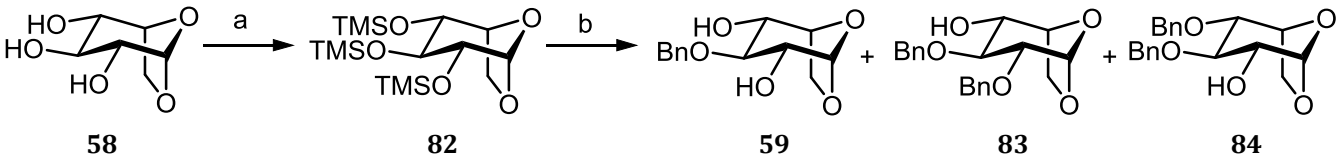


Scheme 14: Work published by Hung *et. al.*[45,59] Reagent and conditions (a) 2M HCl, EtOH (b) i: TMSCl, Et_3N; ii: PhCHO, TMSOTf, Et_3SiH (c) BzCl, DCM, py, Tf_2O (d) $NaNO_2$ (e) Tf_2O, py, BzCl (f) $NaNO_2$ (g) Tf_2O, py (h) $NaNO_2$ (i) BzCl, py (j) Ac_2O, $Sc(OTf)_3$

The key features that were of interest for application in the synthesis of L-AltNAcA were the protections using benzyl and benzoyl groups sequentially in **59** and **60** and selective inversion *via* a triflate intermediate to form **61** (Scheme 14). Other research suggested that stannylene-mediated chemistry might achieve selectivities in benzylations[63] and benzoylations[64]. In addition, similar selectivity has been observed in acetylation reactions[65], modified Mitsunobu reactions[66,67] and also in selective deprotection of fully benzylated 1,6-anhydro D-sugars[68]. The aforementioned literature provided the rationale for the studies that were done on the selectivity in 1,6-anhydro-β-L-idopyranose.

2.2.1 Attempted inversion of configuration at C-4 following Hung's route[59]

In the first instance, selective benzylation was attempted *via* the TMS ethers as reported by Hung *et al.*[59] This approach is in line with that proposed by Hung *et al.*[59] who argued that the selectivity arose from differences in steric strain in the molecule imposed by the substitutions at the different hydroxyl groups.



58 **82** **59** **83** **84**

Scheme 15: Reagents and conditions (a) TMSCl, Et_3N, DCM, RT, 4 h, 84% (b) i: TMSOTf, PhCHO, MS, Et_3SiH, DCM, -78°C, 1 h; ii: TBAF, THF, RT, 1 h, 43% major product **59**, 20% **83** and 7% **84**

The results are shown in Scheme 15. The tris-trimethylsilyl ether was successfully prepared in an 84% yield when **58** was reacted with excess TMSCl in DCM under basic conditions. The structure was confirmed by NMR analysis which correlated with that in the literature.[59] The three new 9 proton singlets at 0.14, 0.14 and 0.17 ppm confirmed the presence of the TMS groups. Selective benzylation was then attempted with encouraging results. Initial attempts led to the 3-*O*-benzyl compound **59** as a major product with the two dibenzyl ethers **83** and **84** as minor products (Scheme 15). However, the yield of the desired compound **59** was increased to 90% on a 0.2 g scale and 60% on 1.0 g scale by maintaining the reaction temperature at -78°C during and after addition of catalytic amount of TMSOTf. The reaction mixture was only allowed to warm to room temperature after addition of Et_3SiH in order to effect the reduction of the TMS-acetal to form the desired benzyl group at O-3. The proposed mechanism for this reaction sequence is outlined in Figure 11. The remaining TMS groups at positions 2 and 4 were then cleaved using TBAF.

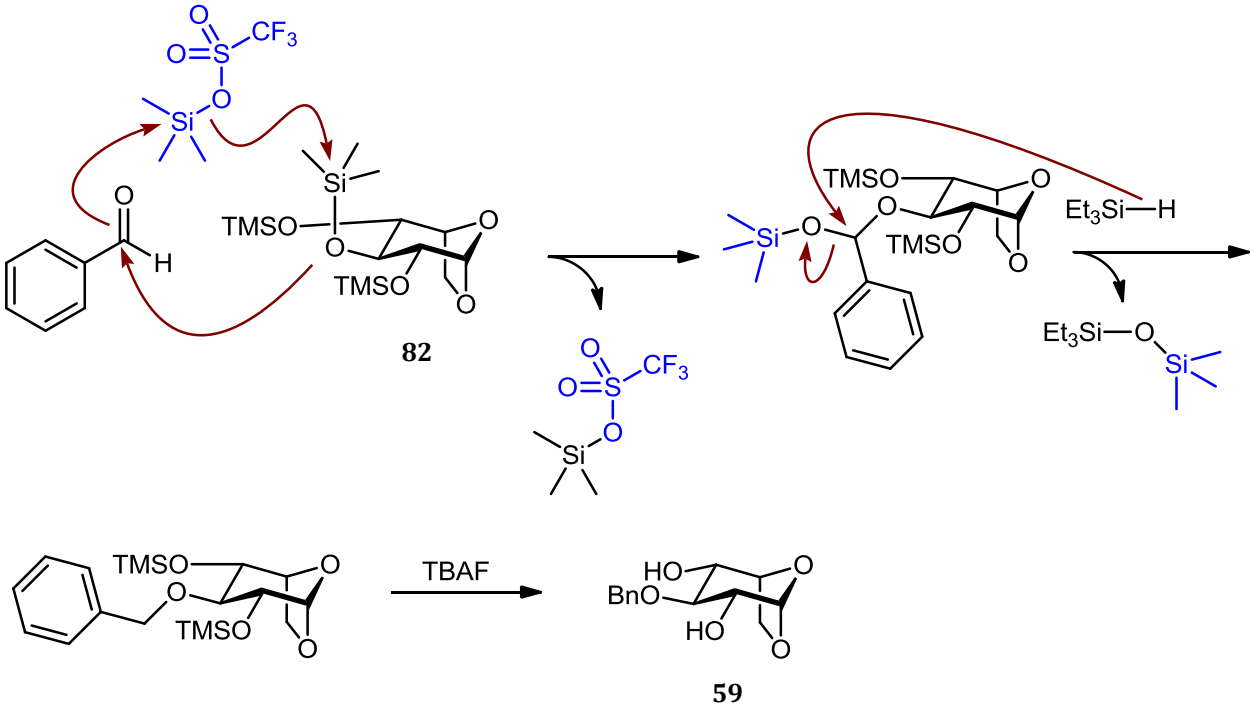


Figure 11: Proposed mechanism for the formation of **59** *via* TMS ether **82**

This structure **59** was confirmed by the appearance of two key signals in the ^{1}H NMR spectrum (Figure 12), confirming the presence of a single benzyl group. These were a multiplet in the aromatic region which integrated for 5 protons, and an AB quartet resonating at 4.85 ppm which is characteristic of the benzylic CH_2. The latter splitting pattern, from a signal that would ordinarily be a singlet, results from each proton being in a slightly different chemical environment due to the rigidity of the molecule. In addition there were two doublets at 4.67 and 4.27 ppm corresponding to the free hydroxyl groups at C-4 and -2 respectively, with assignments confirmed by the COSY spectrum. These data correlated with that in the literature.[59]

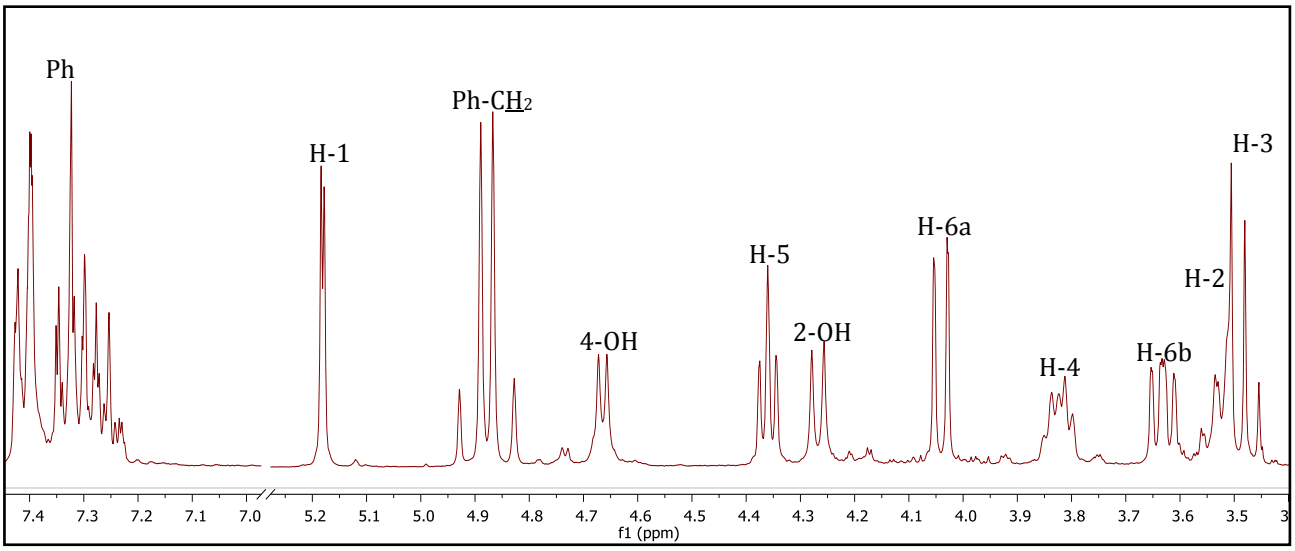
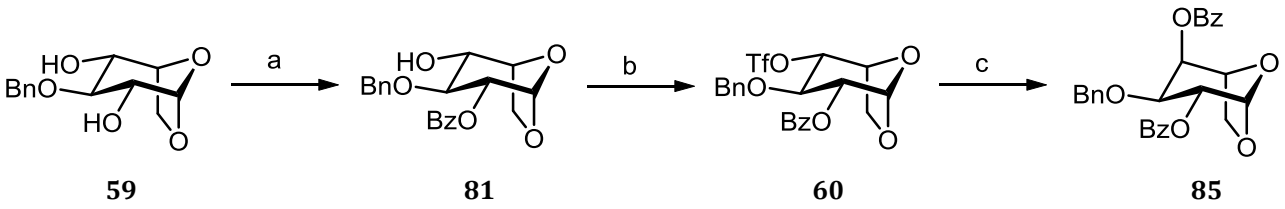


Figure 12: ^{1}H NMR spectrum of compound **59**

After successfully achieving selectivity in the benzylation of O-3, the inversion of configuration at C-4 was investigated. This required initial selective protection of the 2-OH, preferably using a different group to that at O-3. To this end an adaptation of the route described by Lee *et al.*[59] was attempted as shown in Scheme 16. The selective benzoylation at O-2 was first carried out independently to test the viability of this route with the intention of then attempting a sequential one-pot benzoylation and triflation. To this end, diol **58** was treated with BzCl and pyridine at low temperature. The reaction did

not go to completion and gave monobenzoate **81** in 20% yield together with recovered starting material (28%).



59 **81** **60** **85**

Scheme 16: Reagents and conditions (a) BzCl, DCM, py, 0°C, 4 h, 20% (b) Tf_2O, py, RT, 1 h, 59% (d) NaOBz, DMF, 0→20→90°C

The structure of **81** was confirmed by ^{1}H NMR (Figure 13). Three additional signals in the aromatic region integrating for 5 protons in total indicated the presence of the benzoyl group. Its location was confirmed by the downfield shift in the signal for H-2 which now resonated at 4.92 ppm while the signal for H-4 remained further upfield. Although the yield for this reaction was low, the proof of concept was demonstrated through the fact that none of the 4-*O*-benzoyl sugar formed. The one-pot benzoylation at O-2 and triflation at O-4 was consequently pursued.

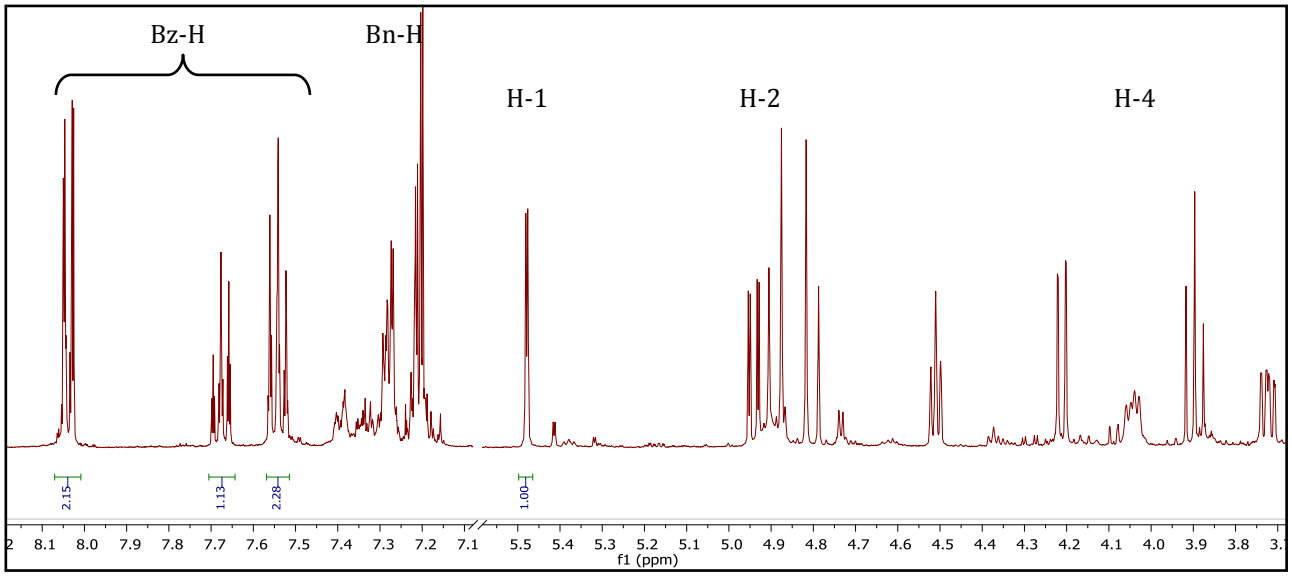


Figure 13: ^{1}H Spectrum of compound **81**

BzCl was added to a solution of **59** in DCM and pyridine and stirred for 4 h at 0°C. Tf_2O was then added to the reaction mixture and this was stirred at room temperature for 1 h after which it was quenched with MeOH. A product was isolated and analysed by NMR which showed that, unfortunately, two products had formed. This was most evident in the ^{1}H spectrum in Figure 14 where a doubling of signals in a 1:2 ratio could be seen. Since the products were present in different amounts, it was possible to

determine their structures through careful analysis of the 2D spectra of this mixture and comparison to reported literature.[59]

The major product was identified as **60** which correlated with literature.[59] The diagnostic peaks confirming this are highlighted in Figure 14. The signal for H-2 resonates at 5.13 ppm, a similar chemical shift to that in **59** while the signal for H-4 has shifted downfield, consistent with triflation at O-4. Coupling between the signal for H-2 and a carbonyl signal in the HMBC proves that the benzoyl group is indeed at O-2. Final confirmation of the presence of the triflate was found in the ^{13}C spectrum. Although it is proton decoupled, the presence of the fluorine is evident from the appearance of a quartet for $\underline{C}F_3$, due to C-F coupling.

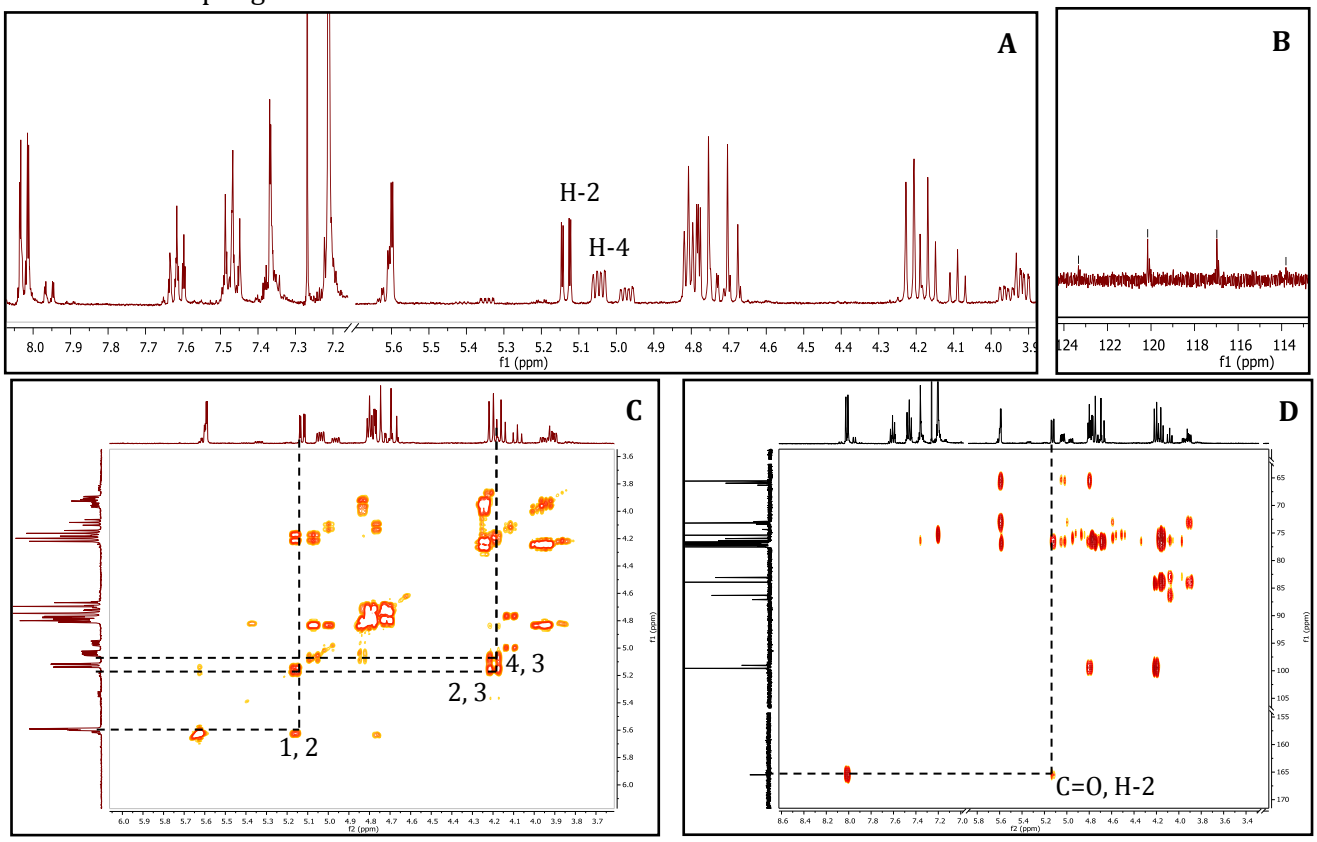


Figure 14: NMR Spectra for mixture of compound **60** and minor product **(A)** ^{1}H **(B)** ^{13}C **(C)** COSY **(D)** HSQC

Even though these results show that the reaction was not highly selective compared to literature which reported an 88% yield of **60**, the desired compound had formed and was the major product. However, before optimising this step the feasibility of the inversion of the triflate with NaOBz was first tested. The

presence of a good leaving group at C-4 would allow for an S_N2 inversion, leaving a protected hydroxyl group at this position.

When triflate **60** was treated with NaOBz in DMF at 0°C or at room temperature there was little reaction progress as judged by TLC. However, when the reaction mixture was heated to 90°C all of the starting material appeared to be consumed after 3 h. After workup and purification by column chromatography two compounds were isolated, the less polar of which was recovered smarting material (34%) while the more polar compound was shown by NMR to be a mixture of compounds. Attempts to separate these by preparative TLC were not successful and no evidence emerged to support the formation of the desired product. With this reaction being unsuccessful, the previous step only moderately selective and the O-2 benzoylation low yielding, it was decided that this direct route to the selective C-4 inversion was not feasible.

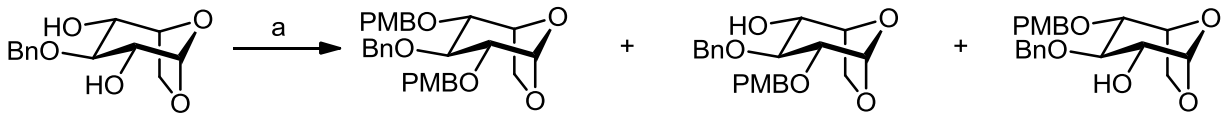
Returning to the 1,6-anhydro-3-*O*-benzyl-β-L-idopyranose moiety, a modified Mitsunobu reaction[66,67] was attempted in the hope that one equivalent of reagents would achieve a selective inversion at either C-2 or C-4. DIAD was added to a mixture of **59**, PPh_3 and PhCOOH in anhydrous THF which had been cooled to 0°C. There was no reaction progress 0°C or at room temperature as judged by TLC. Even after increasing the temperature to 60°C no reaction occurred and only starting material was recovered from the reaction mixture.

2.2.2 Investigation of reactivity differences between hydroxyl groups in triol 58

After failing to replicate the selectivity achieved by Hung *et al.*[45,59] an investigation into the possible differences in reactivity of the hydroxyl groups in triol **58** was conducted by doing reactions under standard, uncatalysed conditions as low temperatures. Although not all of Hung's work could be repeated, the selective benzylation at O-3 was successful and thus compound **59** was investigated first. Failing that, further investigations into the differences in reactivity of 2-, 3- and 4-OH in **58** would be carried out.

It was hypothesized[59] that the inductive effects of the two oxygens at C-1 increased the acidity of 2-OH therefore making it more reactive to electrophiles than 4-OH under basic conditions. The first attempt at a selective protection was to use PMB as the electrophile since it can be selectively removed in the presence of the benzyl at O-3. **59** was treated with one equivalent of PMBCl and NaH in DMF at 0°C. After 4 h at this temperature TLC showed little to no progress so the reaction mixture was warmed to room temperature. After 12 h it was clear that no more starting material was being consumed so the reaction was quenched with MeOH and purified by column chromatography. Two products were recovered together with starting material (27%). The less polar product was identified easily by NMR as the di-PMB ether **86** (50.1%). A single anomeric signal resonated at 5.35 ppm, additional aromatic peaks integrating for 8 protons appeared between 7.42 and 7.19 ppm, two additional CH_2 peaks had appeared as well as two singlets integrating for 3 protons each at 3.78 ppm. These data were consistent with the presence of two PMB groups thus confirming that compound **86** had formed.

The more polar compound migrated on TLC as a single product, recovered in 19% yield. However, two anomeric signals were seen in the ^1H NMR spectrum indicating that a 1:1 mixture of two distinct products with the same R_f had formed. Although these were inseparable, information on their structures was obtained by careful analysis of the NMR spectra of the mixture. The aromatic signals between 7.42 and 6.80 ppm integrated for 18 protons in total which suggested that the two compounds each had one benzyl and one PMB group and suggesting the presence of a mixture of the two alcohols **87** and **88**.

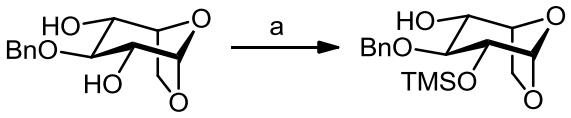


58 **86** **87** **88**

Scheme 17: Reagents, conditions and products for the PMB reaction (a) PMBCl, NaH, DMF, 0°C, 4 h, RT, 12 h

Four CH_2 signals were present which agreed with the proposed structures. The six-proton doublet resonating at 3.78 ppm, suggesting two overlapping methoxyl singlets, and signals in the ring proton region integrating for 14 protons provided further strong evidence for the formation of the two products **87** and **88**. Although as noted earlier, it proved impossible to separate and fully characterize each product.

The next attempt at selectively protecting O-2 was to use TMS which is a less bulky group than PMB creating less steric strain in the product and potentially increasing the yield. A smaller group could react faster allowing for reduced reaction time which might also improve selectivity. **59** was treated with Et_3N and TMSCl in DCM at 0°C. However, no reaction took place even after it was warmed to room temperature and the reaction time increased to 48 h. These results therefore demonstrate that even at low temperature there is no significant difference in the reactivity of the 2-OH and 4-OH.

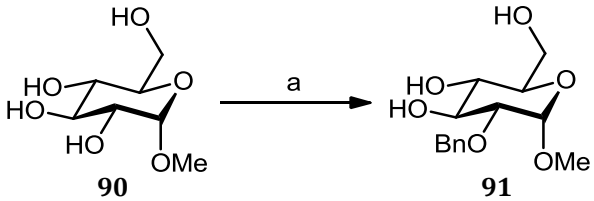


59 **89**

Scheme 18: Reagents and conditions for attempted silylation of **59** (a) TMSCl, Et_3N, DCM, RT, 48 h

2.2.3 Tin mediated protections of 1,6-anhydro-β-L-idopyranose

It was then hypothesised that if O-2 was selectively protected by a benzyl or benzoyl group first, the subsequent protection of O-3 would be easier as it is under less steric strain than O-2. Although there has been reported success in regioselective benzoylations simply through the use of BzCl in pyridine[69], previous attempts on **58** suggested that this would not give adequate selectivity. High regioselectivity had been described when using Bu_2SnO as a catalyst in benzylations[63] (Scheme 19) and benzoylations[64]. Since there was already some observed selectivity at O-2, the tin catalyst could potentially enhance that feature. The formation of stannylene complexes (Scheme 20) with compound **58** shows the potential products from a tin mediated reaction with either benzyl or benzoyl protecting group.

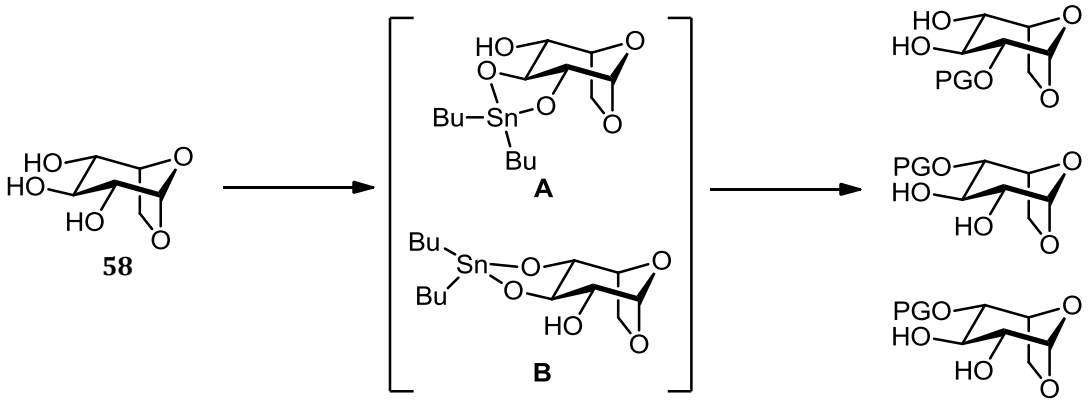


90 **91**

Scheme 19: Reaction reported by Zhou *et. al.*[63] showing regioselectivity through tin mediated benzylation (a) 1.1 equiv Bu_2SnO, 0.5 equiv TBAB, 1.2 equiv BnBr, 100°C, 8 h, 83%

Two experiments were carried out according to the reaction conditions detailed in the aforementioned literature and are summarized in Table 1. In both reactions Bu_2SnO was added to **58** and refluxed for at least 30 mins before adding the other reagents, in order to allow the stannylene complexes to form and possibly influence the regioselectivity. In the benzoylation reaction three compounds were collected after purification by column chromatography. Two of these were in fact mixtures of two products as

shown in Table 1. The 2-*O*-benzoate **92** and 4-*O*-benzoate **93** had a combined yield of 56% and were in a 1:4 ratio determined by NMR. Di-*O*-benzoylated products **94** and **95** were also collected as a mixture (13%) and tri-*O*-benzoylated **96** was isolated in an 11% yield.

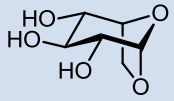
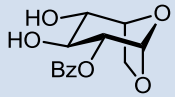
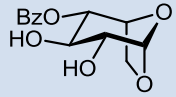
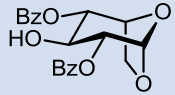
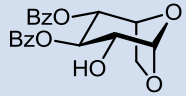
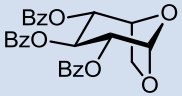
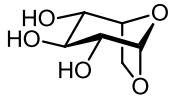
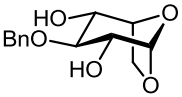
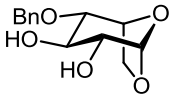
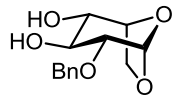


Scheme 20: Stannylene complexes formed with **66** and their potential products

In contrast, in the stannylene-mediated benzylation reaction, the three monobenzylated compounds **59** (30%), **97** (22%) and **98** (22%) were formed in the second reaction in approximately equal proportions, with the 3-*O*-benzyl ether **59** slightly dominating. The variety of products formed in these reactions was most likely the result of stannylene complexes forming both intra- and intermolecularly.[64]

The structures of the products formed in these reactions were determined from NMR experiments using a combination ^{1}H, ^{13}C, COSY, HSQC and HMBC. The COSY spectrum (Figure 15) clearly shows the 1:4 mixture of **92** and **93**. Although the aromatic region showed overlapping signals, the signals for the independent ring systems of the two compounds were sufficiently resolved to allow full assignment of both structures with the assistance of this spectrum.

Table 1: Summary of reaction conditions for tin oxide mediated protections

	Starting Material	Scale (mg)	Reagent(s)	Equivalents	Solvent(mL)	Temperature (°C)	Time (h)	Product(s)
Benzoylation		80	Bu_2SnO BzCl	2.0 2.1	CH_3CN	110 RT	0.5 18	 **92**  **93** 56% as a 1:4 mixture of **92** and **93**  **94**  **95** 13% as a 2:3 mixture of **94** and **95**  **96** 11%
Benzylation		183	Bu_2SnO BnBr TBABr	1.1 1.2 0.5	CH_3CN DMF	110 98	2 8	 **59** 30%  **97**  **98** 40% as a 1:1 mixture of **97** and **98**

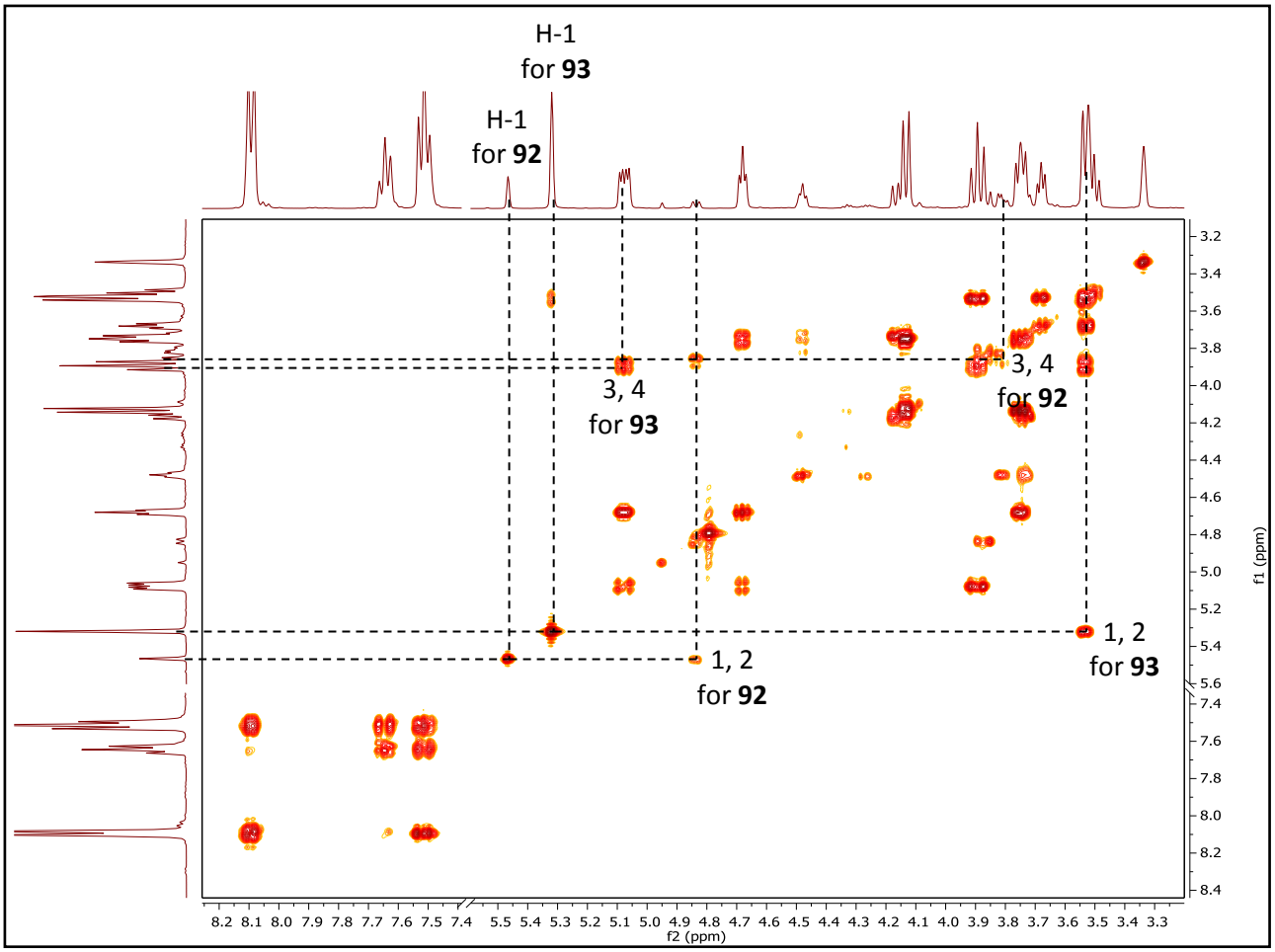


Figure 15: COSY Spectrum for compounds **92** and **93**, with two anomeric signals highlighted, as well as correlations to the respective H-2's and H-4's, which differ dramatically in chemical shift due to the presence or absence of the benzoate ester

The position of the signal for H-2 in **92** indicates that it is significantly deshielded compared to that for **93** supporting the proposed structures. The same is true for H-4 for **92** which is shifted further downfield than in compound **92**. The HMBC spectrum provided further confirmation of the positions of the benzoates in the two compounds. As highlighted in Figure 16, there are very distinct cross peaks between the benzoate carbonyl signals and H-2 of **92** and H-4 of **93** respectively, proving that the benzoyl groups are indeed at those positions.

The structures of the two di-benzoylated compounds **94** and **99** were determined in a similar fashion as they were also recovered as an inseparable mixture after chromatography. It was again possible to estimate from ^{1}H NMR that **94** and **95** were present in a 2:3 ratio, on the basis of analysis of the two clearly resolved spin systems with the aid of their COSY spectrum (Appendix). The signal for H-2 in **94**

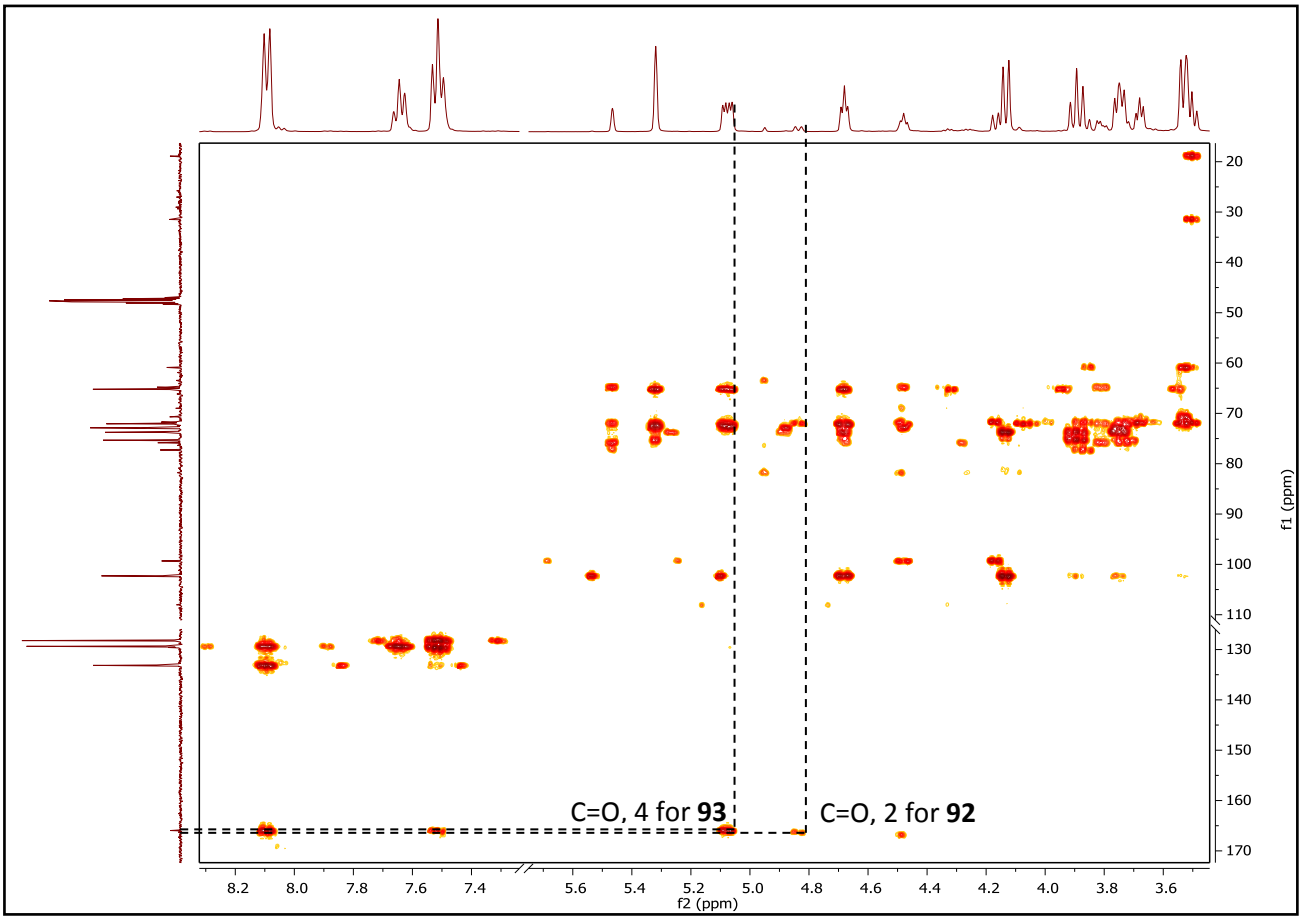


Figure 16: HMBC spectrum for compounds **92** and **93**

is significantly deshielded compared to that for **95** suggesting that **94** has a benzoyl group at O-2 but **95** does not. The same is true for H-3 for **95** which is shifted further downfield than in compound **94**. The signal for H-4 in each compound was deshielded compared to the starting material **58**. In addition, the increased integral for the aromatic peaks indicated that there were two phenyl groups present for each compound confirming the proposed structures.

The tri-benzoylated product **96** was the least polar compound from this reaction and was isolated and characterised easily since it was clear from its ^{1}H NMR spectrum (Figure 17) that only one product was present. The signals for H-2, H-3 and H-4 were all shifted downfield from their positions in the spectrum of the starting material **58**. The aromatic region showed signals correlating to 15 protons and the lack of hydroxyl signals confirmed that this was indeed the fully protected carbohydrate.

The range of products formed was most likely due to the wide number of stannylene complexes that could form between **58** and Bu_2SnO which can bridge two compounds forming a type of dimer. This

opens up a variety of selectivities ultimately leading to the complex mixtures observed. In addition to this, it has been reported that acyl groups such as benzoyl can undergo migration to adjacent hydroxyls in the presence of tin oxide mediators.[64] Unfortunately, while this outcome does show selectivity it is not useful synthetically since it was not able to be isolated as a pure compound.

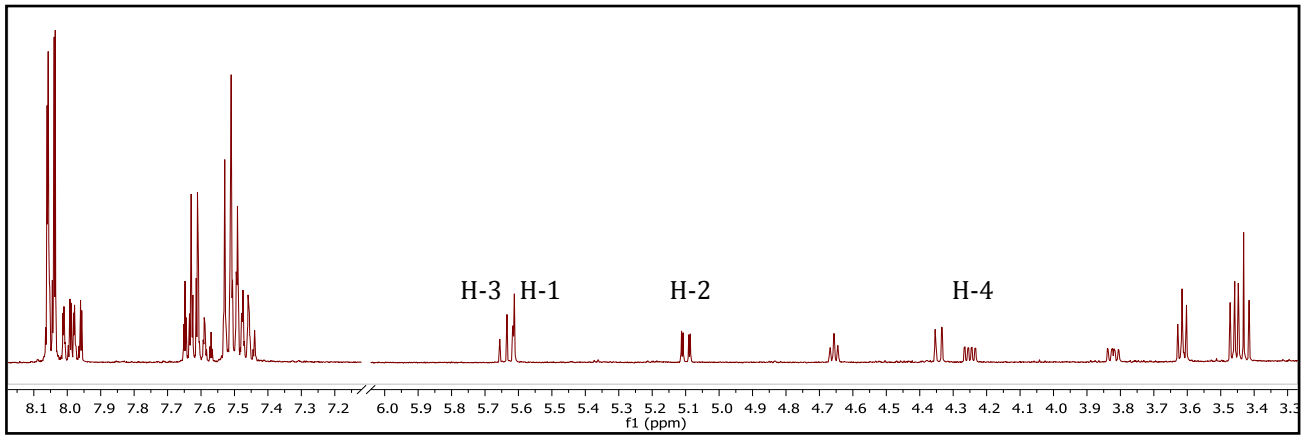


Figure 17: ^{1}H NMR spectrum of compound **96**

The results from the tin mediated benzylation were less encouraging. Although three monoprotected products were formed there was no clear selectivity. As in the case of the benzoylation experiment, the least polar compound was isolated as a single pure compound. There was a single anomeric peak in the ^{1}H NMR spectrum, which was identical to that of the previously synthesised benzyl compound, and the structure therefore assigned as 1,6-anhydro-3-*O*-benzyl-β-L-idopyranose, **59**.

The two mono-benzyl ethers **97** and **98** were recovered as an inseparable mixture, as became apparent from their NMR analysis. The spectrum showed two signals for anomeric protons and twinning of all the other signals between 3.2 and 4.9 ppm. From the integrals it was evident that there were two compounds present in a 1:1 ratio, and this, together with the overlap of some signals, made resolution of the respective spin systems difficult. However, through careful examination of the COSY spectrum, (Figure 18), the two systems could be resolved and it was evident that two mono-protected compounds were present.

Evidence for the presence of two benzyl groups came from the presence of two overlapping AB quartets integrating for four protons, and the presence of 10 protons in the aromatic region. The location of these protecting groups was determined through cross peaks in the HMBC spectrum between the benzylic $C\underline{H}_2$ signals and C-2 of **98** and C-4 of **97**. In addition, the presence of signals correlating to hydroxyl groups at the respective positions in the two products confirmed the proposed structures for the two products **97** and **98**.

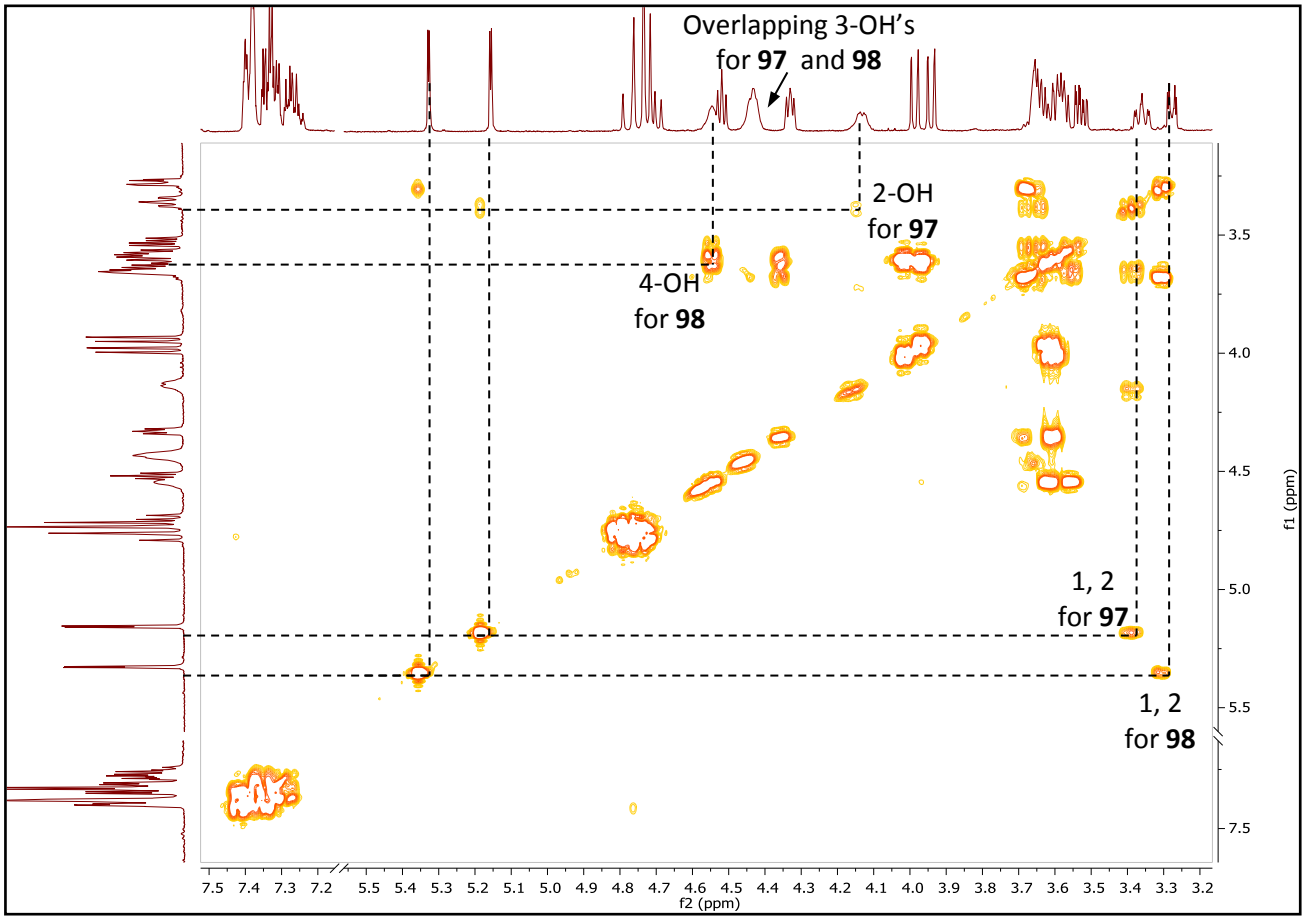


Figure 18: COSY spectrum of compounds **97** and **98,** with two anomeric signals highlighted, as well as the 2- and 3-OH signals for **97** and 3- and 4-OH signals for **98**

After reviewing the results from these attempted regioselective protections and inversion, it was concluded that little progress was being made towards the total synthesis of L-AltNAcA. The steric and stereoelectronic environments in 1,6-anhydro-β-L-idopyranose were simply not sufficiently distinct to achieve good selectivity between C-2, C-3 and C-4. The second proposed route starting from readily available N-acetyl-D-glucosamine was thus investigated as proposed in Chapter 1.

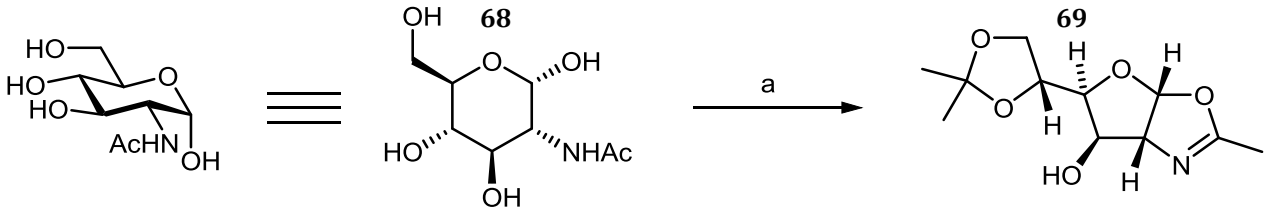
CHAPTER 3

Synthetic Route 2:

In light of the failure to efficiently discriminate between the 2-, 3- and 4-OH groups in triol **58**, the focus was shifted to the second proposed route (Scheme 12) starting from readily available N-acetyl-D-glucosamine, which already has the desired acetamido group at C-2 in the correct configuration.

3.1 Preparation of Oxazoline

The first step in this alternative route involved the formation of the *cis*-fused furano-oxazoline **69**.[60] This reaction traps the carbohydrate in the furanose form while simultaneously protecting all but the 3-hydroxyl group (Scheme 21).



Scheme 21: Reagents and conditions (a) $BF_3.Et_2O$, $(CH_3)_2C=O$, MS, RT, 3.5h, Et_3N, 90% [60]

This is achieved by treating **68** with $BF_3.Et_2O$ and refluxing in acetone. The reaction proceeds *via* the mechanism outlined in Figure 19. The probable sequence is that after the sugar equilibrated to the open-chain form **H**, the 5,6-diol is trapped by the activated acetone to form the acetal, followed by formation of the furanose form and finally formation of the 1,2-oxazoline *via* acid-catalysed formation of an intermediate oxocarbenium ion. An excess of lewis acid $BF_3.Et_2O$ was therefore required as it was involved in the formation of both the isopropylidene and oxazoline rings. Although furanose is thermodynamically less stable than the 6 membered pyranose[70], its formation is encouraged by two factors. Firstly, the formation of the isopropylidene ring in which O-5 is constrained and is therefore unable to reform the pyranose ring. And secondly, in the fused bicyclic system, the oxazoline ring exists

preferentially in the *cis* configuration which would not be possible in the pyranose from with the nitrogen and anomeric oxygen in equatorial and axial positions respectively.[70]

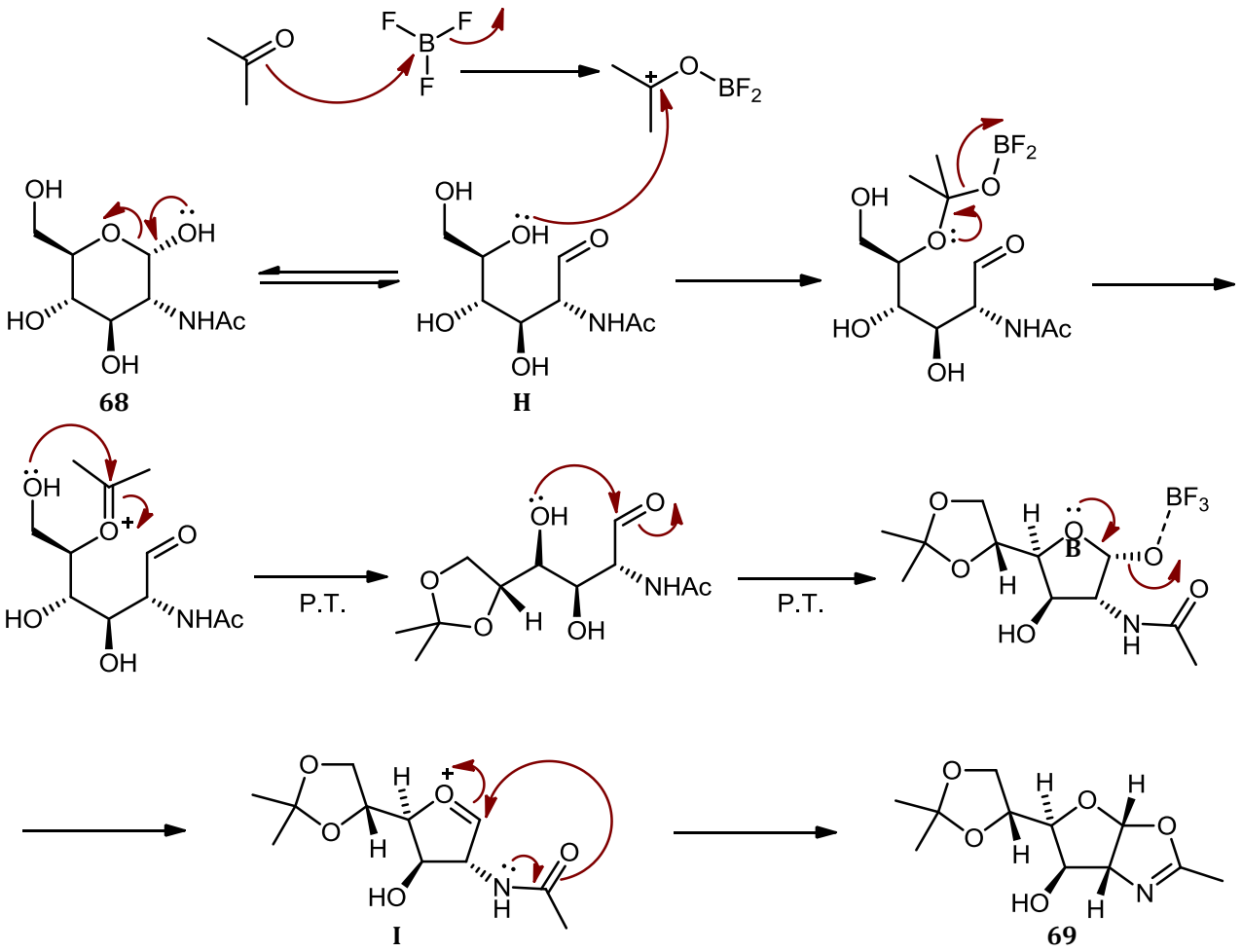


Figure 19: Proposed mechanism for the formation of oxazoline **69**

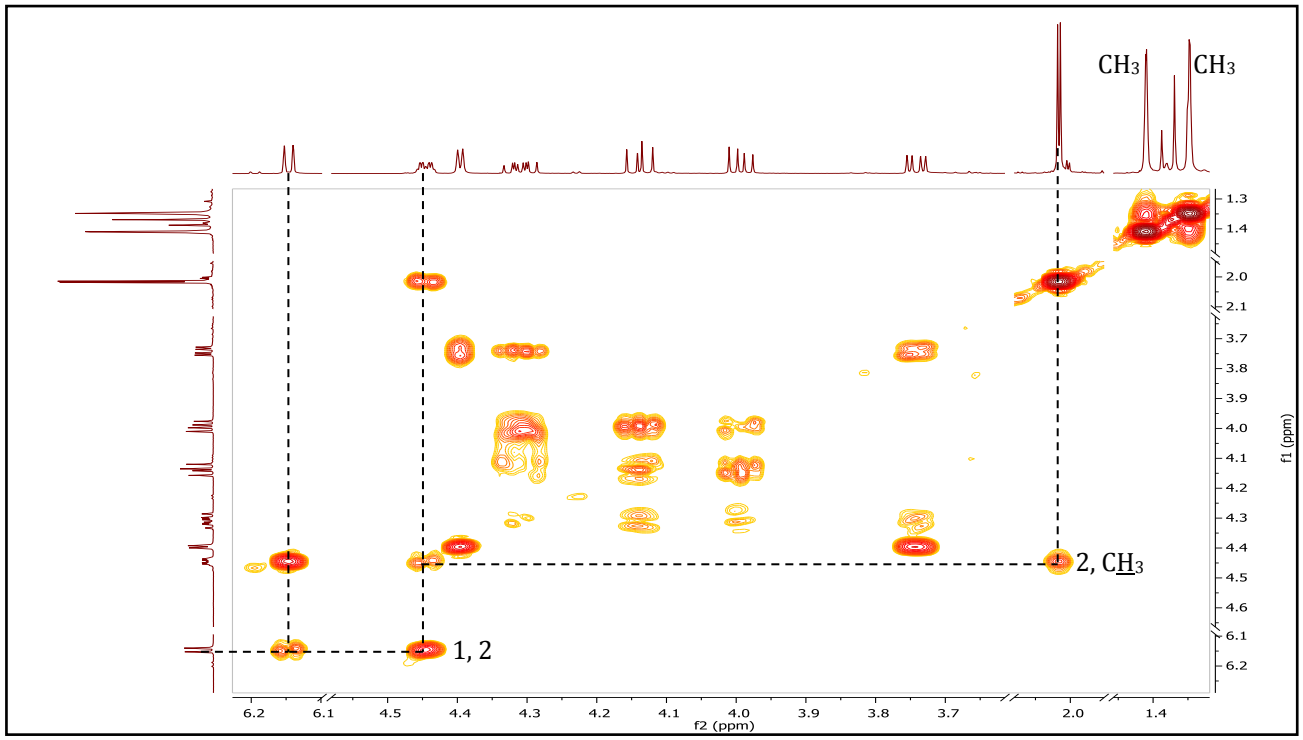
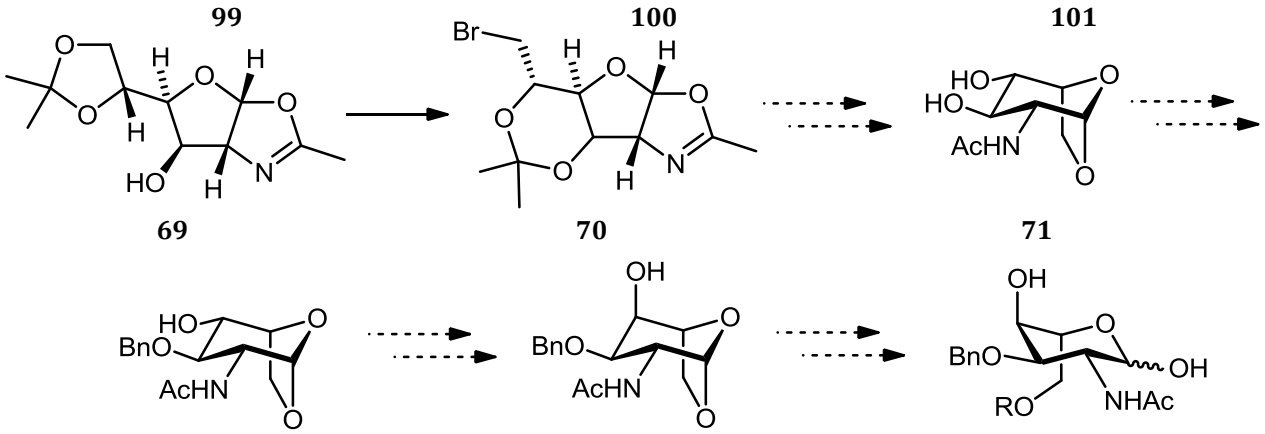


Figure 20: COSY spectrum of compound **69**

The NMR analysis of **69** was in agreement with reported literature.[60,71] A doublet integrating for 3 protons at 2.00 ppm in the ^{1}H spectrum with a cross peak to H-2 in the COSY spectrum (Figure 20) confirmed the presence of the methyl group on the oxazoline ring. This appears as a doublet due to the long range coupling to H-2 (Figure 20) which is as a result of the angles introduced by the bicyclic ring system bringing the protons close enough in space to observe long range coupling. Two other methyl peaks at 1.39 and 1.33 ppm confirmed the presence of the isoprolylidene ring.

3.2 Attempted Synthesis of 70

With **69** in hand, the possibility of carrying out the PPh_3-mediated bromination and rearrangement of the isopropylidene acetal to form **70** was investigated. Compound **70** could then be further modified to **99** (Scheme 22) following the same sequence used to form **58.**

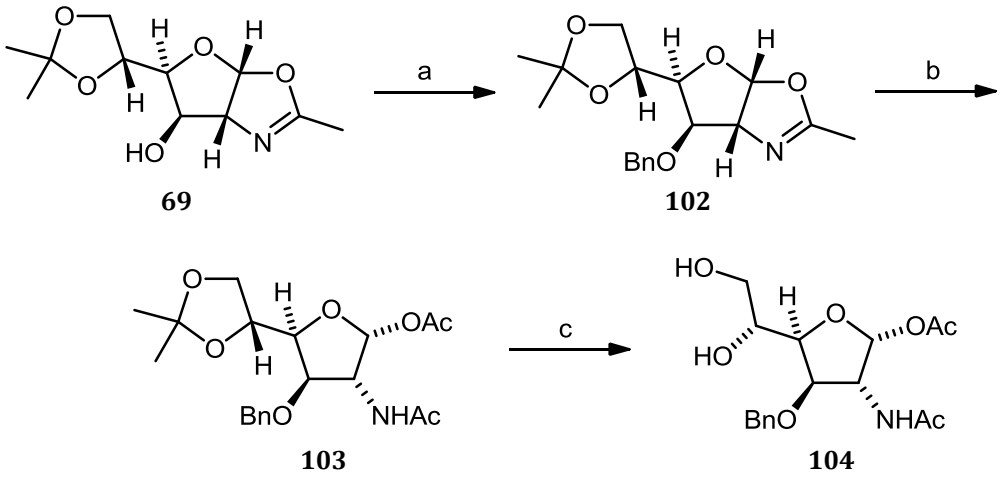


Scheme 22: Envisioned synthetic route from **69** to **99** analogous to that in Chapter 2

This reaction was first attempted using the optimised reaction conditions from the diacetone-D-glucose (**20**) reaction. TLC indicated that the starting material was being consumed but no product was apparent and NMR of the crude reaction products gave no evidence of the expected structures. The reaction was attempted in a number of different solvents while varying the temperature and reagent equivalents (Table 2, Appendix), none of which were successful. Although this reaction was not investigated further, it seems likely that the nitrogen in the oxazoline ring co-ordinates to the phosphonium species, triggering the opening of the ring and further degradation.

3.3 Selective Protection and Rearrangement of Oxazoline 69

Focus was thus shifted to the alternative proposed route (Scheme 12/23) elaborated below. This reaction sequence started with the protection of O-3 followed by selective opening of the oxazoline and isopropylidene rings effectively leaving O-5 and O-6 unprotected.



Scheme 23: Reagents and conditions (a) BnBr, NaH, DMF, RT, 18 h 61% (b) i: H_2O, 100°C, 18 h; ii: Ac_2O, DMAP, DCM, RT, 17 h, 95% (c) 70% AcOH aq., RT, 18 h, 71%

The benzylation of O-3 was successfully achieved by treating **69** with BnBr and NaH in DMF to produce **102** in a 61% yield after purification.[36,71] NMR evidence for the formation of **102** is shown in Figure 21 with the key signals highlighted which correlated to reported literature data.[71] The signal for H-3 has shifted upfield from 4.37 ppm to 4.10 ppm due to shielding effects of the benzyl while the other carbohydrate signals remain at similar chemical shifts to **69**.

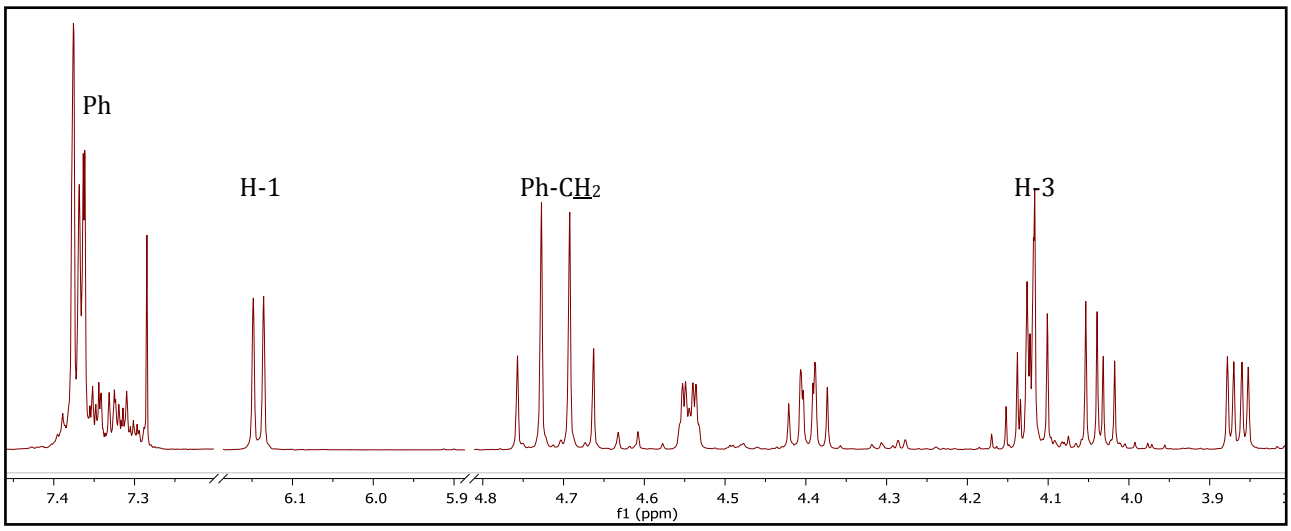


Figure 21: ^1H NMR spectrum for compound **102**

The presence of the benzyl group was confirmed by aromatic peaks between 7.23 and 7.39 ppm which integrate for 5 protons and the characteristic AB quartet at 4.71 ppm which integrated for 2 protons. This AB quartet splitting pattern is of particular importance especially in interpretation of the NMR spectra of products further along in this synthetic sequence.

With the protected oxazoline **102** in hand, the challenge of selectivity cleaving the oxazoline and isopropylidene rings was addressed. A very interesting green chemistry article was published in 2013[72] which used H_2O as both reagent and solvent to achieve acetal cleavage without the use of catalysts or organic solvents. It was conjectured that the lability of the oxazoline ring, compared to that of the isopropylidene, could be exploited to achieve the desired selectivity particularly under such mild reaction conditions.

Compound **102** was dissolved in a large excess of H_2O and heated under reflux overnight producing a more polar compound, apparent from TLC. This was directly acetylated, for ease of purification and to be able to distinguish O-1 from O-5 and O-6, producing compound **103** in a 95% yield. The structure was confirmed through the analysis of ^{1}H, ^{13}C, COSY, HSQC and HMBC NMR spectra. Several identifying features of are shown in Figure 22.

One of the most notable peaks is a broad doublet at 5.82 ppm in **A**, which couples to H-2 in **B**, and also couples to a quaternary carbon and a CH_3 in **C**, suggesting the presence of an NHAc group at C-2. This is supported by the signal for H-2 appearing further downfield than in **102**. The anomeric doublet has shifted slightly downfield (**A**) and cross peaks to a quaternary carbon and a CH_3 signal (**C**) confirm that there is an acetate group at O-1. The acetate groups present at positions 1 and 2 prove that the oxazoline ring was indeed cleaved and two methyl signals at 1.40 and 1.35 ppm verified that the isopropylidene ring is still intact. Another cross peak shown in **C** shows the coupling between C-3 and the benzylic CH_2 group and is again confirmation that the benzyl group is indeed at position 3. These data supports the proposed structure for compound **103**.

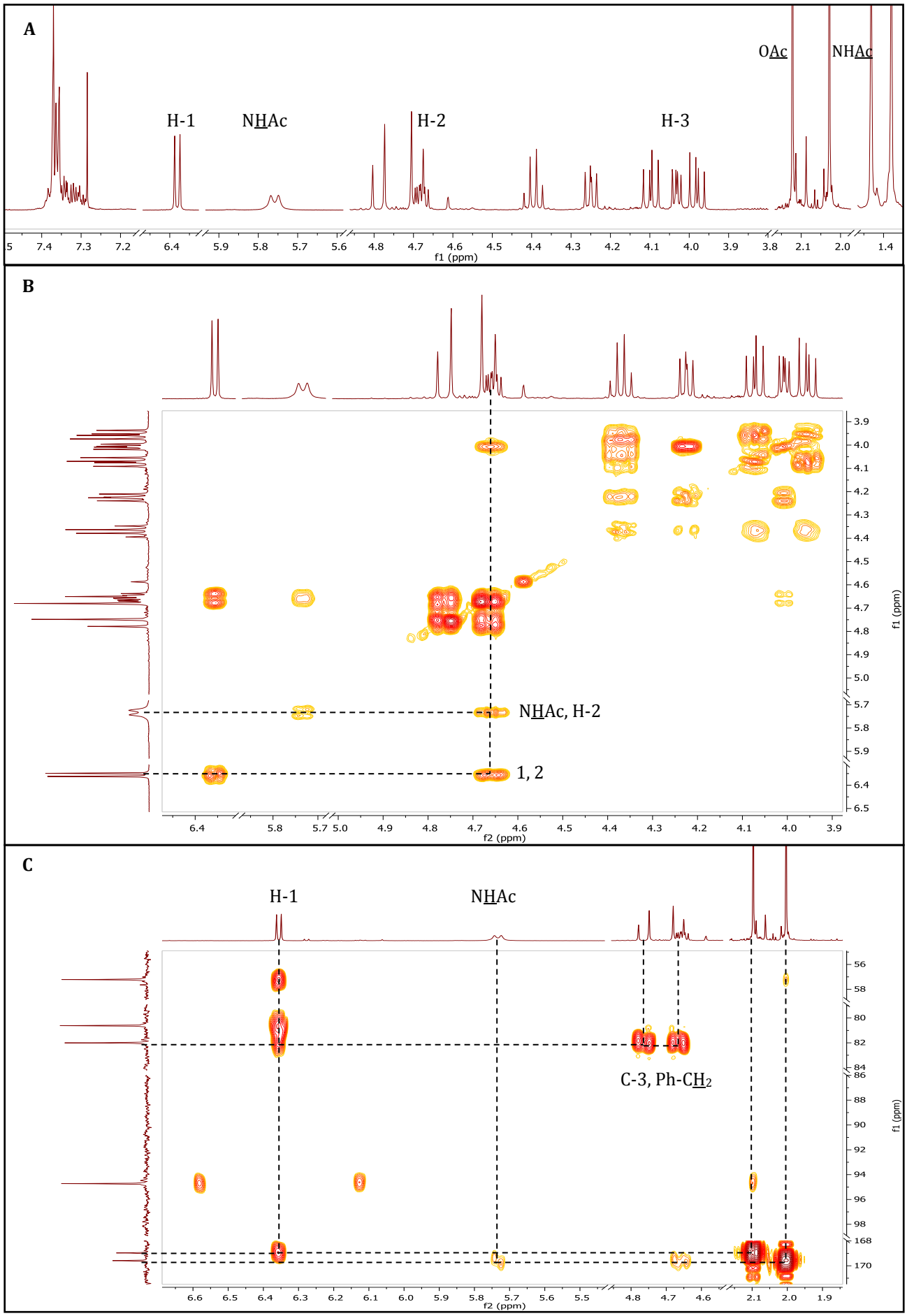
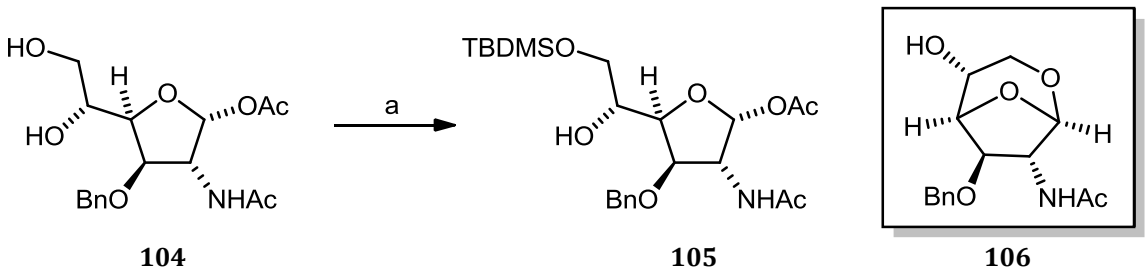


Figure 22: NMR spectra for compound **103 (A)** ^{1}H **(B)** COSY **(C)** HSQC

With C-1, C-2 and C-3 protected, the cleavage of the isopropylidene protecting group was investigated in order to prepare the molecule for selective protection of the primary hydroxyl at C-6 and inversion of configuration at C-5. This was accomplished by acid hydrolysis[71] using 70% AcOH to produce diol **104** in a 71% yield after column purification. The ^{1}H NMR spectrum of this product showed that the two methyl peaks were absent and the signals for H-5 and both H-6 protons were shifted upfield. These data correlate to the loss of the isopropylidene group and confirm the formation of **104**.

3.4 Protection of Primary Hydroxyl

In an initial attempt by a student, in the Gammon research group, to selectively protect O-6 of compound **104** with a TMDBS group, an unusual product was isolated with no evidence for the 6-silyl ether **105**. The product was identified as 1,6-anhydro glucofuranose **106** (Scheme 24) by analysis of a series of 2D NMR experiments (Figure 22). The most interesting and prominent feature was the anomeric signal which appeared as a singlet, shifted significantly further upfield than usual at 5.02 ppm. The COSY spectrum in showed that the typical H-1, H-2 coupling is not seen. However, long range W-coupling from H-1 to H-4 and H-6b is observed as well as U-coupling (couplings across U-shaped HCCCH fragments[73]) to H-3. These coupling patterns are typical of bicyclic systems which suggested that this product was a 1,6-anhydro sugar. Increased splitting of the benzylic CH_2 signals and the fact that the H-6 protons give two distinct peaks support the notion since the molecule is more rigid. H-2 only couples to H-3 and N<u>H</u>Ac suggesting that is has a 90° dihedral angle with H-1. These data in combination with the loss of the anomeric acetate signal suggests that the product was bicyclic compound **106**.



104 **105** **106**

Scheme 24: Reagents and conditions for attempted synthesis of **105** showing unusual isolated product **106** (a) TBDMSCl, DCM, DMAP

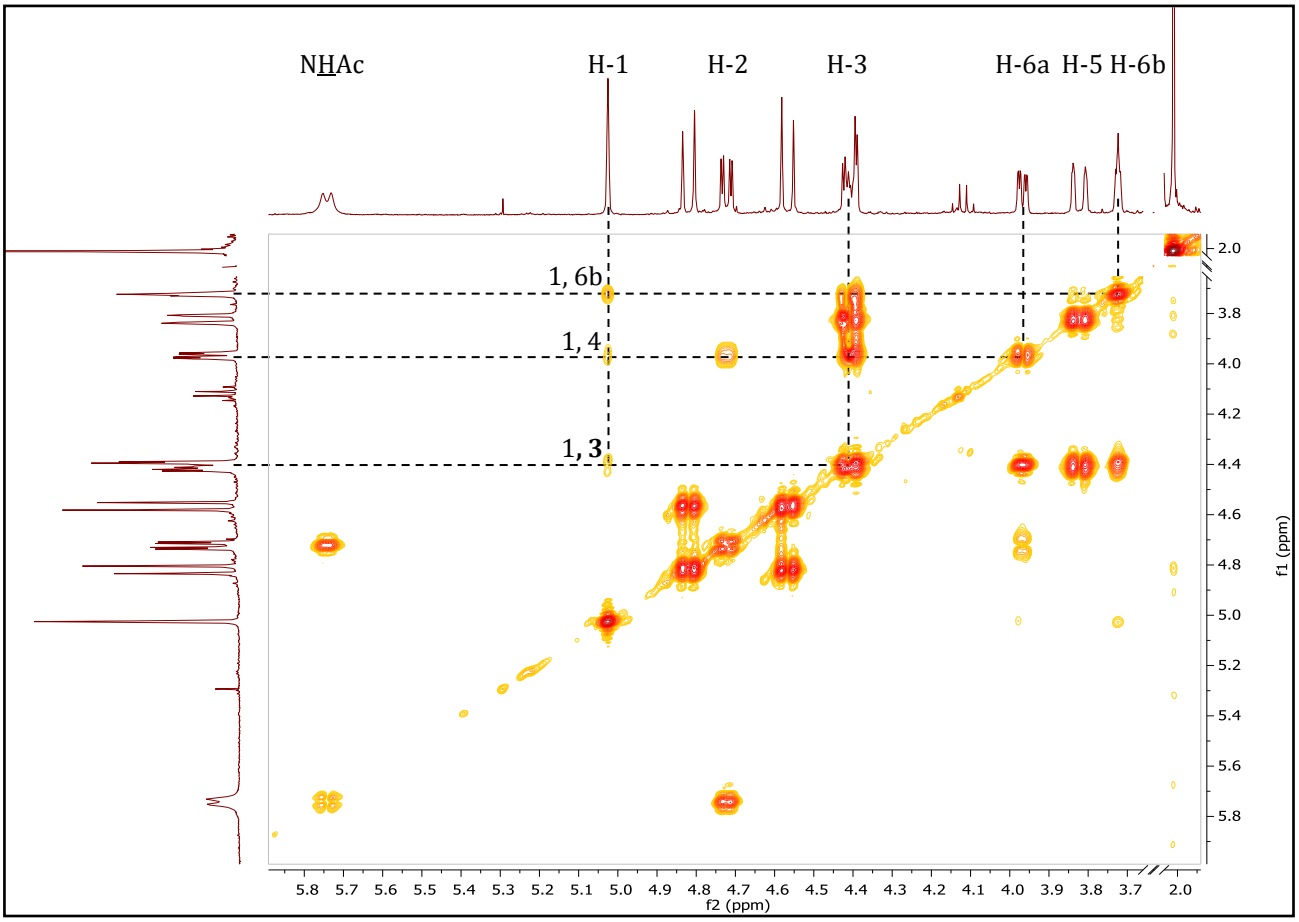


Figure 23: COSY spectrum for unusual minor product **106**

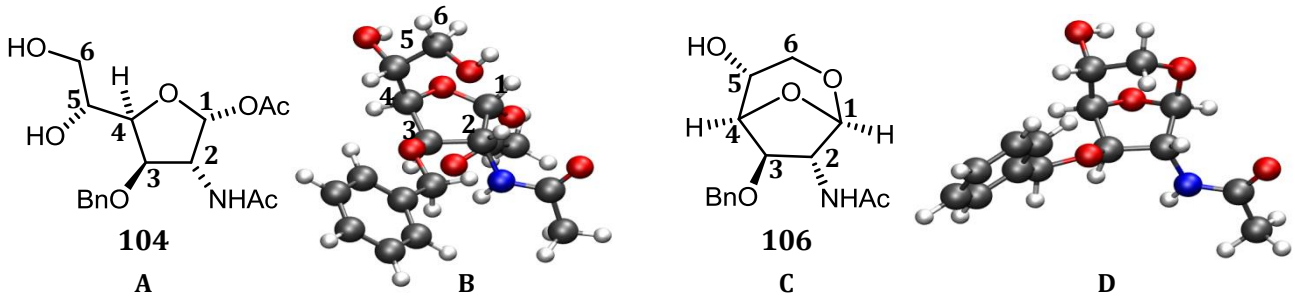


Figure 24: (A) Skeletal structure of **104 (B)** ball and stick model of **104 (C)** skeletal structure of **106 (D)** ball and stick model of **106**

As shown in Figure 24 **B** the ball and stick model of **104**, O-6 and C-1 are in fact ideally lined up for the ring closure to occur forming **106**. Although only 10% of this product was obtained, the structure could potentially be useful in the inversion of C-5 since it is left exclusively unprotected. The optimal conditions for the formation **106** were thus investigated.

The reaction was repeated under the same reaction conditions treating **104** with TBDMSCl in DCM and DMAP, a substitute for pyridine. After purification two compounds were islolated and it was found that

both **105** and **106** had formed. Interestingly, the major product was the silyl ether **105** while again only 10% of **106** formed.

It was initially thought that the ring closure was being mediated by the base DMAP. The reaction was therefore attempted under the same conditions but without the TBDMSCl (Table 3, Appendix). However, after two attempts only starting material was recovered from the reaction. A stronger base, NaH, was employed and after 18 h a very polar compound had formed which was directly acetylated for ease of purification. This product was not the desired **106** but the diol had been acetylated giving a tri-acetylated product. A review of the literature showed that lewis acid mediated formations of 1,6-anhydro rings in sugars[74–83] are generally more common than base mediated reactions. A series of reactions using TMSOTf, $BF_3.Et_2O$ and $SnCl_4$ were therefore attempted (Table 3, Appendix).

All three lewis acids were successful in converting **104** to **106** in varying yields however, unreacted starting material also remained which made the purification very difficult due to their similar polarities. The product from the TMSOTf reaction was isolated in a 12% yield and 29% starting material recovered. NMR analysis of the product from the $BF_3.Et_2O$ reaction indicated a mixture of starting material and 1,6-anhydro product with a combined yield of 68% (by mass). Attempts to separate these were unsuccessful. The reaction using $SnCl_4$ also produced an inseparable mixture of **104** and **106** in a 1:1 ratio with a yield of 35% (by mass). Finally, it was postulated that the bulky TBDMS group at O-6 in compound **105** might have assisted in the ring closure. Thus **105** was treated with TMSOTf which was the most successful reaction forming **106** in a 26% yield and recovering starting material (43%) and diol **104** which had lost the TBDMS group (22%).

Although the desired 1,6-anhydro compound **106** was successfully synthesised the yield was not able to be increased and was very difficult to isolate as a pure product ultimately making this route unfeasible for the synthesis of L-AltNAcA. Attention was therefore shifted to improving the yield of the silyl ether **105**, which also leaves C-5 selectively unprotected which can then be selectively inverted.

The silylation was repeated using pyridine as the solvent since the DMAP and DCM combination lead to the formation of **106**. Diol **104** was treated with TBDMSCl in pyridine and stirred for 24 h after which starting material was still present and so a second equivalent of TBDMSCl was added. This resulted in the successful synthesis of mono-protected silyl ether **105** in a 98% yield.

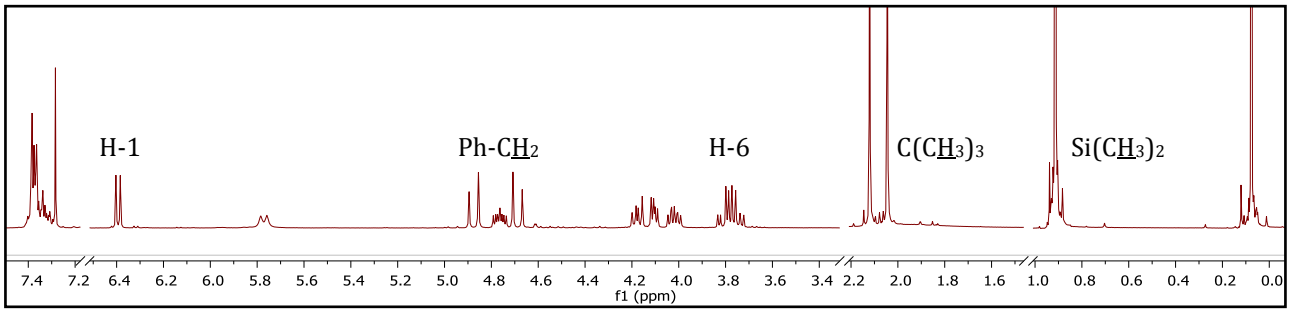
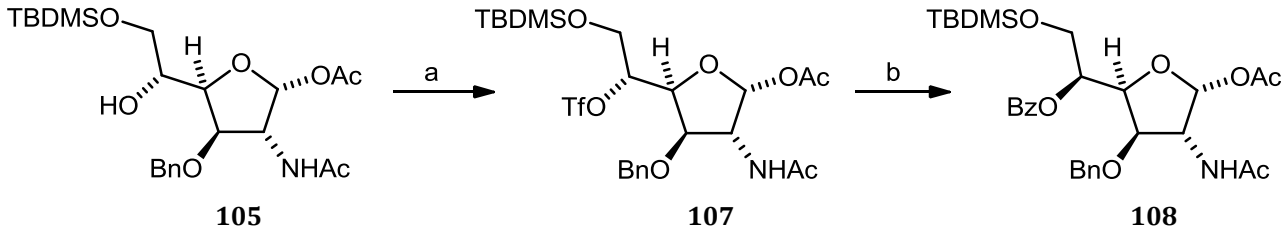


Figure 25: ^{1}H NMR spectrum for compound **105**

The structure of **105** was quickly confirmed by two identifying signals in its ^{1}H NMR spectrum (Figure 25) corresponding to the TBDMS structure. A singlet at 0.89 ppm integrating for 9 protons correlated to *t*-butyl. The singlet further upfield integrated for 6 protons confirming the presence of the two methyls on Si. A notable feature of this spectrum is the splitting of the AB quartet signal from the benzylic CH_2 group. This kind of signal separation occurs when the free rotation of the molecule is reduced. The bulky TBDMS group at O-6 adds a significant amount of steric strain to the molecule which reduces the amount of free rotation that can occur resulting in the increased separation of the AB quartet to the point where two chemical shifts are observed for each half of the quartet.

3.5 Attempted Inversion of C-5

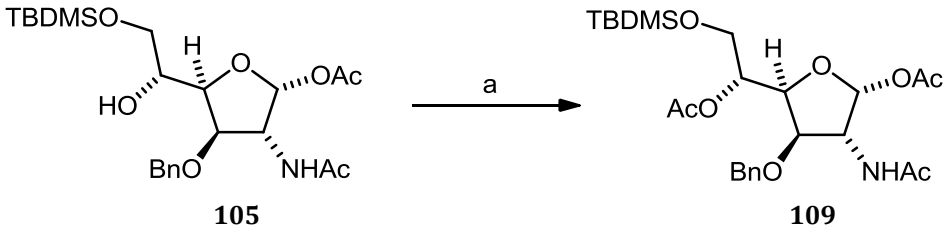
After efficiently and selectively protecting O-6, the inversion of configuration at C-5 was investigated. The proposed sequence (Scheme 25) follows a trilfation and nucleophilic substitution with NaOBz.



105 **107** **108**

Scheme 25: Reagents and conditions (a) Tf_2O, py (b) NaOBz, DMF

Since the molecule is already under steric strain, the likelihood of adding another bulky group at O-5 was tested before attempting the trilfation. Since an acetylation reaction follows a similar mechanism to the triflation it was used as a feasibility test for this sequence



105 **109**

Scheme 26: Reagents and conditions for acetylation (a) Ac_2O, py, 60°C, 18 h, 91%

105 was treated with Ac_2O in pyridine and was successfully acetylated at O-5 after heating the reaction to 60°C overnight. Fully protected compound **109** was produced in a 91% yield. NMR analysis indicated an additional CH_3 peak at 1.91 ppm and a downfield shift in the signal for H-5 while the other peaks remain at very similar chemical shifts to **105**. This proved that O-5 could be manipulated even though the molecule is fairly crowded. This reaction also verified that the TBDMS group was indeed at position 6.

3.5.1 Attempted triflation of O-5

Since the ultimate goal was to invert C-5, the hydroxyl needed to be converted to a good leaving group preparing the molecule for an S_N2 substitution which will effectively invert the configuration.[36,54,55,57,58] Silyl ether **105** was treated with Tf_2O in pyridine and after increasing the reaction temperature to 60°C (Table 4, Appendix) a single, less polar product formed.

NMR analysis of the product was encouraging indicating a key downfield shift in the signal for H-5 (Figure 26) to a similar position to its occurrence in the spectrum of **109**. However, there appeared to be a pyridine contamination in the product due to three signals in the aromatic region of the ^1H NMR spectrum, the doublet and two triplets are typical of a pyridine molecule (Figure 26). In an attempt to remove the pyridine, the work up conditions were changed (Table 4, Appendix) and toluene was used to remove it azeotropically but the signals were still present. It was then noted that the pyridine was

present in a stoichimetric amount, integrating for 5 protons, and the chemical shift values of 8.92, 8.36 and 7.88 ppm did not match literature values for pyridine. Another prominent feature seen in the ^{1}H NMR spectrum is the wide separation of the two benzylic protons which appear as a split AB quartet indicating that they are in quite different chemical environments, suggesting increased steric strain in the product. This suggested that a larger group has been added to O-5. The TBDMS, NHAc and OAc groups at positions 6, 2 and 1 respectively were all present as evident from ^{1}H NMR and confirmed by COSY and HSQC.

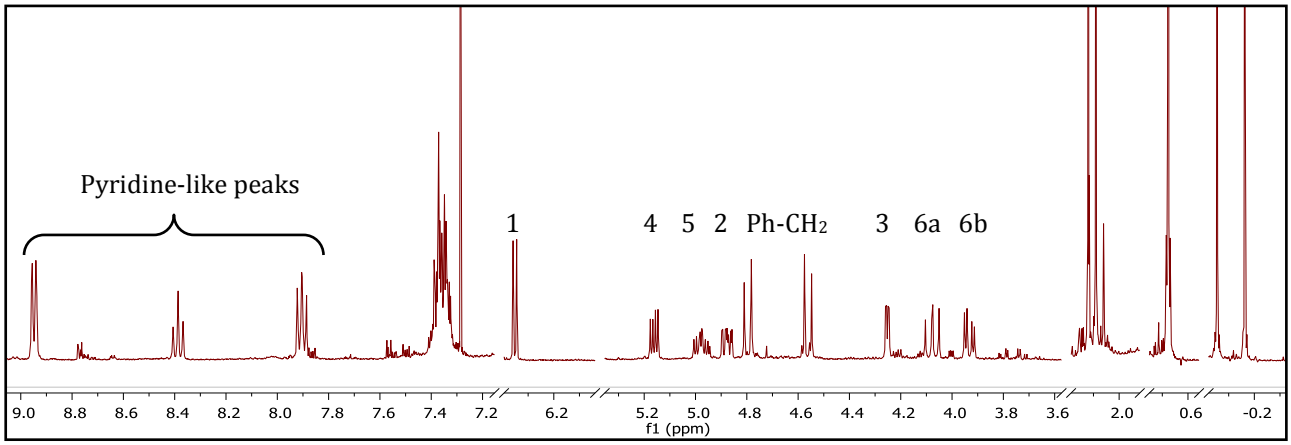


Figure 26: ^{1}H NMR for unknown product

Further structural analysis using 2D NMR lead to proposing the unusual structure **110** (Figure 27). Of particular significance was a clear connection between the proton and carbon signals for pyridine and position 5. These are highlighted in an overlay of the HSQC (orange) and HMBC (grey) spectra in Figure 28. There is a cross peak between C-5 and the pyridine protons 'H-2', and another cross peak between H-5 and the pyridine carbons 'C-2' seen in the grey HMBC spectrum. Since there is such a strong correlation it is very unlikely that there is an oxygen at C-5 and the proposed structure therefore has the pyridine nitrogen bonded directly to C-5. ^{19}F NMR indicated a single peak at -78.27 ppm consistent with the presence of a triflate, suggesting that ^{-}OTf was the counter ion. HRMS analysis supported the proposed structure with the M+ ion at 529.2735 correlating to the cation component of **110**.

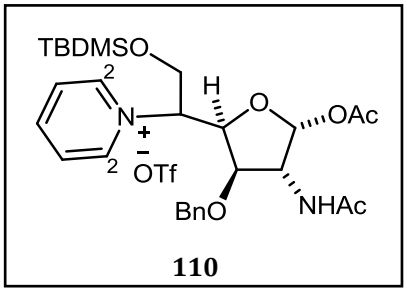


Figure 27: Proposed structure

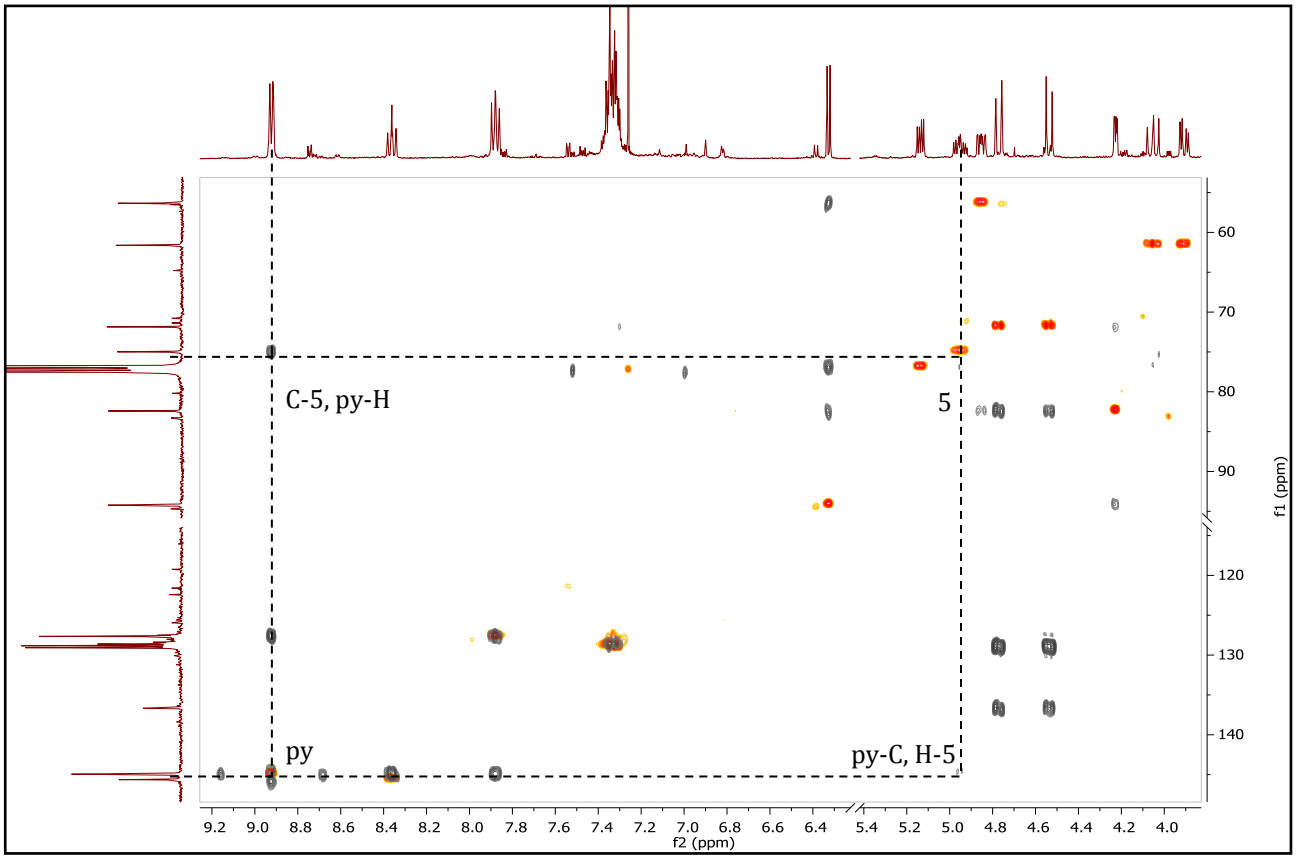


Figure 28: Spectra overlay showing HSQC (orange) and HMBC (grey) for compound **110**

It seems likely that the triflate formed by the reaction at O-5 is highly reactive due to the sterically crowded environment. It therefore departs immediately either in an S_N1 or S_N2 process, to be replaced by the pyridine, which is present in large excess.

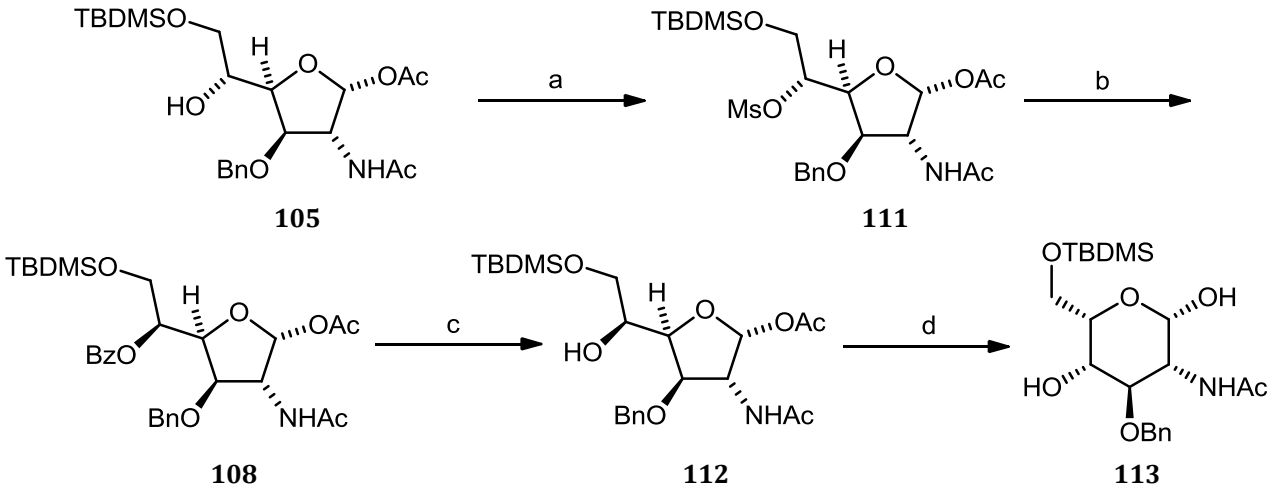
It was evident from product **110** that an alternate solvent to pyridine was necessary. Four other solvent systems were tried namely DMAP in DCM, 2,6-lutidine, N-methylmorpholine and THF, but none were successful in synthesising triflate **107**. The evidence from this set of experiments illustrates that the triflation does work but it immediately departs or is displaced by another nucleophile. It was therefore proposed that if NaOBz was added to the reaction at the correct time then it could displace the triflate forming the desired compound **108**.

3.5.2 Attempted one-pot triflation and inversion at C-5

In this set of experiments (Table 5, Appendix) DMF and DCM were used as solvents and only a minor amount of pyridine added as a base in order to avoid displacement of the triflate with pyridine. The

reaction was initially attempted in DMF in which dissolved starting material **105** was treated sequentially with Tf_2O and NaOBz. The crude product was analysed by NMR but there was no evidence for the formation of compound **108** but rather the TBDMS group at O-6 had been removed. The reaction was then repeated using DCM for the triflation and DMF for the nucleophilic substitution but this resulted in complete degradation of the carbohydrate material. This was observed in the NMR spectrum of the crude product which showed no carbohydrate residue but peaks that resembled TBDMS, acetate and benzyl group were seen which suggested that the compound fragmented. The reaction was tried at different temperatures and varying work up conditions but none were successful. In a final attempt, the pyridine compound **110** was treated with NaOBz in DMF to try and displace the pyridine at C-5 with a benzoyl group. Since the configuration of at C-5 in this compound is unknown the product could be the desired compound **108** or its diastereomer. Unfortunately this reaction also degraded leaving no evidence of a carbohydrate residue.

At this point the synthetic route was re-evaluated as the triflate was evidently too reactive and unstable to effect the desired inversion. A mesylate was selected since it is also an effective leaving group but not as reactive as a triflate which would make the product stable enough to isolate and use in subsequent steps (Scheme 27).



Scheme 27: Reagents and conditions (a) MsCl, py, -10°C, 8 h, 61% (b) NaOBz, DMF (c) NaOMe, MeOH, (d) TFA

Alcohol **105** was treated with MsCl in pyridine and the desired mesylate **111** was isolated in a 61% yield after purification. The structure was confirmed by NMR analysis and attention is drawn to two features (Figure 29). A peak resonating at 3.01 ppm which integrated for 3 protons had appeared in the ^{1}H spectrum corresponding to the mesylate CH_3. A significant downfield shift in the signal for H-5 from 4.00 to 4.94 ppm was observed which is similar to that in the acetylated **109** confirming the position of the mesylate. In addition to this, the further separation of the benzylic CH_2 AB quartet was observed which is expected as the steric congestion in molecule increases.

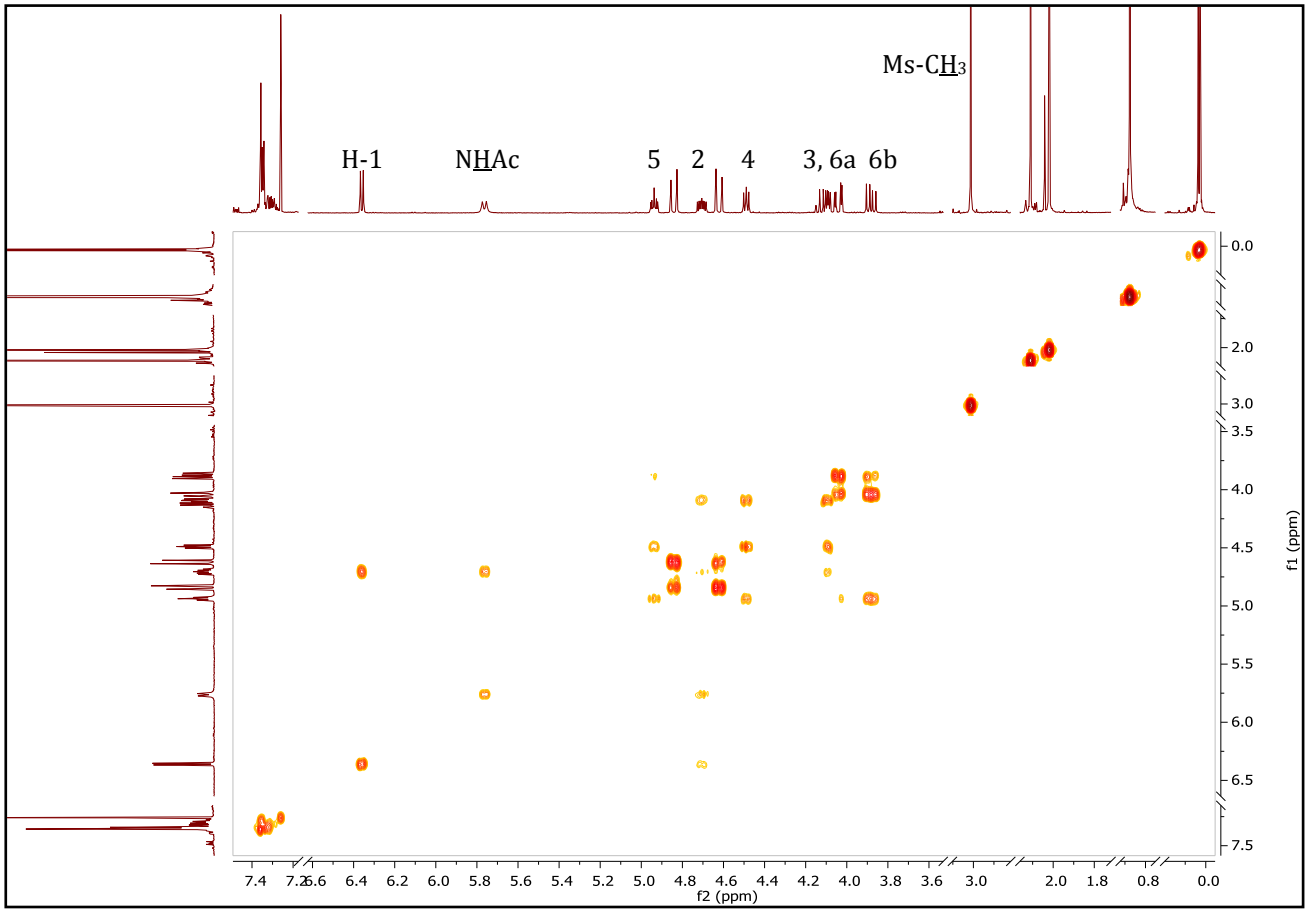


Figure 29: COSY spectrum of compound **111**

3.5.3 Attempted inversion of C-5

With the much desired mesylate **111** in hand the inversion at C-5 was reattempted. **111** was dissolved in DMF and treated with NaOBz at RT but after no reaction occurred and all the starting material was recovered. The reaction was repeated at 100°C but still no reaction occurred (Table 6). A solubility test was done with NaOBz as it appeared not to dissolve in DMF even at raised temperatures. Although it did

not dissolve completely in any standard organic solvent, acetone and pyridine were the most promising. The experiment was repeated under reflux conditions but neither solvent was successful in effecting the desired substitution. The more reactive LiOBz, which was formed *in situ* using n-BuLi, was therefore used in an attempt to make the benzoyl anion more accessible. The reaction was unsuccessful leading not to the desired product but rather complete degradation of the starting material.

It was possible that the bulky benzoyl group simply would not fit in the already congested molecule so a final attempt to invert the centre was done using NaOPiv, a smaller nucleophile. This reaction was performed in DMF at RT and when no reaction occurred, heated to 100°C, but unfortunately this again resulted in complete degradation of the carbohydrate, evident from NMR.

Despite the fact many groups have published successful inversions by this method on both furanose and pyranose rings,[36,54,55,57,58] after these experiments it was apparent that the inversion of configuration *via* and S_N2 mechanism was not working. This suggests that this particular molecule made the inversion unsuccessful. A more thorough analysis of mesylate **111** indicated that C-5 is simply under too much steric strain to allow for an S_N2 substitution to occur at that centre. The *cis* orientation of C-1 and -2, and C-3 and-4 respectively creates steric strain around the furanose ring. In addition to the fact that the substituents at C-3 and C-4 are eclipsed, they are also very bulky. The steric congestion at C-5 is probably influenced more by the TBDMS group at the adjacent carbon 6 than the benzyl at O-3 but both contribute to the amount of steric strain in the molecule. In addition, both the benzoyl and pivaloate groups are bulky nucleophiles which only add to the steric issues in this system. These factors make the inversion of configuration at C-5 by means of an S_N2 substitution impracticable.

CHAPTER 4

Conclusions and Future Work

The aim of this project was to contribute to the preparation of a glycoconjugate vaccine candidate for *S. sonnei* by synthesising a suitable derivative of the acidic sugar, L-AltNAcA, found in the repeating unit of *S. sonnei*. Two synthetic routes were proposed starting from readily available D-sugars diacetone-D-glucose (**20**) and N-acetal-D-glucosamine (**69**) which introduced the challenge of inverting the configuration at C-5.

The first route followed an established synthesis[45,47,59] which successfully converted **20** to L-sugar 1,6-anhydro-3-*O*-benzyl-β-L-idopyranose (**59**) in 6 steps with an overall yield of 20%. The inversion of configuration at C-4 was then attempted *via* a triflation and substitution with a benzoyl group[59] but this was unsuccessful. Alternative methods for efficiently discriminating between the three hydroxyl groups in **58** were then investigated which would allow for the selective inversion of the configuration at C-4 and converting the hydroxyl at C-2 to the desired acetamido group. The most selective reaction in this investigation was a tin-mediated benzoylation in which a major product 1,6-anhydro-3-*O*-benzyl-4-*O*-benzoyl-β-L-idopyranose (**93**) was produced in a 45% yield compared to 11% or less for the other benzoylated products. Unfortunately while this outcome showed good selectivity it was not useful synthetically since it was not able to be isolated as a pure compound. While this synthetic route successfully inverted the configuration at C-5 affording the desired L-configuration, the three hydroxyl groups in **58** could not be sufficiently differentiated to convert it to the desired L-AltNAcA.

The emphasis in the second route was placed on the inversion of C-5 since the 2-acetamido group was already in place. **69** was converted to 2-acetamido-2-deoxy-3-*O*-benzyl-6-*O*-α-D-glucofuranosyl acetate **104** in a 41% yield over four steps which was a key synthetic intermediate towards the inversion of

configuration at C-5. Although this inversion was not achieved, good insight into the reactivity of the diol **104** was gained. The selective protection of the primary hydroxyl O-6 with a TBDMS group was achieved in a 98% yield which left 5-OH selectively unprotected (**105**). During this study, an unusual minor side-product was isolated and identified as 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**106**). This product formed from both **104** and **105** under acidic conditions but its formation was promoted by the presence of the TBDMS group at O-6. While this was a potentially useful intermediate, also having 5-OH selectively unprotected, it was only able to be synthesised in minor amounts and very difficult to isolate as a pure compound and was thus not synthetically useful. Inversion of configuration at C-5 in the 6-*O*-silylated glucofuranose was attempted *via* the 5-*O*-triflate and 5-*O*-mesylate: the triflate formed but was immediately displaced by the solvent pyridine to give an unusual 5-pyridinium derivative (**110**), while the mesylate was stable but unreactive towards subsequent S_N2 inversion. These outcomes were attributed to the steric congestion imposed by the combination of the 3,4-*cis*-disubstitution of the furanose ring and the very bulky silyl substituent at O-6. The presence of the silyl ether was a common characteristic of the formation of both unusual products **106** and **110**, and the failure of the S_N2 substitution at C-5. This protecting group creates a significant amount of steric strain that the molecule which encourages the elimination of a group in order to reduce this high energy state. This was observed in the formation of the 1,6-anhydro ring in **106** which eliminated the TBDMS group, in the immediate displacement of the triflate from C-5 in the attempted synthesis of **107** and in the failure of the displacement of the mesylate in **111** by the benzoyl group. Therefore, this bulky silyl group was ultimately identified as a hindrance in the attempts to invert the configuration at C-5.

While the goal of preparing L-AltNAcA was not achieved *via* these approaches, useful insights have been contributed towards the ongoing study. In future studies, diol **104** is a useful intermediate that could be converted to the desired L-sugar by a stereoselective reduction at C-5 and oxidation, or a triflation and inversion which would essentially only invert C-5 since C-6 is a primary hydroxyl, or by a Mitsunobu reaction. Once the inversion of configuration at C-5 is achieved the compound could be converted back to the pyranose form and the inversion of configuration at C-4 and oxidation at C-6 could be addressed.

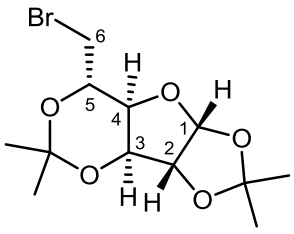
CHAPTER 5

Experimental Procedures

General Procedures

All reagents were used as received from Sigma Aldrich or Carbosynth unless stated otherwise. Anhydrous pyridine, CH_3CN, DMF, and THF were used as acquired from Sigma Aldrich; all other solvents were dried and purified by distillation before use. Aluminium backed pre-coated Merck TLC plates of silica gel (60 F_{254}) were used to monitor reactions and determine suitable solvents systems for column purification. The compounds were visualised on the TLC plates using UV light and/or by spraying them with CAS or anisaldehyde solutions and then heated. Column chromatography for purification of products was either performed manually or by flash chromatography using a Biotage Isolera™ Spektra System with ACI™. Both types of column used Merck Silica Gel (60, 70-230 mesh) and were eluted with mixtures of EtOAc and hexane or MeOH and DCM. Further separations were carried out using glass backed preparative TLC plates using mixtures of EtOAc and hexane or MeOH and DCM. ^{1}H, ^{13}C, COSY, HSQC and HMBC NMR spectra were recorded on Bruker Avance III 600 MHz NMR spectrometer with BBO Prodigy cryoprobe, Bruker BiospinGmbH 400 MHz, Varian Unity XR400 MHz and Varian Mercury XR300 MHz instruments as $CDCl_3$ solutions unless stated otherwise. All chemical shifts are reported in ppm, with reference to the methine signal (δ 7.26 ppm) for residual $CHCl_3$ in the case of ^{1}H spectra or the $CDCl_3$ signal (δ 77.16 ppm) in the case of the ^{13}C spectra unless stated otherwise.

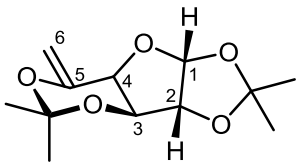
6-Bromo-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranoside (21)



Compound **20** (3.01 g, 11.5 mmol) was dissolved in CH_3CN (70 mL) under Ar, powdered MS (4 Å) were added and the mixture stirred for 5 min. NBS (4.52 g, 25.4 mmol) and PPh_3 (6.66 g, 25.4 mmol) were then added and the reaction refluxed at 80°C for 20 h. The reaction mixture was diluted with Et_2O and filtered through celite® twice. The product was purified by flash column chromatography using 10-30% EtOAc in hexane as eluent to give 6-bromo-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranoside (**21**) as a yellow oil (2.59 g, 69.6%).

R_f in 40% EtOAc/hexane is 0.69. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.99 (d, *J* = 3.7 Hz, 1H, H-1), 4.58 (d, *J* = 3.7 Hz, 1H, H-2), 4.32 (dd, *J* = 7.2, 3.9 Hz, 1H, H-4), 4.23 (d, *J* = 3.9 Hz, 1H, H-3), 3.74 (td, *J* = 7.3, 3.3 Hz, 1H, H-5), 3.61 (dd, *J* = 10.9, 3.3 Hz, 1H, H-6a), 3.43 (dd, *J* = 10.9, 7.4 Hz, 1H, H-6b), 1.50 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.33 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 112.5 (C), 106.5 (C-1), 101.55(C), 84.1 (C-2), 81.7 (C-4), 75.2 (C-3), 72.1 (C-5), 33.2 (C-6), 27.3 (CH_3), 26.7 (CH_3), 24.1 (CH_3), 24.0 (CH_3). These data are in agreement with literature data.[59]

1,2:3,5-Di-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranoside (22)

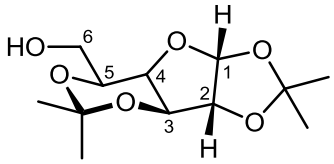


Compound **21** (1.76 g, 5.44mmol) was dissolved in dry DMF (10 mL) under Ar and cooled to 0°C. NaH, 60% mineral oil dispersion, (0.87 g, 21.7 mmol) was added to the reaction mixture which was then stirred for 16 h while slowly warming to RT. The reaction mixture was then cooled to 0°C again and brine added to the flask. The aqueous layer was then extracted with EtOAc (3 x 30 mL) and dried over anhydrous Na_2SO_4. Purification of the product was carried out by flash column chromatography in a gradient of 2 to 30% EtOAc in hexane as eluent to afford 6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranoside (**22**) as a light yellow oil (1.25 g, 95.1%).

R_f in 40% EtOAc/hexane is 0.54. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.98 (d, *J* = 3.7 Hz, 1H, H-1), 4.76 (d, *J* = 0.7 Hz, 1H, H-6a), 4.69 (d, *J* = 0.7 Hz, 1H, H-6b), 4.56 (dd, *J* = 3.8 Hz, 1H, H-2), 4.37 (d, *J* = 2.4 Hz, 1H,

H-3), 4.34 (d, *J* = 2.4 Hz, 1H, H-4), 1.53 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.33 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.3 (C-5), 111.8 (C), 105.2 (C-1), 101.3 (C-6), 100.5 (C), 84.3 (C-2), 74.7 (C-4), 72.4 (C-3), 28.0 (CH_3), 26.7 (CH_3), 26.1 (CH_3), 21.2 (CH_3). These data are in agreement with literature data. [59]

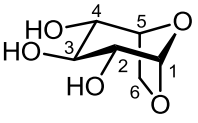
1,2:3,5-Di-*O*-isopropylidene-β-L-idofuranoside (23)



Compound **3** (5.89 g, 24.3 mmol) was dissolved in THF and a 1M BH_3.THF complex (26.2 mL, 26.8 mmol) was added to this under Ar at RT. The reaction was stirred for 3.5 h and was then cooled to 0°C before slowly adding a mixture of 30% H_2O_2 (v/v) and 3M NaOH (1:1, 20 mL). The aqueous layer was then extracted with EtOAc (3 x 50 mL). The combined organic layers were subsequently washed with brine and dried over anhydrous Na_2SO_4. The product was purified by flash column chromatography in a gradient from 5 to 50% EtOAc in hexane as eluent to give 1,2:3,5-di-*O*-isopropylidene-β-L-idofuranoside (**23**) as a colourless oil (4.49 g, 71.0%).

R_f in 40% EtOAc/hexane is 0.33. ^1H NMR (400 MHz, $CDCl_3$) δ 5.94 (d, *J* = 3.7 Hz, 1H, H-1), 4.48 (d, *J* = 3.7, 1H, H-2), 4.30 (d, *J* = 2.3 Hz, 1H, H-3), 4.11 (m, 1H, H-5), 4.01 (t, *J* = 2.2 Hz, 1H, H-4), 3.88 (ddd, *J* = 11.5, 6.9, 3.2 Hz, 1H, H-6a), 3.79 (ddd, *J* = 11.6, 8.5, 4.7 Hz, 1H, H-6b), 1.48 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.31 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 111.9 (C), 105.4 (C-1), 98.3 (C), 84.0 (C-2), 74.1 (C-3), 71.9 (C-4), 69.2 (C-5), 63.3 (C-6), 29.3 (CH_3), 26.8 (CH_3), 26.2 (CH_3), 19.3 (CH_3). These data are in agreement with literature data.[59]

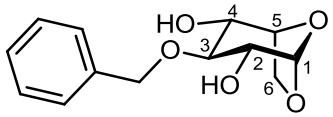
1,6-Anhydro-β-L-idopyranose (58)



A solution of **23** (1.23 g, 4.72 mmol) in 0.2 M ethanolic HCl (40 mL) was refluxed at 95°C for 17 h. After cooling the reaction mixture to RT it was neutralized using $NaHCO_3$ (0.80 g) and filtered through celite® to remove residual salts. The filtrate was concentrated and purified by flash column chromatography in a gradient from 2 to 10% MeOH in EtOAc as eluent to afford the 1,6-anhydro-β-L-idopyranose (**58**) as a colourless oil (0.62 g, 80.0%).

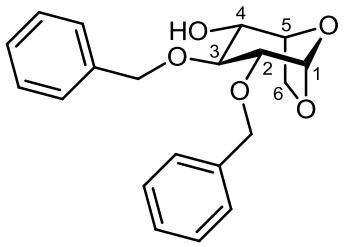
R_f in 10% MeOH/EtOAc is 0.14. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 5.15 (d, *J* = 1.8 Hz, 1H, H-1), 4.45 (d, *J* = 4.4 Hz, 1H, 4-OH), 4.32 (t, *J* = 5.0 Hz, 1H, H-5), 4.24 (d, *J* = 4.9 Hz, 1H, 3-OH), 4.04 (d, *J* = 6.7 Hz, 1H, 2-OH), 3.96 (d, *J* = 7.4 Hz, 1H, H-6a), 3.64 (m, 1H, H-4), 3.58 (m, 1H, H-6b), 3.47 (td, *J* = 8.0, 4.8 Hz, 1H, H-3), 3.33 (ddd, *J* = 8.2, 6.6, 1.8 Hz, 1H, H-2). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 103.2 (C-1), 76.6 (C-5), 76.5 (C-2), 76.5 (C-3), 72.8 (C-4), 65.4 (C-6). These data are in agreement with literature data.[59]

Attempted synthesis of 1,6-anhydro-3-*O*-benzyl-β-L-idopyranose (59)

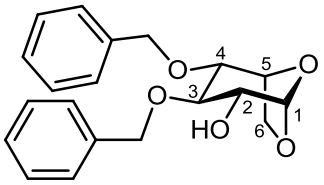


Silyl ether **82** (1.01 g, 2.65 mmol) was dissolved in DCM (20 mL) under Ar at RT and stirred for 5 min. This was cooled to 0°C and 4 Å MS added and the mixture stirred for 30 min after which benzaldehyde (0.32 mL, 3.18 mmol) was added. The reaction was cooled to -78°C and TMSOTf (0.05 mL, 0.26 mmol) was added. The reaction was stirred at that temperature for 1 h. $SiHEt_3$ (0.12 mL, 0.75 mmol) was added and the reaction allowed to warm to RT over 1 h with stirring. A 1M solution of TBAF in THF (0.58 mL, 2.01 mmol) was added dropwise and the reaction stirred for a further 1.5 h. Solid NH_4Cl (0.1 g) was added and the resultant mixture filtered through celite. The filtrate was washed with brine and the combined organic layers concentrated under reduced pressure. The crude product showed three UV active spots on TLC that stained with CAS. These were separated by column chromatography in a gradient solvent system of 20-65% EtOAC in hexane to product; 1,6-anhydro-3-O-benzyl-β-L-idopyranose (**59**) (0.28 g, 42.5%) as a white solid, 1,6-anhydro-2,3-di-*O*-benzyl-β-L-idopyranose (**83**) (0.184.3 g, 20.3%) as white powder and 1,6-anhydro-3,4-di-*O*-benzyl-β-L-idopyranose (**84**) (0.07 g, 7.2%) as a pale yellow powder.

59: R_f in 40% EtOAc/hexane run twice is 0.32. m.p. 140-144°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H, Ph-H), 5.27 (d, *J* = 2.1 Hz, 1H, H-1), 4.85 (AB q, *J* = 11.6 Hz, 2H, CH_2-Ph), 4.44 (t, *J* = 4.6 Hz, 1H, H-5), 4.06 (dd, *J* = 7.7, 0.8 Hz, 1H, H-6a), 3.88 (ddd, *J* = 8.2, 4.3, 1.1 Hz, 1H, H-4), 3.68 (ddd, *J* = 7.9, 5.1, 1.1 Hz, 1H, H-6b), 3.63 (dd, *J* = 7.7, 2.0 Hz, 1H, H-2), 3.42 (t, *J* = 7.9 Hz, 1H, H-3). ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 7.43 – 7.36 (m, 2H, Ph-H), 7.35 – 7.23 (m, 3H, Ph-H), 5.17 (d, *J* = 1.7 Hz, 1H, H-1), 4.86 (AB q, *J* = 11.8 Hz, 2H, CH_2-Ph), 4.55 (td, J = 4.8, 0.9 Hz, 1H, 4-OH), 4.34 (d, *J* = 4.4 Hz, 1H, H-5),), 4.14 (d, *J* = 6.9 Hz, 1H, 2-OH), 4.03 (dd, *J* = 7.5, 0.8 Hz, 1H, H-6a), 3.81 (ddd, *J* = 7.5, 4.3, 1.1 Hz, 1H, H-4), 3.62 (ddd, *J* = 7.6, 5.1, 1.1 Hz, 1H, H-2), 3.51 (dd, *J* = 7.4, 1.8 Hz, 1H, H-6b), 3.48 (t, *J* = 7.5 Hz, 1H, H-3). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 140.7 (Ph-C), 128.8 (Ph-CH), 128.3 (PhCH), 127.9 (Ph-CH), 103.2 (C-1), 84.9 (C-3), 76.6 (C-5), 76.3 (C-2), 75.0 (Ph-CH_2), 72.4 (C-4), 65.4 (C-6). These data are in agreement with literature data. [59]

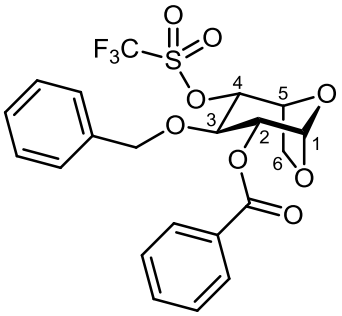


83: R_f in 40% EtOAc/hexane run twice is 0.56. m.p. 102-105°C. ^{1}H NMR (600 MHz, $(CD_3)_2CO$) δ 7.40 – 7.36 (m, 4H, Ph-H), 7.34 – 7.29 (m, 4H, Ph-H), 7.29 – 7.24 (m, 2H, Ph-H), 5.40 (d, *J* = 1.7 Hz, 1H, H-1), 4.85 (AB q, *J* = 11.8 Hz, 2H, Ph-CH_2), 4.72 (AB q, *J* = 12.0 Hz, 2H, Ph-CH_2), 4.63 (d, *J* = 4.3 Hz, 1H, 4-OH), 4.35 (td, *J* = 4.6, 0.9 Hz, 1H, H-5), 4.07 (dd, *J* = 7.5, 0.9 Hz, 1H, H-6a), 3.89 – 3.81 (m, 1H, H-4), 3.62 (ddd, *J* = 8.2, 5.4, 1.4 Hz, 1H, H-6b), 3.59 (t, *J* = 8.1 Hz, 1H, H-3), 3.43 (dd, *J* = 8.0, 1.7 Hz, 1H, H-2).^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 140.4 (Ph-C), 139.8 (Ph-C), 129.0 (Ph-CH), 128.9 (Ph-CH), 128.5 (Ph-CH), 128.3 (Ph-CH), 128.2 (Ph-CH), 127.9 (Ph-CH), 100.3 (C-1), 83.5 (C-2), 83.5 (C-3), 76.6 (C-5), 75.2 (Ph-CH_2), 72.9 (Ph-CH_2), 72.7 (C-4), 65.5 (C-6).



84: R_f in 40% EtOAc/hexane run twice 0.63. m.p. 71-73°C. ^{1}H NMR (600 MHz, $(CD_3)_2CO$) δ 7.46 – 7.20 (m, 10H, Ph-H), 5.18 (d, *J* = 1.9 Hz, 1H, H-1), 4.86 (AB q, *J* = 12.0 Hz, 2H, Ph-CH_2), 4.73 (s, 2H, Ph-CH_2), 4.59 (t, = 4.7 Hz, 1H, H-5), 4.24 (dd, *J* = 7.1, 0.9 Hz, 1H, 2-OH), 4.01 (d, *J* = 7.5 Hz, 1H, H-6a), 3.67 (ddd, *J* = 8.0, 4.2, 1.1 Hz, 1H, H-4), 3.63 (ddd, *J* = 7.5, 5.2, 1.1 Hz, 1H, H-6b), 3.59 (t, *J* = 7.9 Hz, 1H, H-3), 3.54 (td, *J* = 7.5, 1.9 Hz, 1H, H-2). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 140.4 (Ph-C), 139.7 (Ph-C), 129.1 (Ph-CH), 128.9 (Ph-CH), 128.6 (Ph-CH), 128.3 (Ph-CH), 127.9 (Ph-CH), 103.1 (C-1), 83.9 (C-3), 80.0 (C-4), 76.5 (C-2), 75.1 (Ph-CH_2), 73.7 (C-5), 73.1 (Ph-CH_2), 65.7 (C-6).

1,6-Anhydro-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-trifluoromethanesulfonyl-β-L-idopyranose (60)

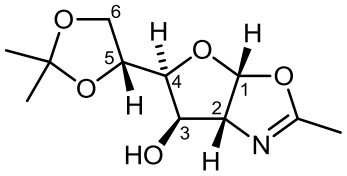


Compound **59** (0.06 g, 0.24 mmol) was dissolved in pyridine (0.5 mL) under Ar and stirred for 5 min and cooled to 0°C. A solution of freshly distilled BzCl (0.03 mL, 0.25 mmol) in DCM (0.6 mL) was added to the reaction mixture and stirred at this temperature for 4 h. Tf_2O (0.12 mL, 0.73 mmol) was added and the reaction kept stirring at RT for 1 h after which it was quenched with MeOH (0.5 mL). This was concentrated under reduced pressure and dissolved in water (4 mL) and then extracted with EtOAc (3 x 5mL). The combined organic layers were washed with 2M HCl then brine and subsequently dried over anhydrous Na_2SO_4. The concentrated residue was purified by column chromatography in a gradient from 20 – 30% EtOAc/hexane to afford two products. The desired 1,6-anhydro-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-trifluoromethanesulfonyl-β-L-idopyranose (**60**) (0.07 g,

59.0%) as a yellow powder and a yellow oil (0.02 g) appeared to be degraded material containing no carbohydrate residue.

R_f in 50% EtOAc/hexane run twice is 0.19. m.p. 76-80°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (dd, *J* = 8.5, 1.3 Hz, 2H, Bz-H), 7.61 (tt, *J* = 7.5, 1.2 Hz, 1H, Bz-H), 7.46 (td, *J* = 8.0, 0.3 Hz, 2H, Bz-H), 7.20 (d, *J* = 0.8 Hz, 5H, Bn-H), 5.59 (d, *J* = 1.8 Hz, 1H, H-1), 5.13 (dd, *J* = 8.1, 1.8 Hz, 1H, H-2), 5.04 (ddd, *J* = 8.4, 4.4, 1.0 Hz, 1H, H-4), 4.80 (t, *J* = 4.6 Hz, 1H, H-5), 4.76 (d, *J* = 11.0 Hz, 1H, Bn-CH_2), 4.68 (d, *J* = 11.1 Hz, 1H, Bn-CH_2), 4.21 (d, *J* = 8.7 Hz, 1H, H-6a), 4.16 (t, *J* = 8.3 Hz, 1H, H-3), 3.91 (ddd, *J* = 8.5, 4.9, 1.0 Hz, 1H, H-6b). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.4 (C), 136.8 (C), 133.8 (CH), 130.0 (CH), 128.8 (C), 128.7 (CH), 128.5 (CH), 128.2 (CH), 128.1 (CH), 118.5 (q, *J* = 319.7, 319.2 Hz, CF_3), 99.5 (C-1), 83.9 (C-3), 76.9 (C-4), 76.6 (C-2), 75.4 (C-6), 73.2 (C-5), 65.5 (Bn-CH_2). These data are in agreement with literature data. [59]

2-Methyl-(1,2-dideoxy-5,6-*O*-isopropylidene-α-D-glucofurano)-[2,1-d]-2-oxazoline[60] (69)

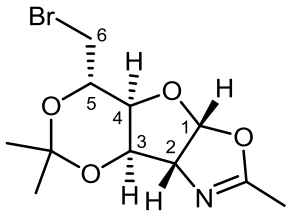


Compound **68** (5.03 g, 22.7 mmol) was dissolved in acetone (100 mL) dried over MS beads under Ar at RT. MS were added and stirred for 10 min before adding $BF_3.Et_2O$ (8.42 mL, 68.2 mmol). The reaction was stirred under reflux for 3.5 h, cooled to RT and diluted with Et_3N (31.9 mL, 227.5 mmol). This mixture was concentrated under reduced pressure and added to a solution of Na_2CO_3 (37.02 g) in water (250 mL), extracted with DCM (5 x 60 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give 2-methyl-(1,2-dideoxy-5,6-*O*-isopropylidene-α-D-glucofurano)-[2,1-d]-2-oxazoline (**69**) (4.99 g, 90.1%) as a brown solid.

R_f in 15% MeOH/EtOAc is 0.54. m.p. 112-116°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 (d, *J* = 5.1 Hz, 1H, H-1), 4.43 (dd, *J* = 5.1, 1.5 Hz, 1H, H-2), 4.37 (d, *J* = 2.8 Hz, 1H, H-3), 4.30 (ddd, *J* = 7.8, 6.2, 4.9 Hz, 1H,

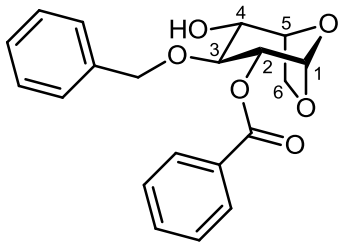
H-5), 4.12 (dd, *J* = 8.7, 6.2 Hz, 1H, H-6a), 3.98 (dd, *J* = 8.7, 4.9 Hz, 1H, H-6b), 3.73 (dd, *J* = 7.8, 2.9 Hz, 1H, H-4), 2.00 (d, *J* = 1.6 Hz, 3H, C(C$\underline{H}_3$)$_2$), 1.39 (d, *J* = 0.7 Hz, 3H, C(C$\underline{H}_3$)$_2$), 1.33 (d, *J* = 0.7 Hz, 3H, NC(C$\underline{H}_3$)O). ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.9 (N$\underline{C}$(CH$_3$)O), 124.3 ($\underline{C}$(CH$_3$)$_2$), 107.2 (C-1), 82.0 (C-4), 78.3 (C-2), 74.9 (C-3), 73.19(C-5), 67.5 (C-6), 26.9 (C($\underline{C}$H$_3$)$_2$),25.27 (C($\underline{C}$H$_3$)$_2$), 14.22 (NC($\underline{C}$H$_3$)O). HRMS calcd for $C_{11}H_{17}NO_5$: 243.1107; found: 244.1186 [*M*+H].

Attempted synthesis of 6-Bromo-2-methyl-(-1,2,6-trideoxy-3,5-*O*-isopropylidene-α-D-glucofurano)-[2,1-d]-2-oxazoline[61] (70)



General procedure, see Table 2 for details. Oxazoline **28** was dissolved in solvent under Ar and stirred for 10 mins. NBS and PPh_3 were added and the reaction stirred under reflux. Once TLC indicated the starting material was consumed, the reaction mixture was cooled to RT. It was then diluted with Et_2O, filtered through celite and concentrated under reduced pressure. NMR of the crude material indicated that the no carbohydrate material was present, predominant peaks were aromatic, most likely PPh_3O. R_f in 50% EtOAc/Hexane is 0.0.

1,6-Anhydro-2-*O*-benzoyl-3-*O*-benzyl-β-L-idopyranose (81)

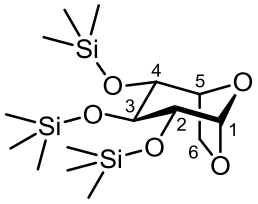


Compound **59** (0.11 g, 0.43 mmol) was dissolved in DCM (4 mL) under Ar, anhydrous pyridine (0.5 mL) was added to increase solubility and the mixture cooled to 0°C. A solution of freshly distilled BzCl (0.05 mL, 0.45 mmol) was added to the reaction mixture slowly and stirred at this temperature for 4 h. The reaction was quenched with MeOH (1 mL), concentrated under reduced pressure, and the

residue dissolved in water (4 mL) and then extracted with EtOAc (3 x 5mL). The combined organic layers were washed with 2N HCl, saturated $NaHCO_3$, water and then brine and subsequently dried over anhydrous Na_2SO_4.The concentrated residue was purified by flash column chromatography in a gradient from 20 to 70% EtOAc in hexane as eluent to afford two compounds. The desired product, 1,6-anhydro-2-*O*-benzoyl-3-*O*-benzyl-β-L-idopyranose (**81**) (0.03 g, 19.8%) as a yellow powder and recovered starting material (**59**) (0.04 g, 28.0%).

R_f in 50% EtOAc/hexane is 0.36. m.p. 82-90°C. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 8.02 (dd, *J* = 8.4, 1.3 Hz, 2H, Bz-H), 7.66 (tt, *J* = 7.4, 1.4 Hz, 1H, Bz-H), 7.52 (t, *J* = 7.8, 7.3 Hz, 1H, Bz-H), 7.30 – 7.24 (m, 2H, Bn-H), 7.22 – 7.16 (m, 3H, Bn-H), 5.46 (d, *J* = 1.8 Hz, 1H, H-1), 4.92 (dd, *J* = 8.4, 1.8 Hz, 1H, H-2,), 4.82 (AB q, *J* = 11.7 Hz, 2H, Bn-CH_2), 4.49 (t, *J* = 4.5 Hz, 1H, H-5), 4.19 (dd, *J* = 7.6, 0.9 Hz, 1H, H-6a), 4.06 – 3.99 (m, 1H, H-4), 3.88 (t, *J* = 8.2 Hz, 1H, H-3), 3.70 (ddd, *J* = 7.7, 5.1, 1.1 Hz, 1H, H-6b). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 166.3 (C), 139.9 (C), 134.2 (CH), 130.8 (CH), 130.4 (C), 129.4 (CH), 128.9 (CH), 128.4 (CH), 128.1 (CH), 100.0 (C-1), 80.9 (C-3), 77.5 (C-4), 76.8 (C-2), 75.0 (C-6), 72.8 (C-5), 65.7 (Bn-CH_2). These data are in agreement with literature data.[59]

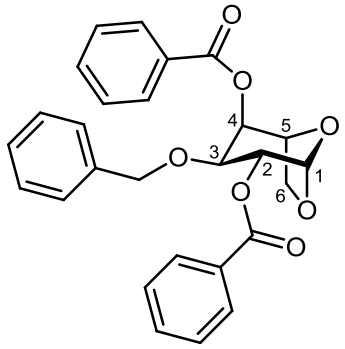
1,6-Anhydro-2,3,4-tri-*O*-trimethylsilyl-β-L-idopyranose (82)



Triol **58** (0.33 g, 2.04 mmol) was dissolved in DCM under Ar and cooled to 0°C before adding Et_3N (1.42 mL, 10.2 mmol). After slowly adding TMSCl (1.03 mL, 8.15 mmol) the reaction mixture was allowed to warm to RT and stirred for 4 h. The reaction mixture was concentrated and the residue dissolved in hexane before filtering through celite®. The residue was recrystallized from EtOH to produce 1,6-anhydro-2,3,4-tri-*O*-trimethylsilyl-β-L-idopyranose (**82**) as white crystals (0.65 g, 83.9%).

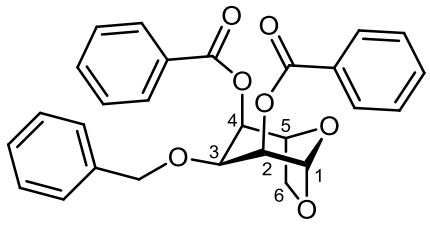
R_f in 40% EtOAc/hexane is 0.74. m.p. 68-71°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.18 (d, *J* = 1.6 Hz, 1H, H-1), 4.25 (t, *J* = 4.6 Hz, 1H, H-5), 4.07 (d, *J* = 7.6 Hz, 1H, H-6a), 3.68 (m, 1H, H-4) 3.67 (m, 1H, H-6b), 3.56 (t, *J* = 7.6 Hz, 1H, H-3), 3.46 (dd, *J* = 7.6, 1.6 Hz, 1H, H-2), 0.17 (s, 9H, Si(C$\underline{H}_3$)$_3$), 0.14 (s, 9H, Si(C$\underline{H}_3$)$_3$), 0.14 (s, 9H, Si(C$\underline{H}_3$)$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 102.3 (C-1), 76.6 (C-2), 76.6 (C-3), 75.9 (C5), 73.2 (C-4), 65.2 (C6), 1.1 (CH_3), 0.5 (CH_3), 0.5 (CH_3). These data are in agreement with literature data.[59]

Attempted synthesis of 1,6-anhydro-2,4-di-*O*-benzoyl-3-*O*-benzyl-β-L-altropyranose[50] (85)



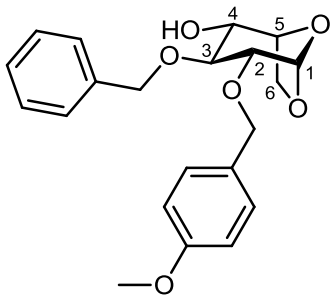
Compound **60** (0.07 g, 0.15 mmol) was dissolved in DMF (4.5 mL) under Ar and cooled to 0°C. NaOBz (0.03 g, 0.23 mmol) was added and the reaction stirred at 0°C for 1 h, TLC showed little progress. The reaction was allowed to warm to RT for 5 h, but there was still very little reaction progress. It was then heated to 90°C for 3 h after which the TLC indicated that the reaction was finished. Brine solution was added to the reaction mixture and the resulting slurry extracted with DCM (3 x 10 mL). The combined DCM phases were dried over anhydrous Na_2SO_4, concentrated and then purified by flash chromatography in a gradient solvent system of 5 to 35% EtOAC in hexane, from which two compounds were identified. The less polar of the two was recovered starting material (0.03 g, 34%) which was confirmed by NMR. The more polar compound (0.02 g) was still not pure (NMR) so it was chromatographed further on a preparative TLC plate. Three separate UV active bands were seen on the plate and these were collected, extracted and analysed by NMR. The middle band (0.009 g) was the only one showing signals consistent with a carbohydrate derivative, though the sample was still clearly not a single compound. More purification lead to further degradation so this route was abandoned due to low yield and difficulty in purification.

Attempted synthesis of 1,6-Anhydro-2,4-di-*O*-benzoyl-3-*O*-benzyl-β-L-altropyranose[84]



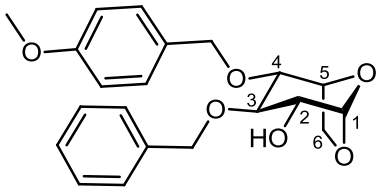
Compound **59** (0.06 g, 0.23 mmol) was dissolved in anhydrous THF (5 mL) under Ar. PPh_3 (0.073 g, 0.281 mmol) and benzoic acid (0.031g, 0.256 mmol) were added and the reaction mixture cooled to 0°C. DIAD (0.05 mL, 0.28 mmol) was added dropwise and the reaction mixture stirred at RT for 18 h. TLC showed little reaction progress and so the temperature was increased to 60°C for 2 h. Almost no reaction progress was observed so the reaction was diluted with Et_2O, the ether layer was washed twice with aqueous $NaHCO_3$ and the separated aqueous portion back-extracted with Et_2O. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was redissolved in Et_2O and hexane then added in order to precipitate the solid PPh_3O which was removed by filtration through celite. This organic filtrate was concentrated, and then purified by flash chromatography in a solvent gradient system of 25 to 65 EtOAc in hexane to recover only unreacted starting material (0.06 g, 100%).

Attempted synthesis of 1,6-Anhydro-2-*O*-*p*-methoxybenzyl-3-*O*-benzyl-β-L-idopyranose[45] (87)

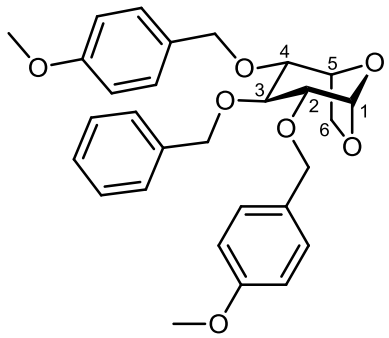


Compound **59** (0.08 g, 0.33 mmol) was dissolved in DMF (1 mL) at RT under Ar. The reaction was cooled to 0°C and NaH, 60% mineral oil dispersion, (0.03 g, 0.7 mmol) added. PMBCl (0.05 mL, 0.37 mmol) was

added and the reaction mixture stirred at 0°C for 4 h. TLC showed slow reaction progress so the reaction was warmed to RT for 12 h. It was then quenched with MeOH (2 mL) and concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with brine and saturated $NaHCO_3$ and dried over anhydrous Na_2SO_4. Three UV active spots that also stained with CAS were identified on TLC of the crude product mixture. These were separated by column chromatography in a gradient solvent system of 20-65% EtOAc in hexane. Starting material was recovered as a white powder (0.02 g, 27.3%), 1,6-Anhydro-2,4-di-*O*-*p*-methoxybenzyl-3-*O*-benzyl-β-L-idopyranose (**86**) (0.08 g, 50.1%) afforded as a clear oil and a mixture of the 2 and 4-PMB products (**87** and **88**) (0.02 g, 19.3%) afforded as a pale yellow powder.

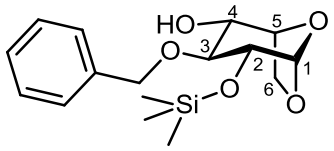


87 and **88**: R_f in 50% EtOAc/hexane run twice is 0.36. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 7.42 – 7.21 (m, 14H, Ar), 6.93 – 6.80 (m, 4H, Ar), 5.35 (d, *J* = 1.7 Hz, 1H. H-1), 5.17 (d, *J* = 1.6 Hz, 1H, H-1'), 4.92 – 4.78 (m, 4H, Bn-CH_2), 4.67 – 4.60 (m, 4H, PMB-CH_2), 4.55 (ddd, *J* = 5.1, 4.2, 0.8 Hz, 1H), 4.39 – 4.30 (m, 1H), 4.22 (d, *J* = 6.3 Hz, 1H), 4.05 (dd, *J* = 7.5, 0.8 Hz, 1H), 3.98 (dd, *J* = 7.4, 0.9 Hz, 1H), 3.78 (d, *J* = 1.0 Hz, 6H, Ph-OCH_3), 3.63 (ddt, *J* = 6.5, 3.7, 1.2 Hz, 3H), 3.60 – 3.51 (m, 3H), 3.40 (dd, *J* = 8.0, 1.7 Hz, 1H).



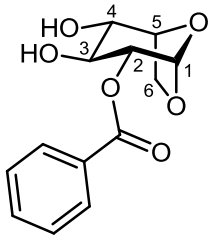
86: R_f in 50% EtOAc/hexane run twice is 0.29. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 7.42 – 7.19 (m, 8H, PMB-H), 6.95 – 6.82 (m, 5H, Bn-H), 5.35 (d, *J* = 1.7 Hz, 1H, H-1), 4.81 (s, 2H, Bn-CH_2), 4.66 – 4.61 (m, 4H, PMB-CH_2), 4.55 (ddd, *J* = 4.9, 3.7, 0.8 Hz, 1H, H-5), 4.01 (dd, *J* = 7.5, 0.9 Hz, 1H, H-6a), 3.78 (d, *J* = 1.1 Hz, 6H, PMB-OCH_3), 3.67 – 3.57 (m, 2H, H-3, H-4), 3.43 – 3.37 (m, 1H, H-6b), 2.76 (d, *J* = 9.5 Hz, 1H, H-2).

Attempted synthesis of 1,6-Anhydro-3-*O*-benzyl-2-*O*-trimethylsilyl-β-L-idopyranose[59] (89)



Compound **59** (0.05 g, 0.21 mmol) was dissolved in DCM (1 mL) under Ar and cooled to 0°C. NEt_3 (0.037 mL, 0.27 mmol) was added to the reaction mixture followed by freshly distilled TMSCl (0.03 mL, 0.23 mmol). The reaction was stirred at 0°C for 5.5 h and then concentrated under reduced pressure. The residue was then dissolved in a minimum amount of DCM and, on addition of hexane the product crystallised and was recovered by filtration through fluted filter paper. NMR confirmed that it was recovered starting material (0.05 g, 99%). The reaction was repeated at RT with the same result.

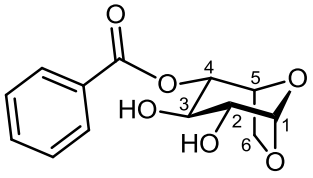
Attempted synthesis of 1,6-anhydro-2-*O*-benzoyl-β-L-idopyranose[64] (92)



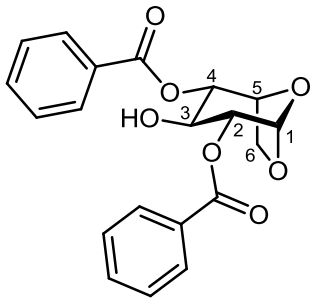
Triol **58** (0.08 g, 0.49 mmol) and Bu_2SnO (0.24 g, 0.98 mmol) were dissolved in dry $PhCH_3$ (2 mL) and refluxed for 30 min under N_2 after which time the water was azeotropically removed under reduced pressure. BzCl (0.12 mL, 1.04 mmol) in $PhCH_3$ (3 mL) was added to the reaction mixture and stirred at RT for 18 h. The reaction mixture was then cooled to 0°C, quenched with EtOAc and filtered through celite. The solvents were removed under reduced pressure and the residue purified by column chromatography using 30 to 100% EtOAc in hexane as eluent to give 1,6-anhydro-2-*O*-benzoyl-β-L-idopyranose (**92**) (0.02 g, 11%) and 1,6-anhydro-4-*O*-benzoyl-β-L-idopyranose (**93**) (0.06 g, 45%) as a 1:4 mixture, and 1,6-anhydro-3,4-di-*O*-benzoyl-β-L-idopyranose (**94**) (0.02 g, 8%) and 1,6-anhydro-2,4-di-*O*-benzoyl-β-L-idopyranose (**95**) (0.009 g, 5%) as a 3:2 mixture, and pure 1,6-anhydro-2,3,4-tri-*O*-

benzoyl-β-L-idopyranose (**96**) (0.03 g, 11%) which were all obtained as white solids. In addition, starting material was recovered (0.01 g, 15%).

92: R_f in 100% EtOAc is 0.44. m.p. 143-148°C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.09 – 8.06 (m, 2H, Ph-H), 7.61 (d, *J* = 7.4 Hz, 1H, Ph-H), 7.51 – 7.46 (m, 2H, Ph-H), 5.44 (d, *J* = 1.8 Hz, 1H, H-1), 4.81 (dd, *J* = 8.2, 1.8 Hz, 1H, H-2), 4.45 (t, *J* = 4.4 Hz, 1H, H-5), 4.14 (dd, *J* = 7.6, 0.8 Hz, 1H, H-6a), 3.84 (t, *J* = 8.3 Hz, 1H, H-3), 3.78 (ddd, *J* = 8.3, 4.2, 1.1 Hz, 1H, H-4), 3.71 (ddd, *J* = 7.7, 5.1, 1.1 Hz, 1H, H-6b). ^{13}C NMR (101 MHz, CD_3OD_4) δ 167.6 (C=O), 134.4 (Ph-C), 130.7 (Ph-C), 129.5 (Ph-C), 100.6 (C-1), 78.6 (C-2), 77.2 (C-5), 73.3 (C-3), 73.0 (C-4), 66.1 (C-6). HRMS calcd for $C_{13}H_{14}O_6$: 266.0790; found: 267.0871 [*M*+H].

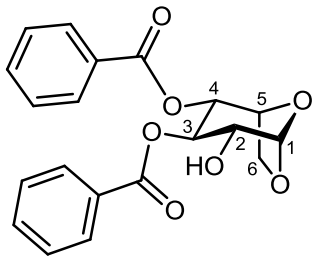


93: R_f in 100% EtOAc is 0.44. m.p. 121-126°C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.09 – 8.06 (m, 2H, Ph-H), 7.61 (d, *J* = 7.4 Hz, 1H, Ph-H), 7.51 – 7.46 (m, 2H, Ph-H), 5.32 (d, *J* = 1.9 Hz, 1H, H-1), 5.07 (ddd, *J* = 8.7, 4.2, 1.1 Hz, 1H, H-4), 4.68 (t, *J* = 4.6 Hz, 1H, H-5), 4.13 (dd, *J* = 7.9, 0.8 Hz, 1H, H-6a), 3.89 (t, *J* = 8.3 Hz, 1H, H-3), 3.75 (ddd, *J* = 8.0, 5.0, 1.2 Hz, 1H, H-6b), 3.68 (t, *J* = 5.0 Hz, 1H, 2-OH), 3.53 (dd, *J* = 8.4, 1.8 Hz, 1H, H-2). ^{13}C NMR (101 MHz, CD_3OD) δ 165.9 (C=O), 133.1 (Ph-C), 129.3 (Ph-C), 128.2 (Ph-C), 102.2 (C-1), 75.3 (C-2), 73.7 (C-4), 72.8 (C-5), 72.0 (C-3), 65.2 (C-6). HRMS calcd for $C_{13}H_{14}O_6$: 266.0790; found: 267.0871 [*M*+H].

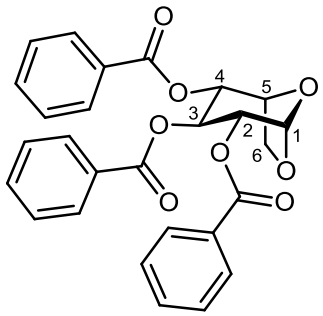


94: R_f in 100% EtOAc is 0.58. m.p. 95-100°C. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.09 – 8.02 (m, 4H, Ph-H), 7.66 – 7.62 (m, 2H, Ph-H), 7.53 – 7.49 (m, 4H, Ph-H), 5.59 (d, *J* = 1.8 Hz, 1H, H-1), 5.24 (ddd, *J* = 8.7, 4.3,

1.2 Hz, 1H, H-4), 5.00 (dd, *J* = 8.4, 1.8 Hz, 1H, H-2), 4.84 (t, *J* = 4.7 Hz, 1H, H-5), 4.43 (t, *J* = 4.9 Hz, 1H, H-3), 4.32 (dd, *J* = 8.0, 0.8 Hz, 1H, H-6a), 3.81 (ddd, *J* = 8.1, 4.9, 1.3 Hz, 1H, H-6b), 3.76 (t, *J* = 5.0 Hz, 1H, 3-OH). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 167.5 (C=O), 160.72 (C=O), 133.7 (Ph-C), 130.4 (Ph-C), 129.3 (Ph-C), 100.3 (C-1), 78.4 (C-2), 74.8 (C-4), 73.8 (C-5), 66.6 (C-6), 64.9 (C-3). HRMS calcd for $C_{20}H_{18}O_7$: 370.1053; found: 371.1125 [*M*+H].



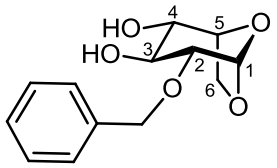
95: R_f in 100% EtOAc is 0.58. m.p. 134-138°C. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.09 – 8.02 (m, 4H, Ph-H), 7.66 – 7.62 (m, 2H, Ph-H), 7.53 – 7.49 (m, 4H, Ph-H), 5.29 (d, *J* = 1.8 Hz, 1H, H-1), 5.23 (t, *J* = 8.4 Hz, 1H, H-3), 4.49 (t, *J* = 4.7 Hz, 1H, H-5), 4.19 (dd, *J* = 7.7, 0.8 Hz, 1H, H-6a), 4.02 (ddd, *J* = 8.4, 4.3, 1.1 Hz, 1H, H-4), 3.74 – 3.71 (m, 1H, H-6b), 3.70 (d, *J* = 1.6 Hz, 1H, H-2). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 167.5 (C=O), 143.8 (C=O), 133.7 (Ph-CH), 130.4 (Ph-CH), 129.3 (Ph-CH), 129.2 (Ph-CH), 103.1 (C-1), 79.2 (C-3), 76.6 (C-5), 74.4 (C-2), 70.7 (C-4), 65.6 (C-6). HRMS calcd for $C_{20}H_{18}O_7$: 370.1053; found: 371.1125 [*M*+H].



96: R_f in 100% EtOAc is 0.68. m.p. 90-93°C. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.09 – 8.01 (m, 6H, Ph-H), 7.65 – 7.60 (m, 3H, Ph-H), 7.53 – 7.51 (m, 6H, Ph-H), 5.65 (d, *J* = 8.6 Hz, 1H, H-3), 5.61 (d, *J* = 1.6 Hz, 1H, H-1), 5.10 (dd, *J* = 8.7, 1.8 Hz, 1H, H-2), 4.66 (t, *J* = 4.5 Hz, 1H, H-5), 4.34 (dd, *J* = 7.8, 0.8 Hz, 1H, H-6a), 4.25 (ddd, *J* = 8.5, 4.4, 1.1 Hz, 1H, H-4), 3.85 – 3.80 (m, 1H, H-6b). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 167.5

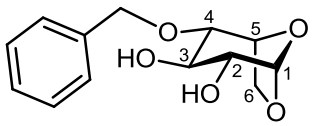
(C=O), 160.7 (C=O), 143.8 (C=O), 133.7 (Ph-C), 130.4 (Ph-C), 129.3 (Ph-C), 100.1 (C-1), 76.7 (C-5), 76.2 (C-2), 75.2 (C-3), 70.6 (C-4), 66.0 (C-6). HRMS calcd for $C_{27}H_{22}O_8$: 474.1315 found: 371.1134 [*M*-Bz].

Attempted synthesis of 1,6-Anhydro-2-*O*-benzyl-β-L-idopyranose[63] (98)



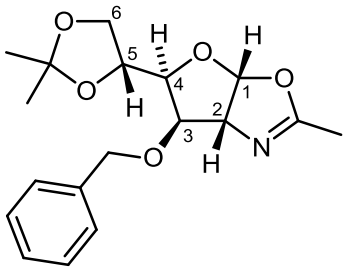
Triol **58** (0.18 g, 1.13 mmol) and Bu_2SnO (0.31 g, 1.24 mmol) were dissolved in anhydrous $PhCH_3$ (40 mL) and refluxed at 110°C under Ar for 2 h after which DMF (1 mL) was added to improve solubility. This mixture was refluxed for a further 2 h after which all the solvent was removed under high pressure vacuum. The reaction mixture was then re-dissolved in $PhCH_3$ (4 mL), and BnBr (0.16 mL, 1.36 mmol) and TBABr (0.18 g, 0.57 mmol) were added to the reaction under Ar. This was then heated at 98°C for 8 h. The reaction mixture was purified directly by flash column chromatography in a gradient from 20 to 65% EtOAc in hexane as eluent.1,6-Anhydro-3-*O*-benzyl-β-L-idopyranose (**59**) (0.03 g, 30.0 %) was obtained pure as a white solid. The next two products 1,6-anhydro-2-*O*-benzyl-β-L-idopyranose (**98**) and 1,6-anhydro-4-*O*-benzyl-β-L-idopyranose (**97**) (0.13 g, 44.3 %) were eluted as an inseparable mixture and recovered as a white powder after drying.

98: R_f in 50% EtOAc/hexane run twice is 0.19.m.p. 66-70°C. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 7.42 – 7.37 (m, 2H, Ph-H), 7.36 – 7.30 (m, 2H, Ph-H), 7.30 – 7.24 (m, 1H, Ph-H), 5.33 (d, *J* = 1.7 Hz, 1H, H-1), 4.80 – 4.67 (AB q, *J* = 11.8 Hz, 2H, Ph-CH_2), 4.54 (d, *J* = 3.6 Hz, 1H, 4-OH), 4.52 (td, *J* = 4.4, 0.8 Hz, 1H, H-5), 4.43 (d, *J* = 5.2 Hz, 1H, 3-OH), 3.94 (dd, *J* = 7.4, 0.8 Hz, 1H, H-6a), 3.67 – 3.61 (m, 1H, H-3), 3.61 – 3.56 (m, 1H, H-6b), 3.53 (ddd, *J* = 8.1, 4.1, 1.1 Hz, 1H, H-4), 3.28 (dd, *J* = 8.2, 1.7 Hz, 1H, H-2).^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 140.1 (Ph-C), 129.04(Ph-CH), 128.4 (Ph-CH), 128.2 (Ph-CH), 100.7 (C-1), 83.7 (C-2), 80.3 (C-4), 75.4 (C-3), 74.1 (C-5), 73.3 (Ph-CH_2), 65.8 (C-6).



97: R_f in 50% EtOAc/hexane run twice is 0.19.m.p. 66-70°C. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 7.42 – 7.37 (m, 2H, Ph-H), 7.36 – 7.30 (m, 2H, Ph-H), 7.30 – 7.24 (m, 1H, Ph-H), 5.16 (d, *J* = 1.9 Hz, 1H, H-1), 4.80 – 4.67 (AB q, *J* =11.8 Hz, 2H, Ph-CH_2), 4.43 (d, *J* = 5.2 Hz, 1H, 3-OH), 4.33 (td, *J* = 3.9, 0.7 Hz, 1H, H-5), 4.13 (d, *J* = 4.4 Hz, 1H, 2-OH), 3.99 (dd, *J* = 7.4, 0.8 Hz, 1H, H-6a), 3.67 – 3.61 (m, 2H, H-4 and H-3), 3.61 – 3.56 (m, 1H, H-6b), 3.36 (td, *J* = 6.2, 1.7 Hz, 1H, H-2).^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 139.9 (Ph-C), 128.9 (Ph-CH), 128.4 (Ph-CH), 128.1 (Ph-CH), 103.0 (C-1), 76.7 (C-2), 76.4 (C-5), 75.8 (C-3), 73.0 (Ph-CH_2), 72.9 (C-4), 65.4 (C-6).

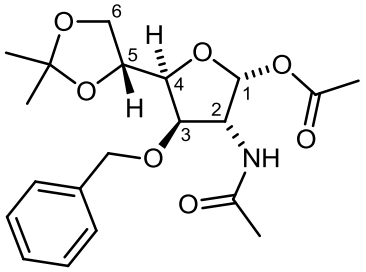
2-Methyl-(3-*O*-benzyl-1,2-dideoxy-5,6-*O*-isopropylidene-α-D-glucofurano)-[2,1-d]-2-oxazoline[36,71] (102)



NaH, 60% mineral oil dispersion, (0.90 g, 22.5 mmol) and compound **69** (3.66 g, 15.1 mmol) were dissolved in DMF (10 mL) at 0°C under Ar and stirred for 10 min. BnBr (1.96 mL, 16.5 mmol) was added to the reaction, the ice bath removed and the reaction stirred at RT for 18 h. The reaction mixture was quenched with MeOH (20 mL) and concentrated under reduced pressure. The residue was taken up in water (50 mL), extracted with DCM (5 x 50 mL), dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash chromatography in a solvent gradient from 20 to 70% EtOAc in hexane affording 2-methyl-(3-*O*-benzyl-1,2-dideoxy-5,6-*O*-isopropylidene-α-D-glucofurano)-[2,1-d]-2-oxazoline (**102**) (3.06 g, 61.2%) as a yellow solid.

R_f in 65% EtOAc/hexane is 0.45. m.p. 102-105°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 5H, Ph-H), 6.12 (d, *J* = 5.1 Hz, 1H, H-1), 4.71 (AB quartet, *J* = 11.8 Hz, 2H, Ph-CH_2), 4.51 (dd, *J* = 5.1, 1.5 Hz, 1H, H-2), 4.36 (dd, *J* = 13.0, 6.0 Hz, 1H, H-5), 4.10 (m, 1H, H-3) 4.05 (m, 1H, H-6a), 4.00 (dd, *J* = 8.6, 5.7 Hz, 1H, H-6b), 3.84 (dd, *J* = 7.1, 3.1 Hz, 1H, H-4), 2.01 (d, *J* = 1.5 Hz, 3H, NC(C<u>H</u>$_3$)O), 1.40 (d, *J* = 0.7 Hz, 3H, C(C<u>H</u>$_3$)$_2$), 1.35 (d, *J* = 0.7 Hz, 3H, C(C<u>H</u>$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (<u>C</u>(CH_3)$_2$), 137.6 (N<u>C</u>(CH_3)O), 128.4 (Ph-CH), 127.8 (Ph-CH), 127.7 (Ph-CH), 109.0 (Ph-C), 107.1 (C-1), 81.6 (C-4), 75.3 (C-2), 72.6 (C-3), 72.2 (C-5), 67.0 (C-6), 26.7 (C(<u>C</u>H$_3$)$_2$), 25.3 (C(<u>C</u>H$_3$)$_2$), 14.1 (NC(<u>C</u>H$_3$)O). HRMS calcd for $C_{18}H_{23}NO_5$: 333.1576; found: 334.1651 [*M*+H].

2-Acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-5,6-*O*-ispropylidene-α-D-glucofuranose[71,72] (103)

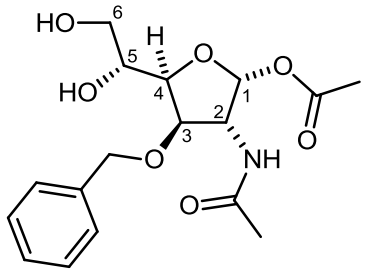


Oxazoline **102** (6.35 g, 19.1 mmol) was dissolved in H_2O (25 mL) and heated under reflux for 18 h. The reaction mixture was extracted with Et_2O (3 x 50 mL), dried over Na_2SO_4 and concentrated. This intermediate was dried for 4 h before continuing (R_f in 20% MeOH/EtOAc is 0.51). Ac_2O (7.09 mL, 75.2 mmol) and DMAP (0.46 g, 3.76 mmol) were added to a solution of the intermediate (6.61 g, 18.8 mmol) in freshly distilled DCM (40 mL). The reaction mixture was stirred at RT for 17 h after which TLC showed all the starting material had been consumed. The reaction was quenched with $NaHCO_3$(aq), extracted with DCM (5 x 35 mL), dried over anhydrous Na_2SO_4 and concentrated.NMR of the crude product, a dark yellow syrup, confirmed the formation of the desired product, 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-5,6-*O*-ispropylidene-α-D-glucofuranose (**103**) (7.11 g, 94.8%).

R_f in 10% MeOH/EtOAc is 0.41. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.30 (m, 5H, Ph-H), 6.35 (d, *J* = 5.4 Hz, 1H, H-1), 5.82 (d, *J* = 7.7 Hz, 1H, N<u>H</u>$COCH_3$), 4.71 (AB quartet, *J* = 4.5 Hz, 2H, Ph-CH_2),

4.69 – 4.62 (m, 2H, H-2), 4.37 (q, *J* = 6.3 Hz, 1H, H-5), 4.23 (t, *J* = 5.4 Hz, 1H, H-4), 4.07 (dd, *J* = 8.6, 6.3 Hz, 1H, H-6a), 4.01 (dd, *J* = 5.1, 3.6 Hz, 1H, H-3), 3.95 (dd, *J* = 8.7, 6.1 Hz, 1H, H-6b), 2.09 (s, 3H, OCOC$\underline{H}_3$), 2.00 (s, 3H, NHCOC$\underline{H}_3$), 1.40 (s, 3H, CH_3), 1.35 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7 (NH$\underline{C}$OCH$_3$), 169.0 (O$\underline{C}$OCH$_3$), 137.1 (Ph-C), 128.4 (Ph-CH), 127.9 (Ph-CH), 127.8 (Ph-CH), 109.0 ($\underline{C}$(CH$_3$)$_2$), 94.6 (C-1), 81.9 (C-3), 80.5 (C-4), 73.3 (C-5), 72.2 (Ph-CH$_2$), 66.5 (C-6), 57.1 (C-2), 26.7 (C($\underline{C}$H$_3$)$_2$), 25.4 (C($\underline{C}$H$_3$)$_2$), 23.3 (NHCO$\underline{C}$H$_3$), 21.2 (OCO$\underline{C}$H$_3$). HRMS calcd for $C_{20}H_{27}NO_7$: 393.1788; found: 394.1874 [*M*+H].

2-Acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose[71] (104)

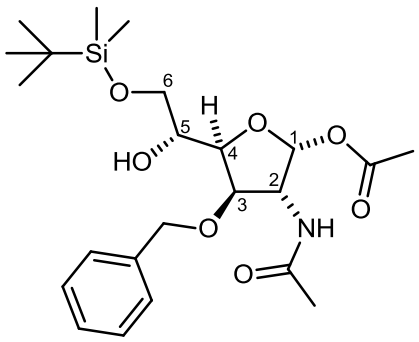


Compound **103** (7.11 g, 18.1 mmol) was dissolved in 70% aqueous AcOH (90 mL) and stirred at RT for 18 h. It was quenched with aqueous $NaHCO_3$ and cooled in an ice bath before being extracted with DCM (5 x 50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography in a gradient solvent system from 100% EtOAc a 15% MeOH/EtOAc mixture. The desired product, 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**104**) was afforded as a yellow foam (4.46 g, 71.1%).

R_f in 15% MeOH/EtOAc is 0.33. m.p. 25-26°C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.31 (m, 5H, Ph-H), 6.32 (d, *J* = 5.3 Hz, 1H, H-1), 5.84 (d, *J* = 8.2 Hz, 1H,N$\underline{H}$COCH$_3$), 4.85 (d, *J* = 11.7 Hz, 1H, Ph-CH$_2$), 4.79 (ddd, *J* = 8.3, 5.3, 4.1 Hz, 1H, H-2), 4.59 (d, *J* = 11.8 Hz, 1H,Ph-CH$_2$), 4.20 (dd, *J* = 8.1, 5.8 Hz, 1H, H-4), 4.16 (dd, *J* = 5.8, 4.1 Hz, 1H, H-3), 3.97 (ddd, *J* = 8.3, 5.1, 3.5 Hz, 1H, H-5), 3.78 (dd, *J* = 11.5, 3.4 Hz, 1H, H-6a), 3.66 (dd, *J* = 11.5, 5.2 Hz, 1H, H-6b), 2.11 (s, 3H, OCOC$\underline{H}_3$), 2.03 (s, 3H,NHCOC$\underline{H}_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6 (NH$\underline{C}$OCH$_3$), 169.1 (O$\underline{C}$OCH$_3$), 137.1 (Ph-C), 128.9 (Ph-CH), 128.4 (Ph-CH), 128.2 (Ph-CH), 94.6 (C-1), 82.3 (C-3), 78.6 (C-4), 72.1 (Ph-CH$_2$), 70.0 (C-5), 63.8

(C-6), 56.7 (C-2), 23.4 (NHCO<u>C</u>H$_3$), 21.2 (OCO<u>C</u>H$_3$). ^{1}H NMR (600 MHz, (CD$_3$)$_2$CO) δ 7.42 (d, *J* = 8.4 Hz, 1H, N<u>H</u>COCH$_3$), 7.39 (ddd, *J* = 8.0, 1.9, 1.3 Hz, 2H, Ph-H), 7.36 – 7.31 (m, 2H, Ph-H), 7.32 – 7.24 (m, 1H, Ph-H), 6.29 (d, *J* = 5.4 Hz, 1H, H-1), 4.82 (d, *J* = 11.7 Hz, 1H, Ph-CH$_2$), 4.76 (ddd, *J* = 8.6, 5.4, 3.3 Hz, 1H, H-2), 4.66 (d, *J* = 11.7 Hz, 1H, Ph-CH$_2$), 4.23 – 4.16 (m, 2H, H-3 and H-4), 3.95 (bs, 1H, H-5), 3.74 – 3.68 (m, 2H, H-6a), 3.58 – 3.52 (m, 1H. H-6b), 2.03 (s, 3H, OCOC<u>H</u>$_3$), 1.93 (d, *J* = 0.8 Hz, 3H, NHCOC<u>H</u>$_3$). ^{13}C NMR (101 MHz, (CD$_3$)$_2$CO) δ 170.0 (NH<u>C</u>OCH$_3$), 169.8 (O<u>C</u>OCH$_3$), 139.3 (Ph-C), 129.13 (Ph-CH), 128.6 (Ph-CH), 128.3 (Ph-CH), 95.4 (C-1), 83.4 (C-3), 80.2 (C-4), 72.5 (Ph-CH$_2$), 70.4 (C-5), 64.47 (C-6), 58.0 (C-2), 22.8 (NHCO<u>C</u>H$_3$), 20.9 (OCO<u>C</u>H$_3$). HRMS calcd for $C_{17}H_{23}NO_7$: 353.1475; found: 354.1537 [*M*+H].

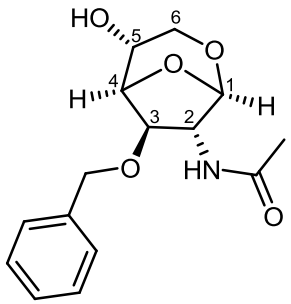
2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucofuranose[85] (105)



Optimised route: TBDMSCl (1.10 g, 7.31 mmol) was dissolved in anhydrous pyridine (10 mL) under Ar. This solution was added to diol **104** (2.58 g, 7.31 mmol) and stirred at RT for 24 h. Another equivalent of TBDMSCl (1.10 g, 7.31 mmol) was added to the reaction using 6 mL pyridine and stirred for a further 24 h at RT. The reaction was washed with brine and extracted with DCM (3 x 50 mL), dried over Na_2SO_4 and concentrated. Residual pyridine was removed azeotropically using $PhCH_3$. No further purification was necessary. NMR confirmed that the white powder was the desired product, 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucofuranose (**105**) (3.35 g, 98%).

R_f in 5% MeOH/EtOAc is 0.54. m.p. 116-120°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.32 (m, 5H, Ph-H), 6.37 (d, *J* = 5.6 Hz, 1H, H-1), 5.73 (d, *J* = 8.0 Hz, 1H, N<u>H</u>COCH$_3$), 4.85 (d, *J* = 12.0 Hz, 1H, Ph-CH$_2$), 4.74 (ddd, *J* = 8.4, 5.6, 3.1 Hz, 1H, H-2), 4.67 (d, *J* = 11.9 Hz, 1H, Ph-CH$_2$), 4.15 (dd, *J* = 7.9, 5.1 Hz, 1H, H-4), 4.08 (dd, *J* = 5.1, 3.2 Hz, 1H, H-3), 4.00 (dd, *J* = 8.2, 3.7 Hz, 1H, H-5), 3.79 (dd, *J* = 10.4, 3.6 Hz, 1H, H-6a), 3.73 (dd, *J* = 10.3, 4.8 Hz, 1H, H-6b), 2.72 (d, *J* = 6.0 Hz, 1H, 5-OH), 2.10 (s, 3H, OCOC<u>H</u>$_3$), 2.02 (s, 3H, NHCOC<u>H</u>$_3$), 0.89 (s, 9H, OSi(CH$_3$)$_2$C(C<u>H</u>$_3$)$_3$), 0.05 (s, 6H, OSi(C<u>H</u>$_3$)$_2$C(CH$_3$)$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4 (NH<u>C</u>OCH$_3$), 168.9 (O<u>C</u>OCH$_3$), 137.7 (Ph-C), 128.6 (Ph-CH), 128.1 (Ph-CH), 128.0 (Ph-CH), 94.7 (C-1), 82.7 (C-3), 79.1 (C-4), 72.15(Ph-CH$_2$), 69.3 (C-5), 64.1 (C-6), 57.3 (C-2), 26.0 (OSi(CH$_3$)$_2$C(<u>C</u>H$_3$)$_3$), 23.4 (NHCO<u>C</u>H$_3$), 21.2 (OCO<u>C</u>H$_3$), 18.5 (OSi(CH$_3$)$_2$<u>C</u>(CH$_3$)$_3$), -5.3 (OSi(<u>C</u>H$_3$)$_2$C(CH$_3$)$_3$). ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO) δ 7.38 (d, *J* = 8.4 Hz, 1H, N<u>H</u>COCH$_3$), 7.41 – 7.24 (m, 5H, Ph-H), 6.31 (d, *J* = 5.5 Hz, 1H, H-1), 4.82 (d, *J* = 11.9 Hz, 1H, Ph-CH$_2$), 4.75 (ddd, *J* = 8.8, 5.5, 3.6 Hz, 1H, H-2), 4.65 (d, *J* = 11.8 Hz, 1H, Ph-CH$_2$), 4.22 (dd, *J* = 7.9, 5.4 Hz, 1H, H-4), 4.17 (dd, *J* = 5.4, 3.7 Hz, 1H, H-3), 4.00 – 3.92 (m, 1H, H-5), 3.83 (dd, *J* = 10.5, 3.0 Hz, 1H, H-6a), 3.75 – 3.69 (m, 1H, H-6b), 3.55 (d, *J* = 5.9 Hz, 1H, 5-OH), 2.02 (s, 3H, OCOC<u>H</u>$_3$), 1.92 (s, 3H, NHCOC<u>H</u>$_3$), 0.90 (s, 9H, OSi(CH$_3$)$_2$C(C<u>H</u>$_3$)$_3$), 0.06 (d, *J* = 0.4 Hz, 6H, OSi(C<u>H</u>$_3$)$_2$C(CH$_3$)$_3$). ^{13}C NMR (101 MHz, (CD$_3$)$_2$CO) δ 169.8 (NH<u>C</u>OCH$_3$), 169.7 (O<u>C</u>OCH$_3$), 139.4 (Ph-C), 129.1 (Ph-CH), 128.6 (Ph-CH), 128.3 (Ph-CH), 95.3 (C-1), 83.5 (C-3), 79.9 (C-4), 72.5 (Ph-CH$_2$), 70.3 (C-5), 65.6 (C-6), 58.4 (C-2), 26.3 (OSi(CH$_3$)$_2$C(<u>C</u>H$_3$)$_3$), 22.8 (NHCO<u>C</u>H$_3$), 21.0 (OCO<u>C</u>H$_3$), 19.0 (OSi(CH$_3$)$_2$<u>C</u>(CH$_3$)$_3$), -5.1 (OSi(<u>C</u>H$_3$)$_2$C(CH$_3$)$_3$). HRMS calcd for $C_{23}H_{37}NO_7Si$: 467.2339; found: 408.2206 [*M*-OAc].

Attempted synthesis of 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (106)

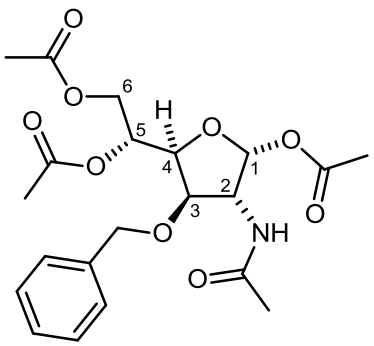


Method 1: Diol **104** (0.26 g, 0.72 mmol) was dissolved in DCM (10 mL) at RT under Ar. DMAP (0.02 g, 0.14 mmol) was added with the reaction and stirred at RT for 10 mins. TBDMSCl (0.120 g,

0.79 mmol) was then added to the reaction and allowed to stir for a 24 hours. Another 0.2 equivalents of DMAP was added. After a further 24 hours at RT reaction mixture was washed with water and back extracted with DCM (3 x 35 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. This residue was purified by flash column chromatography in a gradient solvent system from 70% to 100% EtOAc in hexane. The product was afforded as a pale yellow solid identified as a mixture of two products by NMR. A second purification separated the two products. The major product was identified as 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucofuranose (**35**) (0.160 g, 47.2%) and the minor product was 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**38**) (0.02 g, 10.1%).

Method 2: Diol **104** (0.17 g, 0.49 mmol) was dissolved in freshly distilled DCM (8 mL). DMAP (0.12 g, 0.99 mmol) was added with the reaction stirred at RT for 20 hours. TLC showed no reaction had occurred. Some reaction appeared to be taking place but there was still residual starting material. The reaction mixture was washed with water and back extracted (5 x 35 mL) with DCM. The combined organic layers were dried of Na_2SO_4 and concentrate under reduced pressure. NMR of the crude material was checked and only starting material was recovered (0.17 g, 97.1%).[71]

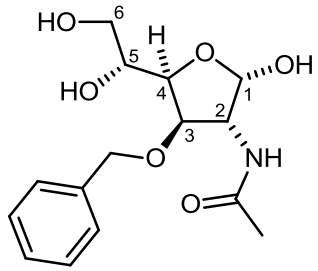
Method 3: **104** (0.16 g, 0.45 mmol) was dissolved in anhydrous THF (10 mL). NaH (0.03 g, 0.91 mmol) was added with the reaction and stirred at room temperature for 18 hours. TLC showed a more polar spot and the disappearance of all the starting material. Ac_2O (0.17 mL, 1.81 mmol) was added to the reaction mixture and stirred for 17 hours after. The reaction mixture was washed with brine and back extracted with EtOAc (3 x 35 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. This residue was purified by flash column chromatography in a gradient solvent system from 100% EtOAc to 2% MeOH/EtOAc. The product was afforded as a white yellow solid and identified by NMR as 2-acetamido-1,5,6-tri-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (0.09 g, 45.2%).



R_f in 5% MeOH/EtOAc is 0.48. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 – 7.30 (m, 5H, Ph-H), 5.74 (d, *J* = 8.6 Hz, 1H, H-1), 5.47 (d, *J* = 9.0 Hz, 1H, N<u>H</u>COCH$_3$), 4.87 – 4.71 (m, 2H, Ph-CH$_2$), 4.57 – 4.49 (m, 1H), 4.37 (t, *J* = 7.0 Hz, 1H), 4.27 (dd, *J* = 12.4, 1.5 Hz, 1H), 4.03 – 3.85 (m, 1H), 3.73 – 3.61 (m, 1H), 3.58 (d, *J* = 2.2 Hz, 1H), 2.13 (s, 3H, OCOC<u>H</u>$_3$), 2.10 (s, 3H, OCOC<u>H</u>$_3$), 2.09 (s, 3H, NHCOC<u>H</u>$_3$), 1.88 (s, 3H, OCOC<u>H</u>$_3$).

Method 4: **104** (0.17 g, 0.48 mmol) was dissolved in DCM (10 mL) under Ar. TMSOTf (0.09 mL, 0.49 mmol) was added to the reaction mixture and stirred at room temperature for 4 hours. The reaction was quenched with aqueous $NaHCO_3$ and extracted with DCM (3 x 35 mL) and dried over anhydrous Na_2SO_4.The residue was purified by column chromatography in a gradient solvent system from 100% EtOAc to 8% MeOH/EtOAc from which two products were eluted. The major product was deacetylated starting material, 2-acetamido-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (0.05 g, 29.7%). The minor product was the desired 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**38**) (0.02 g, 12.1%) although it was still impure even after purification by column chromatography.

106: R_f in 8% MeOH/EtOAc is 0.36. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 5H, Ph-H), 5.76 (d, *J* = 8.8 Hz, 1H, N<u>H</u>COCH$_3$), 5.03 (s, 1H, H-1), 4.82 (d, *J* = 12.0 Hz, 1H, PhCH$_2$), 4.72 (dd, *J* = 8.7, 2.5 Hz, 1H, H-2), 4.57 (d, *J* = 12.0 Hz, 1H, PhCH$_2$), 4.41 (dt, *J* = 9.8, 2.5 Hz, 2H, H-4), 4.41 (dt, *J* = 9.8, 2.5 Hz, 2H, H-6a), 3.97 (ddd, *J* = 6.8, 2.5, 0.9 Hz, 1H, H-3), 3.82 (dt, *J* = 12.7, 1.3 Hz, 1H, H-6b), 3.72 (tt, *J* = 2.3, 0.9 Hz, 1H, H-5), 2.01 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 128.6 (Ph-CH), 128.2 (Ph-CH), 127.8 (Ph-CH), 104.1 (C-1), 84.9 (C-3), 72.9 (Ph-CH$_2$), 69.7 (C-4), 67.1 (C-6), 64.8 (C-5), 57.6 (C-2).



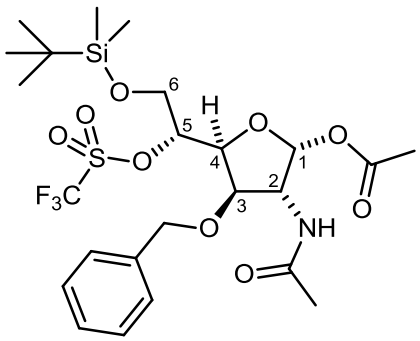
R_f in 8% MeOH/EtOAc is 0.33. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 5H, Ph-H), 6.18 (d, *J* = 4.8 Hz, 1H, H-1), 4.77 (d, *J* = 11.8 Hz, 1H, Ph-CH_2), 4.59 (d, *J* = 11.9 Hz, 1H, PhCH_2), 4.59 (d, *J* = 11.9 Hz, 2H, H-2), 4.19 (d, *J* = 3.1 Hz, 1H), 4.04 (ddd, *J* = 8.3, 5.3, 3.3 Hz, 1H), 3.83 (s, 1H), 3.78 (d, *J* = 3.4 Hz, 1H), 3.67 (dd, *J* = 11.4, 5.4 Hz, 1H), 2.04 (s, 3H, NHCOC$\underline{H}_3$).

Method 5: **104** (0.43 g, 1.20 mmol) was dissolved in DCM (12 mL) under Ar at RT. BF_3Et_2O (0.01 mL, 0.12 mmols) was added to the reaction mixture and stirred at RT for 20 hours. No progress was clear from TLC so more $BF_3.Et_2O$ (0.07 mL, 0.60 mmol) was added. After 8 hours the reaction was worked up in aqueous $NaHCO_3$ and extracted with DCM (3 x 35 mL) and dried over anhydrous Na_2SO_4. No further purification was done. NMR of the crude product showed a mixture of the desired 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**106**) and starting material (**104**) (0.29 g).

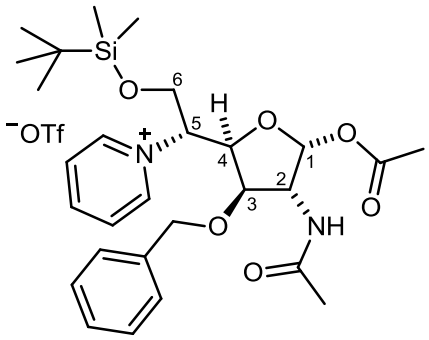
Method 6: **104** (0.05 g, 0.14 mmol) was dissolved in DCM under Ar. $SnCl_4$ (0.019 mL, 0.17 mmol) was added dropwise to the reaction mixture with vigorous stirring which then stirred at RT for 2 h. TLC showed no reaction, therefore equivalent of $SnCl_4$ (0.02 mL, 0.17 mmol) was added and the reaction stirred for a further 18 hours. TLC appeared to indicate that all the starting material had been consumed. The reaction was therefore diluted with DCM, cold aqueous $NaHCO_3$ added and stirred for 30 mins. This was extracted with DCM (3 x 30 mL), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography in a solvent gradient from 100% EtOAc to 10% MeOH in EtOAc. A single spot was eluted and isolated as a yellow solid which was identified as a mixture of the desired 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**106**) and starting material (**104**) (0.02 g).

Method 7: **105** (0.05 g, 0.11 mmol) was dissolved in DCM (8 mL) under Ar. TMSOTf (0.004 mL, 0.02 mmol) was added to the reaction and stirred at room temperature for 48 hours until the reaction appeared complete on TLC. It was quenched with aqueous $NaHCO_3$ and extracted with DCM (3 x 20 mL), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography in a solvent system from 100% EtOAc to 8% MeOH in EtOAc. Three separate products were collected. Recovered starting material (0.02 g, 43.3%), the desired 1,6-anhydro-2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**106**) (0.008 g, 26.0%) and 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**104**) the TBDMS protecting group having been removed from O-6 (0.008 g, 22.1%). R_f's in 5% MeOH/EtOAc are 0.63, 0.37 and 0.27 respectively.

Attempted synthesis of 2-Acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-5-*O*-triflate-α-D-glucofuranose[59] (107)



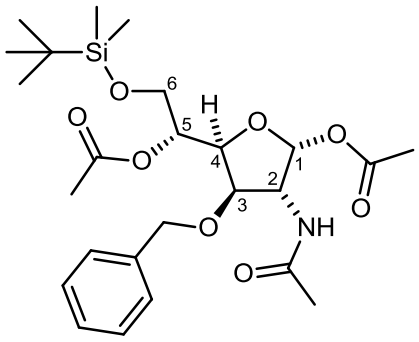
Method 1: General method, see Table 4 for details. **105** was dissolved in anhydrous pyridine under Ar. Tf_2O was added to the reaction mixture and stirred at a variety of temperatures. Once TLC indicated that all the starting material had been consumed the reaction was quenched with H_2O and extracted with EtOAc. The organic layer was washed sequentially with dilute HCl, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated and dried under reduced pressure. No further purification was carried out due to the sensitivity of triflates on silica gel, as observed on TLC while monitoring the reaction. NMR of the crude material showed a single carbohydrate product which was identified as 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-5-*O*-pyridine-α-D-glucofuranose (**110**) was afforded as a light brown syrup (0.15 g, 97.6%).



R_f in 80% EtOAc/hexane is 0.12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (dd, *J* = 6.8, 1.2 Hz, 2H, py), 8.36 (tt, *J* = 7.8, 1.3 Hz, 1H, py), 7.88 (dd, *J* = 7.7, 6.9 Hz, 2H, py), 7.37 – 7.31 (m, 5H, Ph-CH), 7.30 (d, *J* = 1.9 Hz, 2H, N<u>H</u>$COCH_3$), 6.33 (d, *J* = 5.6 Hz, 1H, H-1), 5.14 (dd, *J* = 8.0, 4.0 Hz, 1H, H-4), 4.99 – 4.90 (m, 1H, H-5), 4.85 (ddd, *J* = 8.8, 5.6, 1.6 Hz, 1H, H-2), 4.77 (d, *J* = 11.1 Hz, 1H, Ph-CH_2), 4.54 (dd, *J* = 11.6, 3.6 Hz, 1H, Ph-CH_2), 4.23 (dd, *J* = 4.0, 1.7 Hz, 1H, H-3), 4.05 (dd, *J* = 11.5, 9.8 Hz, 1H, H-6a), 3.91 (dd, *J* = 11.5, 4.0 Hz, 1H, H-6b), 2.10 (s, 3H, OCOC<u>H</u>$_3$), 2.07 (s, 3H, NHCOC<u>H</u>$_3$), 0.65 (s, 9H,OSi$(CH_3)_2$C(C<u>H</u>$_3)_3$), -0.08 (s, 3H, OSi(C<u>H</u>$_3)_2$C$(CH_3)_3$), -0.19 (s, 3H, OSi(C<u>H</u>$_3)_2$C$(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8 (NH<u>C</u>OCH_3), 169.9 (O<u>C</u>OCH_3), 145.6 (py), 144.9 (py), 136.6 (Ph-C), 129.0 (Ph-CH), 128.8 (Ph-CH), 128.6 (Ph-CH), 127.6 (py), 122.3 (q, *J* = 379.6, 296.7 Hz, CF_3), 94.2 (C-1), 82.4 (C-3), 76.9 (C-4), 74.9 (C-5), 71.8 (Ph-CH_2), 61.6 (C-6), 56.3 (C-2), 25.5 (OSi$(CH_3)_2$C(<u>C</u>$H_3)_3$), 22.9 (NHCO<u>C</u>H_3), 21.0 (OCO<u>C</u>H_3), 17.9 (OSi$(CH_3)_2$<u>C</u>$(CH_3)_3$), -5.6 (OSi(<u>C</u>$H_3)_2$C$(CH_3)_3$), -5.8 (OSi(<u>C</u>$H_3)_2$C$(CH_3)_3$). ^{19}F NMR (377 MHz, $CDCl_3$) δ -78.2 (s, 3F, OSO_2CF_3). HRMS calcd for $C_{28}H_{41}N_2O_6Si$: 529.2736; found: 529.2735 [*M*+H^+].

Method 2: The method described above was attempted using different solvents namely; a catalytic amount of DMAP in DCM, 2,6-lutidine, NMM and THF. The reactions in 2,6-lutidine and NMM did not progress and all the starting material was recovered. The DCM and THF reactions recovered no carbohydrate material, the starting material had degraded completely.

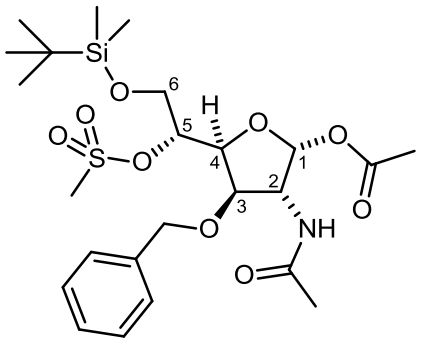
2-Acetamido-1,5-di-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucofuranose[36] (109)



Silyl ether **105** (0.10 g, 0.21 mmol) was dissolved in anhydrous pyridine (3 mL) under Ar. Ac_2O (0.03 mL, 0.33 mmol) was added to the reaction mixture and stirred at RT for 24 h. No reaction was occurring therefore a further portion of Ac_2O (0.09 mL, 0.98 mmol) was added to the reaction and the temperature was increased to 60°C for 18 h. The reaction was washed with water and extracted with DCM (3 x 30 mL), dried over Na_2SO_4 and concentrated. NMR of the crude product indicated that no further purification was needed and confirmed that the dark yellow syrup was the desired 2-acetamido-1,5-di-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucofuranose (**109**) (0.099 g, 90.8%).

R_f in 100% EtOAc is 0.54. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 5H, Ph-H), 6.39 (d, *J* = 5.6 Hz, 1H, H-1), 5.74 (d, *J* = 7.5 Hz, 1H, N<u>H</u>COCH$_3$), 5.22 – 5.10 (m, 1H, H-5), 4.78 (d, *J* = 12.0 Hz, 1H, Ph-CH$_2$), 4.72 – 4.66 (m, 1H, H-2), 4.62 (d, *J* = 12.1 Hz, 1H, Ph-CH$_2$), 4.40 (dd, *J* = 7.5, 4.9 Hz, 1H, H-4), 3.96 (dd, *J* = 5.0, 2.8 Hz, 1H, H-3), 3.92 (d, *J* = 2.3 Hz, 1H, H-6a), 3.81 (dd, *J* = 11.5, 4.3 Hz, 1H, H-6b), 2.10 (s, 3H, 1-OCOC<u>H</u>$_3$), 2.03 (s, 3H, NHCOC<u>H</u>$_3$), 1.91 (s, 3H, 5-OCOC<u>H</u>$_3$), 0.87 (s, 9H,OSi(CH$_3$)$_2$C(C<u>H</u>$_3$)$_3$), 0.01 (d, *J* = 1.2 Hz, 6H, OSi(C<u>H</u>$_3$)$_2$C(CH$_3$)$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9 (5-O<u>C</u>OCH$_3$), 169.5 (NH<u>C</u>OCH$_3$), 168.8 (1-O<u>C</u>OCH$_3$), 137.6 (Ph-C), 128.5 (Ph-CH), 128.4 (Ph-CH), 127.9 (Ph-CH), 94.6 (C-1), 81.6 (C-3), 77.4 (C-4), 71.9 (C-5), 71.6 (Ph-CH$_2$), 61.6 (C-6), 57.0 (C-2), 25.9 (OSi(CH$_3$)$_2$C(<u>C</u>H$_3$)$_3$), 23.4 (NHCO<u>C</u>H$_3$), 21.1 (1-OCO<u>C</u>H$_3$), 21.1 (5-OCO<u>C</u>H$_3$), 18.4 (OSi(CH$_3$)$_2$<u>C</u>(CH$_3$)$_3$), -5.3 (OSi(<u>C</u>H$_3$)$_2$C(CH$_3$)$_3$). HRMS calcd for $C_{25}H_{39}NO_8Si$: 509.2445; found: 450.2297 [*M*-OAc].

2-Acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-5-*O*-mesyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucofuranose[36] (111)

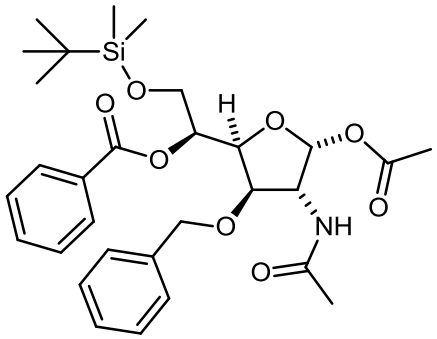


Silyl ether **105** (0.33 g, 0.70 mmol) was dissolved in anhydrous pyridine (3 mL) under Ar and cooled to -10°C. MsCl (0.11 mL, 1.40 mmol) was added to 3 mL anhydrous pyridine and this mixture added to the carbohydrate mixture dropwise. The reaction was stirred for 4 hours while the ice/acetone bath warmed to RT. The reaction was cooled to -10°C and another equivalent of MsCl added and stirred for a further 4 h. The reaction was then diluted with EtOAc and the solid filtered off through filter paper. The filtrate was then washed sequentially with dilute HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. This residue was purified by flash chromatography in a gradient solvent system from 50 to 100% EtOAc in Hexane. 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-5-*O*-mesyl-α-D-glucofuranose (**111**) (0.23 g, 60.5%) was afforded as a light yellow syrup.

R_f in 80% EtOAc/Hexane is 0.33. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H, Ph-H), 6.36 (d, *J* = 5.6 Hz, 1H, H-1), 5.76 (d, *J* = 7.9 Hz, 1H, N<u>H</u>COCH$_3$), 4.94 (ddd, *J* = 6.7, 4.5, 2.2 Hz, 1H, H-5), 4.84 (d, *J* = 11.7 Hz, 1H, Ph-C<u>H</u>$_2$), 4.71 (ddd, *J* = 8.1, 5.6, 3.4 Hz, 1H, H-2), 4.62 (d, *J* = 11.7 Hz, 1H, Ph-C<u>H</u>$_2$), 4.52 – 4.45 (m, 1H, H-4), 4.14 – 4.07 (m, 1H, H-3), 4.04 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6a), 3.88 (dd, *J* = 11.9, 6.6 Hz, 1H, H-6b), 3.01 (s, 3H, Ms-CH$_3$), 2.11 (s, 3H, OCOC<u>H</u>$_3$), 2.02 (s, 3H, NHCOC<u>H</u>$_3$), 0.88 (s, 9H, OSi(CH$_3$)$_2$C(C<u>H</u>$_3$)$_3$), 0.03 (d, *J* = 3.5 Hz, 6H, OSi(C<u>H</u>$_3$)$_2$C(CH$_3$)$_3$).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.5 (NH<u>C</u>OCH$_3$), 168.9 (O<u>C</u>OCH$_3$), 137.4 (Ph-C), 128.6 (Ph-CH), 128.2 (Ph-CH), 128.1 (Ph-CH), 94.2 (C-1), 82.1 (C-3), 81.6 (C-5), 79.5 (C-4), 72.2 (Ph-CH$_2$), 62.4 (C-6), 57.0 (C-2), 38.6 (Ms-<u>C</u>H$_3$), 25.9

(OSi(CH_3)$_2$C($\underline{C}H_3$)$_3$), 23.4 (NHCO$\underline{C}H_3$), 21.1 (OCO$\underline{C}H_3$), -5.3 (OSi($\underline{C}H_3$)$_2$C(CH_3)$_3$). HRMS calcd for $C_{24}H_{39}NO_9SSi$: 545.2115; found: 546.2212 [*M*+H].

Attempted synthesis of 2-Acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-5-*O*-benzoyl-6-*O*-*t*-butyldimethylsilyl-β-L-idofuranose (108)



Method 1: **105** (0.09 g, 0.19 mmol) was dissolved in DMF (3 mL) and pyridine (0.03 mL, 0.37 mmol) under Ar. The reaction mixture was cooled to -20°C and Tf_2O (0.125 mL, 0.742 mmol) added dropwise over 30 minutes. The reaction was then stirred for two hours while allowing the ice/acetone bath to warm to room temperature. Once all the starting material had been consumed (as monitored by TLC), NaOBz (0.05 g, 0.37 mmol) was added to the reaction. It was stirred at RT for a further 6 hours. The reaction was diluted with EtOAc and washed sequentially with aqueous $NaHCO_3$, 1M HCl and brine. The EtOAc layer was dried over anhydrous Na_2SO_4. This residue was purified by flash chromatography in a gradient solvent system from 40 to 100% EtOAc in Hexane. A single product was eluted from the column with an R_f in 100% EtOAc of 0.39, 2-acetamido-*O*-acetyl-2-deoxy-3-*O*-benzyl-α-D-glucofuranose (**104**) (0.05 g, 78%).

Method 2: **105** (0.10 g, 0.22 mmol) was dissolved in DCM (6 mL) and pyridine (0.06 mL, 0.44 mmol) under Ar. The reaction mixture was cooled to -20°C and Tf_2O (0.15 mL, 0.88 mmol) added dropwise over 20 minutes. The reaction was then stirred for 2 hours at that temperature. The DCM was removed on the schlenk line and DMF (1.5 mL) was added to round bottom flask. NaOBz (0.03 g, 0.22 mmol) in DMF (1 mL) was added to the reaction and stirred for 4 hours at RT. The reaction was diluted with EtOAC, washed with water and brine, dried over Na_2SO_4 and concentrated. NMR showed that no carbohydrate

material remained fragments of TBDMS and some aromatic peaks from the benzyl group were seen indicating that the compound had fragmented. This reaction was repeated to include a work up between the steps. After the triflation the reaction mixture was washed sequentially with aqueous $NaHCO_3$, 1M HCl and brine and extracted with DCM (3 x 20 mL). This was dried over anhydrous Na_2SO_4, concentrated and the residue dissolved in DMF (2.5 mL). The second part of the reaction was carried out in the same way. The result was the same as the first attempt.

Method 3: **110** (0.16 g, 0.31 mmol) and NaOBz (0.17 mg, 1.14 mmol) were dissolved in anhydrous pyridine under Ar. The reaction mixture was heated at 90°C for 24 hours. The reaction was cooled, washed with water and the aqueous layer extracted with Et_2O (3 x 25 mL). The combined organic layers were back extracted with brine, dried over anhydrous Na_2SO_4 and concentrated. No further purification was done. NMR of the crude material showed complete degradation of the carbohydrate material.

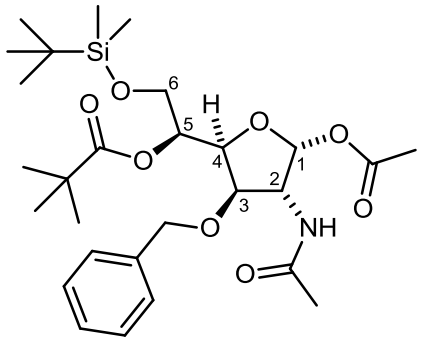
Method 4: **111** (0.08 g, 0.17 mmol) and NaOBz (0.08 g, 0.56 mmol) were dissolved in anhydrous pyridine (4 mL) under Ar. The reaction was stirred at RT 18 hours. The reaction was then diluted with EtOAc and the excess NaOBz filtered off through filter paper. The filtrate was washed with water and the aqueous layer extracted with EtOAc (3 x 25 mL). The organic layers were then back extracted with brine, dried over anhydrous Na_2SO_4 and concentrated. NMR of the crude material showed only starting material was recovered as a yellow syrup (0.06 g, 75%), none of the desired product had formed. R_f in 80% EtOAc/Hexane is 0.31. This reaction was repeated at 100°C with the same result.

Method 5: **111** (0.11 g, 0.19 mmol) and NaOBz (0.11 g, 0.74 mmol) were dissolved in freshly distilled acetone (5 mL) under Ar. The reaction was refluxed at 60°C for 40 h after which TLC indicated no reaction had taken place. The reaction was then diluted with EtOAc and the excess NaOBz filtered off through filter paper. The collected filtrated was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. NMR of the crude material showed that only starting material was recovered (0.10 g, 97.1%) R_f in 80% EtOAc/Hexane is 0.39.

Method 6: **111** (0.10 g, 0.18 mmol) and NaOBz (0.10 g, 0.69 mmol) were dissolved in anhydrous pyridine (3 mL) under Ar. The reaction was heated to 60°C for 14 h after which TLC indicated no reaction was taking place. The temperature was then increased to 90°C and the reaction stirred for a further 24 h. The reaction was then diluted with EtOAc and the solid filtered off through celite. It was then washed sequentially with dilute HCl, saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure.NMR of the crude material showed that only starting material was recovered (0.09 g, 91.4%).R_f in 80% EtOAc/Hexane is 0.39.

Method 7: Benzoic acid (0.13 g, 1.02 mmol) was dissolved in THF (2 mL) under nitrogen and cooled to -78°C. n-Butyllithium (1.6M, 0.88 mL, 1.22 mmol) was added dropwise to the reaction mixture and stirred for 30 mins while warming to 0°C. The reaction was cooled to -78°C again and **111** (0.13 g, 0.231 mmol) in THF (2 mL) was added and the reaction stirred at this temperature for 1 hour. TLC showed no reaction was taking place at that temperature and the reaction was warmed to 0°C for 4 hours. The reaction was not progressing at this temperature and it was warmed to RT for a further 12 hours. The reaction was quenched with cold NH_4Cl and extracted with EtOAc (3 x 25 mL). The combined EtOAc layers were washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. This residue was purified on a preparative TLC plate and three UV active bands collected. It was clear from their NMR spectra that these samples contained no carbohydrate material, indicating that the compound had degraded.

Attempted synthesis of 2-Acetamido-*O*-acetyl-2-deoxy-3-*O*-pivaloyl-5-*O*-benzoyl-6-*O*-*t*-butyldimethylsilyl-β-L-glucofuranose[49]



111 (0.12 g, 0.23 mmol) and NaOPiv (0.12 g, 0.99 mmol) were dissolved in anhydrous DMF (4 mL) under Ar. The reaction was stirred at RT for 4 hours but TLC showed that no reaction was taking place to it was heated to 100°C and stirred at this temperature for 14 hours. The reaction was then cooled to room temperature and diluted with EtOAc, washed with water and the aqueous layer extracted with EtOAc (3 x 25 mL). The combined organic layers were then back extracted with brine, dried over Na_2SO_4 and concentrated. This residue was purified by flash chromatography in a gradient solvent system from 35 to 100% EtOAc in Hexane. Only one compound was eluted from the column but it was clear from the NMR that it contained no carbohydrate material, indicating that the compound had degraded.

CHAPTER 6

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CHAPTER 7

Appendix

7.1 Tabulated Details for Experimental Series

Table 2: Reaction conditions and reagents for the attempted bromination and rearrangement of **70**

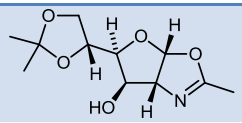
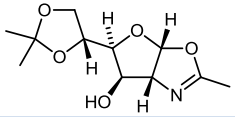
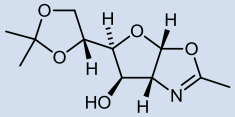
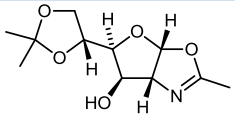
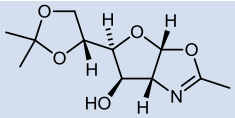
	Starting Material	Scale (mg)	Reagent(s)	Equivalents	Solvent (mL)	Temperature (°C)	Time (h)	Work Up Conditions	Product(s)
1		4985	PPh_3 NBS	2.4 2.4	CH_3CN	80	17	Diluted with Et_2O Filtered through celite	Degraded
2		224	PPh_3 NBS	2.2 2.2	CH_3CN	80	3	Diluted with Et_2O Filtered through celite	Degraded
3		243	None	-	CH_3CN	RT	48	-	Starting material and degraded material
4		211	PPh_3 NBS	2.0 2.0	$PhCH_3$	110	2	Diluted with Et_2O Filtered through celite	Degraded
5		197	PPh_3 NBS	2.0 2.0	DMF	100	2.5	Diluted with Et_2O Filtered through celite	Degraded

Table 3: Reaction conditions and reagents for the attempted 1,6-anhydro ring formation

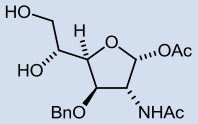
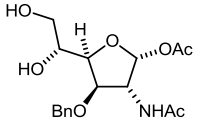
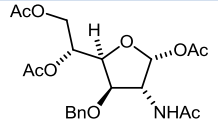
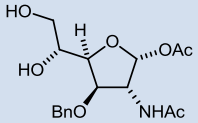
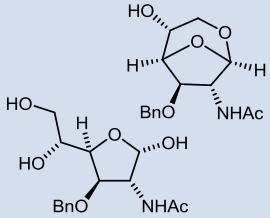
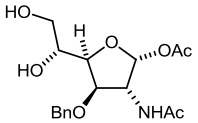
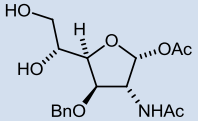
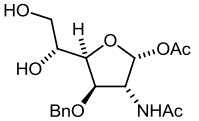
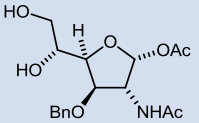
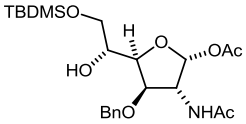
	Starting Material	Scale (mg)	Reagent(s)	Equivalents	Solvent (mL)	Temperature (°C)	Time (h)	Work Up Conditions	Product(s)
1		255	DMAP	2.2	DCM 8	RT	20	AcOH (neutralize DMAP) H_2O and DCM	Starting Material
2		160	NaH Ac_2O	2 4	THF 10	RT	18 17	Brine Extract with EtOAC	
3		206	TMSOTf	0.2 0.8	DCM 10	RT	16 6	$NaHCO_3$ (sat.) Extract with DCM	
4		173	TMSOTf	1	DCM 10	RT	4	$NaHCO_3$ (sat.) Extract with DCM	The same product mixture as reaction 3
5		425	$BF_3.Et_2O$	0.1 0.5	DCM 12	RT	20 8	$NaHCO_3$ (sat.) Extract with DCM	Mixture of starting material and product **106**
7		67	$SnCl_4$	1.2	DCM 4	RT	2	$NaHCO_3$ (sat.) Extract with DCM	Mixture of starting material and product **106**
6		49	$SnCl_4$	1.2 1.2	DCM 3	RT	2 3.5	$NaHCO_3$ (sat.) Extract with DCM	Mixture of starting material and product **106**
8		49	TMSOTf	0.2	DCM 8	RT	48	$NaHCO_3$ (sat.) Extract with DCM	Mixture of starting material, **104** and **106**

Table 4: Reaction conditions and reagents for the attempted triflation of **105**

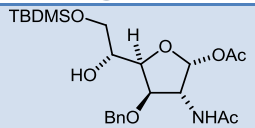
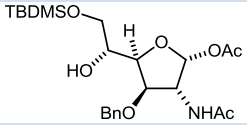
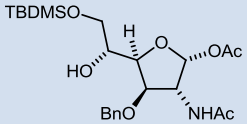
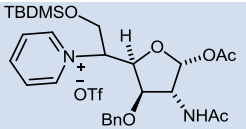
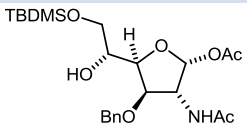
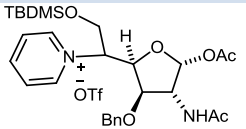
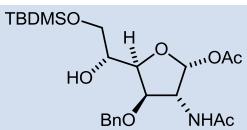
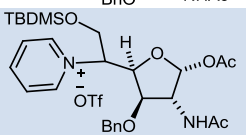
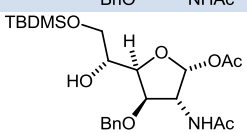
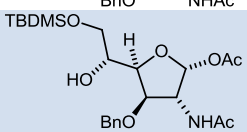
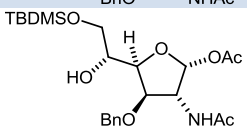
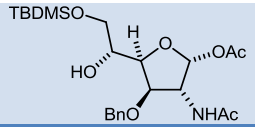
	Starting Material	Scale (mg)	Reagent(s)	Equivalents	Solvent(mL)	Temperature (°C)	Time (h)	Work Up Conditions	Product(s)
1		96	Tf_2O	1.2	Pyridine 2	0	0.5	$NaHCO_3$ (sat.) Extract with DCM	Mostly starting material Minor product
2		100	Tf_2O	1.6	Pyridine 2.5	RT	12	$NaHCO_3$ (sat.) Extract with DCM	Mostly starting material Minor product
3		105	Tf_2O	4	Pyridine 3	60	24	Water Extract with EtOAc	
4		101	Tf_2O	3	Pyridine 2.5	60	20	1M HCl $NaHCO_3$ (sat.) Brine	
5		102	Tf_2O	3	Pyridine 5	RT	16	2M HCl $NaHCO_3$ (sat.) Brine	
6		120	Tf_2O DMAP	3 2	DCM 8	RT	20	1M HCl $NaHCO_3$ (sat.) Brine	Degradation No carbohydrate residue in NMR
7		82	Tf_2O	4	2,6-Lutidine 2.5	0 to RT	20	$NaHCO_3$ (sat.) 1M HCl Brine	Starting material
8		89	Tf_2O	4	NMM 3	RT	20	$NaHCO_3$ (sat.) 1M HCl Brine	Starting material
9		107	Tf_2O	3	THF 5	RT	20	$NaHCO_3$ (sat.) 1M HCl Brine	Degradation No carbohydrate residue in NMR

Table 5: Reaction conditions and reagents for the attempted inversion of C-5

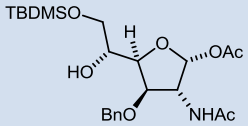
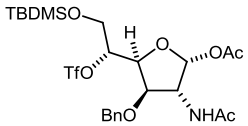
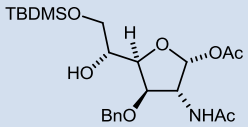
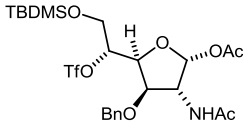
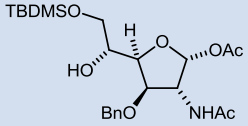
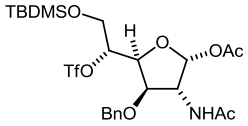
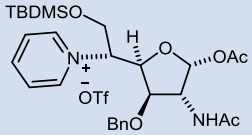
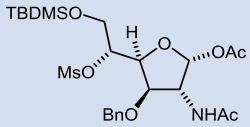
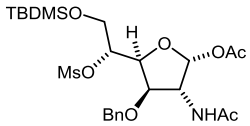
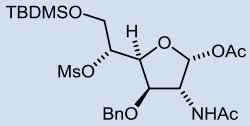
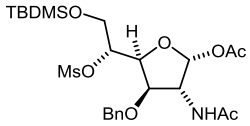
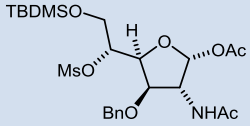
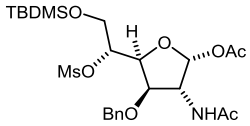
	Starting Material	Scale (mg)	Reagent(s)	Equivalents	Solvent (mL)	Temperature (°C)	Time (h)	Work Up Conditions	Product(s)
1A		87	Tf_2O pyridine	4 2	DMF 3	-10	2	None	Carried over to next step
1B		-	NaOBz	2	DMF 3	RT	6	Water Brine Extract with EtOAc	Final product appears to have lost TBDMS on O-6
2A		103	Tf_2O pyridine	4 2	DCM 3	-19	4	Remove DCM	Carried over to next step
2B		-	NaOBz	2	DMF 2.5	RT	4	Water Brine Extract with DCM	Degradation No carbohydrate residue in NMR
3A		103	Tf_2O pyridine	4 2	DCM 3	-19	4	$NaHCO_3$ (sat.) 1M HCl Brine Extract with DCM	Carried over to next step
3B		-	NaOBz	2	DMF 2.5	RT	4	Water Brine Extract with DCM	Degradation No carbohydrate residue in NMR
4		164	NaOBz	5	DMF 4	90	24	Water Extract with Et_2O Back extract, brine	Degradation No carbohydrate residue in NMR

Table 6: Reaction conditions and reagents for the reattempted inversion of C-5

	Starting Material	Scale (mg)	Reagent(s)	Equivalents	Solvent (mL)	Temperature (°C)	Time (h)	Work Up Conditions	Product(s)
1		80	NaOBz	3.2	DMF 4	RT	18	Water Brine	Starting Material
2		60	NaOBz	3.8	DMF 3	100	18	Water Brine	Starting Material
3		105	NaOBz	3.8	Acetone 5	60	40	Water Brine	Starting Material
4		100	NaOBz	3.8	Pyridine 3	60 90	14 24	1M HCl $NaHCO_3$ (sat.) Brine	Starting Material
5		126	LiOBz	4.4	THF 4	-78 0 RT	1 4 14	NH_4Cl $NaHCO_3$ (sat.) Brine	Degradation
6		123	NaOPiv	4.4	DMF 3	RT 100	4 14	Water Brine	Degradation

7.2 NMR Spectra and HRMS for all compounds

A full set of spectra are available as supplementary material on request.