Towards the Synthesis of the Unusual Monosaccharides Found in the *Shigella sonnei* O-Antigen and Analysis of *Shigella flexneri* 2a Glycoconjugate Vaccine Samples

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Declaration

I declare that "Towards the Synthesis of the Unusual Monosaccharides Found in the *Shigella sonnei* O-Antigen and Analysis of *Shigella flexneri* 2a Glycoconjugate Vaccine Samples" is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references.

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Abstract

Shigellosis (bacillary dysentery) is a severe inflammatory diarrhoeal disease in humans caused by the Gram-negative bacteria belonging to the *Shigella* species. Despite over 60 years of vaccine research, no licensed vaccine to prevent shigellosis is commercially available. Bioconjugate vaccines based on the O-antigen against various *Shigella* serotypes are under development. *Shigella sonnei* and *Shigella flexneri* 2a are the most prevalent serotypes in industrialised and developed countries respectively and is the subject of this study.

This project involves the design and evaluation of alternative synthetic routes to derivatives of 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose (FucNAc4N/AAT) and 2-acetamido-2-deoxy-α-L-altruronic acid (AltNAcA), the two unusual monosaccharides found in the repeating unit of the *Shigella sonnei* O-antigen. Since these sugars are not commercially available, synthetic derivatives are required as authentic standards for the analysis of the bioconjugate.

Various routes to the FucNAc4N derivative were investigated and evaluated. Routes proceeding either through 1,6-anhydro-D-glucose or cyclohexyl-2-acetamido-1-thioglucoside were shown to have potential, but ultimately both were rejected on the basis of inefficient conversions in the early stages of the synthetic sequence. However, important insights were gained into the crucial challenge of differentiating *O*-3 and *O*-4, common to any approach involving starting materials with the D-*gluco* configuration. This led to preparation in good yield of phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-*β*-D-glucopyranoside as a key oxazolidinone-protected intermediate, which allowed for successful preparation of a FucNAc4N derivative in the form of a 4-azido-β-thioglycoside. This was achieved in 10 steps from the commercially available 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in an overall yield of 17%.

Synthesis of an AltNAcA derivative was initially investigated *via* a sequence starting from a glucofuranurono-3,6-lactone. This involved initial inversion at C-5 followed by opening of the lactone and migration of the substituent at *O*-5 to *O*-3, to form an idofuranuronate which, however, could not be readily converted to the required pyranose form. A more successful route utilized a 6-iodo-2,3-oxazolidinone derivative of D-glucose, prepared as a key intermediate in the synthesis of FucNAc4N. The crucial epimerization at C-5 was attempted through initial formation of the 5-ene, followed by a hydroboration/oxidation, but this led exclusively to the D- rather than the L-sugar. Computer modelling and literature precedent suggested that the anomeric configuration strongly influenced the face selectivity of the hydroboration step. An α-analogue of the 6-iodo-2,3-oxazolidinone derivative was therefore prepared *via* an efficient Lewis acid catalysed anomerization of a β-thioglucoside. However attempts to carry out a base-

mediated elimination to the corresponding 5-enopyranoside were not successful, giving rise instead to a product in which the oxazolidinone had been cleaved followed by intramolecular substitution of the 6-iodide to form a 3,6-anhydro derivative. On the basis of these results and observations, an alternative synthetic route to AltNAcA has been proposed, which incorporates early formation of an α-glycoside and removal of the useful 2,3-oxazolidinone protecting group, thus setting the substrate up for effective elimination followed by selective hydroboration from the less hindered β-face to give the L-sugar.

This study also incorporates a spectroscopic analysis of *Shigella flexneri* 2a glycoconjugate and glycopeptide samples. A full set of nuclear magnetic resonance (NMR) spectra were recorded and analysed, resulting in the unambiguous determination of the structure and integrity of the O-antigen saccharide component.

To my nomadic parents, Calum, Meghan and Heinrich.

May the adventure continue.

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And lastly JC, for His grace and strength.

Abbreviations

9-BBN	9-borabicyclo[3.3.1]nonane
Ac	acetyl
ACCN	1,1′-azobis(cyclohexanecarbonitrile)
ACN	acetonitrile
ADH	adipic acid dihydrazide
AltNAcA	2-acetamido-2-deoxy-α-L-altruronic acid
An	anisoyl
aq.	aqueous
Ar	aryl
BIAB	[bis(acetoxy)iodo] benzene
Bn	benzyl
BnBr	benzyl bromide
Boc	*tert*-butoxycarbonyl
bs	broad singlet
bt	broad triplet
Bz	benzoyl
Cbz	carboxybenzyl
CD	circular dichroism
COSY	correlation spectroscopy
CPS	capsular polysaccharide
CSA	10-camphorsulfonic acid
Cycl	cyclohexyl
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhancement by polarization transfer
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DT	diphtheria toxoid
E_1	unimolecular elimination
E_2	bimolecular elimination
EE	enteric enteropathy
ELISA	enzyme linked immunosorbent assay
eq.	molar equivalents

ESI	electrospray ionisation
FDA	US Food and Drug Administration
FucNAc4N	2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose
g	gram(s)
Gal	galactose
GalNAc	*N*-acetyl galactosamine
Glc	glucose
GlcNAc	*N*-acetyl glucosamine
HIV/AIDS	human immunodeficiency virus/acquired immune deficiency syndrome
HMBC	heteronuclear multiple bond correlation
HPAEC	high performance anion exchange chromatography
HPLC	high performance liquid chromatography
HPSEC	high performance size exclusion chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	hertz
IgG	immunoglobulin G
IR	infrared spectroscopy
J	coupling constant
LAH	lithium aluminium hydride
L-AltNAcA	2-acetamido-2-deoxy-α-L-altropyranuronic acid
LPS	lipopolysaccharide
L-Rha	L-rhamnose
m	multiplet
M	molarity (moles per cubic decimeter)
M^+	molecular ion
MALLS	multi angle laser light scattering
Me	methyl
mg	milligrams
MHz	mega hertz
min	minute(s)
mL	millilitre(s)
mmol	millimole(s)
mp	melting point
PMP	*p*-methoxyphenyl
Ms	mesyl
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
O-PS	O-specific polysaccharide
OST	oligosaccharyltransferase

p	para
PAD	pulsed-amperometric detection
PG	protecting group
Ph	phenyl
Phth	phthaloyl
Piv	pivaloyl
ppm	parts per million
PTFACI	*N*-phenyltrifluoroacetimidoyl chloride
q	quartet
r.t.	room temperature
rEPA	recombinant genetically detoxified exotoxin A from *Pseudomonas aeruginosa*
RI	refractive index
rxn	reaction
s	singlet
SM	starting material
S_N2	bimolecular nucleophilic substitution
S_NAc	nucleophilic acyl substitution
t	triplet
TBAF	tetra-n-butylammonium fluoride
TBAI	tetra-n-butylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxy
tert	tertiary
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TIPS	triisopropylsilyl ether
TMS	trimethylsilyl
Ts	*p*-toluenesulfonyl (tosyl)
TT	tetanus toxoid
UV	ultraviolet
WHO	World Health Organization
wt	weight
ZPS	zwitterionic polysaccharide
δ	chemical shift in ppm

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Chapter 1: Introduction

1.1 Enteric diseases

Enteric diseases are a major cause of death worldwide.[1] Diarrhoea is defined as the passing of loose or watery stool more than three times per day and if left untreated, leads to dehydration and can eventually result in death.[2] Dysentery is characterised by the addition of abdominal cramps, blood in the stool and fever, and is associated with a higher mortality rate than diarrhoea.[3,4] Diarrhoea and dysentery are caused by bacterial, viral or protozoan pathogens and of these Rotavirus causes the most cases, with other major pathogens including *Vibrio cholera,* a variety of *Salmonella enterica* serotypes, *Campylobacter* (especially *Campylobacter jejuni*), a variety of enteropathogenic *Escherichia coli* serotypes, and *Shigella*, which accounts for 5 – 15% of all cases.[5,6]

1.1.1 Shigellosis

Shigellosis (bacillary dysentery) is a severe inflammatory diarrhoeal disease in humans confined to the lower intestine and is caused by the Gram-negative, non-motile, rod-shaped, anaerobic bacteria belonging to the *Shigella* species.[4,7,8] For the infected person, this results in further developmental problems, including stunted growth and fitness, and cognitive impairments.[9–13]

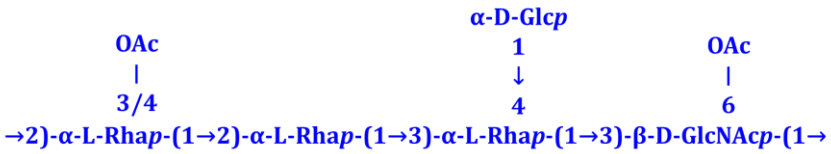
Infection occurs after the ingestion of contaminated food or water, which may be a result of transmission through the common housefly, or passed from human-to-human *via* the oral-faecal route or through personal contact.[3,14–16] It is a low-inoculum contagion requiring between 10-1000 microorganisms to cause infection in adults.[15,17] Shigellosis is common in confined populations, especially where there are poor hygiene practises and a lack of sanitation.[14,15,18] The most effective methods for controlling shigellosis are the promotion of good hygiene practices, provision of ample and safe drinking water, and effective faeces disposal.[2] But in the developing world, these public health measures are unfortunately still long term goals. Although oral rehydration therapy has hugely reduced the number of deaths from diarrhoeal dehydration in developing countries since their introduction in the 1970's,[6,19–22] it has been less effective against the mucosally invasive *Shigella*.[23] Antibiotic treatment of diarrhoeal diseases has been successful, however, in the era of multi-drug resistance, various *Shigella* strains have become impervious to treatment with fluoroquinolones (ciprofloxacin), sulfonamides, tetracyclines, ampicillin, and the trimethoprim/sulfamethoxazole combination, which were successful as first line therapies in the past.[6,24–28] *Shigella* poses a huge problem for current and future treatment, especially in developing countries where health care resources are limited.[6,24,25,29–31]

The greatest group at risk of shigellosis infection are children under the age of five where the symptoms are far more severe compared to adults.[32] In addition, travelers, people in areas of political upheaval, areas affected by natural disaster and deployed military personnel are also at risk.[6,33–38] A multitude of studies have shown that people infected with HIV/AIDS or other immunosuppressed states are also at higher risk,[39–48] and may develop persistent or recurrent *Shigella* infections.[6,46] People suffering from malnutrition, diabetes and malignancy have also been found to be at an increased risk of infection.[2,29,41,42,47,49,50] Furthermore, in both developing and industrialised countries, shigellosis persists in places where there is communal living or where personal hygiene can be sub-optimal, such as daycare centres, old age homes and among institutionalised patients.[51–54]

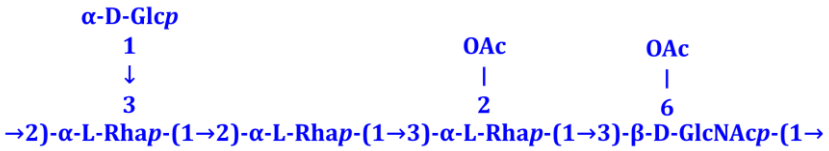
In 1999 the World Health Organisation (WHO) reviewed the literature published on *Shigella* infection between 1966 and 1997 and estimated the total number of *Shigella* episodes worldwide to be 164.7 million each year with 1.5 million cases in industrialised countries and 163.2 million cases in developing countries, resulting in an estimated 5 million hospitalisations and 1.1 million deaths. Of the total 163.2 million cases of shigellosis in developing countries approximately 113.2 million of these cases occur in children under the age 5 (69% of all episodes).[6] Although more recent reviews estimate the number of episodes and mortalities to be lower.[55]

There are four main serogroups of *Shigella* which have been grouped according to their "distinctive" antigens and are currently further subdivided into various serotypes: Serogroup A (*S. dysenteriae*, 15 serotypes); Serogroup B (*S. flexneri*, 17 serotypes); Serogroup C (*S. boydii*, 20 serotypes); and Serogroup D (*S. sonnei*, 1 serotype).[6,55–57] Serogrouping is based on the production of antibodies, generally in animals, when challenged with a particular antigen.[58,59] Antigenic structures within the same serogroup generally have a similar repeating unit.[58,59] The five most prevalent serotypes are *S. flexneri 2a, 3a, 6, S. dystenteriae type 1 and S. sonnei* (**Figure 1.1**).[23,30,60]

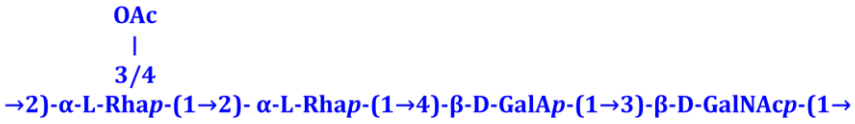
***S. flexneri* 2a**



***S. flexneri* 3a**



***S. flexneri* 6**



***S. dysenteriae* type 1** →3)-α-L-Rhap-(1→3)-α-L-Rhap-(1→2)-α-D-Galp-(1→3)-α-D-GlcNAcp-(1→

S. sonnei →4)-α-L-AltNAcA-(1→3)-β-D-FucNAc4N-(1→

Figure 1.1: Repeating units of the 5 most prevalent *Shigella* serotypes. L-Rha = L-rhamnose, D-GlcNAc = *N*-acetyl-D-glucosamine, D-GalA = D-galacturonic acid, L-AltNAcA = 2-acetamido-2-deoxy-α-L-altruronic acid, FucNAc4N = 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose.

Of these five serotypes, *S. sonnei* is composed of the two uncommon sugars – the 2-acetamido-2-deoxy-α-L-altruronic acid (AltNAcA) and 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose (FucNAc4N) both of which are not commercially available. The AltNAcA unit is found only in the *S. sonnei* repeating unit,[61] while FucNAc4N (also referred to as AAT) is found in the capsular polysaccharides (CPS) of *Streptococcus pneumoniae* type 1,[62] and *Bacteroides fragilis*,[63,64] *Streptococcus mitis*,[65] expressed by the organism *Plesiomonas shigelloides type* 17,[66,67] as well as in the O-specific polysaccharide (O-PS) of *Providencia alcalifaciens* O8,[68] *Providencia alcalifaciens* O22[69] and *Proteus vulgaris* strain TG 276-1.[70]

Figure 1.2 shows that *S. flexneri* is the most prevalent in developing countries, while *S. sonnei* is the most prevalent in industrialised countries and is a common cause of traveler's diarrhoea.[35] Shigellosis due to *S. boydii* is uncommon and exists only on the Indian sub-continent, while infections with *S. dysenteriae* other than type 1 are also uncommon.[6,55,71]

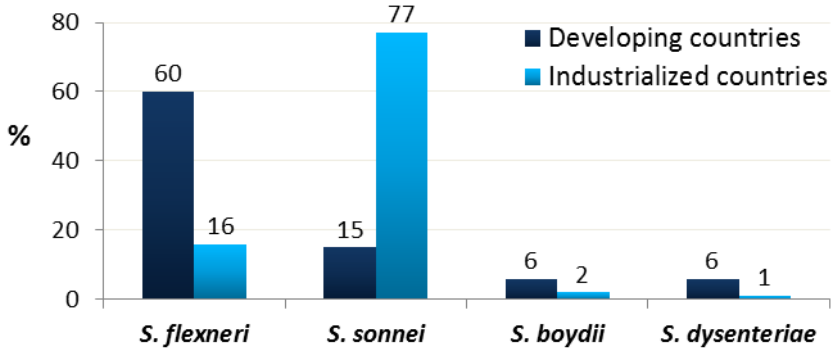


Figure 1.2: WHO estimates of *Shigella* serogroup isolates.[6]

From these statistics it is clear that shigellosis is endemic throughout the world, causing many cases of morbidity and mortality. With this knowledge, the WHO has placed the development of a safe, affordable and efficacious vaccine as a global health priority.[6]

1.2 Bacterial surface polysaccharides

The bacterial cell envelope of Gram-negative bacteria comprises an inner membrane or cytoplasmic membrane and an outer membrane separated by the periplasmic space. This mainly contains peptidoglycans and other smaller molecules like mono- and oligosaccharides, amino acids and peptides. The outer membrane may be covered by a capsular polysaccharide (CPS) or a lipopolysaccharide (LPS) as seen in **Figure 1.3**. The CPS is found in both Gram-negative and Gram-positive bacteria, while only Gram-negative bacteria are covered by LPS. This is also the component of the bacterial cell envelope that is virulent (in the case of toxic bacteria) to a eukaryotic host and is hence also known as the endotoxin.[72] The LPS is composed of three structurally distinct regions – the toxic lipid A, the core polysaccharide region and the external O-specific polysaccharide (O-PS) which is the highly antigenic structure and is therefore also known as the O-antigen.[72] The LPS can either be smooth (S-) LPS or rough (R-) LPS. S-LPS is a higher molecular mass and is normally found in wild-type strains, while R-LPS is a lower molecular mass as it lacks the O-PS and is thus mainly found in laboratory derived strains.[72]

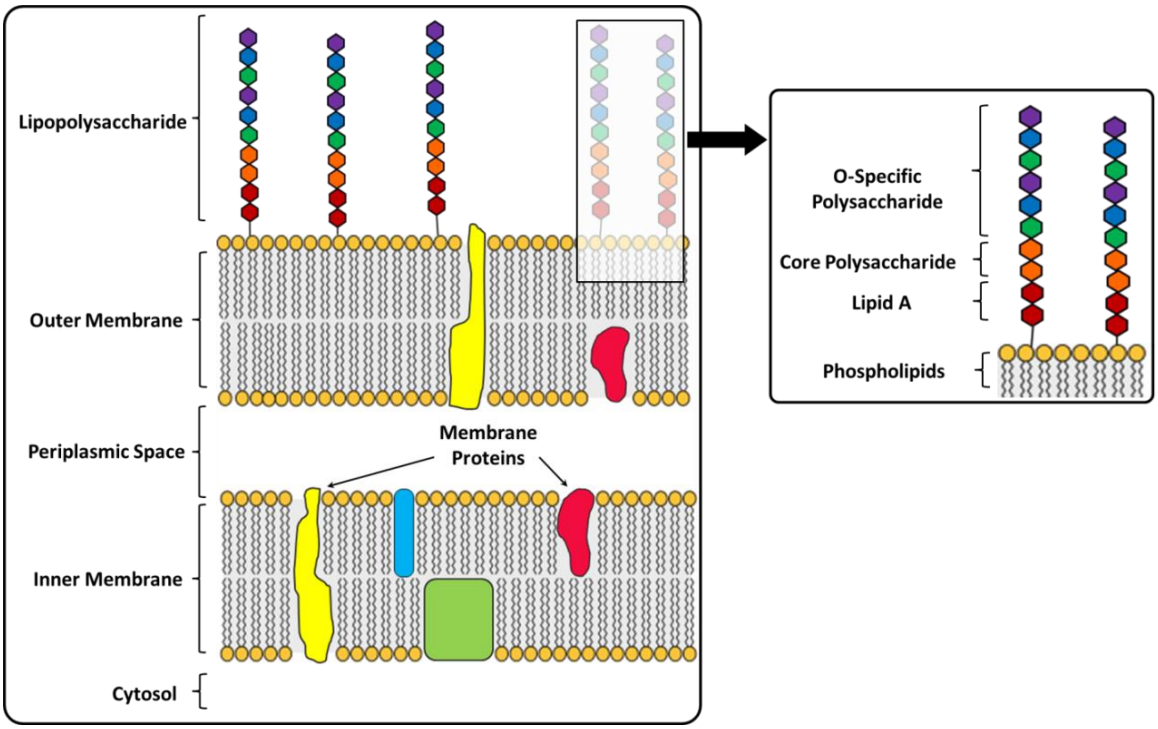


Figure 1.3: Schematic depicting the cell envelope of the Gram-negative bacteria.[72]

The lipid A anchors the LPS to the outer membrane and represents the endotoxically active moiety of toxic LPS. The toxicity is highly dependent on the structural properties, and it should be noted that not all LPSs are toxic molecules. The core region is covalently linked to lipid A and comprises an oligosaccharide of up to 15 sugars. The O-PS is covalently linked to the core region and is mostly composed of repeating units containing 2–8 monosaccharide residues. The core region and O-PS can act as receptors for bacteriophages, thereby contributing indirectly to the destruction of the bacterial cell.[72] The O-PS is an essential virulence factor, and it is this portion of the bacteria that certain vaccines are based on.[73]

1.3 Vaccination

Vaccines are an efficient and cost effective way of preventing human diseases. The advent of antibiotics overshadowed the need for vaccine development,[74] but with the large increase in antibiotic resistant strains of various pathogens in recent years, the need for vaccine development has never been greater.[74,75] Edward Jenner's studies of what we now call vaccination against cowpox in 1796 was a landmark discovery.[76] Jenner essentially showed that by exposing the immune system of a human to a pathogen, in this case the smallpox virus, an immune response can be induced. These studies were successfully carried out without any fundamental knowledge of the immune system.[77] With the advance of technology, and in turn an

advance in scientific knowledge and a more thorough understanding of the human immune system, the development of successful vaccines against polio, rabies, smallpox, meningococcus, pneumococcus, *Haemophilus influenzae* type b, hepatitis, rubella, measles and mumps, has reduced the burden of most of these diseases.[77] However, despite these advances, various diseases such as cancer, HIV/AIDS, tuberculosis and dysentery still remain unpreventable through vaccination.[75,78] Achieving effective results against enteric infections is particularly problematic, as inducing immunity against pathogens that mainly colonise the gut is difficult through traditional vaccine methods.[1,79]

The majority of vaccines developed to date can be broadly divided into three categories, depending on whether they contain live attenuated microorganisms, inactivated whole microorganisms, or subunit vaccines, which contain purified components of the microorganism.[80] Vaccines developed using the live attenuated approach involve the weakening of the microorganism through deleting or modifying genes.[80] The advantage of this method is that no thorough understanding of their attenuation was needed. Today, vaccines developed using this method would be difficult to license.[80] However, successful vaccines against tuberculosis (BCG vaccine),[81,82] polio (the Sabin approach),[83] measles, mumps, rubella,[84] and varicella[80,85,86] have all been licensed using this method. On the other hand, inactivated whole cell vaccines are treated with either heat or chemicals (formalin or glycerine phenol)[77] and an advantage of this method is that all antigens are present, so there is no need to know which is responsible for the immune response. Conversely, other antigens present may be responsible for side effects or may be toxic.[80] Successful vaccines against pertussis,[87] polio (the Salk approach),[88] influenza,[89] rabies[90] and tick-borne encephalitis[91] have been licensed using this method. Subunit vaccines consist of one or more antigens which are purified from the microorganism or produced by recombinant DNA technology and can consist of proteins, polysaccharides conjugated to proteins (glycoconjugate vaccines) or virus-like particles (VLP). Current licensed subunit vaccines using this method include the tetanus and diphtheria vaccine,[92] pertussis,[93] *Neisseria meningitidis;*[94] *Streptococcus pneumoniae,*[95,96] *H. influenzae* type b,[94,97] and *Salmonella typhi*.[80]

1.4 Glycoconjugate vaccines

The studies in 1929 by Avery and Goedel[98] paved the way for glycoconjugate vaccine development. Their studies showed that conjugation of the polysaccharide to a carrier protein induced an enhanced immunogenicity in rabbits compared to that of the polysaccharide only vaccine. Today, glycoconjugate vaccines have superseded their polysaccharide only counterparts. Glycoconjugate vaccines are composed of an antigenic oligo- or polysaccharide

covalently linked to an immunogenic protein carrier and are classed as subunit vaccines.[80,99] The advantage of this method is that only well-defined antigens are present. However, the exact protective antigens need to be identified and production and purification on a large scale needs to be efficient.[80] Glycoconjugates induce a narrower range of immune responses characterized mainly by the production of serum IgG to the O-antigen, while live attenuated oral vaccines confer a wider immune response.[30]

1.5 Immune response

The common principle behind these vaccines is the exposure of a particular epitope that exists on an invading microorganism to the human immune system. This in turn elicits an immune response which can be activated through two different systems: the innate immune system and the adaptive or acquired immune system, both of which involve humoral and cell mediated immunity.[100] The innate immune system involves the immediate recognition of antigenic structures and is generally short lived.[100,101] The adaptive immune response is longer lasting and is made up of B and T lymphocytes which have receptors on them that are unique for various microbial antigens and are only created through a complex cellular cascade upon exposure to a pathogen.[100] It is these antigen-specific cells that are responsible for producing antibodies and therefore immunity when re-exposed to a microbial antigen, and is thus the principle behind vaccination.[100]

Different microbial antigens elicit different immune responses. For example, capsular polysaccharides (CPS) elicit B-cell responses, and are therefore termed T-cell independent responses.[101] The production of antibodies in this case is rapid (days), but does not elicit long-lasting antibody memory (months).[100,101] CPS vaccines like the *H. influenza* type b vaccine[97] make them poor vaccines for infants and young children, the elderly and immunocompromised patients.[74,102] Any memory responses elicited by CPS vaccines are due to the high tendency of an antibody to bind to a specific epitope at the surface of an antigen.[74,101]

On the other hand, glycoconjugate vaccines elicit a T-cell dependent immune response. This is due to the presence of the protein carrier of the glycoconjugate which the immune system recognizes.[100,101] In addition to glycoconjugate vaccines, zwitterionic polysaccharides (ZPS), which are rare and contain both a negative (e.g. phosphate or carboxylate) and a positive (e.g. free amine) charge within the repeating unit are also capable of eliciting T-cell dependent immune responses.[103] T-cell dependent immune responses are generally slow (weeks) to activate antibody production, but elicit long-lasting (years) immune memory due to the formation of memory B- and T-lymphocytes.[74,101] In addition they successfully create long-

lasting antibody production in children under the age of 2, the elderly and immunocompromised patients.[104,105]

1.6 The production and control of glycoconjugate vaccines

The production and control of glycoconjugate vaccines is complex and expensive. The purity and structure of all of the intermediates involved in the process of making the final formulated glycoconjugate vaccine needs to be controlled and characterized at each step as seen in **Figure 1.4**.

The process and testing is performed following the regulatory guidelines and good manufacturing practices (GMP) established by the WHO and pharmacopoeia to ensure consistent production and immunogenicity.[75,106–112]

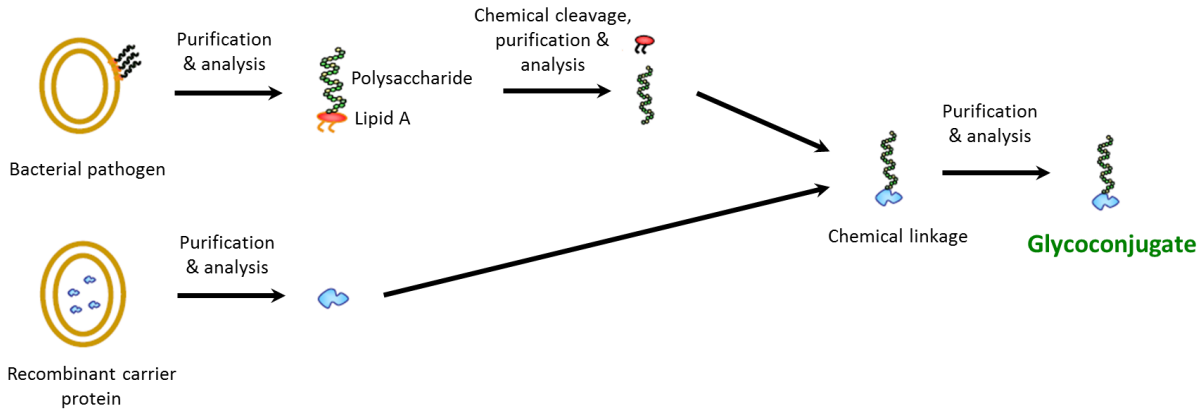


Figure 1.4: Process control and characterization required for the production and control of glycoconjugate vaccines.

1.6.1 Preparation and characterization of the polysaccharide and protein carrier

Traditionally, the CPS and protein carrier have to be purified from fermented bacteria in several steps. Glycoconjugate vaccines produced from LPS require an extra step in order to cleave the Lipid A from the O-antigen and must be achieved without any effect on the O-antigen structure. The polysaccharide is purified using various techniques depending on the nature of the repeating unit and can include selective precipitation, filtration, various types of chromatography (e.g. size exclusion or hydrophobic chromatography), and enzyme treatment.[75,99,113] After purification, the polysaccharide identity and structure need to be determined, which can be achieved through the application of various physicochemical techniques.[75,110–112,114,115] Of these, nuclear magnetic resonance (NMR) spectroscopy (see **Ch. 6** for further details) is by far the most effective and easiest method of characterisation.[116–120] This

also gives an indication of purity, as well as the degree of *O*- and *N*-acetylation, which can be quantified using an internal standard.[121] Immunological identity methods can be used to distinguish between closely related polysaccharide antigens.[106,122] Composition and quantitation of the polysaccharide then needs to be determined and can be achieved through the use of colorimetric assays[123] and various chromatography techniques. For example, gas chromatography coupled to a mass spectrometer (GC-MS) can be used after derivatisation (methanolysis followed by re-*N*-acetylation and trimethylsilylation),[124] and high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) can be used after the hydrolysis of the polysaccharide and can be compared to a commercially available standard.[125–129] However, hydrolysis of certain glycosidic linkages often leads to the degradation of certain labile monomers and is therefore product-specific and may not be quantitative for all components. In addition, HPAEC connected to a conductivity detector (HPAEC-CD) can be used to identify charged components such as uronic acids, phosphates and acetates, which can likewise be detected using colorimetric assays or capillary ion electrophoresis.[130,131]

Once the structure and composition of the polysaccharide have been identified, the polysaccharide molecular size distribution needs to be determined. This is an important factor as it ensures manufacturing consistency between batches. In the case of polysaccharide vaccines, the molecular size is a correlate of vaccine potency.[75,106,132,133] Various studies highlight the importance of the length of the saccharide chain as it has a direct effect on the immunogenicity of the vaccine, but is of course, dependent on the antigen.[134–138] Again, chromatography techniques such as high performance size exclusion chromatography coupled to either a refractive index or multi-angle laser light scattering detector (HPSEC-RI/MALLS) can be used to determine this.[118,139,140]

The protein carrier must be safe in humans and is responsible for eliciting a T-cell-dependent immune response. Common protein carriers used in licensed vaccines include tetanus toxoid (TT), diphtheria toxoid (DT) or the genetically toxoided, single amino acid variant of diphtheria toxin (CRM_{197}). Other less common protein carriers include recombinant genetically detoxified exotoxin A from *Pseudomonas aeruginosa* (rEPA);[141–145] protein D; a designed recombinant protein, N19; keyhole limpet hemocyanin and virus-like particle Qβ, with the latter three mainly being used in vaccines against non-infectious dieases.[146–150] Likewise with the polysaccharide, the purity and identity of the carrier protein needs to be characterized for licensure and can be done using various immunochemical and physicochemical techniques.[122]

1.6.2 Conjugation and characterization of the conjugate vaccine

Coupling of the protein carrier to the polysaccharide is through chemical activation of certain functional groups and needs to be achieved without any modification of immunological integrity of the O-antigen.[75,145,151] Activation of the polysaccharide is generally random and can be achieved through a variety of different strategies depending on the functional group. For example, periodate oxidation of vicinal hydroxyl groups to aldehydes can be conjugated to the protein through reductive amination[75,102,152,153] or through oxime formation.[154] On the other hand, hydroxyl groups can be selectively activated through cyanogen bromide.[155] Carboxylic acids can be coupled through carbodiimide-mediated condensation[156] or various thio/thiol chemistry can be employed to obtain the conjugate.[135,157–160] Alternatively, the reducing end of a short polysaccharide (where size reduction has been carried out), which is a masked carbonyl group, can be coupled directly through reductive amination.[161–164] Conjugation can also be formed directly through the use of spacer groups, such as adipic acid dihydrazide (ADH),[165] or alkyl type maleimido spacers.[138,157] Spacer groups can also be used to reduce steric hindrance between the antigen and protein carrier.[99,102,104,166]

Once conjugation has been achieved and proved through the use of SDS-PAGE, size exclusion chromatography or capillary electrophoresis,[122] the structure, composition and molecular size of the conjugate needs to be determined using various physicochemical techniques already mentioned. These tests are critical for assuring batch consistency. In addition, the saccharide:protein ratio must be determined, as well as the free saccharide content and amount of unconjugated protein carrier.[138,167,168] Unreacted functional groups as a result of the coupling chemistry employed need to be capped in order to prevent further reactions *in vivo* and any residual reagents determined. Finally, stability testing of the conjugate must be carried out and this can be achieved, for example, through the use of HPAEC-PAD and SEC-HPLC-RI/UV/MALLS and NMR spectroscopy.[75,117,169]

1.7 Vaccines against *Shigella*

Efforts to develop a vaccine against *Shigella* have been ongoing for over half a century with several vaccine candidates making it to various stages of clinical trials (summarised in **Table 1.1**). Despite this, there are currently no WHO approved vaccines commercially available.

Table 1.1: Summary of vaccine candidates against shigellosis. [1,3,5,23,30,170–173]

Vaccine candidate	Gene mutations / description	Development stage	Route	References
Live attenuated				
SmD	Serial passage *in vitro*	Phase III	Oral	Mel *et al.*[174] Mel *et al.*[175] Mel *et al.*[176]
S. flexneri T32-Istrati	Serial passage *in vitro*	Phase III - IV	Oral	Meitert *et al.*[177]
S. flexneri 2a	*E. coli* K12, aroD	Phase II	Oral	Cohen *et al.*[178]
S. flexneri 2a SC602	*virG*, *iuc*	Phase I–II	Oral	Sansonetti *et al.*[179] Coster *et al.*[180] Katz *et al.*[181] Rahman *et al.*[182]
S. sonnei WRSs1	*virG*	Phase I-II	Oral	Kotloff *et al.*[183] Orr *et al.*[184]
S. flexneri 2a CVD 1204 and CVD1208	*guaBA*	Phase I	Oral	Kotloff *et al.*[185]
S. dysenteriae 1WRSd1	*virG*, *stxAB*	Phase I	Oral	Venkatesan *et al.*[186] McKenzie *et al.*[187]
S. flexneri 2a CVD 1208S	*guaBA, set, sen*	Phase II	Oral	Kotloff *et al.*[188]
S. flexneri 2a WRSf2G11, 12, 15	*virG*, *senA*, *senB*, *msbB2*	Preclinical	Oral	Ranallo *et al.*[189] Ranallo *et al.*[190] Ranallo *et al.*[191]
S. sonnei WRSs2, 3	*virG*, *senA*, *senB*, *msbB2*	Preclinical NHP	Oral	Collins *et al.*[192] Barnoy *et al.*[193] Bedford *et al.*[194] Barnoy *et al.*[195]
S. dysenteriae 1 CVD 1256	*guaBA*, *sen*, *stxA*, *virG*	Preclinical	Oral	Wu *et al.*[196]
Inactivated – Whole Cell				
S. sonnei	Formalin inactivation	Preclinical	Oral	McKenzie *et al.*[197]
S. flexneri, S. sonnei, S. dysenteriae	Formalin inactivation	Preclinical	Oral or intranasal	Osorio *et al.*[198]
Subunit vaccines				
S. sonnei	Ribosome-LPS	Preclinical	Intranasal	Levenson *et al.*[199]
S. flexneri 2a	Ribosome-LPS	Preclinical	Intranasal	Shim *et al.*[200]
S. flexneri 2a	Proteosome-LPS	Phase II	Intranasal	Fries *et al.*[201]
S. flexneri 2a, *S. sonnei*	Invaplex (LPS plus IpaB, IpaC and IpaD)	Phase I	Intranasal	Oaks *et al.*[202] Tribble *et al.*[203] Riddle *et al.*[204]
S. flexneri 2a	IcsP, SigA	Preclinical	Mucosal	Czerkinsky *et al.*[205]
S. sonnei	GMMA vesicles	Preclinical	Intranasal	Berlanda Scorza *et al.*[206]
S. flexneri 2a, *S. sonnei*	IpaB, IpaD	Preclinical	Intranasal	Martinez-Becerra *et al.*[207] Martinez-Becerra *et al.*[208]

Table 1.1 continued

Vaccine candidate	Gene mutations / description	Development stage	Route	References
Subunit vaccines - Glycoconjugates				
S. dysenteriae 1 LPS-rEPA	Chemical conjugate	Preclinical	Parenteral	Chu *et al.*[209]
S. flexneri 2a LPS-rEPA, *S. sonnei* LPS-rEPA	Chemical conjugate	Phase I–III	Parenteral	Cohen *et al.*[210] Cohen *et al.*[211] Cohen *et al.*[212] Ashkenazi *et al.*[213] Passwell *et al.*[214]
S. flexneri 2a LPS-rEPA , *S. sonnei LPS-CRM*$_9$	Chemical conjugate	Phase I–III	Parenteral	Passwell *et al.*[215]
S. dysenteriae 1 synthetic O-PS-*human serum albumin*	Synthetic oligosaccharide	Preclinical	Parenteral	Pozsgay *et al.*[216]
Synthetic *S. flexneri* 2a O-PS-TT	Synthetic oligosaccharide	Preclinical	Parenteral	Phalipon *et al.*[138] Theillet *et al.*[217]
S. dysenteriae 1 O-PS-rEPA	Bioconjugate	Phase I	Parenteral	Dro *et al.*[218]

Of the vaccines developed, the majority of the live attenuated and glycoconjugate vaccines, as well as the proteosome vaccines have shown efficacy in field trials, while the nuclear protein–ribosomal parenteral vaccine, the Invaplex vaccine and the inactivated *S. sonnei* administered orally are still in preclinical testing (see **Table 1.1**). The live attenuated vaccine approach has had the greatest amount of research invested and has achieved the greatest success by making it to Phase IV clinical trials. Unfortunately, the vaccine developed by Mel *et al*. encountered scale-up and process control problems.[219] Nevertheless, this vaccine paved the way for future development as it showed that a multivalent *Shigella* vaccine is possible and that serotype specific protection can be induced.[174,220] On the other hand, glycoconjugate vaccines have also successfully made it to Phase III clinical trials.[214]

1.7.1 Enteric enteropathy

An overall challenge for live attenuated vaccines has been in achieving the correct balance of safety and immunogenicity.[23] Another major concern, which is poorly understood, has been the ability of an oral live attenuated vaccine to induce immunogenicity in *both* developed and developing nation populations.[221,222] This has been seen with the *S. flexneri 2a* SC602 vaccine which was immunogenic in a North American population, but failed to induce an immune response in Bangladeshi adults.[181,182] Similar complications with oral live attenuated vaccines against polio, rotavirus and cholera have also been observed.[223–228] This phenomenon is referred to as enteric enteropathy (EE) and is a condition thought to be caused by the continual exposure to faecal pathogens often found in individuals from developing countries.[221,222] In addition,

malnutrition, genetic factors and interference by maternal antibodies are also thought to affect the vaccine's efficacy. This results in an increase in intestinal permeability, impaired gut immune function and ultimately efficacy problems with oral live attenuated vaccines.[221,222] Since EE is not yet well understood and the link between oral live attenuated vaccines unclear, the use of glycoconjugate vaccines may provide the ideal alternative. This is because they are administered parenterally and are thus not subjected to the intestinal barrier. This, along with the implications of enteric enteropathy and vaccine efficacy need to be fully investigated.

1.7.2 Additional challenges for *Shigella* vaccine development

As well as the complicated and poorly understood effects of EE, there have been additional hurdles encountered for producing a licensed *Shigella* vaccine. These include:[23]

- An unsatisfactory animal model that accurately represents shigellosis. Non-human primates offer a reasonable model, however, are costly and present additional challenges;[229–231]
- An inadequate understanding of the pathogenesis and effect on immunity;
- Insufficient funding and low profit gains for pharmaceutical companies;[79]
- Manufacturing limitations (as with synthetic vaccines);
- Views that good hygiene and sanitation practises, and safe drinking water are easier to attain;
- Concerns for the ability to provide broad coverage of many serotypes, despite this being shown in various studies.[23,30,60]

Despite all of the above challenges, a novel method for vaccine development is currently being investigated by the biotech company, GlycoVaxyn.

1.8 GlycoVaxyn technology

GlycoVaxyn's novel technology for the production of glycoconjugate vaccines uses recombinant, non-toxic *Escherichia coli* to synthesis the glycoconjugate *in vivo*.

The biosynthesis of the glycoconjugate is achieved through the transfer of the general *N*-linked protein glycosylation system found in *Campylobacter jejuni* to *E. coli.*[232] This glycosylation machinery is encoded by 12 genes in a single gene cluster called *pgl* (protein glycosylation),[233] and is responsible for the synthesis of lipid-linked oligosaccharides and flipping these into the periplasmic space.[234] The mechanism by which the glycoconjugate is assembled is shown in **Figure 1.5**. It involves the biosynthesis and assembly of the O-antigen, which is possible *via* two different means – the Wzy-dependent mechanism and the ABC transporter dependent

pathway,[235] both of which can be initiated in *E. coli*.[236] The Wzy-dependent mechanism synthesizes repeating units on the undecaprenyl-pyrophosphate (Und-PP) lipid carrier at the cytoplasmic side of the inner membrane and flips these across the cytoplasmic membrane, where Wzy polymerase polymerizes the repeating units of the O-antigen in the periplasmic space, and transfers this to the lipid A core by the WaaL ligase. Similarly, the ABC transporter-dependent pathway forms the O-antigen by reactions occurring at the cytosolic face of the cytoplasmic membrane. An ATP-binding cassette transporter then moves the O-antigen across the inner membrane, where it is then attached to the Lipid A core.[237] The integral membrane protein, PglB, is the key enzyme (oligosaccharyltransferase (OST)) in the *C. jejuni N*-glycosylation system and is responsible for the glycosylation of a range of proteins.[233,238] In *C. jejuni*, the *pgl* enzymes synthesize a heptasaccharide, GalNAc-a1,4-GalNAc-a1,4-(Glc-b1,3)-GalNAc-a1,4-GalNAc-a1,4-GalNAc-a1,3-Bac, where Bac is 2,4-diacetamido-2,4,6-trideoxy-D-Glc on an Und-PP lipid carrier where it is transferred to an asparagine side chain present on a protein carrier.[233,234] When PglB is expressed in a *waaL* mutant strain of *E. coli*, it can efficiently accept a diverse range of Und-PP-linked glycans in a similar manner.[234] This allows for the transfer of the O-antigen on the Und-PP lipid carrier to the side chain of site-specific asparagine residues in the consensus sequence N-X-S/T (where X can be any amino acid except proline) on the periplasmic carrier proteins, which is either AcrA from *C. jejuni* or a genetic toxoid form of *Pseudomonas aeruginosa* exotoxin type A (rEPA).[141,234,239,240]

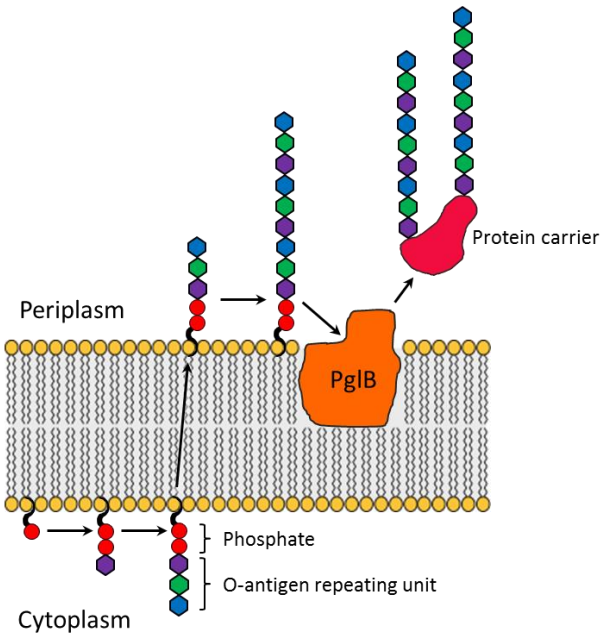


Figure 1.5: Schematic depicting the *in vivo* biosynthesis of a glycoconjugate.[239] The polysaccharide is assembled on the Und-PP lipid carrier and conjugated to a side chain of site-specific asparagine residue of the protein carrier by the oligosaccharyltransferase (OST) PglB from *C. jejuni*.

The relaxed specificity of PglB means that a variety of glycan structures can be used[241] and since the prerequisite for transfer of a polysaccharide by PglB is the presence of a consensus sequence on the carrier protein and a *N*-acetylhexosamine residue at the reducing end of the sugar,[239] this should be possible for various *Shigella* serotypes. rEPA was chosen as the protein carrier for the *Shigella* bioconjugates due to its safety and efficacy as shown in previous clinical trials.[67,242] There are five potential sites of *N*-glycosylation sites in rEPA that follow the N-X-S/T sequence, but only two of these have been glycosylated. The first site of glycosylation is DQNRTK and the second is DNNNST.

This method of developing glycoconjugates is obviously advantageous. It requires no culturing of pathogenic or slow growing bacteria; no chemical treatments, such as removal of endotoxin; no modification or derivatisation of the polysaccharide and protein in order to achieve conjugation.[234] In addition the polysaccharide chain length is controlled *in vivo* yielding reproducible results. Furthermore, this method allows for the production of acid-labile conjugates which cannot be produced by traditional methods.[234]

1.8.1 GlycoVaxyn purification and derivatisation for analysis of bioconjugates

GlycoVaxyn performs various purification and analysis of intermediates and final bioconjugates which are in accordance with regulatory recommendations set out by the WHO and pharmacopoeia, this information is confidential, but will be briefly outlined below.

Intermediate analysis of the polysaccharide linked to the Und-PP lipid carrier is carried out to ensure the correct O-antigen is being synthesised and the methodology has been specially adapted by GlycoVaxyn for this purpose. This is of relevance for this project, as it will give an indication of the ideal form that the *S. sonnei* disaccharide should be in (see subsequent sections). Mild acid hydrolysis of the sugar from the lipid carrier is carried out as reported by Glover *et al*.[243] followed by C-18 Sep-Pak purification, and fluorescent labelling of the polysaccharide at the reducing end by reductive amination using 2-aminobenzamide.[244] The labelled polysaccharide can be separated by HPLC and analysed by MALDIMS/MS, which will confirm if the correct O-antigen has been synthesised.

Once the bioconjugate has been synthesised *in vivo*, it is extracted from the *E. coli* cells by osmotic shock and then purified by various types of chromatography. Monosaccharide analysis, HPLC, total protein mass spectrometry and NMR analysis are carried out on glycoconjugate samples in order to determine structure, composition and molecular size of the conjugate, as well as the saccharide:protein ratio, free saccharide content and amount of unconjugated

protein carrier. For analytical purposes, a portion of the glycoconjugate is treated with pronase, yielding a glycopeptide. The glycopeptide sample is purified using C-18 Sep-Pak, followed by SEC chromatography and analysed as above. This makes analysis simpler, especially in the case of NMR spectroscopy.

1.8.2 Development of *S. flexneri* 2a and *S. sonnei* glycoconjugates using GlycoVaxyn technology

GlycoVaxyn has embarked on developing a multivalent glycoconjugate vaccine against *Shigella* using their technology. The consensus is that an effective vaccine need only cover the 5 most prevalent strains in order to prevent or significantly reduce several important and clinically relevant types of *Shigella* disease.[6,30,57,60] As mentioned, these are *S. flexneri* 2a, 3a and 6, *S. dysenteriae* type 1 and *S. sonnei*. To date, GlycoVaxyn has developed a *S. dysenteriae* O1 conjugate vaccine which has been successfully tested in Phase I of human clinical trials. At the start of this project the *S. flexneri* 2a and *S. sonnei* strains were the next targets, with the *S. flexneri* 2a glycoconjugate currently being tested in human subjects at the time of writing.

The *S. sonnei* serotype presents specific challenges owing to the nature of the unusual monosaccharides in the repeating unit (**Figure 1.6**) which consists of two unusual sugars: 2-acetamido-2-deoxy-α-L-altruronic acid (AltNAcA)[61] and 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose (FucNAc4N or AAT) which is labile.[68,70,245,246] In addition to this, the zwitterionic nature of the disaccharide repeating unit complicates not only biosynthesis, but also analysis.

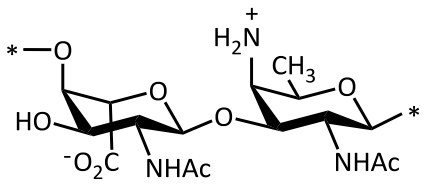


Figure 1.6: The zwitterionic repeating unit of *S. sonnei* comprises two unusual sugars: 2-acetamido-2-deoxy-α-L-altruronic acid (AltNAcA)[61] and 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose (FucNAc4N or AAT), both of which preferentially adopt the $^4C_1$ conformation.[247]

The development and licensure of glycoconjugate vaccines relies on a combination of biological testing and the detailed physicochemical characterization at various stages of vaccine development to evaluate manufacturing consistency and vaccine integrity. In order to carry out this testing, monosaccharide standards are required. The monosaccharides found in the repeating units of four out of the five prevalent *Shigella* serotypes are commercially available

(D-glucose, D-galactose, D-glucosamine and L-rhamnose). However, AltNAcA and FucNAc4N are not, and will need to be chemically synthesised. Owing to the charged nature and lability of each of these sugars,[68,70,245–247] they will need to be synthesised in a suitable form.

1.9 Chemical synthesis of carbohydrates

Oligosaccharides can be obtained in reasonable quantities either through isolation from natural products,[248] through enzymatic synthesis[249,250] or through chemical synthesis. The latter remains the most efficient method to obtaining well defined fragments of the desired saccharide.[251] However, even chemical synthesis presents an array of challenges, despite the years of research in the field. Synthesis of the required carbohydrate building blocks is complicated by the presence of the many hydroxyl groups in the monosaccharide,[252] where the reactivity/nucleophilicity varies according to the location and stereochemistry.[253] These hydroxyls require orthogonal functionalisation and various regioselective protecting group manipulations, which are often difficult to achieve despite the considerable advances in methodology.[253,254] However, it is also possible to differentiate between groups of similar reactivity by exploiting subtle differences in chemical environment, with various authors reporting these seemingly straightforward transformations.[255–257,‡]

The assembly of complex oligosaccharides is also complicated by the formation of glycosidic linkages.[258] Here, the desired stereochemistry in the product, whether a 1,2-*trans* or 1,2-*cis* linkage, needs to be investigated prior to glycosylation, as the effect of the various protecting groups in the donor and acceptor pair often affects not only the stereoselectivity, but the reactivity as well.[258] In addition, the most suitable promoter system and reaction conditions for this step requires careful consideration, as this can also affect the desired stereochemistry.[258–260] Furthermore, the protecting groups can either enhance or reduce the overall reactivity of the monosaccharide building block, and a prominent example of this has been the advances in reactivity tuning by using armed and disarmed approaches, which have greatly improved synthetic efficiency.[251,261–263] Along with these aspects of reactivity tuning, a significant number of programmable one-pot glycosylation strategies have also been published,[264,265] making the reactivity of certain glycosyl donor and acceptor pairs fairly predictable.

A further aspect that hampers carbohydrate synthesis is the effect of the substituent configuration on the overall reactivity of the monosaccharide, not only in glycosylation reactions but also in modifications of the carbohydrate skeleton itself. Here, for example, *gluco*-

‡ See **Ch. 2** for various examples of references which include a list of methods using additional reagents or catalysts to carry out the regioselective protection.

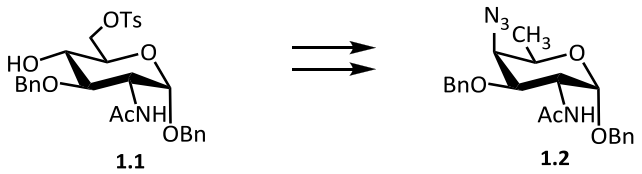
vs. galacto-configurations are known to affect reactivity, but such effects cannot always be predicted.[253,266] The configuration of substituents clearly influences the possible conformations of the monosaccharide building block, which in turn influences overall reactivity and selectivity.[263,267]

Despite all of the advances in chemical synthesis, the overall effect of various protecting groups and/or differentially functionalized positions of the monosaccharide is still difficult to predict. These effects are generally poorly understood and an empirical approach remains the only way to unambiguously determine whether the desired outcome is possible.

1.10 Published FucNAc4N (AAT) syntheses

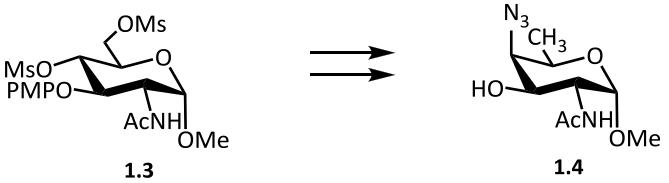
As a result of its occurrence in numerous naturally occurring polysaccharides,[62–66,68–70,268] the synthesis of FucNAc4N derivatives has been the focus of many attempts over the years.[269–282]

The earliest report of a FucNAc4N synthesis is by Sharon *et al.*[269] where the protected 2-acetamido-4-azido-2,4,6-trideoxy-D-galactose **1.2** was synthesised *via* the key intermediate **1.1** (**Scheme 1.1**).[283]



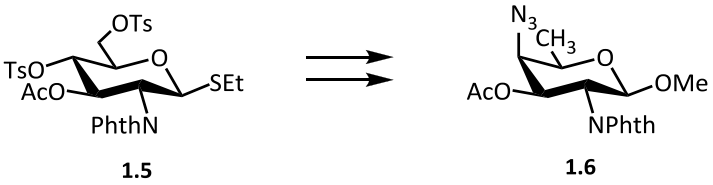
Scheme 1.1: Sharon's (1978)[269] synthesis of the protected sugar **1.2**.
This was synthesised in 13 steps from D-glucosamine.

Lönn and Lönngren[270] then published their method, where the di-mesylate **1.3** was selectively reduced to yield the 6-deoxy sugar, after which the 4-position was inverted with NaN_3 and the 3-position deprotected to yield the methyl glycoside **1.4** (**Scheme 1.2**).



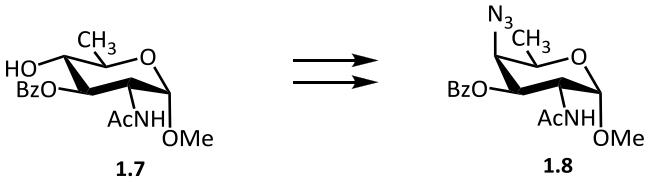
Scheme 1.2: Lönn and Lönngren's (1984)[270] synthesis of the glycosyl acceptor **1.4**.
This was synthesised in 8 steps from *N*-acetyl D-glucosamine. PMP = *p*-methoxyphenyl.

In 1997, Pozsgay *et al.*[273] published their synthesis of the protected FucNAc4N glycosyl donor *via* the 4,6-*O*-di-tosylate intermediate **1.5** (**Scheme 1.3**). This was selectively reduced to the 6-deoxy sugar and inversion of the 4-position was achieved by displacement of the 4-*O*-tosylate with NaN_3, followed by methyl trifluoromethanesulfonate-assisted methanolysis gave **1.6**.‡



Scheme 1.3: Pozsgay's (1997)[273] synthesis of the protected glycosyl acceptor **1.6**. This was synthesised in 12 steps from D-glucosamine in an overall yield of 8%.

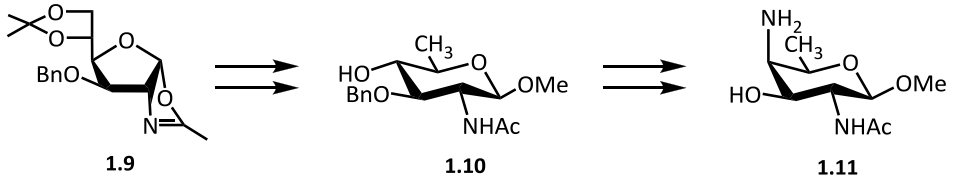
The key step in the synthesis reported by Liang and Grindley was the regioselective benzoylation at *O*-3 using 1.1 eq. of BzCl in pyridine at -35°C for 2 hours, giving the 6-deoxy intermediate **1.7**, which was then converted to the 4-azido sugar **1.8** *via* a Mitsunobu-type reaction with diphenylphosphoryl azide (**Scheme 1.4**).*



Scheme 1.4: Liang & Grindley's (2004)[274] synthesis of the protected glycosyl acceptor **1.8**. This was synthesised in 7 steps from D-glucosamine in an overall yield of 39%.

Bundle's approach is one of the shortest and most efficient for obtaining the FucNAc4N glycosyl acceptor (**Scheme 1.5**). The 3-*O*-benzyl protected oxazoline **1.9** was easily converted to the 6-deoxy sugar **1.10** and then the 4-amino methyl glycoside **1.11**.#

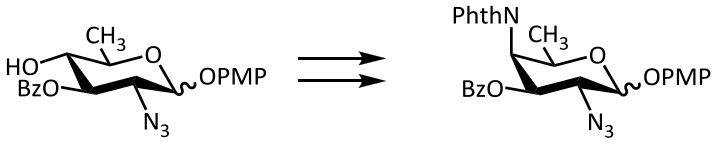
‡ The trehalose-dimer was also obtained.
* The *gluco*-configured 4-azido diastereomer is also obtained in a 18% yield.
# Treatment with of **1.10** with excess Tf_2O followed by NaN_3 resulted in the formation of tetrazole functionality at C-2.



1.9 1.10 1.11

Scheme 1.5: Bundle's (2009)[276] approach to the glycosyl acceptor **1.11**.
This was obtained in 6 steps from oxazoline **1.9** in an overall yield of 22%.

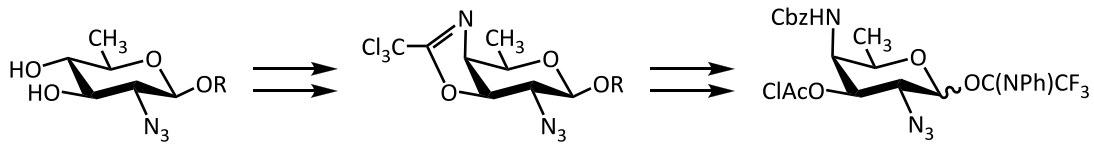
In 2010, Schmidt and co-workers published two papers[277,278] that used a similar approach to obtain **1.12** which was converted to various differentially protected glycosyl donors and acceptors (**Scheme 1.6**). As with Liang and Grindley, the key step in the synthesis was the regioselective benzoylation at *O*-3. Conversion and inversion to the protected 4-amino sugar was achieved with triflation at *O*-4 followed by treatment with potassium phthalimide salt giving **1.13**.



1.12 1.13

Scheme 1.6: Schmidt's (2010)[277] synthesis of the protected sugar **1.13**.
This was obtained in 10 steps from D-glucosamine in an overall yield of 25%.

In a similar approach to previous methods reported by the group,[275] van der Marel's Cbz protected imidate donor **1.16** was obtained from **1.14** *via* the regioselective 3-*O*-trichloroacetimidate formation, triflation at *O*-4, and intramolecular substitution (to give **1.15**) in a total of 14 steps (**Scheme 1.7**).[§]

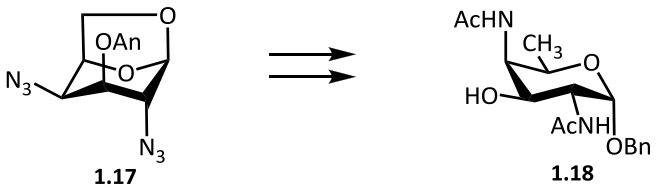


1.14, R = TBDPS **1.15**, R = TBDPS **1.16**

Scheme 1.7: van der Marel's (2007 & 2012)[275,281] synthesis of the imidate donor **1.16**.
This was obtained over 14 steps from D-glucosamine in an overall yield of 15%.

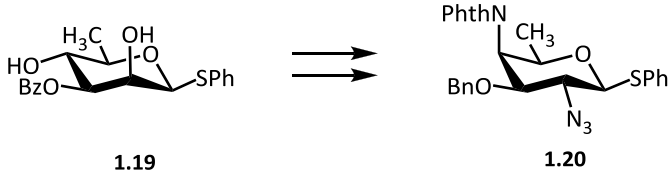
[§] The *allo*-configured oxazoline diastereomer was also obtained in a 23% yield.

Methods for obtaining FucNAc4N derivatives from other starting materials have also been developed. Starting from a mannose derivative, van Boom[271] synthesised the 1,6-anhydro sugar **1.17** through a stepwise introduction of the two azido groups (**Scheme 1.8**), implying that one azide could be selectively modified before addition of the other azide. Acetolysis followed by a Barton-McCombie radical deoxygenation reaction gave the 6-deoxy sugar **1.18**. This would otherwise be difficult to obtain using conventional methods (OTs → I → H) as a result of the *galacto*-configuration.



Scheme 1.8: van Boom's (1987)[271] synthesis of the protected FucNAc4N derivative **1.18.** This is obtained over 12 steps starting from the 1,6-anhydro-2,3-*O*-(4-methoxybenzylidene)-β-D-mannose.[284] An = anisoyl.

The Kulkarni[282,285] group synthesised the D-rhamnosyl diol **1.19** from D-mannose (**Scheme 1.9**). Here C-6 deoxygenation was achieved by regioselective tosylation followed by reduction with $LiAlH_4$. Their one-pot triflation and highly regioselective S_N2 displacement with $TBAN_3$ (at C-2) and then potassium phthalimide (at C-4) gave **1.20**. This is also one of the most efficient routes to the FucNAc4N derivative.

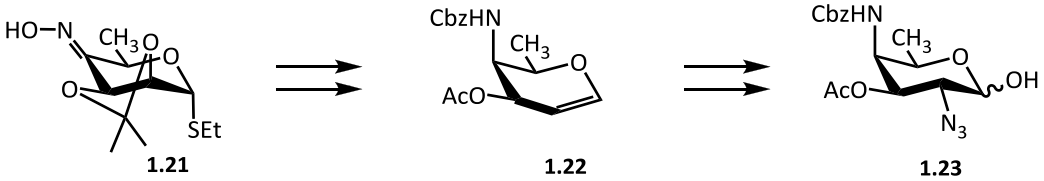


Scheme 1.9: Emmandi and Kulkarni's (2014)[285] highly regioselective synthesis of the FucNAc4N glycosyl donor **1.20**. This was obtained in 5 steps from an unprotected D-mannose thioglycoside in an overall yield of 44%.

The method developed by van Boom and co-workers, is by far the longest, but makes use of some interesting chemistry (**Scheme 1.10**). The oxime **1.21** is synthesised from D-mannose and upon reduction with $NaCNBH_3$ and $TiCl_3$, a 4-amino *talo*-derivative is obtained.‡ *N*-Cbz

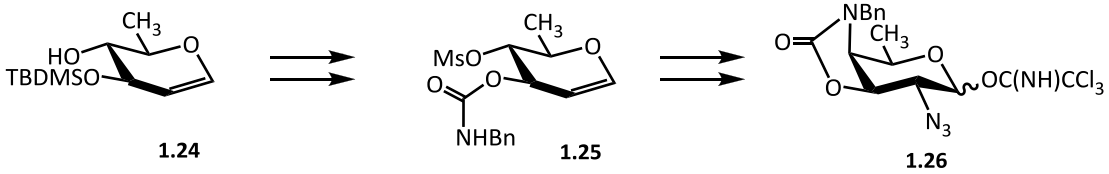
‡ The *manno*-derivative was also obtained in a 5% yield.

protection, acid hydrolysis, bromination and treatment with zinc dust gave the D-galactal precursor **1.22**. Azidonitration, followed by denitration gave the free sugar **1.23**.[#]



Scheme 1.10: van Boom's (1992)[272] synthesis of the free sugar **1.23**.
This was obtained over 13 steps from D-mannose.

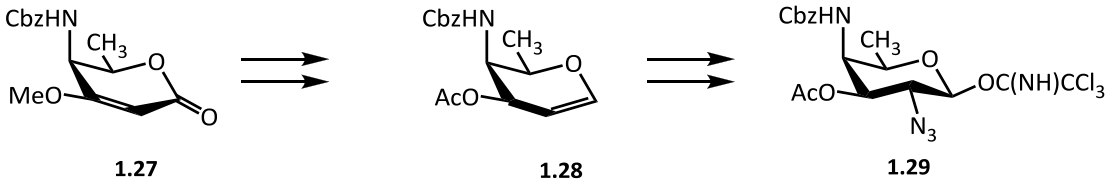
In 2010, Bundle's group published another route which began with commercially available tri-*O*-acetyl-D-glucal (**Scheme 1.11**).[279] The benzylcarbamate **1.25** was obtained from the regioselectively 3-*O*-silylated sugar **1.24**. Azidonitration of the glucal, deprotection and conversion to the trichloroacetimidate donor gave **1.26**.



Scheme 1.11: Bundle's (2010)[279] synthesis of the donor **1.26**.
This was obtained over 13 steps from tri-*O*-acetyl-D-glucal in an overall yield of 24%.

And lastly, Seeberger's *de novo* synthesis of the FucNAc4N derivative **1.29** (**Scheme 1.12**) is by far one of the most elegant. Beginning with the *O*-acetate of the *N*-Cbz-L-threonine methyl ester, the enone **1.27** was obtained *via* a Dieckmann cyclization followed by methylation of the intermediate β-keto-ester. From there an initial 1,2-reduction of the lactone using DIBAL, followed by acid work-up gave the 1-en-3-one, which upon Luche reduction and acetylation gave galactal derivative **1.28**. Azidonitration followed by anomeric deprotection and treatment with trichloroacetonitrile gave the donor **1.29**.

[#] After azidonitration the talopyranoside was obtained in a minor amount.



Scheme 1.12: Seeberger's (2010)[280] synthesis of the donor **1.29**.
This was obtained over 10 steps from *N*-Cbz-L-threonine in an overall yield of 22%.

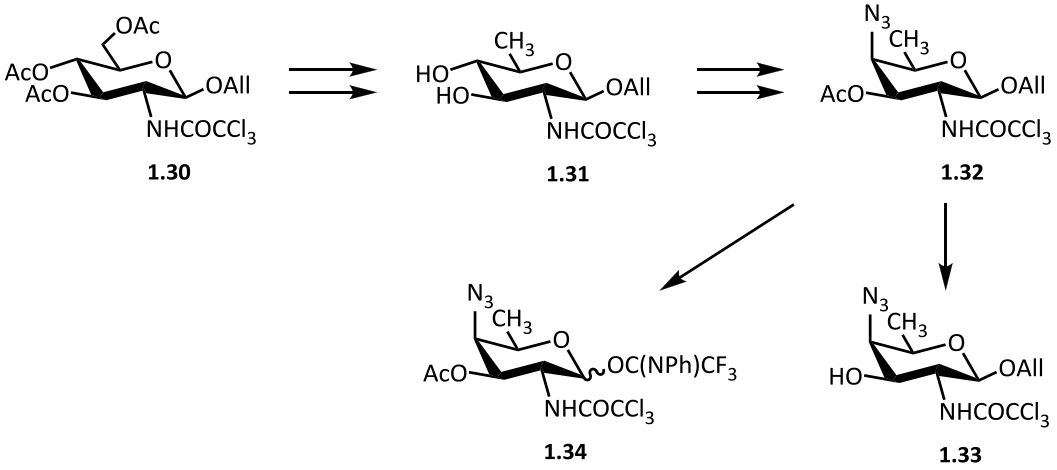
The majority of these approaches obtain the 6-deoxy sugar by conversion to a 6-iodo derivative *via* a 6-*O*-tosylate,[273,277,279,281,286] followed by conversion of 4-hydroxy group to a leaving group and S_N2 inversion with NaN_3 or potassium phthalimide to get the 4-amino functionality in the *galacto*-configuration.[271,273,276,277,282,286] C-3 and C-4 are generally differentiated by a seemingly straightforward regioselective protection.[274,277,279,281,282,286] These conversions seem the easiest, most efficient and robust way of obtaining the 4-amino-6-deoxy sugar. In many of these routes the 2-position remains masked as an azide and requires further manipulation in order to obtain the 2-acetamido group or other *N*-protecting groups which are capable of anchimeric assistance. Many research groups synthesized the glycosyl acceptor only, with immediate installation of the acceptor group. Further manipulation of this position is difficult, but not impossible.[273,274,276,286] The key steps in some of the more efficient and original routes suffer from diastereoselectivity issues, where the desired compound is obtained in a less than ideal yield.

1.11 Published *S. sonnei* disaccharide synthesis

Despite the years of interest in the two unusual sugars found in *S. sonnei* and the need for a well-defined fragment of the repeating unit, there has only been one report for the synthesis of the protected form of the *S. sonnei* di- and tri-saccharides, which was published recently by Pfister and Mulard.[286] Prior to this, Pozsgay and co-workers published the synthesis of the methyl glycoside derivatives of both FucNAc4N and AltNAcA,[273] and later reported glycosylations using a derived FucNAc4N glycosyl donor and acceptor with readily available analogues of AltNAcA.[287]

Pfister and Mulard's approach for the FucNAc4N glycosides (**Scheme 1.13**) began from the allyl glycoside **1.30** of D-glucosamine, where the amine is protected as the trichloroacetate. Since both FucNAc4N and AltNAcA are β-linked, the *N*-acyl group would be required for anchimeric assistance in the glycosylation reactions.[288–290] De-*O*-acetylation of **1.30**, regioselective tosylation and *O*-acetylation, C-6 halogen substitution followed by reductive elimination with $NaCNBH_3$ and de-*O*-acetylation yielded the diol **1.31**. Regioselective acetylation at *O*-3 was achieved after considerable optimization, and then esterification at C-4 with triflic anhydride

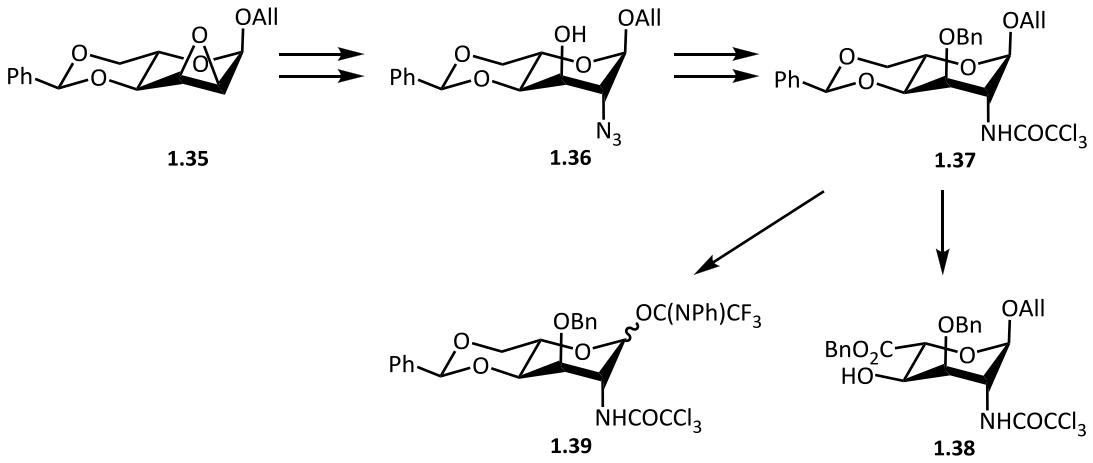
followed by S_N2 inversion with NaN_3 provided the fully protected 4-azido precursor **1.32**. Selective acetate removal yielded the acceptor **1.33** in 9 steps from the allyl glycoside **1.30**, while deallylation and treatment of the hemiacetal with *N*-phenyltrifluoroacetimidoyl chloride (PTFACl) gave the imidate donor **1.34** in 10 steps.



Scheme 1.13: Pfiste and Mulard's synthesis of the protected FucNAc4N glycosyl acceptor **1.33** and donor **1.34**.[286]

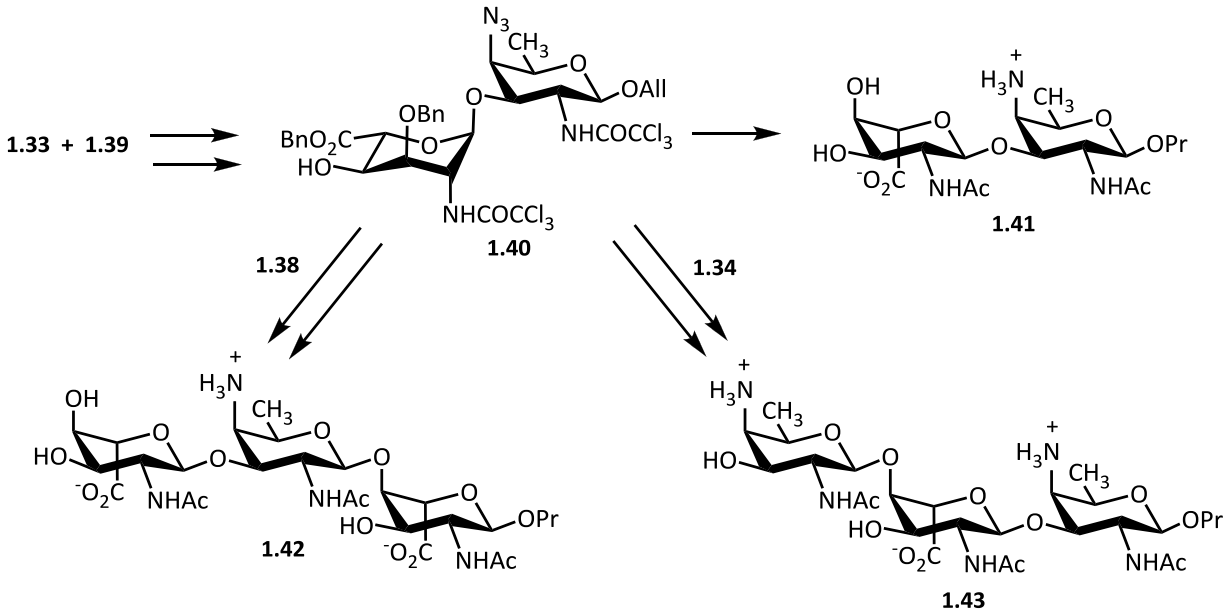
Synthesis of the AltNAcA glycosyl acceptor and donor was based on methods published previously by Pozsgay *et al.*[273] and Walvoort *et al.*[291] (**Scheme 1.14**) The expensive commercially available L-glucose was chosen as the starting material for the synthesis as it contains the desired configuration at C-4 and C-5. The epoxide **1.35** was obtained by conversion of L-glucose to the allyl glycoside, followed by 4,6-*O*-benzylidene protection and 2,3-di-*O*-mesylation.‡ Treatment of **1.35** with NaN_3 yielded the trans-di-axial C-2 amino altropyranoside precursor **1.36**.§ Benzylation, selective reduction of the azide and conversion to the trichloroacetamide# gave **1.37**. From here acetal acidolysis and selective TEMPO/BAIB oxidation[292] of C-6 and subsequent benzyl esterification gave the acceptor **1.38** in 11 steps, while the donor **1.39** was obtained in 10 steps as described above for the conversion of **1.33** to **1.34**.

‡ Medgyes *et al.*[273] synthesised the methyl 2,3-anhydro-4,6-*O*-benzylidene-α-L-allopyranoside *via* the 2,3-di-*O*-tosyl intermediate instead.
§ Partial trans-diequatorial opening of the epoxide ring into allyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-L-glucopyranoside could not be avoided.
# Optimisation of this step was required in order to reduce formation of the carbodiimide dimer.



Scheme 1.14: Pfiste and Mulard's synthesis of the AltNAcA glycosyl acceptor **1.38** and donor **1.39**.[286]

The partially protected disaccharide **1.40** was then synthesised from FucNAc4N acceptor **1.33** and AltNAcA donor **1.39**, after which global deprotection yielded the disaccharide **1.41** (**Scheme 1.15**). Chain elongation of the disaccharide **1.40** was then evaluated. Protection and conversion of **1.40** to the *N*-phenyltrifluoroacetimidate donor, followed by TMSOTf-mediated coupling with **1.38** and global deprotection yielded the trisaccharide **1.42**, while in a similar manner, acceptor **1.40** was coupled with donor **1.34** and deprotected to yield **1.43**. The NMR data for the disaccharide **1.41** and trisaccharides **1.42** and **1.43** suggests that the AltNAcA adopts the $^4C_1$ chair conformation.



Scheme 1.15: Synthesis of the disaccharide **1.41** and trisaccharides **1.42** and **1.43** which show that chain elongation on either end of the disaccharide **1.40** is possible.

This route is the first and only synthesis of the *S. sonnei* disaccharide, which also shows that chain elongation at both ends is possible.

1.12 Methods for synthesising L-sugars

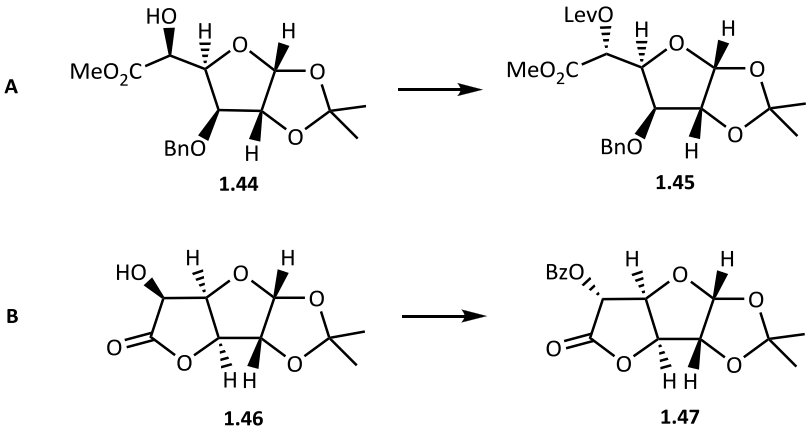
Pozsgay and Mulard's approaches to synthesizing the AltNAcA derivative both begin with the commercially available, yet very expensive L-glucose.[273,286] This seemed an unfeasible starting point for our synthesis and so an alternative was explored. Beginning with a different L-sugar also seemed impractical as only L-arabinose, L-fucose and L-rhamnose are commercially available in reasonable quantities. These are still expensive and suffer from the obvious drawback of requiring extensive manipulations to obtain an AltNAcA derivative. Since isolation and purification of L-sugars that are useful in this synthesis are almost impossible from natural sources, chemical synthesis remains the only viable route to obtaining sufficient and well defined amounts of an AltNAcA derivative.[293]

To this end, a review of the literature reveals an extensive array of L-sugar syntheses. These methods include a *de novo* approach,[294–296] where key steps involve enantioselective dihydroxylation[297–299] or a selective aldol reaction;[300,301] synthesis of L-sugars from chiral building blocks,[302–305] or ascorbic acid;[306,307] or through the use of enzymes;[308–310] and from various D-carbohydrate starting materials.[311–313] In the latter case, the L-sugar has been obtained through a radical tandem decarboxylation-cyclization reaction,[314] or *via* a stereocontrolled cyanohydrin reaction,[315] or *via* inversion at C-5 through various strategies to give the required *R*-configuration which is present in the L-sugar.

Many of the above methods for L-sugar synthesis exploit the use of non-carbohydrate starting materials, and were not considered for this synthesis of an AltNAcA derivative given our intent to use a readily available carbohydrate material. As there are a multitude of publications describing the synthesis of L-altruronic acid and L-iduronic acid, these were explored as potential starting points, noting that these would both require subsequent introduction of the amino group at C-2, and, in the case of the L-iduronic acid, an inversion of configuration at C-4. The published approaches to these L-sugars generally start from a form of D-glucose and can broadly be divided into 2 categories: those proceeding through the formation of 4-deoxypentenosides [316,317] and those proceeding through epimerization of C-5. The methods in the latter category are summarised below:

- S_N2 Inversion at C-5 of a glucopentose derivative:

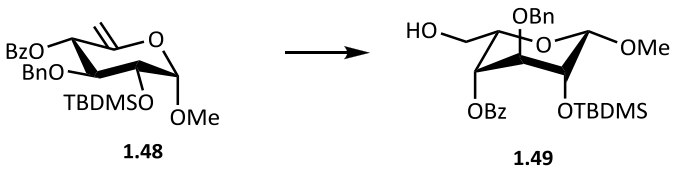
Synthesis of an L-iduronic acid derivative *via* S_N2 inversion was carried out by Seeberger *et al.*[318] (**Scheme 1.16A**) and Ojeda *et al.*[319] (**Scheme 1.16B**) on different substrates, both of which were obtained from D-glucose. Triflation of **1.44** or **1.46** followed by S_N2 inversion with NaOLev or NaOBz gave the desired *R*-configuration at C-5 (**1.45** or **1.47**), which is required in the L-sugar.



Scheme 1.16: The S_N2 inversion of C-5 as published by Seeberger *et al.*[318] and Ojeda *et al.*[319]

- Inversion at C-5 *via* hydroboration/oxidation of an exocyclic olefin in 5-enopyranosides[320–322]

An example of this is seen in **Scheme 1.17** where Rochepeau-Jobron and co-workers[320] achieved inversion through hydroboration of the 5-enopyranoside **1.48** with 9-BBN followed by oxidative work-up to give the L-*ido* derivative **1.49** which was obtained as the major product.

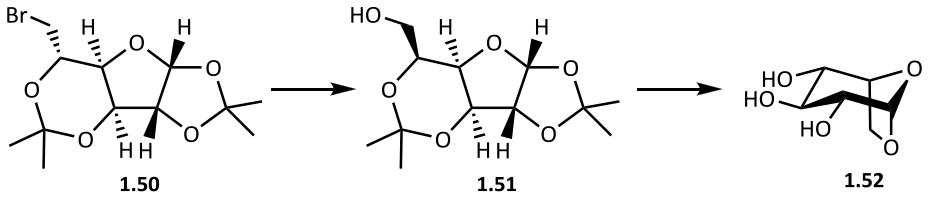


Scheme 1.17: Inversion of C-5 *via* hydroboration/oxidation.[320]

- Inversion *via* bromination, rearrangement, elimination and hydroboration/oxidation.

The synthesis of the 1,6-anhydro sugar **1.52**, an L-idose precursor described by Hung and co-workers is summarized in **Scheme 1.18**.[323,324] Starting with diacetone-α-D-glucose, one-pot S_N2 NBS-mediated bromination and rearrangement gave **1.50**, which was subjected to E_2 elimination and hydroboration/oxidation to yield the 1,2:3,5-di-*O*-isopropylidene-β-L-idofuranose (**1.51**) which after acid hydrolysis afforded **1.52**. The synthesis of AltNAcA using

the 1,6-anhydro sugar (**1.52**) as a starting point is currently being explored in this research group.

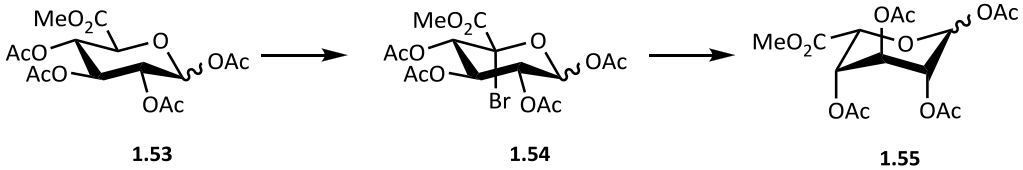


1.50 **1.51** **1.52**

Scheme 1.18: Inversion *via* bromination, rearrangement, elimination and hydroboration/oxidation which after acid hydrolysis yields the L-idose derivative **1.52**.[323,324]

- Inversion *via* free radical bromination at C-5 of a D-glucuronic acid derivative, followed by reduction.

Wong and co-workers[325] described the epimerization of C-5 *via* the free radical bromination of **1.53** yielding **1.54,** which after isomerization and free radical reduction yielded the D-*gluco* and L-*ido* derivative **1.55** in a 1:3 ratio (**Scheme 1.19**).

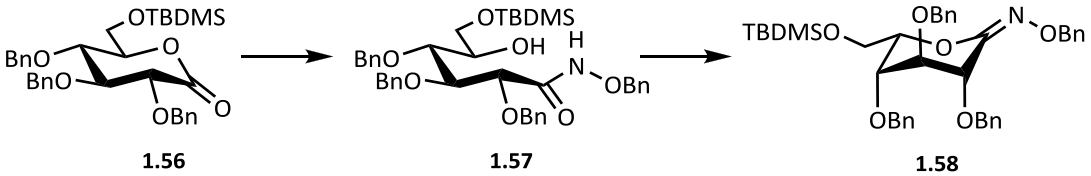


1.53 **1.54** **1.55**

Scheme 1.19: Epimerization of C-5 *via* the free radical bromination, isomerization and reduction.[325]

- Inversion *via* δ-hydroxybenzyloxamate.

The synthesis of an L-*ido* derivative published by Takahashi *et al*.[326,327] was obtainable from the 6-silylated lactone **1.56** *via* the alkoxyamidation to give the δ-hydroxyalkoxamate **1.57**. Cyclization under Mitsunobu conditions yielded the L-sugar **1.58** (**Scheme 1.20**).



1.56 **1.57** **1.58**

Scheme 1.20: C-5 Inversion *via* δ-hydroxybenzyloxamate **1.57**.[326,327]

1.13 Synthetic challenges and strategy

From the strategies for synthesizing FucNAc4N and AltNAcA (or other L-sugars) outlined above, it was decided that a cheap and readily available D-sugar would be a good starting point. The challenges beginning with, for example, a D-glucosamine precursor for the synthesis of these two unusual sugars are highlighted in **Figures 1.7A** and **1.7B**. The choice of D-galactosamine was considered, but thought to be unsuitable due to the unfavorable orientation of O-4 in respect of synthetic manipulations required at C-5 and C-6.

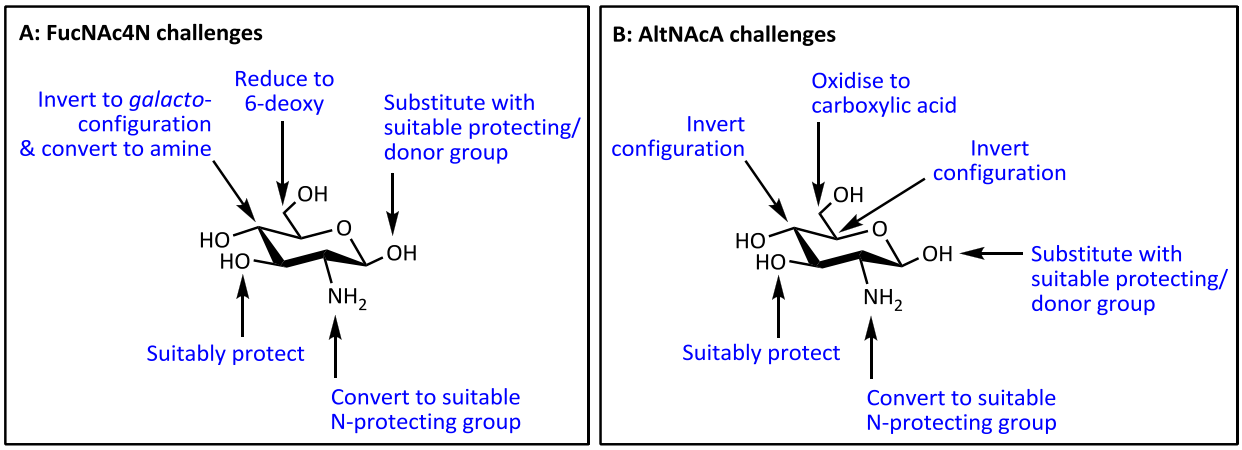
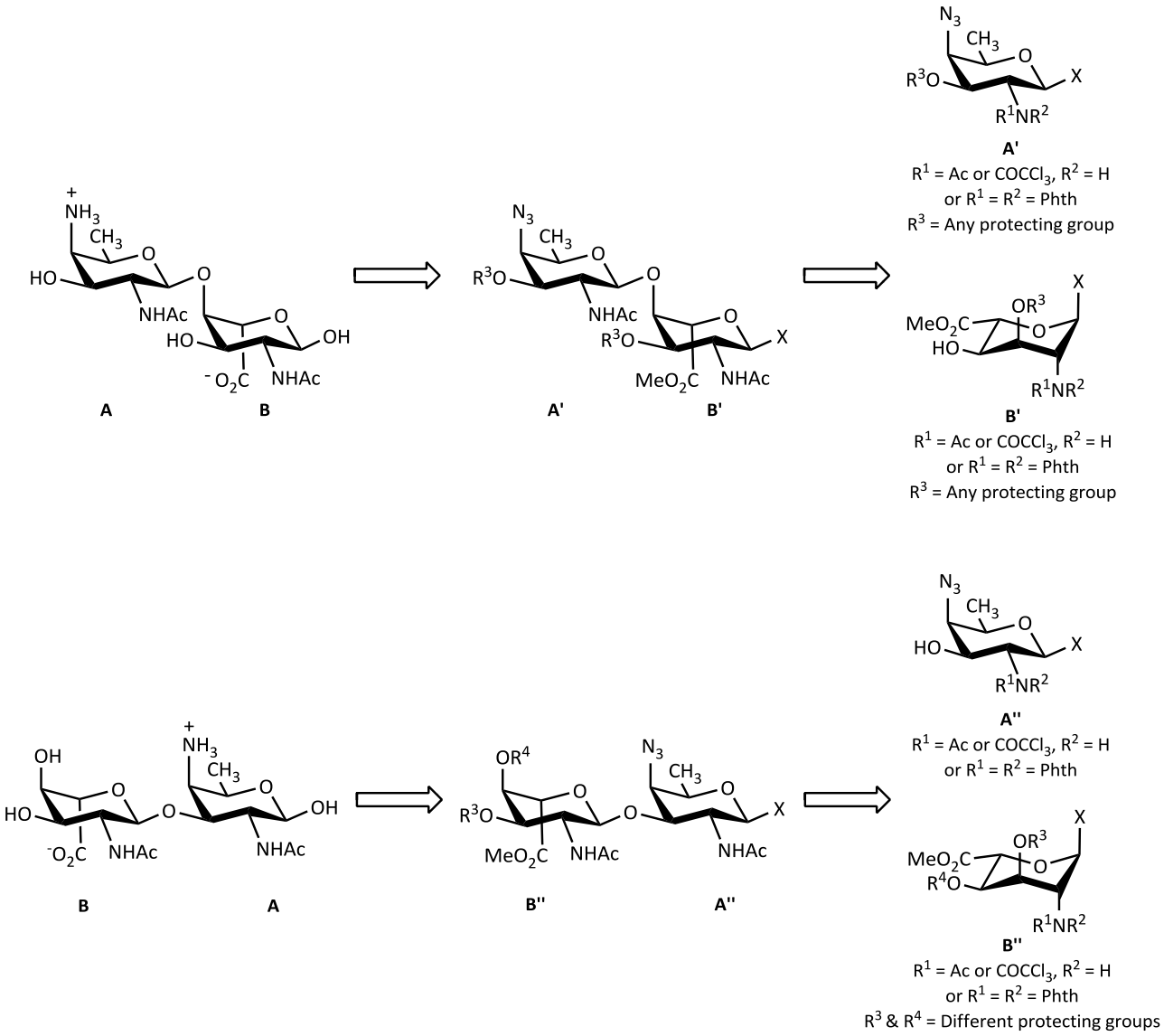


Figure 1.7: Synthetic challenges faced when synthesising the two monosaccharides, FucNAc4N and AltNAcA from an inexpensive and commercially available starting material such as D-glucosamine.

Retrosynthetic analysis of the two monosaccharides (**Scheme 1.21**) linked either in an **AB** or **BA** fashion reveals that both can be synthesised from the FucNA4N derivatives designated **A'** and **A''** and the AltNAcA derivatives designated **B'** and **B''**.



Scheme 1.21: Retrosynthetic analysis for the synthesis of the *S. sonnei* disaccharide in a suitably protected form. The disaccharides **AB** or **BA** can be obtained from the differentially protected monosaccharides **A'**, **A''**, **B'** and **B''**. The AltNAcA derivatives **B'** and **B''** should adopt the $^4C_1$ conformation as reported by Pozsgay[273] and Mulard[286] who noted this conformation in their 2-acetamido derivatives. This is also the case in the *S. sonnei* di- and tri-saccharides synthesised by Pfiste and Mulard.[286]

For the synthesis of the **A'**, **A''**, **B'** and **B''** sugars, the anomeric position (designated as X) needs to be suitably or differentially protected and easily converted to the free sugar post-glycosylation. From a literature survey of the vast array of strategies for simultaneously protecting the anomeric position and selectively priming it for activation, the alkyl or aryl thioglycosides seemed to be a suitable and versatile class of donors and acceptors.[258,328,329] Not only is their preparation generally straightforward, with the Lewis acid-mediated thiolysis of peracetylated sugars being the most direct method,[329,‡] but they act as anomeric protecting

‡ Refer to the Handbook of Chemical Glycosylations for an extensive list of methods.[258]

groups which can be selectively activated by soft electrophiles and can be used directly in glycosylation reactions, or indirectly through the selective transformation to an alternative donor group.[329] For example, a thioglycoside can be converted to a trichloroacetimidate donor *via* the hemiacetal,[329–332] which is obtained using *N*-bromosuccinimide (NBS) or *N*-iodosuccinimide (NIS) in wet acetone.[329,333–337] Alternatively, the so called 'two-step strategy' can be used to selectively transform a thioglycoside to the glycosyl halide which permits glycosylation in association with a variety of possible promoters.[329,338–342] An active-latent strategy can also be employed, which involves a modification of the donor group instead of its substitution. For example, an alkyl or aryl thioglycoside can be oxidized to yield a sulfoxide donor which can be selectively activated with Tf_2O.[343–347] Alternatively, a pre-activation strategy could be employed, This involves pre-activation of the thioglycoside donor and coupling this with a glycosyl acceptor incorporating the thioglycoside in an un-activated form.[329,348] In addition, these can also act as a glycosyl acceptor.[329,338,349–352] All of these transformations occur under different reaction conditions, thereby allowing for the chemoselective glycosylation to give either the **A'B'** or **B''A''** disaccharide. Furthermore, the reactivity of the thioglycosyl donor can be "tuned" by taking various factors into account – solvent, activating/deactivating protecting groups, C-2 neighboring group participation and activator systems, all of which can be investigated at the time of glycosylation.[329] This strategy will also allow for the possibility of chain elongation at each end.

Next, the 2-position must be suitably protected for glycosylation reactions in order to achieve the correct reactivity and selective formation of the 1,2-*trans* glycosidic linkage as desired in the **AB** and **BA** products (**Scheme 1.21**). The 2-acetamido functionality is present in both AltNAcA and FucNAc4N monosaccharides and is of particular concern as its presence in glycosyl donors and acceptors is known to reduce their reactivity.[353–360] For example, in the case of the donor, under glycosylation conditions, the oxocarbenium ion can rearrange to form the cyclic (2-methyl)oxazoline intermediate, which can be unreactive under harsh Lewis acid conditions.[360] In the case of glycosyl acceptors, the reactivity of the 4-hydroxyl group in *N*-acetyl glucosamine derivatives is generally low due to the suspected intermolecular hydrogen bonding from the *N*-H of the amide.[355,357–359] Although there is evidence for poorer reactivity of 2-acetamido derivatives, there are various reports that they can be used without manipulation in glycosylation reactions.[361–364] The advantage of starting with a 2-acetamido precursor is that no manipulations pre- and post-glycosylation are required, and this warrants an investigation in this study. Alternatively, the *N*,*N*-diacetyl or *N*-acetyl-*N*-benzyl derivatives could also be synthesised.[359] Most commonly however, *N*-trichloroacetyl or *N*-phthaloyl protecting groups have been employed to eliminate the poor reactivity of donors and acceptors. Both protecting

groups are capable of anchimeric assistance, *via* the more reactive oxazoline, which is made more reactive by the electron withdrawing chlorine atoms,‡ and the oxazolinium intermediate respectively.[288,289,365–369] These can be installed if necessary.[360]

In the case of the FucNAc4N monosaccharides **A'** and **A''**, the 4-position can be masked as an azide, which is compatible with almost all conditions and can be installed *via* a Mitsunobu-type reaction or by esterification at *O*-4 with triflic anhydride, followed by S_N2 substitution with NaN_3 to give the correct *galacto*-configuration if starting with a *gluco*-derivative. Alternatively, the azide can be reduced and protected as the *N*-Boc derivative, which is easily removed under acidic conditions. The 3-position of the **A'** unit can be protected with an ether or ester group (R_2 = Bn or Bz). The benefit of a benzyl ester protecting group is that it is removed during the reduction of the 4-azido group.

In the case of the AltNAcA monosaccharides **B'** and **B''**, the carboxylic acid moiety at C-6 can be protected as a methyl ester, and since discrimination between the *O*-3 and *O*-4 positions is required for the alternate coupling patterns, these will be protected with different groups, selected from esters, ethers or silyl ethers.

The proposed strategy for the synthesis of both sugars will be discussed in the subsequent chapters.

‡ Various authors report that this derivative makes excellent glycosyl donor intermediates,[288,365–368] while the persistent presence of this intermediate as a by-product in glycosidation reactions has also been reported.[289,369]

1.14 Aims of the project:

The aims of the project are twofold and involve:

- The synthesis of the two unusual sugars found in *S. sonnei*, 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose (FucNAc4N/AAT) and 2-acetamido-2-deoxy-α-L-altruronic acid (AltNAcA) in the suitably protected form of **A'**, **A''**, **B'**, **B''**. These will then be coupled to form the required disaccharides **AB** and **BA** which can be used as standards for the physicochemical characterization of the *S. sonnei* glycoconjugate vaccine being developed by GlycoVaxyn.
- The analysis of glycoconjugate and glycopeptide samples derived from *S. flexneri* 2a obtained from GlycoVaxyn using NMR spectroscopy. Detailed 1D and 2D NMR spectroscopy will be used to determine the structural identity and integrity of the conjugated carbohydrate antigen.

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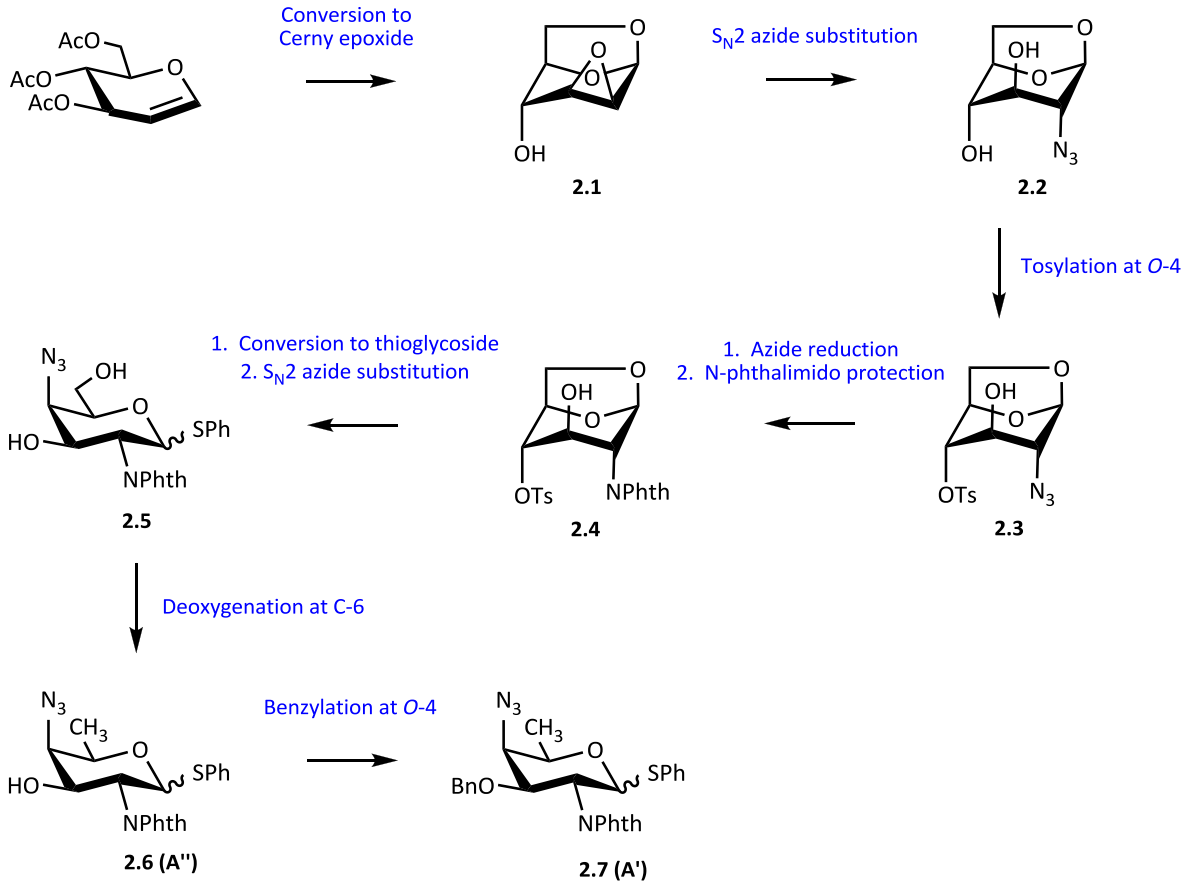
Chapter 2: Design and evaluation of alternative synthetic routes to derivatives of 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose (FucNAc4N)

2. 1 Proposed synthesis of the protected FucNAc4N derivative *via* the 1,6-anhydro sugar route

On the basis of considerations discussed in **Ch 1.13**, and the challenge of pursuing some alternative routes towards a versatile, robust synthesis, a survey of the literature revealed the 1,6-anhydro-2-azido-2-deoxy-4-*O*-tosyl-β-D-glucopyranose **2.3** synthesised by Karban *et al*.[1] to be a suitable starting point (**Scheme 2.1**). This would serve as an ideal intermediate as the 2-azido group can be reduced and protected as any acylamino functionality, for later conversion to the 2-acetamido group, while the tosyl group at *O*-4 can later be substituted to give the 4-azido D-*galacto*-derivative **2.5**. In addition, the 1,6-anhydro bridge provides effective temporary protection of the anomeric position, which can later be converted to a suitable glycosyl donor or the free sugar.

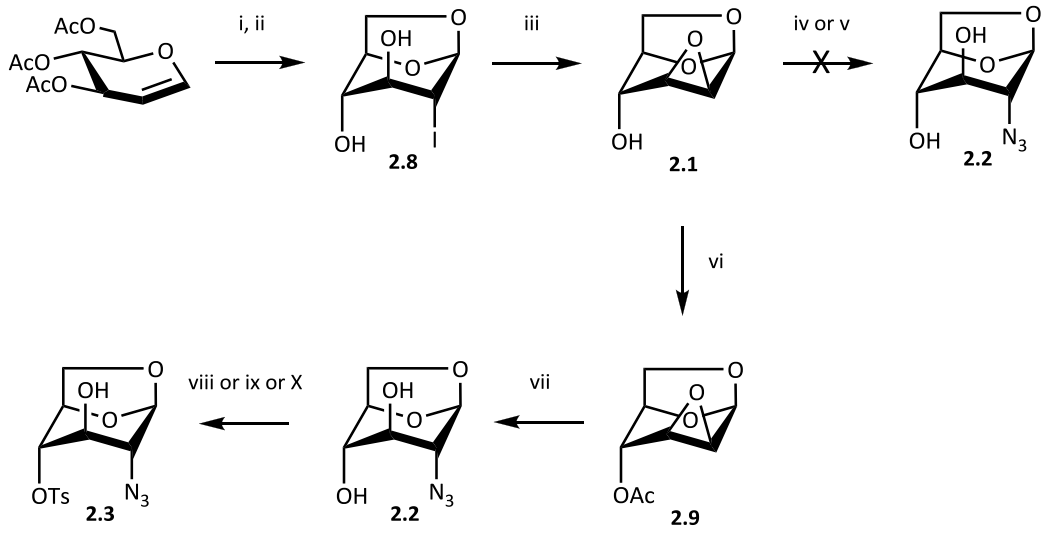
A plausible route, based on literature precedent, is outlined in **Scheme 2.1**. The key intermediate **2.3** can be obtained in two steps from D-glucal *via* the Ćerný epoxide **2.1.**[2–5] Selective nucleophilic opening of the epoxide with NaN_3 should then give the diol **2.2**,[6] which has the 2,3-*trans*-diaxial substituent geometry in place, and regioselective tosylation at the less hindered hydroxyl group would give access to the key intermediate **2.3**.[1] Reduction of the azide and protection with phthalic anhydride should yield **2.4**. Cleavage of the anhydro ring with TMSSPh[7] should then afford the thioglycoside, while C-4 azide substitution should then yield **2.5**, with the desired *galacto*-configuration as required in the FucNAc4N derivative. From here, conversion to the 6-deoxy sugar could be achieved by the Barton-McCombie radical deoxygenation method[8–10] yielding **2.6** (**A''**), which after benzylation at *O*-3 can give the protected derivative **2.7** (**A'**).



Scheme 2.1: Proposed synthesis of a protected FucNAc4N derivative *via* the 1,6-anhydro-2-azido-2-dideoxy-4-*O*-tosyl-β-D-glucopyranose (**2.3**).

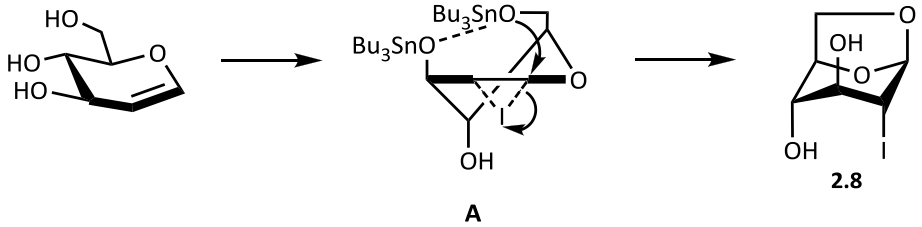
2.2 Towards the synthesis of the protected FucNAc4N derivative *via* the 1,6-anhydro sugar

The synthesis began with the commercially available 3,4,6-tri-*O*-acetyl-D-glucal (**Scheme 2.2**). Deacetylation followed by treatment with bis(tributyltin)oxide and iodine in ACN gave the 2-iodo-1,6-anhydroglucose (**2.8**) in an excellent yield.[4]



Scheme 2.2: Towards the synthesis of a protected FucNAc4N derivative *via* the 1,6-anhydro sugar route. Reagents & conditions: (i) MeOH/H_2O/Et_3N (10:10:1), r.t., 30 mins (ii) a.) $(Bu_3Sn)_2O$, CH_3CN, reflux, 3 hrs b.) I_2, r.t., 2 hrs, 85% over 2 steps (iii) $NaHCO_3$, DMF/H_2O (10:3), 120°C, 4 hrs, 85% (iv) NaN_3, NH_4Cl, MeOH/H_2O, reflux, overnight (v) NaN_3, DMF/H_2O, 80°C, 9 hrs (vi) Ac_2O, pyridine, r.t., overnight, 84% (vii) NaN_3, DMF/H_2O (9:1), 110°C, 9 hrs, 66% (viii) *p*-TsCl, pyridine, r.t., 48 hrs (ix) *p*-TsCl, Et_3N, DCM, 0°C to r.t., overnight, 19% (x) a.) $(Bu_3Sn)_2O$, toluene, reflux, 3 hrs b.) *p*-TsCl, DMAP, 1,4-dioxane, 0°C to r.t., 27 hrs, 25%.

The formation of **2.8** is thought to proceed *via* the tin-mediated 1,6-iodocyclization of the D-glucal (**Scheme 2.3**) as postulated by Czernecki and co-workers.[11] The D-glucal forms the cyclic iodonium cation intermediate **A** which probably adopts the $^5H_4$ conformation due to intramolecular chelation of the pseudo-axial *O*-3 and the tin atom at *O*-6, with additional stabilization by the co-ordination of the stannylene complex to acetonitrile. The iodonium cation forms exclusively on the bottom face of the molecule, and upon nucleophilic substitution of *O*-6 at the electrophilic C-1, opens up to form the rigid 1,6-anhydro sugar **2.8**.[11]

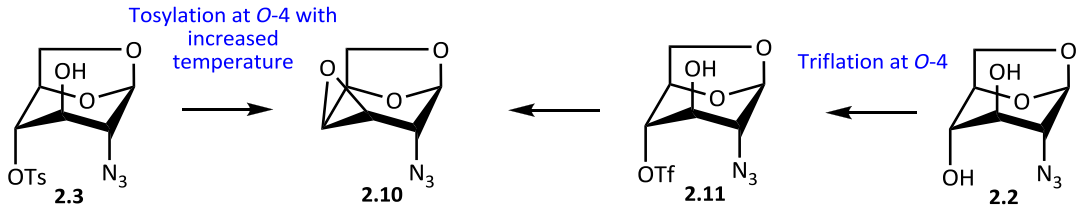


Scheme 2.3: Proposed mechanism for the formation of the 1,6-anhydro-2-deoxy-2-iodo-β-D-glucopyranose (**2.8**) *via* the tin-mediated 1,6-iodocyclization of D-glucal.[11]

The Ćerný epoxide **2.1** was then obtained *via* the S_N2 displacement of the iodine.[5] However, subsequent nucleophilic addition of an azide to form the 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**2.2**) was not achieved, despite attempts under various conditions.[6] However, as

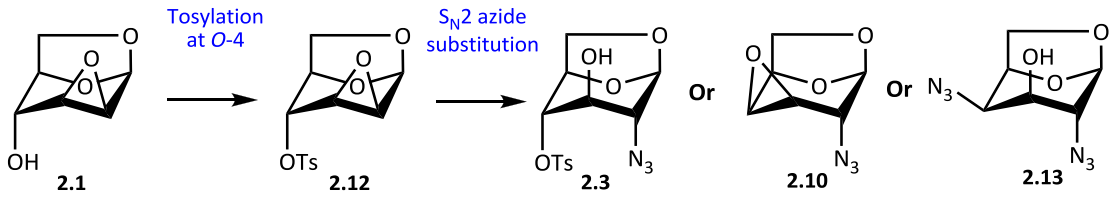
an alternative, it was found that acetylation‡ of **2.1** at *O*-4 to form acetate **2.9** followed by treatment with NaN_3 at a slightly higher temperature yielded the desired 2-azido sugar **2.2**,[5] which had evidently also undergone de-*O*-acetylation, possibly due to the basicity of NaN_3.[12] The next step required regioselective tosylation at *O*-4. Treatment of **2.2** with 1.1 eq. of freshly recrystallized *p*-TsCl in pyridine in an attempt to form the mono-tosylate **2.3** as described by Karban *et al.*,[1] yielded no result, while use of *p*-TsCl in DCM containing Et_3N gave **2.3** in a low yield (19%), with evidence for trace amounts of the di-tosylated product. In an attempt to optimize the yield and maintain regioselectivity, **2.2** was treated with dibutyltinoxide in refluxing toluene, followed by azeotropic removal of the toluene and treatment with *p*-TsCl and DMAP.[15] After 27 hours the yield of **2.3** was only increased to 25%. The limited success of this reaction was surprising in view of the literature precedent as well as the reported regioselective benzylation at *O*-4 of **2.2**.[6,16]

At this point, an increase in temperature of the tosylation reaction was contemplated, but it was anticipated that this could lead to the formation of the 1,6:3,4-di-anhydro sugar **2.10** as shown in **Scheme 2.4**. Triflation of the 4-*O*-position would most likely have led to the same result (**Scheme 2.4**).[17] Similarly, it was anticipated that tosylation at *O*-4 of the Ćerný epoxide **2.1** (**Scheme 2.5**) giving **2.12**, followed by treatment with NaN_3 could yield the desired 4-*O*-tosylate **2.3**, but could also form the 1,6:3,4-di-anhydro sugar (**2.10**) under the high temperatures required for azide substitution, and in fact this was found to be the case.[18] Alternatively, the di-azide **2.13** could also form.



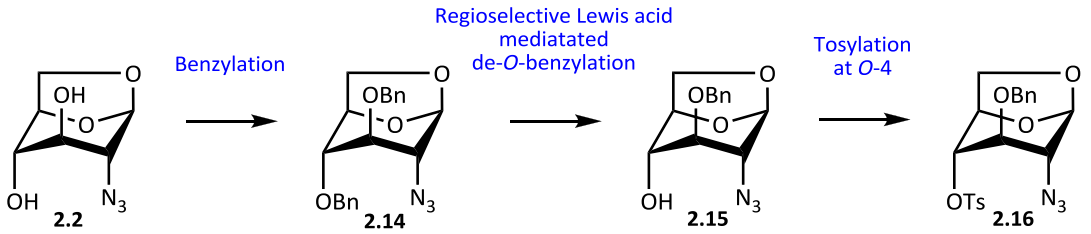
Scheme 2.4: Potential formation of the 2-azido-1,6:3,4-dianhydro sugar **2.10,** either from **2.3** or from 4-*O*-triflation (**2.11**) of **2.22**.

‡ Under strong basic conditions the Ćerný epoxide **2.1** has been known to undergo a Payne rearrangement.[13,14]



Scheme 2.5: Potential side products (**2.10** and **2.13**) that could be formed from attempting to synthesis the 1,6-anhydro-2-azido-2-deoxy-4-*O*-tosyl-β-D-glucopyranose (**2.3**) *via* the 4-*O*-tosyl-1,6:2,3-di-anhydro derivative (**2.12**).

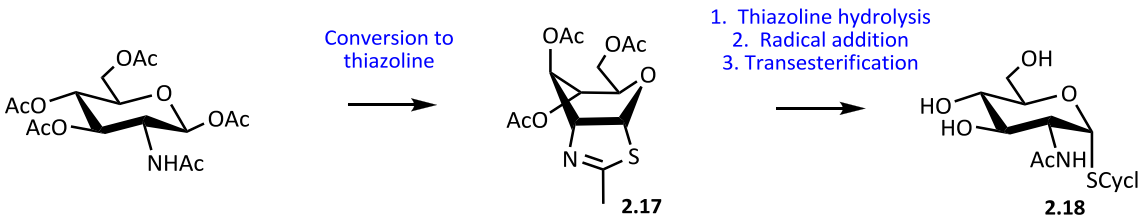
In view of the difficulties encountered with the formation of the key 4-*O*-tosyl derivative **2.3**, this overall route to the FucNAc4N derivative was abandoned at this point. With hindsight, an alternative worth exploring would be to convert diol **2.2** to the di-benzyl ether **2.14** (**Scheme 2.6**) with a view to carrying out a regioselective de-*O*-benzylation using a Lewis acid as described by Hori *et al.*,[19] where the 3-*O*-benzyl ether **2.15** was obtained in an excellent yield. This could then be treated with excess *p*-TsCl to give **2.16** which incorporates the desired 4-tosyloxy group.



Scheme 2.6: An alternative reaction sequence that could be carried out to obtain the 4-*O*-tosyl derivative **2.16**.

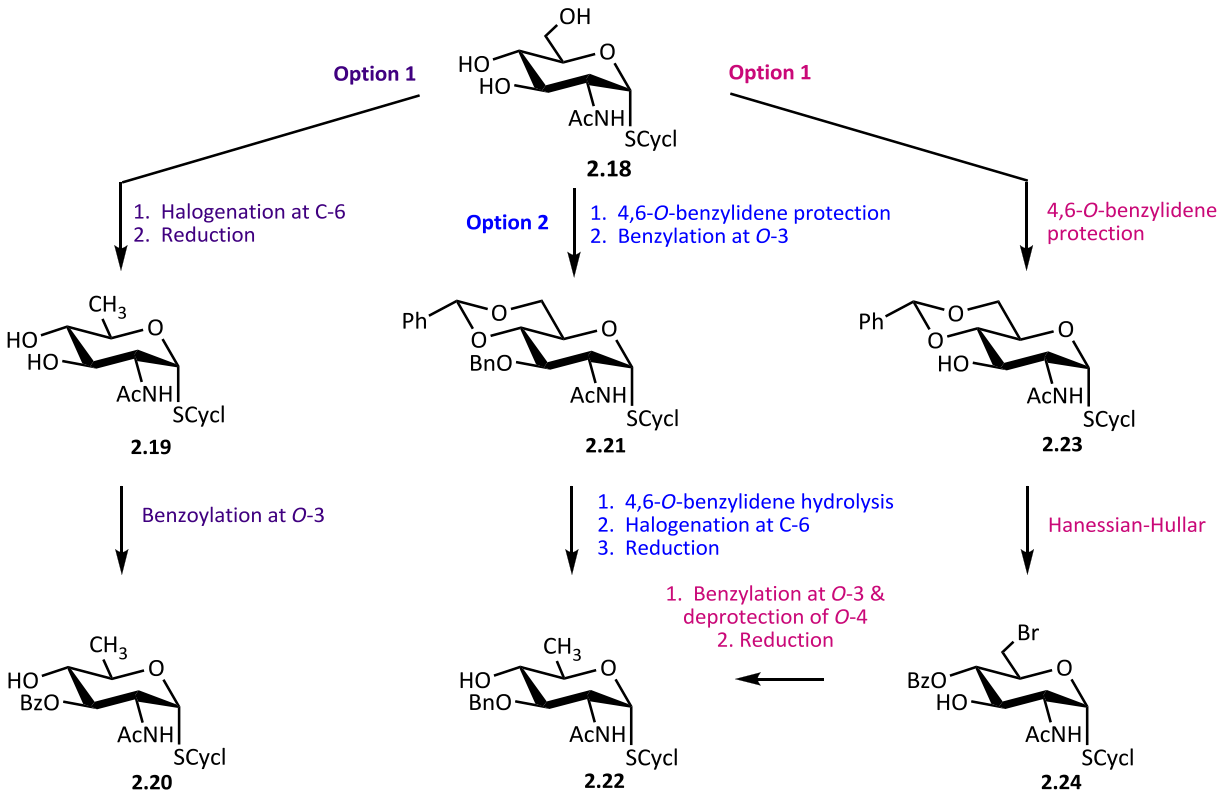
2.3 Proposed synthesis of the protected FucNAc4N derivative *via* a sugar thiazoline

An alternative route to the FucNAc4N derivative was then considered, based on work published by Knapp and co-workers and involving the synthesis of the thiazoline **2.17** and its conversion to thioglycoside **2.18** (**Scheme 2.7**) for further elaboration.[20–24] As demonstrated by Knapp *et al.* the commercially available 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucose can be readily converted to the thiazoline **2.17** using Lawesson's reagent. Acid hydrolysis of **2.17**, followed by radical addition to cyclohexene and transesterification will yield the thioglycoside **2.18** in exclusively the α-configuration.



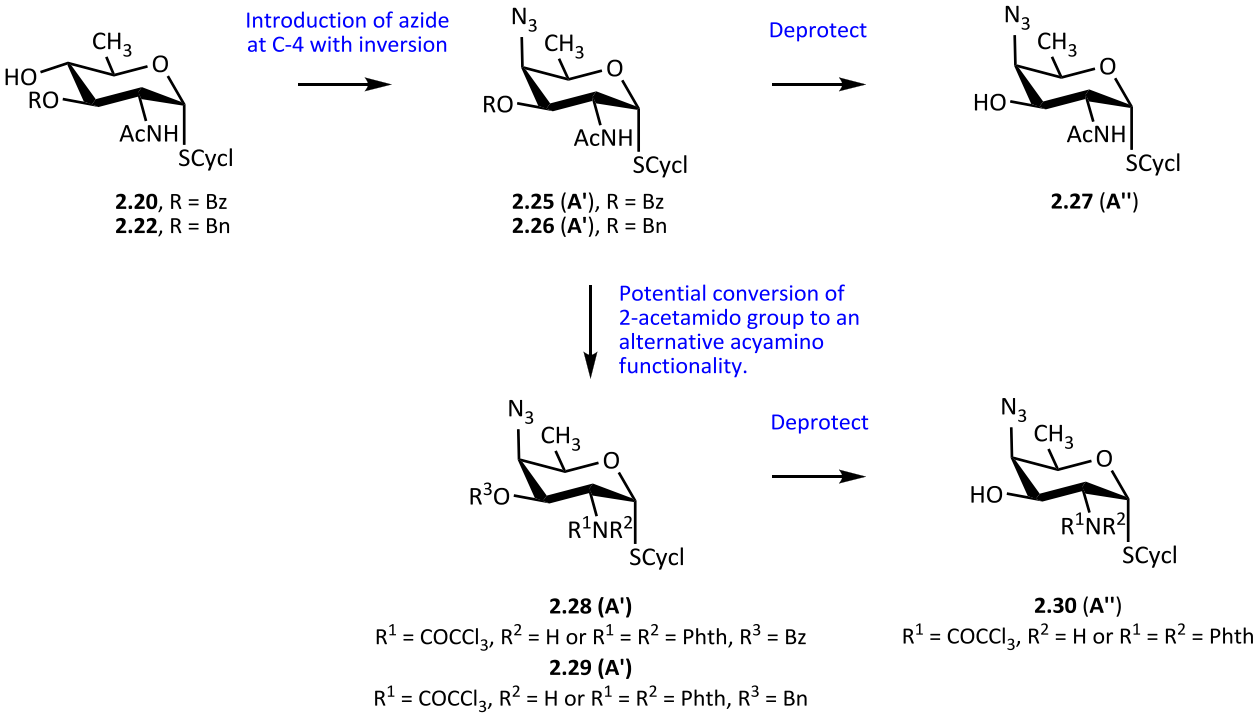
Scheme 2.7: Proposed synthesis of the triol **2.18**.[20–24]

At this point, various strategies could be employed to provide access to the regioselectively protected 6-deoxy sugars (**2.20**) and (**2.22**) (**Scheme 2.8**), with three options selected for further exploration. The first alternative (option 1) would involve halogenation at C-6 followed by reduction to yield the diol **2.19**. Then using the same approach as Liang and Grindley,[25] regioselective protection of *O*-3 should then give the benzoyl ester **2.20**. A second approach (option 2) would involve the synthesis of the 4,6-*O*-benzylidene acetal, followed by benzylation at *O*-3 to yield **2.21**. Acetal hydrolysis and regioselective halogenation, followed by reduction would then give **2.22**. The third option would utilize the 4,6-*O*-benzylidene acetal **2.23** which when subjected to the Hanessian-Hullar radical-mediated acetal fragmentation reaction conditions, could directly give the 6-bromo-4-*O*-benzoyl derivative **2.24.** Protection at *O*-3 and deprotection of *O*-4, followed by C-6 reduction could then yield the 6-deoxy sugar **2.22**. This last option easily differentiates the 3-*O* and 4-*O* positions, but would require an extra protection/deprotection step.



Scheme 2.8: Three possible options for the synthesis of the regioselectively protected sugars **2.20** or **2.22**.

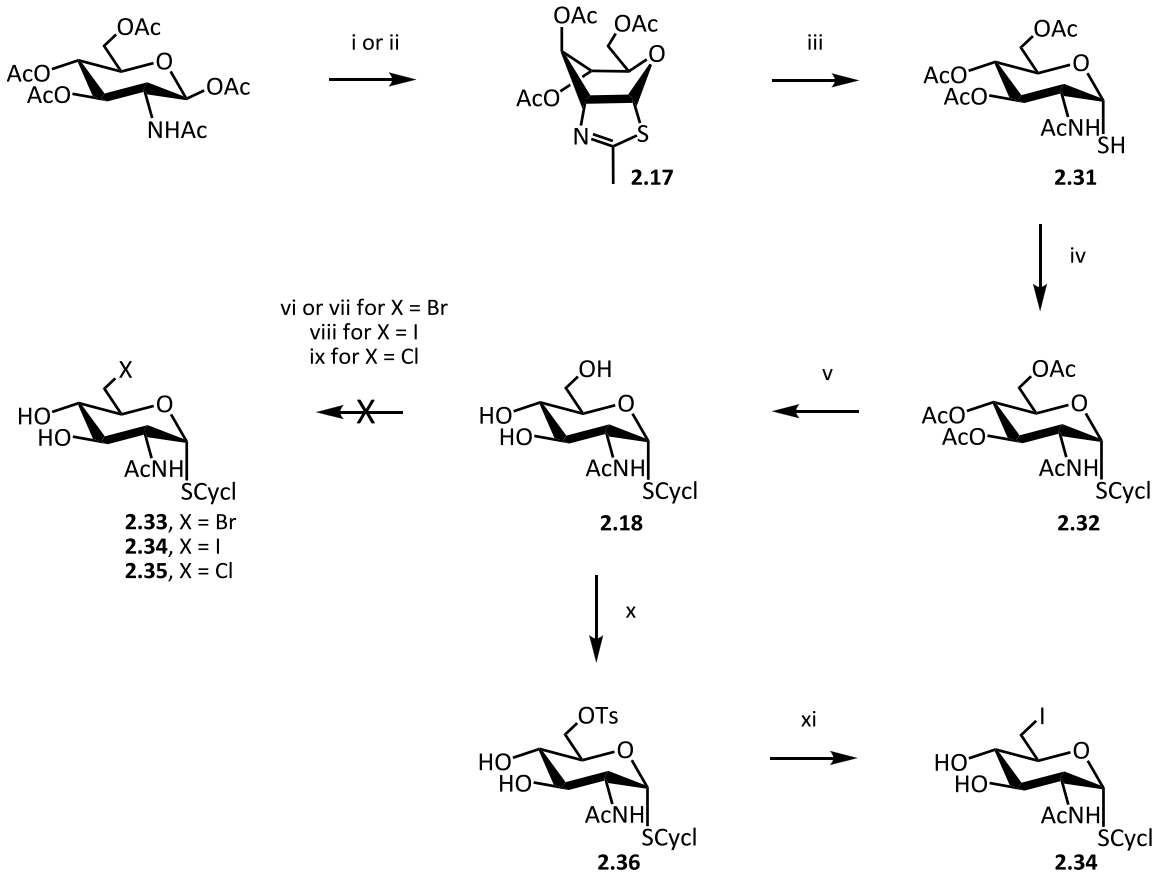
The final steps (**Scheme 2.9**) would involve initial introduction of an azide at C-4 of **2.20** or **2.22** with inversion of configuration, either under Mitsunobu-type conditions or *via* a triflation/azide displacement sequence to yield **2.25** or **2.26** both in the required **A'** form, which after deprotection could yield **2.27** (**A''**). At this point, the conversion of the 2-acetamido group to an alternative acylamino functionality could also be investigated, yielding the alternative derivatives **2.28** and **2.29** (**A'**) and **2.30** (**A''**).



Scheme 2.9: Proposed synthesis of the differentially protected FucNAc4N derivatives **2.25, 2.26, 2.28** or **2.29** (**A'**) and **2.27** or **2.30** (**A''**).

2.4 Towards the synthesis of the protected FucNAc4N derivative *via* the thiazoline sugar – evaluation of option 1

Starting with the commercially available 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, the thiazoline **2.17** was synthesised using either Lawesson's reagent (LR)[20] or phosphorus pentasulfide (P_4S_{10})[26] as the thiolating agent which proceeds *via* the thiophosphine ylide (**Scheme 2.10**).[27–29] LR is costly and purification is troublesome on larger scales, so in this instance it was used on small scale reactions (< 2 g) while P_4S_{10} was used on larger scale (> 2 g). In both cases, the reaction proceeded smoothly under anhydrous conditions to give 82% and 96% yields respectively of the thiazoline **2.17**. The use of hexamethyldisiloxane (HMDO) was found by Curphey *et al.*,[30] to reduce the formation of phosphorus and sulfur by-products, eliminating any purification complications. In addition, HMDO is also thought to quench acetic acid formed as a result of cyclization.

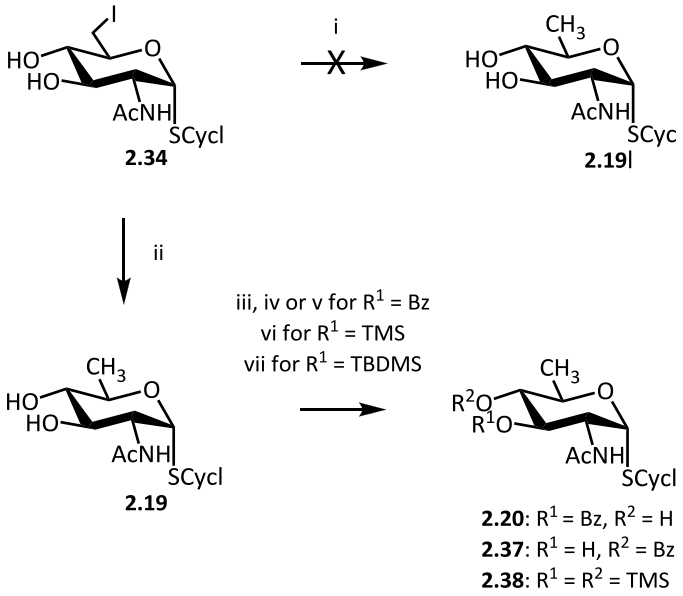


Scheme 2.10: Towards the synthesis of theFucNAc4N derivative *via* the thiazoline route (Option 1). Reagents & conditions: (i) Lawesson's reagent, toluene, 3Å MS, reflux, 4.5 hrs, 82% (ii) P_2S_{10}, hexamethyldisiloxane, $CH(CH_3)_2OAc$, 3Å MS, 100°C, 12 hrs, then r.t., 24 hrs, 96% (iii) TFA, H_2O, MeOH, 0°C to 5°C overnight, r.t., 4 hrs, 57% (iv) ACCN, cyclohexene, $CHCl_3$, 75°C, 37 hrs, 66% (v) NaOMe, MeOH, 0°C to r.t., 30 mins, 63% (vi) CBr_4, PPh_3, pyridine, 3Å MS, 0°C to 35°C, 23 hrs (vii) CBr_4, PPh_3, pyridine, 3Å MS, 50°C, 13 hrs (viii) I_2, PPh_3, imidazole, THF, reflux, 5 hrs (ix) $SOCl_2$, DMF, 3Å MS, 0°C to r.t., 1 hr. (x) *p*-TsCl, pyridine, 3Å MS, 0°C, 2 hrs, 81% (xi) NaI, 2-butanone, reflux, 7 hrs, then r.t., 18 hrs, 97%.

Following this, **2.17** was hydrolysed to the 2-acetamido-1-thiol **2.31** in yields ranging between 20% and 57%.[20,21,‡] This was then set up for the free-radical addition to cyclohexene using ACCN as an initiator. Knapp and co-workers only ever carried out these reactions on small scale (~200 mg), and so considerable optimisation was required in order to achieve reasonable yields (66%) on a large scale. This involved altering the ratio of dry and degassed cyclohexene and chloroform as co-solvent, adjusting the rate of addition of the radical initiator, which was found to be critical, and monitoring the reaction time to decrease degradation. Once the cyclohexyl thioglycoside **2.32** had been synthesised, transesterification under Zemplén conditions gave the triol **2.18**.[24]

‡ No other products forming as judged by TLC.

Various attempts at conversion to the C-6 halo derivative were then explored. Unfortunately, all attempts at direct formation of halides **2.33** – **2.35** failed and so in the end, a two-step sequence was adopted in which the 6-OH was selectively tosylated to give **2.36** (81%), which after treatment with NaI in refluxing 2-butanone gave the 6-iodo analogue **2.34** (97%) in a yield of 78% over the two steps. Confirmation of the introduction of iodine at C-6 was seen in the ^{13}C NMR spectrum (**Appendix 2**), where C-6 was now significantly shielded with a signal upfield at 9.26 ppm.[31] With the halogen in place at C-6, reduction was required (**Scheme 2.11**). Metal hydrides such as $LiAlH_4$ and $NaBH_4$ could not be used due to the presence of the 2-acetamido group. So instead Zn/AcOH was used as described by Medgyes *et al*.[32] but this led to the formation of multiple unidentified products. However, simple reduction using H_2 over Pd/C at 4 bar for 6 hours cleanly gave the 6-deoxy sugar **2.19**, with the appearance of the shielded three-proton doublet at 1.24 ppm in the ^1H NMR spectrum (**Figure 2.1**) providing clear evidence of this transformation. This set the stage for the challenge of differentiating the *trans*-vicinal equatorial hydroxyl groups at C-3 and C-4, presumably characterized by only very subtle differences in environment and reactivity.



Scheme 2.11: Towards the synthesis of the protected FucNAc4N derivative *via* the thiazoline route (Option 1) continued. Reagents & conditions: (i) Zn, AcOH, DCM, r.t., 5.5 hrs (ii) H_2, Pd/C, MeOH, 4 bar, r.t., 6 hrs, 97% (iii) BzCl (1.1 eq.), pyridine, 3Å MS, -35°C, 3.5 hrs, 5°C, 14 hrs (iv) BzCl, (1.1 eq., drop wise), Et_3N (1.1 eq.), 3Å MS, DCM, 5°C, 22 hrs (v) BzCl (1.2 eq.), pyridine, 0°C, 2 hrs, **2.20**: 35%, **2.37**: 24% (vi) TMSCl (1.1 eq. + 0.6 eq.), Et_3N (3 eq.), THF, 0°C to 5°C, 99 hrs, 49% (vii) TBDMSCl (1.1 eq.), imidazole (1.5 eq.), DMF, 0°C, 2.5 hrs, r.t., 17 hrs.

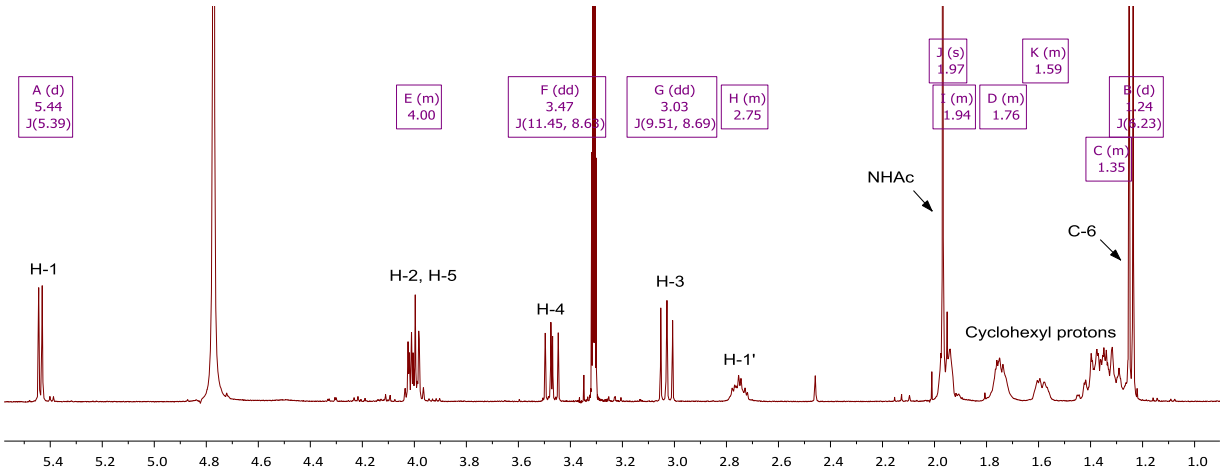


Figure 2.1: ^{1}H NMR spectrum of the cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (**2.19**) in MeOD.

The simple, direct regioselective protection of *trans*-vicinal diols in α- and β-6-deoxy sugars using 1.1 equivalents of protecting reagent at low temperatures (0°C to -80°C) has been described by various authors,[25,33–35] with many others reporting similar selective *O*-3 protection on other sugar substrates.[36–41] In light of this, several reaction conditions were investigated. Firstly, direct benzoylation of *O*-3 was attempted, using one equivalent of benzoyl chloride at low temperatures in either pyridine or dichloromethane with trimethylamine as base. No reaction was observed under either conditions. A slight increase in the number of equivalents of BzCl resulted in formation of two products in low yield, duly identified as isomeric mono-benzoates **2.20** and **2.37**. Here the increased reactivity of the 4-*O*-position in **2.19** may be attributed to the electron withdrawing nature of the acetamido functionality at C-2,[41] or alternatively may be due to intramolecular hydrogen bonding between the 3-OH and acetamido carbonyl group, although this contradicts the reported literature findings. On the other hand, acyl migration of an initially selectively formed 3-*O*-benzoate may be possible. As an alternative, treatment of **2.19** with TMSCl yielded a single product together with unreacted starting material as judged by TLC, with ^{1}H NMR revealing the disilylated derivative **2.38** to have formed. The more bulky silylating reagent TBDMSCl proved unreactive in these conditions, with no evidence of formation of a silyl ether.

Various methods have been explored for obtaining selectively protected carbohydrates using reagents or catalysts that enhance the reactivity at one of several hydroxyl groups. One such approach is the use of a stannylene compounds.[42–50] Other examples include the use of chiral and achiral diamines;[40,51] boronic acid catalysts[52–55] (those reported by Lee and Taylor[55,56] are only effective on *cis*-diols); iodine;[57] bis(2-oxooxazolidin-3-yl)phosphinic chloride (BOP-CI)[38]

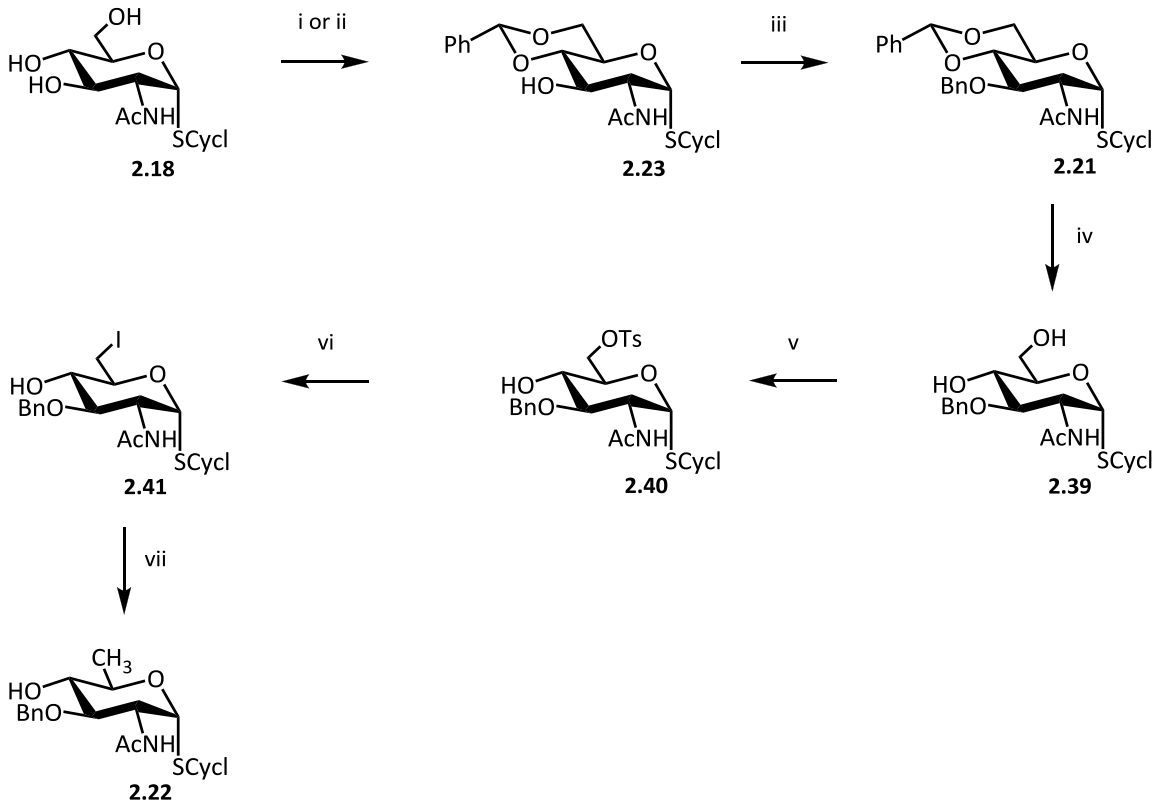
and silver carbonate;[58] $MoCl_5$[59] (limited scope on diols); $CuCl_2$;[53,60] $ZnCl_2$;[61] and the *in situ* generation of 1-acyloxy-1-*H*-benzotriazoles.[62,63] In addition, a complementary approach is to proceed *via* full protection followed by regioselective deprotection.[64–66]

While we were aware of all of these approaches, the results at this point indicated a very subtle difference in reactivity and stereoelectronic environment of *O*-3 and *O*-4 in **2.19**. The decision was therefore taken to explore the alternative options which provided the more unambiguous differentiation, utilizing the simultaneous protection of *O*-4 and *O*-6 to set up selective protection of the 3-OH.

2.5 Towards the synthesis of the protected FucNAc4N derivative *via* a 4,6-*O*-benzylidene derivative of the thiazoline sugar – evaluation of option 2

In order to prepare the 4,6-*O*-benzylidene acetal, triol **2.18** was treated with a catalytic amount of CSA and benzaldehyde dimethyl acetal in DMF to give the desired sugar acetal **2.21**[67] in low yield. The use of benzaldehyde and $ZnCl_2$ gave higher yields and despite a lengthier and more complicated purification procedure, was chosen as the method of choice for this conversion (**Scheme 2.12**).[68] Benzylation at *O*-3 proceeded smoothly to yield **2.21**, which after treatment with TFA/H_2O yielded the diol **2.39**. This was then regioselectively tosylated (**2.40**) and then further converted to the 6-iodo analogue **2.41** as before. Since hydrogenation of **2.41** could potentially remove the 3-*O*-benzyl group, alternative conditions were required for the reduction at C-6. So, iodide **2.41** was treated with Bu_3SnH and ACCN,[69] yielding the 6-deoxy sugar **2.22** in a low yield of 20%, accompanied by other products as judged by TLC.

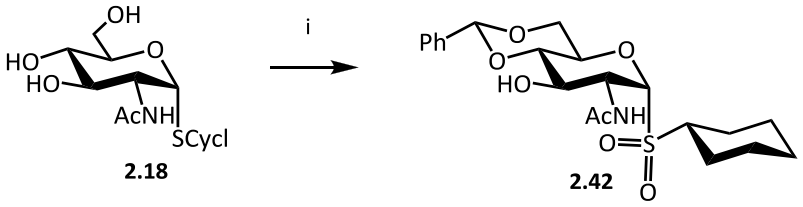
At this point, the regioselective reductive cleavage[70] of the 4,6-*O*-benzylidene acetal **2.23** would indeed have been another option to explore, but it was overlooked at the time.



Scheme 2.12: Alternative synthesis towards the protected FucNAc4N derivative *via* the thiazoline route (Option 2). Reagents & conditions: (i) CSA, benzaldehyde dimethyl acetal, DMF, 3Å MS, 0°C to 50°C, 16 hrs, 34% (ii) $ZnCl_2$, benzaldehyde, 3Å MS, r.t., 17.5 hrs, 76% (iii) NaH, BnBr, TBAI, THF, 0°C to 2.5 hrs, 72% (iv) TFA/H_2O, DCM, 0°C ,1.5 hrs, 75% (v) *p*-TsCl, pyridine, 3Å MS, 0°C, 2 hrs, 42% (vi) NaI, 2-butanone, reflux, 7 hrs, then r.t., 18 hrs. 90% (vii) ACCN, Bu_3SnH, toluene, 3Å MS, 75°C, 2 hrs, 20%.

It is pertinent to note at this point that in the process of preparing a further sample of the 4,6-*O*-benzylidene **2.23**, by repeating the sequence outlined above, the sulfone **2.42** (**Scheme 2.13**) was instead obtained.‡ It is not at all clear how oxidation of the sulfur occurred, although it was not viewed as problematic since glycosyl sulfones are capable of being activated with $MgBr_2$ etherate in glycosylation reactions, and is therefore a viable masked glycosyl donor.[71,72] Since enough of the sulfone **2.42** was available, it was used to evaluate option 3.

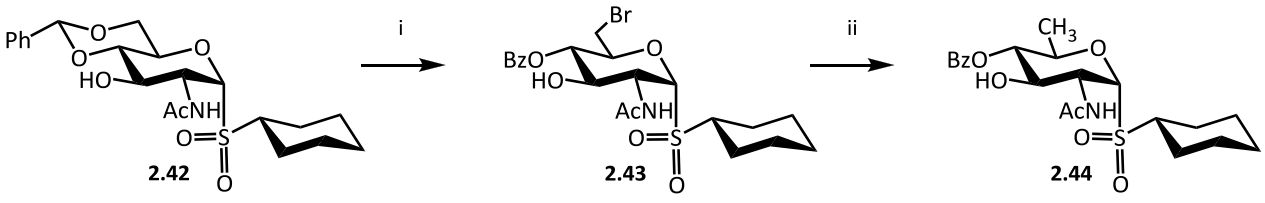
‡ The only difference in the preparation of **2.23** and **2.42** was in the work-up: in the case of **2.23**, work-up involved washing the reagent mixture with water, before concentrating the organic phase and chromatographing the residue, whereas in the formation of **2.42**, the water wash was omitted and solvents removed directly to form a residue which was chromatographed.



Scheme 2.13: Oxidation of **2.18** forming the sulfone **2.42**. Reagents and conditions: (i) $ZnCl_2$, benzaldehyde, 3Å MS, r.t., 19 hrs, 61%.

2.6 Towards the synthesis of the protected FucNAc4N derivative *via* alternative processing of the 4,6-*O*-benylidene derivative of the thiazoline sugar – evaluation of option 3

Next, the Hanessian-Hullar radical-mediated acetal fragmentation reaction was explored (**Scheme 2.14**). This potentially offered an efficient way of obtaining the desired C-6 halogenated derivative, while simultaneously allowing for differentiation of *O*-3 and *O*-4. This one-step transformation should reduce the amount of manipulations in the overall sequence despite requiring the extra protection at *O*-3 and deprotection of *O*-4. So, treatment of benzylidene acetal **2.42** with NBS and $BaCO_3$ successfully yielded the 4-*O*-benzoyl-6-bromo-6-deoxy intermediate **2.43** in a 70% yield (**Scheme 2.14**). Evidence for the formation of **2.43** was seen in the ^{1}H NMR spectrum (**Figure 2.2**), with the disappearance of the signal for the benzylic methine proton, while a more deshielded H-4, resonating at 4.56 ppm (*vs*. 3.58 ppm for **2.42**) was noted. The HMBC experiment definitively confirmed 4-*O*-benzoylation by virtue of the observable long-range coupling between the benzoyl carbonyl carbon and H-4. With **2.43** in hand, it was possible to reduce C-6 by hydrogenation as before to yield the 4-*O*-benzoyl-6-deoxy sugar **2.44.**



Scheme 2.14: Alternative synthesis towards the protected FucNAc4N derivative *via* the thiazoline route (Option 3). Reagents & conditions: (i) NBS, $BaCO_3$, CCl_4, 3Å MS, reflux, 2 hrs, 70% (ii) H_2, Pd/C, MeOH, 4 bar, r.t., 7.5 hrs, 86%.

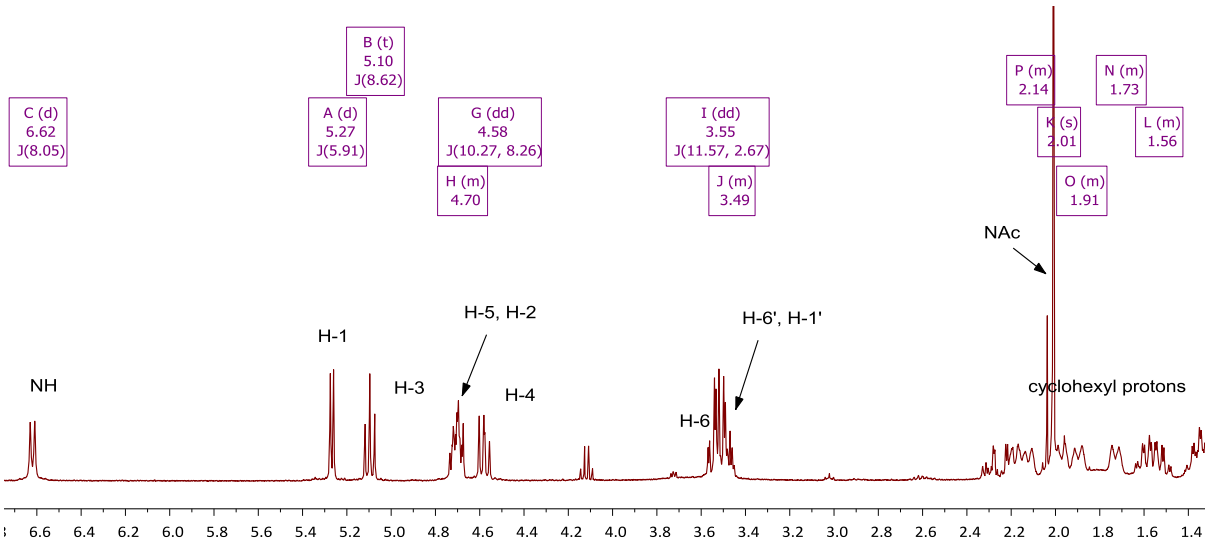
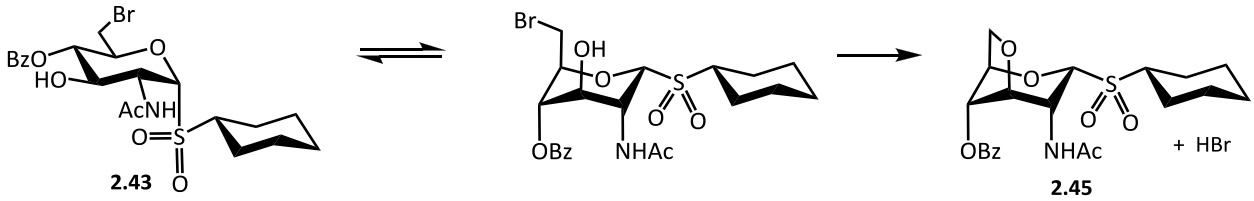


Figure 2.2: ^{1}H NMR spectrum of the cyclohexyl 2-acetamido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-1-sulfonyl-α-D-glucopyranoside (**2.43**).

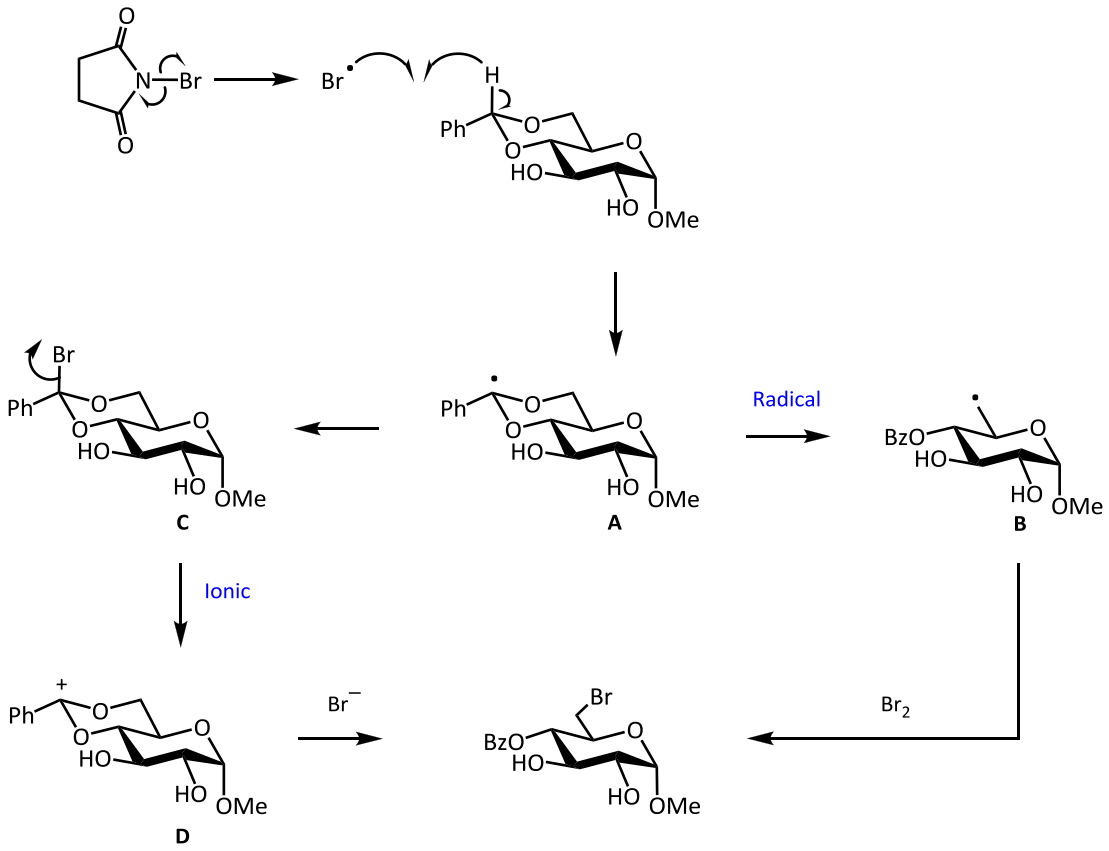
It is worth noting that no acyl migration or intramolecular substitution to give the 3,6-anhydro sugar **2.45** was seen (**Scheme 2.15**) as judged by TLC and ^{1}H NMR.



Scheme 2.15: Potential formation of the 3,6-anhydro sugar **2.45**.

Two different mechanisms have been proposed for the Hanessian-Hullar reaction (**Scheme 2.16**), both of which begin with the initial abstraction of the benzylic hydrogen by the bromine radical to give **A**.[73–75] In 1966, Hullar proposed a radical fragmentation to form **B**, even though this primary radical is less stable than the radical that could form at C-4. **B** then undergoes radical bromination to form the 4-*O*-benzoyl-6-bromo-6-deoxy-β-D-glucopyranoside.[74] At the same time, Hanessian proposed that the reaction could proceed *via* an ionic termination mechanism where the unstable bromoacetal intermediate **C** forms and then collapses to give the benzoxonium ion **D**, which upon S_N2-like displacement by the bromide at the least hindered carbon gives the 4-*O*-benzoyl-6-bromo-6-deoxy-β-D-glucopyranoside product.[73] Although subsequent publications by Hanessian[75–77] proposed that the reaction could also proceed *via* a radical displacement type of reaction, the ionic fragmentation reaction is generally accepted to

be the most plausible mechanism[78] with indirect support of this pathway published by McNulty *et al*.[79] In both instances, the reaction proceeds almost always with the bromine atom attacking at the least hindered position, allowing for complete regiocontrol of the reaction.[73–77,79]

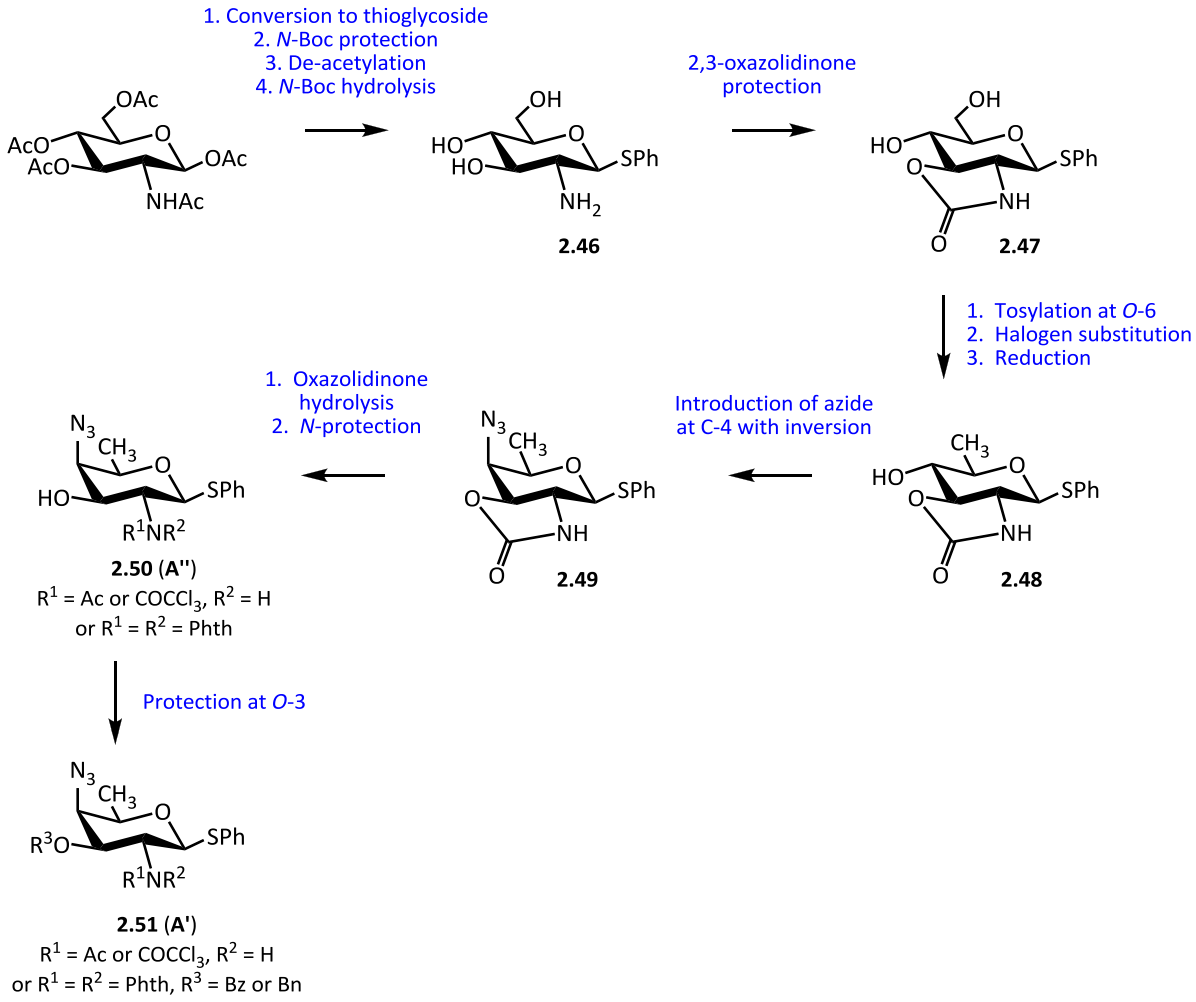


Scheme 2.16: The two proposed mechanisms for the mediated fragmentation reaction of 4,6-*O*-benzylidene acetals.[78]

The successful conversion of the 4,6-*O*-benzylidene (**2.42**) to the 6-deoxy sugar **2.44** represents a proof of concept that this as a viable method of obtaining the FucNAc4N derivative. However, at this stage the efficiency of the overall route was re-evaluated: the earlier steps of the synthesis, in particular the hydrolysis of the thiazoline **2.17** to form the 2-acetamido-1-thiol **2.31** and the radical-mediated formation of the thioglycoside (**2.32**), were not amenable to efficient scale-up and were not reproducible, thus limiting the material available for subsequent steps. An additional drawback was the unexpected oxidation of the sulfur during the formation of the benzylidene acetal, and in view of all of these factors, and results in a parallel study of an alternative approach (see below), it was decided not to proceed with this route.

2.7 Proposed synthesis of the FucNAc4N derivative *via* the 2,3-oxazolidinone sugar route

An alternative route to the FucNAc4N derivative is illustrated in **Scheme 2.17**. In this approach, the 2,3-oxazolidinone **2.47**, efficiently synthesised by Benakli and co-workers[80] *via* the free amine **2.46**, was seen as an ideal intermediate for further manipulations, as the *O*-3 and *O*-4 positions are differentiated. The 2,3-oxazolidinone **2.47** could easily be converted to the 6-deoxy sugar **2.48** by methods previously employed in the thiazole route, leaving the 4-position free for inversion and conversion to 4-azido sugar **2.49** (using Mitsunobu conditions or *via* triflation followed by S_N2 substitution with NaN_3) to give the desired *galacto*-configuration. Hydrolysis of the oxazolidinone, although reportedly not without complications, could then yield the FucNAc4N derivative **2.50** (**A''**), which after C-3 protection can give **2.51** (**A'**). If successful, this route would be much more efficient than any of the above routes.



Scheme 2.17: Proposed synthesis of the protected FucNAc4N derivative *via* the 2,3-oxazolidinone (**2.47**).

2.8 Scope of 2,3-oxazolidinones

The use of 2,3-oxazolidinones as protecting groups in carbohydrate synthesis gives rise to highly functionalised and selectively protected building blocks. Their synthesis on a large scale and in high yield has also been possible.[80–88] Furthermore, 2,3-oxazolidinone-protected glycosyl donors stereoselectively yield 1,2-*cis* glycosides (see discussion in **Ch. 3.3**)[80,82–86] although 1,2-*trans* glycosides can be also be obtained by altering the promoter conditions, as found by the Oscarson research group.[83,86] Glycosyl acceptors incorporating the 2,3-oxazolidinone have also been found to have enhanced reactivity at the 4-position.[41,87,88] This is from the prevention of intermolecular hydrogen bond formation, which is generally the cause of poor reactivity of 2-acetamido glycosyl acceptors[88,89] while the 'tied back nature' of the protecting group reduces steric hindrance around the 4-position.[87,88]

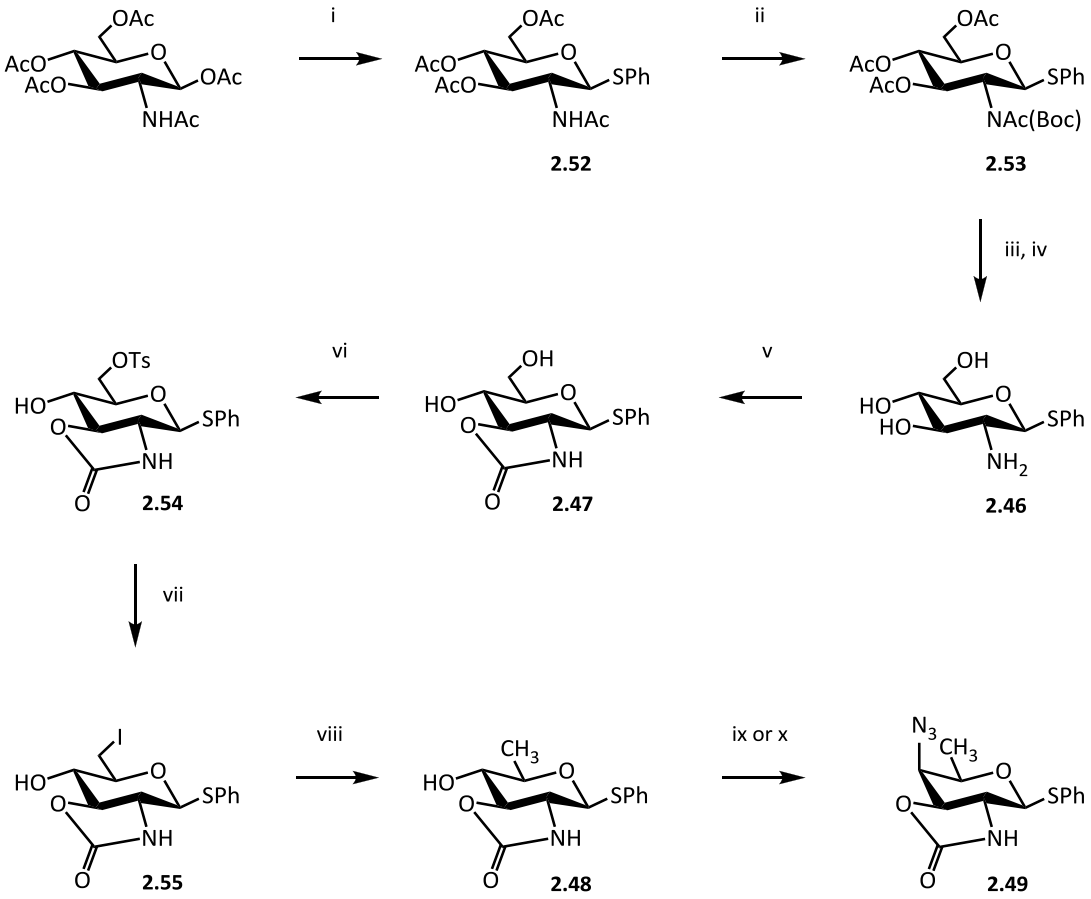
2.9 Synthesis of the protected FucNAc4N derivative *via* the 2,3-oxazolidinone

The thioglycoside **2.52** (**Scheme 2.18**) was obtained from the commercially available 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose. The β-anomer is obtained exclusively due to the participation of the 2-acetamido group, blocking the α-face of the oxocarbenium ion. Thioglycoside **2.52** was easily transformed to the free amine **2.46** *via* a sequence involving initial *N*-Boc protection to give **2.53**, (amide rotamers) followed by global deacetylation under Zemplén conditions, and finally *N*-deprotection with TFA. The common method of removing an *N*-acetate involves refluxing in NaOH for several days, and involves a complicated extraction and purification procedure.[90] In contrast, this method uses only catalytic amounts of base and easily removable TFA.[80] De-*N*-acetylation occurs due to the enhanced electrophilicity of the *N*-acetate carbonyl group which results from the delocalization of electrons that extends into the *N*-Boc protecting group.

The free amine **2.46** was then readily converted in an excellent yield to the 2,3-oxazolidinone **2.47** using *p*-nitrophenyl chloroformate. At this point, separation of the oxazolidinone **2.47** from the concomitantly formed *p*-nitrophenol presented no purification problems as described by Nagai *et al*.[41] Transformation to the 6-deoxy sugar **2.48** proceeded smoothly as previously discussed (*via* the 6-*O*-tosylate **2.54** and the 6-iodo-derivative **2.55**), with no purification required after tosylation of *O*-6.

In the first attempt at inversion of configuration at C-4 to give the *galacto*-derivative, **2.48** was treated under Mitsunobu-type conditions with diphenylphosphoryl azide, but proved unreactive even after 20 hours reaction time. However, the 4-azido sugar **2.49** was readily obtained through triflation at *O*-4 followed by treatment with NaN_3, with ^1H NMR confirming

inversion as seen from the change in the three-bond homonuclear coupling constants (**2.48**: $J_{3,4}$ = 9.7 Hz, $J_{4,5}$ = 8.4 Hz *vs.* **2.49**: $J_{3,4}$ = 2.9 Hz, $J_{4,5}$ = 1.7 Hz), while the presence of the azide was confirmed by the sharp peak in the IR spectrum at 2116 cm^{-1}.

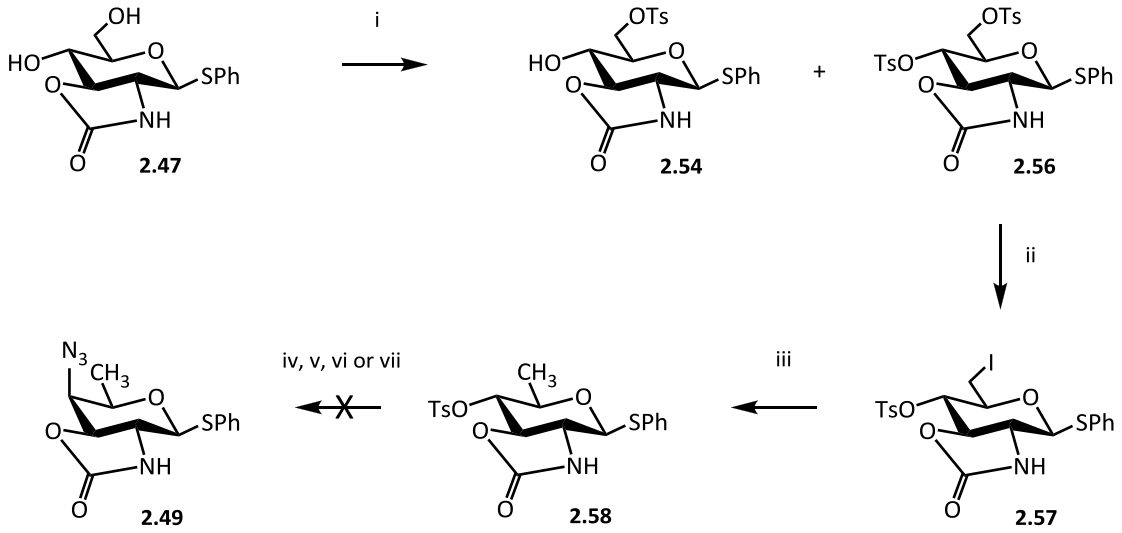


Scheme 2.18: Towards the synthesis of the protected FucNAc4N derivative *via* the 2,3-oxazolidinone sugar route. Reagents & conditions: (i) HSPh, $SnCl_4$, DCM, 3Å MS, reflux, 18 hrs, 86% (ii) Boc_2O, DMAP, THF, 70°C, 3 hrs, 98% (iii) NaOMe, MeOH, r.t., 45 mins (iv) TFA, 0°C to r.t., 3 hrs, 83% (v) 4-nitrophenyl chloroformate, $NaHCO_3$, ACN, H_2O, 0°C, 2.5 hrs, 94% (vi) *p*-TsCl, pyridine, 0°C, 4.5 hrs, 89% (vii) NaI, 2-butanone, reflux, overnight, 79% (viii) H_2, Pd/C, MeOH, 4 bar, r.t., 25 hrs, 60% (ix) PPh_3, DIAD, diphenylphosphoryl azide, THF, r.t., 20 hrs (x) a.) Tf_2O, pyridine, 3Å MS, -30°C, 1 hr b.) NaN_3, DMF, r.t., 18 hrs, 77%.

During the first large scale attempt at selective formation of the 6-tosylate **2.54** from diol **2.47**, the di-tosylate **2.56** in addition to the desired mono-tosylate **2.54** was formed (**Scheme 2.19**).‡ The possibility of conversion of this di-tosylate to the desired FucNAc4N derivative was therefore evaluated by selectively converting it to the 6-iodo-4-*O*-tosyl intermediate **2.57** as described by Medgyes *et al.*[32] and then reducing to give the 6-deoxy-4-*O*-tosyl sugar **2.58**.

‡ 1.5 eq. of *p*-TsCl was used for this reaction and from this point onwards only 1.2 eq. of *p*-TsCl was used. Starting material was also recovered.

However, all attempts to carry out the subsequent S_N2 displacement of the tosyl group to yield azide **2.49** failed. The first three attempts using NaN_3 in DMF and H_2O with conventional or microwave heating returned only starting material, while the last attempt with NaN_3 in DMF at a higher temperature for an extended period of time revealed the formation of multiple polar products as judged by TLC. It was assumed that degradation as well as hydrolysis of the 2,3-oxazolidinone was occurring possibly due to the basicity of NaN_3.[12] Since **2.49** had already been synthesised *via* triflation, no further attempts were made to achieve this conversion using the 6-deoxy-4-*O*-tosyl sugar **2.58**.



Scheme 2.19: Towards the synthesis of the protected FucNAc4N derivative *via* the di-tosylate **2.56**. Reagents & conditions: (i) *p*-TsCl, pyridine, 3Å MS, 0°C to r.t., 2 hrs, 54% mono-tosylate, 24% di-tosylate (ii) NaI, 2-butanone, reflux, 16.5 hrs, 71% (iii) H_2, Pd/C, MeOH, 4 bar, 20 hrs, 52% (iv) NaN_3, DMF, H_2O, r.t. to 70°C, 39.5 hrs (v) NaN_3, DMF, H_2O, MW, 90°C, 3 mins (vi) NaN_3, DMF, H_2O, MW, 100°C, 10 mins (vii) NaN_3, DMF, 120°C, 16 hrs.

At this point *N*-acetylation of the 4-azido sugar **2.49** would yield a potential glycosyl donor as reported by Oscarson and co-workers.[83,86] However, hydrolysis (deprotection) of the 2,3-oxazolidinone group was a priority in order to establish the appropriate conditions for this step and eliminate a potentially difficult deprotection of the disaccharide at a later stage of the synthesis.

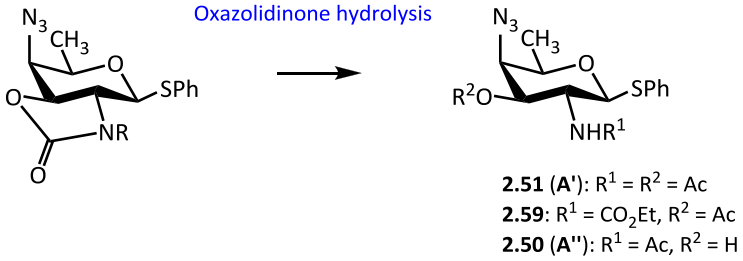
A review of the literature revealed that base hydrolysis or mild reducing agents have been used to deprotect the oxazolidinone.[41,80,83,87,88,91–93] Benakli and co-workers[80] treated their 2,3-oxazolidinone derivative with 1M NaOH in THF/H_2O at room temperature to yield the free

amine.‡ However, treatment of **2.49** with 1M NaOH in THF (**Table 2.1**, entry 1) gave unreacted starting material after 1 hour together with various more polar products, whereas after 4 hours no starting material remained, but a range of products could be seen from TLC, with no major product identifiable. Doubling the concentration of NaOH (entry 2) yielded the same results.‡ When **2.49** was heated at 60°C with 2M NaOH in THF (entry 3) a major product was evident from TLC after 20 mins (together with other minor polar products), and so the reaction was terminated and after workup the crude products were immediately acetylated in order to facilitate isolation and characterization. The major product was in due course identified as the diacetate **2.51** (**A'**), though unfortunately in a low yield of 35%.

Based on the limited success of the attempted deprotection, an alternative method reported by Calveras *et al*.[93] was investigated. They found that treatment of their β-*cis*-2,3-oxazolidinone with LiOH in refluxing EtOH, followed by acidification with AcOH and then acetylation using Ac_2O yielded the desired *N*-acetylated, 3-hydroxy intermediate. Here, AcOH was used to collapse the carbamic acid lithium salt and prevent re-formation of the cyclic carbamate. However, upon treatment of **2.49** with LiOH in refluxing ethanol, followed by full acetylation (entry 4) the carbamate **2.59** was formed, probably through the addition of ethanol to the isocyanate intermediate.[94] Interestingly, the carbamate **2.59** could potentially be used as an *N*-protecting group in the desired glycosyl donor, since this functional group is capable of anchimeric assistance through the formation of an oxazolinium intermediate, which should be more reactive than the (2-methyl)oxazoline as discussed in **Ch. 1.13.**

‡ Treatment of their derivative in the presence of a mild base (Cs_2CO_3) in alcohol deprotected the 3-position with simultaneous formation of the C-2 carbamate.
‡ In both these cases TLC did not show degradation, but the formation of distinct products. These were not isolated and analysed, or the reaction mixture acetylated due to time constraints.

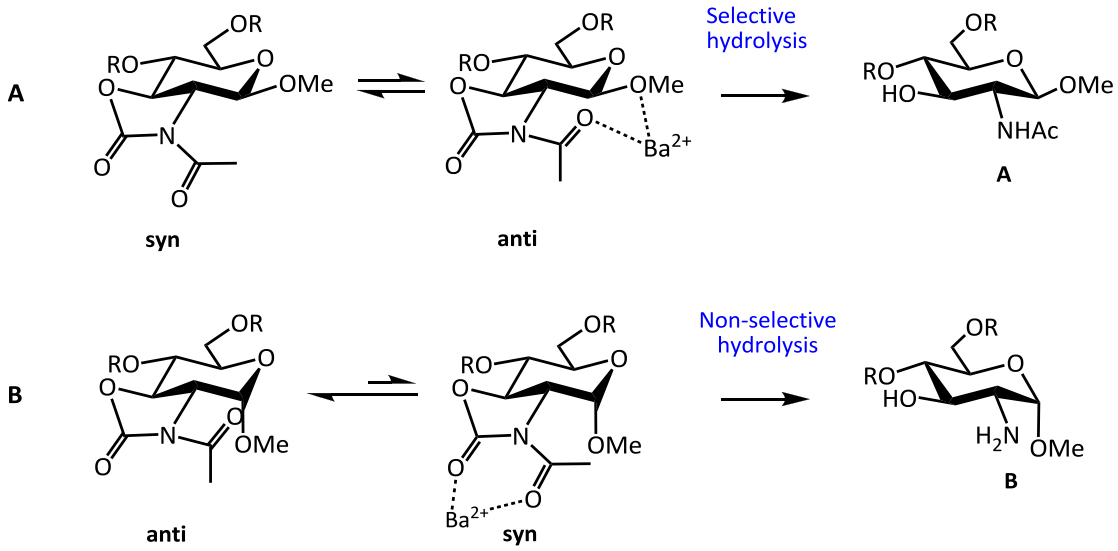
Table 2.1: Reagents & conditions for the hydrolysis of the 2,3-oxazolidinone protecting group.



2.51 (**A'**): $R^1 = R^2 = Ac$
2.59: $R^1 = CO_2Et$, $R^2 = Ac$
2.50 (**A''**): $R^1 = Ac$, $R^2 = H$

Entry	R	Reagents & conditions	Product	Yield
1	H	NaOH (1M), THF, r.t., 4 hrs	Multiple products	-
2	H	NaOH (2M), THF, r.t., 2.5 hrs	Multiple products	-
3	H	i.) NaOH (2M), THF, 60°C, 25 mins ii.) Amberlist IR 120, 20 mins iii.) Ac_2O, DMAP, pyridine, r.t., 2 hrs	**2.51** (**A'**)	35%
4	H	i.) $LiOH.H_2O$, EtOH, reflux, 18 hrs ii.) AcOH, r.t., 1 hr iii.) Ac_2O, r.t., 22 hrs, no rxn iv.) Ac_2O, pyridine, r.t., 6 hrs	**2.59**	75%
5	Ac	LiCl, LiOH, THF/H_2O, r.t., 30 mins	**2.50** (**A''**)	90%

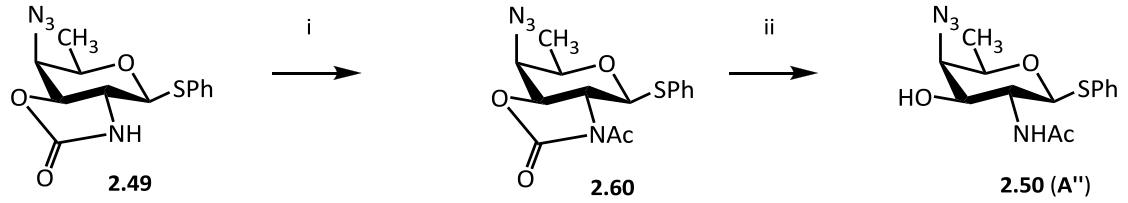
Since the conditions used in entries 1 – 4 (**Table 2.1**) had not been successful, attention next turned to the possibility of chemoselectively hydrolysing the *N*-acetyl oxazolidinone. A review of the literature revealed that the methods used to deprotect the *N*-acetyl 2,3-oxazolidinone are not of the 'one hat fits all' nature. Instead, the rate of cleavage is very much dependent on *cis* or *trans* configuration of the oxazolidinone,[92,93] as well as the anomeric configuration of the sugar derivative. Crich and Vinod reported an example of the latter, where the use of $Ba(OH)_2$ in refluxing ethanol chemoselectively cleaved their methyl *N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-β-D-pyranoside to give the 2-acetamido derivative (**A**) (**Scheme 2.20A**), while the α-anomer under the same conditions yields the 2-amino sugar (**B**) (**Scheme 2.20B**).[88] This selectivity can be explained by the chelation of the Lewis acidic barium cation and the oxygen of the anomeric methoxyl in the β-anomer, making the oxazolidinone group more exposed and therefore susceptible to attack. Alternatively, the barium cation chelates between the two syn orientated carbonyl groups of the oxazolidinone and *N*-acetyl group in the α-anomer.



Scheme 2.20: Crich's explanation for the observed chemoselectivity for *N*-acetyl or *N*-oxazolididone hydrolysis in the α- and β-series.[88]

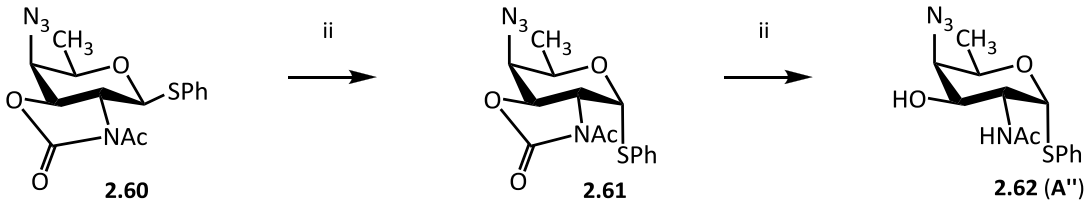
Contrasting results were however reported by Nagai *et al.*[41] where treatment of their 1,6-di-*O*-*tert*-butyldiphenylsilyl-2-*N*,3-*O*-carbonyl-2-deoxy-β-D-glucopyranose with LiCl and LiOH in an alcohol (MeOH or EtOH) with H_2O and THF yielded the C-3 carbonate. Strangely, no reaction was reported to occur without the alcohol, potentially due to solubility issues. Again, these results are in contrast with those reported by Wei and Kerns who conducted a helpful study in the chemoselective deprotection of the 2,3-oxazolidinones, where treatment of their 4-*O*-allyloxycarbonyl-1-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-*N*,3-*O*-carbonyl-2-deoxy-β-D-glucopyranose with either NaOMe or Cs_2CO_3 in methanol yielded the *N*-acetyl methyl carbamate. Successful results were however obtained when their oxazolidinone was treated with either $LiOH/H_2O_2$ in THF/H_2O at -40 to 0°C or LiCl and LiOH in $EtOH/H_2O$ at room temperature.

These observations suggested that carrying out the reaction in the absence of an alcohol would prevent any carbamate or carbonate formation, and consequently the *N*-acetyl 2,3-oxazolidinone **2.60** (**Scheme 2.21**) was treated with LiCl and LiOH in THF/H_2O (**Table 2.1**, entry 5) which yielded the desired *N*-acetyl intermediate **2.50** (**A''**) cleanly and in an excellent yield (90%). Here the addition of LiCl prior to LiOH is crucial, and it is suspected that the weakly Lewis acidic Li^+ chelates with the carbonyl carbon of the acetate and the anomeric sulfur as seen with Crich's β-anomers, resulting in selective cleavage of the oxazolidinone.



Scheme 2.21: Synthesis of the protected FucNAc4N derivative **2.50** (**A''**). Reagents & conditions: (i) AcCl, DIPEA, DCM, 0°C, 2.5 hrs, 87% (ii) LiCl, LiOH,THF, H_2O, r.t., 1 hr 20 mins, 90%.

In a separate investigation which parallels reactions carried out in **Ch. 3.3**, the anomerization of **2.60** was also investigated (**Scheme 2.22**). Treatment of **2.60** with $SnCl_4$ yielded the α-anomer **2.61**, which after hydrolysis of the 2,3-oxazolidinone with LiCl/LiOH yielded the α-FucNAc4N derivative **2.62**, again in the form of **A''**. These results indicate that, in contrast to Crich's conclusions[88] discussed above, the anomeric configuration has no bearing on chemoselectivity.‡ The reasons for this are not clear. There are key differences between the substrates in this study and those in the study reported by Crich, notably in the anomeric substituents (SPh *vs.* OMe), the configuration at C-4 (*galacto vs. gluco*), and the substituents at C-4 and C-6. The nature and configuration of C-4 and C-6 substituents may well have a decisive influence on the conformation and hence ring-strain in the fused system, so that the anomeric configuration plays less of a role. Alternatively, or in addition, it is possible that the larger sulfur atom can effectively co-ordinate with the Li^+ together with the carbonyl oxygen of the *N*-acetyl group in its anti-conformation (**Scheme 2.20**) in both anomeric configurations, as opposed to only the β-configuration.

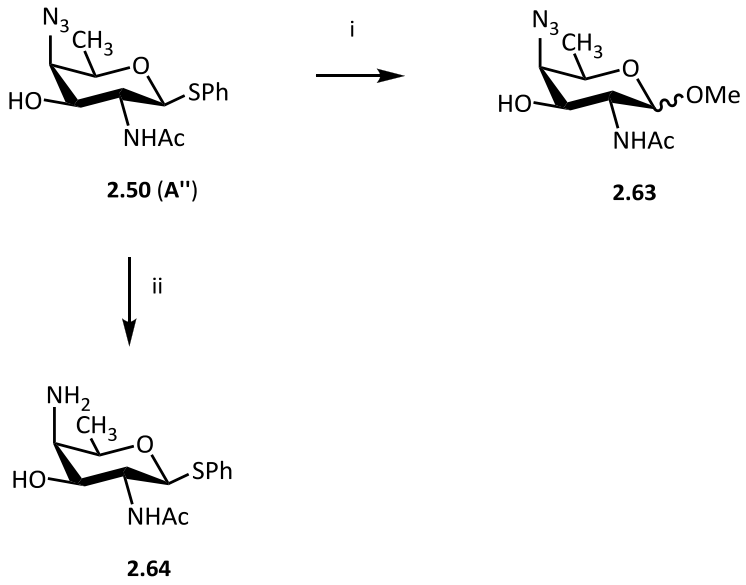


Scheme 2.22: Synthesis of the α-FucNAc4N derivative **2.62** (**A''**). Reagents & conditions: (i) $SnCl_4$, DCM, r.t., 16 hrs, 71% (ii) LiCl, LiOH,THF, H_2O, r.t., 45 mins, 82%.

‡ The hydrolysis of the 2,3-oxazolidinone of the α-anomer **2.61** is however, faster, taking almost half of the time to go to completion.

2.10 Synthesis of alternative FucNAc4N derivatives

With the FucNAc4N derivative **2.50** (**A''**) and its corresponding *O*-acetyl **2.51** (**A'**) successfully synthesised, attention turned to synthesising the glycosyl acceptor **2.63** for potential use as a standard (**Scheme 2.23**). Treatment of **2.50** (**A''**) with NCS in methanol and DCM gave the methyl glycoside **2.63** in a moderate yield (55%, α/β 3.3:1). In addition, it was worth investigating the conditions for azide reduction, and after optimisation using hydrogenation, the free amine **2.64** was obtained in an excellent yield.

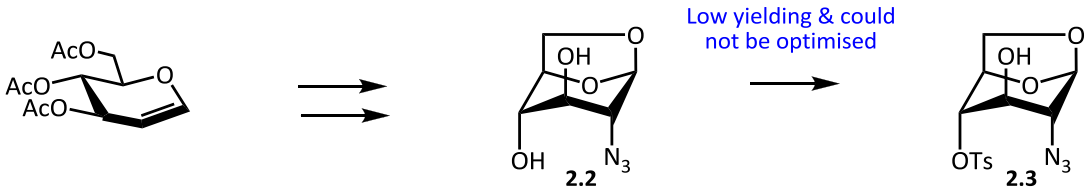


Scheme 2.23: Synthesis of alternative the protected FucNAc4N derivative. Reagents & conditions: (i) NCS, MeOH, DCM, r.t., 20 mins, 55% (α/β 3.3:1) (ii) H_2, Pd/C, MeOH, DCM, r.t., 4 hrs, 80%.

The full scope of **2.50** (**A''**) and **2.51** (**A'**) as glycosyl donors could have been explored further, but at this point the primary focus of the project was on establishing efficient routes to the required monosaccharide derivatives.

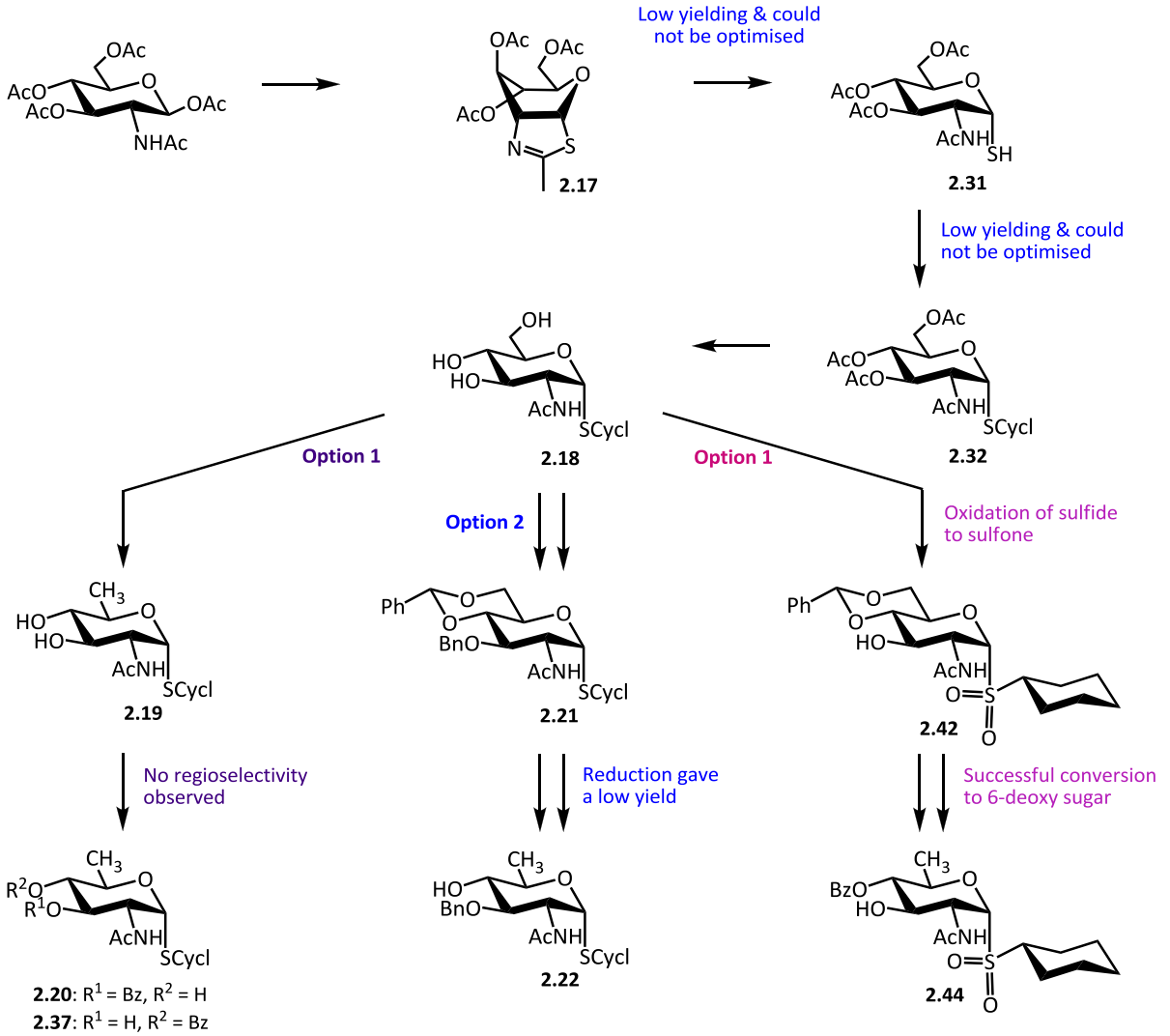
2.11 Summary

Five different routes to the FucNAc4N derivative were investigated and evaluated. The first route *via* the 1,6-anhydro sugar involved the synthesis of the key intermediate **2.3** which after further manipulations could yield the desired FucNAc4N derivatives, **A'** and **A''**. However, obtaining the regioselectively protected derivative **2.3** in a reasonable yield could not be optimised, and so the synthesis *via* this route was not investigated any further (**Scheme 2.24**).



Scheme 2.24: Summary of synthesis *via* the 1,6-anhydro sugar route.

Instead, attention then turned to synthesising the triol **2.18** *via* the thiazoline **2.17** (**Scheme 2.25**) as described by Knapp and co-workers.[20–24] This intermediate allowed for further elaboration and exploration of three different alternatives to obtaining the FucNAc4N derivative. However, the low yields for the synthesis of the 2-acetamido-1-thiol **2.31** and the radical-mediated formation of the thioglycoside (**2.32**) were not amenable to efficient scale-up, thus limiting the material available for subsequent steps. Regardless, all three options were partially investigated. In the first instance, attempts to regioselectively benzoylate *O*-3 instead yielded a mixture of the 3-*O* and 4-*O*-benzoyl derivatives (**2.20** and **2.37** respectively). Next, option 2 was evaluated, but reduction to yield the 6-deoxy intermediate **2.22** gave poor yields and upon resynthesizing the 4,6-*O*-benzylidene derivative **2.23**, the sulfonyl derivative **2.42** was obtained. This was instead used to evaluate option 3 which made use of the Hanessian-Hullar radical-mediated acetal fragmentation reaction and successfully yielded the 6-bromo-derivative **2.43** which after reduction gave the 6-deoxy sugar **2.44**. Options 2 and 3 both had potential; however, after a complete evaluation of the synthesis *via* the thiazoline sugar route, it was decided that an alternative route to the FucNAc4N derivative rather be pursued.

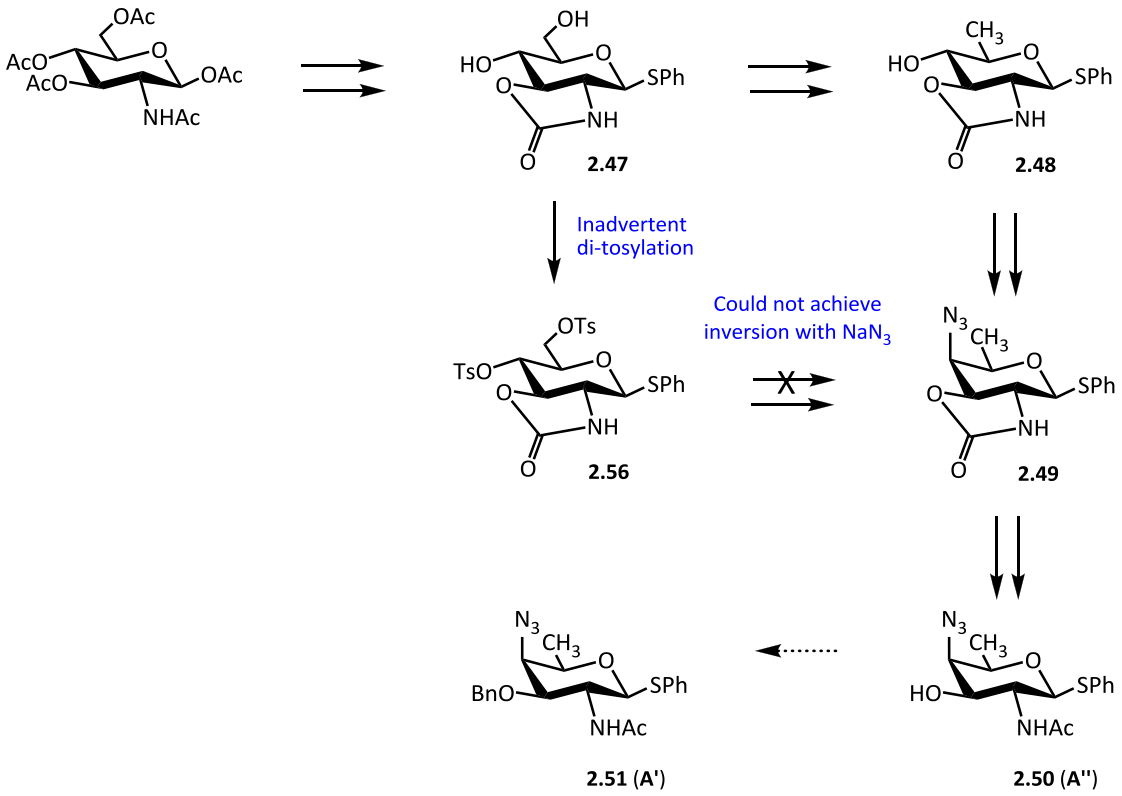


Scheme 2.25: Summary of synthesis *via* the thiazoline sugar route.

The synthesis of the FucNAc4N derivative *via* the 2,3-oxazolidinone route efficiently yielded the phenyl 2-acetamido-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.50**) (**A''**) in 10 steps from the commercially available 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**Scheme 2.26**) in a 17% overall yield. This compares well with previous FucNAc4N synthesis where overall yields for a similar FucNAc4N derivative range between 8-39%.

The inadvertent di-tosylation of **2.47** to give **2.56** and hydrolysis of the 2,3-oxazoldinone protecting group of the 4-azido sugar **2.49** (**Table 2.1**) presented the only challenges in the synthesis. Di-tosylation was further prevented by reducing the number of equivalents of *p*-TsCl, while 2,3-oxazolidinone hydrolysis was easily overcome by *N*-acetylation followed by 2,3-oxazolidinone hydrolysis with LiCl and LiOH to yield the 2-acetamido derivative **2.50** (**A''**) (**Scheme 2.21**). With the FucNAc4N derivative **2.50** (**A''**) in hand, the methyl glycoside **2.63** and

free amine **2.64** were also obtained. Subsequent protection of **2.50** (**A''**) could then yield the FucNAc4N derivative **2.51** in the **A'** form.



Scheme 2.26: Summary of synthesis *via* the 2,3-oxazolidinone sugar route.

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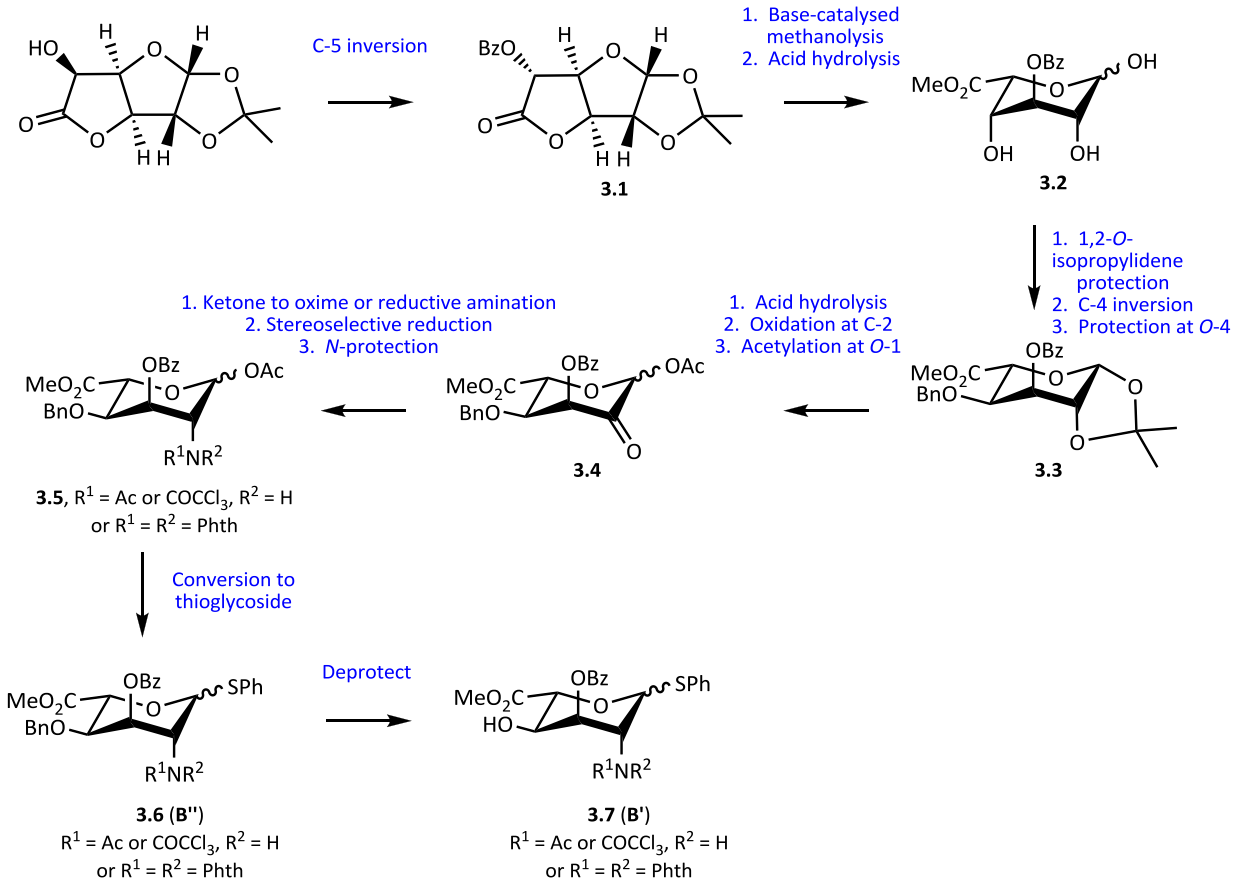
Chapter 3: Design and evaluation of alternative synthetic routes to derivatives of 2-acetamido-2-deoxy-α-L-altruronic acid (AltNAcA)

3.1 Towards the synthesis of a protected AltNAcA derivative: first approach *via* a fused furanolactone

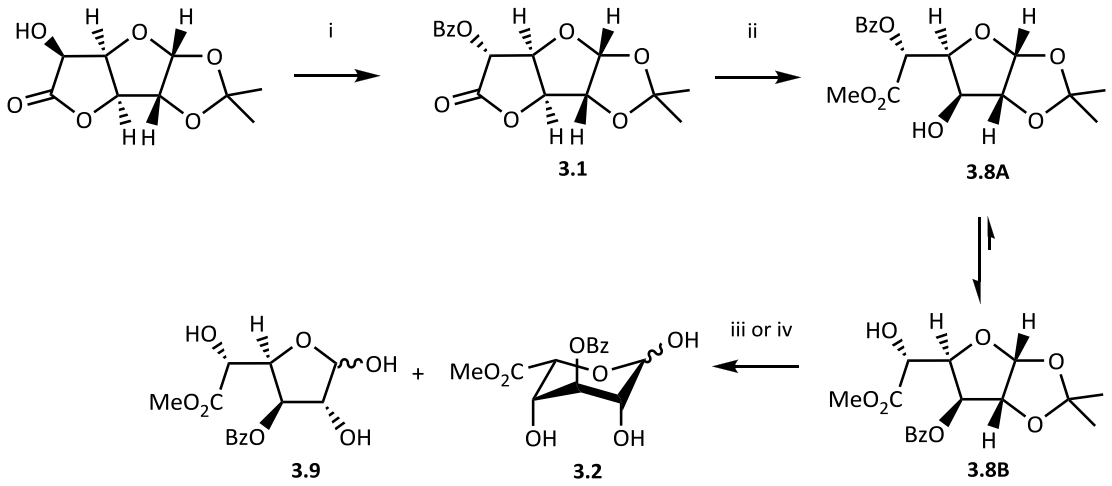
This part of the study involved the development of a synthetic route to the unusual AltNAcA sugar which would combine inherent synthetic interest and a reasonable degree of efficiency. The approach was chosen on the basis of recent literature reports on the viability of strategies for preparing L-sugars[1] *via* inversion of configuration at C-5 of the D-sugars, and was based on the initial preparation of L-iduronic acid derivative **3.2** as reported by Ke and Whitefield.[2] The proposed synthetic route is outlined in Scheme **3.1**. It involved the initial key inversion at C-5 of the readily available protected glucuronolactone to yield the 5-*O*-benzoyl furanolactone **3.1** (**Scheme 3.1**), *via* triflation and displacement with benzoate.[2,3] This would be followed by base-catalysed methanolysis of the lactone, accompanied by migration of the benzoate from *O*-5 to *O*-3, and then acid hydrolysis to give iduronic acid derivative **3.2.** This derivative then requires the following key transformations: inversion of configuration at C-4, introduction of the acetamido functionality at C-2 with retention of configuration and installation of a suitable anomeric substituent, allowing for selective activation at a later stage. One possible strategy to achieve all of this would start with preparation of the *cis*-1,2-*O*-isopropylidene derivative using Seeberger's methodology,[4] which after an inversion sequence at C-4 using the nitrite-mediated substitution of the 4-*O*-triflate,[5] followed by benzylation at *O*-4 would give the altruronic acid derivative **3.3**. After removal of the isopropylidene group, selective oxidation of the 2-hydroxy group could be attempted using the cupric acetate methodology of Hanaya *et al*.[6,7] followed by simple acetylation to protect the anomeric position (**3.4**). The final steps would involve an oxidation-oximation-reduction sequence or oxidation-reductive amination sequence to form **3.5** with the 2-acetamido functionality in the correct configuration. The stereoselective reduction of the oxime could be a challenge and it would depend, in part, on the preferred conformation of this altrose derivative, as well as the extent to which the substituents at C-1, C-3 and C-4 influence the face selectivity of the addition of hydrogen to the oxime. The use of a variety of reducing agents (e.g. $LiAlH_4$; H_2, PtO_2, HCl;[8] MoO_3, $NaBH_4$;[9] $NiCl_2$, $NaBH_4$;[10] Zn/HCOONH;[11] SmI_2[12]) may need to be investigated in order to obtain the desired 2,3-*trans* stereochemistry in **3.5.** Earlier inversion of the 4-position will also helpfully reduce any 2,4-steric interactions. Conversion of **3.5** to the thioglycoside would then yield **3.6** in the form of **B''**, while hydrogenolysis would cleave the benzyl ether at *O*-4 to give **3.7** (**B'**).



Scheme 3.1: Proposed synthesis of the protected AltNAcA derivatives **3.6** (**B''**) and **3.7** (**B'**) *via* the 5-*O*-benzoyl furanolactone (**3.1**).

Beginning with the commercially available 1,2-*O*-isopropylidene-β-D-glucofuranurono-6,3-lactone (**Scheme 3.2**), the first sequence, with the objective of achieving the crucial inversion of configuration at C-5, involved esterification of the free 5-OH using triflic anhydride followed by S_N2 inversion with benzoate to give the key 5-*O*-benzyl furanolactone (**3.1**), with the assumed *R*-configuration at C-5.[2,3] This proceeded in good yield, but there was no direct proof of this inversion from the ^{1}H NMR of the product, as the *J*-coupling constants in the starting material and product were virtually the same, as is expected from bond orientations in substituents on the furanosyl ring. This result was, however, consistent with that reported in the literature, where the authors also report use of sodium pivaloate as an alternative.[2,13] The next step involved the base-catalysed cleavage of the lactone with subsequent migration of the benzoyl group to form (**3.8B**). Ojeda *et al.*[3] originally reported the selective cleavage of the lactone ring using methanolic 1% Et_3N at low temperatures (-60°C to -40°C), but found that increasing the amount of Et_3N or increasing the temperature led to the undesired (in their case) migration of the pivaloyl group from the 5-*O*-position to the 3-*O*-position. By exploiting this side reaction, Ke and Whitfield reported the complete migration of the pivaloyl protecting group to the 3-position

upon treatment with Et_3N (8 eq.) in methanol at 0°C for 1 hour.[2,‡] However, in our hands under these conditions on both a small and larger scale, some starting material was recovered, together with an almost inseparable mixture of the 5-benzoate (**3.8A**) and 3-benzoate (**3.8B**). Nevertheless, after repeated column chromatography on silica, the desired product **3.8B** was isolated in moderate yield. The failure to achieve a clean, efficient lactone opening and benzoyl migration led to repeating the procedure starting with the 5-pivaloate, which gave similar results.



Scheme 3.2: Towards the synthesis of the protected AltNAcA derivative *via* the fused furanolactone route. Reagents and conditions: (i) a.) Tf_2O, pyridine, -40°C, 1.5 hrs, 98% b.) NaOBz, DMF, 0°C to r.t., 6 hrs, 88% (ii) Et_3N, MeOH, 0°C, 1.5 hrs, 15% (**3.8A**), 36% (**3.8B**) (iii) 90% TFA, 0°C, 15 mins, 78% (iv) 90% TFA, 3 hrs, r.t., 45%.

In an attempt to better understand the conversion of **3.1** to **3.8B**, the reaction was repeated in an NMR tube, in order to monitor progress by NMR and acquire evidence for the proposed sequence of steps. The reactants were combined at room temperature using 5 eq. of Et_3N (a reduced amount of Et_3N was used so as not to saturate the spectra) in anhydrous MeOD and $CDCl_3$, with ^1H NMR spectra recorded at the intervals indicated (**Figure 3.1**). **Figure 3.1** shows the set of spectra obtained, with the spectrum of the starting material **3.1** shown at the bottom, and those of the two products **3.8A** and **3.8B** shown at the top. The spectra of **3.1**, **3.8A** and **3.8B** are of the purified compounds and have been run in $CDCl_3$ only, hence the differences in chemical shifts of these three spectra *vs.* spectra 1-9. The most diagnostic signals in all the spectra are from H-3 and H-5 in compounds **3.1**, **3.8A** and **3.8B**, and the shifts of these can be monitored. The spectrum of **3.1** shows the relatively shielded H-3 and H-5 signals, while H-5 in

‡ Procedure was emailed from corresponding author, but reaction time differed from published conditions.

the spectrum of **3.8A** is shifted significantly downfield due to the combined electron-withdrawing effects of the attached benzoate ester, and the adjacent carboxyl group. On the other hand, H-3 of **3.8B** is significantly more deshielded due to the attached benzoate ester, with H-5 appearing upfield and giving evidence for the migration of the benzoate to *O*-3. After the addition of the reagents, changes in the spectra can already be seen after only 10 minutes (spectrum 1), with the noticeable appearance of H-5 of **3.8A** and H-3 of **3.8B**. In the period from 30 minutes to 270 minutes (spectra 2-8) the decrease in intensity of the H-2, H-3, H-4 and H-5 signals of **3.1** can be seen, while a subsequent increase in the amount of the 3-benzoate, **3.8B** is accompanied by a reduction in the amount of **3.8A** as seen from the decrease in intensity of the H-5 signal. Finally, after 24 hours (spectrum 9) only a minor amount of the starting material (**3.1**) can be seen, while the desired 3-benzoate **3.8B** was formed in majority as seen from H-3 and the most deshielded anomeric signal, with the persistent presence of the 5-benzoate **3.8A**, indicating that there is an equilibrium between this and **3.8B**.

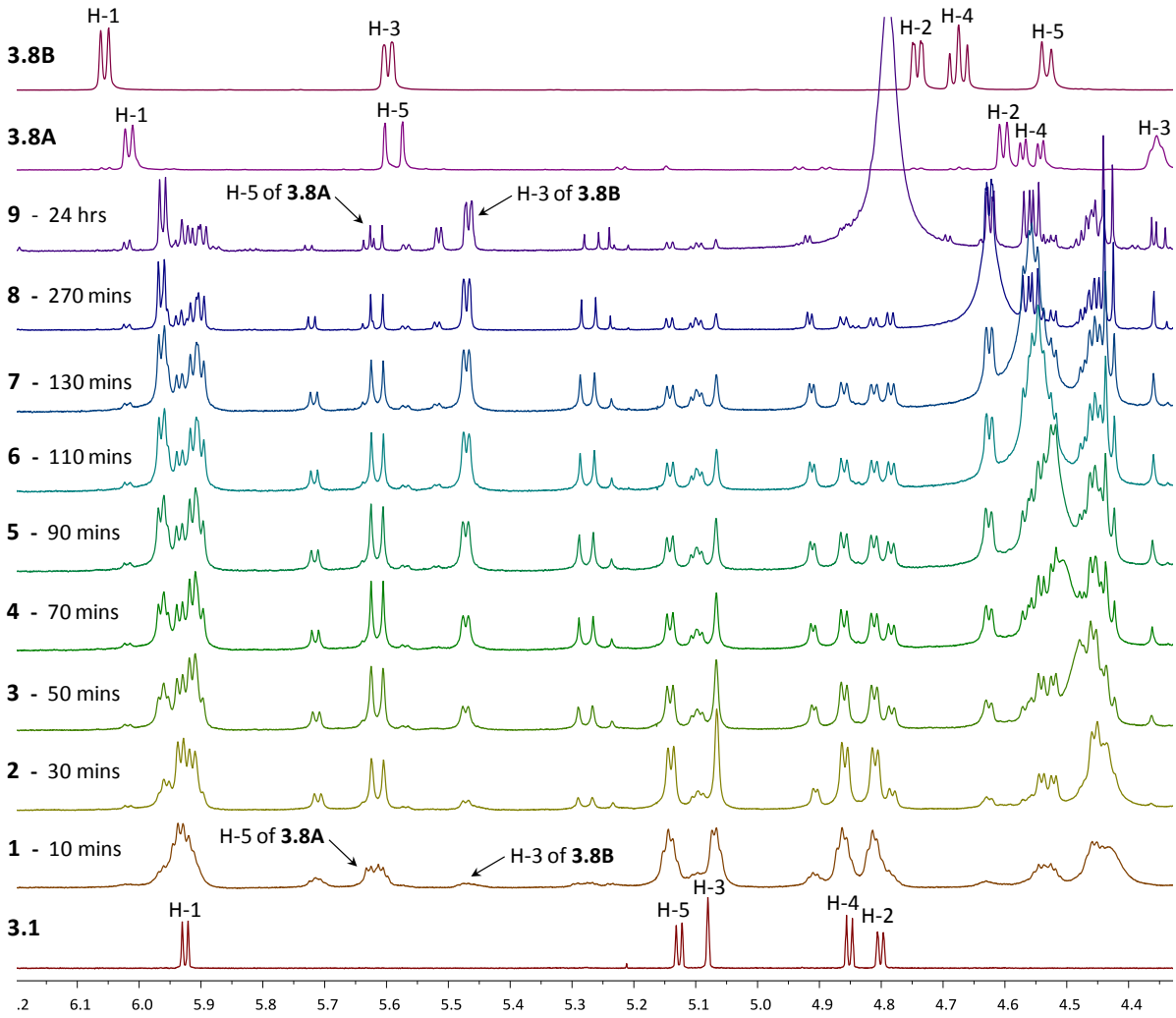


Figure 3.1: NMR-monitoring of the reaction of **3.1** with MeOD under basic conditions. Reagents & conditions: Et_3N (dry) (5 eq.), MeOD (dry) (10 eq.), $CDCl_3$ (dry), spectra recorded at 30°C, NMR tube kept at r.t.. Concentration of starting material = 74 mg/mL in $CDCl_3$. Chemical shifts (δ) referenced relative to the internal TMS standard. . The spectra of **3.1**, **3.8A** and **3.8B** are of the purified compounds and have been run in $CDCl_3$ only, hence the differences in chemical shifts of these three spectra *vs.* spectra 1-9.

An investigation was then carried out to determine the effect of various bases and temperatures, but this gave similar results in all instances. With hindsight, the use of a stronger base (such as NaOMe in MeOH) may have been required in order to effect the required conversion, although this would also potentially have deprotected the benzoyl ester.[14]

Once a substantial amount of pure **3.8B** had been obtained from these experiments, attention turned to the deprotection of the 1,2-*O*-isopropylidine acetal and rearrangement to the pyranoside **3.2**. Following literature precedent, **3.8B** was treated with 90% aqueous TFA for 15 minutes at 0°C yielding a single more polar product, as judged by TLC, but shown by ^{1}H NMR to be a mixture of the α/β-furanosyl (**3.9**) and α/β-pyranosyl glycoses (**3.2**) (**Figure 3.2**).[15] This

was confirmed, after isolation of the products from the reaction mixture, by a detailed 2D NMR study with the COSY in particular confirming the presence of the distinct spin systems and revealing the very deshielded H-3 protons, consistent with the presence of the 3-*O*-benzoyl ester. The HSQC spectrum allowed full assignment of carbon signals, and the complex HMBC spectrum gave evidence for both α- and β-pyranose (**3.2-α and 3.2-β**) forms, with clear crosspeaks between C-1_α and H-5_α, and C-1_β and H-5_β, while no C-1 to H-4 crosspeaks could be seen in either cases (**Figure 3.3**). Evidence for the α-furanose (**3.9-α**) is likewise seen in the HMBC, where a C-1_α to H-4_α crosspeak can be seen clearly, but no C-1 to H-5 crosspeak. Unfortunately, neither C-1 to H-5 nor C-1 to H-4 crosspeaks could be seen for the β-furanose (**3.9-β**), and these assignments were based on COSY and HSQC data only. Assignments of furanose anomeric protons were based on ^{13}C NMR chemical shifts, where carbons appear more deshielded when their C-1 and C-2 substituents are *trans*-oriented.[16–18] The most deshielded ^{13}C signal at 103.77 ppm was therefore assigned to the α-furanose. The assignments for the anomeric protons in the α- and β-pyranose sugars were based on *J*-coupling values from the ^{1}H NMR spectrum.

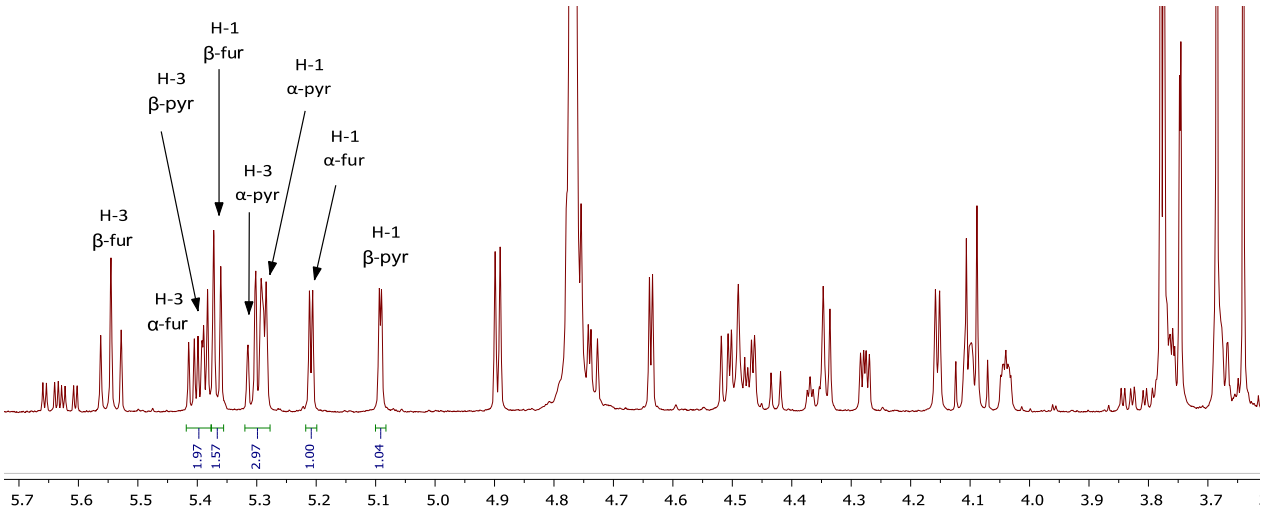


Figure 3.2: ^{1}H NMR spectrum of the ring region of the α/β-pyranose sugar (**3.2**) and α/β-furanose sugar (**3.9**).

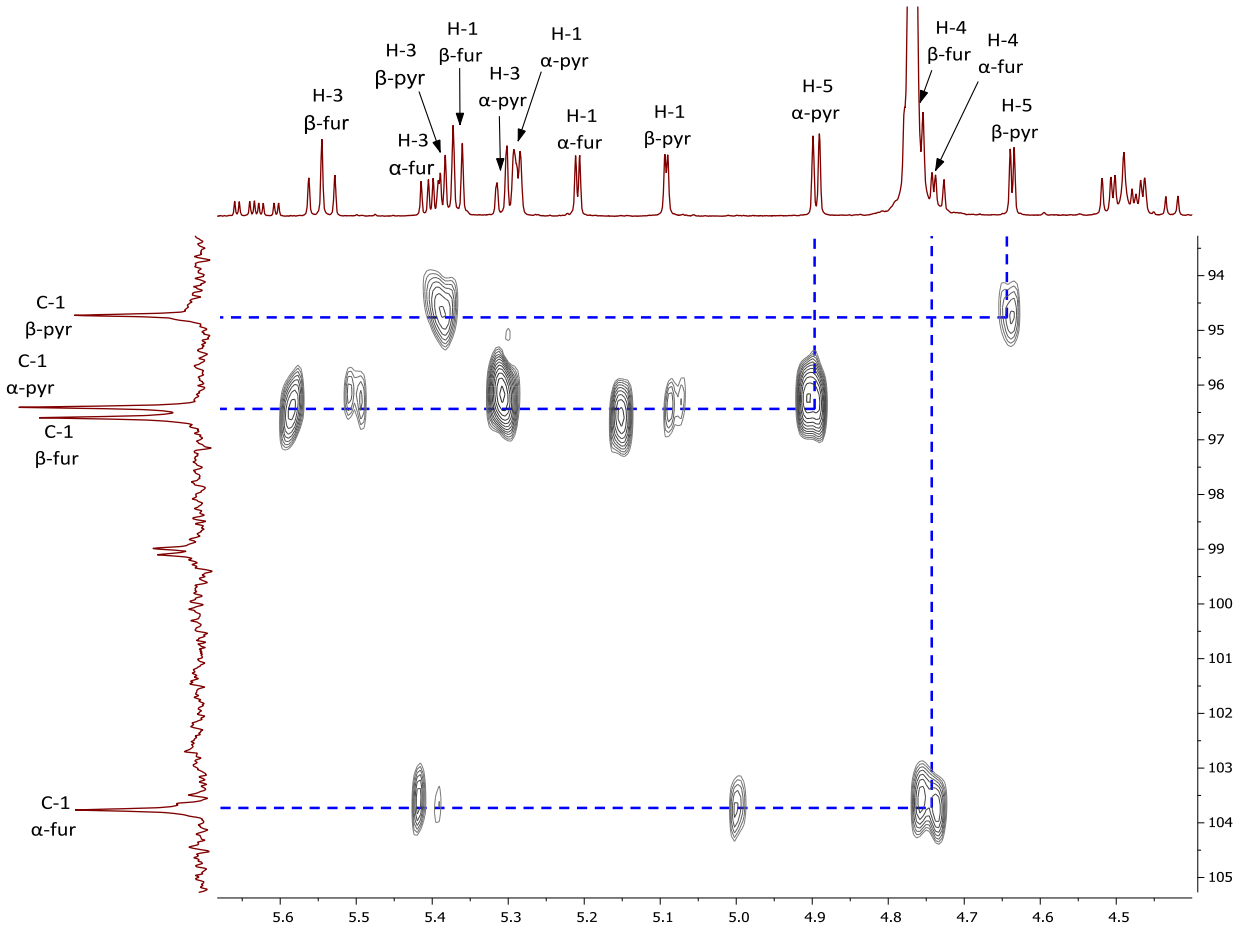


Figure 3.3: HMBC spectrum of anomeric region showing distinctive C-1 to H-5 crosspeaks for α- and β-pyranose sugars (**3.2-α** and **3.2-β**) and C-1 to H-4 for the α-furanose (**3.9-α**), whole no C-1 to H-4 crosspeak could be seen for the β-furanose (**3.9-β**).

From the integration of the protons in the anomeric region, the ratio of the different forms of the sugars were found to be approximately 1.5 : 1 : 1 : 1.5 (**3.2-α** : **3.2-β** : **3.9-α** : **3.9-β**). Lastly, the presence of these proposed structures was confirmed by HRMS which gave a molecular ion at 330.1185 corresponding to $[M+NH_4]^+$.

Following these observations, a further survey of the literature revealed that application of similar conditions to the hydrolysis of analogous substrates achieved exclusive formation of the α- and β-pyranoses, with no furanoses detected, with reaction times ranging from 15 minutes[15,19] to 3 hours.[2,20–22] This is expected since the furanoses are formed faster (kinetic product) but are generally less stable.[23] The persistence of the furanose sugars in our case was therefore somewhat surprising. Repeating the reaction over 3 hours at room temperature gave the same mixture of all forms as discussed above, but in a lower yield. In a final experiment, the reaction was carried out in an NMR tube using 90% TFA in D_2O at room temperature, and ^1H

NMR spectra recorded at intervals. It was clear from the results (**Appendix 2**) that the 1,2-*O*-isopropylidene group was rapidly cleaved, with the α- and β-furanoses and pyranoses forming almost instantly, and that there was no detectable change in the product distribution after 2 hours. A further exhaustive review of the literature revealed a paper by Dilhas *et al*.[24] and showed that when a corresponding 3-*O*-benzyl ether (and not a 3-*O*-benzoyl ester, as in this case) was treated with 90% TFA, it resulted in formation of an equilibrium mixture of both the furanose and pyranose forms, with the α-pyranose the major product after only 3 minutes, followed by subsequent equilibration to a more-or-less equimolar mixture of the four compounds after a 120 minute interval. These findings are in close agreement with the results described above. In order to obtain increased amounts of the pyranose sugar (**3.2**), acetylation of the crude reaction mixture after hydrolysis could have been explored at various temperatures as described by Dilhas and co-workers, where their β-pyranose (**3.2-β**) was trapped in an 83% yield after recrystallization.[24]

Thus, although new insights into this fascinating sequence of reactions were obtained by careful monitoring of reaction progress by ^{1}H NMR, as well as by using detailed 2D NMR analysis of the products of the final hydrolysis step, there were already clear indications that this would not be an efficient route to the AltNAcA derivative. The sequence of lactone opening and acyl migration was not completely selective, and prospects of obtaining high yields of the desired glycopyranose **3.2** in the hydrolysis step seemed low. Together, these would have serious implications for the challenging steps that were to come in the proposed sequence. It was therefore decided to abandon this route and focus on an alternative strategy to obtain the desired AltNAcA derivative.

3.2 Towards the synthesis of a protected AltNAcA derivative: second approach *via* the 2,3-oxazolidinone derivative

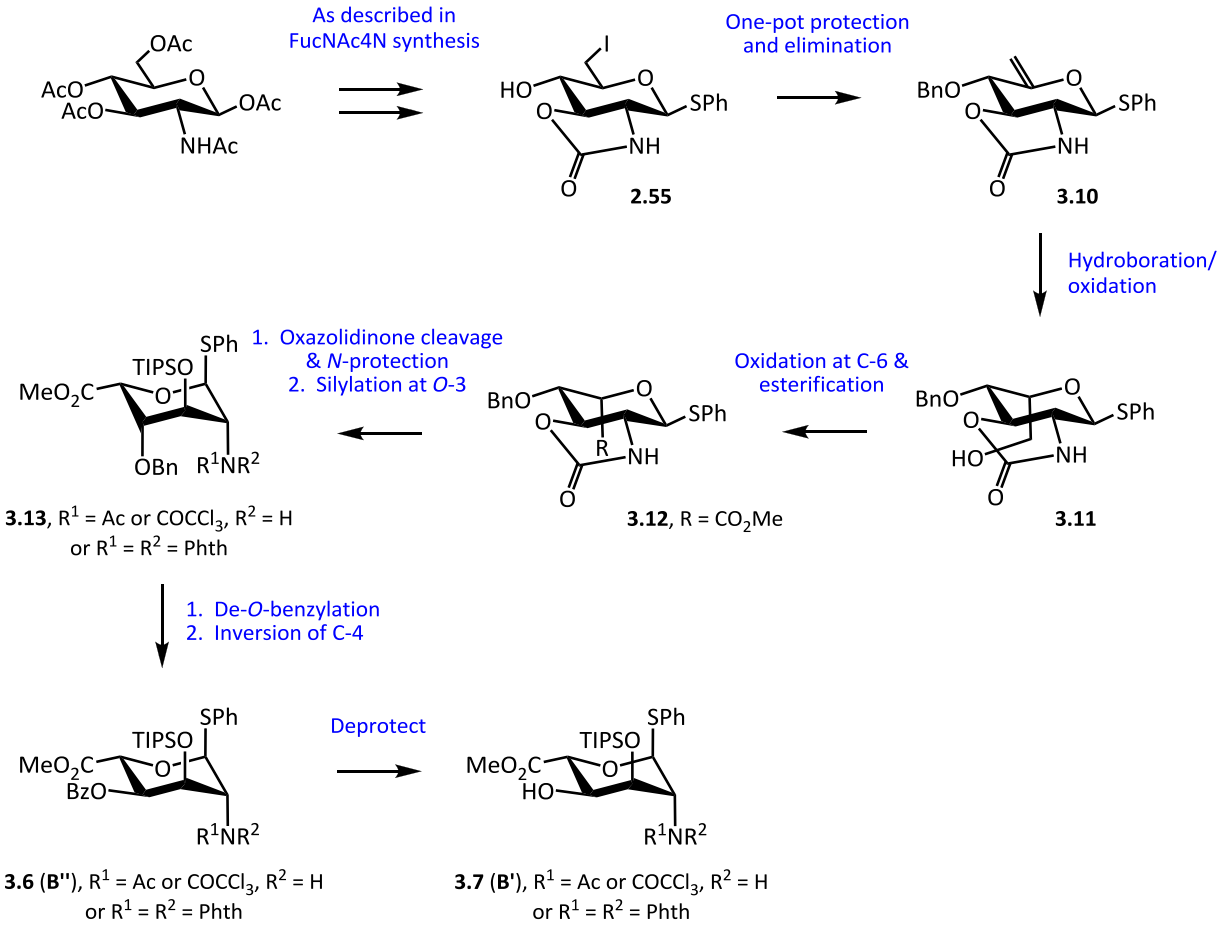
Based on our findings in the attempt to synthesize the AltNAcA derivative *via* a glucofuranolactone, and observations in our parallel attempts to synthesize a FucNAc4N derivative (**Ch. 2**), a potential alternative synthetic route to an AltNAcA derivative emerged, involving a key intermediate used in the FucNAc4N synthesis. One obvious advantage of this option lay in having the 2-amino group already in place and in the correct configuration. It also presented a simple solution to the challenge of selective protections of *O*-3 and *N*-2, and allowed for creative modifications at C-4, C-5 and C-6. In addition, it appeared to offer a considerably shortened and more efficient route to the AltNAcA derivative, compared with those reported by Pozgay *et al.* and Mulard and Pfiste and previously discussed in **Ch. 1.13**.[25,26] An intriguing final

implication of this option is that it would provide access, from the same chemical intermediate, to derivatives of both components of the repeating unit of *S. sonnei*.

The proposed synthetic route is outlined in **Scheme 3.3**. The starting point would be the 6-iodo derivative **2.55** which is a key intermediate in the accompanying synthesis of FucNAc4N (**Ch. 2.9**). It was envisaged that a one-pot, sodium hydride-mediated benzylation at *O*-4 and elimination at C-5/6 would give the 5-enopyranoside **3.10**, which upon face-selective hydroboration/oxidation[27–30] would give the desired *R*-configuration at C-5 (**3.11**), which is required in an L-sugar. In order to ensure high diastereoselectivity in the hydroboration/oxidation step, various borane complexes could be evaluated, in different concentrations, taking into account the observation of Takahashi *et al*.[29] The early protection of the 4-position would be required in order to ensure selective oxidation at C-6 in a subsequent step,[#] whereas a late-stage inversion to a C-4 axial substituent was envisaged to cause an unfavourable influence on the facial selectivity of the hydroboration step, as also seen in studies by Takahashi *et al*.[29] Oxidation, followed by methylation, would then yield ester **3.12**. It was then reasoned that the inversion at C-4 would be best achieved after removal of the 2,3-oxazolidinone group, since the latter is removed under basic conditions which would not be compatible with an ester protecting group at *O*-4, potentially installed during a Mitsunobu inversion or triflation/S_N2 benzoate substitution. Therefore the plan involved cleavage of the 2,3-oxazolidinone group,[‡] followed by *N*-protection (with HAc, Phth or $HCOCCl_3$) and silylation at *O*-3 to give **3.13**, which upon de-benzylation at *O*-4 is set up for inversion to yield the fully protected AltNAcA derivative **3.6** (**B''**) and **3.7** (**B'**) after deprotection.

[#] Selective oxidation can in fact be carried out.

[‡] Cleavage of the 2,3-oxazolidinone at this point in the synthesis, which is prior to any glycosylation reactions, will remove its ability to enhance the glycosyl acceptors reactivity.

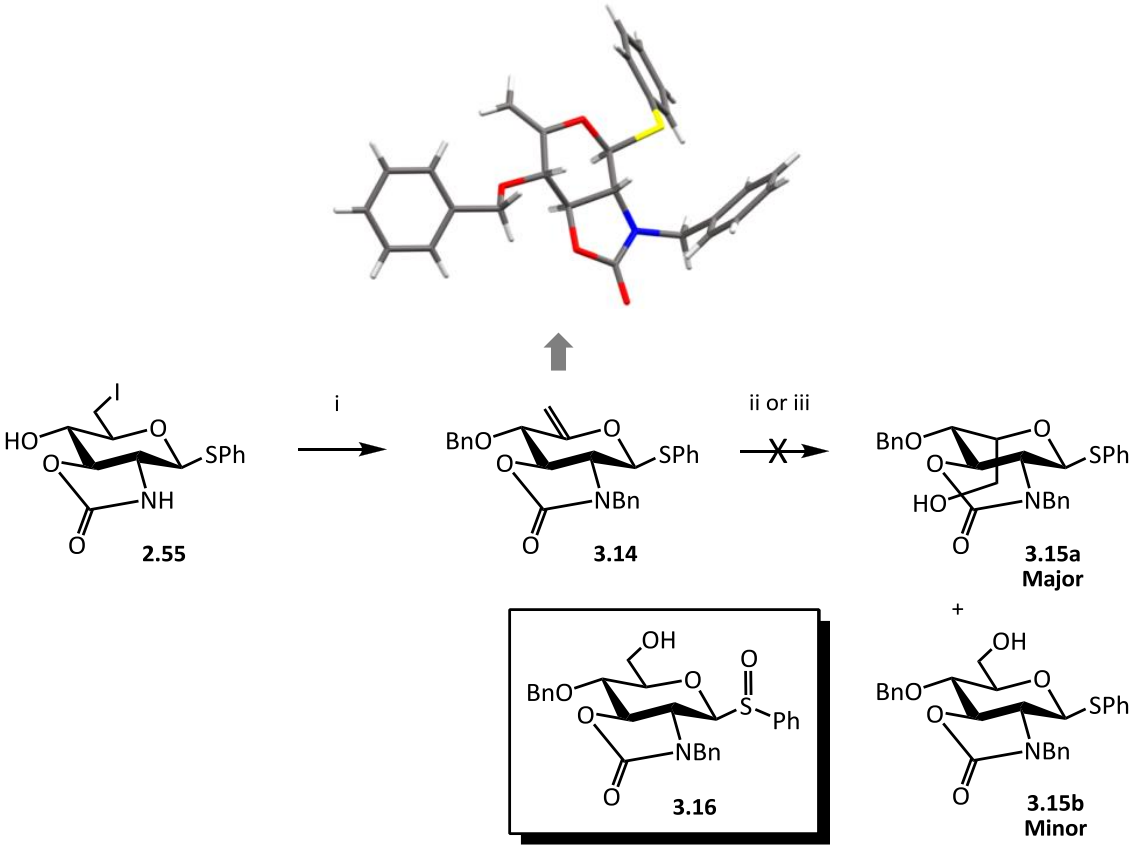


Scheme 3.3: Proposed synthesis of the AltNAcA derivatives **3.6** (**B''**) and **3.7** (**B'**) from the phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-*β*-D-glucopyranoside (**2.55**).

This proposed synthesis of the AltNAcA derivative is much shorter than that proposed earlier (**Ch 3.1**) and offers a novel and more efficient route to the synthesis of protected derivatives of both FucNAc4N and AltNAcA, allowing for assembly of the disaccharide.

The synthesis of the 6-iodo derivative **2.55** was achieved in a 79% yield over 6 steps *via* the methods described in **Ch 2.9**. In the first attempt at simultaneous protection at *O*-4 and elimination of the 6-iodide, **2.55** was treated with NaH and an inadvertent excess of BnBr in DMF for 1.5 hours at low temperature, yielding the *N*-benzyl-*O*-benzyl derivative **3.14** in a 90% yield. The dibenzylation was apparent from the ^{1}H NMR spectrum, with an AB quartet appearing at 4.75 ppm and two AB doublets at 4.75 ppm and 4.62 ppm (**Figure 3.4**) and the structure was confirmed by single crystal x-ray diffraction (**Scheme 3.4** and **Appendix 3**). Although introduction of the *N*-benzyl group had not been part of the plan, it did not seem to present any problems in the ongoing synthesis, and selective preparation of the 4-*O*-benzyl-2,3-oxazolidinone product was therefore not attempted. On re-evaluation of the proposed route, the

N-benzyl derivative was in fact seen as a potential benefit, as hydrolysis of the 2,3-oxazolidinone and *N*-acetylation would result in the *N*-acetamido, *N*-benzyl derivative, which could serve as an alternative to an *N*-acetyl, *N*-phthaloyl or *N*-trichloroacetyl protecting groups for glycosylation reactions.[31]



Scheme 3.4: Towards the synthesis of a protected AltNAcA derivative *via* **2.55**. Reagents and conditions: (i) NaH, BnBr, DMF, 0°C,30 mins, r.t. for 1.5 hrs, 90% (ii) a.) 0.5M 9-BBN, THF, -10°C, to r.t., 2 hrs, then another 7.4 eq. of 9-BBN, r.t., 4 hrs (iii) a.) 1M BH_3.THF, THF, 0°C, 1.5 hrs b.) 30% H_2O_2, 2N NaOH, r.t., 30 mins, 52%.

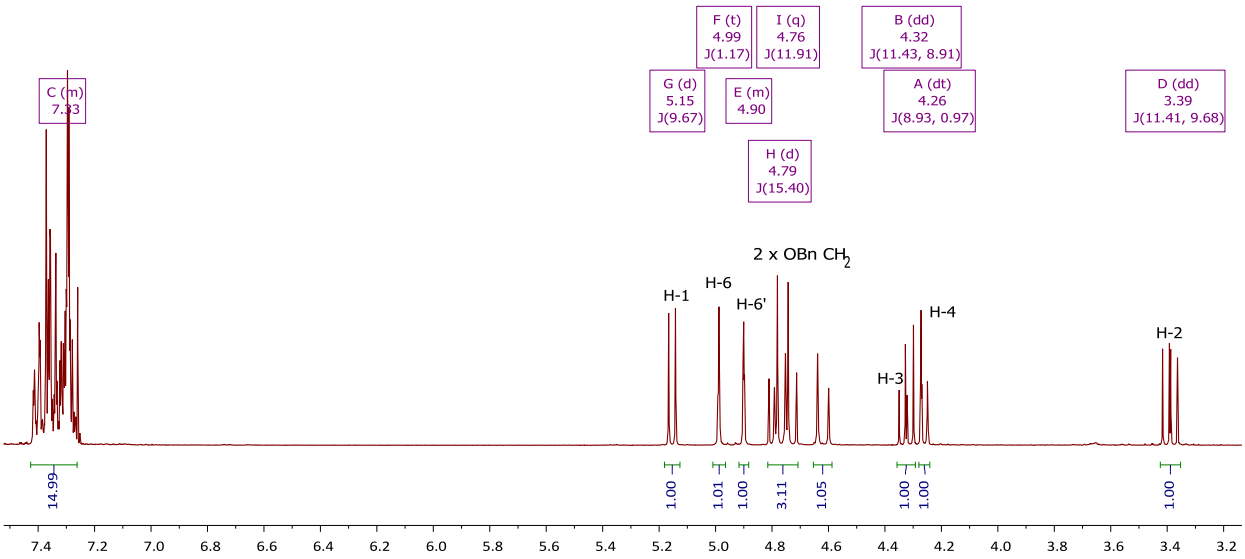


Figure 3.4: ^{1}H NMR spectrum of the 5-enopyranoside **3.14**. This shows the presence of two benzyl CH_2 groups with an AB quartet appearing at 4.75 ppm and two AB doublets at 4.75 ppm and 4.62 ppm.

The selective hydroboration of **3.14** was then attempted using both borane and the more sterically demanding 9-borabicyclo[3.3.1]nonane (9-BBN). In the latter case, even with up to 10 equivalents of 9-BBN, there was no reaction at all after 6 hours, suggesting that both faces of the 5-ene were sterically inaccessible. However, when **3.14** was treated with 10 eq. of BH_3.THF for 1.5 hours at 0°C followed by oxidative work-up, a single oxidation product was formed, although the ^{1}H NMR spectrum did not provide conclusive evidence for the formation of an L-idose derivative **3.15a** (which was anticipated to be the major product for this transformation) as the *J*-coupling constants for H-4 and H-5 were not well resolved. However, the NOESY spectrum (**Figure 3.5**) gives proof of the formation of the D-sugar **3.15b**, with the clear NOE between H-5 and both H-1 and H-3, which is expected as seen in **Figure 3.6**. If an α-L-idose derivative (**3.15a**) had formed, then there would be no NOEs between H-1 and H-5, and H-3 and H-5, since H-5 is on the opposite face, with the only observable NOE crosspeak between the H-1 and H-3 signals (the latter is not detectable in this case since the signals for H-1 and H-3 overlap). It is also noted that the $^{1}C_4$ chair conformer of the L-sugar is not possible due to the conformational restrictions of the 2,3-oxazolidinone group. This therefore gave clear evidence that oxidation had taken place from the α-face to give the D-sugar **3.16** rather than the L-sugar. In addition, the HRMS data suggested that in this process, the sulfide unexpectedly underwent oxidation to the sulfoxide. This provided an explanation for the fact that signals in the ^{1}H NMR spectrum for certain protons were poorly resolved or appeared to indicate two closely similar products. This may be due to the presence of two sulfoxide diastereomers. While the oxidation to the sulfoxide was not anticipated, it presented no problem, as this conversion was part of the

overall strategy for fine-tuning the reactivity of the glycosyl donor and acceptor (discussed in **Ch. 1.13**).[32–37]

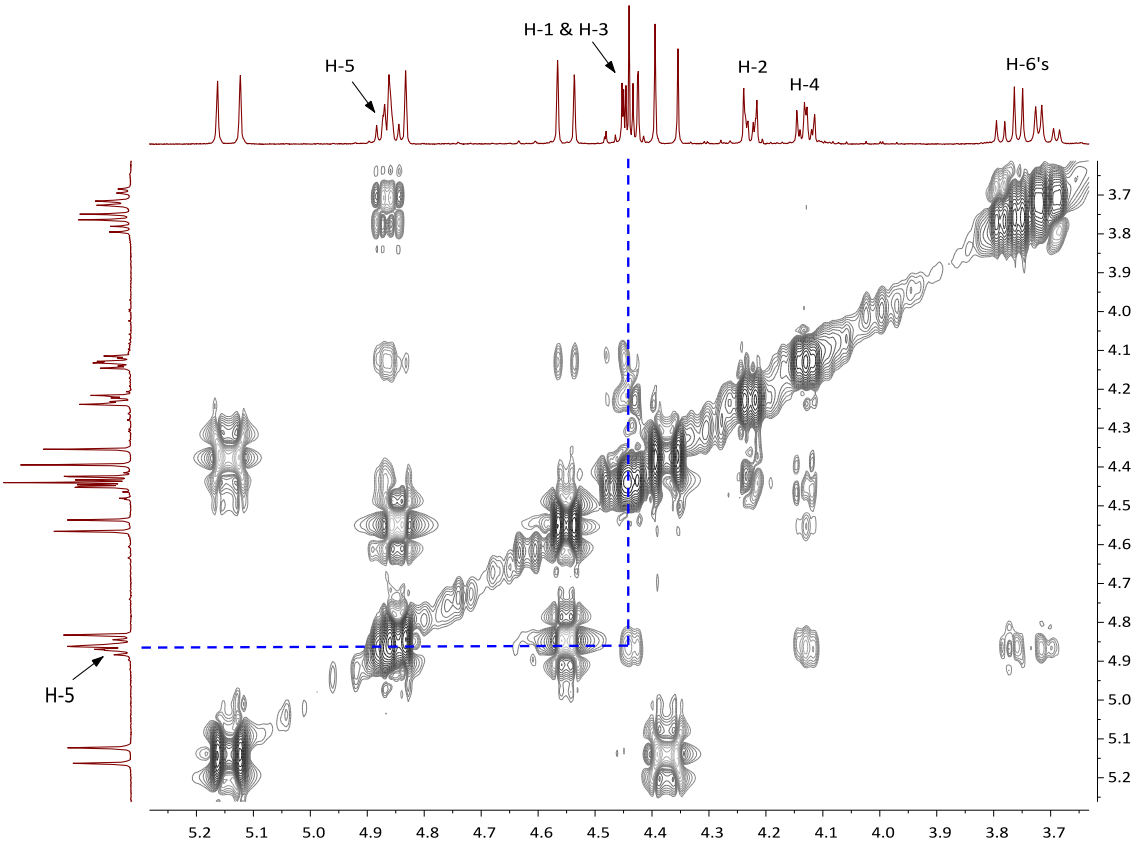


Figure 3.5: NOESY NMR spectrum of compound **3.16**. The distinct NOEs between H-5, H-1 and H-3 confirm that upon hydroboration/oxidation of **3.14**, the D-sugar is formed exclusively.

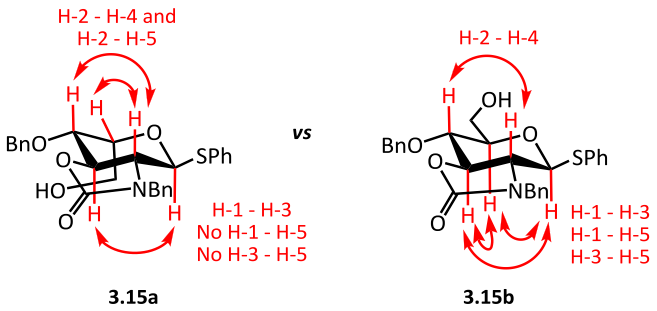
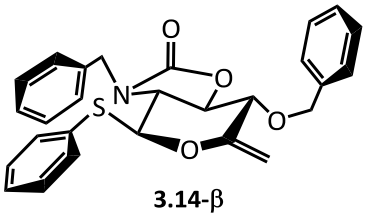


Figure 3.6: The expected NOE interactions of **3.15a** and **3.15b**.

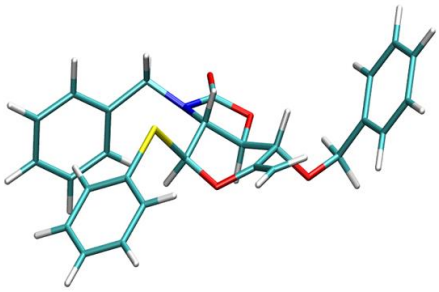
The foregoing result led to a re-evaluation of the proposed route towards the L-sugar, and in particular, the stereo-electronic requirements for facial-selectivity in the hydroboration step. A closer inspection of the literature revealed that the configuration of the anomeric position was

crucial in determining this selectivity in the attempted hydroboration-oxidation sequence.[27,29,38,39] This led to a brief molecular modelling investigation of the conformational preferences of the α- and β-phenylthio-2,3-oxazolidino-5-eno-glucopyranosides (**3.14**) using Spartan (**Figure 3.7**).[40] A conformer distribution search of 100000 conformers using MMFF force field of both **3.14α** and **3.14β** in the gas phase was carried out. The probable facial selectivity of the both anomers was then investigated using the lowest energy conformer. As seen in **Figure 3.7A**, the β-face (top face) in the β-anomer is sterically hindered by the anomeric sulfur substituent, whereas this top face is much less hindered in the α-anomer (**Figure 3.7B**).‡

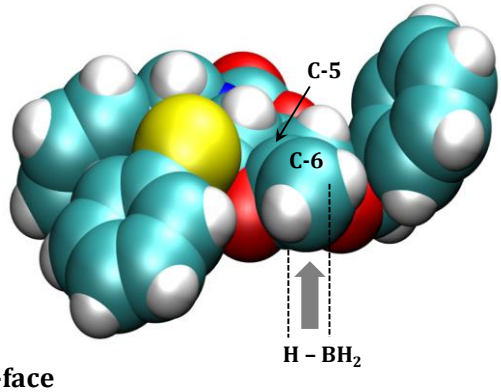
‡ There is free rotation around the C-O-Bn bond of the benzyl group, so steric hindrance from these functional groups is not taken into consideration when using this model for the basis of facial approach. Also, the conformer obtained is assumed to be the global lowest energy conformer, and not a local minima energy conformer.



3.14-β

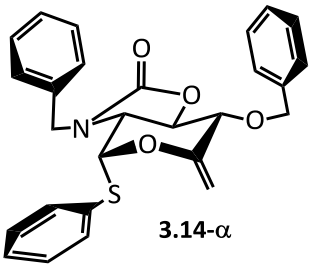


β-face

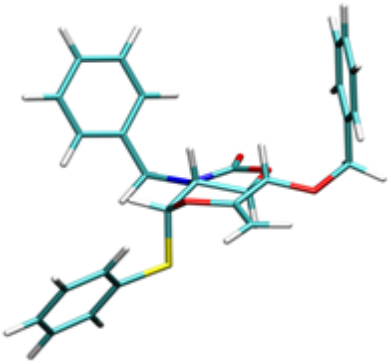


α-face

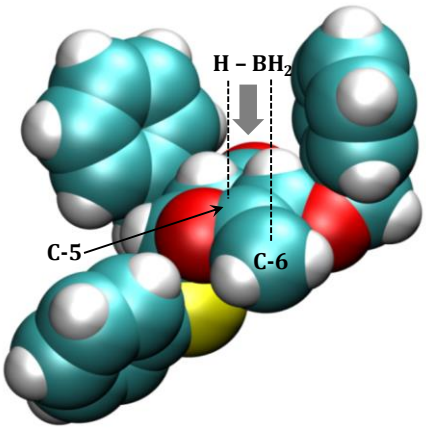
Figure 3.7A: Van der Waals diagram of the experimentally obtained β-anomer (**3.14-β**). This illustrates the less-hindered α-face of the 5-6-ene, and the expected preference for α-facial attack, which corresponds to the experimental result. From the modelling, the β-anomer is in a distorted boat, rather than a chair conformation.



3.14-α



β-face



α-face

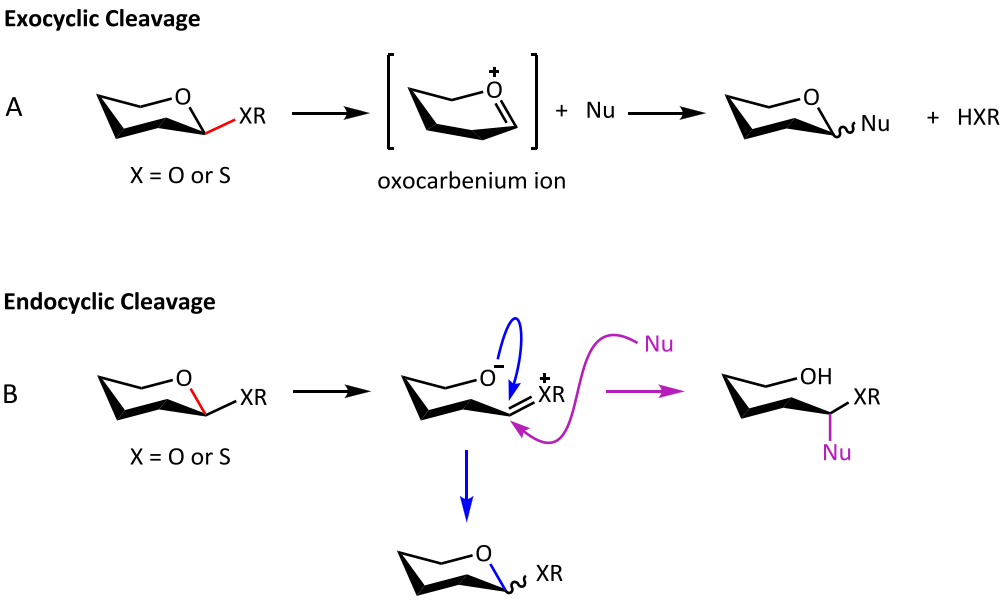
Figure 3.7B: Van der Waals diagram of the the theoretical α-anomer (**3.14-α**) This shows that the less hindered environment should favour the β-facial approach of the borane complex which should yield the L-sugar.

The modelling results suggested that a higher selectivity towards the L-sugar might be possible *via* the α-anomer. This analogue could be prepared in at least two distinctive ways, either starting with the α-anomer and continuing to form the 2,3-oxazolidinone, or by attempting an anomerization of the β-thioglycoside at a strategic point in the synthesis, with the latter being favoured in view of the advantages of using intermediates in hand.

3.3 Towards the synthesis of a protected AltNAcA derivative: third approach *via* the 2,3-oxazolidinone derivative

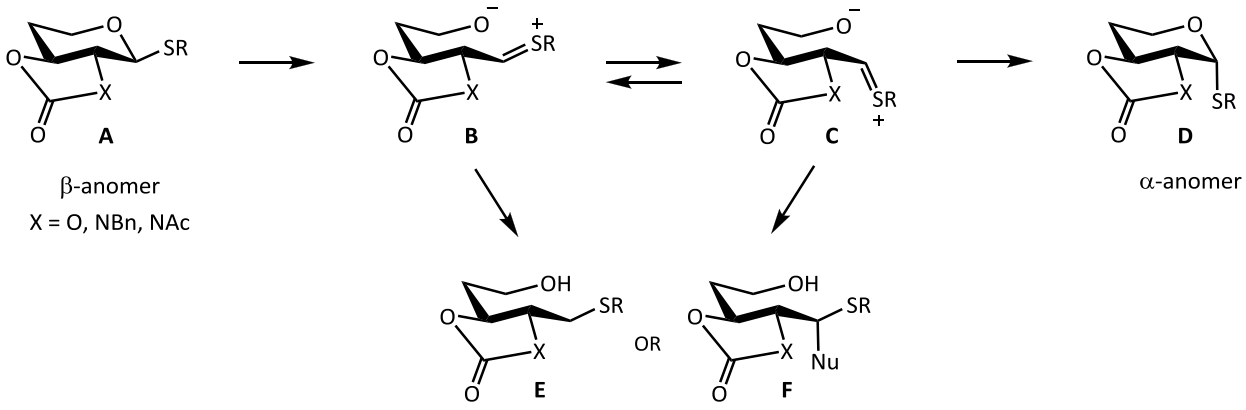
Having identified the α-thioglycoside as a better substrate for the attempted hydroboration/oxidation, consideration was given to whether this should be synthesized *de novo*, or *via* anomerization of the β-thioglycoside which we had in hand. A survey of the literature revealed that Lewis acids such as $FeCl_3$, $SnCl_4$ or $TiCl_4$ could be used to epimerize the anomeric centre of various β-glycosides even when C-2 substituents offering anchimeric assistance were in place.[41–43] Furthermore, a study by Satoh *et al.*[44] found that the presence of the 2,3-oxazolidinone ring in fact facilitates anomerization, with theoretical density functional theory (DFT) calculations showing that 2,3-oxazolidinone protected pyranosides undergo anomerization much more easily than their unprotected counterparts. Intriguing experimental results obtained by Vinod *et al.*[45] while cleaving a 4,6-*O*-benzylidene protecting group using $NaBH_3CN$, HCl/Et_2O show that it is possible to partially anomerize the 2,3-oxazolidinone using a Brønsted acid. Ollson *et al.*[46] were able to anomerize a 2,3-oxazolidinone substrate with AgOTf, while an extensive study carried out by Ito *et al.*[44,47] showed that anomerization was possible using $BF_3.Et_2O$. In addition, various publications by Manabe and Ito studied the effect of the Lewis acid ($BF_3.OEt_2$, $FeCl_3$, $Cu(OTf)_2$ and Tf_2NH),[48] the solvent[49] and various protecting groups[47,48,50] on the degree of anomerization.

This ease of anomerization of the 2,3-oxazolidinone protected pyranosides can be explained through their mode of cleavage. Since carbohydrates are asymmetrical acetals, cleavage can occur *via* two different pathways.[51–56] Exocyclic cleavage (**Scheme 3.5A**) is the more commonly encountered mechanism in carbohydrate chemistry and involves the cleavage of the bond between the exocyclic oxygen and the anomeric carbon atom (bond highlighted in red) to form the cyclic oxocarbenium ion. This is attacked by a nucleophile from either the α- or β-face depending on steric (β-destabilisation), stereoelectronic (anomeric effect) and neighbouring group participation (anchimeric) effects.[57] On the other hand, endocyclic cleavage (**Scheme 3.5B**) occurs when the bond between the ring oxygen and anomeric carbon breaks (bond highlighted in red) to yield an acyclic intermediate. This is less commonly postulated in carbohydrate chemistry despite various authors providing extensive experimental evidence for it,[53,58–62] and being the proposed mechanism among aldofuranosides.[63–65] The acyclic intermediate can then recyclise (blue mechanism), or the exocyclic oxocarbenium ion can be intercepted by a nucleophile (purple mechanism) to yield an acyclic product.



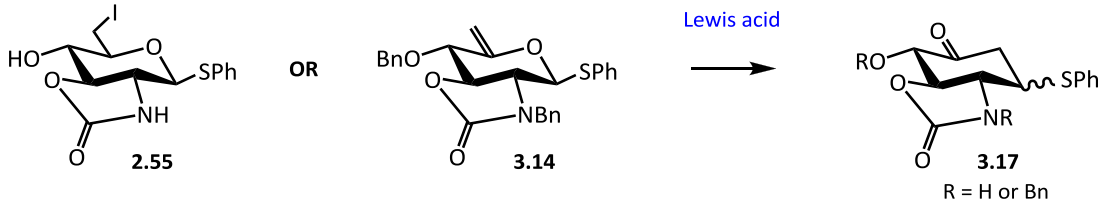
Scheme 3.5: Exocyclic *vs.* endocyclic cleavage of a pyranoside.

Manabe and Ito[44,48,50,66] have comprehensively shown that 2,3-oxazolidinone protected β-glycosides undergo endocyclic cleavage to reform the α-anomer**.** This is illustrated in **Scheme 3.6** where the β-anomer (**A**) when treated with acid forms the charged species **B**. The C1-C2 bond can rotate (**C**) and the ring can re-form in the more thermodynamically stable α-anomeric form (**D**). Both **B** and **C** can be reduced to form **E**, or intercepted by a nucleophile to form **F**. Various adducts of the acyclic cations produced *via* endocyclic cleavage were obtained experimentally by Manabe *et al.*[50] after treatment of their 2,3-oxazolidinone substrates with $NaBH_3CN$, HCl/THF or $BF_3.OEt_2$, Et_3SiH. In addition, intra- and intermolecular Friedel-Crafts products were obtained upon treatment with only $BF_3.OEt_2$, proving that the 2,3-oxazolidinone compounds undergo endocyclic cleavage.[50] In this early report, they postulated that this was due to the locked $^4C_1$ conformer that the bicyclic structure adopts, which together with the β-configuration makes exocyclic cleavage unfavourable stereoelectronically. Various early publications also report this finding, where β-anomers undergo predominantly endocyclic cleavage.[59–61] Later studies by Manabe *et al*. however showed *via* QM calculations that the locked $^4C_1$ conformer is in fact a secondary factor, with the inner ring strain caused by the bicyclic structure being the dominant contribution to endocyclic cleavage.[66]



Scheme 3.6: Anomerization of a β-2,3-oxazolidinone protected pyranoside. Pathway proceeds *via* endocyclic cleavage, which yields the α-anomer **D**. **E** or **F** can also be formed.

Careful consideration was therefore given to the most appropriate stage in the synthesis for the anomerization to be attempted. Firstly, it was noted that attempted anomerization at a later stage in the synthesis, for example of either compound **2.55** or **3.14** (**Scheme 3.7**) under Lewis acid conditions could potentially be in competition with a Ferrier Type II rearrangement,[67,68] forming the rearranged cyclohexanone **3.17**. This has been reported by various authors[69–72] and is also an efficient method of synthesising D-*myo*-inositol from D-sugars.[73]

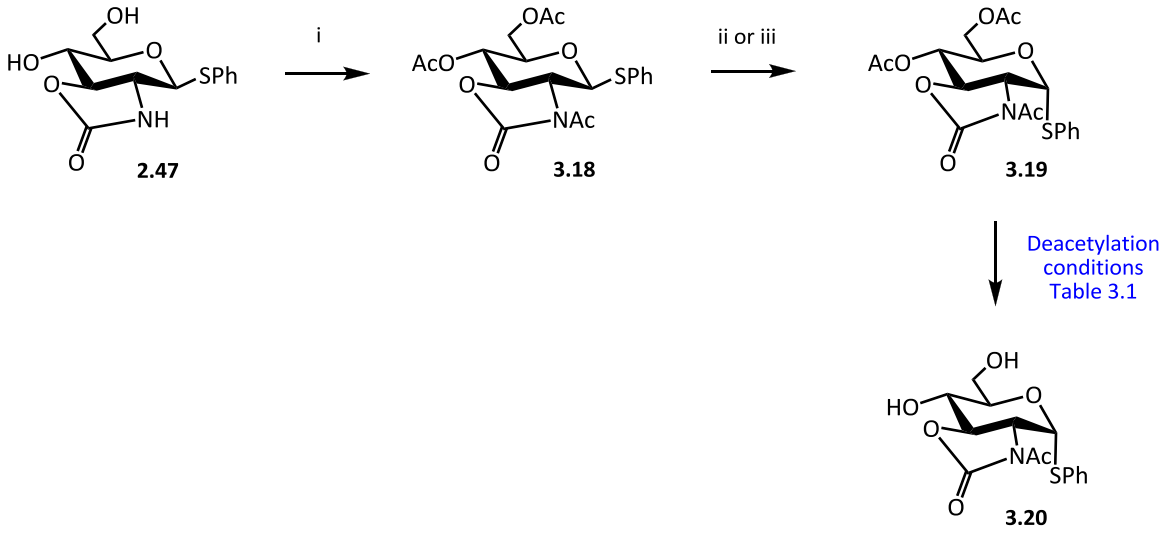


Scheme 3.7: Treatment of either **2.55** or **3.14** with a Lewis acid would undergo Ferrier Type II rearrangement to give the cyclohexanone **3.17**.

Secondly, in considering the role of protecting groups, it was noted that higher ratios of the α-anomer were obtained with *N*-acetylated rather than *N*-benzylated derivatives.[47,48,50] Based on these observations, and the absence of reports in the literature of anomerizing an unprotected 2,3-oxazolidinone, it was decided to first prepare the fully acetylated derivative **3.18** from **2.47** (**Scheme 3.8**).‡[74] In the first attempt at anomerization, the fully acetylated sugar (**3.18**) was treated with 0.02 eq. of AgOTf for 20 hours, but this gave a mixture of α- and β-anomers. However, treatment of **3.20** with 2.5 eq. of $SnCl_4$ for 25 hours gave exclusively the α-anomer

‡ At the time of writing, a publication by Manabe *et al*. showed that anomerization on an unprotected 2,3-oxazolidinone gives very low yields.[78]

(**3.19**) in an 89% yield. Confirmation of the anomeric stereochemistry was gained from the ^{1}H NMR spectrum (**Figure 3.8**) where the *J*-coupling constant for the anomeric proton in the β-anomer (**3.18**) is 8.3 Hz, while the signal for the anomeric proton in the α-anomer (**3.19**) is more deshielded and has a smaller *J*-coupling of 4.6 Hz.



Scheme 3.8: Anomerization of **3.18** followed by de-*O*-acetylation of **3.19**. Reagents and conditions: (i) AcCl, DIPEA, DCM, 0°C, 45 mins, 96% (ii) AgOTf, DCM, r.t., 20 hrs, 77% (9:1 α/β mixture from NMR) (iii) $SnCl_4$, DCM, r.t., 25 hrs, 89% (α-anomer only).

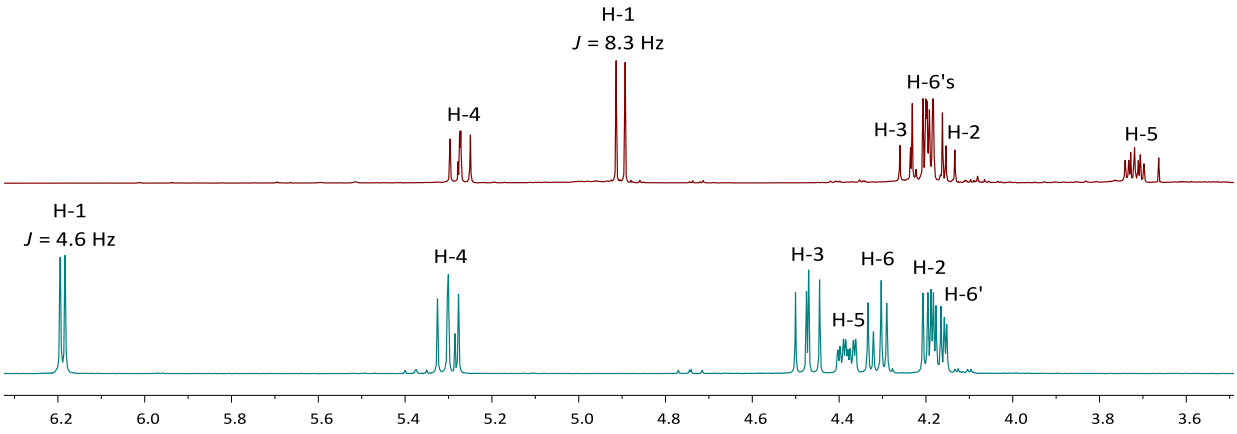
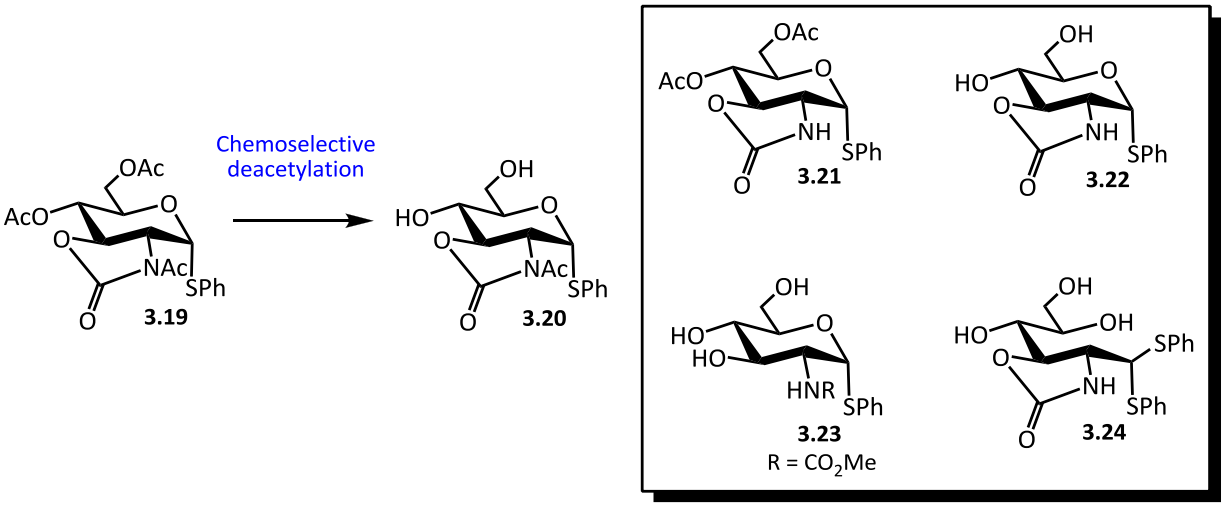


Figure 3.8: ^{1}H NMR spectra of α-anomer **3.19** (top) and the β-anomer **3.18** (bottom), where the clear difference in *J*-coupling and chemical shift is indicative that anomerization has taken place.

Having efficiently achieved the anomerization, the next step required the chemoselective de-*O*-acetylation of **3.19**. In **Ch. 2.9**, the chemoselective cleavage of the 2,3-oxazolidinone ring was explored, whereas in this case it was hoped that under selected basic conditions only de-*O*-acetylation or global deacetylation would occur, leaving the 2,3-oxazolidinone in place to allow

for later differentiation of *O*-3 and *O*-4. To this end, a variety of deacetylation conditions were explored (**Table 3.1**).

Table 3.1: Attempted chemoselective de-*O*-acetylation reagents & conditions.

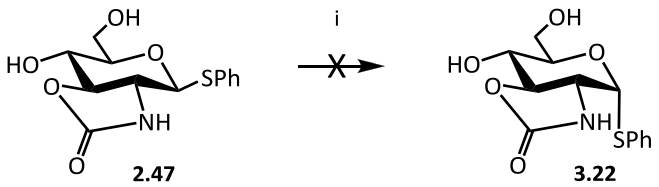


Entry	Reagents & conditions	Product	Yield
1	4.62 M NaOMe (2 drops), MeOH, 0°C to r.t., 30 mins.	**3.21**	55%
2	4.62 M NaOMe (0.4 eq.), MeOH, 0°C to r.t., 30 mins.	**3.22, 3.23 & 3.24**	22 mg recovered from 68 mg SM
3	K_2CO_3, MeOH, H_2O, 10 mins, r.t.	**3.22, 3.23 & 3.24**	36 mg recovered from 100 mg SM
4	LiOH, H_2O_2, THF, H_2O, 0°C, 2 hrs.	Degraded	-
5	LiOH, H_2O_2, THF, H_2O, -40°C, 5 mins.	Multiple products	-
6	NaOH (2M), THF, 0°C to r.t., 2 hrs.	Degraded	-
7	HCl, acetone, H_2O, 65°C, 4.5 hrs, r.t., 18 hrs.	**3.20**	29%
8	HCl, acetone, H_2O, 65°C, 4 hrs.	**3.20**	49%

Initial attempts using NaOMe (entry 1) surprisingly achieved chemoselective cleavage of the *N*-acetate to give the 4,6-di-*O*-acetate (**3.21**) (**Table 3.1**). Another attempt in which the number of equivalents of NaOMe was increased (entry 2) or where K_2CO_3 (entry 3) was used, gave interesting results where the formation of a single product with lower R_f was evident from TLC. Upon purification, the ^{1}H NMR (**Appendix 2**) revealed that it was in fact a complex mixture of compounds with HRMS providing evidence for the presence of compounds **3.22**, **3.23** and **3.24**. The formation of **3.22** and carbamate **3.23** is expected, as seen in the literature[75,76] and by

previous experimental results (**Ch. 2.9**). However, the formation of **3.24** is unexpected, as thiophenol, as well as acidic conditions would be required to give the exo- and endocyclic cleaved products in order to form the di-thioacetyl.‡ So next, LiOH and H_2O_2 was used (entries 4 and 5). Wei *et al*.[76] found these conditions to give the highest chemoselectivity when hydrolysing their *N*-acetyl-2,3-oxazolidinone. Unfortunately, entry 4 conditions led to degradation, while entry 5 conditions gave multiple product spots as seen from TLC. Following this, NaOH in THF (entry 6) was used and again led to degradation of the starting material. Finally, since base hydrolysis yielded futile results, acid was instead used in an attempt to remove the *O*-acetates (entry 7, **Table 3.1**) as described by Khiar *et al*.[77] This yielded the desired de-*O*-acetylated product (**3.20**), albeit in a low yield with evidence for formation of other unidentified minor products (as seen per TLC), possibly resulting from hydrolysis of the phenylthioglycoside.# However, repetition using less HCl over a shorter duration (entry 8) gave a reasonable yield of 49% which was suitable for continuation of the synthesis.

Given the mixed success of the foregoing experiments, anomerization of the un-protected 2,3-oxazolidinone was investigated (**Scheme 3.9**). This would circumvent the problems encountered above. Unfortunately, when **2.47** was treated with $SnCl_4$ at r.t for 20 hours with monitoring by TLC, multiple products had formed. Attempts to repeat this reaction at lower temperatures with alternative Lewis acids and solvents could have been investigated, however, a recent publication by Manabe and Ito[78] reported that the *N*-substituent on the oxazolidinone was critical to anomerization, confirming our own experiences.



Scheme 3.9: Attempts at anomerization of the unprotected 2,3-oxazolidinone. Reagents and conditions: (i) $SnCl_4$, DCM, r.t., 20 hrs.

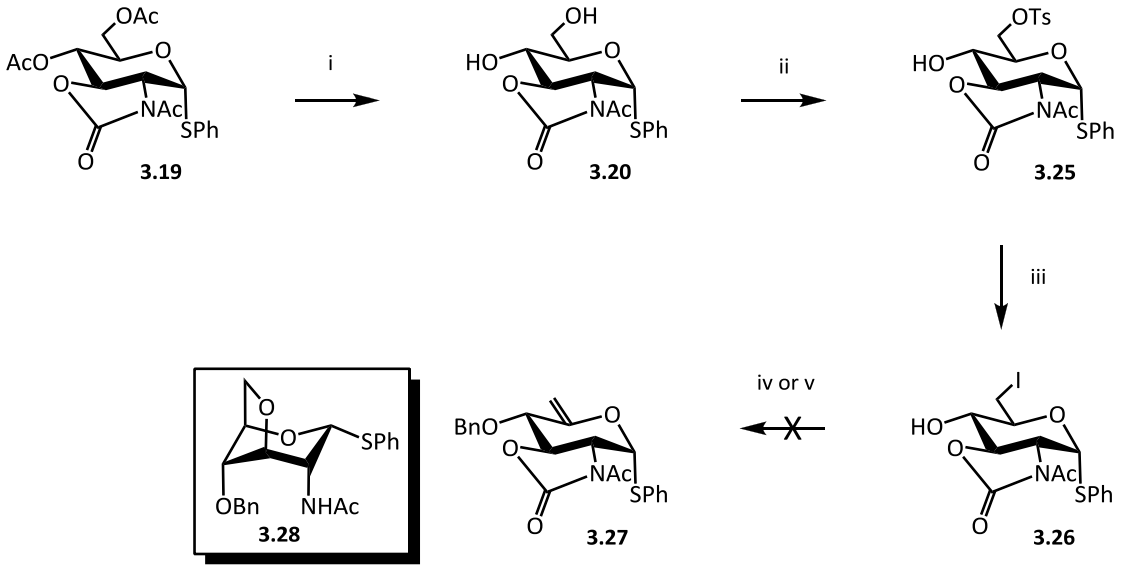
In light of this, the next step required conversion of **3.20** to the 6-iodo derivative in order to set up the elimination to give the 5-enopyranoside. As before, a sequence of regioselective tosylation (**2.25**) (**Scheme 3.10**), followed by substitution by the iodide gave **3.26**. Treatment

‡ The Amberlite used to neutralize the reaction mixture could however have been too acidic and responsible for this. The low mass recovery suggests that degradation also occurred and could possibly be the source of thiophenol.

The smell of thiophenol was detectable during work-up.

of **3.26** with standard benzylation conditions either at room temperature or at 0°C using 2-3 equivalents of NaH produced a single less polar product.[79] However, there was no spectroscopic evidence for the formation of the 5-enopyranoside (**3.27**), with the ^{1}H NMR spectrum (**Figure 3.9**) lacking the distinctive signals for the exocyclic alkene.[#] The COSY spectrum showed a distinct crosspeak between H-5 and H-6, with the H-6's appearing as a doublet and a doublet of doublets respectively (normally both appear as dd), indicating potential conformational restriction at this carbon. Further evidence from the NMR spectra as well as HRMS ($[M+H]^+$ of 386.1428 m/z) pointed towards the proposed 3,6-anhydro sugar **3.28** having formed instead of the expected 5-enopyranoside **3.27**. Compound **3.28** is assumed to adopt the $^1C_4$ conformation due to the 3,6-bridge, where evidence for this is seen in the small equatorial-equatorial couplings between H-3, H-4 and H-5 as opposed to the larger *J*-values (axial-axial) which would be seen if it was in the $^4C_1$ conformer.

The formation of **3.28** can be accounted for by a sequence involving initial protection at *O*-4, followed by hydrolysis of the 2,3-oxazolidinone and subsequent intramolecular S_N2 substitution at C-6 by *O*-3 to give the 3,6-anhydro sugar.[‡] The formation of 3,6-anhydro rings is not uncommon, these having been synthesised by numerous groups in a similar manner.[80–83]



Scheme 3.10: Attempts to obtain the 5-enopyranoside **3.27**. Reagents and conditions: (i) 10.2M HCl, acetone/H_2O (2:1), 65°C for 4.5 hrs, 49% (ii) *p*-TsCl (1.2 eq.), pyridine, 0°C 3 hrs, r.t., 1.5 hrs, 77% (iii) NaI, 2-butanone, reflux, 17 hrs, 67% (iv) BnBr, NaH, DMF, r.t., 6 hrs, 65% (v) BnBr, NaH, DMF, 0°C, 2 hrs, r.t., 6 hrs, 49%

[#] Interestingly, H-1 of **3.20** and **3.26** appear as doublet of doublets.
[‡] H-4 of **3.28** appears as a ddd (*J* = 5.5, 2.6, 0.9 Hz) due to W-coupling between H-2 - C-2 - C-3 - C-4 - H-4.

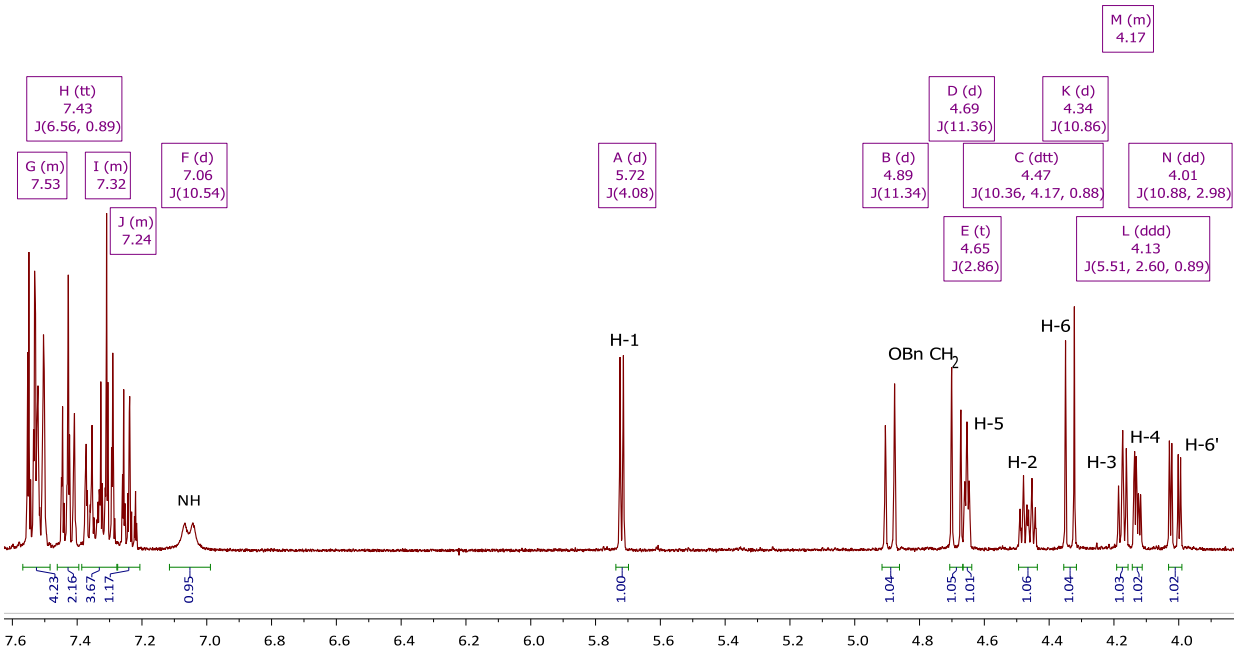
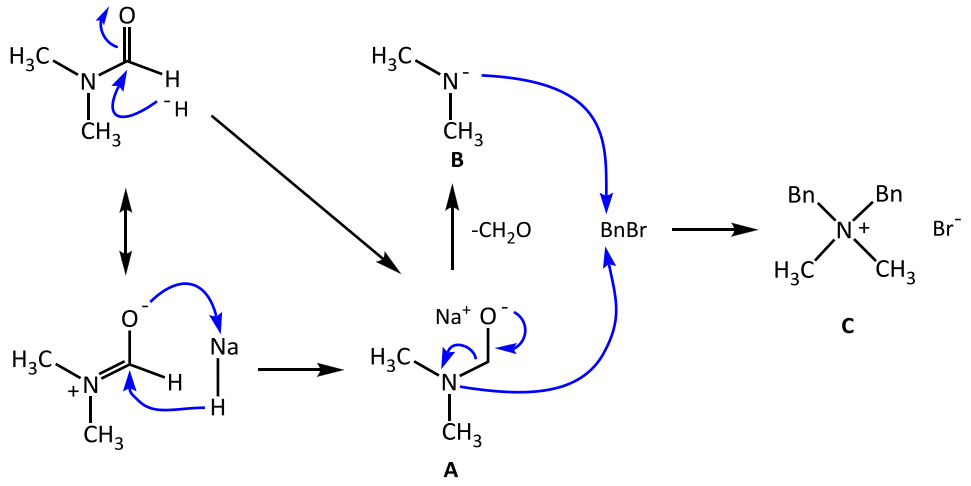


Figure 3.9: ^{1}H NMR of the 3,6-anydro sugar **3.28**.

While this was not the desired result, it presented the intriguing outcome where the 2,3-oxazolidinone is cleaved under these conditions. At first, it was suspected that H_2O may have been present in the reaction mixture, but the reaction was repeated using freshly activated 3Å powdered molecular sieves and under these conditions the same result was obtained. Dimethylamine could potentially be responsible for this, since it can form through the disproportionation reaction of DMF,[84] and is also known to exist in old bottles of DMF.[85] However, the most likely source is probably the dimethylamide anion which forms *in situ* from NaH and DMF, and could be responsible for S_NAc attack at the 2,3-oxazolidinone carbonyl group. Evidence for this has been proposed by Hesek *et al*.[86] who isolated the *N*-dibenzyl-dimethyl bromide salt (**C**) (**Scheme 3.11**), the formation of which can only be explained by the reaction between NaH and DMF, giving either the sodium (dimethylamino)methanolate (**A**) or dimethylamide anion (**B**) which reacts with the excess BnBr present. With this, the sodium (dimethylamino)methanolate (**A**) could also be responsible for cleavage of the 2,3-oxazolidinone ring.



Scheme 3.11: Proposal by Hesek *et al.*[86] for formation of **C** which can form either *via* generation of the sodium (dimethylamino)methanolate (**A**) or the dimethylamide anion (**B**).

In order to probe whether this is in fact the case, these reactions could be repeated by first protecting the 4-*O*-position as the silyl ether or another non-base sensitive protecting group and treating this derivative with NaH in DMF or alternatively repeating this reaction with NaH in anhydrous THF, and then compare the results. However, time did not permit for any of these reactions to be explored further.

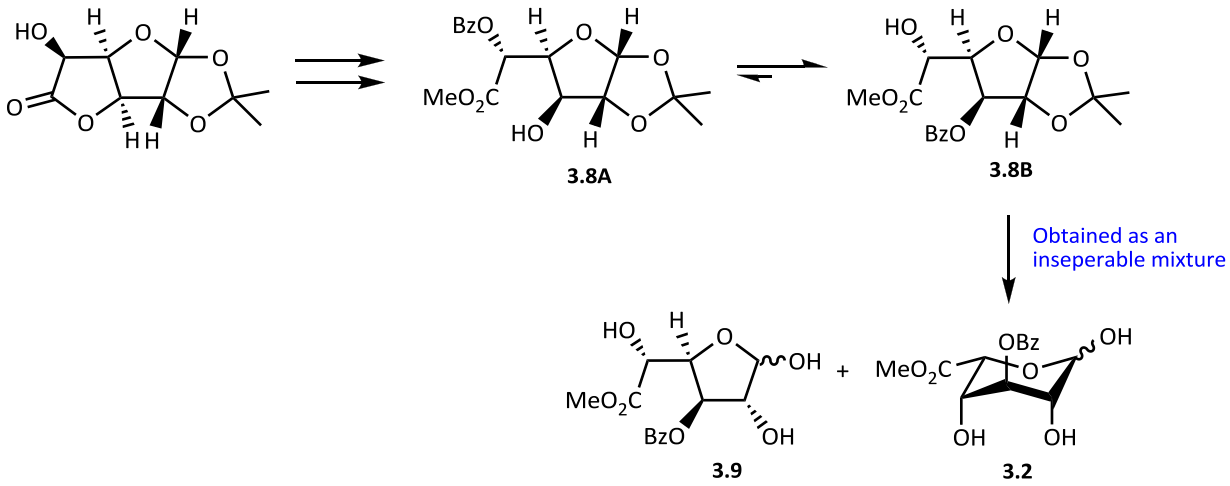
The next intriguing result from this reaction is that complete chemoselectivity in the 2,3-oxazolidinone cleavage is observed. As has been stated earlier, poor chemoselectivity has previously been reported for this attempted transformation,[76,87,88] and our results may contribute to the development of a viable and efficient alternative for this conversion. In this instance, the chemoselectivity can potentially be explained (as in **Ch. 2.9**) by the chelation of the Lewis acidic Na^+ with the carbonyl carbon of the *N*-acetyl group and the anomeric sulfur atom. However, the effect of the anomeric substituent and the configuration will also need to be evaluated.

By this stage, it was apparent that although the anomerization to the α-thioglycoside could be achieved with high efficiency, the resultant α-anomer **3.26** significantly impedes the possible E_1 or E_2 elimination reaction at C-5/6. This may potentially be based on steric interactions from the α-thioglycoside with the *trans*-2,3-oxazolidinone in place. Alternatively, the increased ring strain associated with the elimination product, where an sp^2 centre at C-5 of the 5-enopyranoside **3.27** is formed, and the combination of a fused oxazolidinone ring at C-2/3 as well as an α-substituent at C-1 may be too high an energy barrier to overcome. This result

points to conditions of the reaction and/or the reactivity of the 6-iodo derivative **3.26** not favouring elimination at C-5 to form the 5-enopyranoside **3.27**.

3.4 Summary

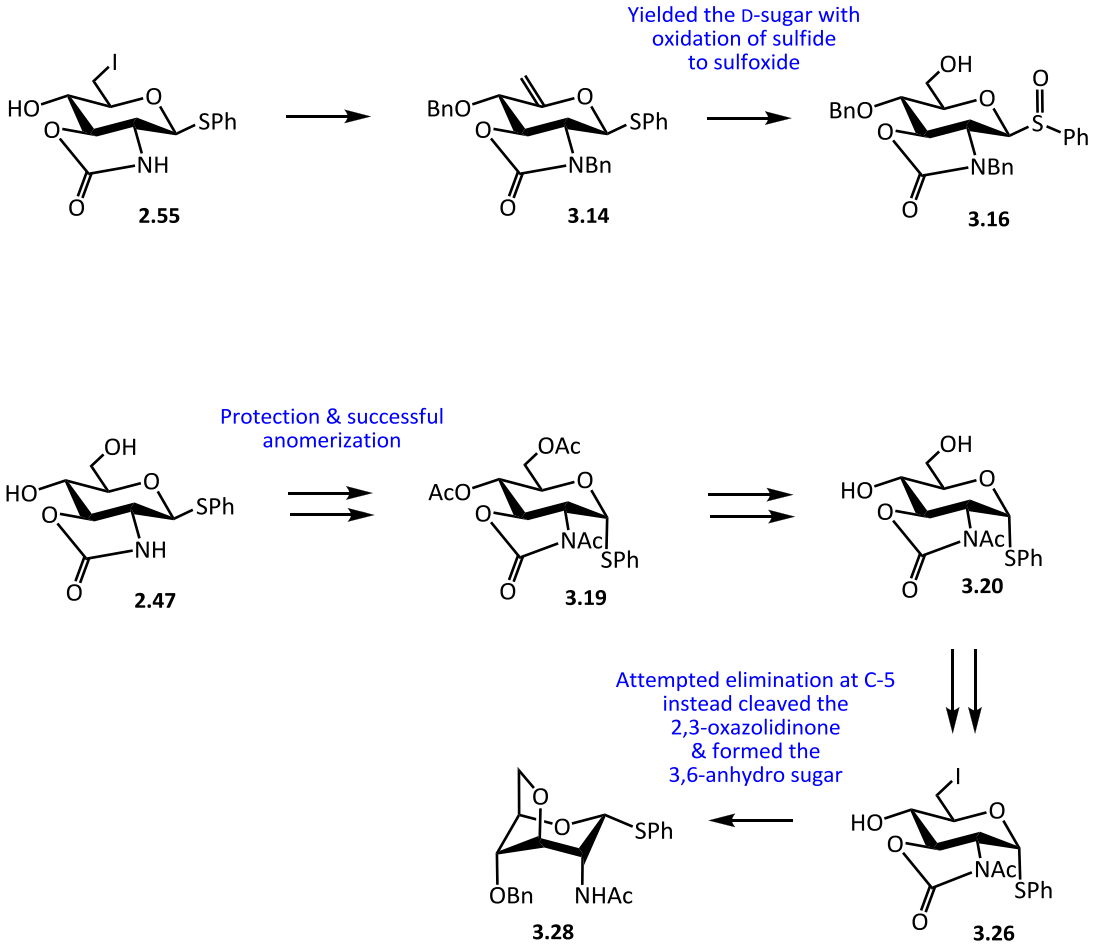
Different approaches to the preparation of the AltNAcA derivative were investigated. In the first instance, starting from a furanolactone offered access to the 5-*O*-benzoyl furanolactone **3.1** (**Scheme 3.12**) and the subsequent L-iduronic acid derivative **3.2** as reported by Ke and Whitefield.[2] This allowed for an interesting and moderately efficient route to obtaining the AltNAcA derivative. However, in the key base-catalysed methanolysis of the lactone **3.1**, it proved difficult to control the migration of the benzoate from *O*-5 to *O*-3, and the 5-benzoate (**3.8A**) and 3-benzoate (**3.8B**) were obtained as an almost inseparable mixture. When a sufficient quantity of **3.8B** had accumulated following careful separation of the benzoates, the subsequent acid hydrolysis step yielded an inseparable mixture of anomers of the desired pyranose **3.2** and the undesired furanose **3.9**. In the light of these inefficiencies, this route was not pursued further.



Scheme 3.12: Summary of synthesis *via* a fused furanolactone sugar.

The idea of synthesising both sugars from an intermediate in the FucNAc4N synthesis was then explored. They key challenge here was to form a 5-enopyranoside derivative, which could be transformed to the L-sugar *via* a selective hydroboration-oxidation. Conversion of the oxazolidinone-protected-6-iodo derivative **2.55** to the 5-enopyranoside **3.14** proceeded smoothly, but upon hydroboration/oxidation yielded the D-sugar **3.16** only (**Scheme 3.13**). From modelling and a survey of the literature,[27,29,38,39] it was postulated that the anomeric configuration in the 5-enopyranoside played a crucial part in determining the facial attack of the

borane complex, and this led to synthesis of an α-anomer. Serendipitously, the presence of the 2,3-oxazolidinone facilitated the efficient anomerization of the β-sulfide to form α-D-glucopyranoside **3.19**, presumably *via* initial endocyclic cleavage of the anomeric thioacetal. Chemoselective hydrolysis to the diol **3.20** was achieved in a moderate yield, and subsequent transformation to the 6-iodo derivative **3.26** was successful. This was then set up for the one-pot benzylation at *O*-4 and elimination step. This however was not achieved, with the 3,6-anhydro sugar **3.28** obtained instead, through the premature cleavage of the 2,3-oxazolidinone under these conditions. It was hoped that having an α-5-enopyranoside in hand would predominantly yield an L-sugar after hydroboration/oxidation, and despite anomerization of the β-derivative **2.47** to give the α-D-glucopyranoside **3.19** working well, it now seemed that the presence of the α-sulfur substituent, along with the *trans*-fused-2,3-oxazolidinone in fact hindered elimination at C-5 under these conditions.

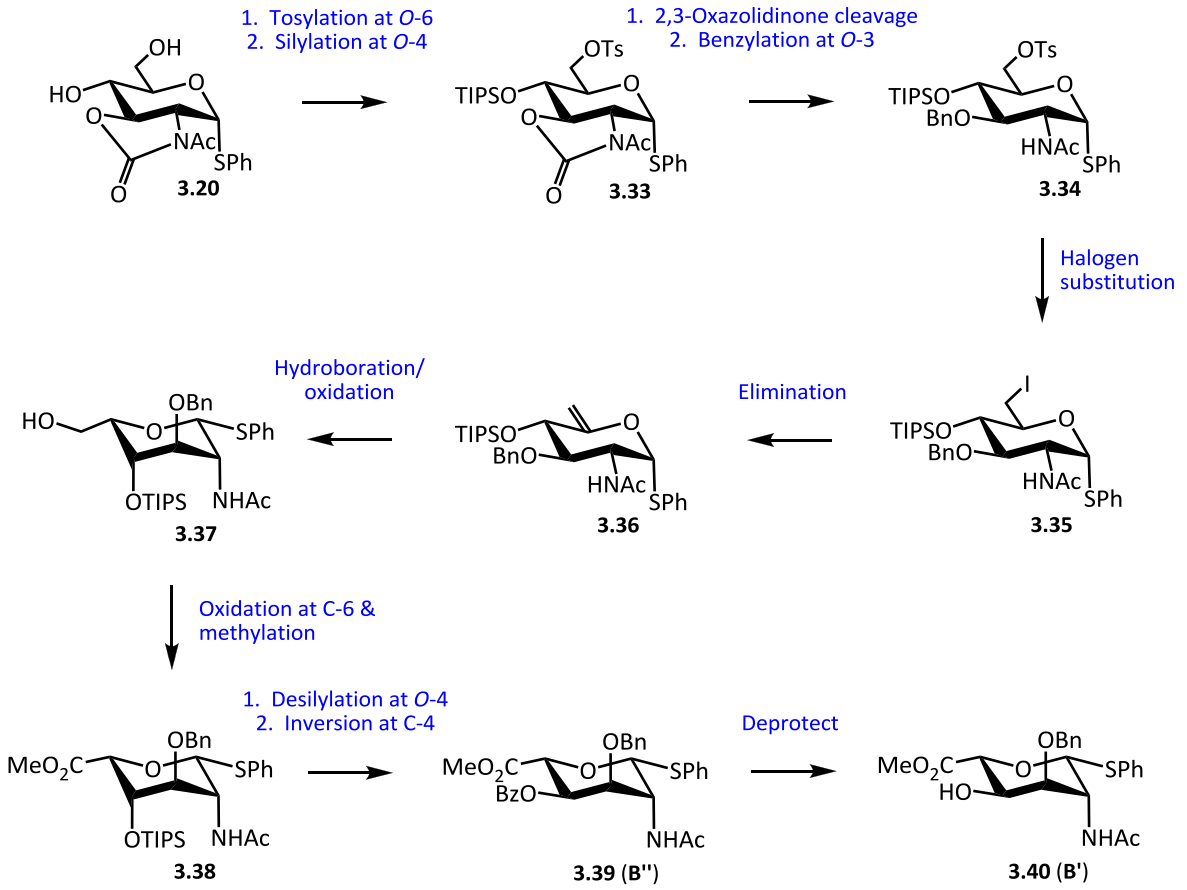


Scheme 3.13: Summary of synthesis *via* the 2,3-oxazolidinone sugar.

The idea of obtaining both sugars from a common intermediate is still worth pursuing. In order to obtain the AltNAcA derivative *via* the 2,3-oxazolidinone route, an alternative has been proposed (**Scheme 3.14**), which will attempt to deal with the limitations exposed from the reactions carried out.

3.5 Proposed synthesis of the protected AltNAcA derivative

Taking stock of the results presented, an alternative route to the AltNAcA derivative is proposed and deals with the premature hydrolysis of the 2,3-oxazolidinone ring, which in turn will prevent the formation of the 3,6-anhydro sugar and hopefully facilitate elimination at C-5 to form a 5-enopyranoside derivative. This is outlined in **Scheme 3.14**. Firstly, the diol **3.20** could be converted to the 6-*O*-tosyl derivative as before and can immediately be protected as the silyl ether, giving **3.33**. At this point, deliberate cleavage of the 2,3-oxazolidinone with LiCl and LiOH, followed by 3-*O*-protection with BnBr can give **3.34,** which will block any 3,6-anhydro formation later. The cleavage of the 2,3-oxazolidinone and 3-protection can also potentially be done in one pot with an excess of BnBr and NaH in DMF as per our observations. Following this, halogen substitution to give **3.35** can be carried out as before, which with the reduced steric interactions and ring strain will hopefully allow for the E_2 elimination reaction to take place giving **3.36**. After hydroboration/oxidation the L-sugar **3.37** should be formed in majority. Subsequent oxidation to the acid and methylation to give the L-*ido* derivative **3.38** then sets the stage for C-4 inversion *via* triflation followed by a benzoate or nitrite-mediated substitution to give the L-*altro* derivative **3.39** (**B''**). Deprotection will then give the AltNAcA derivative **3.40** (**B'**).



Scheme 3.14: Proposed synthesis of the AltNAcA derivatives **3.39** (**B''**) and **3.40** (**B'**) from the phenyl 2-acetamido-2-*N*,3-*O*-carbonyl-1-thio-*β*-D-glucopyranoside (**3.20**).

The proposed synthesis would allow both the FucNAc4n derivatives (**A'** and **A''**) and AltNAcA derivatives (**B'** and **B''**) to be obtained from a common intermediate.

The use of the thiazoline **2.17** (**Scheme 2.10**, **Ch. 2.4**) could be investigated as a suitable starting point, as the conversion to the 2-acetamido-1-thiol **2.28** gives the desired α-configuration, eliminating the need for any anomerization reactions. However, since the yields for the synthesis of **2.28** and **2.29** were low, this would not be as efficient.

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Chapter 4: Conclusions and Outlook

The aim of the project was to synthesize derivatives of the two monomers, 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranose (FucNAc4N/AAT) and 2-acetamido-2-deoxy-α-L-altruronic acid (AltNAcA) found in the *S. sonnei* repeating unit. These were required in the suitably protected forms of **A'**, **A''**, **B'**, **B''** (see **Scheme 1.21**), which could then be coupled to give the desired disaccharides **AB** and **BA**.

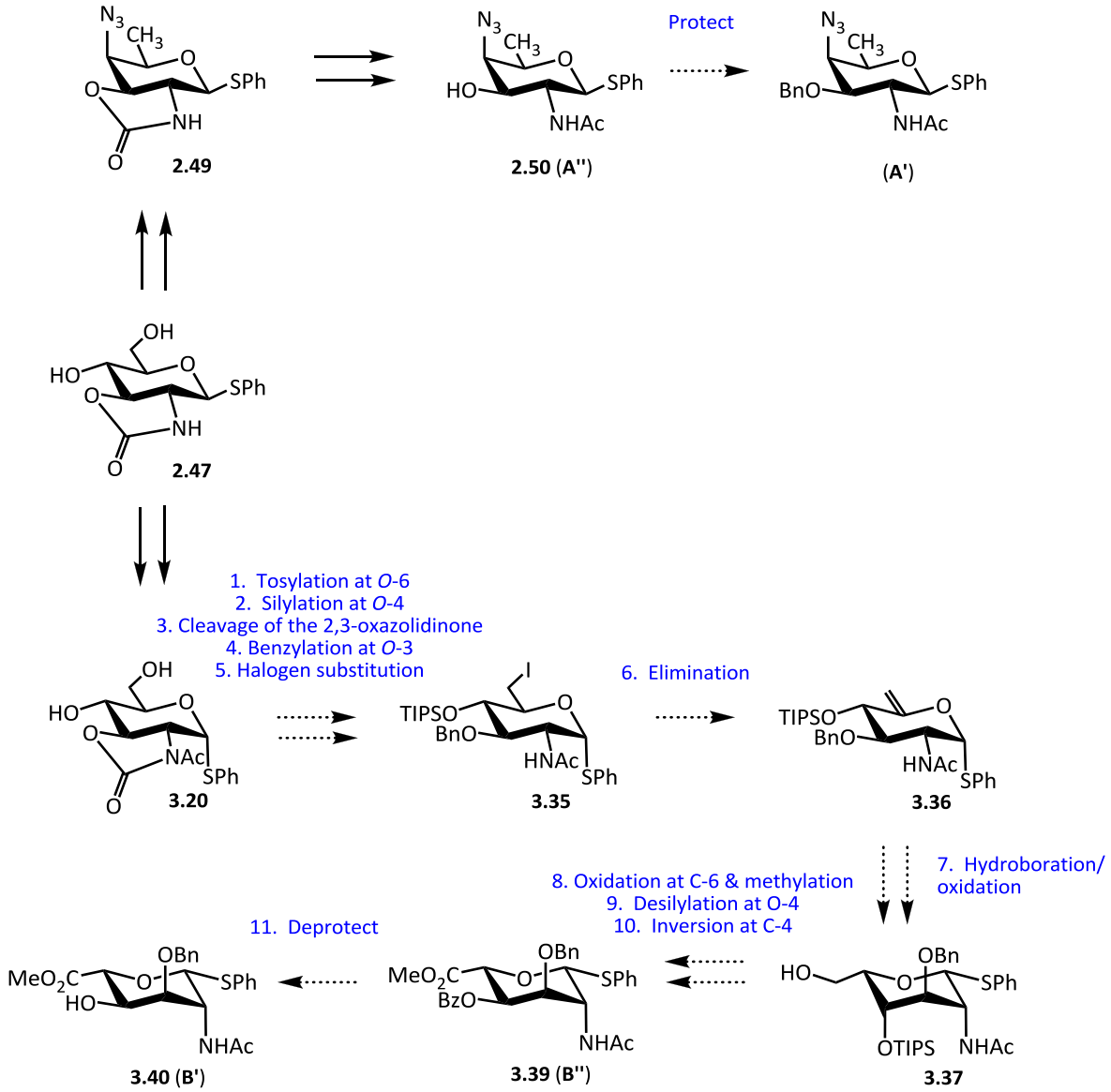
Synthetic derivatives of these unusual monosaccharides can be obtained from a variety of starting materials, some more readily available than others. Although brief attempts were made to modify derivatives of D-glucose, this project focused mainly on the use of D-glucosamine or its simple derivatives, where the amine functionality at C-2 is already installed with the desired stereochemistry. As is common to any modification of a carbohydrate starting material, the task required the selection or development of protection/deprotection strategies which effectively exploited structural and stereoelectronic features of the sugar template to achieve the required stereo-, chemo- and regioselective modifications, in the most efficient way possible. Despite many years of progress in the field of carbohydrate synthesis, the ability to obtain a differentially protected sugar through regioselective modifications generally remains difficult to achieve. In addition, the effect that each protecting group has on the overall reactivity of the monosaccharide building block is often unpredictable and poorly understood, as seen in many instances throughout this study. A further challenge was the difficulties often encountered in repeating literature procedures: a welcome development in recent years in this regard is the emergence of the series, *Carbohydrate Chemistry: Proven Synthetic Methods*, which "addresses concerns to chemists regarding irreproducibility of synthetic protocols, lack of data in many chemical communications, and inflated yields, which has recently become a serious, widely recognized problem".[1]

The first attempt to obtain the FucNAc4N derivative *via* the 1,6-anhydro-2-azido-2,4-dideoxy-4-*O*-tosyl-β-D-glucopyranose **2.3**[2] was abandoned on account of difficulties encountered in its formation and therefore any further efficient transformations. This led to evaluation of a route *via* the cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside **2.18**, an intermediate readily available according to a procedure of Knapp and co-workers.[3–7] Subsequent access to either of the regioselectively protected 6-deoxy sugars **2.20** or **2.22,** could be achieved either *via* a seemingly straightforward regioselective protection or *via* manipulations of a 4,6-*O*-benzylidene acetal. However, after a full assessment, the preparations of both the early

intermediate, 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranose (**2.31**) and the cyclohexyl 2-acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (**2.32**) were low yielding and unreproducible on a large scale. Despite the steps involving the manipulation of the 4,6-*O*-benzylidene acetal showing potential as practicable routes to obtaining the FucNAc4N derivative, this route was not pursued any further. The final, most successful approach involved efficient preparation, even on a large scale, of the 2,3-oxazolidinone **2.47** using procedures first described by Benakli *et al.*[8] This achieved a successful circumvention of the previously encountered problems of differentiating *O*-3 and *O*-4. From this intermediate, the desired derivative of FucNAc4N (**2.50**, **A''**) was obtained over 10 steps in an overall yield of 17% from the commercially available 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose. In many respects, this route is more viable than those previously published and does not yield the diastereomeric mixtures encountered in some of the more efficient published routes. However, the effectiveness of this, or closely related derivatives as either a glycosyl donor or acceptor in the preparation of the disaccharide remains to be evaluated.

The synthesis of the AltNAcA derivative was also attempted by several approaches. In the first instance, the key inversion at C-5 of the benzoyl furanolactone (**3.1**) was achieved, but difficulties were encountered with the subsequent cleavage of the lactone, opening of the furanose ring and equilibration to the desired pyranose. With these early indications that this would not be a practical route to the desired target, attention turned to the possibility of synthesising both the FucNAc4N and AltNAcA derivative from a common intermediate, the 6-iodo-derivative **2.55.** The elimination of the iodide to form the 5-ene was achieved, but hydroboration/oxidation led exclusively to the undesired D-sugar (**3.16**). It was suspected that the anomeric configuration (β- in **3.14**) seriously restricted the desired β-face approach of the borane complex, and this led to a successful preparation of the required 6-iodo-α-thioglycoside by facile anomerization of a β-thioglycoside **3.26** in order to evaluate its elimination to the corresponding 5-ene and subsequent hydroboration. However, base-mediated elimination to the 5-enopyranoside was not possible, and attempts to achieve this simply resulted in cleavage of the 2,3-oxazolidinone, followed by formation of the 3,6-anhydro sugar **3.28**. It appears that formation of the 5-ene is significantly disfavoured in this highly strained *trans*-fused ring system with the bulky, α-oriented anomeric substituent. It became clear from this that the pursuit of this strategy for inversion of configuration at C-5 would require an early removal of the 2,3-oxazolidinone protecting group, to allow for successful elimination and face-selective hydroboration.

Considerations of all of these results and experiences led to the proposal, for future investigation, of an alternative route to both sugars, retaining the attractive option of utilizing a common intermediate, as outlined in **Ch. 3.5** and summarized in **Scheme 4.1**. The upper part of this scheme summarizes the successful synthesis of the FucNAc4N derivative **2.50** from the key intermediate **2.47**. The suggested alternative route to the AltNAcA derivative is then illustrated in the lower part of the scheme. The possibility of efficient formation of the α-thioglycoside **3.20** has been demonstrated, and from this it should be possible to form differentially protected 6-iodo-intermediate **3.35** over steps 1 – 6 which is based on chemistry already well established from this study. The key difference in this strategy is in step 3, involving the deliberate, early cleavage of the 2,3-oxazolidinone using methods previously employed, followed by protection at *O*-3. The presence of the tosyloxy group at C-6 would prevent any intramolecular substitution from occurring. Following this, elimination in step 6 should proceed smoothly, as the steric demands of the α-thiophenol substituent and 2,3-oxazolidinone ring have been removed, to yield the 5-enopyranoside **3.36**. Hydroboration/oxidation could then be attempted in step 7 to yield the desired L-sugar **3.37** as the major product, based on literature precedent and our own observations. Subsequent steps (8 – 11) would be directed towards achieving the desired oxidation at C-6 and inversion of configuration at C-4, yielding fully protected derivative **3.39** as a potential glycosyl donor (**B''**) and **3.40** as a potential glycosyl acceptor (**B'**).



Scheme 4.1: Outline and proposed synthesis for the FucNAc4N (**A'** and **A''**) and AltNAcA (**B'** and **B''**) derivatives.

While the route to AltNAcA outlined in Scheme **4.1** is longer, requiring a further 14 steps from **2.47**, the chemistry for the majority of these reactions is either acknowledged as standard in the literature, or has been established during this study. It also has the advantage of avoiding starting from the expensive L-sugar,[9,10] and the need to install the 2-acetamido functionality. In addition, it incorporates the novel and elegant prospect of preparing both sugars found in the *S. sonnei* repeating unit from a common precursor.

In summary, this work has highlighted the difficulties in differentiating hydroxyl groups of similar reactivity, where seemingly straight-forward regioselective protection strategies have

not worked, showing that despite literature precedent, this still remains a non-trivial task. This work has also revealed the poorly understood effects that protecting groups have on the conformation and stereoelectronics of the ring, and therefore the overall reactivity, which in this case has made certain transformations difficult to achieve. In addition, configurational influences of various functional groups have impacted certain conversions, the effect of which is not predictable, nor initially obvious. More broadly, this leaves the synthetic carbohydrate chemist with the only conclusion that can in fact be drawn – firstly, that serendipity plays a minor part, and secondly, that a great deal of work still needs to be carried out in order to gain a better understanding in the exciting and unpredictable field of synthetic carbohydrate chemistry.

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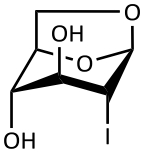
Chapter 5: Experimental

5.1 General Experimental

All commercial chemicals were purchased from Merck (South Africa) or Sigma-Aldrich except for the 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose and 1,2-*O*-isopropylidene-β-D-glucofuranurono-6,3-lactone which were purchased from Carbosynth. Acetonitrile, DCM, isopropyl acetate and toluene were freshly distilled from calcium hydride, $CDCl_3$ was dried with freshly activated 3Å molecular sieves (beads) and anhydrous DMF, MeOH, THF and pyridine were purchased from Sigma Aldrich. Microwave reactions were carried out using a CEM Discoverer microwave (μλ=150 W, P = 17.2 bar, mixing time = 15 minutes). Hydrogenation reactions with reduced pressure were carried out using a Parr Hydrogenator 3911. Molecular sieves were activated in a 400°C furnace for 3 hours prior reaction start time. Reactions were monitored by thin layer chromatography (TLC) using pre-coated 60 F_{254} silica gel alumina plates (Merck) and were visualised under ultraviolet light or by staining with acidicfied *p*-anisaldehyde solution (1:2:37 *p*-anisaldehyde/H_2SO_4 (conc)/EtOH) or acidified ceric ammonium sulphate solution (5g CAS/50 mL EtOH/50 mL 2M H_2SO_4) followed by charring with a heat gun. Silica gel chromatography was performed using Merck silica gel 60, 70–230 mesh for gravity columns. Automated flash chromatography was performed on a Biotage Isolera system, using either Biotage prepacked SNAP columns, or self-packed Biotage SNAP columns with gravity silica. Purification using preparative TLC was carried out using Analtech glass backed (20x20 cm, 1.5 mm thickness) plates. ^1H, ^{13}C and 2D NMR spectra were recorded on a Varian Mercury 300 MHz Spectrometer, a Varian Unity 400 MHz Spectrometer or a Bruker Advance III with Ultra Shield 400 Plus magnet and all spectra were recorded at 303K. All spectra were recorded in deuterated solvent as indicated and referenced with respect to deuterated solvent peaks: $CDCl_3$, δ 7.26 ppm for ^1H and 77.16 ppm for ^{13}C; $(CD_3)_2CO$, δ 2.05 ppm for ^1H and 29.84 ppm for ^{13}C; $(CD_3)_2SO$, δ 2.50 ppm for ^1H and 39.52 ppm for ^{13}C; CD_3OD, δ 3.31 ppm for ^1H and 49.00 ppm for ^{13}C; D_2O, δ 4.76 ppm for ^1H. Assignments were aided by ^1H–^1H and ^1H–^{13}C correlation experiments. HRMS data were obtained using a Waters Synapt G2 with direct injection (1 μl) into a stream of 80% acetonitrile, 0.1% formic acid using a Waters UPLC at flow rate of 0.1 mL/min. Source: Electrospray positive/negative, Capillary voltage 3 kV, Cone Voltage 25 V. Melting points were determined on a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer in the range 4000 – 500 cm^{-1} using thin films of compound on NaCl disks. All care was taken between carrying out reactions to ensure compounds did not degrade and were evaporated to dryness and stored in a -20°C freezer until needed.

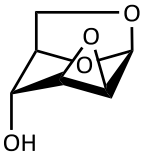
5.2 Chapter 2 Experimental Procedures

1,6-anhydro-2-deoxy-2-iodo-β-D-glucopyranose (2.8)[1]



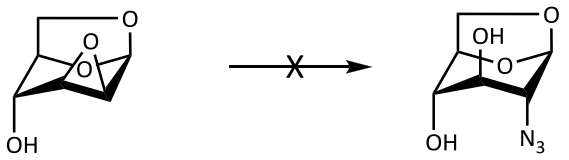
A solution of tri-*O*-acetyl-D-glucal (5.18 g, 19.03 mmol, 1 eq.) in MeOH/H_2O/Et_3N (46.2 mL) (10:10:1) was stirred for half an hour at room temperature, then concentrated by repeated evaporations with EtOH and kept overnight under vacuum in the presence of P_2O_5. The syrupy D-glucal was treated with bis(tributyltin)oxide (7.76 mL,15.22 mmol, 0.8 eq.) and freshly activated 3Å powdered molecular sieves in refluxing dry acetonitrile (35 mL) for 3 hours under argon. The reaction mixture was cooled to 5 °C and iodine (7.25 g, 28.55 mmol, 1.5 eq.) was added in one portion. The dark brown mixture was stirred for 15 minutes at 5 °C, and then at r.t. for 2 hours. The reaction mixture was filtered through celite, concentrated and then stirred with sat. aq. $Na_2S_2O_3$ and hexane (40 mL) for 3 hrs at r.t. This was then transferred to a separating funnel and washed with EtOAc (3 x 60 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (1:1 hexane/EtOAc) to yield 4.39 g (85%) of **2.8** as a clear oil, which was recrystallized to give off-white crystals. R_f = 0.45 (1:1 toluene/acetone); mp 102 – 104°C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 5.62 (s, 1H), 5.42 (d, *J* = 4.3 Hz, 1H), 5.07 (d, *J* = 4.3 Hz, 1H), 4.48 – 4.38 (m, 1H), 4.02 (dd, *J* = 7.1, 1.2 Hz, 1H), 3.99 – 3.89 (m, 1H), 3.87 – 3.79 (m, 1H), 3.53 (dd, *J* = 7.0, 5.8 Hz, 1H), 3.51 – 3.42 (m, 1H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 102.65, 76.06, 74.71, 72.02, 65.22, 30.33. NMR values agree with published values.[1]

1,6:2,3-Dianhydro-β-D-mannopyranose (2.1)[2]



The 1,6-anhydro-2-deoxy-2-iodo-β-D-glucopyranose (**2.8**) (1.52 g, 5.61 mmol, 1 eq.) and $NaHCO_3$ (1.08 g, 12.90 mmol, 2.3 eq.) in DMF/H_2O (1.35 mL) (10:3) was heated to 120 °C for 4 hrs. The reaction mixture was cooled, concentrated and purified using silica gel chromatography (0–10% MeOH/EtOAc) to yield 0.69 g (85%) of **2.1** as a light yellow oil. R_f = 0.71 (9.5:0.5, EtOAc/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 (d, *J* = 3.1 Hz, 1H), 4.46 – 4.38 (m, 1H), 3.91 (s, 1H), 3.77 (dd, *J* = 7.3, 2.2 Hz, 1H), 3.77 – 3.69 (m, 1H), 3.47 – 3.40 (m, 1H), 3.14 (ddd, *J* = 3.7, 1.6, 0.8 Hz, 1H), 2.42 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 97.82, 74.32, 67.22, 65.64, 54.35, 49.39. NMR values agree with published values.[2]

1,6-Anhydro-2-azido-2-deoxy-β-D-glucopyranose (2.2)



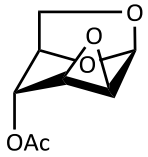
First attempt:[3]

The di-anhydro sugar (**2.1**) (95 mg, 0.66 mmol, 1 eq.) was dissolved in MeOH/H_2O (2 mL) (9:1) and to this the NaN_3 (0.43 g, 6.59 mmol, 10 eq.) and NH_4Cl (0.26 g, 4.94 mmol, 0.75 eq.) was added. The reaction mixture was refluxed overnight after which time TLC indicated multiple products had formed with SM still remaining.

Second attempt:

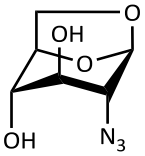
The di-anhydro sugar (**2.1**) (25 mg, 0.18 mmol, 1 eq.) and NaN_3 (0.11 g, 1.80 mmol, 10 eq.) in DMF/H_2O (1.1 mL) (1:0.1) was heated at 80°C for 9 hrs after which time TLC indicated that no reaction had taken place.

1,6:2,3-dianhydro-4-*O*-acetyl-4-deoxy-β-D-mannopyranose (2.9)



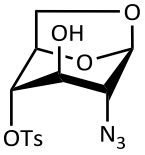
The di-anhydro sugar (**2.1**) (0.69 g, 4.82 mmol, 1 eq.) was dissolved in pyridine (1.55 mL) and to this the acetic anhydride (1.14 mL) was added. The reaction mixture was stirred overnight at r.t., then cooled to 5°C, quenched with MeOH, diluted with DCM and washed with 5% HCl solution. The aqueous layer was washed with DCM (3 x 20 mL), combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (1:1 hexane/EtOAc) to yield 0.75 g (84%) of **2.9** as a white solid. R_f = 0.35 (1:1 hexane/EtOAc); mp 64 – 66°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (d, *J* = 3.1 Hz, 1H), 4.96 (s, 1H), 4.49 – 4.41 (m, 1H), 3.80 (dd, *J* = 7.4, 2.0 Hz, 1H), 3.74 (t, *J* = 7.1 Hz, 1H), 3.47 (td, *J* = 3.4, 0.6 Hz, 1H), 3.15 – 3.10 (m, 1H), 2.20 – 2.13 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 5.72 (d, *J* = 3.2 Hz, 1H), 4.95 (s, 1H), 4.47 – 4.40 (m, 1H), 3.79 (dd, *J* = 7.5, 2.0 Hz, 1H), 3.73 (dd, *J* = 14.1, 0.5 Hz, 1H), 3.48 – 3.43 (m, 1H), 3.15 – 3.08 (m, 1H), 2.16 (d, *J* = 0.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.19, 97.72, 71.54, 68.74, 65.91, 54.30, 47.56, 21.02. NMR values agree with published values.[4]

1,6-Anhydro-2-azido-2-deoxy-β-D-glucopyranose (2.2)[2]



The acetylated di-anhydro sugar (**2.9**) (0.41 g, 2.18 mmol, 1 eq.) and NaN_3 (0.71 g, 10.91 mmol, 5 eq.) in DMF/H_2O (7 mL) (9:1) was heated to 110°C and left to stir for 9 hrs. The reaction mixture was then diluted with EtOAc and sat. aq. NH4Cl, transferred to a separating funnel and washed with EtOAc (3 x 30 mL), combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (1:1 hexane/EtOAc) to yield 0.27 g (66%) of **2.2** as a white solid. R_f = 0.2 (7:3 hexane/EtOAc); mp 114 – 116°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.51 (t, *J* = 1.7 Hz, 1H), 4.58 (d, *J* = 5.5 Hz, 1H), 4.21 (d, *J* = 7.4 Hz, 1H), 3.89 (bs, 1H), 3.80 (dd, *J* = 7.7, 5.4 Hz, 1H), 3.66 (bs, 1H), 3.50 (bs, 1H), 2.58 (bs, 1H), 2.45 (bs, 1H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 99.82, 76.37, 71.51, 70.94, 64.87, 62.37. NMR values agree with published values.[2,3]

1,6-Anhydro-2-azido-2,4-dideoxy-4-*O*-tosyl-β-D-glucopyranose (2.3)



First attempt:[5]

The 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**2.2**) (90 mg, 0.48 mmol, 1 eq.) and freshly recrystallized *p*-TsCl (0.10 g, 0.52 mmol, 1.1 eq.) in dry pyridine (1.3 mL) were stirred under argon at r.t. for 48 hrs after which time TLC indicated no reaction had taken place.

Second attempt:

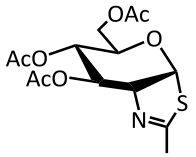
The 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**2.2**) (84 mg, 0.45 mmol, 1 eq.) and Et_3N (63 µL, 0.45 mmol, 1 eq.) in dry DCM (1.5 mL) was cooled to 0°C under argon and to this freshly recrystallized *p*-TsCl (94 mg, 0.49 mmol, 1.1 eq.) was added. This was allowed to slowly warm to r.t. overnight after which time TLC indicated SM and a more non-polar product. The reaction mixture was then quenched with MeOH, diluted with DCM, washed with 5% HCl solution. The aqueous layer was washed with DCM (3 x 10 mL), combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (1:1 hexane/EtOAc) to yield 20 mg (19%) of **2.3** as a clear oil and 41 mg of SM. R_f = 0.25 (1:1 hexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.3 Hz, 2H), 7.37 (d, *J* = 7.9 Hz, 2H), 5.43 (bs, 1H), 4.62 (dd, *J* = 5.5, 1.1 Hz, 1H), 4.43 (dd, *J* = 3.7, 1.5 Hz, 1H), 4.04 (dd, *J* = 7.9, 0.9 Hz, 1H), 3.89 (bs, 1H), 3.71 (dd, *J* = 7.9,

5.5 Hz, 1H), 3.25 (d, *J* = 3.7 Hz, 1H), 3.00 (s, 1H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.73 , 133.15, 130.22, 128.00, 101.11, 79.63, 75.10, 70.38, 66.20, 62.64 , 21.81; IR (NaCl, dry film) ν_{max} (cm^{-1}): 3594 (O-H), 2926 (C-H Ar), 2920 (C-H aliphatic), 2108 (N_3), 1599 (C=C). NMR values agree with published values;[5] HRMS (ESI-TOF): $[M+HCO_2]^-$ Calcd for $C_{14}H_{16}N_3O_8S$: 386.0658 found: 386.0674.

Third attempt:[6]

The 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**2.2**) (55 mg, 0.29 mmol, 1 eq.) and dibutyltin oxide (50 mg, 0.20 mmol, 0.7 eq.) in dry toluene (1.5 mL) was refluxed under argon for 3 hrs. The toluene was then removed under reduced pressure and the reaction mixture dried using a Schlenk line, dissolved in dry 1,4-dioxane (1.4 mL) under argon and cooled to 0°C. To this freshly recrystallized *p*-TsCl (0.12 g, 0.59 mmol, 1.2 eq.) and cat. amount of DMAP was added and left to stir at r.t. for 3 hrs after which time TLC indicated SM and the mono-tosylated product **2.3**. After 18.5 hrs another 47 mg (0.5 eq.) of *p*-TsCl was added and left for a further 5.5 hrs after which time TLC indicated that SM was still present. The reaction mixture was quenched with MeOH, concentrated and purified using silica gel chromatography (1:1 hexane/EtOAc) to yield 26 mg (25%) of **2.3** as a clear oil and 22 mg of SM. R_f = 0.25 (1:1 hexane/EtOAc). Characterized as above.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(Acetoxymethyl-6,7-diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole (2.17).



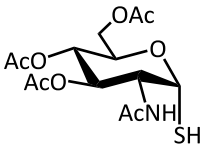
Using Lawesson's reagent:[7,8]

The 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (1.61 g, 4.13 mmol, 1 eq.), Lawesson's reagent (1.80 g, 4.46 mmol, 1.08 eq.) and freshly activated 3Å powdered molecular sieves in dry toluene (20 mL) was refluxed under argon for 4.5 hours after which time the reaction mixture turned a dark orange colour. This was allowed to cool to r.t., neutralized by the addition of 160 mg of sodium bicarbonate, filtered through celite and purified using silica gel chromatography (7:3 EtOAc/hexane) to yield 1.17 g (82%) of **2.17** as a yellow syrup. R_f = 0.58 (9.5:0.5 DCM/MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.24 (d, *J* = 7.1 Hz, 1H), 5.56 (dd, *J* = 3.3, 1.9 Hz, 1H), 4.95 (ddd, *J* = 9.5, 1.9, 1.1 Hz, 1H), 4.53 – 4.42 (m, 1H), 4.12 (app d, *J* = 4.4 Hz, 2H), 3.55 (dt, *J* = 9.1, 4.4 Hz, 1H), 2.32 (d, *J* = 2.2 Hz, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.67, 169.67, 169.39, 168.20, 89.01, 76.91, 70.93, 69.51, 68.65, 63.45, 21.05, 20.97, 20.83, 20.79. NMR values agree with published values.[8]

Using P_4S_{10}:[9]

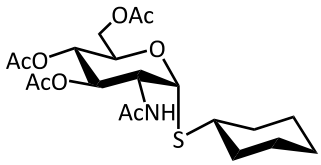
The freshly activated 3Å powdered molecular sieves and anhydrous isopropyl acetate (115 mL) were stirred in a dry round bottom flask under argon at r.t. for 10 mins. To this the phosphorus pentasulfide (8.57g, 19.28 mmols, 0.5 eq.) was added in two lots followed by the addition of hexamethyldisiloxane (29 mL) over 10 mins with vigorous stirring. The reaction mixture was then heated to 100°C for 10 mins. The round bottom flask was removed from the heat, cooled, and to this the 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (15.00 g, 38.55 mmol, 1 eq.) was added in small portions. This was refluxed for 12 hours after which time the reaction mixture turned a dark orange colour and then stirred at r.t. for an additional 24 hours. The reaction mixture was filtered through celite, concentrated to approx. 50 mL, cooled to 0°C and stirred with 5.3 M K_2CO_3 (20 mL) for 30 mins. This was then transferred to a separating funnel, diluted with water (50 mL) and the aqueous layer was washed with EtOAc (4 x 60 mL) and combined organic fractions concentrated and purified using silica gel chromatography (7:3 EtOAc/hexane) to yield 12.87 g (96%) of **2.17** as a yellow syrup. R_f = 0.58 (9.5:0.5 DCM/MeOH). NMR characterization as above.

2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranose (2.31).[7,8]



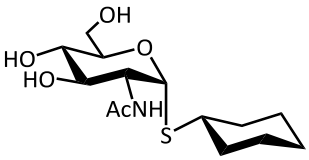
A solution of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(Acetoxymethyl-6,7-diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole (GlcNAc-thiazoline triacetate) (**2.17**) (3.85 g, 11.12 mmol, 1 eq.) in degassed MeOH (40 mL) was cooled to 0°C and treated with trifluoroacetic acid (1.7 mL, 22.25 mmol, 2 eq.) and water (1.7 mL). The reaction was stirred at 5°C overnight followed by stirring at r.t. for 4 hours. The reaction mixture was then concentrated and purified using flash silica gel chromatography (1:1 hexane/DCM) to yield 2.31 g (57%) of **2.31** as a colourless syrup. R_f = 0.36 (6:4 EtOAc/DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (d, J = 8.4 Hz, 1H), 5.78 (dd, J = 6.8, 5.4 Hz, 1H), 5.16 – 5.07 (m, 2H), 4.53 – 4.44 (m, 1H), 4.33 – 4.29 (m, 1H), 4.26 (d, J = 12.3 Hz, 1H), 4.12 (br d, J = 11.9 Hz, 1H), 2.10 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 170.7, 170.1, 169.3, 79.0, 70.8, 69.2, 68.1, 61.9, 52.7, 23.2, 20.8, 20.7. NMR values agree with published values.[8]

Cyclohexyl 2-acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (2.32).[10,11]



The 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranose (**2.31**) (3.58 g, 9.85 mmol, 1 eq.) and activated 3Å powdered molecular sieves were dried using a Schlenk line and dissolved in dry and degassed cyclohexene (50 mL) under argon. To this was added drop wise (using a syringe pump) over 45 mins 1,1′-Azobis(cyclohexanecarbonitrile) (ACCN) (1.44 g, 5.91 mmol, 0.6 eq.) in dry and degassed chloroform (10 mL) (co-solvent, 2:1 cyclohexene/chloroform ratio) and the reaction mixture was stirred at 75°C for 3 hours after which time another 0.6 eq. of ACCN in chloroform (10 mL) was added drop wise over 45 mins. At 15 hours another 0.3 eq. of ACCN in chloroform (5 mL) was added drop wise over 45 mins to the reaction mixture and left to stir until TLC indicated complete conversion of the starting material (total 37 hours). The reaction mixture was filtered through celite, concentrated and purified using silica gel chromatography (6:4 DCM/EtOAc) to yield 2.19 g (66%) of **2.32** as a white solid. R_f = 0.44 (6:4 DCM/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (br d, J = 9.0 Hz, 1H), 5.44 (d, J = 5.4 Hz, 1H), 5.06 (t, J = 9.5 Hz, 1H), 5.03 (t, J = 10 Hz, 1H), 4.47 (ddd, J = 10.7, 9.1, 5.4 Hz, 1H), 4.36 (ddd, J = 9.7, 4.7, 2.3 Hz, 1H), 4.22 (dd, J = 12.3, 4.8 Hz, 1H), 4.05 (dd, J = 12.3, 2.3 Hz, 1H), 2.80 (tt, J = 10.2, 3.6 Hz, 1H), 2.05 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H), 1.95 – 1.91 (m, 2H), 1.76 – 1.67 (m, 2H), 1.60 – 1.52 (m, 1H), 1.45 – 1.19 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.47, 170.66, 169.85, 169.35, 83.65, 71.66, 68.59, 68.44, 62.23, 52.43, 45.09, 34.32, 33.77, 26.00, 25.91, 25.63, 23.30, 20.74, 20.65. NMR values agree with published values.[10]

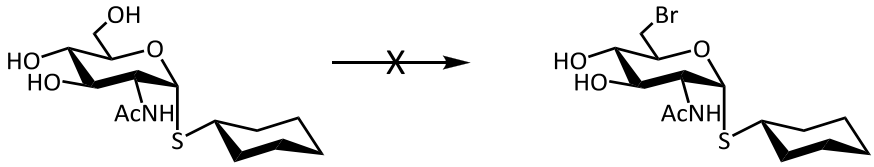
Cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (2.18).[12]



A solution of the cyclohexyl 2-acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (**2.32**) (2.91 g, 6.54 mmol, 1 eq.) in MeOH (30 mL) was cooled to 0°C and treated with NaOMe solution (25 wt. % in MeOH, 0.28 mL, 1.3085 mmol, 0.2 eq.). The reaction mixture was stirred at r.t. for 30 mins. This was then treated with DOWEX 50WX8–100 ion exchange resin, filtered, concentrated and purified using silica gel chromatography (9.5:0.5 DCM/MeOH) to yield 1.32 g (63%) of **2.18** as a white solid. R_f = 0.27 (9:1 DCM/MeOH); mp 207 – 209°C; ^{1}H NMR (400 MHz, CD_3OD) δ 5.52 (d, J = 5.3 Hz, 1H), 4.01 (dd, J = 11.0, 5.3 Hz, 1H), 3.97 (ddd, J = 9.6, 5.2, 2.0 Hz,1H),

3.80 (dd, J = 12.0, 2.5 Hz, 1H), 3.72 (dd, J = 12.0, 5.2 Hz, 1H), 3.54 (dd, J = 11.0, 8.7 Hz, 1H), 3.36 (dd, J = 9.8, 8.7 Hz, 1H), 2.87 – 2.76 (m, 1H), 1.97 (s, 3H), 2.02 – 1.92 (m, 2H), 1.80 – 1.69 (m, 2H), 1.63 – 1.54 (m, 1H), 1.46 – 1.22 (m, 5H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.56, 84.08, 74.36, 72.84, 72.63, 62.62, 55.93, 44.79, 35.30, 34.95, 27.04, 26.86, 26.81, 22.57. NMR values agree with published values.[12]

Cyclohexyl 2-acetamido-6-bromo-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.33).[13]



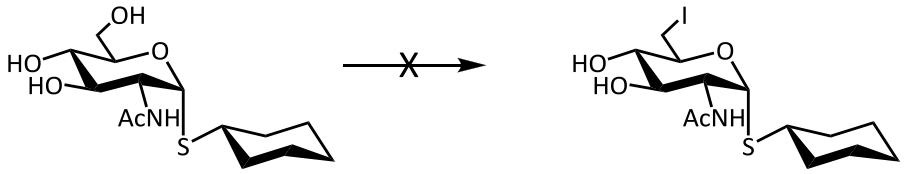
First attempt:

A solution of the cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (85 mg, 0.27 mmol, 1 eq.) and freshly activated 3Å powdered molecular sieves in dry pyridine (1.8 mL) was cooled to 0°C and treated with triphenylphosphine (0.14 g, 0.53 mmol, 2 eq.) and tetrabromomethane (97 mg, 0.29 mmol, 1.1 eq.) under argon. The reaction mixture was heated at 35°C for 23 hours without any conversion of the starting material.

Second attempt:

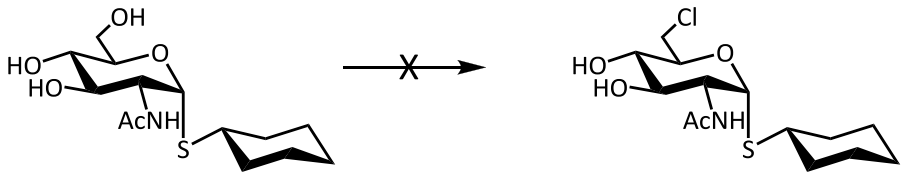
A solution of the cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (65 m g, 0.20 mmol, 1 eq.) and freshly activated 3Å powdered molecular sieves in dry pyridine (1.8 mL) was treated with triphenylphosphine (0.10 g, 0.41 mmol, 2 eq.) and tetrabromomethane (75 mg, 0.22 mmol, 1.1 eq.) under argon at r.t. The reaction mixture was then heated at 50°C for 13 hours without any conversion of the starting material.

Cyclohexyl 2-acetamido-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (2.34).[14]



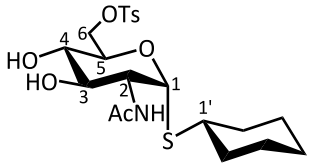
The cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (80 mg, 0.2505 mmol, 1 eq.), imidazole (34 mg, 0.50 mmol, 2 eq.) and triphenylphosphine (99 mg, 0.38 mmol, 1.5 eq.) in THF (2.5 mL) was treated with iodine (95 mg, 0.38 mmol, 1.5 eq.) under argon at r.t. The reaction mixture was heated to reflux for 5 hours without any conversion of the starting material.

Cyclohexyl 2-acetamido-6-chloro-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.35).[15]



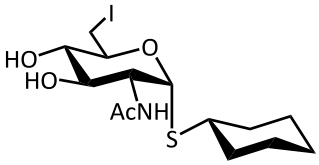
The cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (60 mg, 0.19 mmol, 1 eq.) and 3Å powdered molecular sieves were dried using a Schlenk line. To this dry DMF (0.7 mL) was added under argon, cooled to 0°C and treated with freshly distilled thionyl chloride (16 µL, 0.2254 mmol, 1.2 eq.). This was allowed to slowly warm to r.t. and stirred for 1 hour after which time TLC indicated no conversion of SM, but increasing amounts of degradation.

Cyclohexyl 2-acetamido-2-deoxy-1-thio-6-*O*-tosyl-α-D-glucopyranoside (2.36).[16]



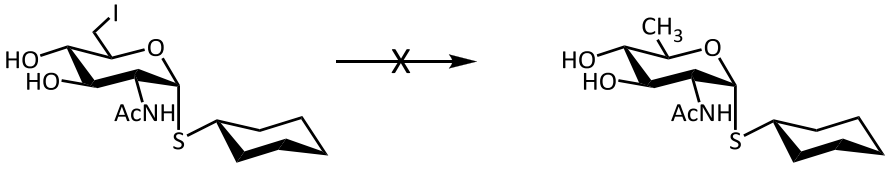
A solution of cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (1.28 g, 4.00 mmol, 1 eq.) and freshly activated 3Å molecular sieves in dry pyridine (10 mL) was stirred under argon and cooled to 0°C. To this freshly recrystallized *p*-toluenesulfonyl chloride (1.14 g, 6.00 mmol, 1.5 eq.) was added and stirred for 2 hours at this temperature. The reaction mixture was filtered through celite, transferred to a separating funnel and washed with 5% HCl solution. The aqueous layer was washed with DCM (4 x 20 mL), combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (9.7:0.3 EtOAc/MeOH) to yield 1.54 g (81%) of **2.36** as a white solid. R_f = 0.41 (9:1 EtOAc/MeOH); mp 145-147°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.33 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.19 (d, *J* = 8.4 Hz, 1H, NH), 5.32 (d, *J* = 5.3 Hz, 1H, H-1), 4.34 (dd, *J* = 10.9, 5.0 Hz, 1H, H-6), 4.26 (dd, *J* = 10.9, 2.0 Hz, 1H, H-6'), 4.22 – 4.13 (m, 2H, H-2, H-5), 3.53 – 3.43 (m, 2H, H-4, H-3), 2.81 – 2.69 (m, 1H, H-1'), 2.43 (s, 3H, CH_3), 2.01 (s, 3H, CH_3CO), 1.97 – 1.87 (m, 2H, cyclohexyl), 1.75 – 1.66 (m, 2H, cyclohexyl), 1.61 – 1.52 (m, 1H, cyclohexyl), 1.42 – 1.18 (m, 5H, cyclohexyl); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.02 (C=O), 145.08 (Ar), 133.13 (Ar), 129.98 (Ar), 128.17 (Ar), 83.87 (C-1), 74.73 (C-3), 71.43 (C-4), 70.24 (C-5), 68.76 (C-6), 53.77 (C-2), 45.31 (C-1'), 34.40 (cyclohexyl), 33.88 (cyclohexyl), 26.01 (2 x cyclohexyl), 25.71 (cyclohexyl), 23.50 (CH_3CO), 21.80 (CH_3); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{21}H_{32}NO_7S_2$: 474.1620 found: 474.1621; IR (NaCl, dry film) ν_{max} (cm^{-1}): 3376 (O-H), 3051 (C-H Ar), 2929 (C-H aliphatic), 1648 (C=O), 1544 (C=C), 1176 (C-O).

Cyclohexyl 2-acetamido-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (2.34).[16]



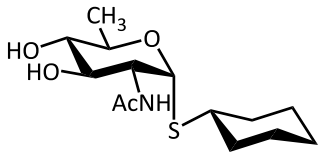
The cyclohexyl 2-acetamido-2-deoxy-1-thio-6-*O*-tosyl-α-D-glucopyranoside (**2.36**) (1.44 g, 3.05 mmol, 1 eq.) and NaI (1.01 g, 6.71 mmol, 2.2 eq.) were dried using a Schlenk line, dissolved in 2-butanone (12 mL) under argon, refluxed for 7 hours and then stirred at r.t. for 18 hrs. The reaction mixture was concentrated, diluted with EtOAc (20 ml) and aq. $Na_2S_2O_3$ (20 mL), transferred to a separating funnel and the aqueous layer was washed with EtOAc (4 x 15 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (9.7:0.3 EtOAc/MeOH) to yield 1.26 g (97%) of **2.34** as a white solid. R_f = 0.40 (9:1 EtOAc/MeOH); mp 169 – 171°C; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.77 (d, *J* = 7.1 Hz, 1H, NH), 5.42 (d, *J* = 5.3 Hz, 1H, H-1), 5.39 – 5.34 (m, 1H, OH-4), 4.88 (d, *J* = 5.9 Hz, 1H, OH-3), 3.79 (ddd, *J* = 11.0, 7.1, 5.3 Hz, 1H, H-2), 3.64 (ddd, *J* = 9.2, 6.6, 2.2 Hz, 1H, H-5), 3.54 (dd, *J* = 10.7, 2.4 Hz, 1H, H-6), 3.44 – 3.34 (m, 1H, H-3), 3.35 (dd, *J* = 10.7, 6.8 Hz, 1H, H-6'), 3.02 (td, *J* = 9.0, 5.8 Hz, 1H, H-4), 2.90 – 2.80 (m, 1H, H-1'), 1.93 – 1.84 (m, 2H, cyclohexyl), 1.82 (s, 3H, CH_3CO), 1.72 – 1.61 (m, 2H, cyclohexyl), 1.57 – 1.48 (m, 1H, cyclohexyl), 1.39 – 1.17 (m, 5H, cyclohexyl); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 169.47 (C=O), 81.97 (C-1), 74.76 (C-4), 71.42 (C-5), 70.19 (C-3), 54.24 (C-2), 42.07 (C-1'), 33.63 (cyclohexyl), 33.20 (cyclohexyl), 25.53 (cyclohexyl), 25.23 (cyclohexyl), 25.14 (cyclohexyl), 22.49 (cyclohexyl), 9.26 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{14}H_{25}INO_4S$: 430.0549 found: 430.0546.

Cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.19).[17]



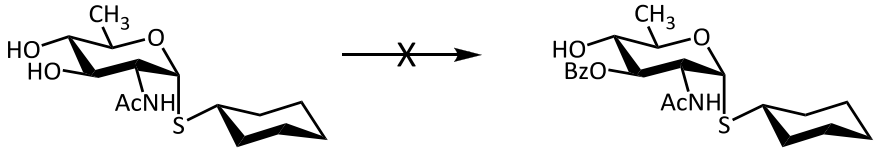
The cyclohexyl 2-acetamido-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (**2.34**) (61 mg, 0.14 mmol, 1 eq.) in DCM (2 mL) and AcOH (68 μL, 1.20 mmol, 8.4 eq.) was treated with 0.20 g of Zn dust. The reaction mixture was vigorously stirred at r.t. for 5.5 hours during which time TLC indicated multiple products forming, with starting material still present.

Cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.19).



The cyclohexyl 2-acetamido-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (**2.34**) (0.10 g, 0.25 mmol, 1 eq.) and $NaHCO_3$ (0.10 g, 1.19 mmols, 2 eq.) was dissolved in dry MeOH and to this a suspension of 10 wt % Pd/C (0.1 g, 0.09 mmol, 2.6 eq.) in dry MeOH was added. The reaction mixture was flushed three times with hydrogen and using a hydrogenator the pressure was increased to 4 bar and left at r.t. for 6 hours. The reaction mixture was filtered through celite, concentrated and columned using flash chromatography (100% EtOAc) to yield 1.26 g (97%) of **2.19** as a white crystalline solid. R_f = 0.44 (9:1 EtOAc/MeOH); mp 200 – 202°C; ^{1}H NMR (400 MHz, CD_3OD) δ 5.44 (d, *J* = 5.4 Hz, 1H, H-1), 4.04 – 3.96 (m, 2H, H-2, H-5), 3.47 (dd, *J* = 11.3, 8.7 Hz, 1H, H-4), 3.03 (dd, *J* = 9.5, 8.7 Hz, 1H, H-3), 2.80 – 2.69 (m, 1H, H-1'), 1.97 (s, 3H, CH_3CO), 1.99 – 1.90 (m, 2H, cyclohexyl), 1.79 – 1.70 (m, 2H, cyclohexyl), 1.64 – 1.55 (m, 1H, cyclohexyl), 1.44 – 1.26 (m, 5H, cyclohexyl), 1.24 (d, J = 6.2 Hz, 3H, C-6); ^{13}C NMR (101 MHz, CD_3OD) δ 173.55 (C=O), 84.33 (C-1), 78.34 (C-3), 72.66 (C-4), 69.68 (C-2), 56.22 (C-5), 45.31 (C-1'), 35.47 (cyclohexyl), 35.00 (cyclohexyl), 27.01 (cyclohexyl), 26.88 (cyclohexyl x2), 22.58 (CH_3CO), 17.99 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{14}H_{26}NO_4S$: 304.1583 found: 304.1590, $[M+Na]^+$ Calcd for $C_{14}H_{25}NNaO_4S$: 326.1402 found: 326.1394.

Cyclohexyl 2-acetamido-3-*O*-benzoyl-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.20).



First attempt:[13]

The cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (**2.19**) (0.14 g, 0.47 mmol, 1 eq.) and freshly activated 3Å powdered molecular sieves in dry pyridine (2.5 mL) was cooled to -35°C under argon. To this was added benzoyl chloride (60 µL, 0.52 mmols, 1.1 eq.) and stirred for 3.5 hrs after which time TLC indicated no reaction had taken place. The reaction mixture was then taken up to 5°C and stirred at this temperature for 14 hrs, after which time TLC still indicated that no reaction had taken place.

Second attempt:[18]

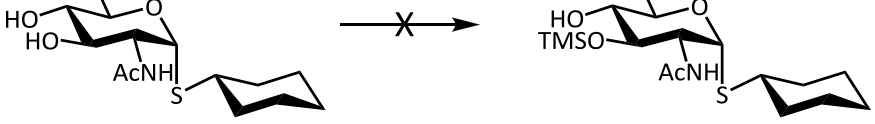
A solution of cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (**2.19**) (87 mg, 0.29 mmol, 1 eq.) and freshly activated 3Å powdered molecular sieves in dry DCM (2 mL) and

Et_3N (44 µL, 0.32 mmol, 1.1 eq.) was cooled to 0°C under argon. To this the BzCl (37 µL, 0.32 mmol, 1.1 eq.) in DCM (1 mL) was added drop wise to the reaction mixture over 10 mins. This was left to stir at 5°C for 22 hrs after which time TLC indicated no reaction had taken place.

Third attempt:

The cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (**2.19**) (61 mg, 0.20 mmol, 1 eq.) and freshly activated 3Å powdered molecular sieves in dry pyridine (2 mL) was cooled to 0°C under argon. To this was added benzoyl chloride (28 µL, 0.24 mmols, 1.2 eq.) and stirred for 2 hrs after which time TLC indicated two more non-polar products. The reaction mixture was quenched with MeOH, diluted with DCM, filtered through celite and washed with 5% HCl solution. The aqueous layer was washed with DCM (3 x 15 mL), combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (8:2 EtOAc/hexane) to yield two products. 3-benzoate (**2.20**): 29 mg (35%) of white solid. R_f = 0.38 (100% EtOAc); mp 132 – 134°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.03 (m, 2H, Ar-H), 7.62 – 7.54 (m, 1H, Ar-H), 7.49 – 7.41 (m, 2H, Ar-H), 5.86 (d, *J* = 8.0 Hz, 1H, NH), 5.41 (d, *J* = 5.4 Hz, 1H, H-1), 4.90 (dd, *J* = 9.7, 9.1 Hz, 1H, H-4), 4.41 (ddd, *J* = 10.8, 8.1, 5.3 Hz, 1H, H-2), 4.34 (dq, *J* = 9.6, 6.1 Hz, 1H, H-5), 3.72 (bt, *J* = 9.8 Hz, 1H, H-3), 2.86 (tt, *J* = 10.4, 3.7 Hz, 1H, H-1'), 2.04 (s, 3H, CH_3CO), 2.04 – 1.97 (m, 1H, cyclohexyl), 1.82 – 1.72 (m, 2H, cyclohexyl), 1.65 – 1.54 (m, 2H, cyclohexyl), 1.47 – 1.28 (m, 5H, cyclohexyl), 1.25 (d, *J* = 6.3 Hz, 3H, H-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{21}H_{30}NO_5S$: 408.1845 found: 408.1845, $[M+Na]^+$ Calcd for $C_{21}H_{29}NNaO_5S$: 430.1664 found: 430.1662. 4-benzoate (**2.37**): 19 mg (24%) of white solid. R_f = 0.55 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 – 7.98 (m, 2H, Ar-H), 7.58 – 7.53 (m, 1H, Ar-H), 7.46 – 7.39 (m, 2H, Ar-H), 5.85 (d, *J* = 9.2 Hz, 1H, NH), 5.38 (d, *J* = 5.4 Hz, 1H, H-1), 5.07 (dd, *J* = 11.1, 8.9 Hz, 1H, H-4), 4.62 (ddd, *J* = 10.9, 9.3, 5.4 Hz, 1H, H-2), 4.19 (dq, *J* = 9.5, 6.2 Hz, 1H, H-5), 3.50 (td, *J* = 9.1, 4.2 Hz, 1H, H-3), 2.84 (tt, *J* = 10.3, 3.6 Hz, 1H, H-1'), 2.04 – 1.95 (m, 2H, cyclohexyl), 1.85 (s, 3H, CH_3CO), 1.67 – 1.56 (m, 2H, cyclohexyl), 1.48 – 1.37 (m, 3H, cyclohexyl), 1.36 (d, *J* = 6.2 Hz, 3H, H-6), 1.32 – 1.23 (m, 3H, cyclohexyl).

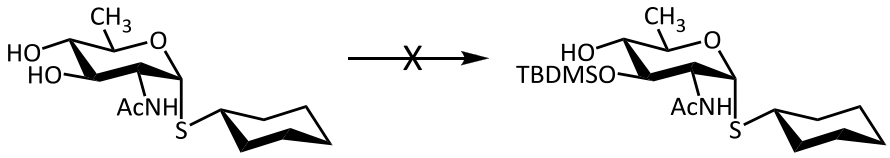
Cyclohexyl 2-acetamido-3-*O*-trimethylsilyl-2,6-dideoxy-1-thio-α-D-glucopyranoside.[19]



The cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (**2.19**) (74 mg, 0.24 mmol, 1 eq.) was dissolved in dry DCM (2 mL) and cooled to 0°C. To this the Et_3N (0.10 mL, 0.73 mmol, 3 eq.) followed by TMSCl (34 µL, 0.27 mmol, 1.1 eq.) was added and stirred at 5°C for 18 hours. After 95 hrs SM was still present and another 0.6 eq. TMSCl (20µL) was added and left for a

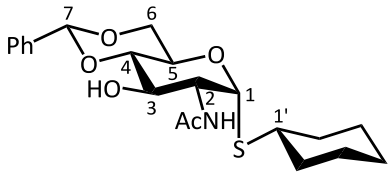
further 4 hrs after which time TLC indicated no further conversion of the SM (total 99 hrs). The reaction mixture was then transferred to a separating funnel and washed with sat. aq. $NaHCO_3$. The aqueous layer was washed with EtOAc (3 x 15 mL), combined organic were dried over Na_2SO_4, concentrated and columned using flash chromatography (100% EtOAc) to yield 53 mg (49%) of the disilyl product **2.38** as a clear oil. R_f = 0.48 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 (d, *J* = 9.6 Hz, 1H), 5.23 (d, *J* = 4.6 Hz, 1H), 4.25 (td, *J* = 9.5, 4.6 Hz, 1H), 4.05 – 3.94 (m, 1H), 3.49 (dd, *J* = 9.2, 7.3 Hz, 1H), 3.26 (t, *J* = 7.6 Hz, 1H), 2.79 (ddd, *J* = 10.2, 7.0, 3.7 Hz, 1H), 1.98 – 1.92 (m, 2H), 1.79 – 1.70 (m, 2H), 1.45 – 1.27 (m, 6H), 1.25 (d, *J* = 6.5 Hz, 3H), 0.15 (s, 9H), 0.14 (s, 9H).

Cyclohexyl 2-acetamido-3-*O*-*tert*-butyldimethylsilyl-2,6-dideoxy-1-thio-α-D-glucopyranoside.[20,21]



The cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (**2.19**) (0.12 g, 0.39 mmol, 1 eq.) was dissolved in dry DMF (2 mL) and cooled to 0°C. To this the imidazole (40 mg, 0.58 mmol 1.5 eq.) followed by TBDMSCl (65 mg, 0.43 mmol, 1.1 eq.) was added and stirred at 0°C for 2.5 hrs after which time TLC indicated only SM present, so was left to warm to r.t. and stirred at this temperature for 17 hrs, after which time TLC indicated no further reaction had taken place.

Cyclohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (2.23)



Using benzaldehyde dimethyl acetal:[22]

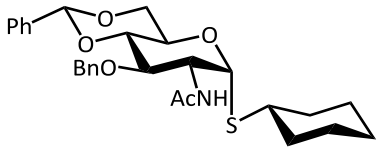
A solution of cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (96 mg, 0.30 mmol, 1 eq.) and activated 3Å powdered molecular sieves in dry DMF (2 mL) was stirred under argon and cooled to 0°C. To this (1S)-(+)-10-camphorsulfonic acid (7 mg, 0.03 mmol, 0.1 eq.) was added, followed by the addition of the benzaldehyde dimethyl acetal (64 μL, 0.42 mmol, 1.4 eq.). The ice bath was removed and the reaction mixture was stirred at 50°C for 16 hours. TLC showed no further conversion of the starting material at this point. The reaction mixture was filtered through celite, concentrated and purified using flash chromatography (5:5 hexane/EtOAc) to yield 35 mg (34%) of **2.23** as a white solid (53% of starting material was

recovered). R_f = 0.60 (100% EtOAc); mp 181 – 183°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.46 (m, 2H, Ar-H), 7.36 (dd, *J* = 5.1, 2.0 Hz, 3H, Ar-H), 5.95 (d, *J* = 8.1 Hz, 1H, NH), 5.55 (s, 1H, H-7), 5.47 (d, *J* = 5.4 Hz, 1H, H-1), 4.37 (ddd, *J* = 10.6, 8.1, 5.4 Hz, 1H, H-2), 4.25 (dd, *J* = 4.9, 3.2 Hz, 1H, H-6), 4.20 (t, *J* = 4.6 Hz, 1H, H-4), 3.81 – 3.73 (m, 2H, H-3, H-6'), 3.56 (t, *J* = 9.2 Hz, 1H, H-5), 2.82 (tt, *J* = 10.1, 3.5 Hz, 1H, H-1'), 2.04 (s, 3H, CH_3CO), 2.00 – 1.88 (m, 2H, cyclohexyl), 1.80 – 1.64 (m, 2H, cyclohexyl), 1.64 – 1.54 (m, 1H, cyclohexyl), 1.45 – 1.22 (m, 5H, cyclohexyl); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.20 (C=O), 137.22 (Ar), 129.38 (Ar), 128.44 (Ar), 126.48 (Ar), 102.18 (C-7), 84.57 (C-1), 82.61 (C-5), 70.50 (C-3), 68.85 (C-6), 63.75 (C-4), 54.38 (C-2), 45.12 (C-1'), 34.07 (cyclohexyl), 33.98 (cyclohexyl), 25.75 (cyclohexyl), 25.72 (cyclohexyl), 25.07 (cyclohexyl), 23.54 (CH_3CO); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{21}H_{30}NO_5S$: 408.1845 found: 408.1841.

Using benzaldehyde:[23]

The cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (1.27 g, 3.97 mmol, 1 eq.) and anhydrous $ZnCl_2$ (1.08 g, 7.94 mmol, 2 eq.) were dried using a Schlenk line. To this Benzaldehyde (8 mL) followed by freshly activated 3Å molecular sieves was added under argon and stirred for 17.5 hrs at r.t. The reaction mixture was diluted with DCM, filtered through celite, transferred to a separating funnel and washed with water. The aqueous layer was washed with DCM (3 x 40 mL), combined organic fractions were dried over Na_2SO_4 and concentrated. The product was recrystallized from hexane and purified using flash chromatography (100% DCM to 9.8:0.2 DCM/MeOH) to yield 1.23 g (76%) of **2.23** as a white solid. R_f = 0.60 (100% EtOAc). Characterisation as above.

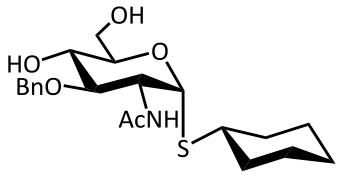
Cyclohexyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (2.21).[24,25]



A solution of the cyclohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (**2.23**) (48 mg, 0.12 mmol, 1 eq.) in dry THF (2 mL) was stirred under argon and cooled to 0°C. To this NaH (23 mg, 0.59 mmol, 5 eq.) was added and the reaction mixture was stirred at 0°C for 30 mins. After this time benzyl bromide (42 µL, 0.35 mmol, 3 eq.) and tetrabutylammonium iodide (9 mg, 0.02 mmol, 0.2 eq.) was added and left to stir at r.t. for 2 hours. The reaction mixture was cooled down to 0°C, quenched with MeOH, concentrated and washed with brine and EtOAc (4 x 15 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (1:1 hexane/EtOAc) to yield 42 mg (72 %) of **2.21** as a white solid. R_f = 0.31 (1:1 hexane/EtOAc); mp 179 – 182°C; ^{1}H NMR (400

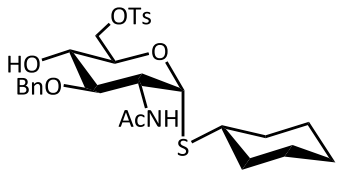
MHz, $CDCl_3$) δ 7.55 – 7.47 (m, 2H, Ar-H), 7.42 – 7.35 (m, 3H, Ar-H), 7.36 – 7.27 (m, 5H, Ar-H), 5.60 (s, 1H, H-7), 5.48 (d, *J* = 5.4 Hz, 1H, H-1), 5.22 (d, *J* = 8.2 Hz, 1H, NH), 4.91 (AB d, *J* = 12.2 Hz, 1H, CH_2OPh), 4.61 (AB d, *J* = 12.2 Hz, 1H, CH_2OPh), 4.37 (ddd, *J* = 10.6, 8.2, 5.5 Hz, 1H, H-2), 4.30 – 4.19 (m, 2H, H-5, H-6), 3.78 (m, 2H, H-6', H-4), 3.58 (dd, *J* = 10.6, 8.9 Hz, 1H, H-3), 2.77 (tt, *J* = 10.1, 3.6 Hz, 1H, H-1'), 1.88 (s, 3H, CH_3CO), 1.70 (m, 2H, cyclohexyl), 1.65 – 1.56 (m, 3H, cyclohexyl), 1.41 – 1.23 (m, 5H, cyclohexyl); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.91 (C=O), 138.47 (Ar), 137.53 (Ar), 129.12 (Ar), 128.64 (Ar), 128.41 (Ar), 128.21 (Ar), 128.03 (Ar), 126.18 (Ar), 101.53 (C-7), 84.82 (C-1), 83.23 (C-4), 75.98 (C-3), 74.15 (CH_2OPh), 68.98 (C-6), 64.05 (C-5), 53.03 (C-2), 44.80 (C-1'), 34.10 (cyclohexyl), 25.78 (cyclohexyl), 25.74 (cyclohexyl), 25.09 (2 x cyclohexyl), 23.51 (CH_3CO); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{28}H_{36}NO_5S$: 498.2314 found: 498.2310.

Cyclohexyl 2-acetamido-3-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside (2.39).[26]



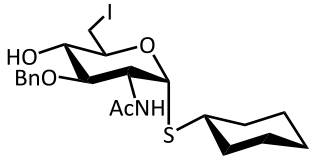
A solution of the cyclohexyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (**2.21**) (50 mg, 0.1004 mmol, 1 eq.) in DCM (1 mL) was cooled to 0°C and to this 0.18 mL TFA/H_2O (3:1) was added stirred at temperature for 1.5 hours. After this time the reaction mixture was transferred to a separating funnel, washed with sat. aq. $NaHCO_3$ and EtOAc (4 x 15 mL), combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (6:4 EtOAc/hexane) to yield 31 mg (75 %) of **2.39** as a clear oil. R_f = 0.27 (100% EtOAc); ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.29 (m, 5H, Ar-H), 5.39 (d, *J* = 5.2 Hz, 1H, H-1), 4.70 (AB q, *J* = 11.8, 6.3 Hz, 2H, CH_2OPh), 4.35 (ddd, *J* = 11.0, 8.7, 5.3 Hz, 1H, H-2), 4.05 (dt, *J* = 9.7, 3.9 Hz, 1H, H-5), 3.83 (app d, *J* = 3.9 Hz, 2H, H-6's), 3.75 (t, *J* = 9.2 Hz, 1H, H-4), 3.44 (dd, *J* = 10.9, 8.7 Hz, 1H, H-3), 2.83 – 2.74 (m, 1H, H-1'), 2.00 – 1.90 (m, 3H, cyclohexyl), 1.88 (s, 3H, CH_3CO), 1.78 – 1.69 (m, 2H, cyclohexyl), 1.63 – 1.54 (m, 1H, cyclohexyl), 1.43 – 1.25 (m, 5H, cyclohexyl); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.29 (Ar), 128.90 (Ar), 128.29 (Ar), 128.27 (Ar), 84.39 (C-1), 80.40 (C-3), 74.05 (CH_2OPh), 72.39 (C-5), 71.26 (C-4), 62.57 (C-6), 52.37 (C-2), 44.94 (C-1'), 33.77 (2 x cyclohexyl), 29.04 (cyclohexyl), 26.10 (cyclohexyl), 25.90 (cyclohexyl), 25.44 (cyclohexyl), 23.16 (CH_3CO).

Cyclohexyl 2-acetamido-3-*O*-benzyl-2-deoxy-1-thio-6-*O*-tosyl-α-D-glucopyranoside (2.40).[16]



A solution of cyclohexyl 2-acetamido-3-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside (**2.39**) (0.23 g, 0.55 mmol, 1 eq.) and freshly activated 3Å powdered molecular sieves in dry pyridine (3 mL) was stirred under argon and cooled to 0°C. To this freshly recrystallized *p*-toluenesulfonyl chloride (0.15 g, 0.83 mmol, 1.5 eq.) was added and stirred for 2 hours at this temperature. The reaction mixture was filtered through celite, transferred to a separating funnel and washed with 5% HCl solution. The aqueous layer was washed with DCM (3 x 20 mL), combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (9.7:0.3 DCM/MeOH) to yield 0.13 g (42%) of **2.40** as a white solid. R_f = 0.60 (19:1 DCM/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.72 (m, 2H, Ar-H), 7.41 – 7.27 (m, 7H, Ar-H), 5.31 (d, *J* = 5.29 Hz, 1H, H-1), 5.28 (d, *J* = 8.8 Hz, 1H, NH), 4.69 (AB q, *J* = 19.0, 11.8 Hz, 2H, CH_2OPh), 4.37 (dd, *J* = 11.1, 4.6 Hz, 1H, H-6), 4.32 – 4.23 (m, 1H, H-2), 4.24 (dd, *J* = 11.1, 2.1 Hz, 1H, H-6'), 4.17 (ddd, *J* = 9.8, 4.7, 2.0 Hz, 1H, H-5), 3.62 (t, *J* = 9.2 Hz, 1H, H-4), 3.38 (dd, *J* = 10.9, 8.7 Hz, 1H, H-3), 2.77 – 2.71 (m, 1H, H-1'), 2.44 (s, 3H, CH_3), 1.94 – 1.87 (m, 2H, cyclohexyl), 1.84 (s, 3H, CH_3CO), 1.75 – 1.64 (m, 2H, cyclohexyl), 1.62 – 1.52 (m, 1H, cyclohexyl), 1.36 – 1.20 (m, 5H, cyclohexyl); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.89 (C=O), 145.04 (Ar), 138.25 (Ar), 133.11 (Ar), 129.92 (Ar), 128.84 (Ar), 128.26 (Ar), 128.13 (2 x Ar), 84.12 (C-1), 80.00 (C-3), 74.14 (CH_2OPh), 70.72 (C-5), 70.36 (C-4), 68.70 (C-6), 52.23 (C-2), 44.83 (C-1'), 34.30 (cyclohexyl), 33.85 (cyclohexyl), 26.04 (cyclohexyl), 25.99 (2 x cyclohexyl), 25.72 (CH_3CO), 23.49 (CH_3).

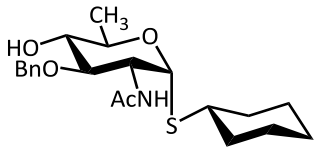
Cyclohexyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (2.41).[16]



The cyclohexyl 2-acetamido-2-deoxy-1-thio-6-*O*-tosyl-α-D-glucopyranoside (**2.40**) (89 mg, 0.16 mmol, 1 eq.) and NaI (52 mg, 0.35 mmol, 2.2 eq.) were dried using a Schlenk line, dissolved in 2-butanone (2 mL) under argon, refluxed for 7 hours and then stirred at r.t. for 18 hrs. The reaction mixture was concentrated, diluted with EtOAc (20 mL) and aq. $Na_2S_2O_3$ (20 mL), transferred to a separating funnel and the aqueous layer was washed with EtOAc (3 x 15 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica

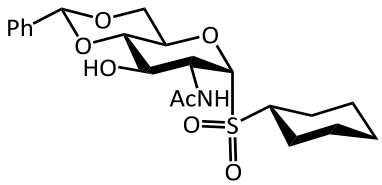
gel chromatography (9:1 EtOAc/hexane) to yield 74 mg (90%) of **2.41** as a white solid. R_f = 0.62 (9:1 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H, Ar-H), 5.38 (d, *J* = 5.3 Hz, 1H, H-1), 5.35 (d, *J* = 9.1 Hz, 1H, NH), 4.68 (AB q, *J* = 11.7, 5.0 Hz, 2H, CH_2OPh), 4.44 (ddd, *J* = 10.6, 9.0, 5.3 Hz, 1H, H-2), 3.81 (ddd, *J* = 8.8, 5.9, 2.6 Hz, 1H, H-5), 3.58 – 3.48 (m, 1H, H-6, H-4), 3.45 (dd, *J* = 10.6, 8.6 Hz, 1H, H-3), 3.39 (dd, *J* = 10.8, 6.1 Hz, 1H, H-6), 2.96 – 2.84 (m, 1H, H-1'), 2.32 (d, *J* = 3.5 Hz, 1H, OH-4), 2.04 – 1.93 (m, 2H, cyclohexyl), 1.89 (s, 3H, CH_3CO), 1.81 – 1.68 (m, 2H, cyclohexyl), 1.64 – 1.52 (m, 1H, cyclohexyl), 1.48 – 1.25 (m, 5H, cyclohexyl); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.79 (C=O), 138.14 (Ar), 128.96 (Ar), 128.39 (Ar), 128.26 (Ar), 84.06 (C-1), 80.42 (C-3), 74.50 (C-4), 74.11 (CH_2OPh), 71.13 (C-5), 52.28 (C-2), 44.64 (C-1'), 34.47 (cyclohexyl), 34.03 (cyclohexyl), 26.23 (cyclohexyl), 26.00 (cyclohexyl), 25.76 (cyclohexyl), 23.62 (CH_3CO), 7.15 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{21}H_{31}INO_4S$: 520.1018 found: 520.1015.

Cyclohexyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.22).[27]



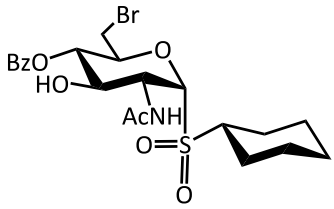
The cyclohexyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (**2.41**) (40 mg, 0.08 mmol, 1 eq.) was dried using a Schlenk line and dissolved in dry degassed toluene (2 mL) under argon. To this this freshly activated 3Å molecular sieves, 1,1′-azobis(cyclohexanecarbonitrile) (ACCN) (14 mg, 0.06 mmol, 0.75 eq.) and Bu_3SnH (0.13 mL, 0.46 mmol, 6 eq.) was added and heated at 75°C for 2 hrs. The reaction mixture was then filtered through celite, concentrated and purified using flash chromatography (9:1 DCM/hexane) to yield 6 mg (20%) of the 6-deoxy sugar **2.22** as an oil. R_f = 0.56 (9:1 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H, Ar-H), 5.39 (d, *J* = 9.1 Hz, 1H, NH), 5.30 (d, *J* = 5.3 Hz, 1H, H-1), 4.69 (A_2 s, 2H, CH_2OPh), 4.41 (ddd, *J* = 10.5, 9.0, 5.2 Hz, 1H, H-2), 4.05 (dq, *J* = 8.8, 6.2 Hz, 1H, H-5), 3.37 (dd, *J* =8.73, 5.55 Hz, 1H, H-4), 3.35 (dd, *J* = 2.8, 1.6 Hz, 1H, H-3), 2.79 (td, *J* = 10.2, 3.7 Hz, 1H, H-1'), 2.18 (d, *J* = 3.2 Hz, 2H, cyclohexyl), 2.01 – 1.91 (m, 2H, cyclohexyl), 1.89 (s, 3H, CH_3CO), 1.79 – 1.70 (m, 6H, cyclohexyl), 1.29 (d, *J* = 6.3 Hz, 3H, H-6).

Cyclohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-1-sulfonyl-α-D-glucopyranoside (2.42).



The cyclohexyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**2.18**) (1.55 g, 4.87 mmol, 1 eq.) and anhydrous $ZnCl_2$ (1.33 g, 9.73 mmol, 2 eq.) were dried using a Schlenk line. To this Benzaldehyde (17 mL) followed by freshly activated 3Å powdered molecular sieves was added under argon and stirred for 19 hrs at r.t. The reaction mixture was wet loaded and purified using flash chromatography (8:2 EtOAc/hexane) to yield 1.30 g (61%) of **2.42** as a white solid. R_f = 0.40 (18.5:1.5 DCM/MeOH); mp 107 – 182°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.44 (m, 2H, Ar-H), 7.37 – 7.30 (m, 3H, Ar-H), 6.81 (d, *J* = 7.7 Hz, 1H, NH), 5.48 (s, 1H, H-7), 5.29 (d, *J* = 6.38, 1H, H-1), 4.58 (ddd, *J* = 10.7, 7.7, 6.3 Hz, 1H, H-2), 4.50 (dd, *J* = 10.7, 8.8 Hz, 1H, H-3), 4.34 (td, *J* = 9.7, 4.9 Hz, 1H, H-5), 4.26 (dd, *J* = 10.3, 4.9 Hz, 1H, H-6), 3.66 (t, *J* = 10.1 Hz, 1H, H-6'), 3.58 (t, *J* = 9.2 Hz, 1H, H-4), 3.00 (tt, *J* = 12.1, 3.4 Hz, 1H, H-1'), 2.15 – 2.01 (m, 2H, cyclohexyl), 1.99 (s, 3H, CH_3CO), 1.96 – 1.90 (m, 1H, cyclohexyl), 1.91 – 1.82 (m, 1H, cyclohexyl), 1.75 – 1.65 (m, 1H, cyclohexyl), 1.58 – 1.44 (m, 2H, cyclohexyl), 1.34 – 1.16 (m, 3H, cyclohexyl) ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.42 (C=O), 136.87 (Ar), 129.38 (Ar), 128.37 (Ar), 126.44 (Ar), 102.10 (C-7), 85.13 (C-1), 81.40 (C-4), 68.53 (C-6), 67.90 (C-3), 67.32 (C-5), 60.48 (C-1'), 52.45 (C-2), 26.07 (cyclohexyl), 25.20 (cyclohexyl), 25.11 (2 x cyclohexyl), 23.04 (cyclohexyl), 22.75 (CH_3CO); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{21}H_{30}NO_7S$: 440.1743 found: 440.1732.

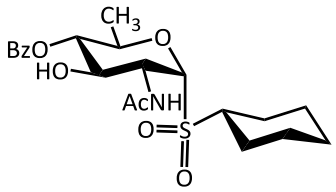
Cyclohexyl 2-acetamido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-1-sulfonyl-α-D-glucopyranoside (2.43).[28]



The cyclohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-1-sulfonyl-α-D-glucopyranoside (**2.42**) (0.52 g, 1.19 mmol, 1 eq.), *N*-bromosuccinimide (0.27 g, 1.54 mmol, 1.3 eq.), $BaCO_3$ (0.10 g, 0.51 mmol, 0.4 eq.) and freshly activated 3Å powdered molecular sieves were dissolved in dry and degassed CCl_4 (20 mL) under argon. The reaction mixture was then refluxed for 2 hours, cooled, filtered through celite, washed 1M NaOH and DCM (3 x 40 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (1:1 hexane/EtOAc) to yield 0.43 g (70%) of **2.43** as a white solid. R_f = 0.56 (7:3 EtOAc/hexane); mp

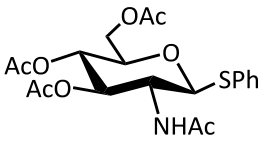
107 – 111°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 7.96 (m, 2H, Ar-H), 7.64 – 7.54 (m, 1H, Ar-H), 7.50 – 7.40 (m, 2H, Ar-H), 6.62 (d, *J* = 8.0 Hz, 1H, NH), 5.27 (d, *J* = 5.9 Hz, 1H, H-1), 5.10 (t, *J* = 8.62, 1H, H-3), 4.75 – 4.62 (m, 2H, H-2, H-5), 4.58 (dd, *J* = 10.3, 8.3 Hz, 1H, H-4), 3.55 (dd, *J* = 11.6, 2.7 Hz, 1H, H-6), 3.53 – 3.43 (m, 2H, H-6', H-1'), 2.20 – 2.07 (m, 2H, cyclohexyl), 2.01 (s, 3H, CH_3CO), 1.94 – 1.84 (m, 2H, cyclohexyl), 1.76 – 1.68 (m, 1H, cyclohexyl), 1.65 – 1.45 (m, 6H, cyclohexyl); ^{13}C NMR (101 MHz, $CCDCl_3$) δ 172.22 (C=O), 166.03 (Ar), 133.93 (Ar), 130.13 (Ar), 128.72 (Ar), 82.65 (C-1), 74.98 (C-5), 73.64 (C-3), 69.03 (C-4), 59.66 (C-1'), 51.95 (C-2), 31.51 (C-6), 26.45 (cyclohexyl), 25.22 (cyclohexyl), 25.17 (cyclohexyl), 24.90 (2 x cyclohexyl), 23.08 (CH_3CO).

Cyclohexyl 2-acetamido-4-*O*-benzoyl-2,6-dideoxy-1-sulfonyl-α-D-glucopyranoside (2.44).



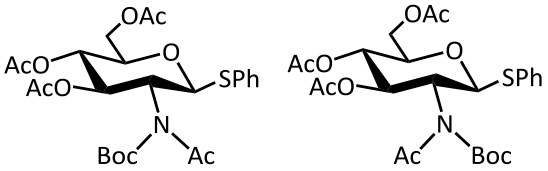
The cyclohexyl 2-acetamido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-1-sulfonyl-α-D-glucopyranoside (**2.43**) (0.18 g, 0.35 mmol, 1 eq.) and $NaHCO_3$ (31 mg, 0.37 mmols, 1.1 eq.) was dissolved in dry MeOH and to this a suspension of 10 wt % Pd/C (0.18 g, 0.17 mmol, 0.5 eq.) in dry MeOH was added. The reaction mixture was flushed three times with hydrogen and using a hydrogenator the pressure was increased to 4 bar and left at r.t. for 7.5 hours. The reaction mixture was filtered through celite, concentrated and columned using flash chromatography (100% EtOAc) to yield 0.13 g (86%) of **2.44** as a clear oil. R_f = 0.56 (7:3 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.02 (m, 2H, Ar-H), 7.63 – 7.55 (m, 1H, Ar-H), 7.49 – 7.42 (m, 2H, Ar-H), 6.50 (d, *J* = 8.3 Hz, 1H, NH), 5.19 (d, *J* = 6.1 Hz, 1H, H-1), 4.96 (dd, *J* = 9.4, 8.8 Hz, 1H, H-4), 4.68 (ddd, *J* = 10.7, 8.3, 6.1 Hz, 1H, H-2), 4.66 – 4.54 (m, 1H, H-5), 4.55 – 4.45 (m, 1H, H-3), 3.11 – 2.97 (m, 1H, H-1'), 2.15 – 2.05 (m, 1H, cyclohexyl), 2.04 (s, 3H, CH_3CO), 2.01 – 1.89 (m, 2H, cyclohexyl), 1.78 – 1.69 (m, 1H, cyclohexyl), 1.66 – 1.53 (m, 7H, cyclohexyl), 1.30 (d, *J* = 6.2 Hz, 3H, C-6).^{13}C NMR (101 MHz, $CDCl_3$) δ 171.94 (C=O), 166.52 (Ar), 133.72 (Ar), 130.11 (Ar), 128.67 (Ar), 84.05 (C-1), 76.84 (C-4), 71.37 (C-3), 70.12 (C-5), 60.91 (C-1'), 52.88 (C-2), 26.21 (cyclohexyl), 25.31 (cyclohexyl), 25.22 (cyclohexyl), 25.20 (cyclohexyl), 23.30 (cyclohexyl), 23.00 (CH_3CO), 18.20 (C-6); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{21}H_{30}NO_7S$: 440.1743 found: 440.1736.

Phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (2.52).[29,30]



Thiophenol (7.9 mL, 76.98 mmol, 1.3 eq) and 1 M $SnCl_4$ in DCM (11.8 mL, 11.84 mmol, 0.2 eq) were added to a stirred solution of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (23.06 g, 59.22 mmol, 1 eq) and 3Å powdered molecular sieves in DCM (120 mL) at r.t. The reaction mixture was heated at reflux for 18 hrs, cooled to r.t., filtered through celite and quenched by the addition of saturated aqueous $NaHCO_3$ (40 mL). The aqueous layer was separated and extracted with DCM (3 × 70 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue obtained was recrystallized in DCM/hexane (10:1) to give 22.40 g (86%) of **2.52** as a white solid. R_f = 0.24 (9.5:0.5 DCM/MeOH); mp 210-212°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 – 7.46 (m, 2H, Ar-H), 7.33 – 7.27 (m, 3H, Ar-H), 5.58 (d, *J* = 9.1 Hz, 1H, NH), 5.22 (dd, *J* = 10.1, 9.4 Hz, 1H, H-3), 5.05 (dd, *J* = 10.1, 9.4 Hz, 1H, H-4), 4.85 (d, *J* = 10.4 Hz, 1H, H-1), 4.22 (dd, *J* = 12.2, 5.3 Hz, 1H, H-6), 4.16 (dd, *J* = 12.3, 2.7 Hz, 1H, H-6'), 4.02 (td, *J* = 10.4, 9.3 Hz, 1H, H-2), 3.72 (ddd, *J* = 10.0, 5.2, 2.8 Hz, 1H, H-5), 2.07 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.14 (C=O), 170.71 (C=O), 170.11 (C=O), 169.45 (C=O), 132.68 (Ar), 129.06 (Ar), 128.21 (2 x Ar), 86.82 (C-1), 76.01 (C-5), 73.91 (C-3), 68.65 (C-4), 62.57 (C-6), 53.60 (C-2), 23.45 (CH_3CO), 20.84 (CH_3CO), 20.79 (CH_3CO), 20.71 (CH_3CO).

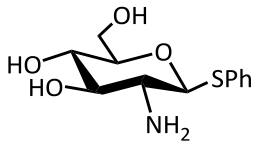
Phenyl 2-(*N*-*tert*-butyloxycarbonylacetamido)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (2.53).[29,30]



To a stirred mixture of phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**2.52**) (22.3 g, 50.97 mmol, 1 eq) and DMAP (1.25 g, 10.19 mmol, 0.2 eq) in THF (100 mL) was added Boc_2O (27.81 g, 127.41 mmol, 2.5 eq) at room temperature. Stirring was continued at 70°C for 3 hours. The reaction mixture was concentrated and purified by flash chromatography (4:6 hexane/EtOAc) to give 26.83 g (98%) of **2.53** (mixture of rotamers) as a yellow oil. R_f = 0.56 (100% EtOAc); ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.49 – 7.38 (m, 4H, Ar-H), 7.30 – 7.21 (m, 6H, Ar-H), 5.82 (dd, *J* = 10.1, 8.8 Hz, 1H, H-3), 5.73 (d, *J* = 10.2 Hz, 1H, H-1), 5.68 (dd, *J* = 10.4, 8.9 Hz, 1H, H-3'), 5.43 (d, *J* = 10.2 Hz, 1H, H-1'), 5.7 (dd, *J* = 8.8, 3.2 Hz, 1H, H-4'), 5.03 (dd, *J* = 8.8, 3.2 Hz, 1H, H-4). 4.96 (t, *J* = 10.3 Hz, 1H, H-2'), 4.35 (t, *J* = 10.1 Hz, 1H, H-2), 4.24 (dd, *J* =

5.6, 1.8 Hz, 1H, H-6a), 4.21 (dd, *J* = 5.7, 1.9 Hz, 1H, H-6'a), 4.14 (dd, *J* = 12.2, 2.5 Hz, 1H, H-6'b), 4.09 (dd, *J* = 12.2, 2.4 Hz, 1H, H-6b), 3.77 (ddd, *J* = 10.3, 5.8, 2.4 Hz, 1H, H-5'), 3.69 (ddd, *J* = 10.3, 5.5, 2.5 Hz, 1H, H-5), 2.43 (s, 3H, CH_3CO), 2.35 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 1.99 (s, 6H, 2 x CH_3CO), 1.95 (s, 3H, CH_3CO), 1.92 (s, 3H, CH_3CO), 1.52 (s, 9H, t-butyl), 1.49 (s, 9H, t-butyl); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.95 (C=O), 173.07 (C=O), 170.69 (C=O), 170.64 (C=O), 170.30 (C=O), 170.04 (C=O), 169.73 (C=O), 169.44 (C=O), 153.04 (C=O), 151.81 (C=O), 133.21 (Ar), 132.96 (Ar), 132.26 (Ar), 132.23 (Ar), 129.04 (2 x Ar), 128.11 (Ar), 127.96 (Ar), 86.84 (C-1'), 85.21 (C-1), 76.12 (C-5), 75.78 (C-5'), 72.07 (C-3'), 71.60 (C-3), 69.72 (C-4, C-4'), 62.67 (C-6), 62.63 (C-6'), 60.31 (C-2), 55.72 (C-2'), 28.09 (2 X t-butyl), 28.04 (2 X t-butyl), 28.01 (2 X t-butyl), 27.61 (CH_3CO), 27.90 (CH_3CO), 26.82 (2 x CH_3CO), 20.72 (2 x CH_3CO), 20.64 (CH_3CO), 20.56 (CH_3CO). NMR spectrum agrees with literature, although no chemical shifts published, only the spectra given.[30]

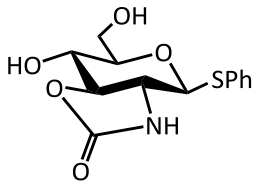
Phenyl 2-amino-2-deoxy-1-thio-β-D-glucopyranoside (2.46).[29,30]



Phenyl 2-(*N*-*tert*-butyloxycarbonylacetamido)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (**3.53**) (10.80 g, 20.02 mmol, 1 eq) in MeOH (60 mL) containing a catalytic amount of freshly prepared 1 M NaOMe solution was stirred for 45 mins at r.t. To this 2 teaspoons of Amberlite IR-120 (H^+ form) was added and stirred for a further 20 mins. The Amberlite was filtered off and solvent removed by evaporation.

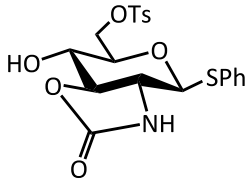
The reaction mixture was cooled to 0°C and to this a total of TFA (100 mL, 1.3 mol, 65 eq) was added in 33.3 mL aquilots over 3 hours. The reaction mixture was left to slowly warm to r.t. over 15 hrs after the last addition and TFA removed. Another 100 mL of TFA was added in a similar fashion and left to stir at r.t until TLC showed the reaction to be complete. The TFA was then removed and the product was purified using a short column of silica gel (9.5:0.5 EtOAc/MeOH) to yield 4.51 g (83%) of the free amine **2.46** as a brown oil. R_f = 0.26 (9.5:0.5 EtOAc/MeOH); ^{1}H NMR (400 MHz, D_2O) δ 7.75 – 7.68 (m, 2H, Ar-H), 7.59 – 7.51 (m, 3H, Ar-H), 5.09 (d, *J* = 10.3 Hz, 1H, H-1), 4.01 (dd, *J* = 12.5, 2.1 Hz, 1H, H-6), 3.85 (dd, *J* = 12.5, 5.2 Hz, 1H, H-6'), 3.79 (dd, *J* = 10.1, 8.5 Hz, 1H, H-3), 3.63 (ddd, *J* = 9.7, 5.3, 2.1 Hz, 1H, H-5), 3.58 (dd, *J* = 9.8, 8.5 Hz, 1H, H-4), 3.30 (t, *J* = 10.3 Hz, 1H, H-2); ^{13}C NMR (101 MHz, D_2O) δ 132.51 (Ar), 130.39 (Ar), 129.66 (Ar), 129.12 (Ar), 83.94 (C-1), 80.32 (C-5), 73.58 (C-3), 69.46 (C-4), 60.62 (C-6), 54.56 (C-2). Note: Reaction proceeds within 3 hrs with 12 eq. TFA on a small scale (between 0.1 and 0.3 g of starting material).

Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-β-D-glucopyranoside (2.47).[30]



An ice-cooled solution of *p*-nitrophenoxycarbonyl chloride (8.37 g, 41.54 mmol, 2.5 eq) in acetonitrile (50 mL) was added over 30 minutes to a stirred mixture of the phenyl 2-amino-2-deoxy-1-thio-β-D-glucopyranoside (**2.46**) (4.51 g, 16.62 mmol, 1 eq) and $NaHCO_3$ (6.98 g, 83.09 mmol, 5 eq) in water (200 mL) at 0°C. This was vigorously stirred at this temperature for 2 hours after the final aliquots of *p*-nitrophenoxycarbonyl chloride was added. The reaction mixture was transferred to a separating funnel and extracted with EtOAc (3 x 50 mL), the combined organic layers were dried over Na_2SO_4, concentrated and purified using flash chromatography (100% hexane to 3:7 EtOAc/hexane) to yield 4.67 g (94%) of **2.47** as a yellow oil. R_f = 0.38 (9:1 EtOAc/MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.62 – 7.57 (m, 2H, Ar-H), 7.37 – 7.28 (m, 3H, Ar-H), 4.93 (d, *J* = 9.6 Hz, 1H, H-1), 4.15 (dd, *J* = 10.5, 9.2 Hz, 1H, H-3), 3.88 (dd, *J* = 12.2, 2.2 Hz, 1H, H-6), 3.83 (dd, *J* = 10.0, 8.8 Hz, 1H, H-4), 3.76 (dd, *J* = 12.2, 5.2 Hz, 1H, H-6'), 3.46 (ddd, *J* = 8.8, 5.2, 2.3 Hz, 1H, H-5), 3.35 (ddd, *J* = 9.6, 6.4, 4.7 Hz, 1H, H-2); ^{13}C NMR (101 MHz, CD_3OD) δ 161.93 (C=O), 134.13 (Ar), 133.57 (Ar), 130.07 (Ar), 129.14 (Ar), 86.12 (C-1), 86.08 (C-5), 83.90 (C-3), 68.53 (C-4), 62.10 (C-6), 60.09 (C-2).

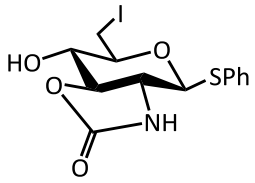
Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-6-*O*-tosyl-β-D-glucopyranoside (2.54).[16]



A solution of the phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-β-D-glucopyranoside (**2.47**) (4.21 g, 14.14 mmol, 1 eq.) and activated 4Å powdered molecular sieves in dry pyridine (20 mL) was stirred under argon and cooled to 0°C. To this freshly recrystallized *p*-toluenesulfonyl chloride (3.26 g, 16.95 mmol, 1.2 eq.) was added and stirred for 4.5 hours at this temperature. The reaction mixture was filtered through celite, transferred to a separating funnel and washed with 1M HCl. The aqueous layer was washed with DCM (4 x 30 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (3:7 EtOAc/hexane to 6:4 EtOAc/hexane) to yield 5.68 g (89%) of **2.54** as a yellow oil. R_f = 0.58 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.78 (m, 2H, Ar-H), 7.50 – 7.43 (m, 2H, Ar-H), 7.40 – 7.26 (m, 5H, Ar-H), 5.33 (s, 1H, NH), 4.70 (d, *J* = 9.6 Hz, 1H, H-1), 4.37 (dd, *J* = 11.2, 4.1 Hz, 1H, H-6), 4.33 (dd, *J* = 11.1, 2.2 Hz, 1H, H-6'), 4.10 (dd, *J* = 11.1, 10.0 Hz, 1H, H-3), 3.97 (td, *J* = 9.8,

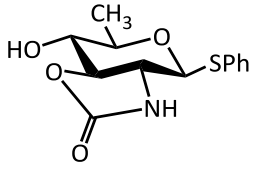
3.6 Hz, 1H, H-4), 3.58 (ddd, *J* = 8.8, 4.1, 2.3 Hz, 1H, H-5), 3.30 (ddd, *J* = 11.0, 9.6, 1.2 Hz, 1H, H-2), 2.42 (s, 3H, CH_3); ^{13}C NMR (101 MHz, CDCl3) δ 158.63 (C=O), 145.36 (Ar), 133.71 (Ar), 132.84 (Ar), 130.15 (Ar), 129.44 (Ar), 129.15 (2 x Ar), 128.18 (Ar), 84.60 (C-1), 84.09 (C-3), 79.19 (C-5), 67.94 (C-6), 67.32 C-4), 58.25 (C-2), 21.67 (CH_3); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{20}H_{22}NO_7S_2$: 452.0838 found: 452.0838; $[M+NH_4]^+$ calc. for $C_{20}H_{25}N_2O_7S_2$: 469.1103 found: 469.1103. Note: The crude **2.54** can be used in the following step.

Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-β-D-glucopyranoside (2.55).[31]



The phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-6-*O*-tosyl-β-D-glucopyranoside (**2.54**) (5.68 g, 12.58 mmol, 1 eq.) and NaI (4.15 g, 27.69 mmol, 2.2 eq.) were dried using a Schlenk line. To this dry butanone (63 mL) was added under argon and refluxed overnight. The reaction mixture was then concentrated and purified using flash chromatography (3:7 EtOAc/DCM to 5:5 EtOAc/DCM) to yield 4.03 g (79%) of **2.55** as a white solid. R_f 0.64 (100% EtOAc); mp 167-169°C; ^1H NMR (400 MHz, $CO(CD_3)_2$) δ 7.74 – 7.65 (m, 2H, Ar-H), 7.40 – 7.31 (m, 3H, Ar-H), 6.89 (s, 1H, NH), 5.23 (d, *J* = 5.4 Hz, 1H, 4-OH), 5.10 (d, *J* = 9.6 Hz, 1H, H-1), 4.27 (dd, *J* = 11.1, 9.9 Hz, 1H, H-3), 3.83 – 3.76 (m, 1H, H-4), 3.72 (dd, *J* = 10.8, 2.5 Hz, 1H, H-6), 3.53 (dd, *J* = 10.8, 6.2 Hz, 1H, H-6'), 3.45 (ddd, *J* = 7.6, 5.1, 1.9 Hz, 1H, H-5), 3.41 (ddd, *J* = 11.1, 6.4, 1.6 Hz, 1H, H-2); ^{13}C NMR (101 MHz, $CO(CD_3)_2$) δ 159.45 (C=O), 133.85 (Ar), 132.42 (Ar), 129.85 (Ar), 129.02 (Ar), 85.09 (C-1), 84.12 (C-3), 81.34 (C-5), 72.31 (C-4), 59.60 (C-2), 6.82 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{13}H_{15}INO_4S$: 407.9766 found: 407.9776; $[M+NH_4]^+$ Calcd for $C_{13}H_{18}IN_2O_4S$: 425.0032 found: 425.0021.

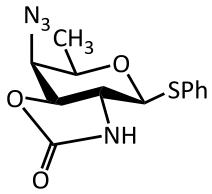
Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-β-D-glucopyranoside (2.48).



The phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-β-D-glucopyranoside (**2.55**) (3.96 g, 9.73 mmol, 1 eq.) and $NaHCO_3$ (0.82 g, 9.73 mmols, 1 eq.) were dissolved in dry MeOH (15 mL) and to this a suspension of 10 wt % Pd/C (2.8 g, 0.7 wt eq.) in dry MeOH (10 mL) was added. The reaction vessel was evacuated and flushed three times with hydrogen and using a hydrogenator the pressure was increased to 4 bar and left at r.t. for 25 hrs. The reaction mixture

was filtered through celite, concentrated and columned using silica gel chromatography (3:7 hexane/EtOAc) to yield 1.63 g (60%) of **2.48** as a white solid. R_f = 0.6 (3:7 hexane/EtOAc); mp 168-170°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.49 (m, 2H, Ar), 7.38 – 7.31 (m, 3H, Ar-H), 5.13 (s, 1H, NH), 4.73 (d, *J* = 9.6 Hz, 1H, H-1), 4.10 (dd, *J* = 11.3, 9.7 Hz, 1H, H-3), 3.64 (td, *J* = 9.6, 3.4 Hz, 1H, H-4), 3.52 (dq, *J* = 8.4, 6.1 Hz, 1H, H-5), 3.38 (ddd, *J* = 11.1, 9.6, 1.2 Hz, 1H, H-2), 2.50 (d, *J* = 3.6 Hz, 1H, OH-4), 1.44 (d, *J* = 6.1 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.72 (C=O), 133.40 (Ar), 130.89 (Ar), 129.43 (Ar), 128.96 (Ar), 84.68 (C-3), 84.58 (C-1), 78.27 (C-5), 72.65 (C-4), 58.97 (C-2), 17.76 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{13}H_{16}NO_4S$: 282.0800 found: 282.0804; $[M+NH_4]^+$ Calcd for $C_{13}H_{19}N_2O_4S$: 299.1066 found: 299.1074.

Phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.49).



First attempt:[13]

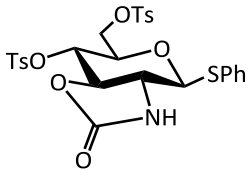
The phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-*β*-D-glucopyranoside (**2.48**) (53 mg, 0.19 mmol, 1 eq.) and PPh_3 (54 mg, 0.20 mmol, 1.1 eq.) were dried using a Schlenk line. To this THF (3.5 mL) was added under argon followed by the addition of Diisopropyl azodicarboxylate (DIAD) (41 µL, 0.20 mmol, 1.1 eq.) and diphenylphosphoryl azide (45 µL, 0.20 mmol, 1.1 eq.). The reaction mixture was left to stir for 20 hrs after which time TLC indicated no reaction had taken place. SM recovered = 51 mg.

Second attempt:

A solution of the phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-*β*-D-glucopyranoside (**2.48**) (1.20 g, 4.27 mmol, 1 eq.) and activated 3Å powdered molecular sieves in dry pyridine (10 mL) was stirred under argon and cooled to -30°C. To this trifluoromethanesulfonic anhydride (0.79 mL, 4.69 mmol, 1.1 eq.) was added and stirred at this for 1 hr. The reaction mixture was diluted with DCM, filtered through celite and washed with 1M HCl. The combined organic fractions were dried over Na_2SO_4, concentrated and used without further purification. R_f = 0.56 (1:1 hexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.47 (m, 2H, Ar-H), 7.43 – 7.30 (m, 2H, Ar-H), 5.78 (s, 1H, NH), 4.73 (d, *J* = 9.6 Hz, 1H, H-1), 4.71 – 4.66 (m, 1H, H-4), 4.33 (dd, *J* = 11.3, 10.1 Hz, 1H, H-3), 3.78 (dq, *J* = 8.6, 6.2 Hz, 1H, H-5), 3.43 (ddd, *J* = 11.4, 9.6, 1.2 Hz, 1H, H-2), 1.49 (d, *J* = 6.2 Hz, 3H, H-6).

The triflate was then dissolved in dry DMF (6 mL) under argon and to this the NaN_3 (0.39 g, 5.97 mmol, 1.4 eq.) was added and left to stir at r.t. for 18 hours. The reaction mixture was then diluted with EtOAc (20 mL) washed with H_2O and the combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (7:3 hexane/EtOAc) to yield 1.01 g (77%) of **2.49** as a white solid. R_f = 0.56 (1:1 hexane/EtOAc); mp 90-92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.49 (m, 2H, Ar-H), 7.39 – 7.31 (m, 3H, Ar-H), 5.09 (s, 1H, NH), 4.65 (d, *J* = 9.6 Hz, 1H, H-1), 4.29 (dd, *J* = 11.3, 2.9 Hz, 1H, H-3), 3.98 – 3.95 (m, 1H, H-4), 3.88 (qd, *J* = 6.3, 1.7 Hz, 1H, H-5), 3.81 (ddd, *J* = 11.0, 9.7, 1.0 Hz, 1H, H-2), 1.43 (d, *J* = 6.3 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.90 (C=O), 133.36 (Ar), 130.86 (Ar), 129.44 (Ar), 129.01 (Ar), 85.77 (C-1), 82.02 (C-3), 75.34 (C-5), 60.97 (C-4), 54.59 (C-2), 17.88 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{13}H_{15}N_4O_3S$: 307.0865 found: 307.0866; $[M+NH_4]^+$ Calcd for $C_{13}H_{18}N_5O_3S$: 324.1130 found: 324.1133; $[M+Na]^+$ Calcd for $C_{13}H_{14}N_4NaO_3S$: 329.0684 found: 329.0688; IR (NaCl, dry film) ν_{max} (cm^{-1}): 3413 (O-H), 3051 (C-H Ar), 2926 (C-H aliphatic), 2116 (N_3), 1769 (C=O), 1640 (C=C), 1265 (C-O), 742 (C-H Ar bend).

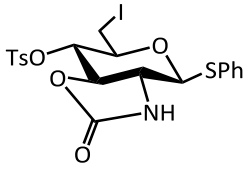
Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-4,6-di-*O*-tosyl-β-D-glucopyranoside (2.56).[16]



A solution of the phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-*β*-D-glucopyranoside (**2.47**) (4.04 g, 13.60 mmol, 1 eq.) and activated 3Å powdered molecular sieves in dry pyridine (20 mL) was stirred under argon and cooled to 0°C. To this freshly recrystallized *p*-toluenesulfonyl chloride (3.89 g, 20.40 mmol, 1.5 eq.) was added and stirred for 2 hours at this temperature. The reaction mixture was filtered through celite, transferred to a separating funnel and washed with 1M HCl. The aqueous layer was washed with DCM (4 x 30 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (3:7 EtOAc/hexane to 6:4 EtOAc/hexane) to yield 2.79 g (45%) of the mono- tosylate **2.54**, 2.04 g (24%) of the di-tosylate **2.56**, both as yellow oils and 0.31 g of starting material. R_f = 0.71 (8:2 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.80 (m, 2H, Ar-H), 7.80 – 7.74 (m, 2H, Ar-H), 7.50 – 7.43 (m, 2H, Ar-H), 7.40 – 7.28 (m, 7H, Ar-H), 5.55 (s, 1H, NH), 4.74 (dd, *J* = 10.0, 9.0 Hz, 1H, H-4), 4.64 (d, *J* = 9.6 Hz, 1H, H-1), 4.41 (dd, *J* = 11.2, 2.0 Hz, 1H, H-6), 4.14 (dd, *J* = 11.3, 5.0 Hz, 1H, H-6'), 4.09 (dd, *J* = 11.3, 10.1 Hz, 1H, H-3), 3.76 (ddd, *J* = 8.8, 4.9, 2.0 Hz, 1H, H-5), 3.26 (ddd, *J* = 11.0, 9.6, 1.0 Hz, 1H, H-2), 2.43 (s, 3H, CH_3), 2.42 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.54 (C=O), 146.04 (Ar), 145.22 (Ar), 134.20 (Ar), 132.78 (Ar), 132.65 (Ar), 130.11

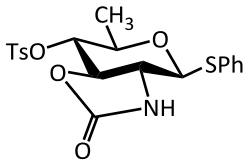
(Ar), 130.08 (Ar), 129.49 (Ar), 129.39 (Ar), 129.21 (Ar), 128.27 (Ar), 128.26 (Ar), 84.12 (C-1), 80.90 (C-3), 76.85 (C-5), 73.14 (C-4), 67.21 (C-6), 58.01 (C-2), 21.83 (CH_3), 21.76 (CH_3); HRMS (ESI-TOF): $[MH]^+$ Calcd for $C_{27}H_{28}NO_9S_3$: 606.0926 found: 606.0926, $[MNH_4]^+$ Cald for $C_{27}H_{31}N_2O_9S_3$: 623.1192 found: 623.1192.

Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-4-*O*-tosyl-β-D-glucopyranoside (2.57).[17,31]



The phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-4,6-di-*O*-tosyl-*β*-D-glucopyranoside (**2.56**) (0.70 g, 1.15 mmol, 1 eq.) and NaI (0,19 g, 1.27 mmol, 1.1 eq.) were dried using a Schlenk line, dissolved in dry 2-butanone (13 mL) under argon and refluxed for 16.5 hrs. The reaction mixture was then concentrated, diluted with EtOAc (40 mL) and aq. $Na_2S_2O_3$ (40 mL), transferred to a separating funnel and the aqueous layer washed with EtOAc (4 x 40 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (1:1 EtOAc/hexane) to yield 0.46 g (71%) of **2.57** as an off white solid. R_f = 0.71 (100% EtOAc); mp 154-157°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.79 (m, 2H, Ar-H), 7.66 – 7.61 (m, 2H, Ar-H), 7.42 – 7.32 (m, 5H, Ar-H), 5.33 (s, 1H, NH), 4.73 (d, *J* = 9.4 Hz, 1H, H-1), 4.72 (dd, *J* = 9.7, 7.7 Hz, 1H, H-4), 4.16 (dd, *J* = 11.3, 10.0 Hz, 1H, H-3), 3.64 (dd, *J* = 10.9, 2.5 Hz, 1H, H-6), 3.47 (ddd, *J* = 8.2, 5.8, 2.5 Hz, 1H, H-5), 3.41 (dd, *J* = 10.9, 5.8 Hz, 1H, H-6'), 3.33 (ddd, *J* = 11.1, 9.6, 1.2 Hz, 1H, H-2), 2.45 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.49 (C=O), 146.01 (Ar), 134.65 (Ar), 132.91 (Ar), 130.13 (Ar), 129.53 (2 x Ar), 129.11 (Ar), 128.40 (Ar), 84.33 (C-1), 80.75 (C-3), 78.01 (C-5), 77.16 (C-4), 58.34 (C-2), 21.89 (CH_3), 4.00 (C-6).

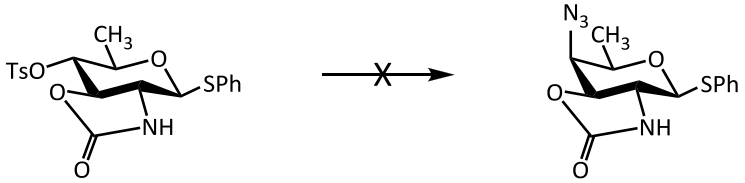
Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-4-*O*-tosyl-β-D-glucopyranoside (2.58).



The phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-4-*O*-tosyl-*β*-D-glucopyranoside (**2.57**) (0.68 g, 1.21 mmol, 1 eq.) and $NaHCO_3$ (0.10 g, 1.21 mmols, 1 eq.) were dissolved in MeOH (10 mL) and dry DCM (2 mL) and to this a suspension of 10 wt % Pd/C (2.8 g, 0.7 wt eq.) in dry MeOH (5 mL) was added. The reaction vessel was evacuated and flushed three times with hydrogen and using a hydrogenator the pressure was increased to 4 bar and

left at r.t. for 20 hrs. The reaction mixture was filtered through celite, concentrated and columned using silica gel chromatography (7:3 hexane/EtOAc) to yield 0.27 g (52%) of **2.58** as a clear oil. R_f = 0.31 (6:4 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.77 (m, 2H, Ar-H), 7.53 – 7.48 (m, 2H, Ar-H), 7.39 – 7.29 (m, 5H, Ar-H), 5.65 (s, 1H, NH), 4.65 (d, *J* = 9.6 Hz, 1H, H-1), 4.54 (dd, *J* = 10.0, 8.5 Hz, 1H, H-4), 4.09 (dd, *J* = 11.3, 10.0 Hz, 1H, H-3), 3.65 (dq, *J* = 8.5, 6.2 Hz, 1H, H-5), 3.33 (app t, *J* = 11.0 Hz, 1H, H-2), 2.43 (s, 3H, CH_3), 1.44 (d, *J* = 6.2 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.00 (C=O), 145.58 (Ar), 133.87 (Ar), 133.42 (Ar), 129.94 (Ar), 129.40 (Ar), 129.16 (2 x Ar), 128.16 (Ar), 84.12 (C-1), 81.24 (C-3), 78.63 (C-4), 76.19 (C-5), 58.88 (C-2), 21.78 (CH_3), 17.69 (C-6); HRMS (ESI-TOF): $[MNH_4]^+$ Calcd for $C_{20}H_{25}N_2O_6S_2$: 453.1154 found: 453.1154.

Phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.49).[17]



First attempt:

The phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-4-*O*-tosyl-*β*-D-glucopyranoside (**2.58**) (57 mg, 0.13 mmol, 1 eq.) and NaN_3 (8.5 mg, 0.14 mmols, 1 eq.) was dissolved in DMF (1 mL) and H_2O (0.7 mL) and stirred at r.t for 18.5 hrs. TLC indicated that no reaction had taken place, so was then heated at 50°C for 4 hrs and then at 70°C for 17 hrs after which time TLC indicated no reaction had taken place.

Second attempt:[32]

The above reaction mixture was then transferred to a microwave tube and heated at 90°C (300W) for 3 mins after which time TLC again indicated no reaction had taken place.

Third attempt:

In a microwave tube the phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-4-*O*-tosyl-*β*-D-glucopyranoside (**2.58**) (21 mg, 0.05 mmol, 1 eq.) and NaN_3 (4.4 mg, 0.0.7 mmols, 1.4 eq.) was dissolved in DMF (0.5 mL) and a drop of H_2O. This was then heated at 100°C (300W) for 10 mins after which time TLC indicated that multiple more polar products had formed.

Fourth attempt:

The phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-4-*O*-tosyl-β-D-glucopyranoside (**2.58**) (57 mg, 0.13 mmol, 1 eq.) and NaN_3 (8.5 mg, 0.14 mmols, 1 eq.) was dissolved in DMF (1 mL) and stirred at 120°C for 16 hrs after which time TLC indicated that multiple more polar products had formed.

Phenyl 2-amino-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside.

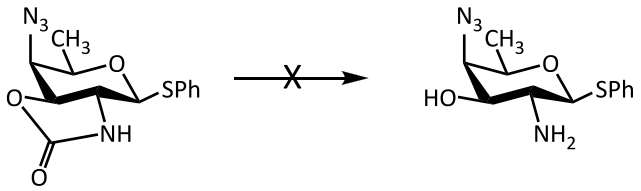


Table 2.2, entry 1:[30]

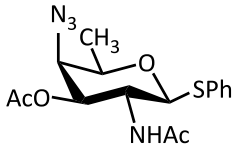
Phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.49**) (71.2 mg, 0.23 mmol, 1 eq.) was dissolved in THF (2 mL) and to this 1M NaOH (0.18 mL, 0.18 mmol, 0.76 eq.) was slowly added at r.t. and left to stir at this temperature for 45 minutes after which time TLC analysis revealed starting material to be present as well the minor formation of multiple polar products. This was then left for a total of 4 hrs at which point TLC analysis no more starting material, but multiple products with no major product visible.

Table 2.2, entry 2:[30]

Phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.49**) (40 mg, 0.13 mmol, 1 eq.) was dissolved in THF (2 mL) and to this 2M NaOH (0.12 mL, 0.24 mmol, 1.8 eq.) was added at r.t. and left to stir at this temperature for 15 mins. At this point TLC analysis revealed starting material to be present as well as the formation of multiple more polar products. This was left for a total of 2.5 hrs hrs at which point TLC analysis no more starting material, but multiple products with no major product visible.

Phenyl 2-acetamido-3-*O*-acetyl-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.51)

Table 2.2, entry 3:

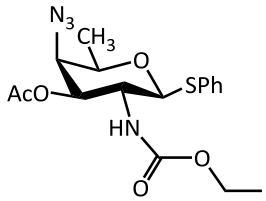


Phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.49**) (57.4 mg, 0.19 mmol, 1 eq.) was dissolved in 2 mL of THF and to this 2M NaOH (0.19 mL, 2 eq.) was added. Stirring was continued at 60°C for 20 mins, removed from the heat, stirred with 20

mg of Amberlite IR-120 H^+ for 20 mins. The Amberlite was filtered off and washed with DCM. The crude material was concentrated and re-dissolved in pyridine (1 mL) and to this DMAP (4.57 mg, 0.0375 mmol, 0.2 eq.) and acetic anhydride (70.8 µL, 0.7492 mmol, 4 eq.) was added and left to stir at r.t. for 2 hours. The reaction mixture was diluted with DCM, transferred to a separating funnel and washed with 5% HCl solution. The aqueous layer was washed with DCM (3 x 20 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (100% EtOAc) to yield 23.3 mg (35%) of **2.51** as a clear oil. R_f = 0.42; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.46 (m, 2H, Ar-H), 7.33 – 7.26 (m, 3H, Ar-H), 5.45 (d, *J* = 8.7 Hz, 1H, NH), 5.38 (dd, *J* = 10.6, 3.6 Hz, 1H, H-3), 4.94 (d, *J* = 10.3 Hz, 1H, H-1), 4.04 (td, *J* = 10.6, 9.0 Hz, 1H, H-2), 3.81 (dd, *J* = 3.6, 1.3 Hz, 1H, H-4), 3.76 (qd, *J* = 6.3, 1.3 Hz, 1H, H-5), 2.11 (s, 3H, CH_3CO), 1.97 (s, 3H, OAc), 1.36 (d, *J* = 6.3 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.70 (C=O), 170.29 (C=O), 133.14 (Ar), 132.41 (Ar), 129.07 (Ar), 127.95 (Ar), 86.54 (C-1), 73.56 (C-3), 73.45 (C-5), 63.84 (C-4), 50.42 (C-2), 23.61 (OAc), 20.72 (CH_3CO), 17.91 (C-6). HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{16}H_{21}N_4O_4S$: 365.1284 found: 365.1285.

Phenyl 3-*O*-acetyl-4-azido-2,4,6-trideoxy-2-ethoxycarbonylamino-1-thio-β-D-galactopyranoside (2.59).

Table 2.2, entry 4:[33]

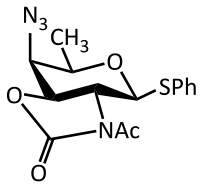


Phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.49**) (52.2 mg, 0.17 mmol, 1 eq.) was dissolved in EtOH (5 mL) and to this $LiOH.H_2O$ (50.04 mg, 1.20 mmol, 7 eq.) was added. Stirring was continued at reflux for 18 hours. The reaction mixture was removed from the heat, allowed to cool to r.t. and then AcOH (0.13 mL, 2.31 mmol, 13.6 eq) was added and stirred at this temperature for 1 hour after which time acetic anhydride (80 µL, 0.85 mmol, 5 eq.) was added and left to stir at this temperature for 22 hrs. This was diluted with EtOAc and H_2O, transferred to a separating funnel and the aqueous layer was washed with EtOAc (3 x 40 mL) and combined organic fractions were dried over Na_2SO_4 and concentrated to yield 39.6 mg (crude) of a white solid.

The crude material (39 mg, 0.11 mmol, 1 eq.) was dissolved in dry pyridine (2 mL) and to this acetic anhydride (1 mL) was added and stirred at r.t. for 6 hrs. The reaction mixture was diluted with DCM, transferred to a separating funnel and washed with 5% HCl solution. The aqueous layer was washed with DCM (3 x 20 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (100% hexane to 8:2

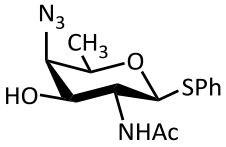
DCM/hexane) to yield 32.7 mg (75%) of **2.59** as a white solid. R_f = 0.67 (100% DCM); mp 177-179 °C; ^{1}H NMR (400 MHz, $CO(CD_3)_2$) δ 7.52 – 7.47 (m, 2H, Ar-H), 7.35 – 7.24 (m, 3H, Ar-H), 6.32 (d, *J* = 8.5 Hz, 1H, NH), 5.29 (d, *J* = 10.6 Hz, 1H, H-3), 5.03 (d, *J* = 10.4 Hz, 1H, H-1), 4.09 – 4.02 (m, 3H, H-4, CH_2), 3.99 – 3.90 (m, 2H, H-5, H-2), 2.05 (s, 3H, CH_3CO), 1.31 (d, *J* = 6.3 Hz, 3H, H-6), 1.19 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CO(CD_3)_2$) δ 170.30 (C=O), 156.95 (C=O), 135.16 (Ar), 132.09 (Ar), 129.70 (Ar), 128.00 (Ar), 87.61 (C-1), 74.85 (C-3), 73.76 (C-5), 64.71 (C-4), 61.09 (CH_2), 51.66 (C-2), 20.52 (CH_3CO), 17.92 (C-6), 15.02 (CH_3); HRMS (ESI-TOF): $[M+Na]^+$ Calcd for $C_{17}H_{22}N_4NaO_5S$: 417.1209 found: 417.1210.

Phenyl 2-*N*-acetyl-2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.60).[34]



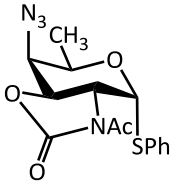
A solution of phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.49**) (0.21 g, 0.70 mmol, 1 eq.) in dry DCM (10 mL) was stirred under argon and cooled to 0°C. To this DIPEA (0.42 mL, 2.43 mmol, 3.5 eq.) followed by AcCl (0.20 mL, 2.43 mmol, 3.5 eq.) was added and stirred for 2.5 hours at this temperature. The reaction mixture was diluted with DCM, transferred to a separating funnel and washed with 5% HCl solution. The aqueous layer was washed with DCM (3 x 20 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (100% hexane to 8:2 DCM/hexane) to yield 0.21 g (87%) of **2.60** as a white solid. R_f = 0.60 (100% DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.45 (m, 2H, Ar), 7.32 – 7.26 (m, 3H, Ar), 4.77 (d, J = 8.6 Hz, 1H, H-1), 4.45 (dd, J = 11.3, 8.6 Hz, 1H, H-2), 4.30 (dd, J = 11.3, 2.6 Hz, 1H, H-3), 4.08 – 4.04 (m, 1H, H-4), 3.80 (qd, J = 6.4, 1.8 Hz, 1H, H-5), 2.58 (s, 3H, CH_3CO), 1.37 (d, J = 6.4 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.05 (C=O), 153.50 (C=O), 134.09 (Ar), 132.75 (Ar), 128.95 (Ar), 128.11 (Ar), 88.18 (C-1), 79.81 (C-3), 75.07 (C-5), 61.28 (C-4), 56.28 (C-2), 24.97 (OAc CH_3), 17.49 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{15}H_{17}N_4O_4S$: 349.0971 found: 349.0976; $[M+Na]^+$ Calcd for $C_{15}H_{16}O_4N_4SNa$: 371.0790 found: 371.0787.

Phenyl 2-acetamido-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.50).[35,36]



Phenyl 2-N-acetyl-2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.60**) (0.61 g, 1.76 mmol, 1 eq.) was dissolved in THF/H_2O (59 mL) (8:2) and to this LiCl (0.33 g, 7.91 mmol, 4.5 eq.) was added at r.t. This was stirred for 5 mins after which time LiOH (0.22 g, 5.27 mmol, 3 eq.) was then added and vigorously stirred for a further 1 hr 15 mins. To this ~100 mg of Amberlite IR-120 (H^+ form) was added and stirred for 20 mins. The reaction mixture was then filtered through celite, THF removed by evaporation, diluted with EtOAc and H_2O and transferred to a separating funnel. The aqueous layer was washed with EtOAc (3 x 60 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (9.5:0.5 DCM/MeOH) to yield 0.51 g (90%) of **2.50** as a white solid. R_f = 0.18 (9.5:0.5 DCM/MeOH); mp 206-209 °C; ^{1}H NMR (400 MHz, $SO(CD_3)_2$) δ 7.79 (d, *J* = 9.0 Hz, 1H, NH), 7.39 – 7.28 (m, 4H, Ar-H), 7.25 – 7.19 (m, 1H, Ar-H), 5.63 (d, *J* = 3.7 Hz, 1H, OH-3), 4.76 (d, *J* = 10.1 Hz, 1H, H-1), 3.92 – 3.82 (m, 1H, H-3), 3.83 (apparent m, 1H, H-4), 3.80 (dd, *J* = 9.3, 8.3 Hz, 1H, H-2), 3.75 (dd, *J* = 6.3, 1.4 Hz, 1H, H-5), 1.83 (s, 3H, CH_3CO), 1.18 (d, *J* = 6.3 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $SO(CD_3)_2$) δ 169.38 (C=O), 135.01 (Ar), 129.20 (Ar), 128.82 (Ar), 126.31 (Ar), 85.85 (C-1), 72.19 (C-5), 71.93 (C-3), 65.61 (C-4), 50.58 (C-2), 23.05 (CH_3CO), 17.55 (C-6); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{14}H_{19}N_4O_3S$: 323.1178 found: 323.1167; $[M+Na]^+$ Calcd for $C_{14}H_{18}N_4NaO_3S$: 345.0997 found: 345.0991; IR (NaCl, dry film) ν_{max} (cm^{-1}): 3290 (O-H), 3055 (C-H Ar), 2984 (C-H aliphatic), 2119 (N_3), 1652 (C=O), 1543 (C=C), 1265 (C-O), 715 (C-H Ar bend).

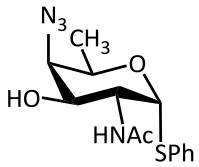
Phenyl 2-*N*-acetyl-2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-α-D-galactopyranoside (2.61).[37]



A solution of phenyl 2-N-acetyl-2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.60**) (48 mg, 0.14 mmol, 1 eq.) and activated 3Å powdered molecular sieves in dry DCM (2 mL) was stirred under argon for 10 minutes. To this 1M $SnCl_4$ (0.35 mL, 034 mmol, 2.5 eq.) was added and stirred at r.t. for 16 hours. The reaction mixture was filtered through celite, diluted with DCM and transferred to a separating funnel and washed with brine and DCM (3 x 10 mL). Combined organic fractions were dried over Na_2SO_4, concentrated and

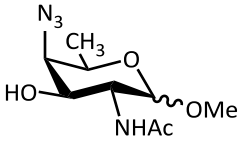
purified using flash chromatography (1:1 hexane/DCM) to yield 34 mg (71%) of **2.61** as a white solid. R_f = 0.62 (1:1.5 hexane/DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.40 (m, 2H, Ar-H), 7.34 – 7.27 (m, 3H, Ar-H), 6.13 (d, *J* = 4.1 Hz, 1H, H-1), 4.56 (dd, *J* = 12.1, 2.5 Hz, 1H, H-3), 4.51 (dd, *J* = 12.2, 4.2 Hz, 1H, H-2), 4.50 – 4.41 (m, 1H, H-5), 4.08 (ddd, *J* = 2.6, 1.8, 0.7 Hz, 1H, H-4), 2.54 (s, 3H, CH_3CO), 1.37 (d, *J* = 6.4 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.37 (C=O), 152.65 (C=O), 132.50 (C=O), 129.35 (2 x Ar), 129.35 (Ar), 128.26 (Ar), 86.96 (C-1), 75.68 (C-3), 67.62 (C-5), 61.81 (C-4), 55.86 (C-2), 23.98 (CH_3CO), 17.28 (C-6).

Phenyl 2-acetamido-4-azido-2,4,6-trideoxy-1-thio-α-D-galactopyranoside (2.62).[35,36]



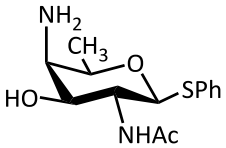
Phenyl 2-N-acetyl-2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-α-D-galactopyranoside (**2.61**) (0.34 g, 0.10 mmol, 1 eq.) was dissolved in THF/H_2O (2.7 mL) (3.5:1) and to this LiCl (18.6 mg, 0.44 mmol, 4.5 eq.) was added at r.t. This was stirred for 5 mins after which time LiOH (12 mg, 0.29 mmol, 3 eq.) was then added and vigorously stirred for a further 40 mins. To this Amberlite IR-120 (H^+ form) (8 mg) was added and stirred for 20 mins. The reaction mixture was then filtered through celite, THF removed by evaporation, diluted with EtOAc and H_2O and transferred to a separating funnel. The aqueous layer was washed with EtOAc (3 x 60 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (100% EtOAc) to yield 28 mg (82%) of **2.62** as a white solid. R_f = 0.22 (100% EtOAc); mp = 213-215 °C; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.98 (d, *J* = 6.9 Hz, 1H, NH), 7.39 – 7.29 (m, 4H, Ar-H), 7.28 – 7.23 (m, 1H, Ar-H), 5.60 (d, *J* = 5.2 Hz, 1H, H-1), 5.55 (d, *J* = 4.7 Hz, 1H, OH-3), 4.33 (qd, *J* = 6.4, 1.1 Hz, 1H, H-5), 4.17 (ddd, *J* = 10.6, 7.0, 5.3 Hz, 1H, H-2), 3.98 – 3.91 (m, 2H, H-3, H-4), 1.86 (s, 3H, CH_3CO), 1.14 (d, *J* = 6.4 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 169.90 (C=O), 134.39 (Ar), 131.02 (Ar), 129.03 (Ar), 126.92 (Ar), 87.42 (C-1), 67.61 (C-3), 66.13 (C-4), 65.70 (C-5), 50.50 (C-2), 22.55 (CH_3CO), 17.13 (C-6); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{14}H_{19}N_4O_3S$: 323.1178 found: 323.1176; $[M+Na]^+$ Calcd for $C_{14}H_{18}N_4NaO_3S$: 345.0997 found: 345.0999.

Methyl 2-acetamido-4-azido-2,4,6-trideoxy-α/β-D-galactopyranoside (2.63).[17,38]



Phenyl 2-acetamido-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.50**) (0.16 g, 0.51 mmol, 1 eq.) was dissolved in dry MeOH/DCM (20 mL) (7:13) under argon and to this NCS (0.13 g, 1.01 mmol, 2 eq.) was added at r.t. This was stirred for 20 mins, quenched by the addition of aq. NaOH, diluted with DCM and transferred to a separating funnel. The aqueous layer was washed with DCM (3 x 30 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using silica gel chromatography (9.5:0.5 DCM/MeOH) to yield 52.2 mg (α) and 15.6 mg (β) (55% overall) of **2.63** as a white solid. α-anomer: R_f = 0.31 (18.5:1.5 DCM/MeOH); mp 186-189 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 4.59 (dd, *J* = 3.7, 0.6 Hz, 1H, H-1), 4.18 (dd, *J* = 11.0, 3.7 Hz, 1H, H-2), 4.04 – 3.97 (m, 2H, H-3, H-5), 3.73 (dd, *J* = 3.6, 1.4 Hz, 1H, H-4), 3.32 (s, 3H, OCH_3), 1.98 (s, 3H, CH_3CO), 1.26 (d, *J* = 6.5 Hz, 3H, C-6); ^{13}C NMR (101 MHz, CD_3OD) δ 173.98 (C=O), 99.98 (C-1), 69.91 (C-3), 68.61 (C-4), 66.11 (C-5), 55.66 (OCH_3), 51.56 (C-2), 22.60 (CH_3CO), 17.65 (C-6). β-anomer: R_f = 0.20 (18.5:1.5 DCM/MeOH); mp 192-194 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 4.26 (d, *J* = 8.3 Hz, 1H, H-1), 3.89 (dd, *J* = 10.6, 3.7 Hz, 1H, H-3), 3.79 (dd, *J* = 10.6, 8.3 Hz, 1H, H-2), 3.72 (qd, *J* = 6.4, 1.4 Hz, 1H, H-5), 3.68 (dd, *J* = 5.0, 2.4 Hz, 1H, H-4), 3.41 (s, 3H, OCH_3), 1.97 (s, 3H, CH_3CO), 1.31 (d, *J* = 6.3 Hz, 3H, H-6); ^{13}C NMR (101 MHz,CD_3OD) δ 174.20 (C=O), 103.55 (C-1), 73.49 (C-3), 70.60 (C-5), 67.77 (C-4), 56.79 (OCH_3), 54.26 (C-2), 22.94 (CH_3CO), 17.67 (C-6). NMR of the β-anomer agrees with published values.[17] Mixture of both anomers: HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_9H_{17}N_4O_4$: 245.1250 found: 245.1251.

Phenyl 2-acetamido-4-amino-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.64).

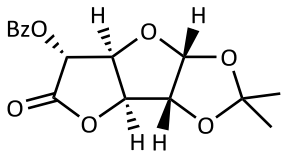


Phenyl 2-acetamido-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**2.50**) (52 mg, 0.16 mmol, 1 eq.) was dissolved in dry MeOH/DCM (10 mL) (1:1) and to this a suspension of 10 wt % Pd/C (34 mg, 0.03 mmol, 0.2 eq.) in dry DCM (2 mL) was added. The round bottom flask was evacuated and flushed three times with hydrogen and was stirred vigorously under a balloon of H_2 at r.t. for 3.5 hrs. The reaction mixture was filtered through celite, concentrated and the solid was washed with acetone to yield 38.3 mg (80%) of **2.64** as a white solid. R_f = 0.20 (15:5 DCM/MeOH); mp 210 – 213°C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 – 7.44 (m, 2H, Ar-H), 7.33 – 7.23 (m, 3H, Ar-H), 4.69 (d, *J* = 10.5 Hz, 1H, H-1), 3.86 (t, *J* = 10.3 Hz, 1H, H-2), 3.73 (qd, *J* = 6.4,

1.5 Hz, 1H, H-5), 3.65 (dd, *J* = 10.1, 4.0 Hz, 1H, H-3), 2.89 (dd, *J* = 4.1, 1.5 Hz, 1H, H-4), 2.00 (s, 3H, CH_3CO), 1.27 (d, *J* = 6.5 Hz, 3H, H-6); ^{13}C NMR (101 MHz, $CO(CD_3)_2$) δ 173.85 (C=O), 135.44 (Ar), 132.77 (Ar), 129.85 (Ar), 128.43 (Ar), 88.32 (C-1), 75.85 (C-5), 74.28 (C-3), 55.94 (C-4), 52.33 (C-2), 22.99 (CH_3CO), 17.54 (C-6); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{14}H_{21}N_2O_3S$: 297.1273 found: 297.1266.

5.3 Chapter 3 Experimental Procedures

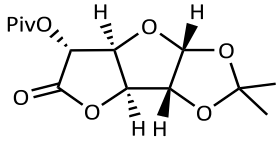
5-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone (3.1).[39–42]



Pyridine (3.6 mL) was added to a stirred solution of 1,2-*O*-isopropylidene-β-D-glucofuranurono-6,3-lactone (3.60 g, 16.68 mmol, 1 eq.) and 3Å powdered molecular sieves in DCM (40 mL) at r.t. under argon and was cooled to -40°C. To this trifluoromethanesulfonic anhydride (3.2 mL, 18.35 mmol, 1.1 eq.) was added drop wise. The reaction mixture was stirred for 1.5 hours at this temperature, diluted with DCM and washed with cold 5% HCl and DCM (3 x 40 mL). Combined organic fractions were dried over Na_2SO_4, filtered and concentrated to yield 5.70 g (98%) of the crude triflate as a brown powder which was used without further purification. R_f = 0.40 (3:2 hexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (d, *J* = 3.5 Hz, 1H, H-1), 5.40 (d, *J* = 4.4 Hz, 1H, H-5), 5.06 (dd, *J* = 4.3, 2.9 Hz, 1H, H-4), 4.92 (d, *J* = 2.9 Hz, 1H, H-3), 4.86 (d, *J* = 3.7 Hz, 1H, H-2), 1.54 (s, 3H, CH_3), 1.36 (s, 3H, CH_3). NMR values agree with published values.[42]

A portion of the crude triflate (1.04 g, 2.97 mmol, 1 eq.) in dry DMF (5 mL) and 3Å powdered molecular sieves was cooled to 0°C under argon and to this was added the NaOBz (0.60 g, 4.16 mmol, 1.4 eq.). This was allowed to slowly warm to r.t. over 6 hours. The reaction mixture was filtered through celite with EtOAc, diluted with H_2O (40 mL), washed with EtOAc (3 x 40 mL) and the combined organic fractions were dried over Na_2SO_4, filtered, concentrated and purified using silica gel chromatography (8:2 hexane/EtOAc) to yield 0.84 g (88%) of **3.1** as a white solid. R_f = 0.63 (6:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 7.98 (m, 2H, Ar-H), 7.66 – 7.57 (m, 1H, Ar-H), 7.51 – 7.42 (m, 2H, Ar-H), 6.01 (d, *J* = 3.7 Hz, 1H, H-1), 5.21 (d, *J* = 3.8 Hz, 1H, H-5), 5.16 (s, 1H, H-3), 4.93 (dt, *J* = 3.9, 0.7 Hz, 1H, H-4), 4.88 (d, *J* = 3.9 Hz, 1H, H-2), 1.50 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.76 (C=O), 165.43 (C=O), 134.28 (Ar), 130.36 (Ar), 128.84 (Ar), 128.25 (Ar), 113.45 (C), 106.49 (C-1), 85.58 (C-5), 82.55 (C-2), 81.89 (C-4), 73.59 (C-3), 34.09 (CH_3), 33.75 (CH_3). NMR values agree with referenced values.[41]

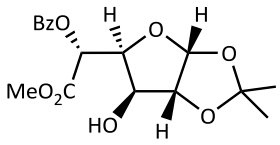
5-*O*-pivaloyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone.[40]



A portion of the crude triflate (0.15 g, 0.43 mmol, 1 eq.) in dry DMF (1.5 mL) and 3Å powdered molecular sieves was cooled to 0°C under argon and to this was added the NaOPiv (58.7 mg, 0.47 mmol, 1.1 eq.). This was allowed to slowly warm to r.t. over 7 hours. The reaction mixture was filtered through celite with EtOAc, diluted with H_2O (20 mL), washed with EtOAc (3 x 20

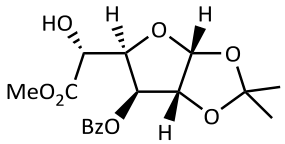
mL) and the combined organic fractions were dried over Na_2SO_4, filtered, concentrated and purified using silica gel chromatography (8:2 hexane/EtOAc) to yield 0.13 g (96%) of the pivaloate as a white solid. R_f = 0.63 (5:1 hexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.97 (d, *J* = 3.8 Hz, 1H), 5.12 (d, *J* = 3.8 Hz, 1H), 4.86 (s, 1H), 4.84 (d, *J* = 3.9 Hz, 1H), 4.75 (d, *J* = 3.9 Hz, 1H), 1.51 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.24 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.60, 171.05, 113.31, 106.33, 85.52, 82.38, 81.99, 73.38, 38.82, 27.34, 27.07, 26.74.

Methyl 5-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (3.8A).[40,41]



The 5-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone (**3.1**) (0.95 g, 0.295 mmol, 1 eq.) in dry MeOH (8 mL) and 3Å powdered molecular sieves was cooled to 0°C and to this was added drop wise Et_3N (3.3 mL, 23.62 mmol, 8 eq.) under argon. The reaction mixture was stirred for 1.5 hours at this temperature, diluted with DCM and washed with 5% HCl and DCM (3 x 30 mL), combined organic fractions were dried over Na_2SO_4, filtered and concentrated and purified using flash chromatography (x 4) (3:1 hexane/EtOAc to 2:1 hexane/EtOAc) to yield a mixture of starting material (0.16 g), unmigrated ester (**3.8A**) (0.13 g, 15%) and the desired product (**3.8B**) (0.31 g, 36%). R_f = 0.45 (1:1 hexane/EtOAc); ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.15 – 8.06 (m, 2H, Ar-H), 7.63 – 7.54 (m, 1H, Ar-H), 7.50 – 7.42 (m, 2H, Ar-H), 6.02 (d, *J* = 3.6 Hz, 1H, H-1), 5.59 (d, *J* = 8.5 Hz, 1H, H-5), 4.60 (dd, *J* = 3.6, 0.46 Hz, 1H, H-2), 4.56 (dd, *J* = 8.6, 2.7 Hz, 1H, H-4), 4.36 (d, *J* = 2.65 Hz, 1H, H-3), 3.81 (s, 3H, CH_3COO), 1.54 (s, 3H, CH_3), 1.34 (s, 3H, CH_3).

Methyl 3-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (3.8B).[40,41]

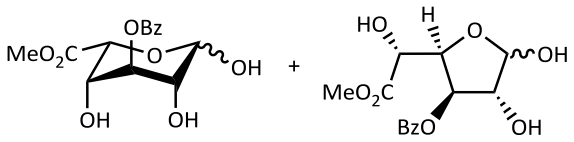


R_f = 0.49 (1:1 hexane/EtOAc); ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.04 – 7.99 (m, 2H, Ar-H), 7.58 – 7.52 (m, 1H, Ar-H), 7.46 – 7.39 (m, 2H, Ar-H), 6.03 (d, *J* = 3.8 Hz, 1H, H-1), 5.57 (ddd, *J* = 3.9, 1.1, 0.5 Hz, 1H, H-3), 4.71 (dd, *J* = 3.8, 1.1 Hz, 1H, H-2), 4.65 (dd, *J* = 4.7, 3.9 Hz, 1H, H-4), 4.51 (d, *J* = 4.7 Hz, 1H, H-5), 3.73 (s, 3H, CH_3COO), 1.52 (d, *J* = 1.2 Hz, 3H, CH_3), 1.33 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.08 (C=O), 165.59 (C=O), 133.52 (Ar), 129.85 (Ar), 129.45 (Ar), 128.57 (Ar), 112.97 (C), 104.97 (C-1), 84.17 (C-2), 79.77 (C-4), 77.24 (C-3), 69.63 (C-5), 53.00 (CH_3COO), 27.09 (CH_3), 26.64 (CH_3). Yields of **3.8A** and **3.8B** calculated by taking the unreacted SM into account.

NMR Experiment with 5-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone (3.1):

The 5-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone (**3.1**) (44.2 mg, 0.16 mmol, 1 eq.) was dissolved in dry CD_3OD (0.06 mL, 1.55 mmol, 10 eq.) and dry $CDCl_3$ (0.6 mL) (dried with freshly activated with 3Å molecular sieves (beads) under argon and added to a Wilmad pressure/vacuum valved NMR tube which had been evacuated and flushed with argon. Concentration of starting material = 74 mg/mL in $CDCl_3$. To this the Et_3N (0.11 mL, 0.78 mmol, 5 eq.)(dried with freshly activated with 3Å molecular sieves (beads)) was added at r.t. and the first spectra recorded at 30°C straight after the addition of Et_3N. Spectra were then recorded every 20 minutes for 2 hours, at 4.5 hrs and 24 hrs after Et_3N addition with shaking beforehand. The reaction mixture was left in the NMR at r.t. in between data collection. δ are in ppm and referenced with respect to TMS.

Methyl 5-*O*-benzoyl-α/β-L-idopyranuronate (3.2) and Methyl 5-*O*-benzoyl-α/β-L-idofuranuronate (3.9).[43]



First attempt:[43]

The methyl 3-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (**3.8B**) (68 mg, 0.19 mmol, 1 eq.) was dissolved in 90% TFA (0.4 mL) at 0°C and vigorously stirred in a sealed round bottom flask for 15 mins, which after time TLC analysis revealed a single more polar product. The TFA was then removed under a stream of air and H_2O removed azeotropically with toluene and purified using flash chromatography (1:1 hexane/EtOAc to 7:3 hexane/EtOAc) to yield 46 mg (78%) of a mixture of compounds as per NMR. R_f = 0.32 (100% EtOAc); ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 – 8.01 (m, 10H, Ar-H), 7.66 – 7.56 (m, 5H, Ar-H), 7.53 – 7.44 (m, 10H, Ar-H), 5.55 (t, *J* = 6.9 Hz, 1H, H-3 β-fur), 5.42 – 5.38 (m, 1H, H-3 α-fur, H-3 β-pyr), 5.37 (d, *J* = 4.8 Hz, 1H, H-1 β-fur), 5.31 (t, *J* = 5.0 Hz, 1H, H-3 α-pyr), 5.29 (d, *J* = 3.4 Hz, 1H, H-1 α-pyr), 5.21 (d, *J* = 2.1 Hz, 1H, H-1 α-fur), 5.09 (d, *J* = 1.5 Hz, 1H, H-1 β-pyr), 4.89 (d, *J* = 3.3 Hz, 1H, H-5 α-pyr), 4.78 – 4.76 (m, 1H, H-4 β-fur), 4.75 – 4.72 (m, 1H, H-4 α-fur), 4.64 (d, *J* = 2.1 Hz, 1H, H-5 β-pyr), 4.54 – 4.46 (m, 1H, H-2 β-fur), 4.34 (d, *J* = 4.5 Hz, 1H, H-5, β-fur), 4.28 (dd, *J* = 3.8, 2.1 Hz, 1H, H-2 α-fur), 4.15 (d, *J* = 2.8 Hz, 1H, H-5 α-fur), 4.12 – 4.08 (m, 1H, H-4 α-pyr), 4.04 (ddd, *J* = 3.5, 2.1, 1.2 Hz, 1H, H-4 β-pyr), 3.78 (s, 3H, CH_3COO, β-pyr), 3.77 (s, 3H, CH_3COO, α-pyr), 3.76 – 3.73 (m, 1H, H-2 β-pyr), 3.69 (s, 3H, CH_3COO, β-fur), 3.68 – 3.67 (m, 1H, H-2 α-pyr), 3.64 (s, 3H, CH_3COO, α-fur); ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.94 (C=O), 173.62 (C=O), 172.03 (C=O), 171.20 (C=O), 134.73 (Ar), 134.57 (Ar), 134.37 (Ar), 134.14 (Ar), 134.10 (Ar), 131.28 (Ar), 130.80 (4 x Ar), 130.73 (Ar), 129.74 (Ar), 129.61 (2 x Ar), 129.51 (Ar), 129.42 (Ar), 103.77 (C-1 α-fur), 96.60 (C-1 β-

fur), 96.41 (C-1 α-pyr), 94.73 (C-1 β-pyr), 81.64 (C-4 α-fur), 81.23 (C-2 α-fur), 79.90 (C-3 α-fur), 79.47 (C-3 β-fur), 78.18 (C-4 β-fur), 75.55 (C-5 β-pyr), 75.47 (C-2 β-fur), 73.32 (C-3 α-pyr), 73.00 (C-3 β-pyr), 71.52 (C-5 β-fur), 71.21 (C-5 α-pyr), 71.03 (C-5 α-fur), 70.14 (C-2 α-pyr), 69.43 (C-2 β-pyr), 69.18 (C-4 α-pyr), 68.47 (C-4 β-pyr), 52.69 (CH_3COO), 52.61 (2 x CH_3COO), 52.55 (CH_3COO); HRMS (ESI-TOF): $[M+NH_4]^+$ Calcd for $C_{14}H_{20}NO_8$: 330.1189 found: 330.1185, $[M+Na]^+$ Calcd for $C_{14}H_{16}NaO_8$: 335.0743 found: 335.0742.

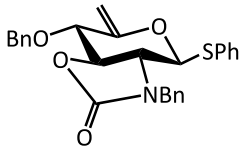
Second attempt:[40,44]

The methyl 3-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (**3.8B**) (89 mg, 0.25 mmol, 1 eq.) was dissolved in 90% TFA (0.5 mL) at r.t. and vigorously stirred in a sealed round bottom flask for 3 hrs, which after TLC analysis revealed a single more polar product. The TFA was then removed under a stream of air and H_2O removed azeotropically with toluene and purified using flash chromatography (1:1 hexane/EtOAc to 7:3 hexane/EtOAc) to yield 36 mg (45%) of a mixture of compounds as per NMR and as characterised above.

NMR experiment with methyl 3-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (3.8B):

The methyl 3-*O*-benzoyl-1,2-O-isopropylidene-β-L-idofuranuronate (**3.8B**) (60 mg) was dissolved in 90% TFA (1 mL) at r.t. in a round bottom flask, immediately transferred to an NMR tube, sealed and the spectrum recorded. The spectra were then recorded at 10 mins, 30 mins, 1 hr, 1 hr 30 mins and 2 hrs after the initial run. δ are in ppm and referenced with respect to acetone (2.22 ppm) which formed from the cleavage of the 1,2-isopropylidene group.

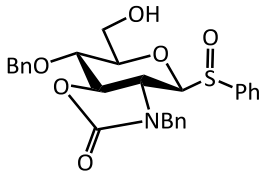
Phenyl 2-*N*-benzyl-4-*O*-benzyl-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-β-D-xylo-hex-5-enopyranoside (3.14).[45]



A solution of phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-β-D-glucopyranoside (**2.55**) (0.23 g, 0.57 mmol, 1 eq.) in dry DMF (5 mL) was stirred under argon and cooled to 0°C. To this benzyl bromide (75 µL, 0.63 mmol, 1.1 eq.) was added, followed by the slow addition of NaH (60% dispersion in mineral oil) (68 mg, 1.71 mmol, 3 eq.) The reaction mixture was stirred at 0°C for 30 mins and then at r.t. for 1.5 hrs. The reaction mixture was cooled down to 0°C, quenched with MeOH, concentrated and washed with brine (40 mL) and EtOAc (4 x 15 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (100% hexane to 8:2 hexane/EtOAc) to yield 0.24 g (90%) of **3.14** as a white

solid. R_f = 0.60 (6:4 hexane/EtOAc); mp 119-121°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.26 (m, 15H, Ar-H), 5.15 (d, *J* = 9.7 Hz, 1H, H-1), 4.99 (t, *J* = 1.5 Hz 1H, H-6), 4.90 (t, *J* = 1.0 Hz, 1H, H-6'), 4.78 (AB q, *J* = 15.24, 11.92 Hz, 2H, CH_2), 4.77 (AB d, *J* = 15.6 Hz, 1H, CH_2OPh), 4.62 (AB d, *J* = 15.6 Hz, 1H, CH_2OPh), 4.32 (dd, *J* = 11.4, 8.9 Hz, 1H, H-3), 4.26 (dt, *J* = 8.9, 0.9 Hz, 1H, H-4), 3.39 (dd, *J* = 11.4, 9.7 Hz, 1H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.18 (C=O), 137.28 (Ar), 136.34 (Ar), 132.50 (Ar), 132.17(Ar), 129.28 (Ar), 128.85 (Ar), 128.67(Ar), 128.59 (Ar), 128.30 (Ar), 128.11 (Ar), 127.99 (Ar), 127.88 (Ar), 103.71 (C-6), 88.50 (C-1), 79.84 (C-3), 76.08 (C-4), 71.60 (CH_2OPh), 59.79 (C-2), 47.99 (CH_2OPh); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{27}H_{26}NO_4S$: 460.1583 found: 460.1583; $[M+NH_4]^+$ Calcd for $C_{27}H_{29}N_2O_4S$: 477.1848 found: 477.1855. Crystal structure data is in **Appendix 3.**

Phenyl 2-*N*-benzyl-4-*O*-benzyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-sulfinyl-β-D-glucopyranoside (3.16).[46,47]



Using 9-BBN:[48]

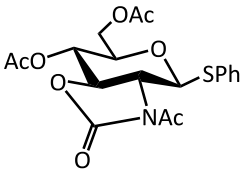
A solution of phenyl *N*-benzyl-4-*O*-benzyl-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-β-D-xylo-hex-5-enopyranoside (**3.14**) (64 mg, 0.14 mmol, 1 eq.) in dry THF (2 mL) and freshly activated with 3Å powdered molecular sieves was stirred under argon and cooled to -10°C. To this 0.5M 9-BBN in THF (0.7 mL, 0.36 mmol, 2.6 eq.) was added and stirred at this temperature for 10 minutes and r.t. for 2 hrs. Another 7.4 eq. of 9-BBN was then added and stirred for a further 4 hrs.

Using BH_3.THF:[49]

A solution of phenyl 2-*N*-benzyl-4-*O*-benzyl-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-β-D-xylo-hex-5-enopyranoside (**3.14**) (90 mg, 0.24 mmol, 1 eq.) in dry THF (3.5 mL) and freshly activated with 3Å powdered molecular sieves was stirred under argon and cooled to 0°C. To this 1M BH_3.THF (2.44 mL, 2.44 mmol, 10 eq.) was added and stirred at this temperature for 1.5 hrs. The reaction mixture was then filtered through celite with cold THF (30 mL), cooled to 0°C and to this was added 30% aq. H_2O_2 (3.48 mL) followed by 2N NaOH (3.48 mL). This was left to slowly warm to r.t. over 1 hr. The reaction mixture was then diluted with EtOAc, transferred to a separating funnel and washed with EtOAc (4 x 35 mL) and NH_4Cl (30 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (1:1 hexane/EtOAc) to yield 62 mg (52%) of **3.16** as a white solid. R_f = 0.42 (100% EtOAc); mp 145-147°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.26 (m, 13H, Ar-H), 7.08 –

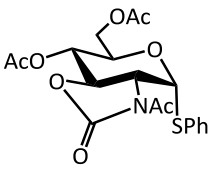
6.93 (m, 2H, Ar-H), 5.14 (AM d, *J* = 16.1 Hz, 1H, CH_2OPh), 4.86 (ddd, *J* = 9.9, 4.5, 1.3 Hz, 1H, H-5), 4.85 (AB d, *J* = 11.66 Hz, 1H, CH_2OPh), 4.55 (AB d, *J* = 11.8 Hz, 1H, CH_2OPh), 4.44 (m, 1H, H-3), 4.43 (d, *J* = 6.5 Hz, 1H, H-1), 4.37 (AM d, *J* = 16.0 Hz, 1H, CH_2OPh), 4.25 – 4.20 (m, 1H, H-2), 4.13 (m, 1H, H-4), 3.77 (ddd, *J* = 12.6, 5.9, 4.4 Hz, 1H, H-6), 3.76 – 3.65 (m, 1H, H-6'), 2.08 (dd, *J* = 9.0, 4.4 Hz, 1H, 6-OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.11 (C=O), 131.41 (Ar), 131.26 (Ar), 129.48 (Ar), 129.04 (Ar), 128.83 (Ar), 128.78 (Ar), 128.43 (Ar), 128.28 (2 x Ar), 127.92 (Ar), 124.32 (Ar), 90.92 (C-2), 80.39 (C-1), 79.27 (C-5), 76.67 (C-4), 72.07 (CH_2OPh), 61.55 (CH_2OPh), 55.20 (C-3), 48.90 (CH_2OPh); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{27}H_{28}NO_6S$: 494.1637 found: 494.1625, $[M+Na]^+$ Calcd for $C_{27}H_{27}NNaO_6S$: 516.1457 found: 516.1464.

Phenyl 2-*N*-acetyl-4,6-di-*O*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.18).[34]



A solution of phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-β-D-glucopyranoside (**2.47**) (3.79 g, 12.75 mmol, 1 eq.) was dissolved in dry DCM under argon and cooled to 0°C. To this the DIPEA (13.3 mL, 76.50 mmol, 6 eq.), followed by freshly distilled AcCl (5.3 mL, 76.5 mmol, 6 eq.) was added and stirred for 45 mins at this temperature. The reaction mixture was diluted with DCM, transferred to a separating funnel and washed with 5% HCl solution (100 mL) and DCM (3 x 60 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (1:1 hexane/DCM) to yield 5.27 g (96%) of **3.18** as a yellow oil. R_f = 0.67 (3:1 DCM/EtOAc); ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.46 (m, 2H, Ar-H), 7.31 – 7.25 (m, 3H, Ar-H), 5.27 (dd, *J* = 9.8, 8.8 Hz, 1H, H-4), 4.90 (d, *J* = 8.3 Hz, 1H, H-1), 4.23 (dd, *J* = 11.4, 9.8 Hz, 1H, H-3), 4.19 (dd, *J* = 6.1, 1.0 Hz, 2H, H-6's), 4.16 (dd, *J* = 11.7, 8.6 Hz, 1H, H-2), 3.72 (ddd, *J* = 8.7, 5.2, 3.3 Hz, 1H, H-5), 2.54 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO).

Phenyl 2-*N*-acetyl-4,6-di-*O*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.19).



First attempt:[34]

A solution of phenyl *N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-*β*-D-glucopyranoside (**3.18**) (47 mg, 0.11 mmol, 1 eq.) and activated 4Å molecular sieves in mL of

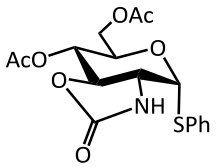
dry DCM (5 mL) was stirred under argon for 10 minutes. To this silver trifluoromethanesulfonate (AgOTf) (5.7 mg, 0.2 mmol, 0.2 eq.) was added and stirred at r.t. for 20 hours. The reaction mixture was filtered through celite, diluted with DCM and transferred to a separating funnel and washed with brine and DCM (3 x 20 mL). Combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (7.5:2.5 hexane/EtOAc to 6:4 hexane/EtOAc) to yield 36.2 mg (77%, 9:1 α/β from NMR) of a mixture of **3.18** and **3.19** as a yellow oil. R_f = 0.67 (3:1 DCM/EtOAc). Crude NMR revealed un-anomerized product still present.

Second attempt:[37]

A solution of phenyl *N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-β-D-glucopyranoside (**3.18**) (3.29 g, 7.73 mmol, 1 eq.) and activated 3Å powdered molecular sieves in dry DCM (20 mL) was stirred under argon for 10 minutes. To this $SnCl_4$ (2.3 mL, 19.32 mmol, 2.5 eq.) was added and stirred at r.t. for 25 hours. The reaction mixture was filtered through celite, diluted with DCM and transferred to a separating funnel and washed with brine and DCM (3 x 20 mL). Combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (7.5:2.5 hexane/EtOAc to 6:4 hexane/EtOAc) to yield 2.94 g (89%) of **3.19** as a yellow oil. R_f = 0.67 (3:1 DCM/EtOAc); ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.49 – 7.43 (m, 2H, Ar), 7.35 – 7.28 (m, 3H, Ar), 6.19 (d, *J* = 4.6 Hz, 1H, H-1), 5.30 (dd, *J* = 10.1, 9.3 Hz, 1H, H-4), 4.47 (dd, *J* = 12.1, 10.1 Hz, 1H, H-3), 4.38 (dddd, *J* = 9.2, 5.1, 2.2, 0.7 Hz, 1H, H-5), 4.31 (dd, *J* = 12.3, 5.1 Hz, 1H, H-6), 4.18 (ddd, *J* = 12.3, 6.4, 3.4 Hz, 2H, H-2, H-6'), 2.53 (s, 3H, CH_3CO), 2.14 (s, 3H, CH_3 CO), 2.06 (s, 3H, CH_3 CO); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.12 (C=O), 170.55 (C=O), 169.22 (C=O), 152.55 (C=O), 132.82 (C-Ar), 131.99 (C), 129.40 (C-Ar), 128.59 (C), 86.29 (C-1), 76.00 (C-3), 70.52 (C-5), 68.18 (C-4), 61.81 (C-6), 60.03 (C-2), 23.83 (CH_3CO), 20.72 (CH_3CO), 20.72 (CH_3CO). NMR values agree with the published values.[50] HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{19}H_{22}NO_8S$: 424.1066 found 424.1056, $[M+NH_4]^+$ Calcd for $C_{19}H_{25}N_2O_8S$: 441.1332 found: 441.1317, $[M+Na]^+$ Calcd for $C_{19}H_{21}NNaO_8S$: 446.0886 found: 446085.

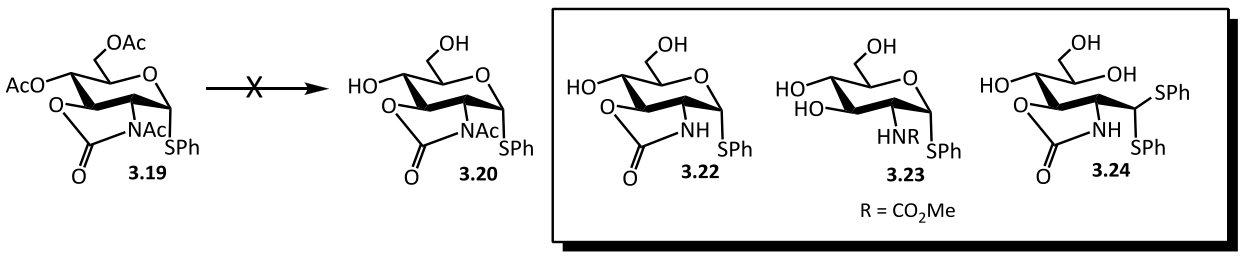
Phenyl 4,6-di-*O*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.21).

Table 3.1, Entry 1:



Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (55 mg, 0.13 mmol, 1 eq.) in MeOH (2 mL) was cooled to 0°C and to this 2 drops of 4.62 M NaOMe was added. This was then taken up to r.t. and left to stir for 30 mins after which time Amberlite IR-120 (H^+ form) was added and stirred for 15 mins. The reaction mixture was filtered and purified using flash chromatography (3:1 DCM/EtOAc) to yield 27 mg (55%) of **3.21** as a white solid. R_f = 0.67 (13:7 DCM/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.47 (m, 2H, Ar-H), 7.37 – 7.30 (m, 3H, Ar-H), 5.68 (d, *J* = 4.89 Hz, 1H, H-1), 5.34 (dd, *J* = 10.1, 9.3 Hz, 1H, H-4), 5.29 (bs, 1H, NH), 4.57 (dd, *J* = 11.8, 10.1 Hz, 1H, H-3), 4.39 (dddd, *J* = 9.3, 5.4, 2.2, 0.6 Hz, 1H, H-5), 4.32 (dd, *J* = 12.24, 5.37 Hz, 1H, H-6), 4.17 (dd, *J* = 12.3, 2.1 Hz, 1H, H-6'), 4.07 (ddd, *J* = 11.7, 4.8, 1.3 Hz, 1H, H-2), 2.14 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.60 (C=O), 169.37 (C=O), 157.97 (Ar), 132.25 (Ar), 129.48 (Ar), 128.45 (Ar), 85.28 (C-1), 78.26 (C-3), 70.59 (C-5), 68.38 (C-4), 62.00 (C-6), 58.68 C-2), 20.80 (d, 2 X CH_3CO).

Table 3.2, Entry 2:



Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (68 mg, 0.16 mmol, 1 eq.) in MeOH (2 mL) was cooled to 0°C and to this 4.62 M NaOMe (14 µL, 0.07 mmol, 0,4 eq.) was added. This was then taken up to r.t. and left to stir for 30 mins after which time Amberlite IR-120 (H^+ form) was added and stirred for 10 mins. The reaction mixture was filtered and purified using flash chromatography (19:1 DCM/MeOH) to yield 36 mg of a mixture of compounds (**3.22**-**3.24**) as seen by ^{1}H NMR. R_f = 0.12 (19:1 DCM/MeOH)

HRMS (ESI-TOF):

3.22: $[M+H]^+$ Calcd for $C_{13}H_{16}NO_5S$: 298.0749 found: 298.0750

3.23: $[M+H]^+$ Calcd for $C_{14}H_{20}NO_6S$: 330.1011 found: 330.1013, $[M+Na]^+$ Calcd for $C_{14}H_{19}NNaO_6S$: 352.0831 found: 352.0833

3.24: $[M+H]^+$ Calcd for $C_{19}H_{21}NO_5S_2$: 407.0861 found: 408.0943

Table 3.1, Entry 3:[51]

Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (0.10 g, 0.24 mmol, 1 eq.) was dissolved in MeOH/H_2O (7.5 mL) (2:1) and to this K_2CO_3 (0.23 g, 1.65 mmol, 7 eq.) was added and stirred at r.t. for 10 mins after which time Amberlite IR-120 (H^+ form) was added and stirred for a further 10 mins. The reaction mixture was filtered and purified using flash chromatography (19:1 DCM/MeOH) to yield 36 mg of a mixture of compounds as seen by ^1H NMR. R_f = 0.12 (19:1 DCM/MeOH). Characterization as above.

Table 3.1, Entry 4:[52]

Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (53 mg, 0.13 mmol, 1 eq.) in THF (2 mL) was cooled to 0°C and to this was added LiOH.H_2O (16 mg, 0.38 mmol, 3 eq.) and 30% aq. H_2O_2 (0.19 mL) and stirred at this temperature for 2 hrs after which time TLC indicated that the starting material had degraded.

Table 3.1, Entry 5:[36]

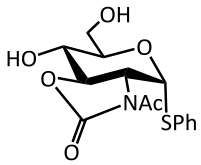
Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (59 mg, 0.14 mmol, 1 eq.) in THF/H_2O (5.6 mL) (3:1) was cooled to -40°C and to this was added 2 drops of 30% aq. H_2O_2 followed by LiOH.H_2O (12 mg, 0.0.28 mmol, 2 eq.) and stirred at this temperature for 5 mins, brought to r.t. and immediately quenched with sat. aq. Na_2SO_3 (2 mL). The reaction mixture was diluted with EtOAc (10 mL) and brine (10 mL) and the aq. layer washed with EtOAc (3 x 20 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and TLC checked to reveal a mixture of compounds which was not worth purifying since no dominant product could be seen.

Table 3.1, Entry 6:

Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (17 mg, 0.04 mmol, 1 eq.) in THF (2 mL) was cooled to 0°C and to this was added 2N NaOH (60 µL) and stirred at this temperature for 2 hrs after which time TLC indicated that the starting material had degraded.

Phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.20).[53]

Table 3.1, Entry 7:

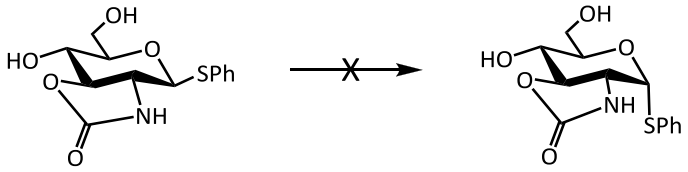


Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (0.95 g, 2.22 mmol, 1 eq.) was dissolved in acetone/H_2O (39 mL) (2:1) and to this 10.2M HCl (5.6 mL) was added and stirred at 65°C for 4.5 hrs and then at r.t. for 18 hrs. The reaction mixture was neutralized with sat. aq. $NaHCO_3$ (50 mL), acetone removed, diluted with EtOAc, transferred to a separating funnel and the aqueous layer washed with EtOAc (3 x 40 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (7:3 hexane/EtOAc) to yield 0.22 g (29%) of **3.20** as a white solid. R_f = 0.29 (7:3 hexane/EtOAc); mp 191-193°C; ^{1}H NMR (400 MHz, $CO(CD_3)_2$) δ 7.56 – 7.52 (m, 2H, Ar), 7.39 – 7.32 (m, 3H, Ar), 6.11 (dd, *J* = 4.4, 0.6 Hz, 1H, H-1), 5.12 (d, *J* = 5.5 Hz, 1H, OH-4), 4.37 (dd, *J* = 12.1, 9.6 Hz, 1H, H-3), 4.23 (dd, *J* = 12.1, 4.4 Hz, 1H, H-2), 4.10 (ddd, *J* = 9.6, 8.8, 5.4 Hz, 1H, H-4), 4.03 – 3.94 (m, 1H, H-5), 3.87 (dd, *J* = 3.4, 2.1 Hz, 1H, H-6), 3.85 (d, *J* = 3.4 Hz, 1H, OH-6), 3.78 (dd, *J* = 7.2, 5.3 Hz, 1H, H-6'), 2.44 (s, 3H, CH_3CO); ^{13}C NMR (101 MHz, Acetone-d_6) δ 171.83 (C=O), 154.21 (C=O), 134.57 (Ar), 133.23 (Ar), 130.06 (Ar), 128.79 (Ar), 87.55 (C-1), 79.76 (C-3), 77.05 (C-5), 68.93 (C-4), 61.33 (C-6), 60.57 (C-2), 23.79 (CH_3CO); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{15}H_{18}NO_6S$: 340.0855 found: 340.0848, $[M+Na]^+$ Calcd for $C_{15}H_{17}NNaO_6S$: 362.0674 found: 362.0669.

Table 3.1, Entry 8:

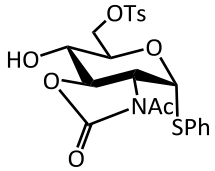
Phenyl 2-*N*-acetyl-2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.19**) (0.47 g, 2.22 mmol, 1 eq.) was dissolved in acetone/H_2O (19.5 mL) (2:1) and to this 10.2M HCl (1.6 mL) was added and stirred at 65°C for 4 hrs. The reaction mixture was neutralized with sat. aq. $NaHCO_3$ (50 mL), acetone removed, diluted with EtOAc, transferred to a separating funnel and the aqueous layer washed with EtOAc (3 x 40 mL). The combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (7:3 hexane/EtOAc) to yield 0.22 g (29%) of **3.20** as a white solid. R_f = 0.29 (7:3 hexane/EtOAc). Characterized as above.

Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.32).[37]



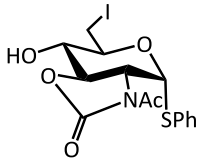
A solution of phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-β-D-glucopyranoside (**2.47**) (44.9 mg, 0.15 mmol, 1 eq.) was dissolved in dry DCM/THF (5 mL) (4:1) and activated 3Å molecular sieves under argon. To this 1M $SnCl_4$ (0.04 mL, 0.38 mmol, 2.5 eq.) was added and stirred at r.t. for 20 hours after which time TLC indicated multiple products had formed.

Phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-6-*O*-tosyl-1-thio-α-D-glucopyranoside (3.25).



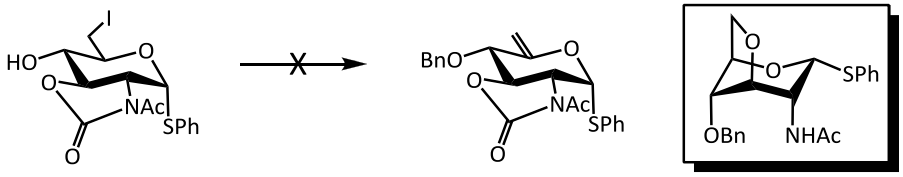
A solution of phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (**3.20**) (0.35 g, 1.02 mmol, 1 eq.) and activated 3Å molecular sieves in dry pyridine (10 mL) was stirred under argon and cooled to 0°C. To this freshly recrystallized *p*-toluenesulfonyl chloride (0.23 g, 1.22 mmol, 1.2 eq.) was added and stirred for 3 hours at this temperature and then at r.t. for 1.5 hours. The reaction mixture was filtered through celite, transferred to a separating funnel and washed with 5% HCl solution (40 mL). The aqueous layer was washed with DCM (4 x 30 mL) and combined organic fractions were dried over Na_2SO_4, concentrated and purified using flash chromatography (9.5:0.5 Hexane/EtOAc to 7:3 hexane/EtOAc) to yield 0.39 g (77%) of **3.25** as an off white solid. R_f = 0.51 (1:1 hexane:EtOAc), mp 165-167°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.73 (m, 2H, Ar-H), 7.43 – 7.36 (m, 2H, Ar-H), 7.33 – 7.27 (m, 5H, Ar), 6.02 (d, *J* = 4.5 Hz, 1H, H-1), 4.51 (dd, *J* = 11.5, 3.4 Hz, 1H, H-6), 4.34 (dd, *J* = 12.1, 9.2 Hz, 1H, H-3), 4.26 (dd, *J* = 11.5, 1.8 Hz, 1H, H-6'), 4.18 – 4.06 (m, 2H, H-5, H-4), 3.97 (dd, *J* = 12.1, 4.5 Hz, 1H, H-2), 3.19 (s, 1H, OH-4), 2.52 (s, 3H, CH_3), 2.43 (s, 3H, CH_3CO); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.28 (C=O), 153.10 (C=O), 145.38 (Ar), 132.78 (Ar), 132.63 (Ar), 132.33 (Ar), 130.03 (Ar), 129.36 (Ar), 128.40 (Ar), 128.14 (Ar), 86.37 (C-1), 78.15 (C-3), 72.54 (C-5), 68.12 (C-4), 67.78 (C-6), 59.72 (C-2), 23.88 (CH_3CO), 21.78 (CH_3); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{22}H_{24}NO_8S_2$: 494.0943 found: 494.0937, $[M+NH_4]^+$ Calcd for $C_{22}H_{27}N_2O_8S_2$: 511.1209 found: 511.1210.

Phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (3.26).



Phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-6-*O*-tosyl-1-thio-α-D-glucopyranoside (**3.25**) (0.100 g, 0.21 mmol, 1 eq.) and NaI (70 mg, 0.46 mmol, 2.2 eq.) were dried using a Schlenk line. To this dry butanone (3 mL) was added under argon and refluxed for 17 hrs. The reaction mixture was then concentrated and purified using flash chromatography (8:2 hexane/DCM to 100% DCM) to yield 63 mg (67%) of **3.26** as a white solid. R_f = 0.56 (18.5:1.5 DCM:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.49 (m, 2H, Ar-H), 7.36 – 7.30 (m, 3H, Ar-H), 6.18 (dd, *J* = 4.5, 0.6 Hz, 1H, H-1), 4.43 (dd, *J* = 12.1, 9.6 Hz, 1H, H-3), 4.08 (dd, *J* = 12.1, 4.5 Hz, 1H, H-2), 3.97 (dd, *J* = 9.6, 8.5 Hz, 1H, H-4), 3.86 – 3.77 (m, 1H, H-5), 3.58 (dd, *J* = 11.0, 3.0 Hz, 1H, H-6), 3.54 (dd, *J* = 11.0, 4.9 Hz, 1H, H-6'), 2.55 (s, 3H, CH_3CO); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.31 (C=O), 153.16 (C=O), 132.97 (Ar), 132.31 (Ar), 129.40(Ar), 128.50 (Ar), 86.61 (C-1), 78.10 (C-3), 72.75 (C-5), 72.71 (C-4), 59.98 (C-2), 23.93 (CH_3CO), 5.87 (C-6); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{15}H_{17}INO_5S$: 449.9872 found: 449.9860, $[M+Na]^+$ Calcd for $C_{15}H_{16}INNaO_5S$: 471.9692 found: 471.9686.

Phenyl 2-acetamido-3,6-anhydro-4-*O*-benzyl-2.6-dideoxy-1-thio-α-D-glucopyranoside (3.28).[45]



First attempt:[45]

A solution of phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (**3.26**) (43 mg, 0.10 mmol, 1 eq.) in dry DMF (2 mL) and 3Å powdered molecular sieves was stirred under argon and cooled to 0°C. To this benzyl bromide (14 µL, 0.12 mmol, 1.2 eq.) was added, followed by the slow addition of NaH (60% dispersion in mineral oil) (11.6 mg, 0.30 mmol, 3 eq.) The reaction mixture was stirred at 0°C for 10 mins and then at r.t. for 6 hrs. The reaction mixture was cooled down to 0°C, quenched with MeOH, concentrated, and washed with brine (15 mL) and EtOAc (4 x 15 mL). The combined organic fractions were dried over Na_2SO_4, concentrated, remaining DMF azeotropically removed with toluene and purified using flash chromatography (100% hexane to 4:6 hexane/EtOAc) to yield 24.5 mg

(65%) of **3.28** as an opaque oil, which upon recrystallization (DCM/hexane) gave a white solid. R_f = 0.22 (9:11 hexane/EtOAc); mp 134-136°C; ^{1}H NMR (400 MHz, $CO(CD_3)_2$) δ 7.57 – 7.48 (m, 4H, Ar-H), 7.43 (tt, *J* = 6.6, 0.9 Hz, 2H, Ar-H), 7.39 – 7.28 (m, 3H, Ar-H), 7.27 – 7.21 (m, 1H, Ar-H), 7.06 (bd, *J* = 10.5 Hz, 1H, NH), 5.72 (d, *J* = 4.1 Hz, 1H, H-1), 4.89 (AB d, *J* = 11.3 Hz, 1H, CH_2OPh), 4.69 (AB d, *J* = 11.4 Hz, 1H, CH_2OPh), 4.65 (t, *J* = 2.9 Hz, 1H, H-5), 4.47 (dtt, *J* = 10.4, 4.2, 0.9 Hz, 1H, H-2), 4.34 (d, *J* = 10.9 Hz, 1H, H-6), 4.19 – 4.16 (m, 1H, H-3),‡ 4.13 (ddd, *J* = 5.5, 2.6, 0.9 Hz, 1H, H-4), 4.01 (dd, *J* = 10.9, 3.0 Hz, 1H, H-6'), 1.75 (s, 3H, CH_3CO); ^{13}C NMR (101 MHz, $CO(CD_3)_2$) δ 169.13 (C=O), 138.86 (Ar), 136.38 (Ar), 130.99 (Ar), 129.77 (Ar), 129.45 (Ar), 128.88 (Ar), 128.61 (Ar), 127.55 (Ar), 83.31 (C-1), 78.71 (C-4), 74.77 (C-5), 72.92 (C-3), 72.72 (CH_2OPh), 69.42 (C-6), 52.23 (C-2), 23.03 (CH_3CO); HRMS (ESI-TOF) $[M+H]^+$ Calcd for $C_{21}H_{24}NO_4S$: 386.1426 found: 386.1428; $[M+Na]^+$ Calcd for $C_{21}H_{23}NNaO_4S$: 408.1245 found: 408.1240.

Second attempt:

A solution of phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (**3.26**) (34 mg, 0.07 mmol, 1 eq.) in dry DMF (2 mL) and 3Å powdered molecular sieves was stirred under argon and cooled to 0°C. To this benzyl bromide (10 µL, 0.08 mmol, 1.1 eq.) was added, followed by the slow addition of NaH (60% dispersion in mineral oil) (9 mg, 0.37 mmol, 5 eq.) The reaction mixture was stirred at 0°C for 2 hrs and then at room temperature for 6 hrs. The reaction mixture was cooled down to 0°C, quenched with MeOH, concentrated, and washed with brine (15 mL) and EtOAc (4 x 15 mL). The combined organic fractions were dried over Na_2SO_4, concentrated, remaining DMF azeotropically removed with toluene and purified using flash chromatography (100% hexane to 4:6 hexane/EtOAc) to yield 14.3 mg (49%) of **3.28** as an opaque oil, which upon recrystallization (DCM/hexane) gave a white solid. Characterised as above.

‡ Assigned as a multiplet, but appears to be a dd.

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Chapter 6: Nuclear Magnetic Resonance (NMR) spectral analysis of *S. flexneri* 2a glycoconjugate and glycopeptide samples

6.1 NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool used for the structural elucidation of molecules. It is a robust, non-destructive method of analysis, requiring easy sample preparation (no derivatisation of the molecule is necessary) and gives an indication of the structural integrity and purity of a compound.[1] NMR is an important analytical tool used by pharmacopoeia, the WHO and U.S. Food and Drug Administration (FDA) for regulation and approval of drugs and vaccines.[2]

6.1.1 NMR spectroscopy for glycoconjugate analysis

In the case of glycoconjugate vaccines, NMR analysis provides a fingerprint spectrum which is characteristic of the antigen. Because of this it can be used to monitor the polysaccharide component of the vaccine at intermediate steps throughout the manufacturing process, making it an ideal tool for the analysis, regulation and licensure of glycoconjugate vaccines.

The number of monosaccharides in the repeating unit can be determined from the anomeric region (^{1}H: δ 4.4 - 5.5 ppm) in the ^{1}H NMR spectrum and the anomeric configuration of these sugars can be identified from the chemical shifts and coupling constants.[3] However, line broadening is often seen in glycoconjugate samples due to the slow tumbling of the conjugate as a result of the large hydrodynamic size, and so determining coupling constants of the attached polysaccharide is not always possible.[3,4] In addition, other functional groups such as methyl groups belonging to *N*-acetyl and *O*-acetyl groups (2.2 - 2.0 ppm) as well as methyl groups belonging to 6-deoxy sugars (1.35 - 1.5 ppm) can easily be identified. Since it is a sensitive tool, changes to inter-sugar linkages or *O*-acetylation patterns of the antigen can also be detected simply from inspecting the 1D ^{1}H NMR spectrum.[5] Furthermore, it may be possible to identify terminal residues of short chains, and since NMR spectroscopy is a quantitative tool, this can sometimes be used to determine the saccharide chain length.

In addition to the 1D ^{1}H NMR experiment, more advanced 1D and 2D homo- and heteronuclear experiments can be used to elucidate the structure of the repeating unit to gain further structural information. The 2D homonuclear (^{1}H-^{1}H) correlation experiment (COSY) reveals correlations between vicinal protons, and by tracing the signals from the anomeric region, the neighboring proton (H-1 to H-2 and H-2 to H-3 and so on) for each constituent monosaccharide can potentially be identified. While the TOCSY experiment can be used to identify all protons

within the same spin system (e.g. H-1 to H-6). This however depends on signal overlap and the magnitude of coupling constants.[6] The heteronuclear correlation experiment (HSQC) identifies ^{1}H-^{13}C correlations and gives the 2D proton-carbon fingerprint. The HMBC and NOESY experiments allow for the determination of inter-sugar linkages and the 3D structure of the saccharide through ^{3}J heteronuclear multiple bond couplings and through-space effects respectively.[7,8] From the above experiments it is possible to fully characterize the structure of the polysaccharide component of glycoconjugate vaccines.

6.2 The structure *S. flexneri* 2a serotype

S. flexneri 2a is one of 17 serotypes in the *S. flexneri* serogroup[9] and accounts for the majority of infections in the developing world.[10–12] All *S. flexneri* serotypes, except type 6 and 6a are composed of the basic linear tetrasaccharide repeating unit (**Figure 6.1**), to which α-D-glucopyranose and/or *O*-acetyl groups are attached at different positions. It is this varying substitution pattern that defines the serological identity of this particular strain.[13–15]

→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→

(Rha III) (Rha II) (Rha I)

Figure 6.1: The basic tetrasaccharide repeating unit found in all *S. flexneri* serotypes except type 6 & 6a.[13]

The *S. flexneri* 2a repeating unit was found to be 100% glucosylated at the RhaI position (α-1→4 linked) with *O*-acetylation quantified,[13,16] but the position and degree of *O*-acetylation was only recently determined by Kubler-Kielb *et al.*,[17] with later reports by Perepelov *et al.*[18] giving evidence for further minor *O*-acetylation at the 4-position of RhaIII (**Figure 6.2**). In other bacteria, the observed pattern of *O*-acetylation may vary with the specific strain used, the growth conditions and method of purification.[19]

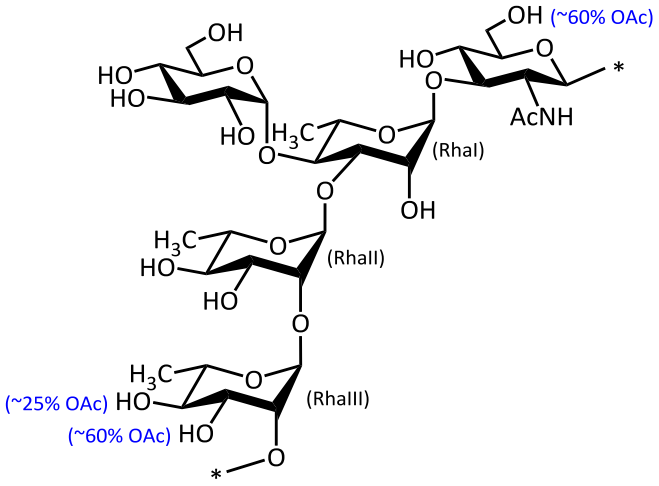


Figure 6.2: The repeating unit of *S. flexneri* 2a.[17,18]

6.3 GlycoVaxyn *S. flexneri* 2a sample analysis

Glycoconjugate vaccines made using traditional methods require the separate culturing of the bacterial pathogen and protein carrier (**Figure 6.3**). In order to obtain the polysaccharide component, it needs to be chemically cleaved from the lipid A, after which, further derivatisation for chemical linkage to the protein may be needed. Each step requires the purification and rigorous analysis of the desired component using various techniques. The step-by-step analysis of the polysaccharide using several assays confirms composition and structure early on in the process and therefore only a subset of assays are required for the final glycoconjugate analysis. On the other hand, by virtue of the GlycoVaxyn method, only one purification step is required to obtain the glycoconjugate and all analysis is carried out at this stage. The use of NMR spectroscopy in characterizing the antigen is therefore essential for this method of vaccine development.

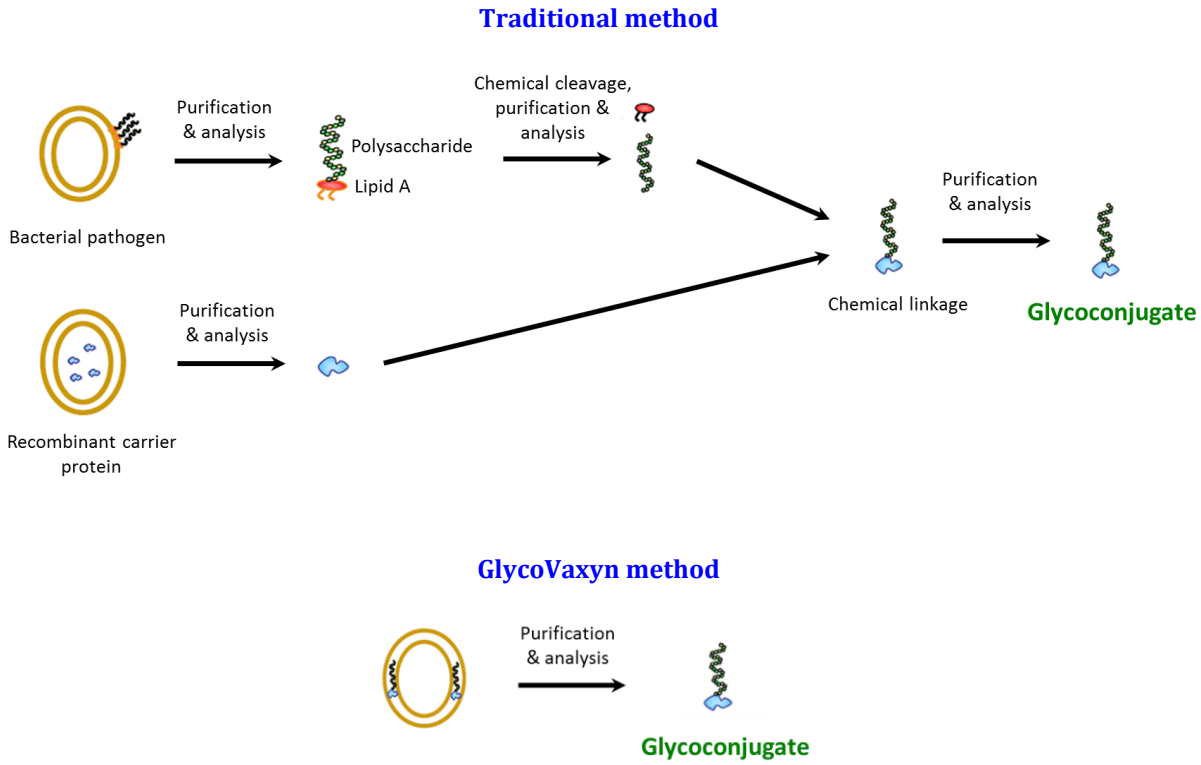


Figure 6.3: Traditional *vs*. GlycoVaxyn methods for glycoconjugate preparation.[20]

GlycoVaxyn is preparing for Phase I clinical trials of the *S. flexneri* 2a serotype glycoconjugate vaccine and this chapter deals with the analysis of the glycoconjugate and glycopeptide samples from development to the GMP test lots for the clinical trial.

6.4 Experimental methods

Samples were received from GlycoVaxyn. Each sample contained between 1 – 2 mg of O-antigen and as a result no ^{13}C NMR experiments could be conducted. The vial contents was dissolved in D_2O and transferred to a pear shaped flask and freeze-dried. After a further exchange with D_2O (0.7 mL), the sample was dissolved in 0.7 mL D_2O and transferred to a NMR tube (Wilmad) for analysis. NMR experiments on the glycoconjugate and glycopeptide development lot samples were conducted using a Bruker 400 MHz NMR spectrometer with the probe temperature set at 303K or 313K, whereas the glycoconjugate and glycopeptide GMP test lot samples were analysed using a Bruker Avance III 600 MHz NMR spectrometer equipped with a BBO Prodigy cryoprobe and the probe temperature was set at 303K or 313K. ^{1}H and ^{13}C spectra were referenced with respect to H-1 and C-1 of the Glc residue (5.20/98.9 ppm). 2D NMR spectra were obtained using standard Bruker software, and MesReNova 8.1 was used to process the NMR data. 1D TOCSY (selmlgp) experiments were conducted with mixing times = 250 ms (400 MHz) and 200 ms (600 MHz); 2D TOCSY (mlevphpr), mixing time = 120 – 180 ms (400/600

MHz); HSQC-TOCSY (hsqcdietgpsisp), mixing time = 120 ms (600 MHz); 2D NOESY (noesyphpr), mixing time = 300 ms (600 MHz).

6.5 Analysis of the glycoconjugate development lot sample

Analysis of the 1D ^{1}H NMR spectrum of the glycoconjugate development lot sample identified various key signals (**Figure 6.4**). This showed the presence of broad rEPA signals together with sharper peaks assignable to the *S. flexneri* 2a antigen. The 5 anomeric signals at 5.20 ppm (Glc), 5.13 ppm (RhaIII), 5.02 ppm (RhaII), 4.84 ppm (RhaI) and 4.73 ppm (GlcNAc), the CH_3 peaks from C-6 of the rhamnose units at 1.35 and 1.29 ppm, and the *N*-acetyl group at 2.07 ppm are all easily identified. No additional *O*-acetyl peaks in the region of 2.0 - 2.2 ppm can be seen (although free acetate at 1.91 ppm is present) and this together with the anomeric shifts agree with the reported chemical shifts of the non-*O*-acetylated antigen.[18]

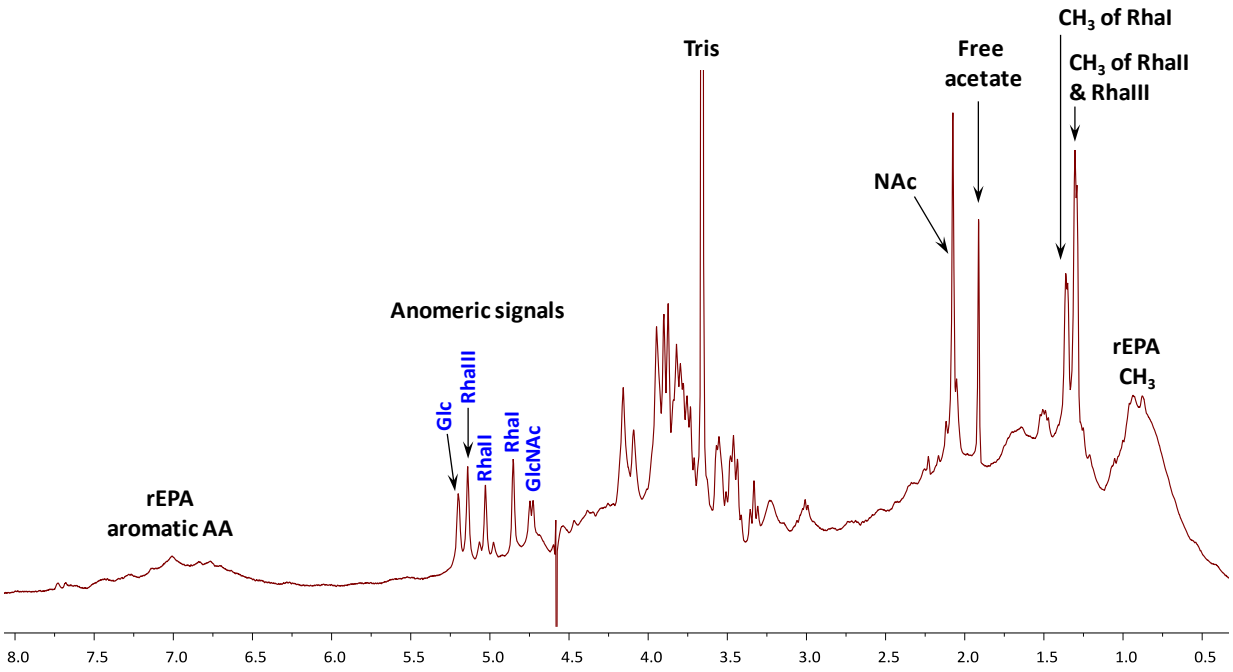


Figure 6.4: 1D ^{1}H NMR spectrum of the glycoconjugate development lot sample with pre-saturation of the HOD peak at 313K.

The spin systems for the five monosaccharide residues were investigated using proton-proton 1D TOCSY experiments. Generally, for TOCSY experiments (1D and 2D) transfer of magnetization from H-1 to all the other protons in the spin system requires large vicinal *J*-couplings between the protons.[6] With Glc and GlcNAc residues it is often possible for magnetisation to be transferred to all protons in the spin system due to the axial-axial couplings between H-2 to H-5 (assuming $^{4}C_{1}$ conformer) and therefore all proton signals can be seen,

while signals beyond H-2 are not usually observed for L-rhamnose. However, irradiating an alternative proton first, such as H-6 of the Rha residue overcomes this. In this instance the 1D TOCSY experiments were performed by irradiating H-6 of RhaI and H-2 of RhaII and RhaIII and this allowed for the identification of all protons in the respective spin systems (**Figure 6.5**), which were in agreement with published data.[18] The 2D COSY and TOCSY experiments further confirmed the assignments for the five residues.

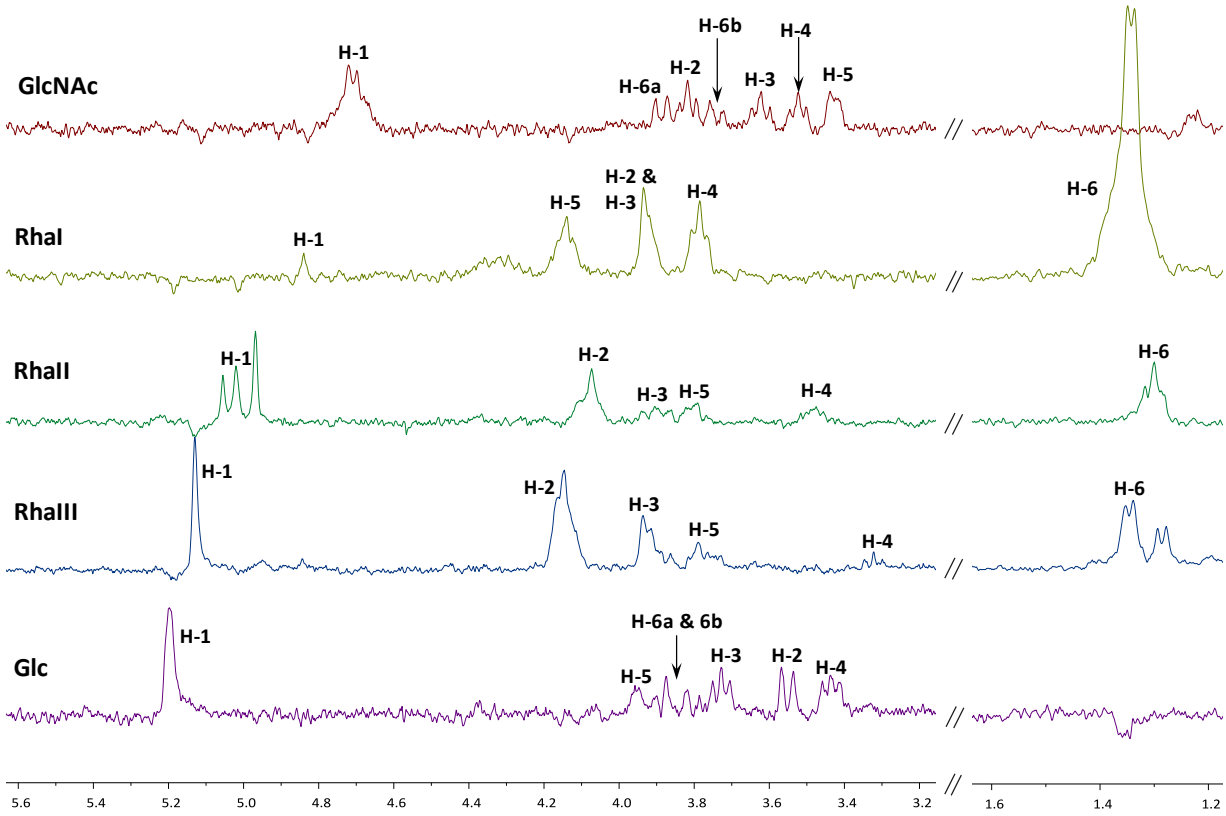


Figure 6.5: 1D TOCSY spectra of each monosaccharide from the glycoconjugate development lot sample.

Next, carbon assignments were confirmed with the aid of the HSQC experiment. The HSQC spectrum (**Figures 6.6**) constitutes a 2D proton-carbon fingerprint of the *S. flexneri* 2a antigen. The area highlighted by the red oval indicates where *O*-acetyl crosspeaks should appear, confirming that this sample contains no *O*-acetylation. Closer inspection of the ring region made the proton-carbon assignments possible for all residues with the aid of literature (**Figure 6.7**). The ^{13}C chemical shifts of C-5 (70.4-70.9 ppm) of all Rha units indicate they are α-linked.[15] While the downfield shifts between 5.91-9.70 ppm of C-2 for RhaIII and RhaII, and C-3 of RhaI and GlcNAc compared to free monosaccharide shifts indicate they are all linked through these carbons.[21]

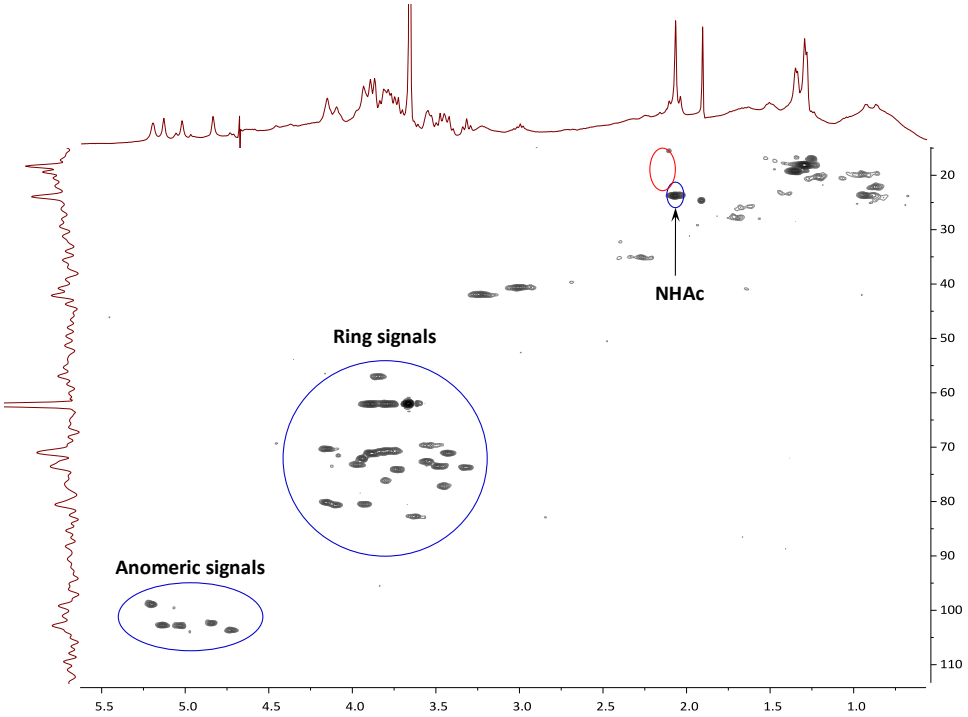


Figure 6.6: HSQC experiment of the glycoconjugate development lot sample. This provides the proton-carbon fingerprint of the antigen. The area highlighted by the red oval indicates where *O*-acetyl crosspeaks should appear, confirming that this sample is not *O*-acetylated.

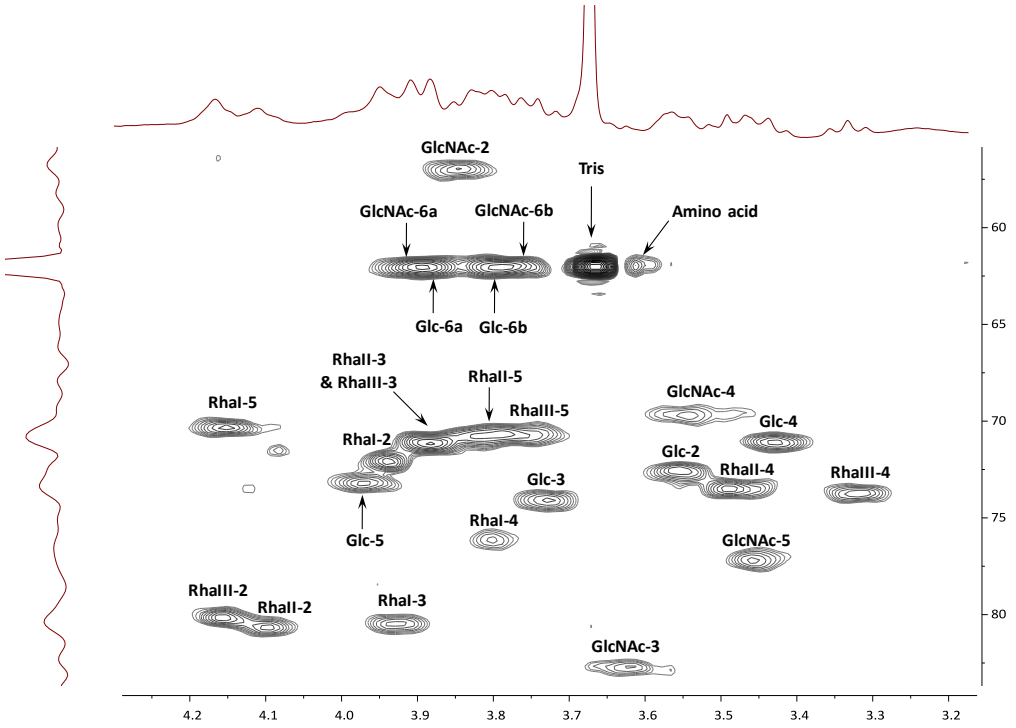


Figure 6.7: HSQC spectrum of the ring region with assignments for the glycoconjugate development lot sample. Crosspeaks are assigned based on the proton assignments made from COSY and TOCSY and literature and agree with published data.[18]

Likewise, analysis of the anomeric region made proton-carbon assignments for the five residues possible (**Figure 6.8**). Interestingly, two additional small crosspeaks were seen, one at 4.97/103.99 ppm and another at 5.07/99.56 ppm. From the crosspeak at 4.97/1013.99 ppm in the TOCSY spectrum (**Figure 6.9**) a corresponding proton and carbon at 4.08/71.56 ppm in HSQC spectrum can be identified. These peaks were assigned to the non-reducing end terminal RhaIII unit (tRhaIII), with H-1 appearing at 4.97 ppm and H-2 at 4.08 ppm. This is in close agreement with reported chemical shifts for this residue.[22]

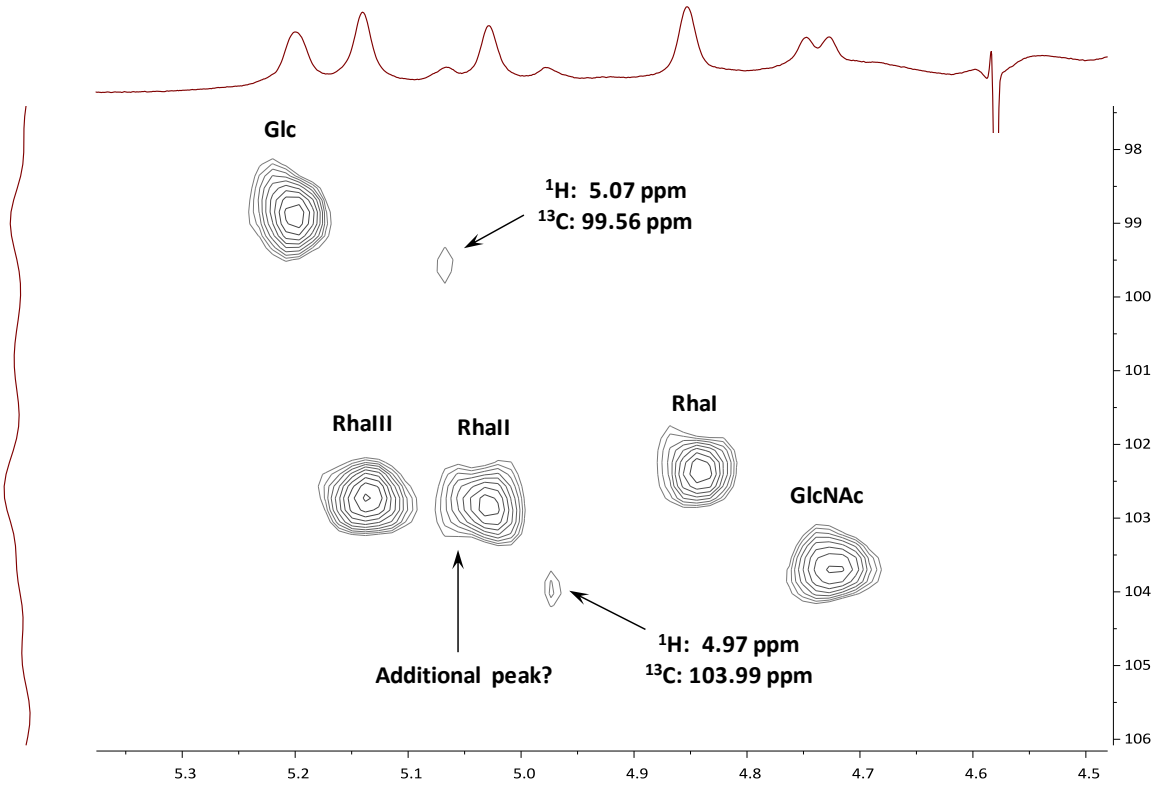


Figure 6.8: HSQC spectra of the anomeric region of the development lot glycoconjugate sample. The crosspeak 4.97/103.99 ppm was assigned to H-1 of tRhaIII, while it was not possible to assign the crosspeak at 5.07/99.56 ppm.

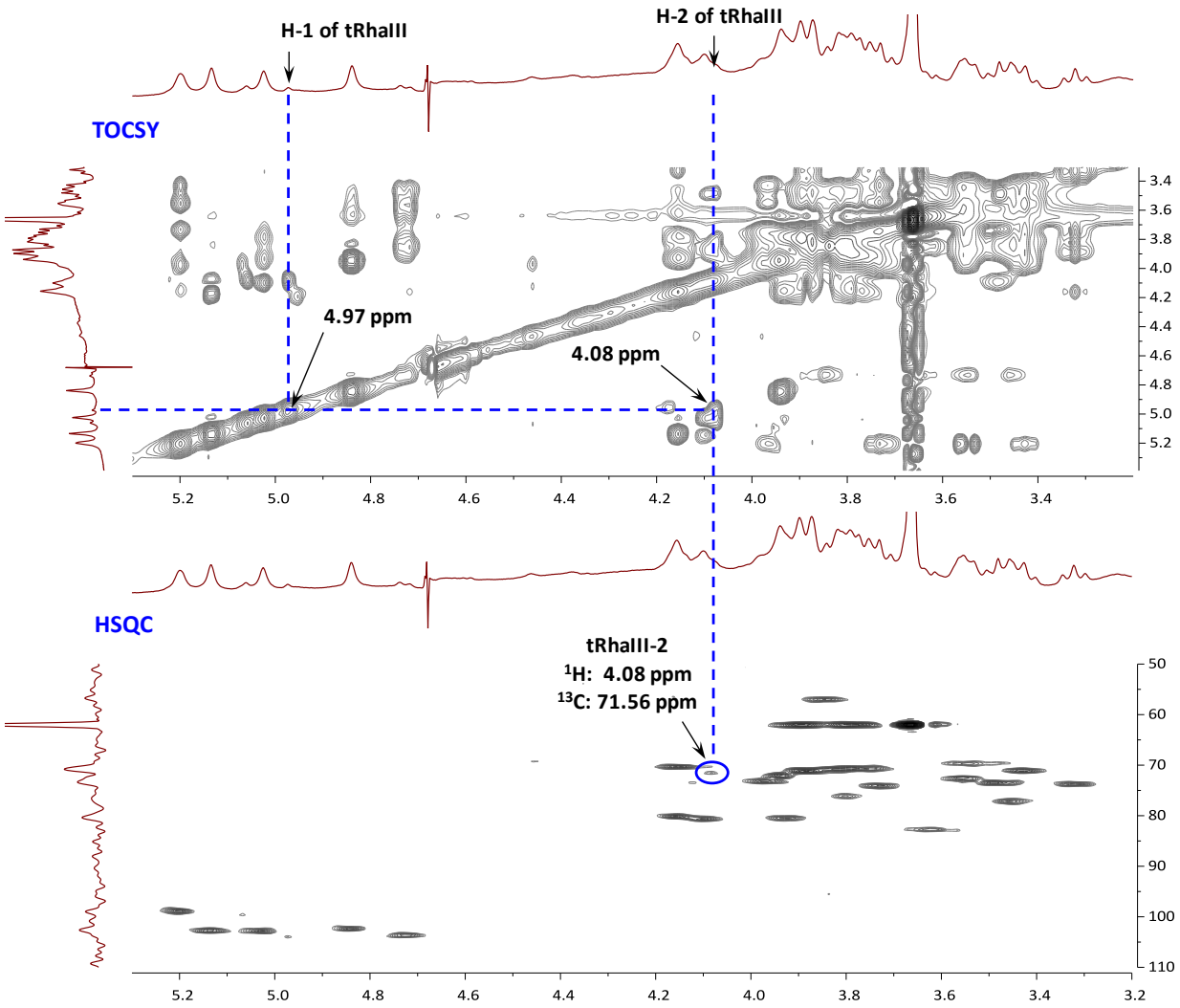
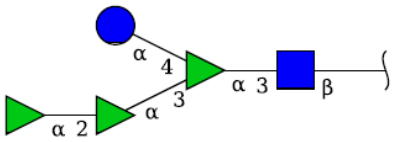


Figure 6.9: TOCSY and HSQC spectra of the glycoconjugate development lot sample indicating the correlation between H-1 and H-2 of the tRhaIII residue which in turn gives the ^{13}C chemical shift of tRhaIII-2.

The ^{1}H and ^{13}C chemical shifts of tRhaIII-1 and tRhaIII-2 are also consistent with CASPER predictions (Computer-Assisted Spectrum Evaluation of Regular polysaccharides, http://www.casper.organ.su.se/casper/).[23] CASPER calculates the chemical shifts of various oligo- or polysaccharides based on the constituent monosaccharides and the glycosylation shifts from the substituents, and can be used to predict chemical shifts of unknown structures. Predictions for tRhaIII can be seen in **Figure 6.10** and gives further evidence that supports the tRhaIII assignment.



α-L-Rha^{v}(1→2)α-L-Rha^{iv}(1→3)[α-D-Glc^{iii}(1→4)]α-L-Rha^{ii}(1→3)β-D-GlcNAc^{i}

→3)β-D-GlcNAc^{i}	1	2	3	4	5	6	6	Me_2	CO_2
Expected Calc. Error: 0.50	95.27	57.43	82.42	69.74	76.85	61.64		23.08	175.00
	4.75	3.76	3.63	3.52	3.48	3.76	3.92	2.07	
→3,4)α-L-Rha^{ii}(1→	1	2	3	4	5	6			
Expected Calc. Error: 6.69	101.67	71.58	77.44	81.24	69.14	17.56			
	4.88	3.89	3.90	3.64	4.16	1.35			
α-D-Glc^{iii}(1→	1	2	3	4	5	6	6		
Expected Calc. Error: 5.17	100.29	72.48	73.76	70.52	72.86	61.41			
	5.07	3.58	3.70	3.45	3.97	3.77	3.84		
→2)α-L-Rha^{iv}(1→	1	2	3	4	5	6			
Expected Calc. Error: 7.53	101.66	79.09	70.97	73.18	70.02	17.57			
	5.16	4.10	3.97	3.52	3.81	1.31			
α-L-Rha^{v}(1→	1	2	3	4	5	6			
Expected Calc. Error: 2.03	102.87	71.03	71.15	72.86	69.83	17.38			
	5.00	4.07	3.81	3.50	3.74	1.26			

Figure 6.10: CASPER predicted chemical shifts for the tRhaIII and Glc residues.

With the assignment of the terminal non-reducing end rhamnose (tRhaIII), it was possible to estimate the average number of repeating units in the polysaccharide. From the integration of the RhaIII signal *vs*. tRhaIII in **Figure 6.11** the antigen is made up of approximately 5 repeats. However, the integration is at best a rough approximation due to various factors which need to be considered: Firstly, the baseline is 'noisy' and the signals are broad. Secondly, there are two sites of glycosylation on the protein (see **Ch 1.7** for details) and integration will represent an average of the repeating units for these two. Lastly, the intensity of the signals for the antigen component may not include the internal residues (those closer to the protein component), leading to an under estimation of chain length. This means that the approximation of the O-antigen chain length using the glycoconjugate sample may in fact be an underestimate.[24]

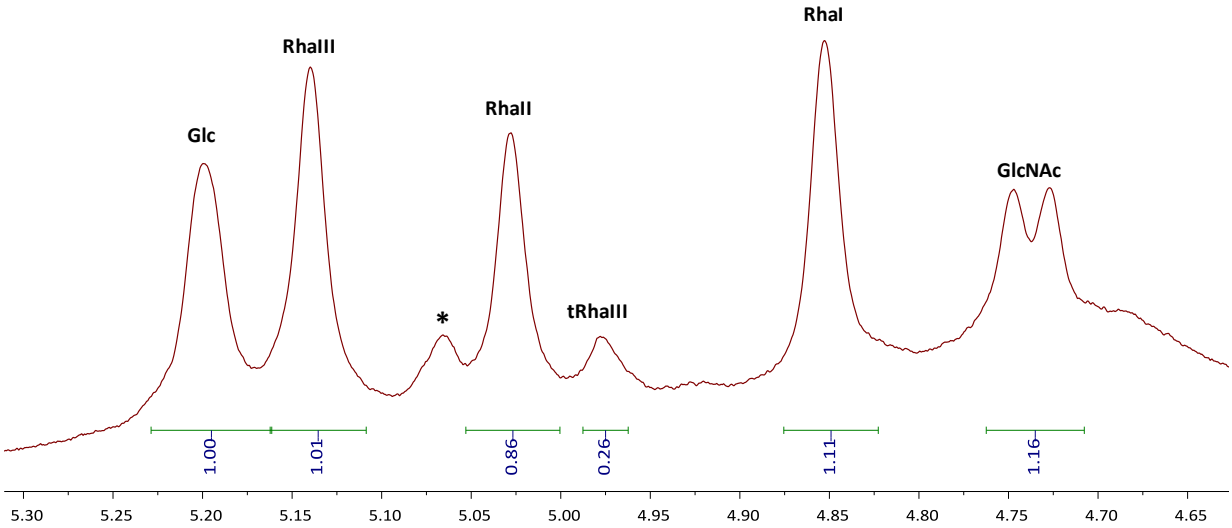


Figure 6.11: ^{1}H NMR spectrum of the anomeric region with integration of the glycoconjugate development lot sample. *The intensity of this peak (5.07 ppm) is due to overlapping signals.

The small crosspeak at 5.07 ppm could possibly be assigned to the terminal Glc residue (tGlc) or RhaII residue (tRhaII) found on the non-reducing end of the antigen.[#] Although crosspeaks from this proton could be seen in the TOCSY and HSQC spectra (**Figure 6.9**), the chemical shifts were too high for it to belong to a sugar residue, and therefore it could potentially belong to a contaminant.[‡]

6.6 Analysis of the non-*O*-acetylated and *O*-acetylated glycopeptide development lot samples

In order to obtain more information about the antigen by diminishing signal overlap from amino acids, two additional glycoconjugate development lot samples were treated with pronase by GlycoVaxyn to give the glycopeptide sample. The remaining amino acid and antigen signals are much sharper in contrast to the glycoconjugate development lot sample (**Figure 6.12**), but are similar in profile showing that pronase treatment had no impact on the attached antigen structure.

[#] If the unknown crosspeak at 5.07/99.56 ppm was Glc then correlations beyond H-2 would be seen in the TOCSY.
[‡] No free amino acids have chemical shifts in this region.

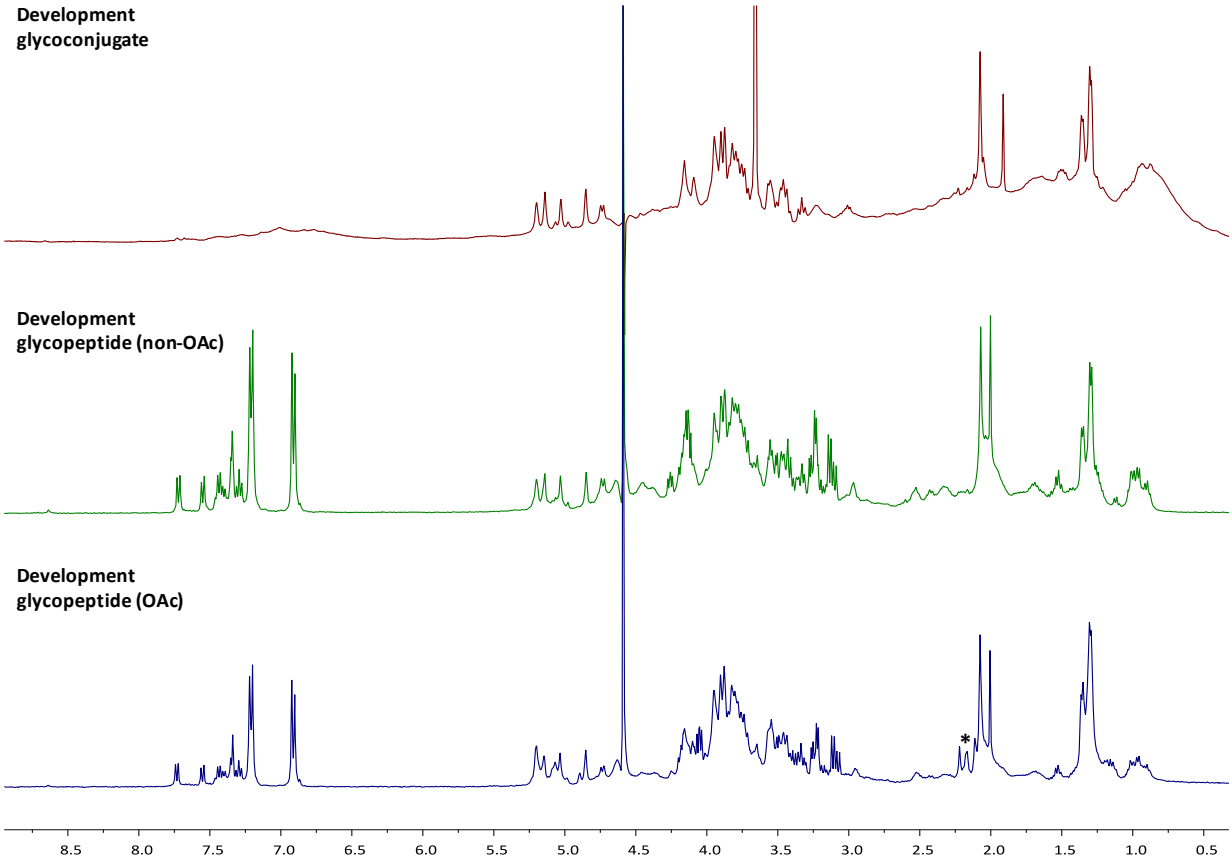


Figure 6.12: Comparison of the ^{1}H NMR spectra of glycoconjugate and glycopeptide development lot samples. *Additional OAc signals.

Since the signals in these spectra are sharper, the baseline is less noisy and protein component is shorter (peptide), integration of the RhaIII *vs*. the tRhaIII unit gives a better indication of the repeating unit length (**Figure 6.13**). Integration indicates that the polysaccharide is approximately 7 repeating units long.

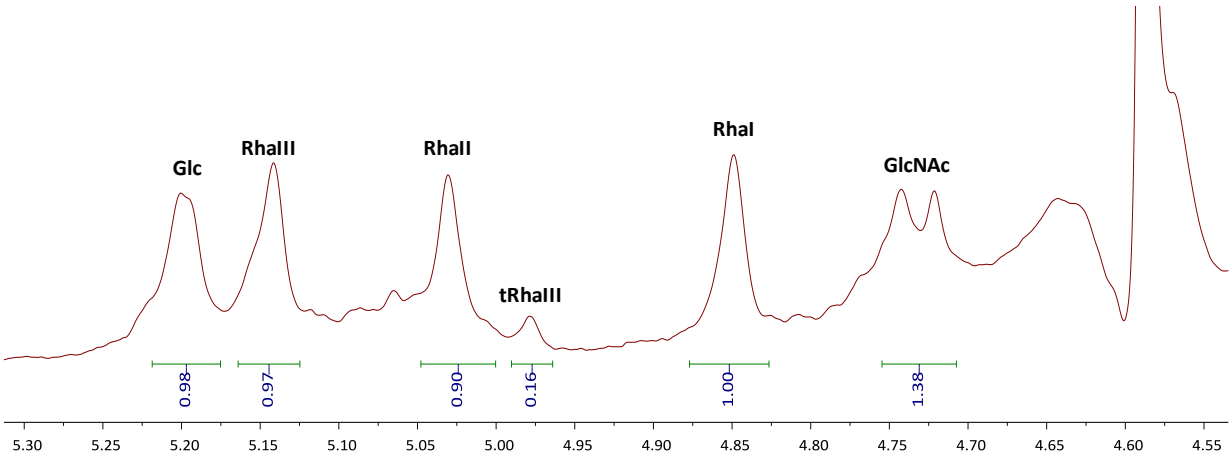


Figure 6.13: ^{1}H NMR spectrum of the anomeric region with integration of the glycopeptide development lot (non-OAc) sample.

The residual amino acids in both glycopeptide samples were identified from the COSY, TOCSY and HSQC experiments and were consistent with literature chemical shifts for the free amino acids.[25] From the aromatic region tryptophan, tyrosine and phenylalanine were identified, while traces from the alkane region identified threonine, leucine, alanine and isoleucine. Proline, arginine and serine could also be identified from the above experiments for both samples. The two prominent distorted doublet of doublets at 3.24 and 3.09 ppm were assigned to asparagine (**Figure 6.14**). This is slightly deshielded compared to the free asparagine reference spectrum (2.94 and 2.85 ppm)[26] indicating a different chemical environment and giving evidence that it is either still attached to the antigen or a part of the peptide.‡

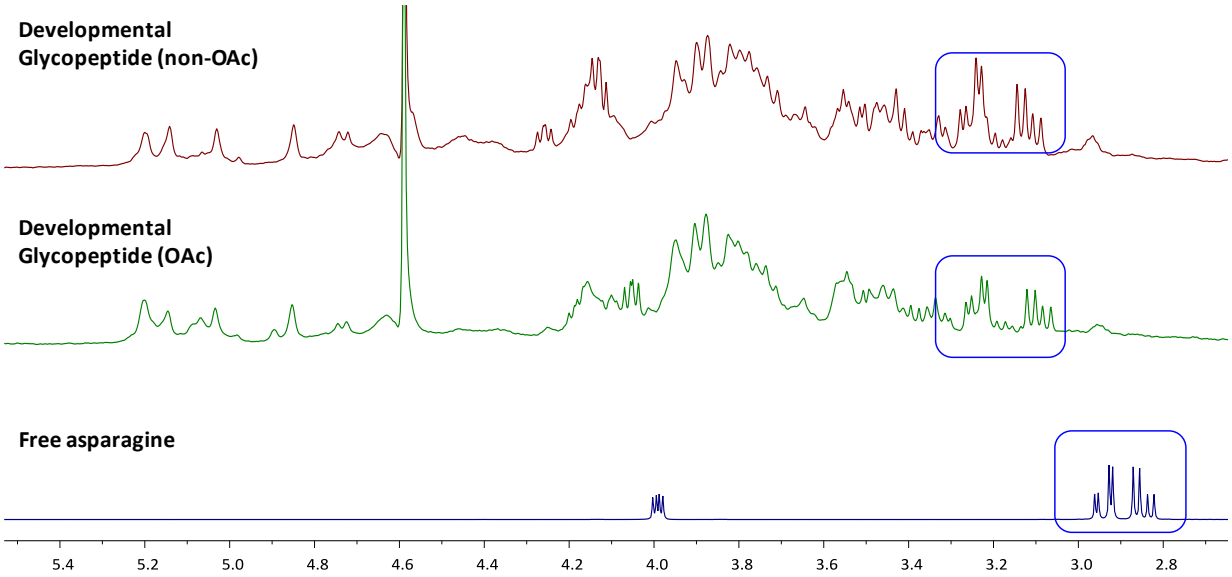


Figure 6.14: Comparison between the ^{1}H NMR spectra of the glycopeptide development lot samples and free asparagine reference spectra.

In order to assess whether the antigen component remained linked to the Asn residue, a diffusion ordered spectroscopy (DOSY) experiment was used. The DOSY experiment is based on the random translational motion of a molecule and can be quantitatively characterized by its diffusion coefficient (D). This is generally related to the size and shape of a molecule, and so this experiment can be used to separate out and identify different compounds in a complex mixture. DOSY is a pseudo 2D experiment where the horizontal axis represents the chemical shift (ppm) and the vertical axis represents the diffusion coefficient in milliseconds (ms).[27] As seen in **Figure 6.15**, the antigen component diffuses at a different rate to the amino acids and particularly, to the Asn residue (3.24 and 3.09 ppm, highlighted), indicating that the antigen and

‡ *J*-couplings of the two peaks are comparable to literature.

peptide are unconjugated. In this instance, the observed chemical shift of Asn compared to the free Asn residue indicates that it remains attached to the peptide and is not linked to the saccharide. The intensity of the antigen signals are small compared to those of the peptide signals, which is not expected if the Asn was attached to the O-antigen, again confirming that the two are not attached.

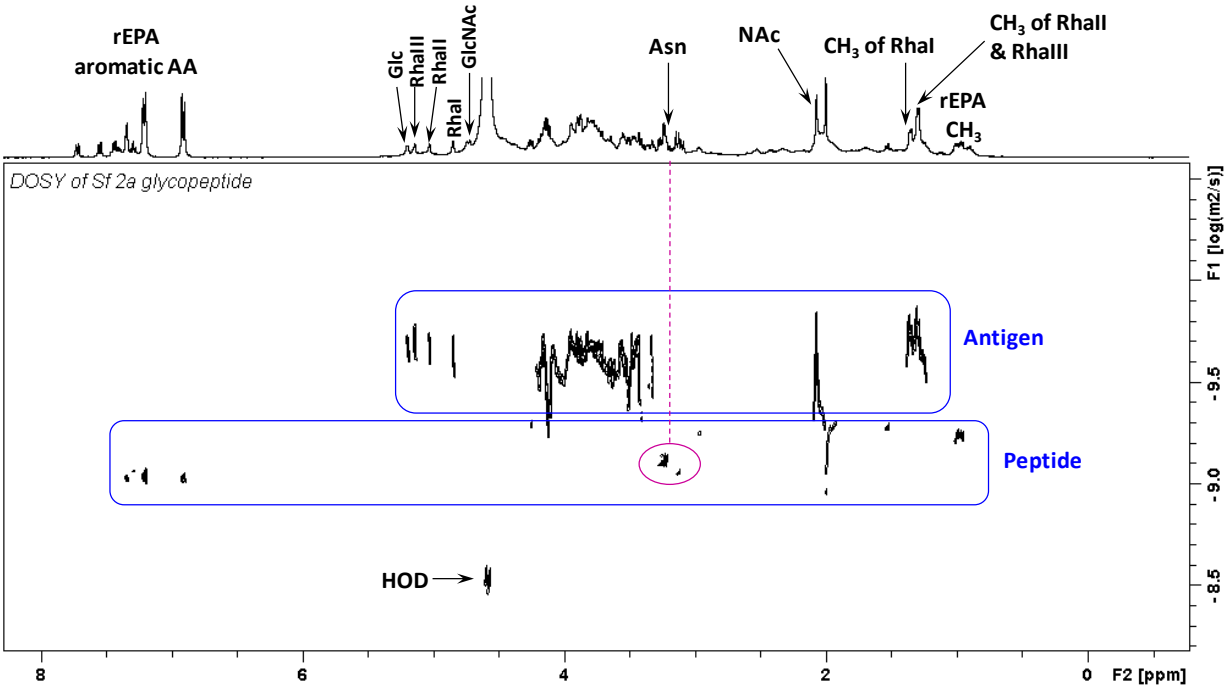


Figure 6.15: DOSY spectrum of the glycopeptide development lot (non-OAc). This shows that the amino acids, in particular Asn (highlighted in purple) is not attached to the antigen as seen from the different diffusion coefficient.

The glycopeptide development lot (OAc) was obtained from wild-type *S. flexneri* and contained partial non-stoichiometric *O*-acetylation as seen from the intensity of the *O*-acetyl peaks between 2.22 and 2.17 ppm in the ^{1}H NMR spectrum; with the position and degree of acetylation unknown. Acetylation of a hydroxyl group results in a deshielded environment, with the α-hydrogen of the OAc carbon shifting significantly downfield in the ^{1}H NMR spectrum and the α-carbon less so in the ^{13}C NMR spectrum. Often the proton of the *O*-acetylated carbon is visible in the proton anomeric region (4.4-5.5 ppm), while the carbon will appear at lower field resonances, and can therefore easily be identified in the HSQC spectrum.[3,28] The β-carbon experiences an upfield shift, while the effects on the γ- and δ-carbons is much less.[28] In fact, the effect of partial *O*-acetylation at various positions of the *S. flexneri* 2a repeating unit is felt across the rest of the repeating unit and can be seen when comparing the anomeric chemical shifts of the non-*O*-acetylated polysaccharide to the partially *O*-acetylated antigen.[17,18] This is seen by the

broader peak at 5.15 ppm and additional lower intensity peaks at 5.09, 5.07 and 4.90 ppm in anomeric region. Closer inspection of the HSQC spectrum (**Figure 6.16**) revealed a small crosspeak at 5.09/73.88 ppm. Based on the more shielded ^{13}C shift and literature, this was assigned to RhaIII-3 (3OAc). Further evidence for *O*-acetylation of the 3-position of RhaIII is seen from the broad peak at 5.15 ppm which correlates to H-1 of RhaIII(OAc) and the more shielded C-2 and C-4 signals as a result of the β-effect of *O*-acetylation.[28] While the crosspeak at 5.07/102.48 ppm and 4.90/102.04 ppm was assigned to RhaII-1 and RhaI-1 respectively of the acetylated polysaccharide. If the RhaIII residue was *O*-acetylated at C-4, then the key crosspeak at 4.80/75.8 ppm should be visible, but it was not observed.[18] Chemical shifts for Glc and GlcNAc remained unchanged, and in the latter residue this is highly indicative of no acetylation at the 6-position of GlcNAc. The lack of a crosspeak in the region of 4.54/103.7 ppm confirms this. From the TOCSY (**Figure 6.17**), crosspeaks from 5.07/4.12 ppm and 5.07/3.88 ppm can be assigned to H-1/H-2 and H-1/H-3 of RhaII(OAc) respectively. A crosspeak at 5.09/3.52 can be assigned to H-3/H-4 of RhaIII(OAc) while a large crosspeak at 4.90/3.96 can be seen and assigned to H-1/H-2 and H-3 of RhaI(OAc).

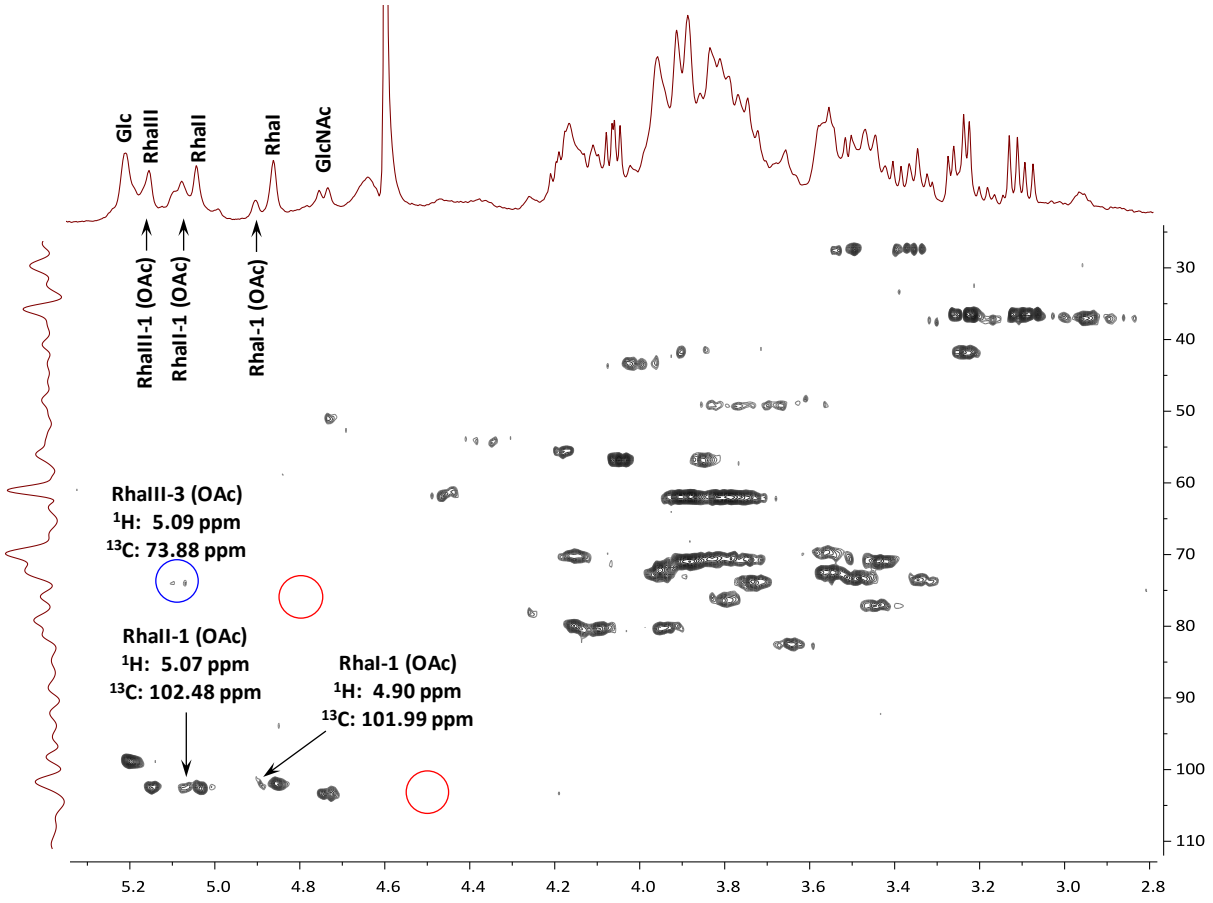


Figure 6.16: The 2D HSQC spectrum of the glycopeptide development lot (OAc) sample. This shows the RhaIII-3 (OAc) crosspeak (highlighted in blue), while the red circles indicate where crosspeaks should be seen if the sample was OAc at C-4 of RhaIII and C-6 of GlcNAc.

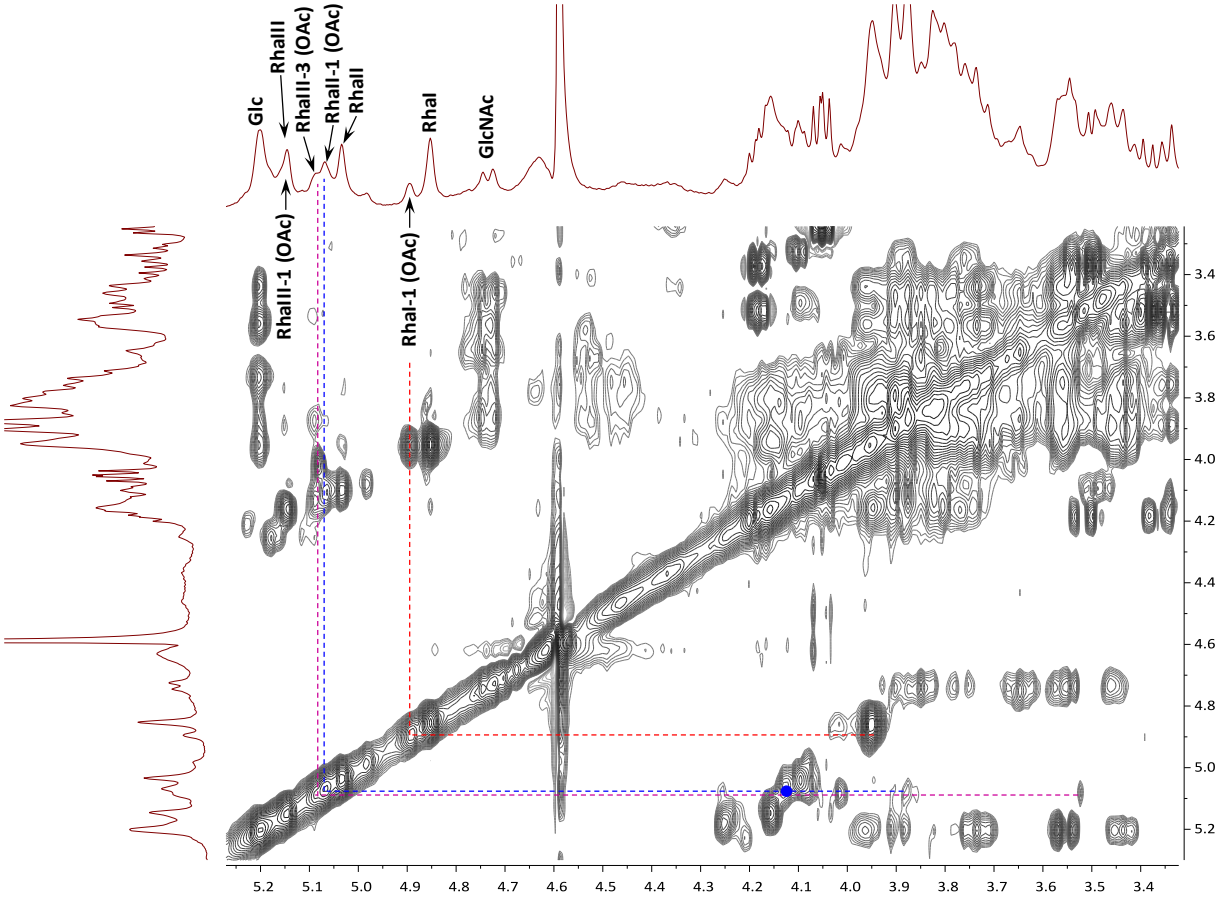


Figure 6.17: TOCSY spectrum of the development lot glycopeptide (OAc) sample showing distinct crosspeaks between RhaIII-3 (OAc) (pink), RhaII-1(OAc) (blue) and RhaI-1(OAc) (red) and the respective ring protons.

Attempts to determine the degree of *O*-acetylation were made by deconvolution of the ^{1}H NMR spectrum. However, since the starting point of the deconvolution is arbitrary, this method was abandoned. On the other hand, integration of the NAc peak of GlcNAc *vs*. the *O*-acetyl of RhaIII at 2.22 ppm suggested that the antigen is approximately 20% acetylated (**Figure 6.18**). Alternatively, integration of the signals in the anomeric region could yield this result, however, there is signal overlap between 5.20 ppm to 4.95 ppm, but integration of RhaI-1(OAc) *vs*. the non-*O*-acetylated RhI-1 unit indicates that RhaIII is 30% *O*-acetylated in the 3-position.

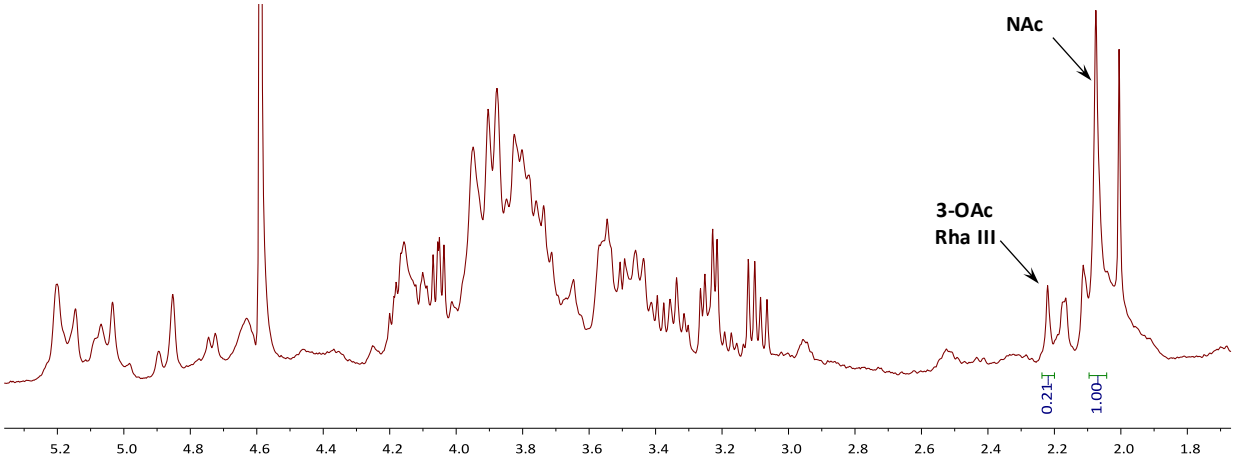


Figure 6.18: Integration of OAc at 2.22 ppm *vs*. GlcNAc NAc suggests the antigen is approximately 20% acetylated at the 3-position of RhaIII.

Alternatively, assays to determine the amount of *O*-acetylation could have been used,[29,30] however, from this point onwards GlycoVaxyn decided to synthesis the non-*O*-acetylated antigen. The degree and position of *O*-acetylation is known to influence immunogenicity,[31–38] however GlycoVaxyn's findings, as well as recent reports, suggest that the lack of non-stoichiometric *O*-acetylation of the *S. flexneri* 2a antigen does not significantly affect immunogenicity.[15,39]

6.7 Analysis of the glycoconjugate GMP test lot sample

These samples were analysed using a Bruker 600 MHz equipped with a cryoprobe which resulted in better quality spectra and permitted additional experiments such as HSQC-TOCSY and NOESY to be recorded with 1 – 2 mgs of saccharide.

The anomeric and ring proton-carbon pairs in the glycoconjugate GMP test lot sample could be assigned from the HSQC spectrum (**Figure 6.19**). The additional crosspeak at 5.06/103.11 ppm was assigned to the H-1 of the terminal non-reducing end RhaII unit (tRhaII-1), which agrees with literature.[22,‡]

‡ In the previous glycoconjugate sample this crosspeak was diminished, but is now distinctly seen here, while the crosspeak at 5.07/99.56 ppm is absent.

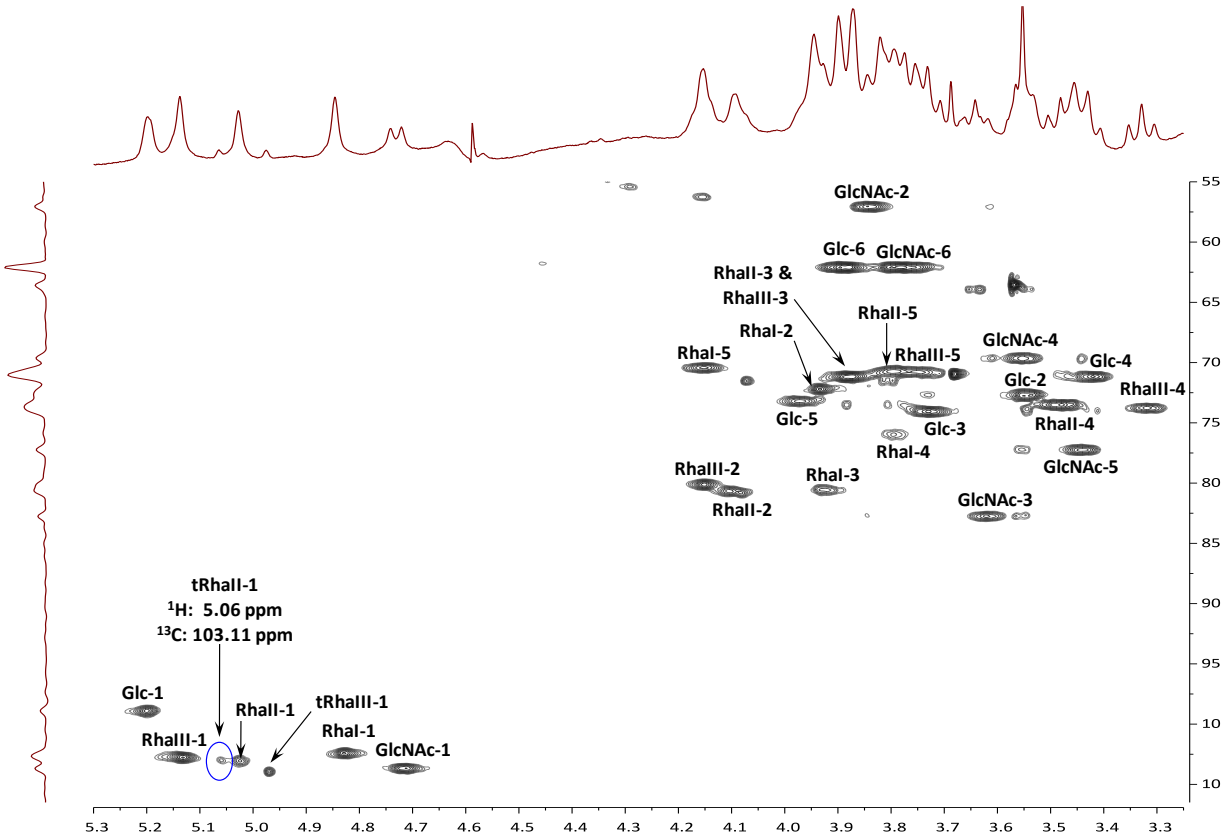


Figure 6.19: HSQC spectrum of the anomeric and ring regions with assignments of the Glycoconjugate GMP test lot sample. Crosspeaks are assigned based on the proton assignments made from COSY and TOCSY experiments and agree with published data.[18] Here the tRhaII-1 is clearly seen, highlighted by the blue oval.

The average number of repeating units can again be determined from comparison of the integration of the tRhaIII and the RhaIII (**Figure 6.20**). In this instance, the polysaccharide was found to be approximately 6 repeating units long.

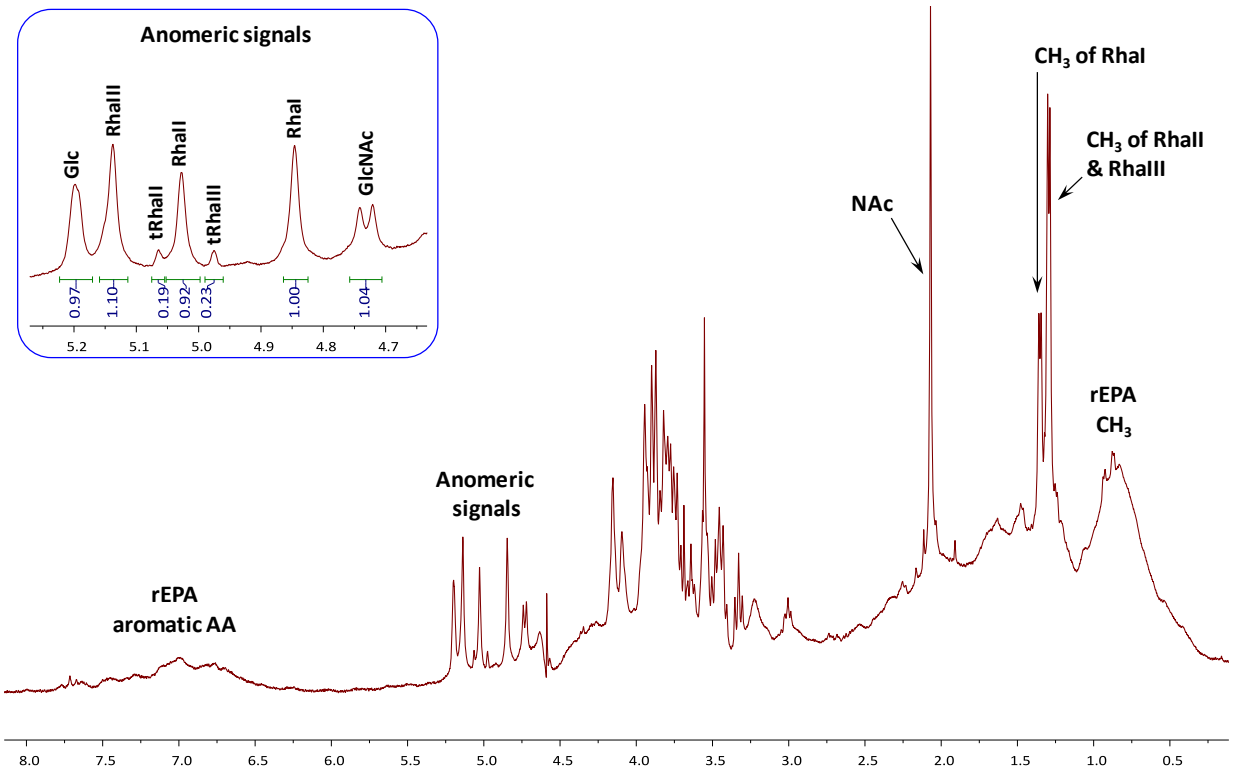


Figure 6.20: 1D ^{1}H NMR spectrum of the glycoconjugate GMP test lot sample with pre-saturation of the HOD peak at 313K. Integration of the anomeric protons is highlighted in the blue box.

6.8 Analysis of the glycopeptide GMP test lot sample

This sample was derived from the above glycoconjugate sample and further structural information was obtained. The coupling constants of the Glc and GlcNAc residues could be determined (**Figure 6.21**) and were found to 3.86 Hz and 8.55 Hz, which is consistent with the corresponding α- and β-configurations respectively.[3] Here, integration of the RhaIII *vs*. the tRhaIII unit shows the polysaccharide to be approximately 7 repeating units long.[#]

[#] There is a slight shoulder on the RhaIII arising die to signal overlap. However, integration of this signal is consistent with the other signals in the anomeric region whose integration is approximately equal to 1.

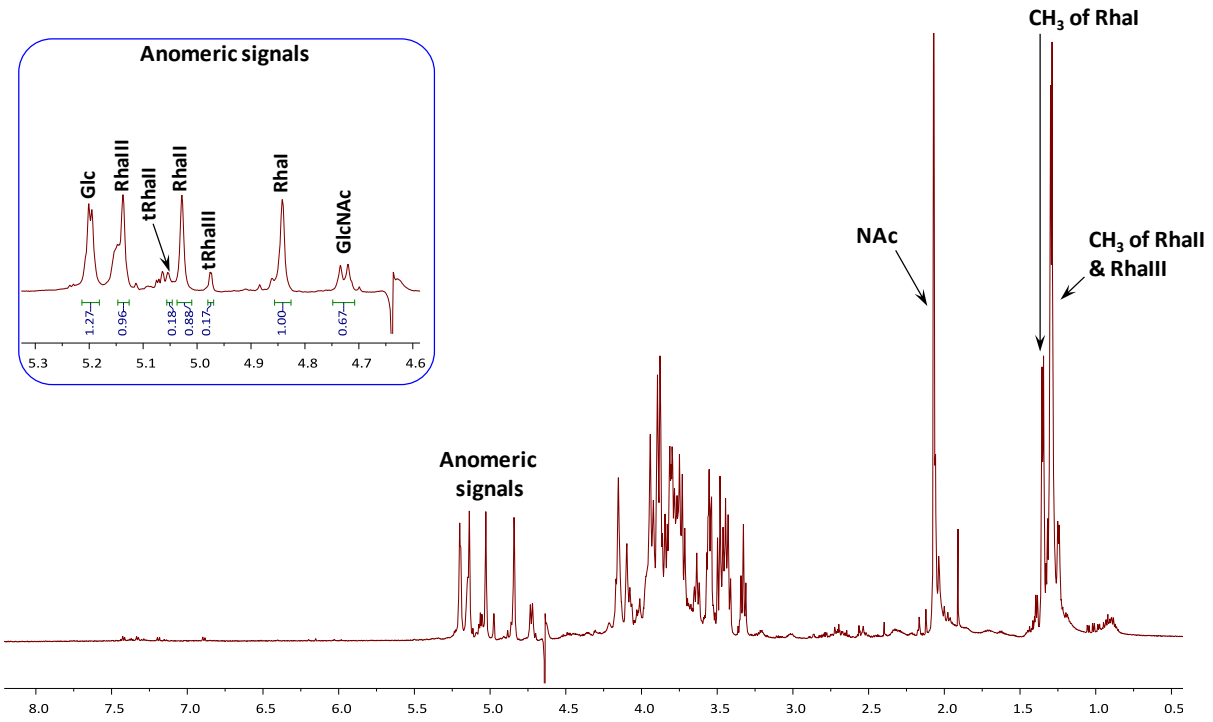


Figure 6.21: 1D ^{1}H NMR spectrum of the Glycopeptide GMP test lot sample at 313K. Integration of the anomeric protons is highlighted in the blue box.

In this case, the 1D TOCSY experiments (**Figure 6.22**) were again performed and all protons from H-1 to H-6 in the Rha residues were seen when irradiating H-1 first. As a result the H-3 to H-6 signals are of a lower intensity. Interestingly, NOEs could also be detected (negative peaks highlighted in blue) as a result of magnetisation transfer through space, instead of through the bonds.[8,40] These correlations were further confirmed from the 2D NOESY experiment.

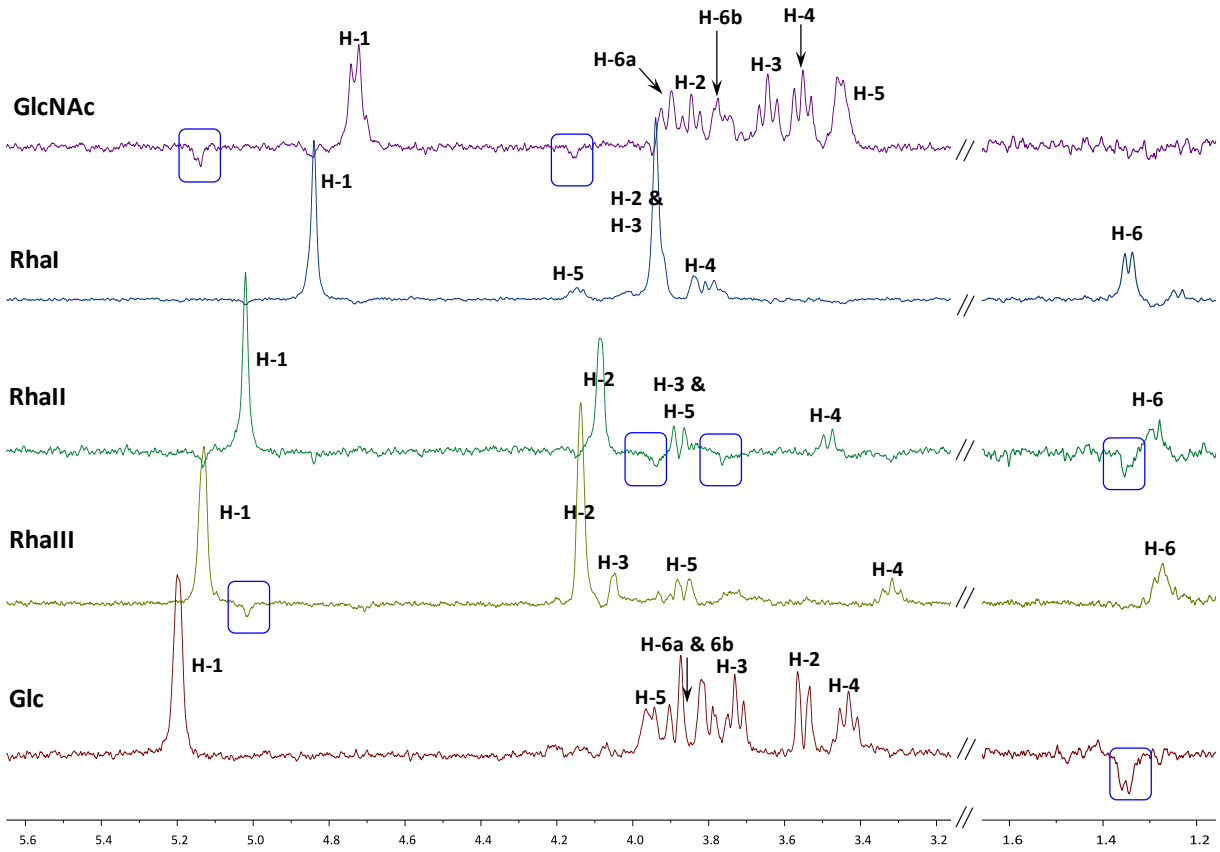


Figure 6.22: 1D TOCSY spectra of each monosaccharide from test GMP glycopeptide sample (600 MHz). The negative peaks highlighted in blue indicate NOEs.

With the 2D NOEs the inter-sugar linkages can be elucidated (**Figure 2.23**) This had previously been assumed based on literature and the downfield shifts of the linked carbons.[18] The detectable crosspeaks are reported in **Table 6.1**, and this gives confirmation that the linkage connections are consistent with literature. For example, the key NOE between H-1 of Glc and H-4 of RhaI indicates the 1→4 linkage, while the NOE between H-1 of RhaIII and H-2 of RhaII confirms the 1→2 linkage.

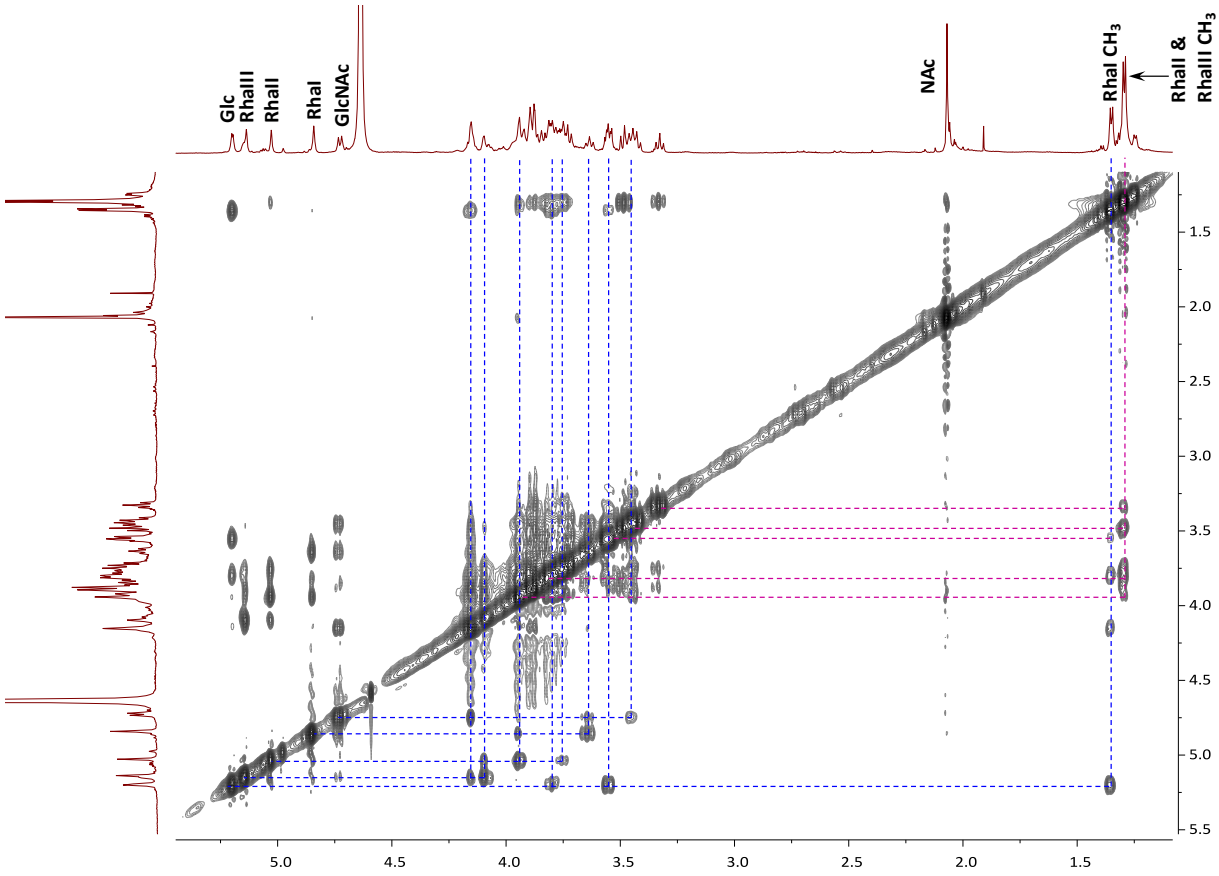


Figure 6.23: NOESY spectrum of the test GMP glycopeptide sample (600 MHz). Distinct crosspeaks from the anomeric region for each residue to neighbouring protons in the ring region can be seen.

Table 6.1: Chemical shifts of NOEs confirming inter-sugar linkages.

From H-1	NOE (ppm)	Assignment
Glc	3.79	RhaI-4
	3.55	Glc-2
	1.35	RhaI-CH_3
RhaIII	4.16	RhaIII-2
	4.11	RhaII-2
RhaII	4.11	RhaII-2
	3.77	RhaI-4
RhaI	3.94	RhaI-2
	3.64	GlcNAc-3
GlcNAc	4.16	RhaI-5

From CH_3	NOE (ppm)	Assignment
RhaII & RhaIII	3.80	RhaII-5, Glc-6
	3.48	RhaII-4
	3.33	RhaIII-3
RhaI	5.20	Glc-1
	4.16	RhaI-5

The HSQC-TOCSY experiment provides additional dispersion as the TOCSY correlations are obtained along the attached carbons. As a result a much more comprehensive proton-carbon map was obtained (**Figure 6.24**).[41] The HSQC-TOCSY highlights the main signals visible in the

anomeric region, but was used to confirm the chemical shifts of all antigen signals through the respective intra-residue correlations. In addition, it made the detection of H-2 of the tRhaIII much easier than before, with the distinct tRhaIII-2 crosspeak at 4.08/71.56 ppm visible. For simplicity only the proton-carbon correlations of the GlcNAc and tRhaIII have been highlighted.

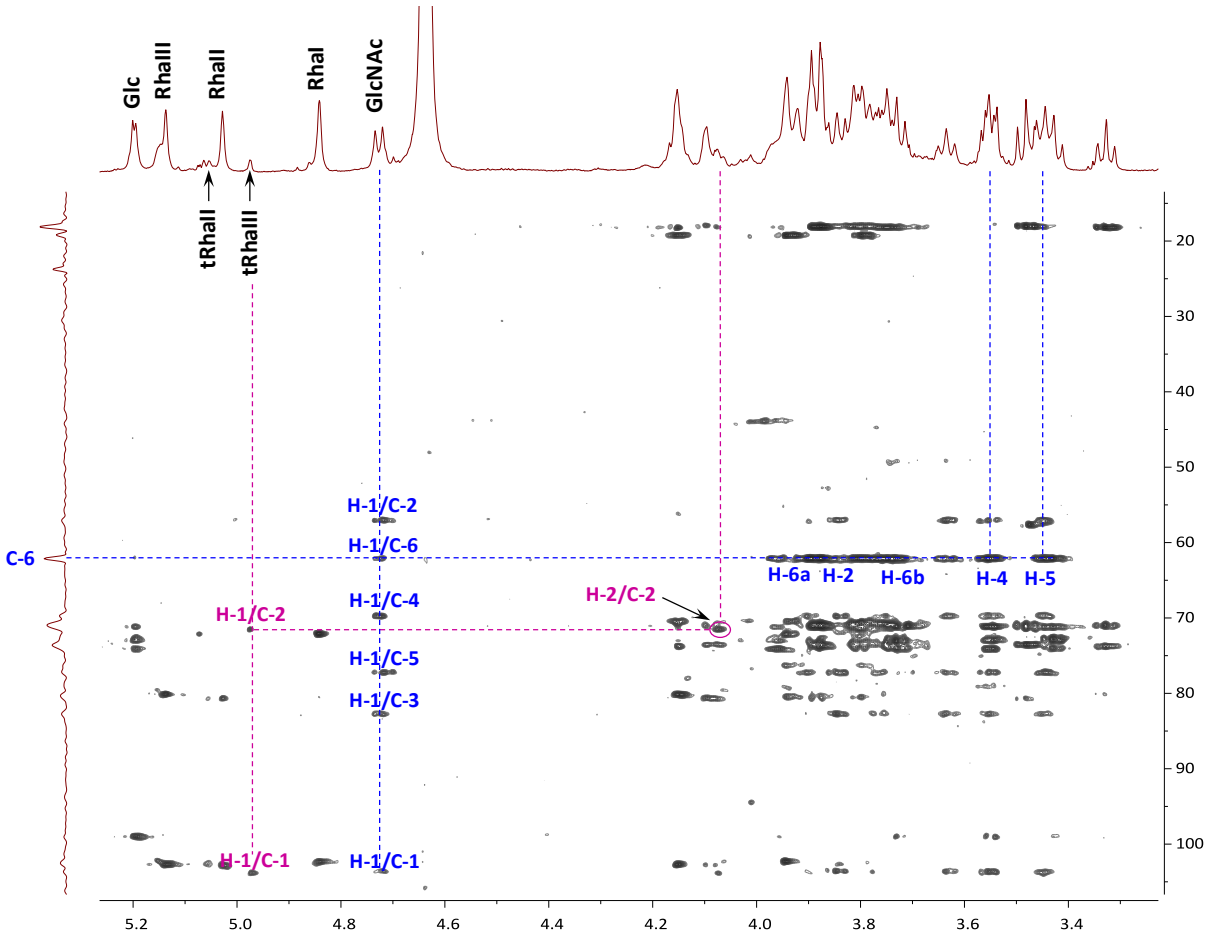


Figure 6.24: HSQC-TOCSY spectrum of the test GMP glycopeptide sample (600 MHz). Identification of the tRhaIII H-2/C-2 crosspeak is much easier (highlighted in purple). For simplicity the crosspeaks between H-1/C-1 to H-1/C-6 and C-6/H-1 to C-6/H6 are highlighted in blue.

Closer inspection of the anomeric region of the HSQC of the glycopeptide GMP test lot sample revealed additional crosspeaks. With the glycopeptide development lot samples it had not been possible to identify any GlcNAc-Asn residue. However, the appearance of an unusual crosspeak at 5.12/79.13 ppm (**Figure 6.25**) was assigned to H-1 of the GlcNAc linked to Asn (rGlcNAc) based on literature precedent.[42,43] No rGlcNAc to Asn-NH crosspeaks in the in the COSY, TOCSY or NOESY spectra were detectable. Additional crosspeaks (highlighted in red) are due to partial hydrolysis of some glycosidic linkages, as a result of prolonged experiments performed at 313K. These were identified as α- and β-L-rhamnose.

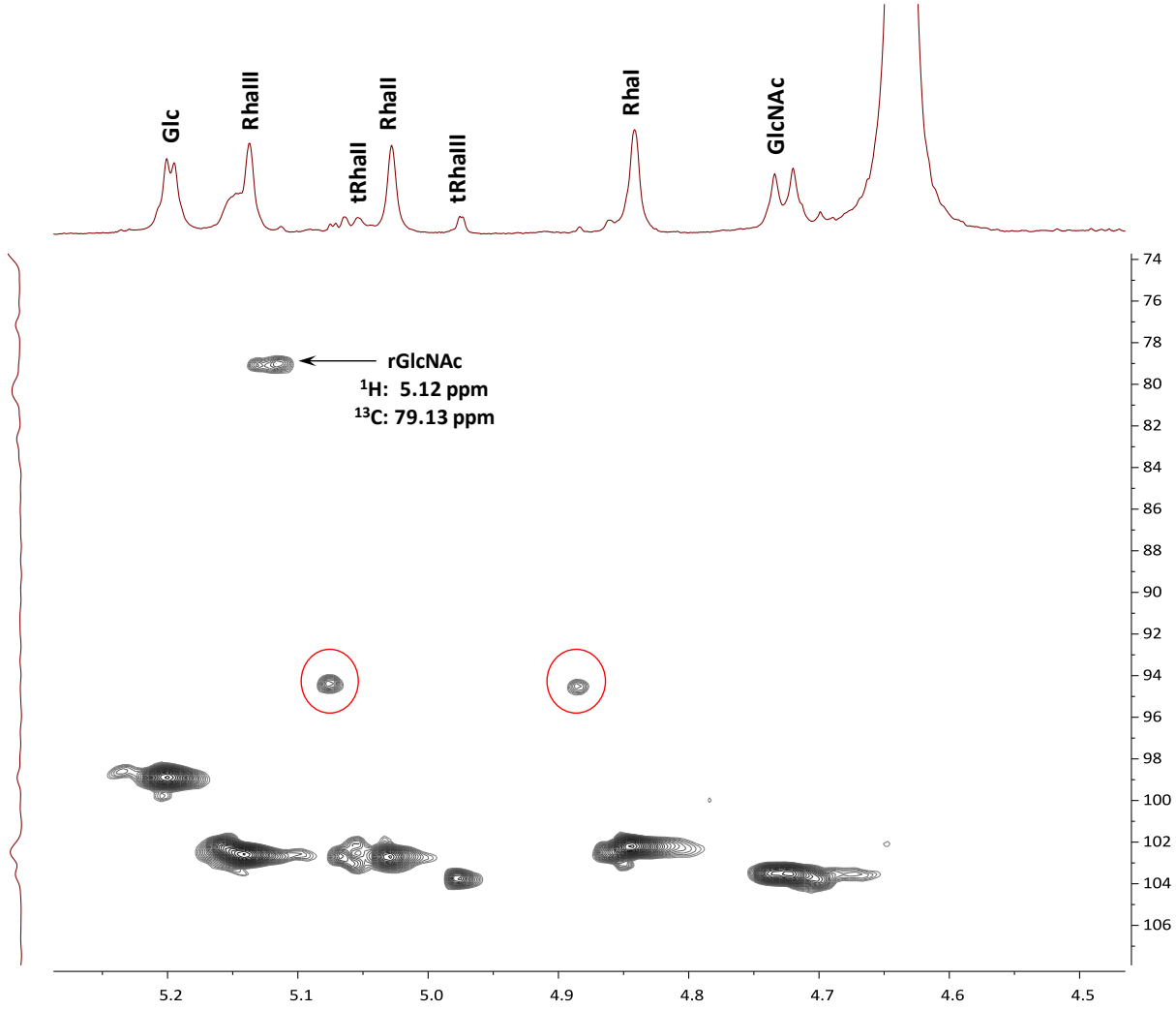


Figure 6.25: HSQC spectrum of the anomeric region of the test GMP glycopeptide sample (600 MHz). Additional crosspeaks (highlighted in red) arose after extensive NMR experiments at 303K and 313K which caused partial hydrolysis of some glycosidic linkages giving rise to free α/β-L-rhamnose residues.

Lastly, a comparison of the ^{1}H NMR spectra of all samples (**Figure 6.26**) from development to GMP test lots shows the consistency of the antigen between the samples.

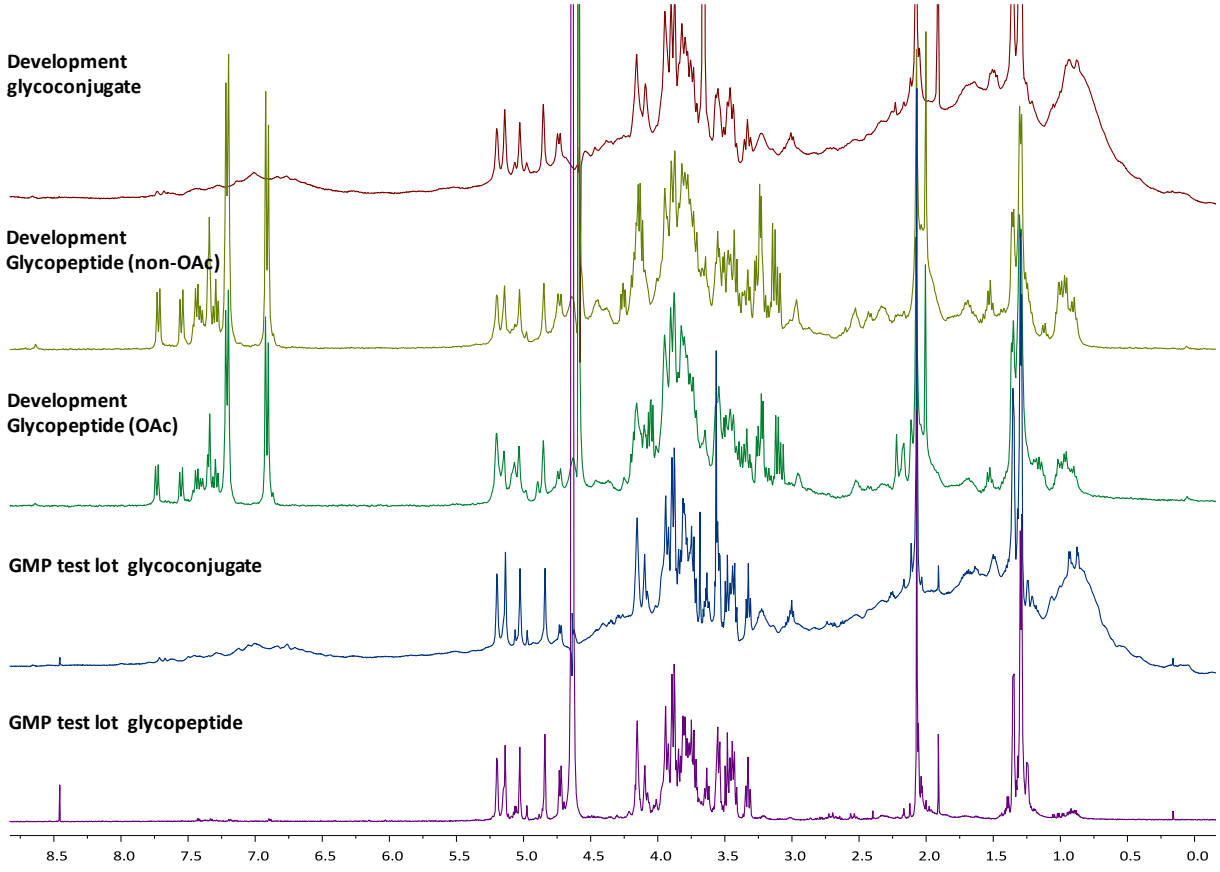


Figure 6.26: Comparison of the ^{1}H NMR spectra of all samples.

6.9 Summary and Conclusion

Analysis of the development and glycoconjugate GMP test lot vaccine using various 1D and 2D experiments confirmed the structure of the repeating unit, which is in agreement with literature.[17,18]

α-D-Glc*p*
1
↓
4
→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-GlcNA*cp*-(1→

The smaller peak at 4.97/103.99 ppm was assigned to the terminal RhaIII unit from non-reducing end (tRhaIII), which is in agreement with literature.[22] Integration of this peak can be estimated and indicated that the antigen was approximately 5-6 repeats long, but this is probably an underestimation.

The position and degree of *O*-acetylation could be determined from the *O*-acetylated development glycopeptide lot, Here, only the 3-*O*-position of RhaIII was found to be approximately 20-30% acetylated. Additional information was also obtained through spin system correlations and NOESY experiments, which proved the inter-sugar linkages. In addition, the GlcNAc residue on reducing end attached to Asn was identified from the unusual position of the crosspeak at 5.12/79.13 ppm.[42,43]

More detailed analysis of development and glycopeptide GMP test lot samples produced spectra with less overlap, sharper lines and better correlations. This again confirmed the structure of repeating unit, with integration indicating a slightly longer polysaccharide, estimated to be approximately 7 repeats long. In addition, H-1 of the reducing end GlcNAc (rGlcNAc) linked to asparagine could be identified.

In conclusion, NMR spectroscopy has been an invaluable tool for the analysis of the glycoconjugate and glycopeptide development and GMP test lot samples. This has shown consistency between batches and has definitively confirmed antigen structure, identity and integrity.

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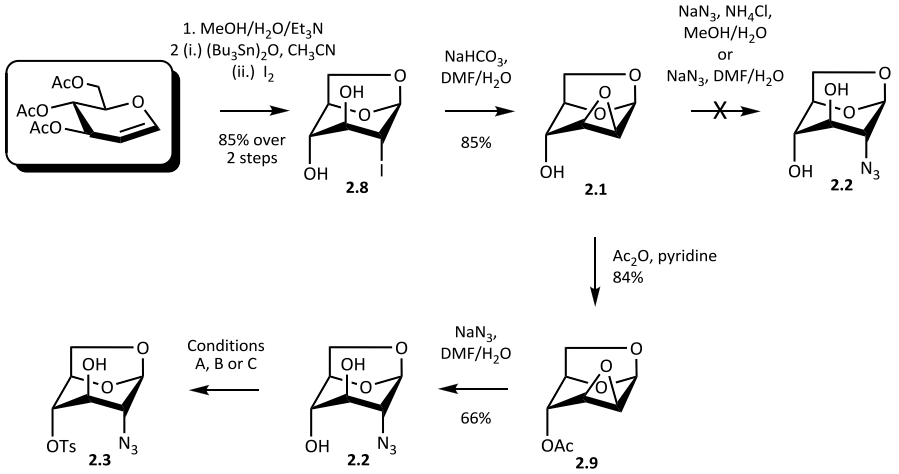
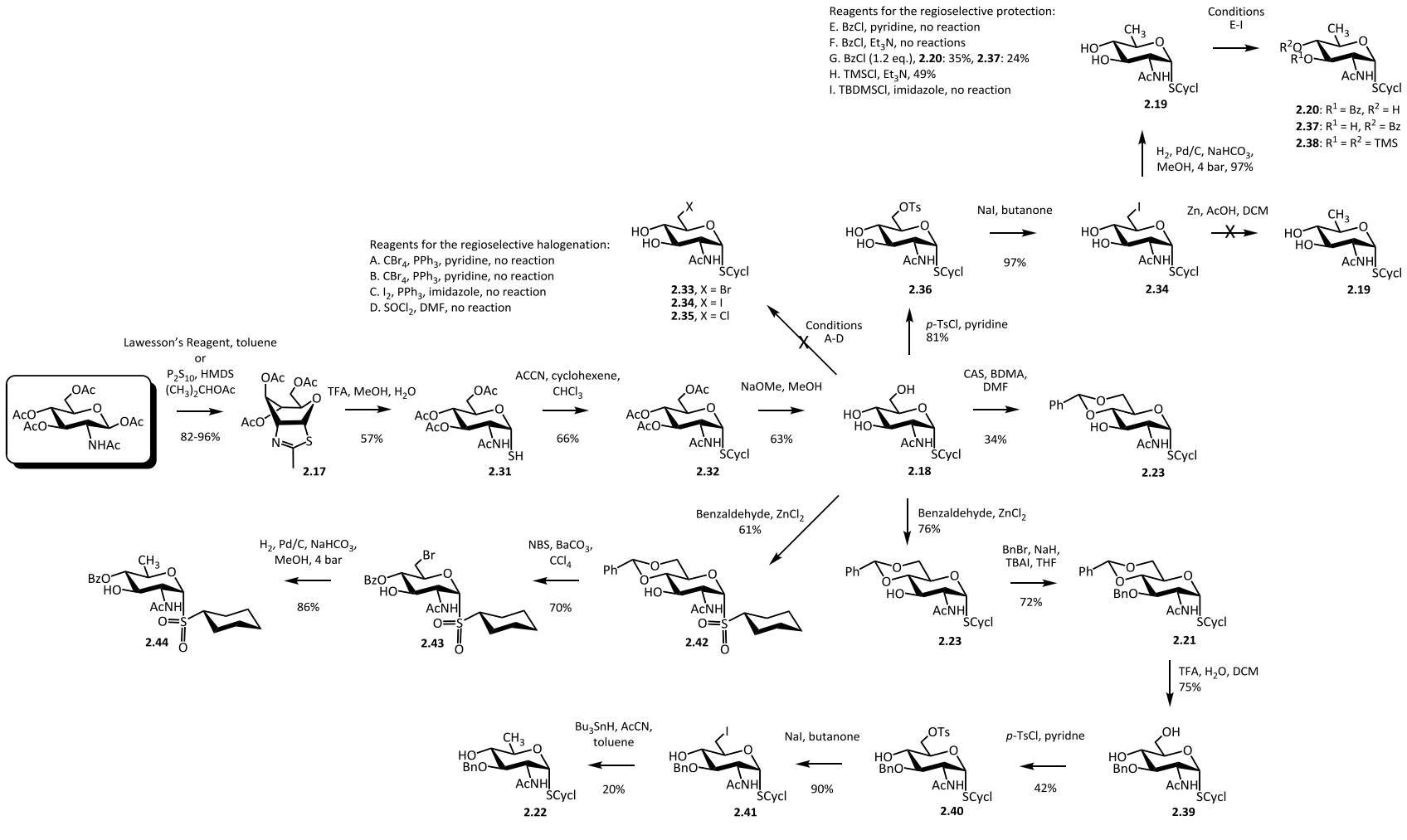
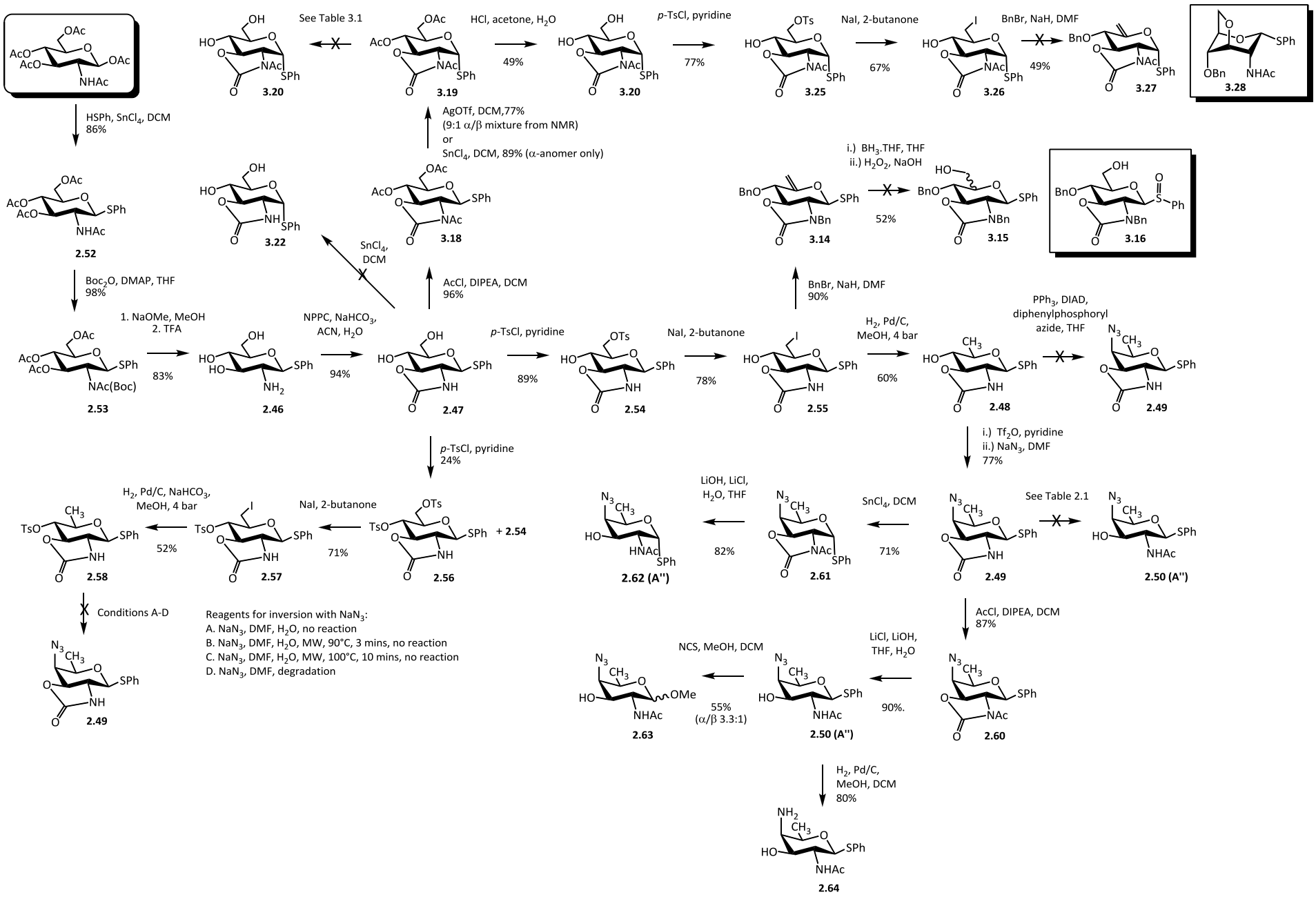
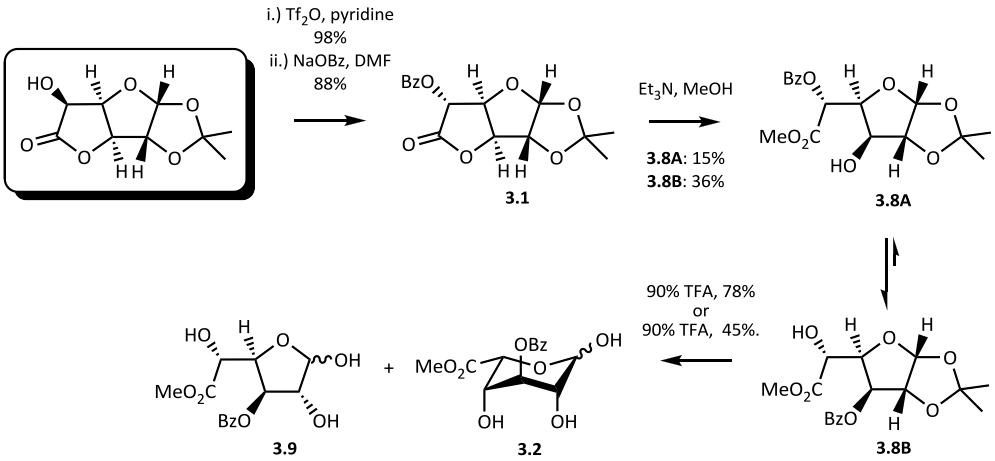
Appendix 1: Summary schemes of compounds synthesised



Reaction conditions for regioselective tosylation:
A. *p*-TsCl, pyridine, no reactions
B. *p*-TsCl, Et_3N, DCM, 19%
C. a.) $(Bu_3Sn)_2O$, toluene b.) *p*-TsCl, DMAP, 25%.


Reagents for the regioselective protection:
E. BzCl, pyridine, no reaction
F. BzCl, Et3N, no reactions
G. BzCl (1.2 eq.), 2.20: 35%, 2.37: 24%
H. TMSCl, Et3N, 49%
I. TBDMSCl, imidazole, no reaction
Conditions E-I
2.19
2.20: R1 = Bz, R2 = H
2.37: R1 = H, R2 = Bz
2.38: R1 = R2 = TMS
H2, Pd/C, NaHCO3, MeOH, 4 bar, 97%
Reagents for the regioselective halogenation:
A. CBr4, PPh3, pyridine, no reaction
B. CBr4, PPh3, pyridine, no reaction
C. I2, PPh3, imidazole, no reaction
D. SOCl2, DMF, no reaction
2.33, X = Br
2.34, X = I
2.35, X = Cl
Conditions A-D
p-TsCl, pyridine 81%
2.36
NaI, butanone
97%
Zn, AcOH, DCM
Lawesson's Reagent, toluene or P2S10, HMDS (CH3)2CHOAc
82-96%
2.17
TFA, MeOH, H2O
57%
2.31
ACCN, cyclohexene, CHCl3
66%
2.32
NaOMe, MeOH
63%
2.18
CAS, BDMA, DMF
34%
2.23
Benzaldehyde, ZnCl2
61%
Benzaldehyde, ZnCl2
76%
BnBr, NaH, TBAI, THF
72%
2.21
TFA, H2O, DCM
75%
2.39
p-TsCl, pyridne
42%
2.40
NaI, butanone
90%
2.41
Bu3SnH, AcCN, toluene
20%
2.22
NBS, BaCO3, CCl4
70%
2.42
H2, Pd/C, NaHCO3, MeOH, 4 bar
86%
2.43
2.44

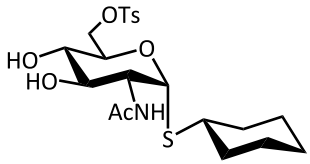

See Table 3.1
HCl, acetone, H2O
49%
p-TsCl, pyridine
77%
NaI, 2-butanone
67%
BnBr, NaH, DMF
49%
3.20
3.19
3.20
3.25
3.26
3.27
3.28
HSPh, SnCl4, DCM
86%
AgOTf, DCM,77%
(9:1 α/β mixture from NMR)
or
SnCl4, DCM, 89% (α-anomer only)
i.) BH3.THF, THF
ii.) H2O2, NaOH
52%
3.14
3.15
3.16
2.52
3.22
3.18
SnCl4, DCM
AcCl, DIPEA, DCM
96%
BnBr, NaH, DMF
90%
Boc2O, DMAP, THF
98%
1. NaOMe, MeOH
2. TFA
83%
NPPC, NaHCO3,
ACN, H2O
94%
p-TsCl, pyridine
89%
NaI, 2-butanone
78%
H2, Pd/C,
MeOH, 4 bar
60%
PPh3, DIAD,
diphenylphosphoryl
azide, THF
2.53
2.46
2.47
2.54
2.55
2.48
2.49
p-TsCl, pyridine
24%
i.) Tf2O, pyridine
ii.) NaN3, DMF
77%
H2, Pd/C, NaHCO3,
MeOH, 4 bar
52%
NaI, 2-butanone
71%
+ 2.54
LiOH, LiCl,
H2O, THF
82%
SnCl4, DCM
71%
See Table 2.1
2.58
2.57
2.56
2.62 (A'')
2.61
2.49
2.50 (A'')
Conditions A-D
Reagents for inversion with NaN3:
A. NaN3, DMF, H2O, no reaction
B. NaN3, DMF, H2O, MW, 90°C, 3 mins, no reaction
C. NaN3, DMF, H2O, MW, 100°C, 10 mins, no reaction
D. NaN3, DMF, degradation
AcCl, DIPEA, DCM
87%
NCS, MeOH, DCM
LiCl, LiOH,
THF, H2O
55%
(α/β 3.3:1)
90%.
2.49
2.63
2.50 (A'')
2.60
H2, Pd/C,
MeOH, DCM
80%
2.64

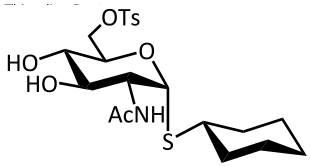

i.) Tf2O, pyridine
98%
ii.) NaOBz, DMF
88%
3.1
Et3N, MeOH
3.8A: 15%
3.8B: 36%
3.8A
90% TFA, 78%
or
90% TFA, 45%.
3.8B
3.9
3.2

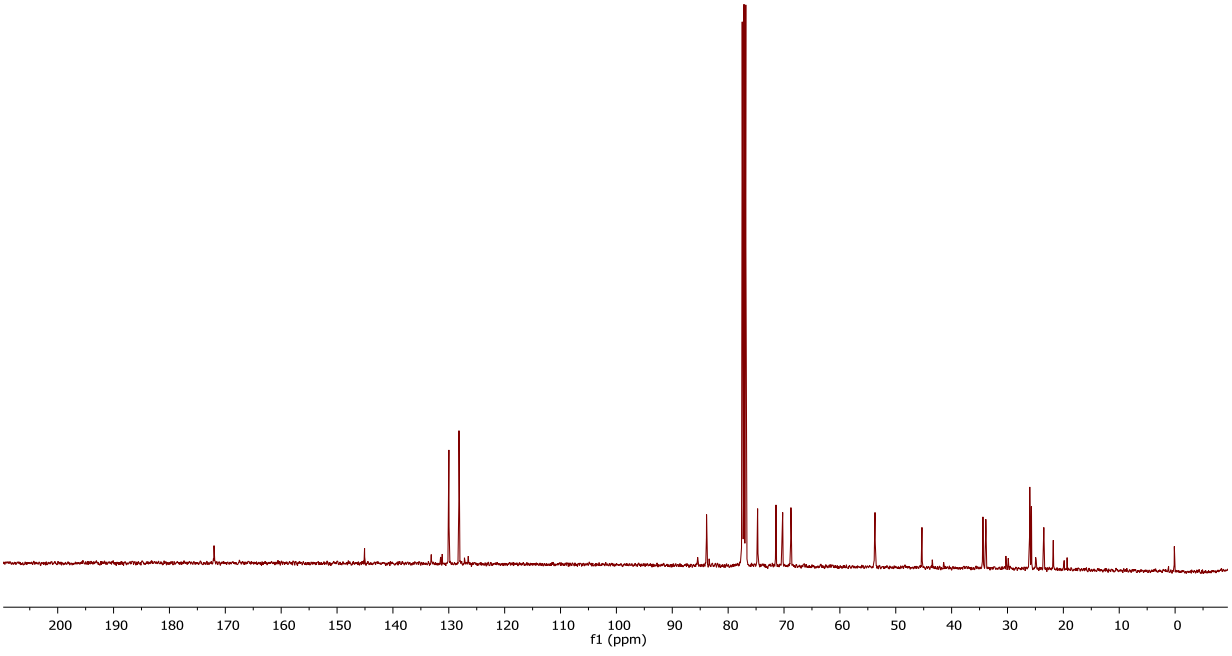
Appendix 2: NMR spectra of compounds synthesised

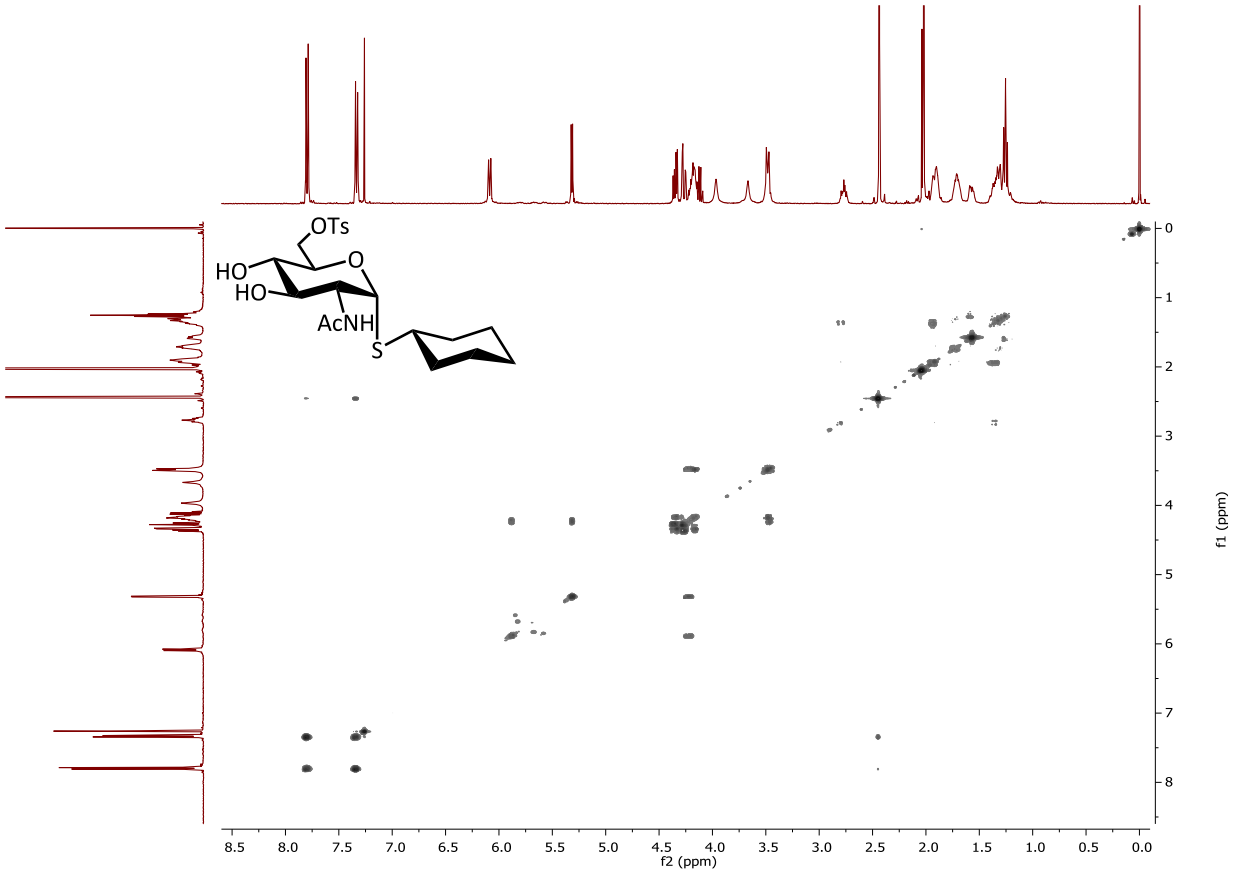
2.1 Chapter 2 NMR spectra

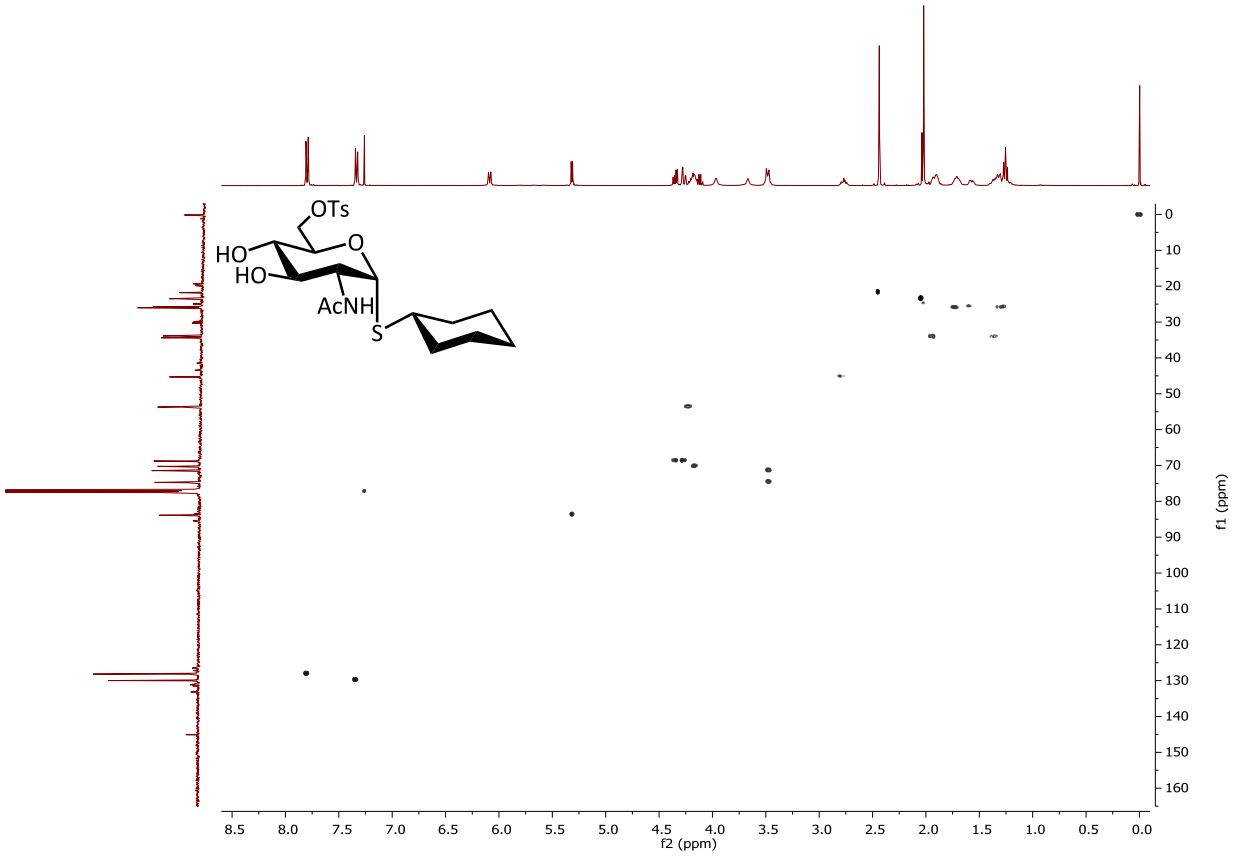
Cyclohexyl 2-acetamido-2-deoxy-1-thio-6-*O*-tosyl-α-D-glucopyranoside (2.36).



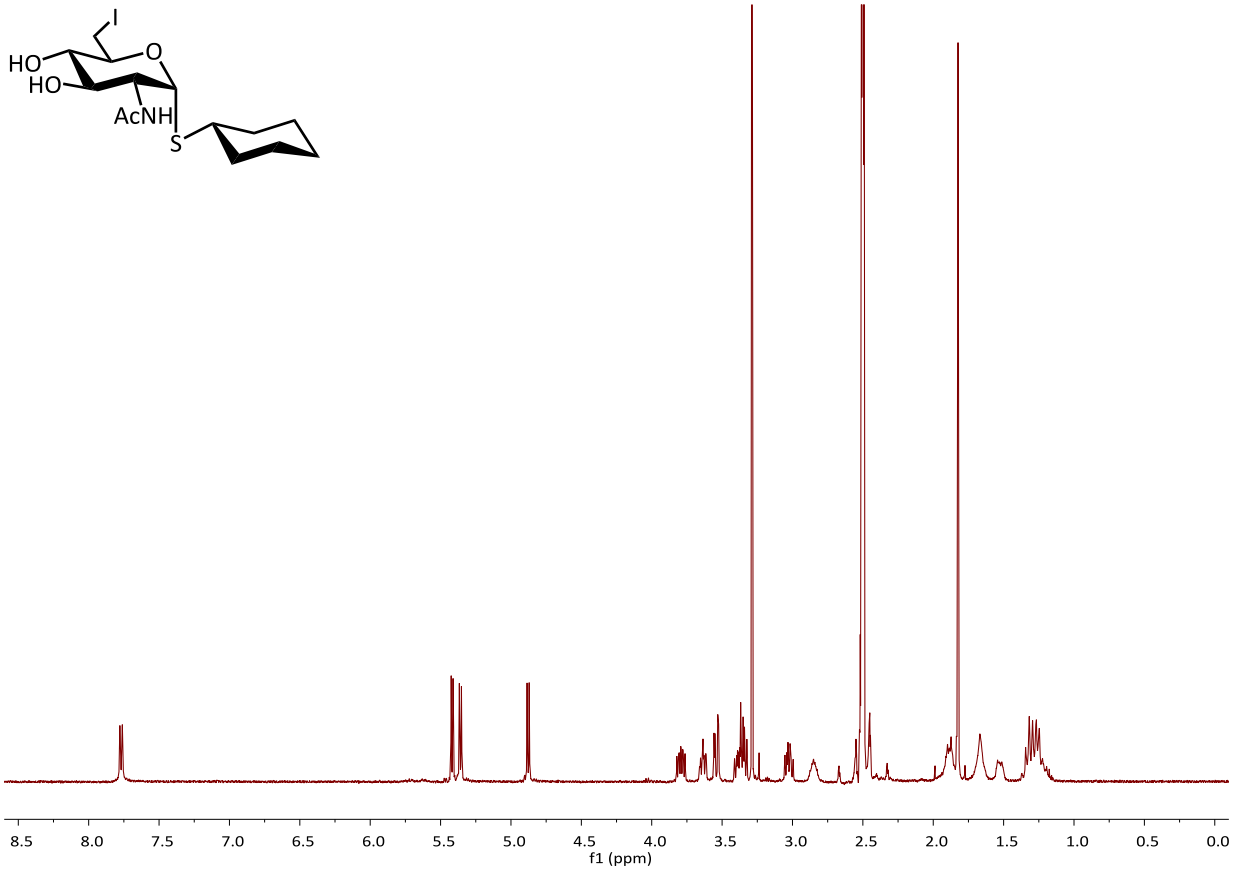


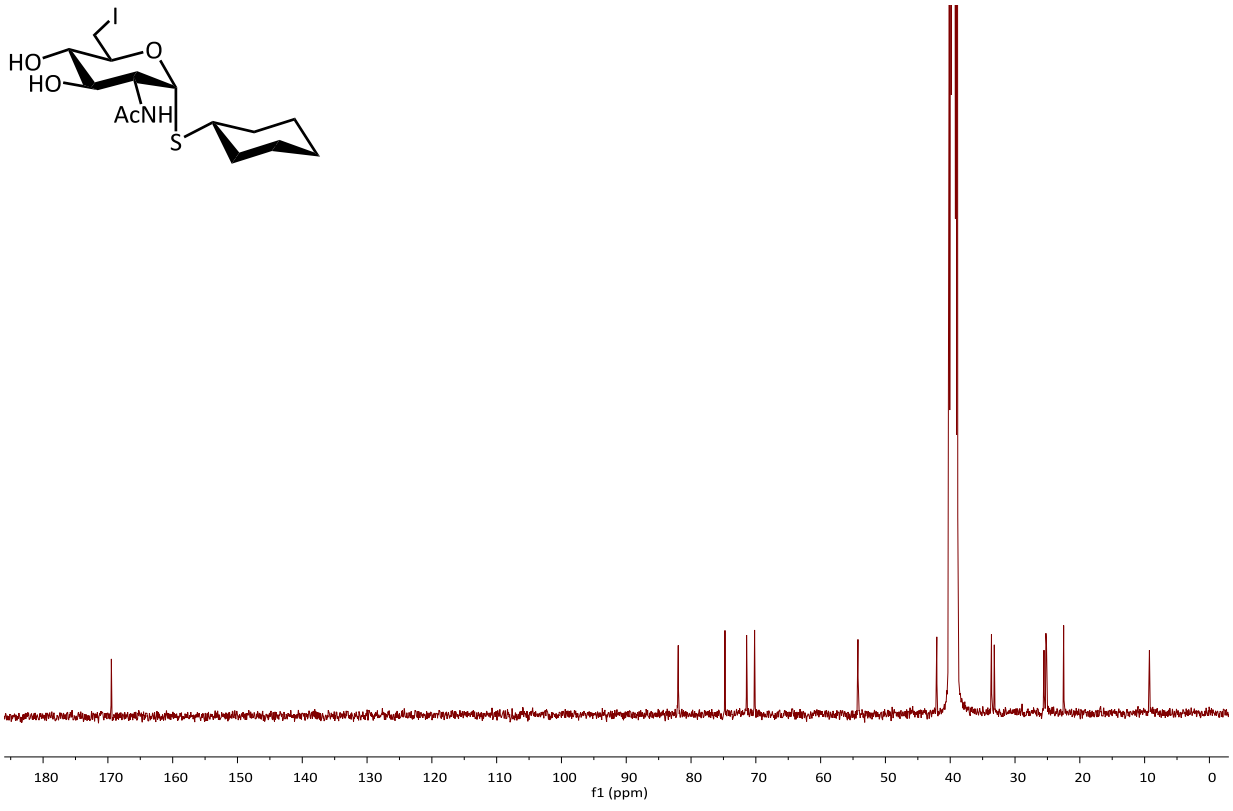


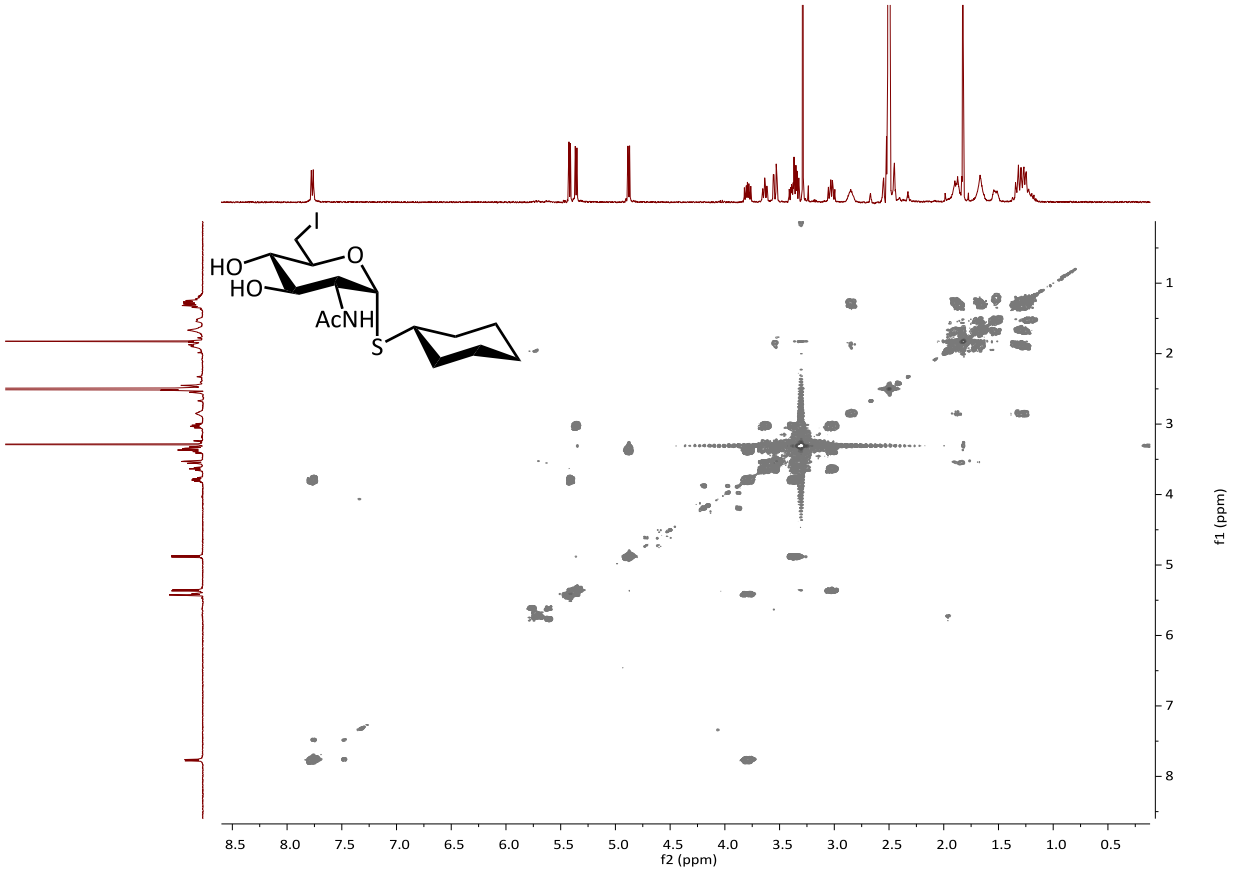

OTs
HO
HO
O
AcNH
S
f2 (ppm)
f1 (ppm)

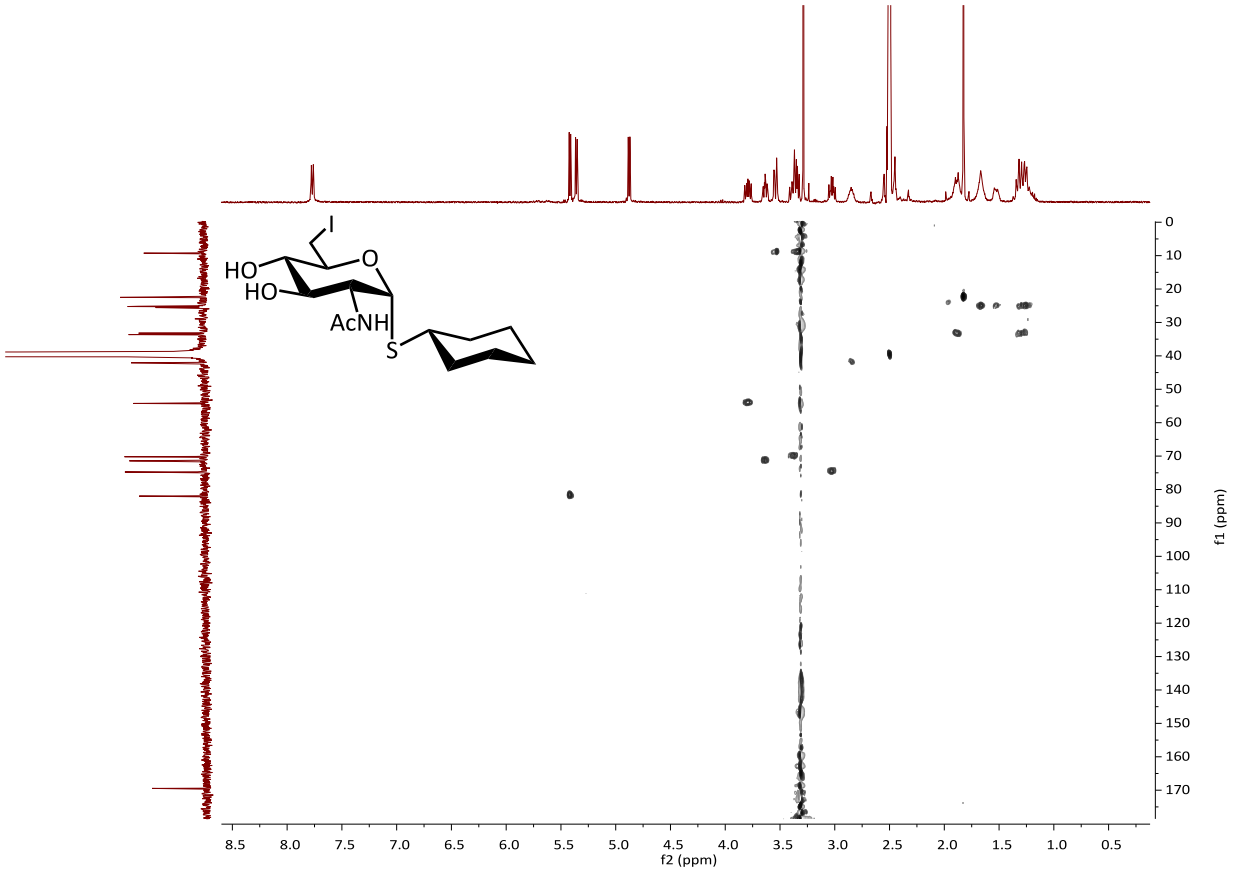

OTs
HO
HO
O
AcNH
S
f2 (ppm)
f1 (ppm)

Cyclohexyl 2-acetamido-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (2.34).

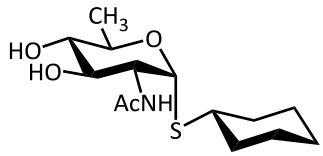


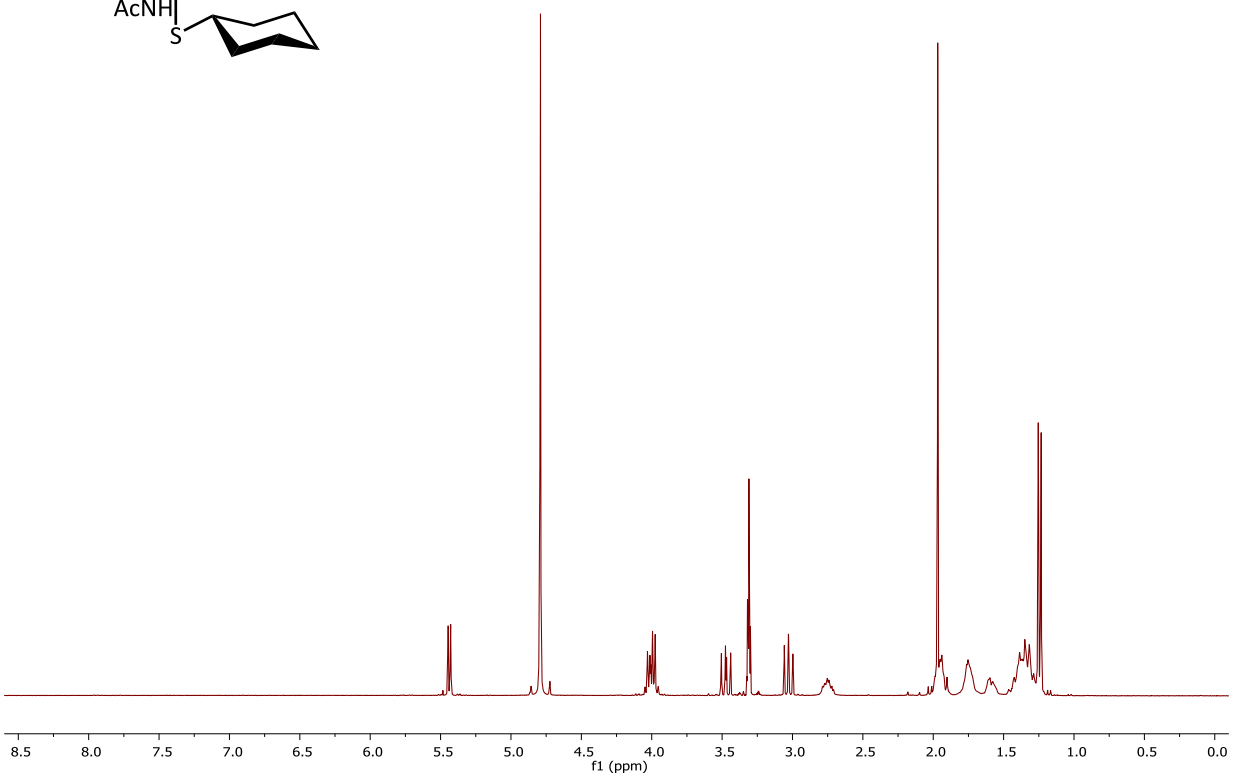


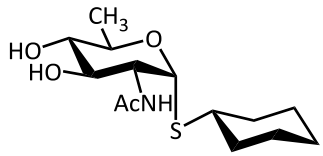

I
O
HO
HO
AcNH
S
f1 (ppm)
f2 (ppm)

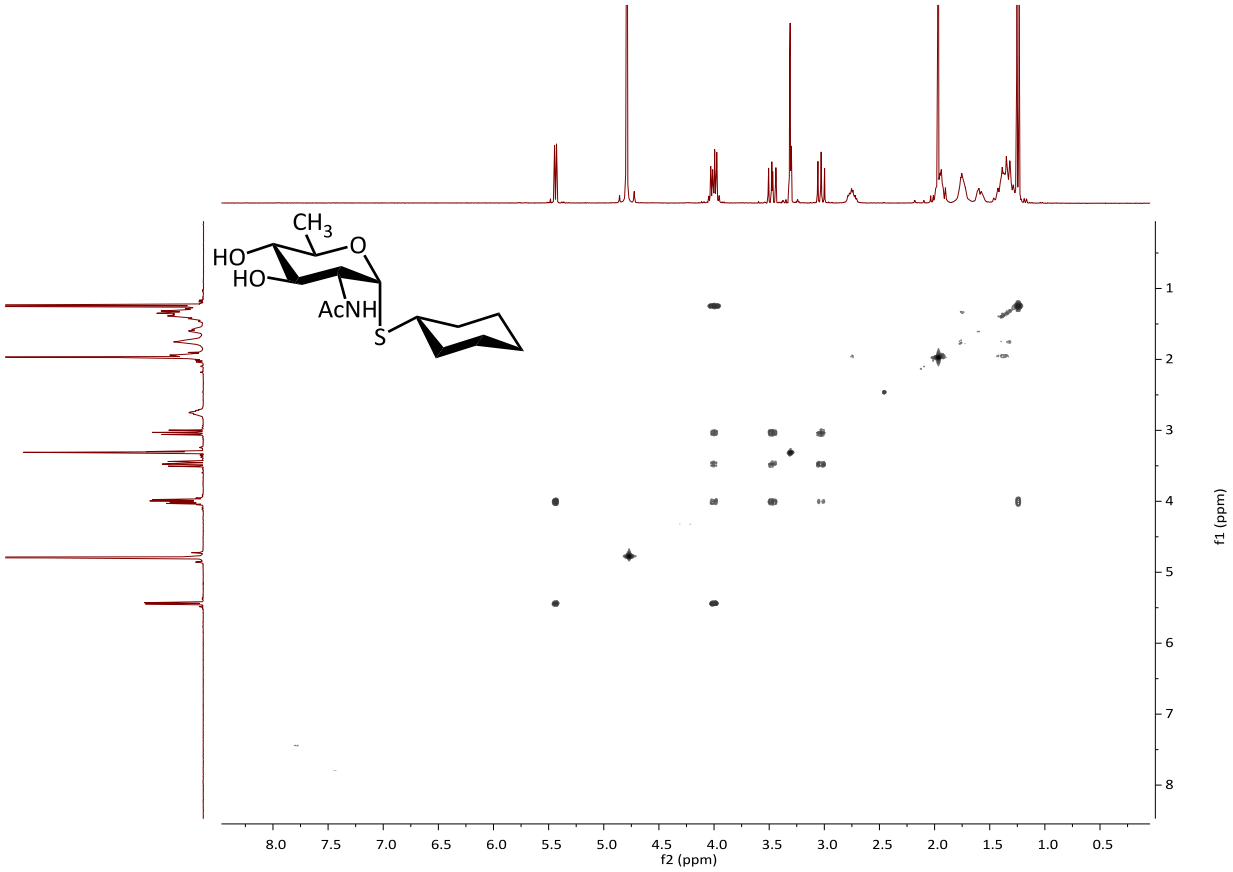

I
O
HO
HO
AcNH
S
f1 (ppm)
f2 (ppm)

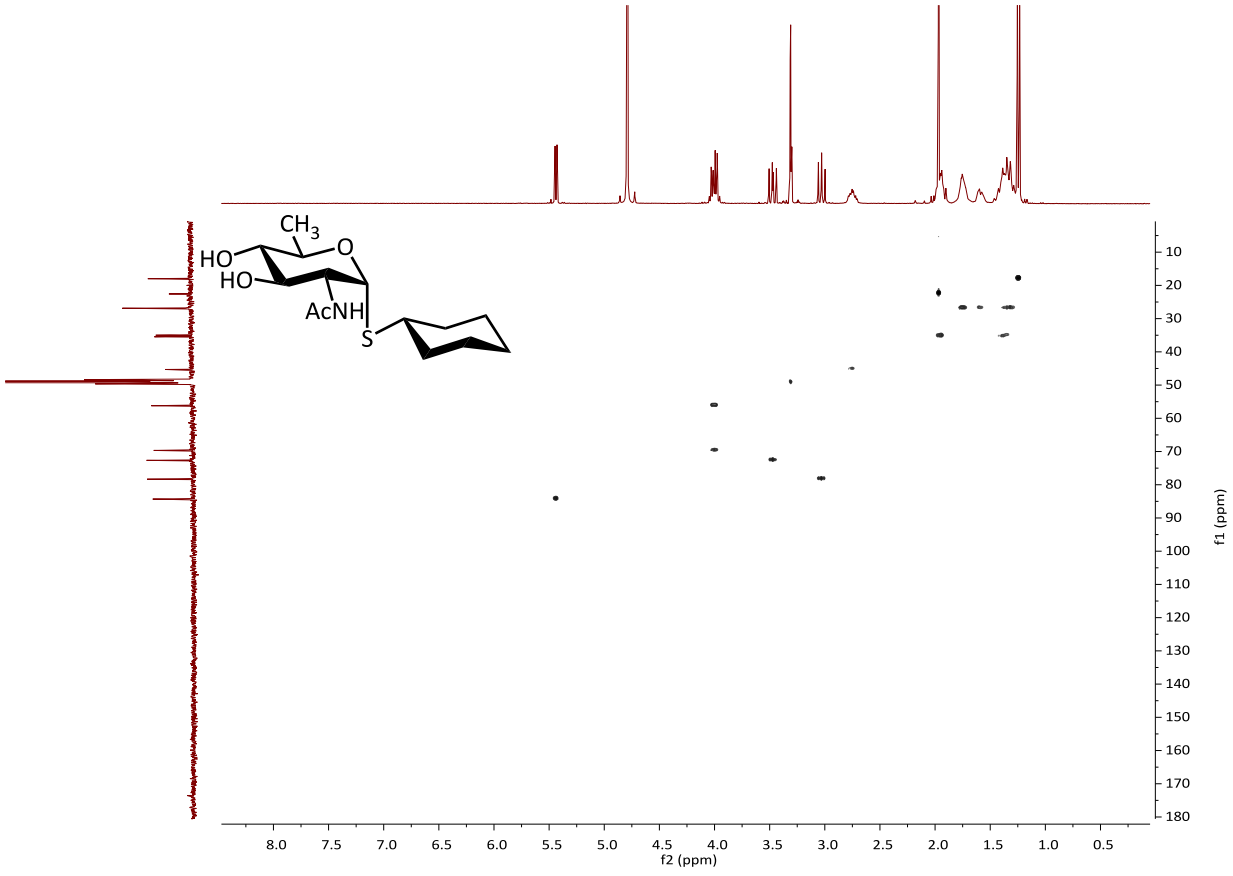
Cyclohexyl 2-acetamido-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.19).



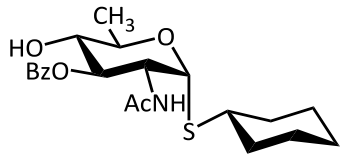




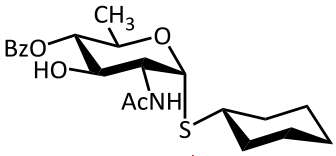

CH3
O
HO
HO
AcNH
S
f2 (ppm)
f1 (ppm)

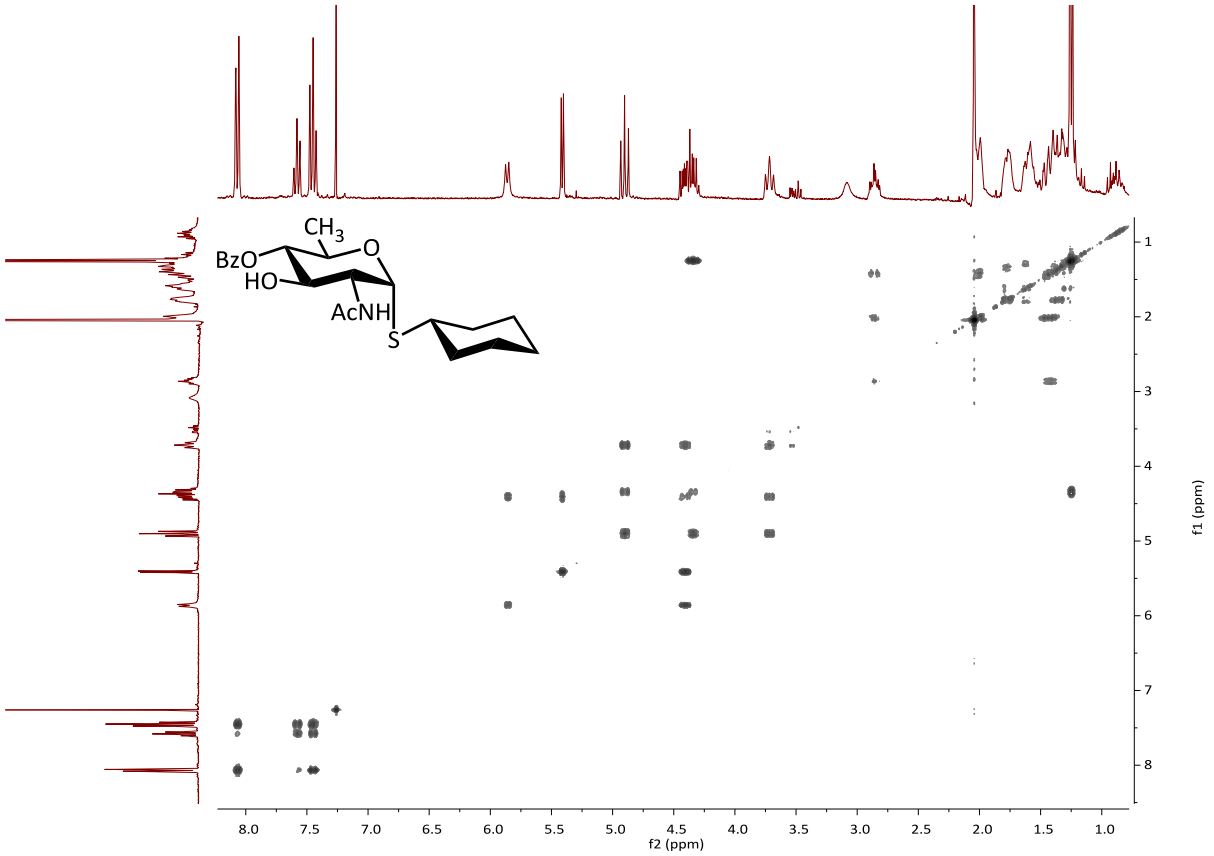

CH3
O
HO
HO
AcNH
S
f2 (ppm)
f1 (ppm)

Cyclohexyl 2-acetamido-3-*O*-benzoyl-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.20).

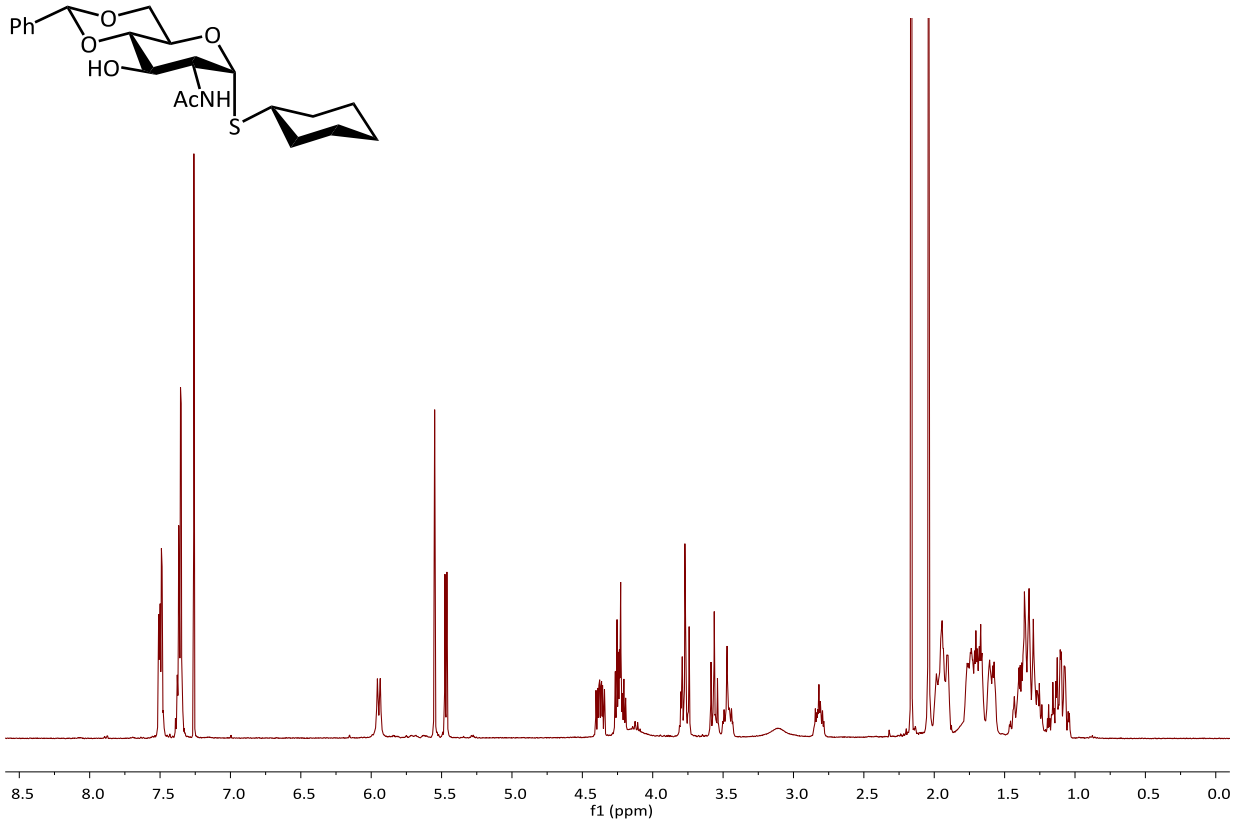


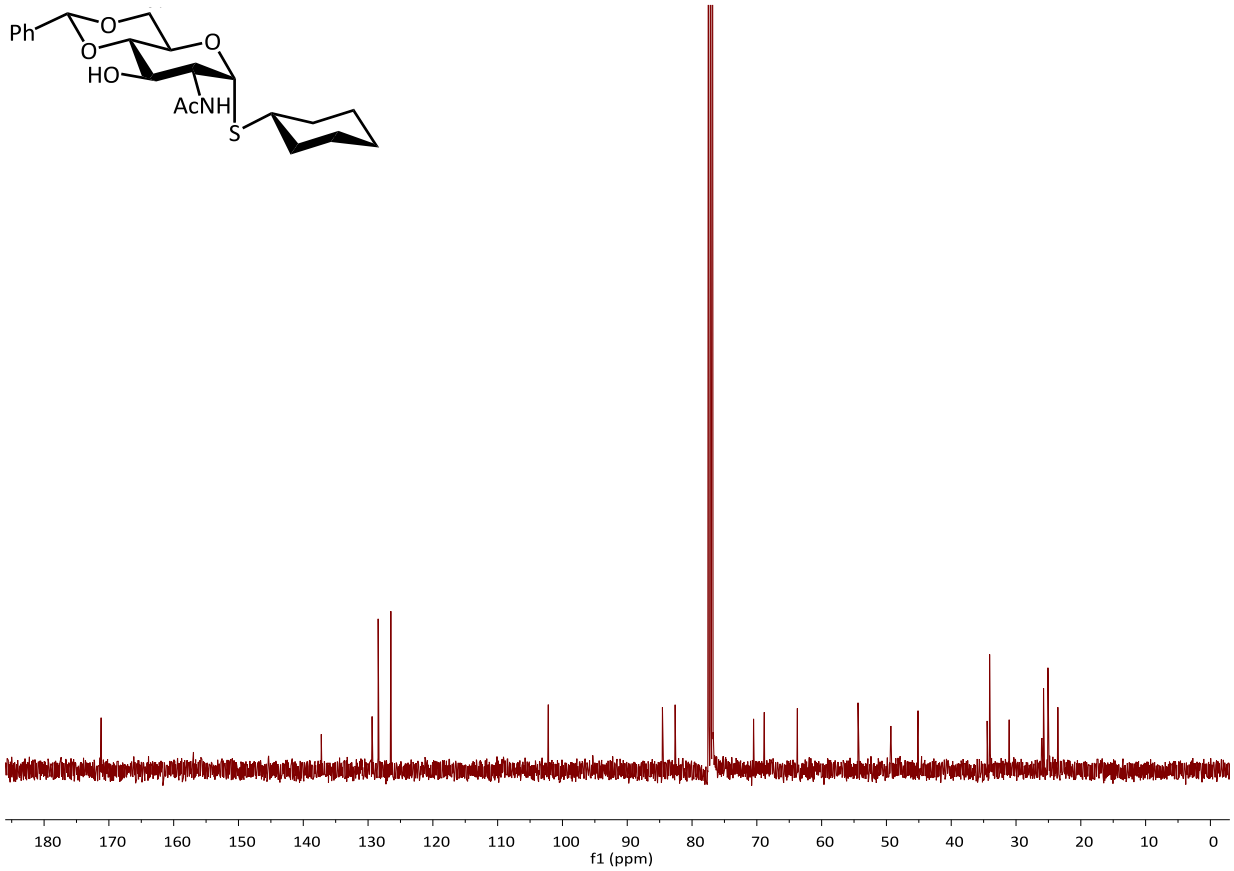
Cyclohexyl 2-acetamido-4-*O*-benzoyl-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.37).

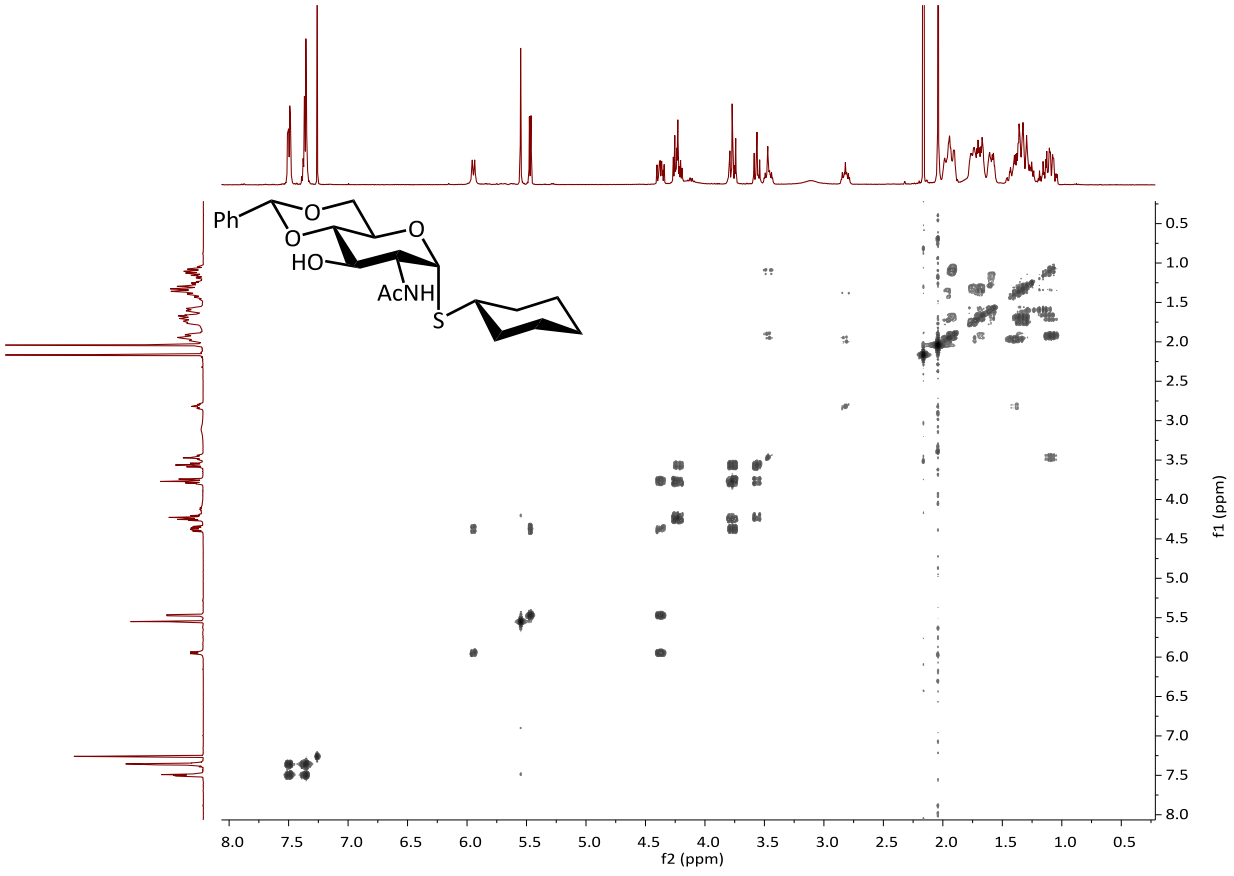


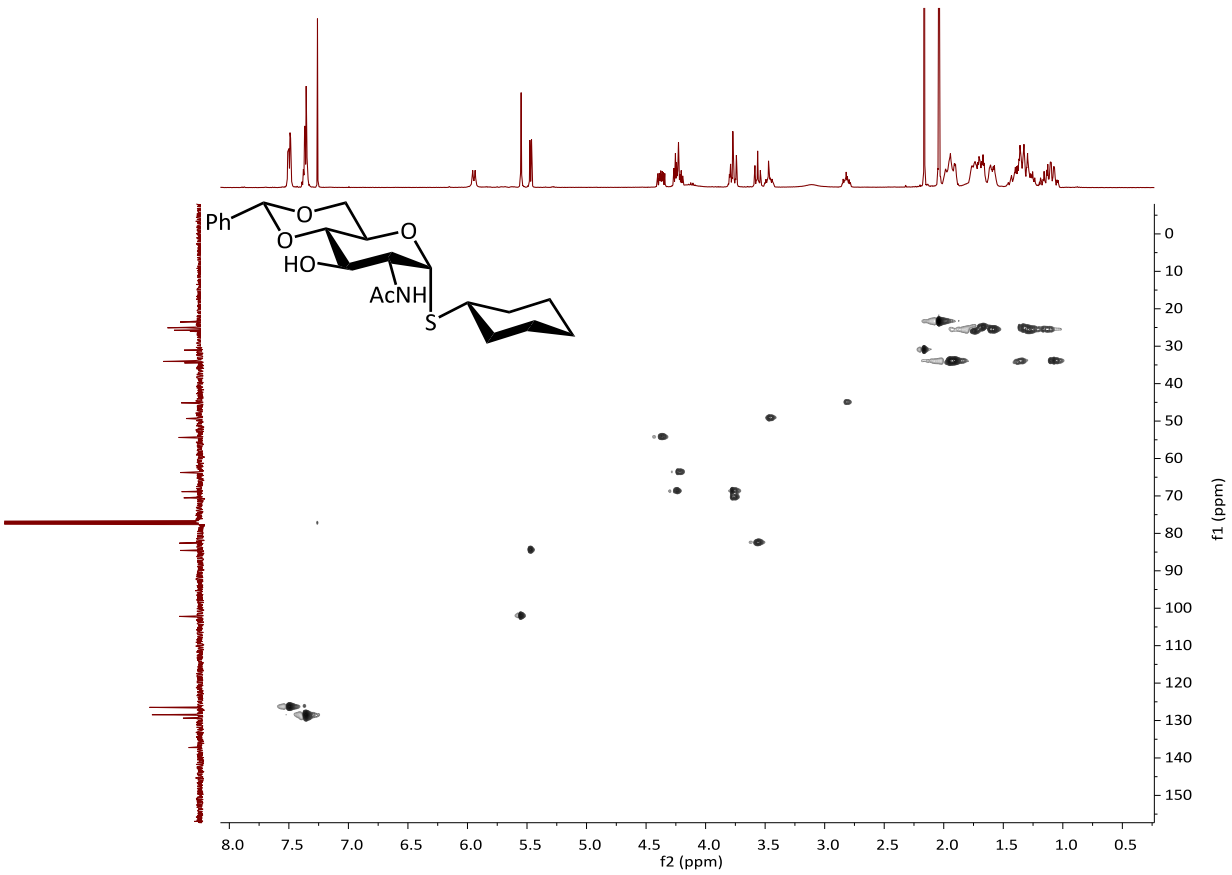


Cyclohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (2.23).

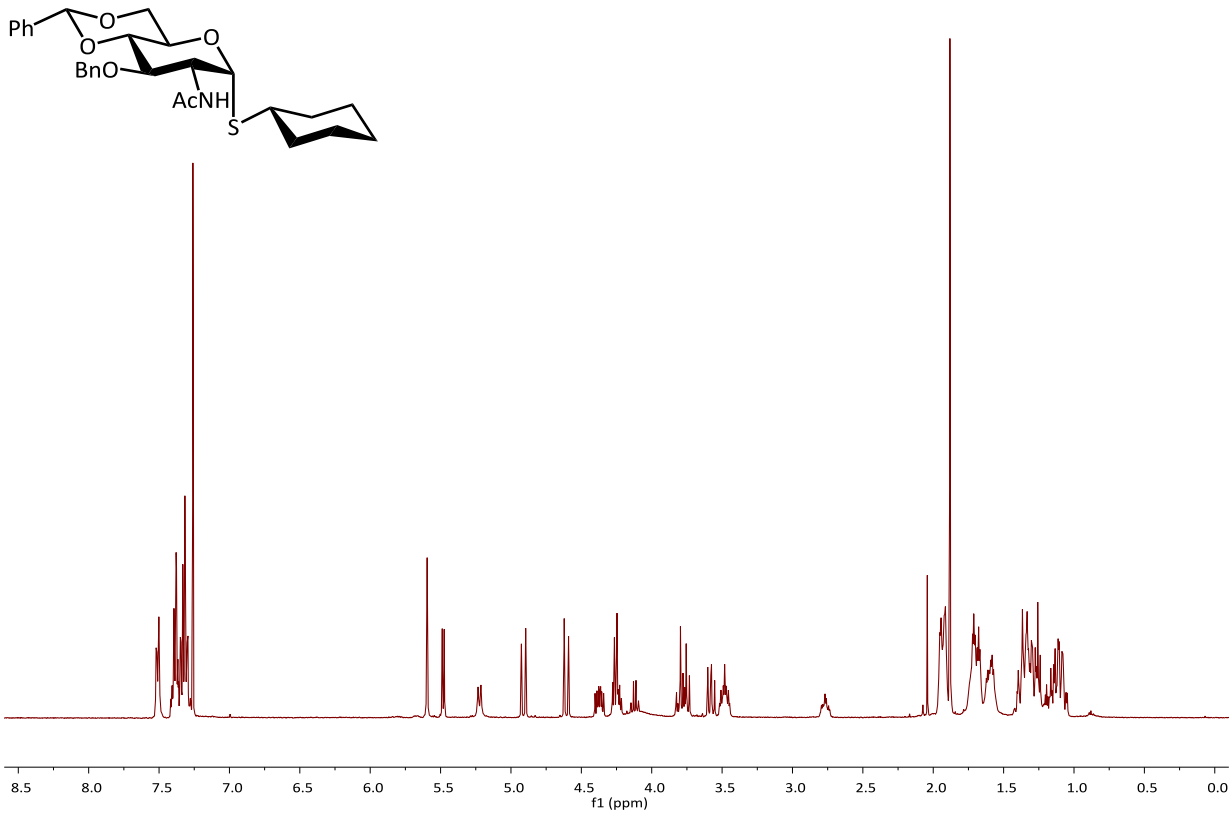


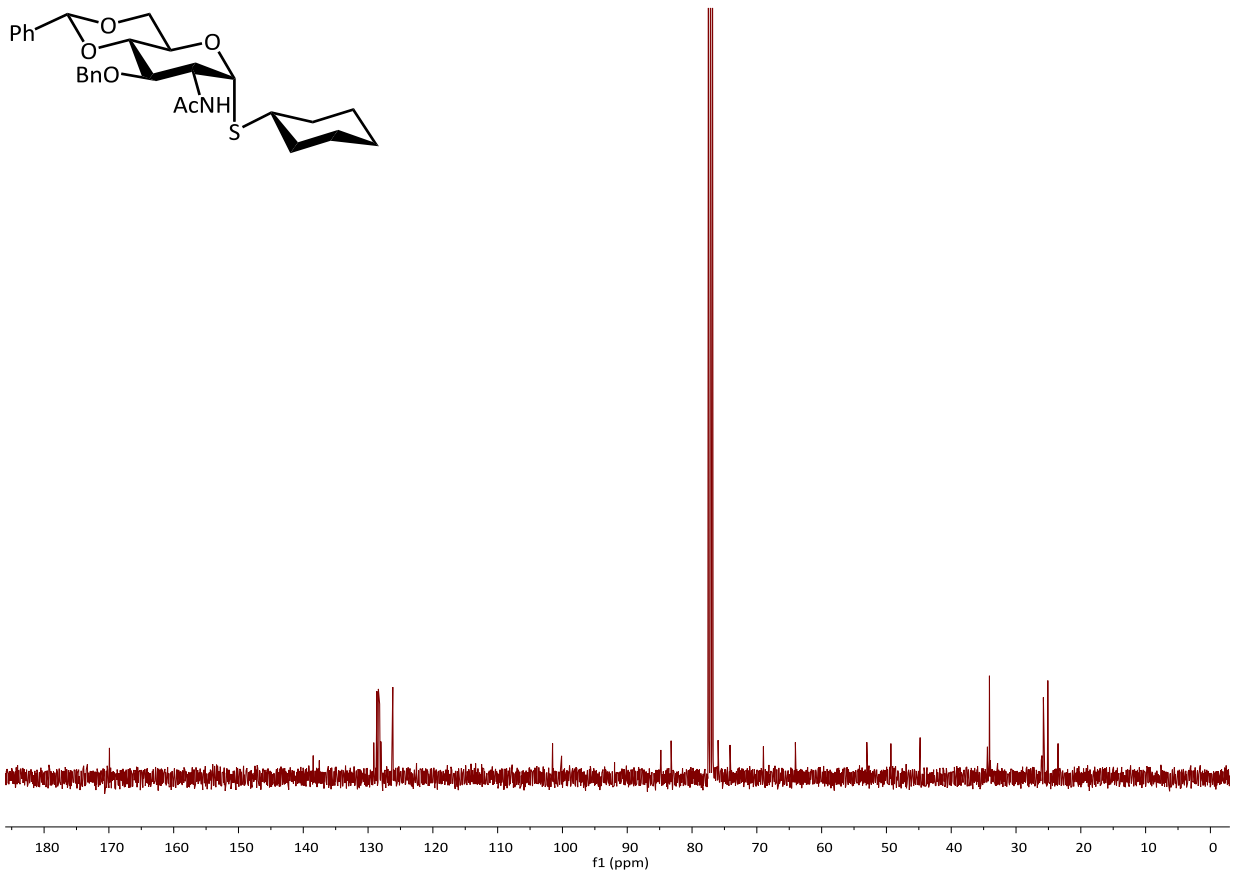

Ph
O
O
O
HO
AcNH
S
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

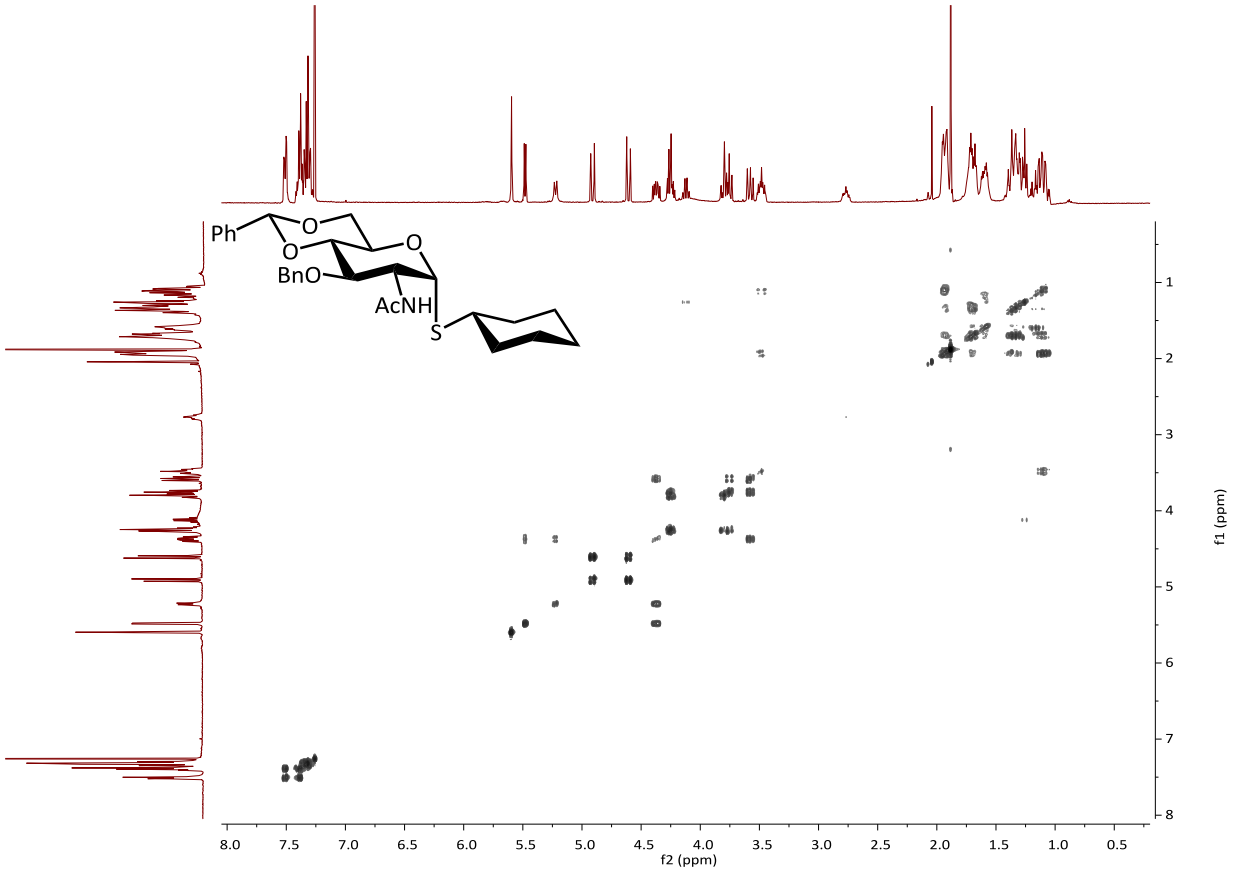

Ph
O
O
O
HO
AcNH
S
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
f1 (ppm)
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)

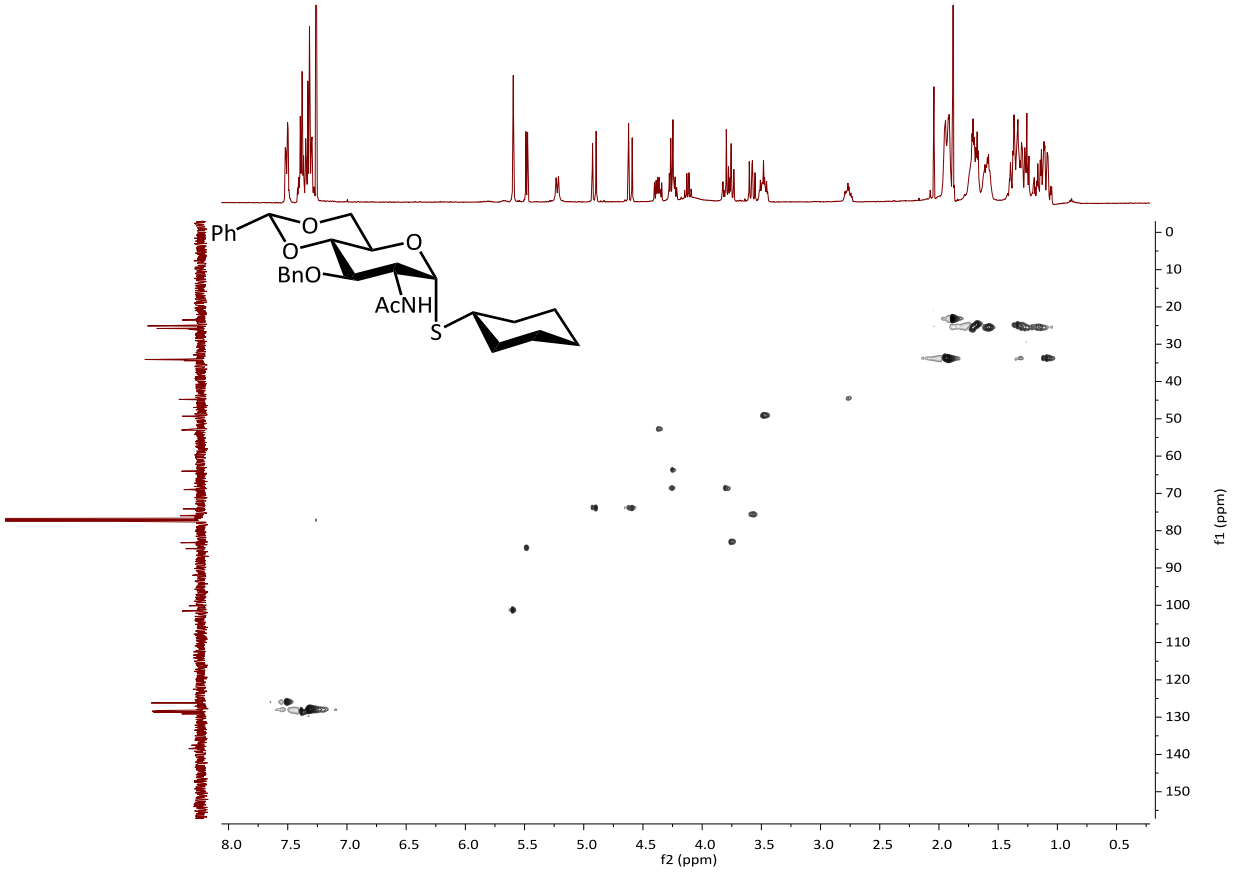


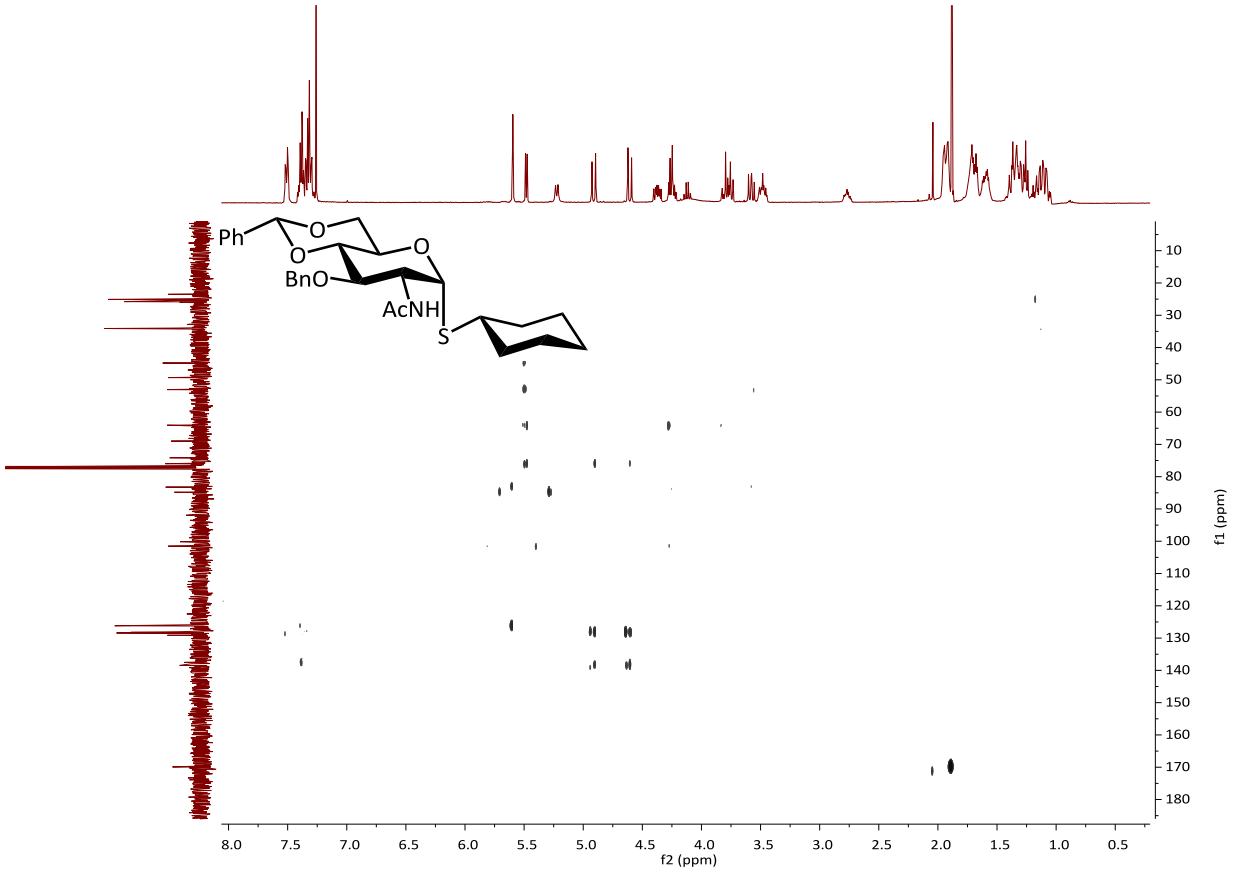
Cyclohexyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (2.21).



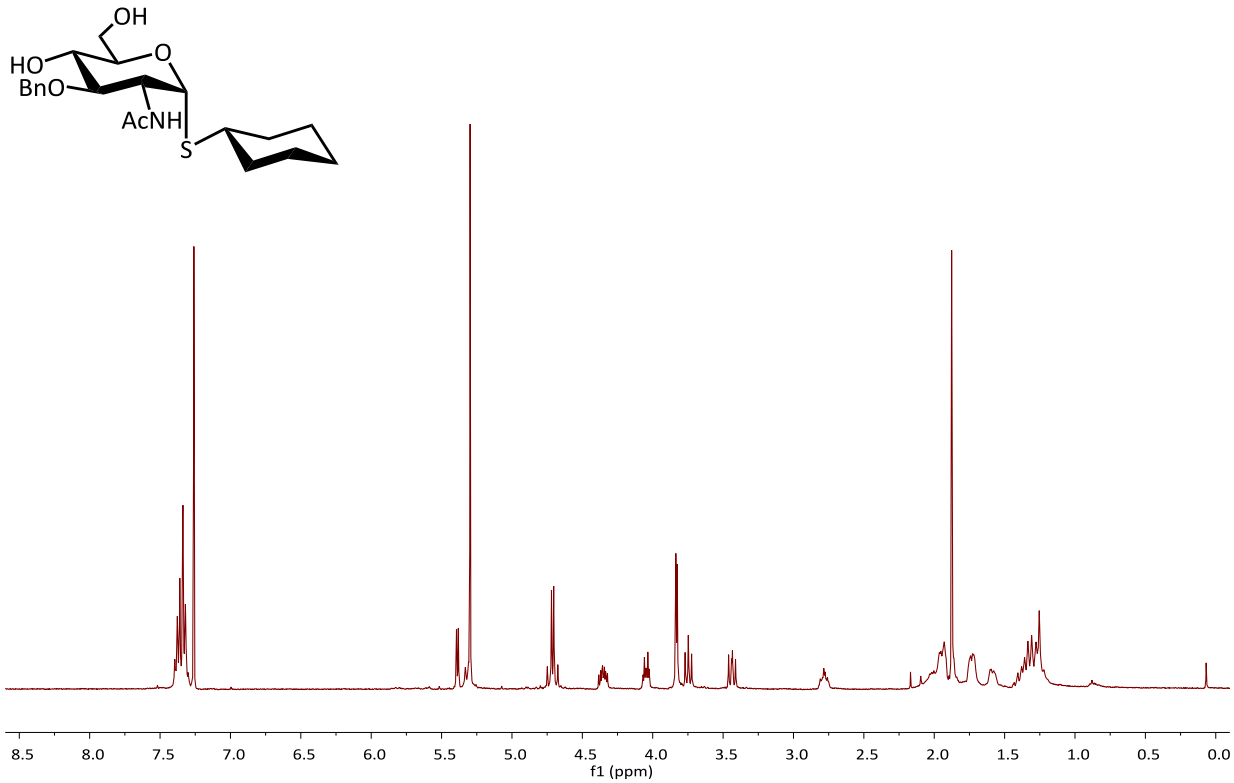

Ph
O
O
O
BnO
AcNH
S
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

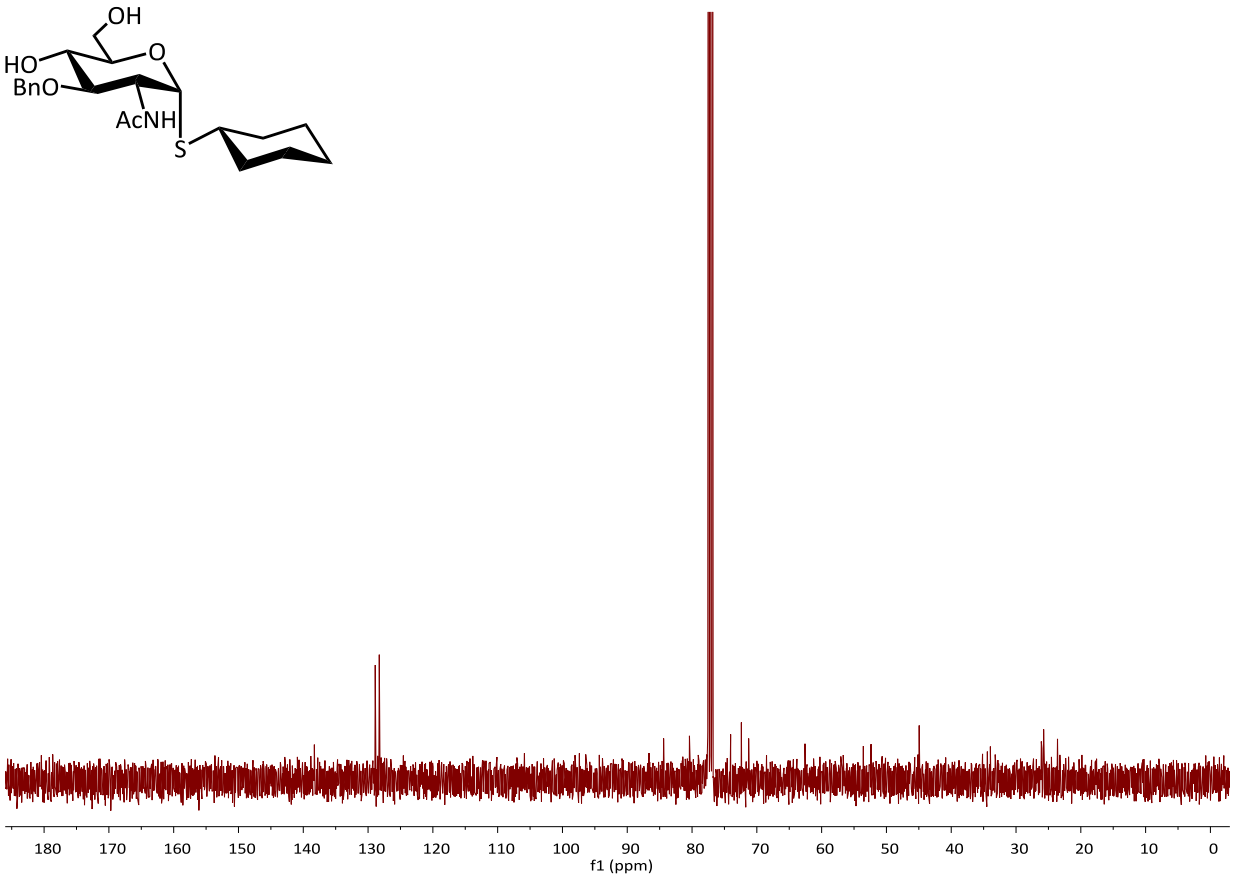

Ph
O
O
O
BnO
AcNH
S
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)
1 2 3 4 5 6 7 8
f1 (ppm)

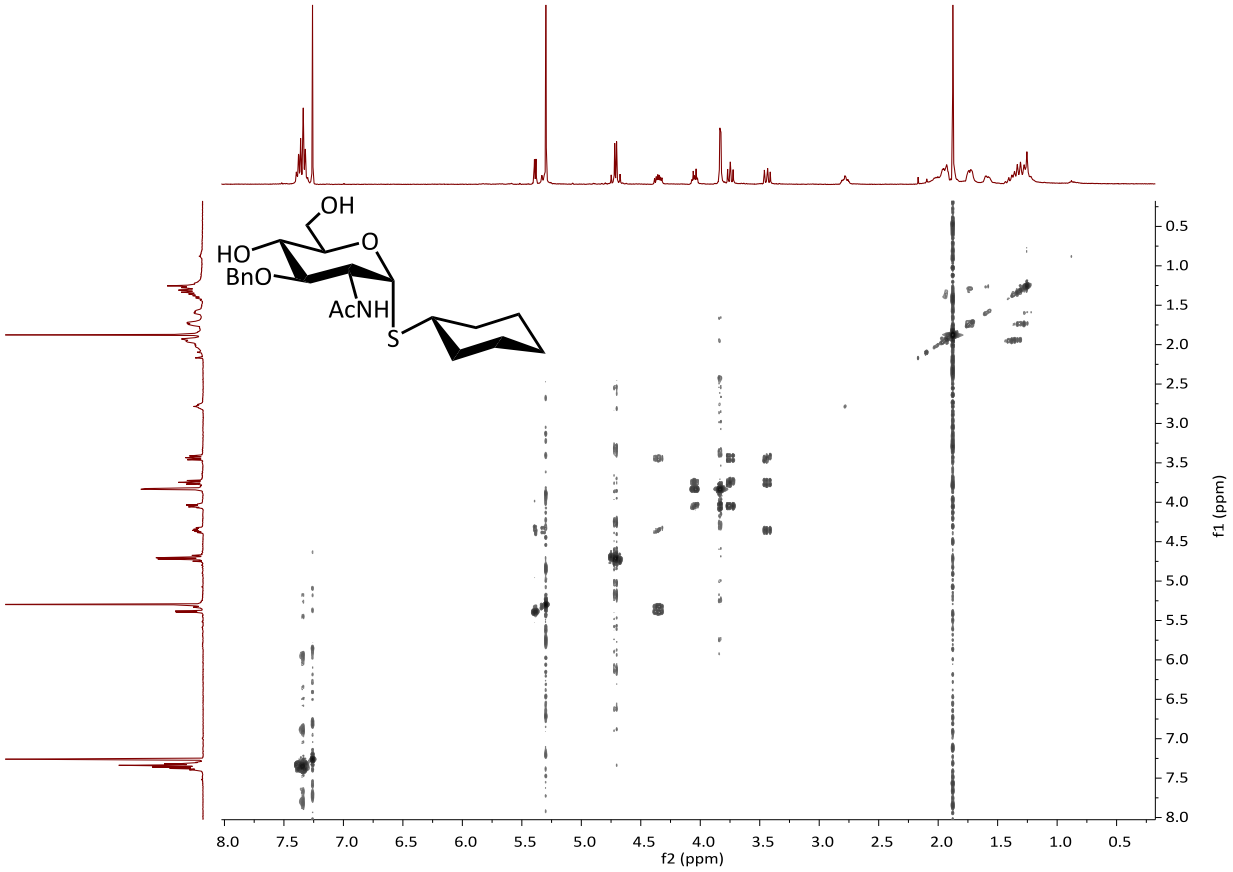

Ph
O
O
O
BnO
AcNH
S
f2 (ppm)
f1 (ppm)

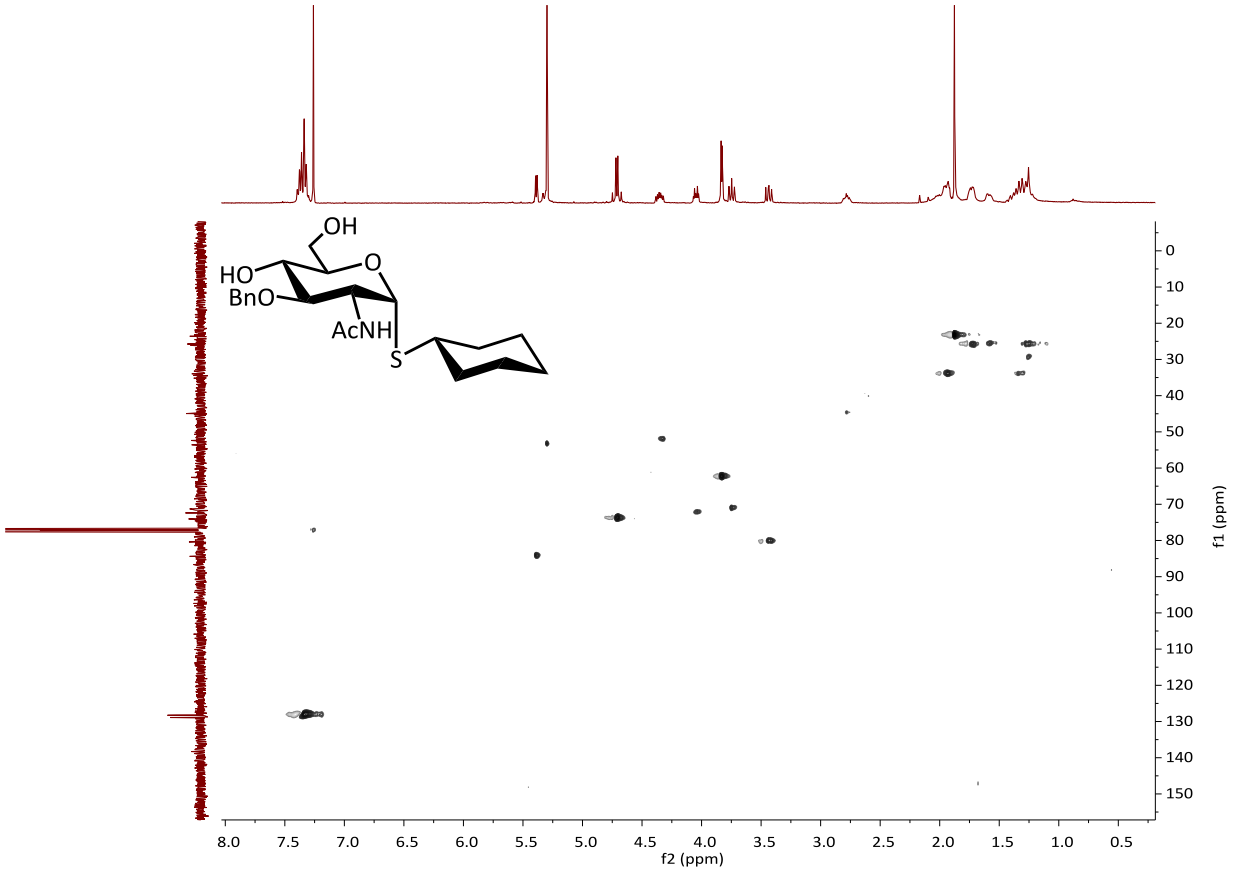

Ph
O
O
O
BnO
AcNH
S
f2 (ppm)
f1 (ppm)

Cyclohexyl 2-acetamido-3-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside (2.39).

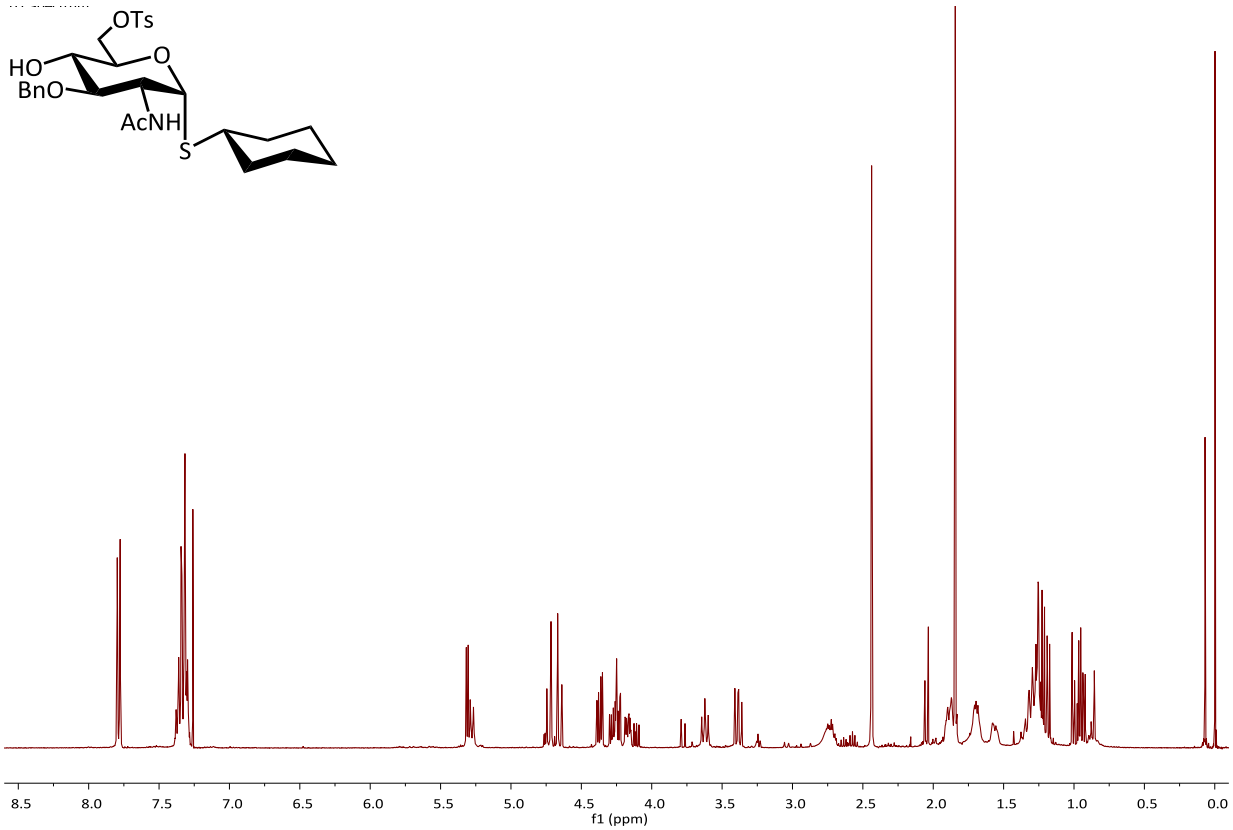


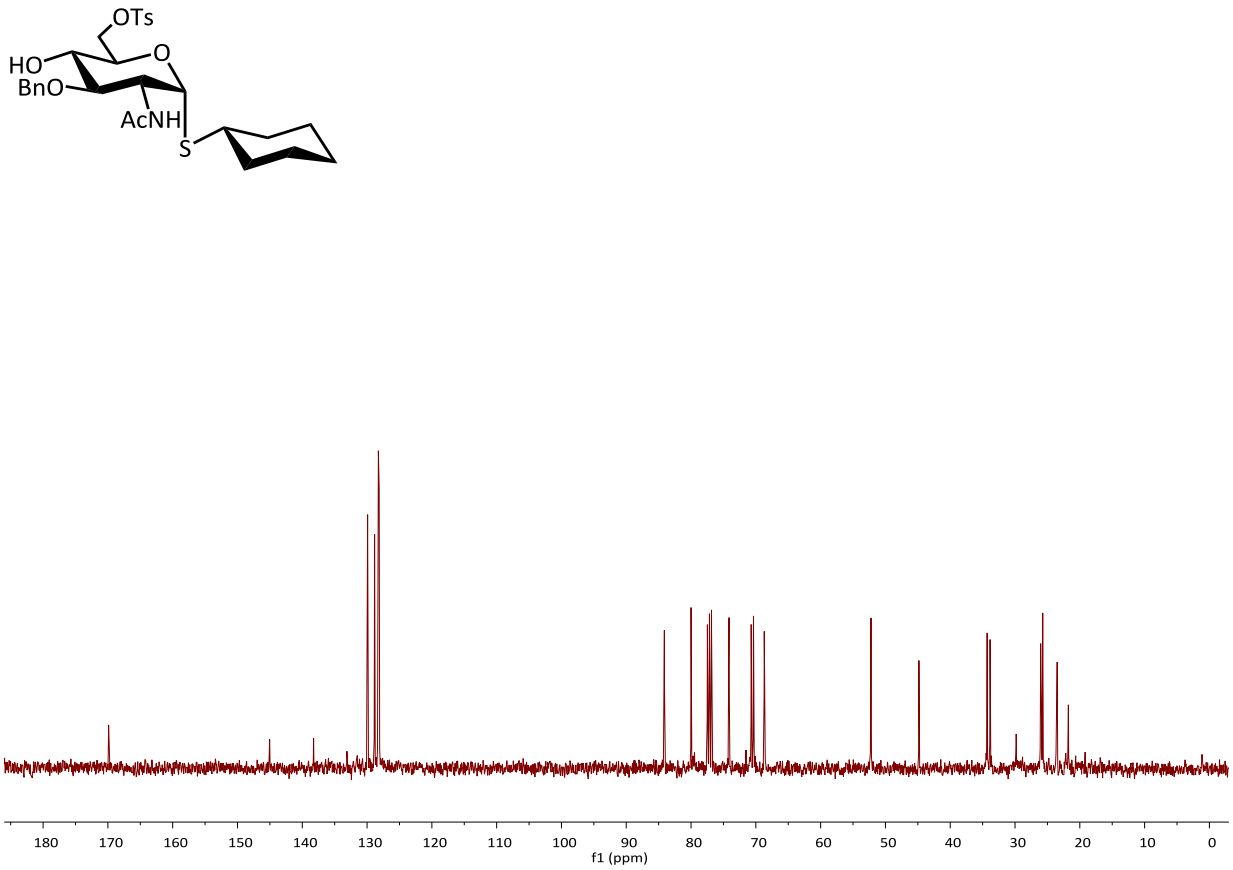


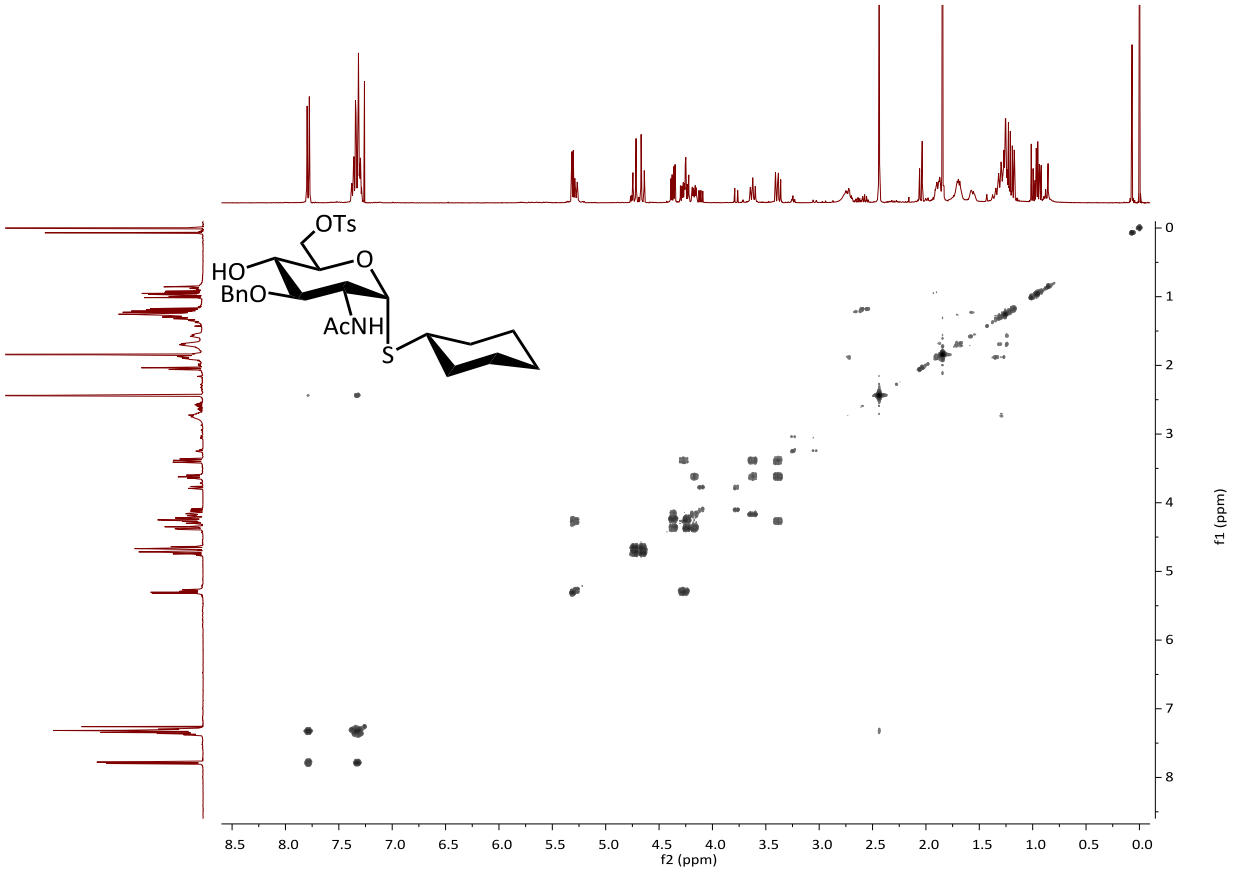

OH
HO
O
BnO
AcNH
S
f2 (ppm)
f1 (ppm)

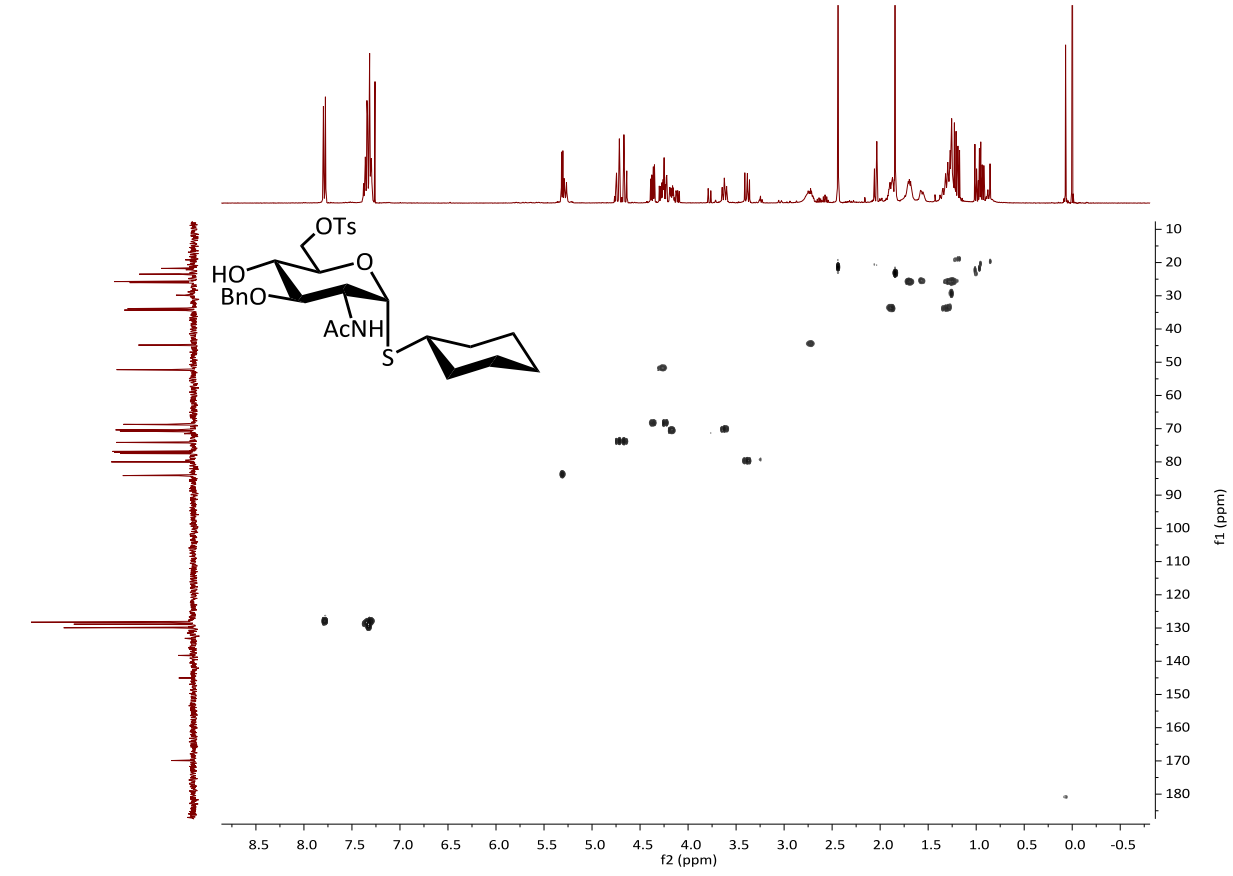

OH
HO
O
BnO
AcNH
S
f2 (ppm)
f1 (ppm)

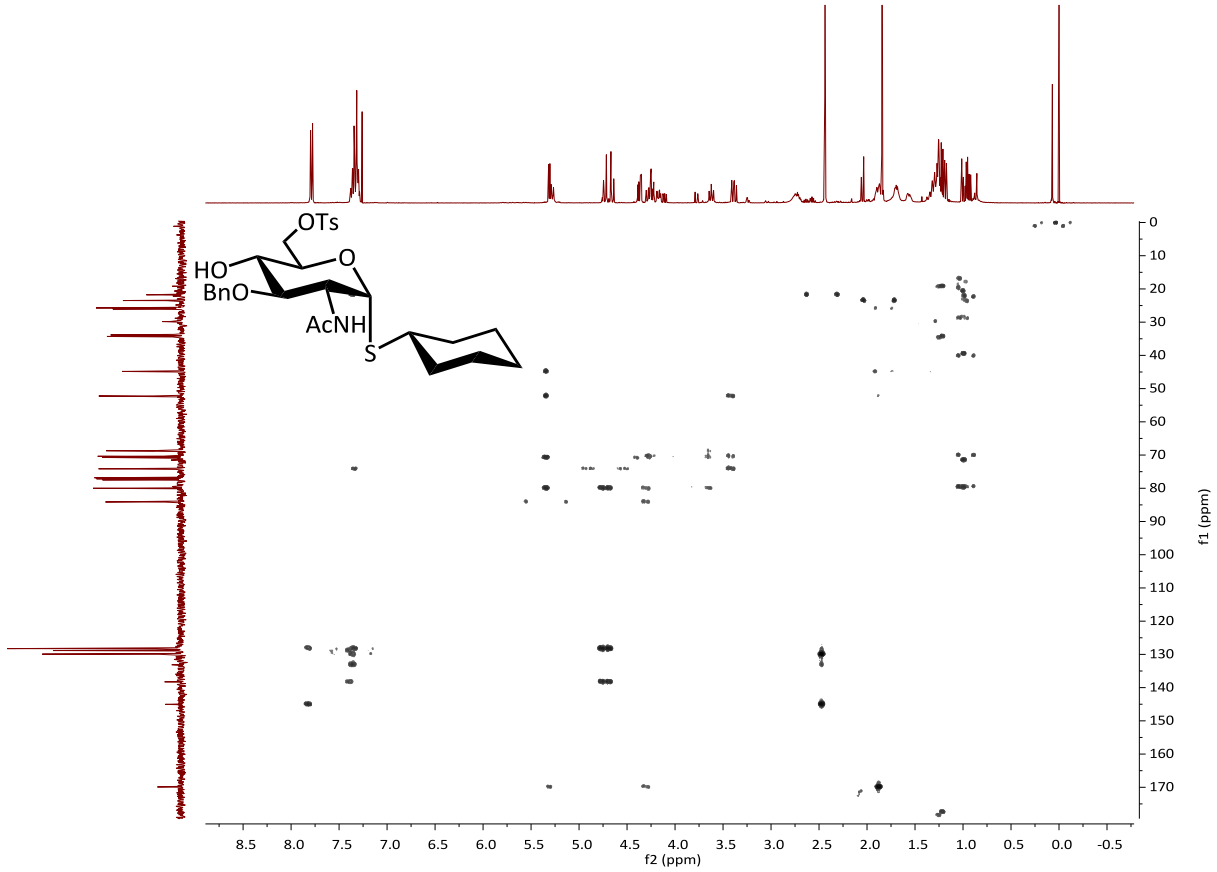
Cyclohexyl 2-acetamido-3-*O*-benzyl-2-deoxy-1-thio-6-*O*-tosyl-α-D-glucopyranoside (2.40).



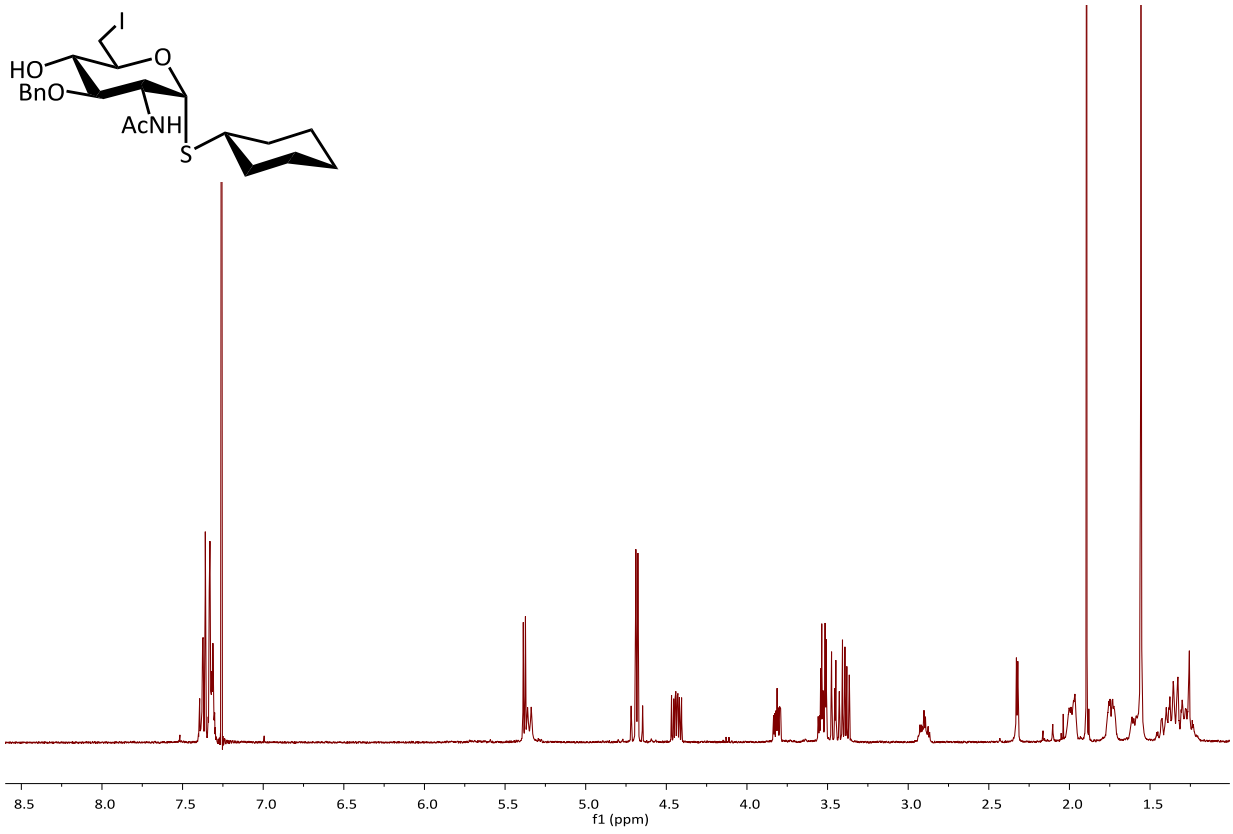


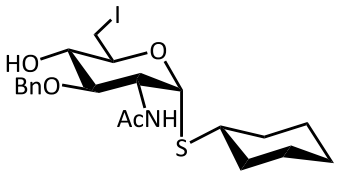

OTs
HO
BnO
AcNH
S
f2 (ppm)
f1 (ppm)

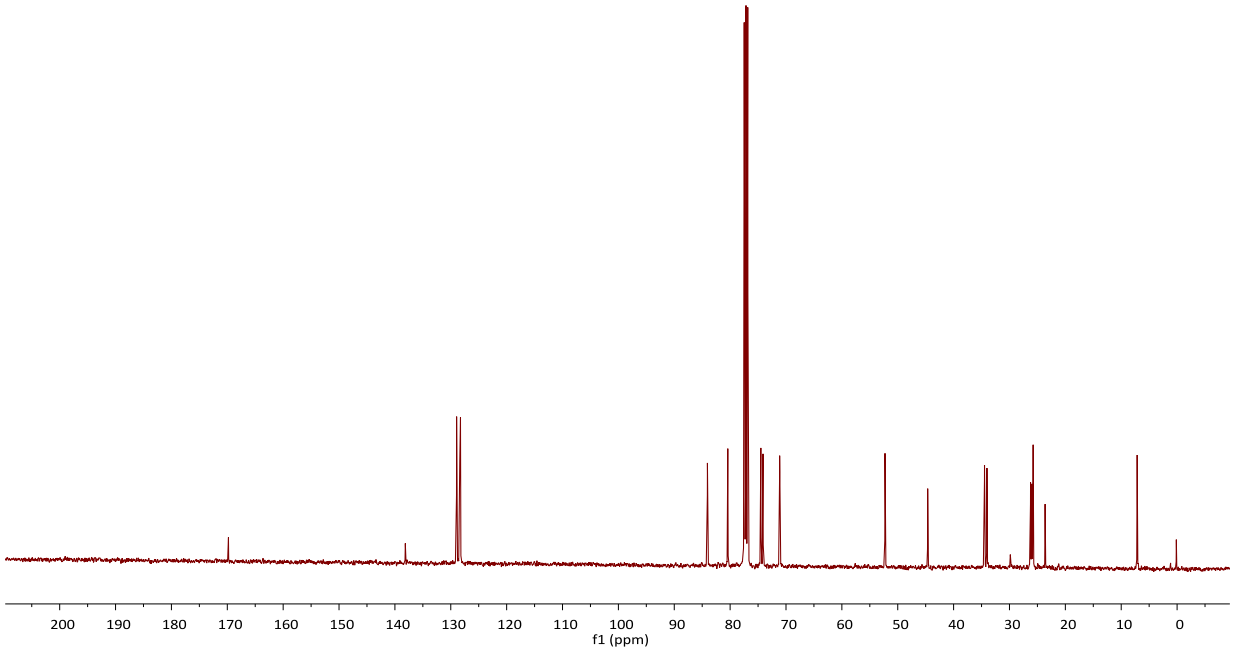

OTs
HO
BnO
AcNH
S
f2 (ppm)
f1 (ppm)

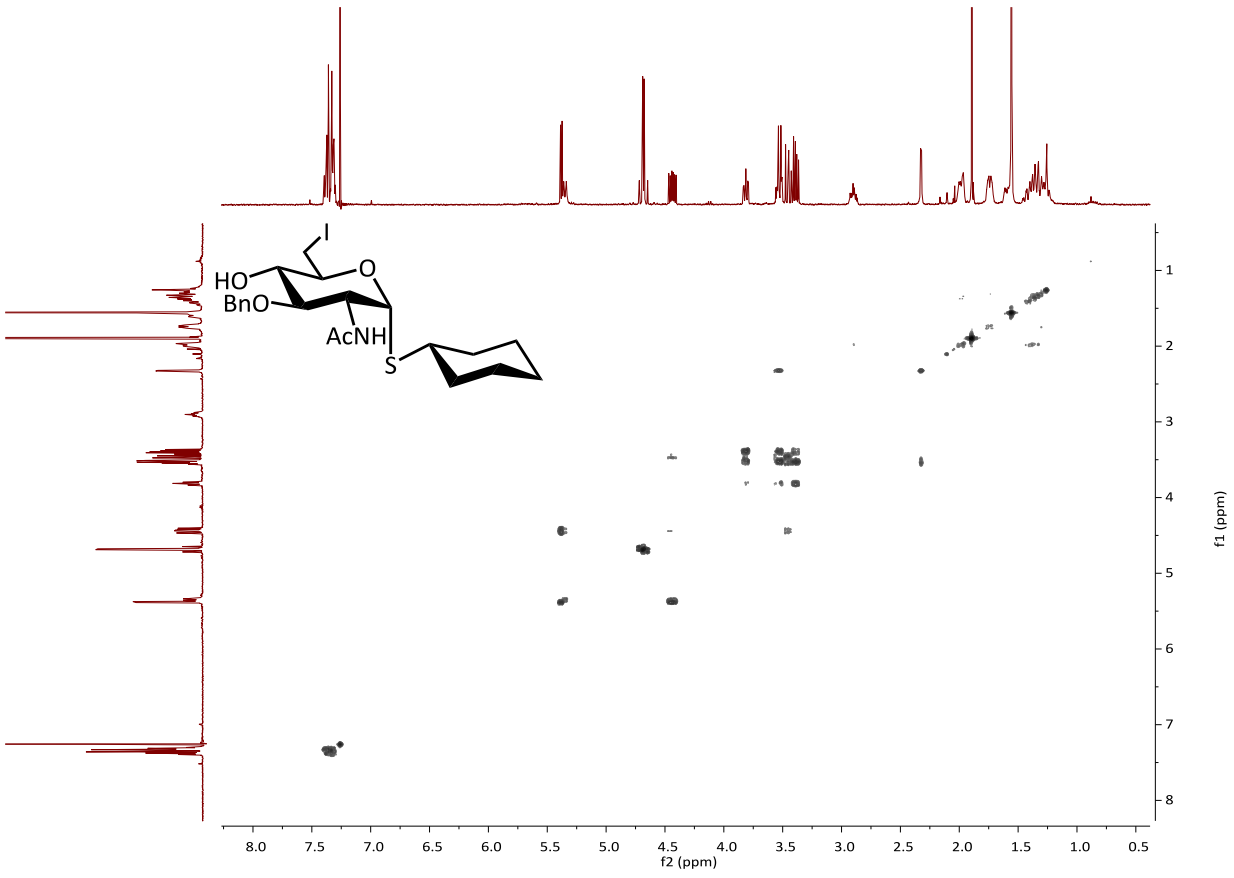


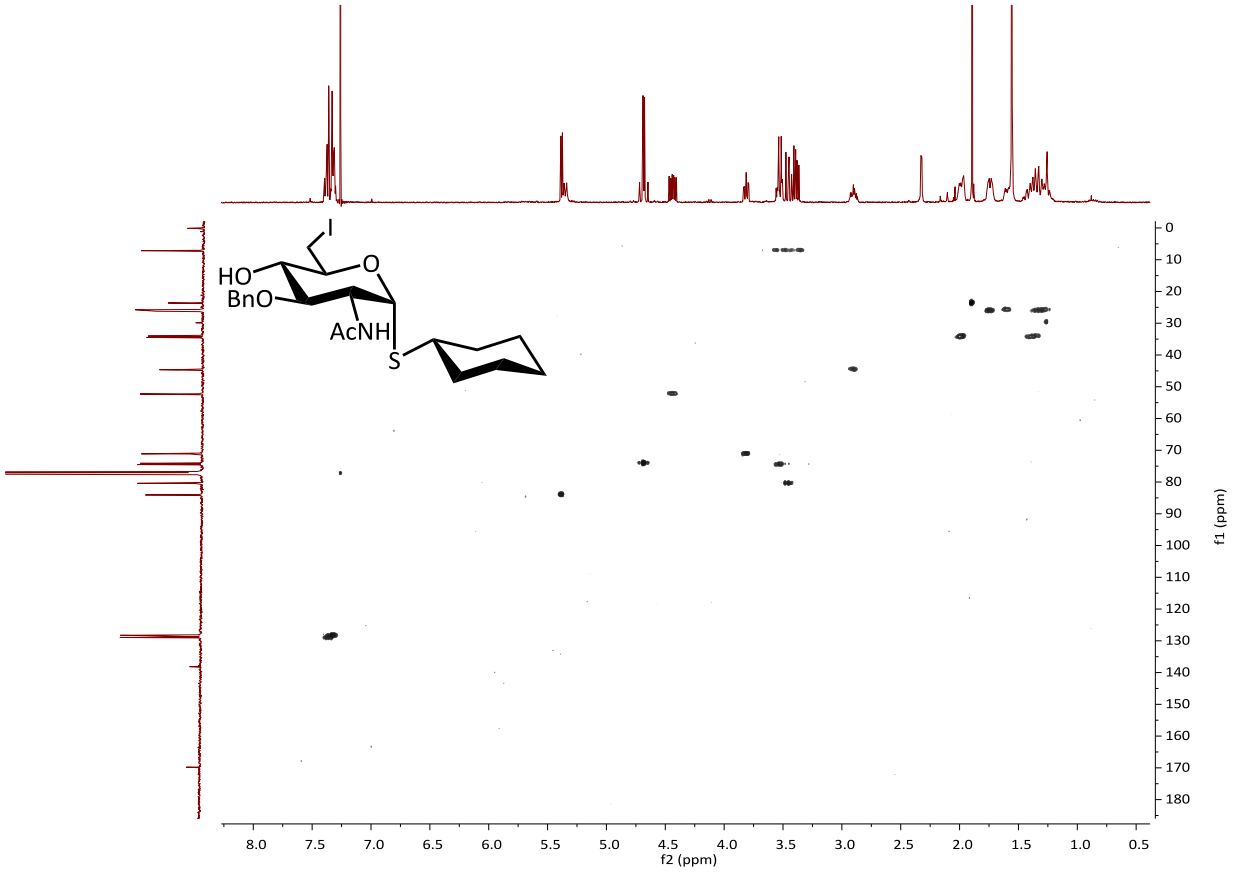
Cyclohexyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (2.41).

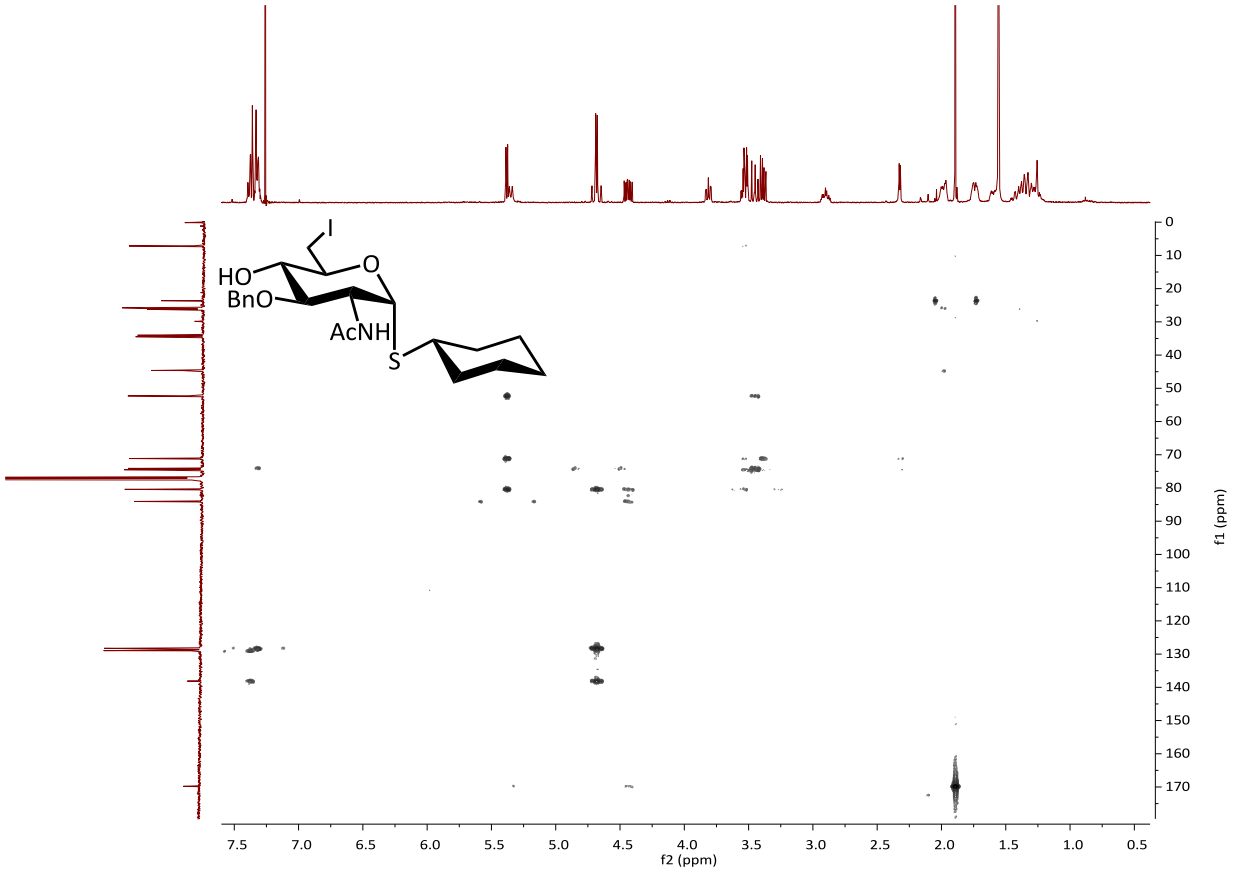



I
HO
O
BnO
AcNH
S

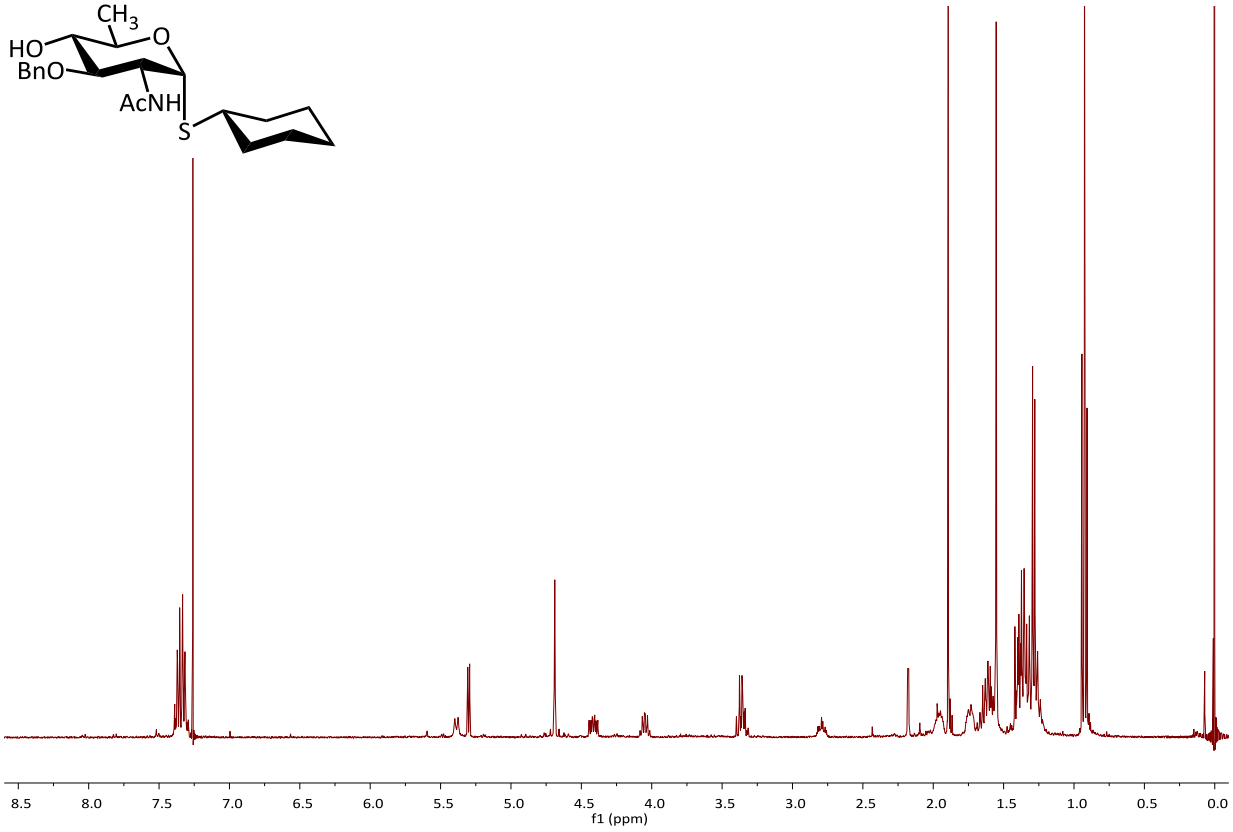

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

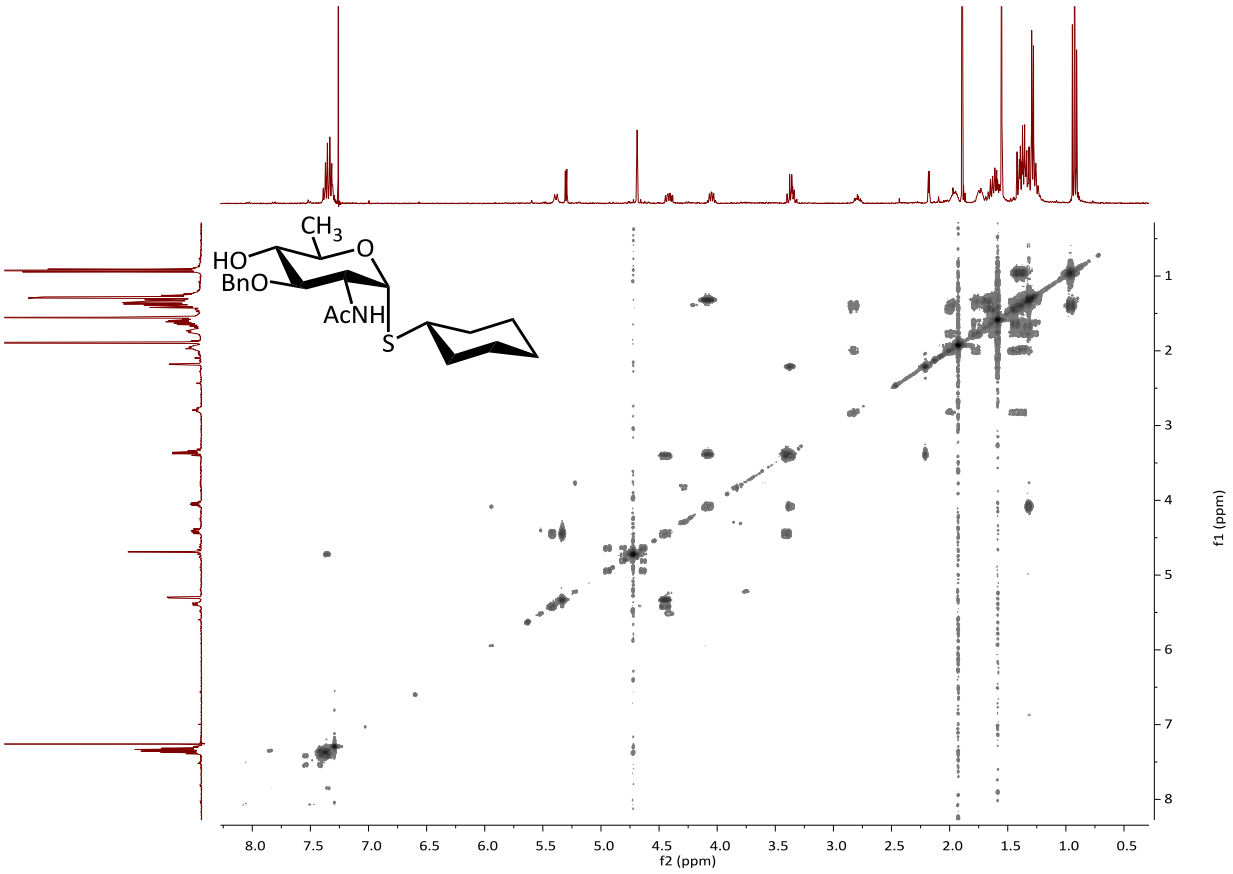

I
HO
O
BnO
AcNH
S
1 2 3 4 5 6 7 8
f1 (ppm)
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)


I
HO
BnO
O
AcNH
S
f2 (ppm)
f1 (ppm)

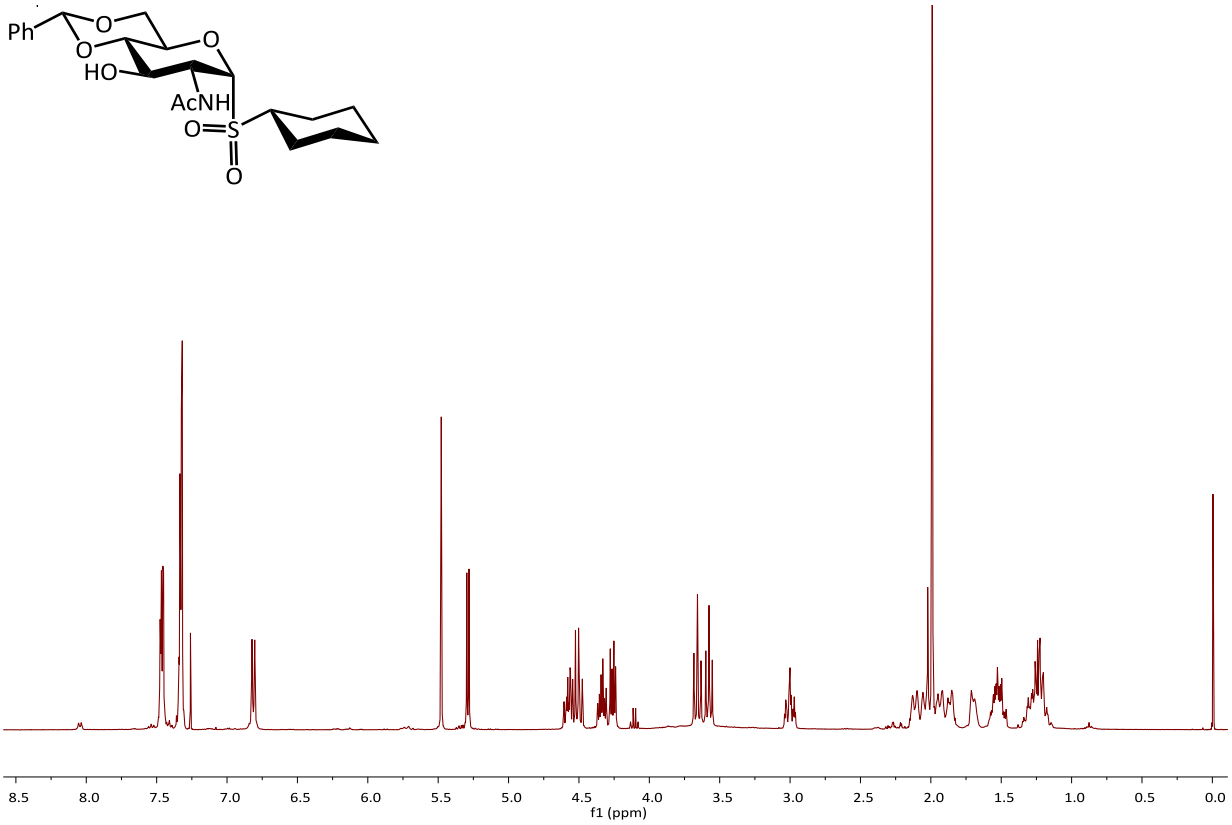

I
HO
BnO
O
AcNH
S
f2 (ppm)
f1 (ppm)

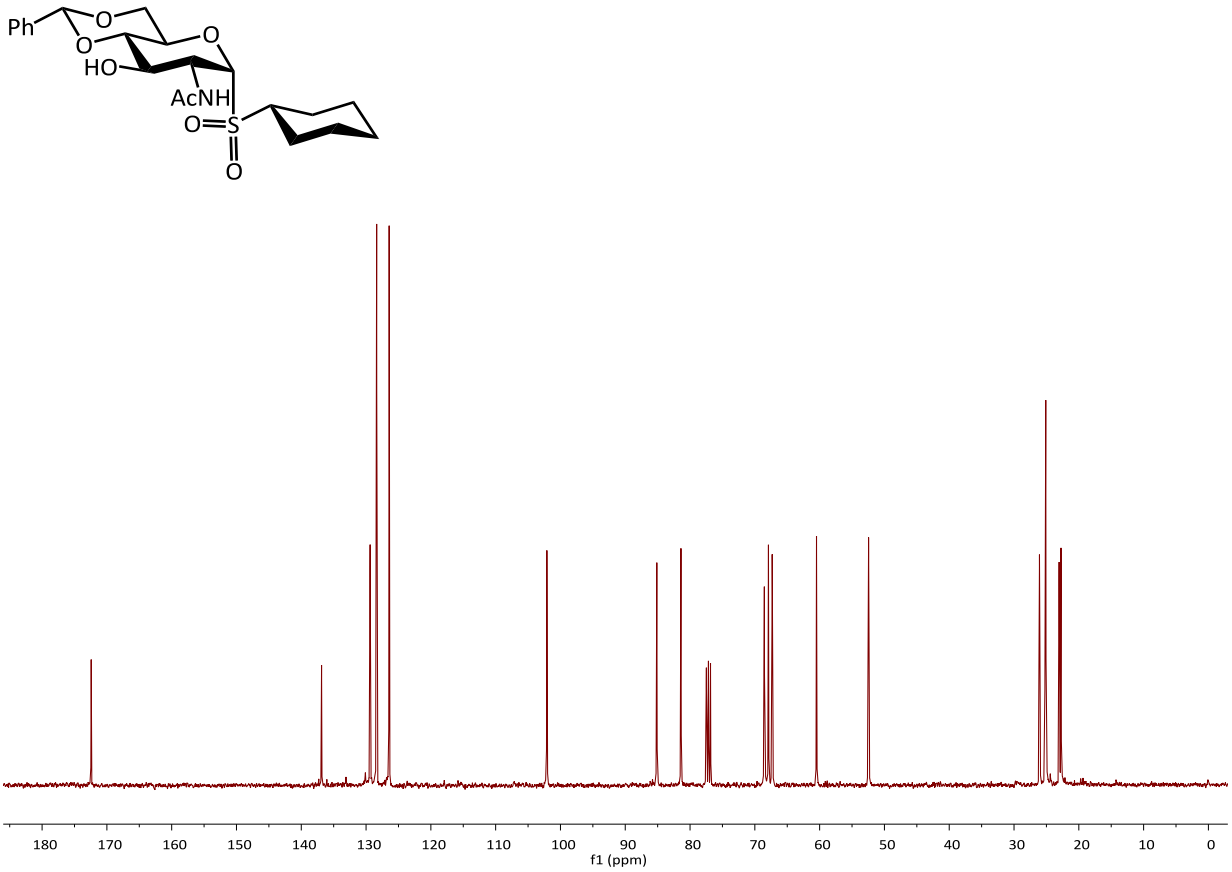
Cyclohexyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-1-thio-α-D-glucopyranoside (2.22).

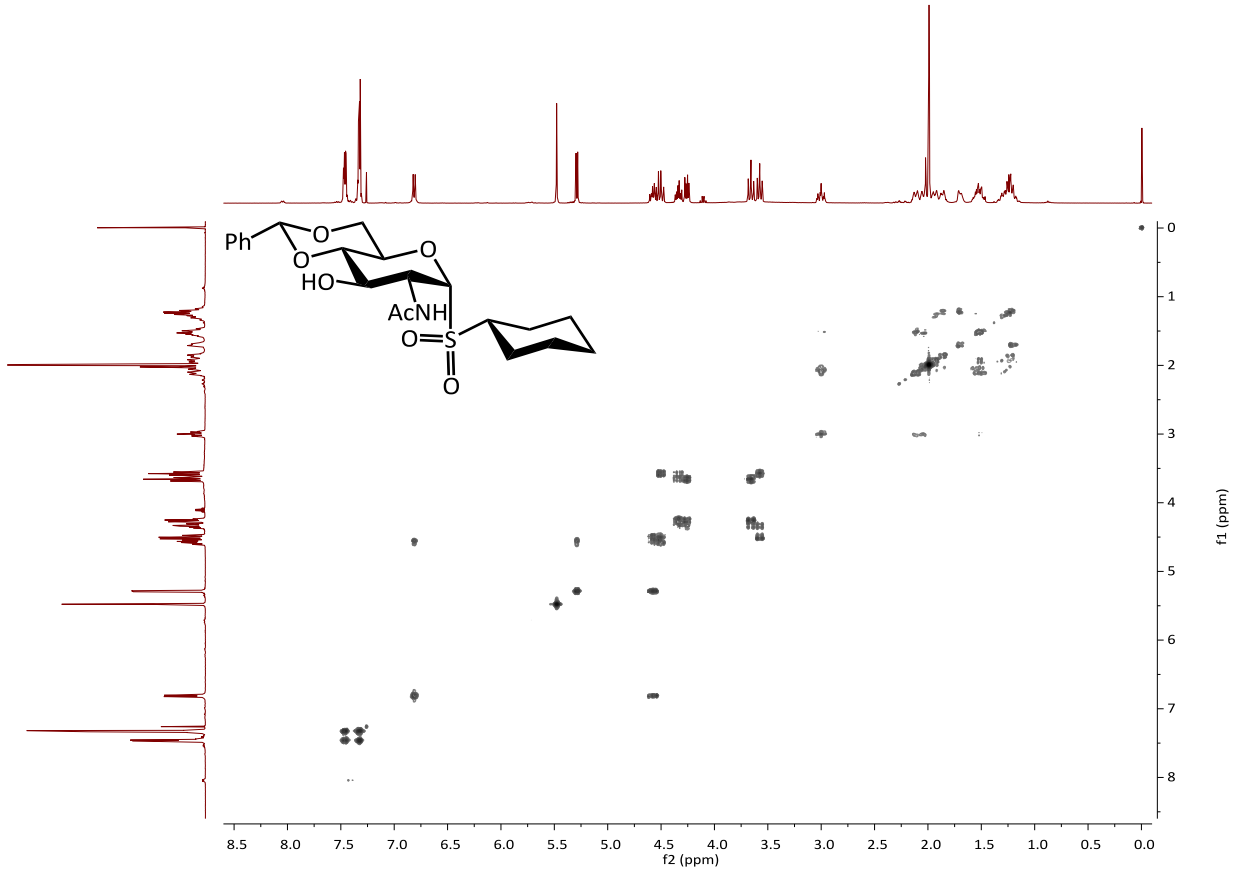


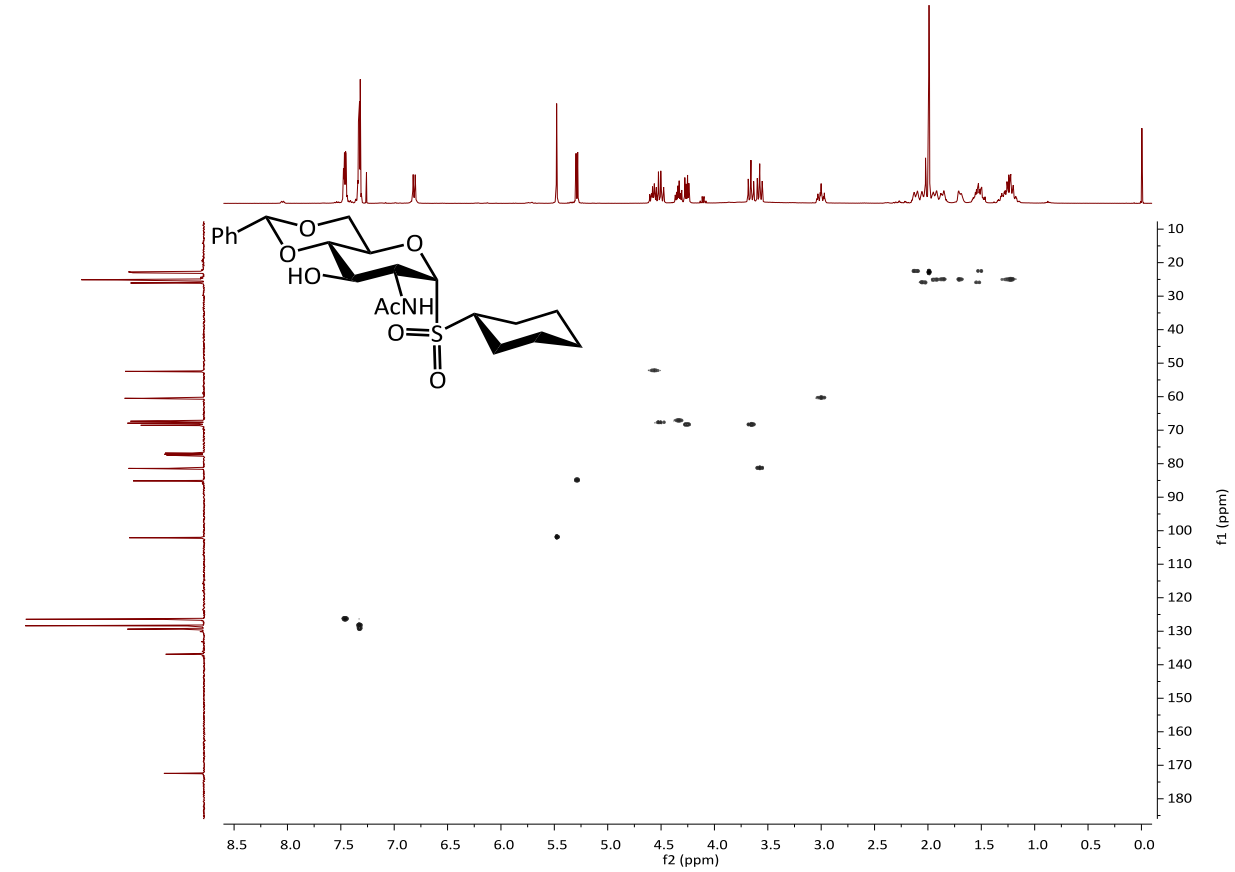


Cyclohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-1-sulfonyl-α-D-glucopyranoside (2.42).

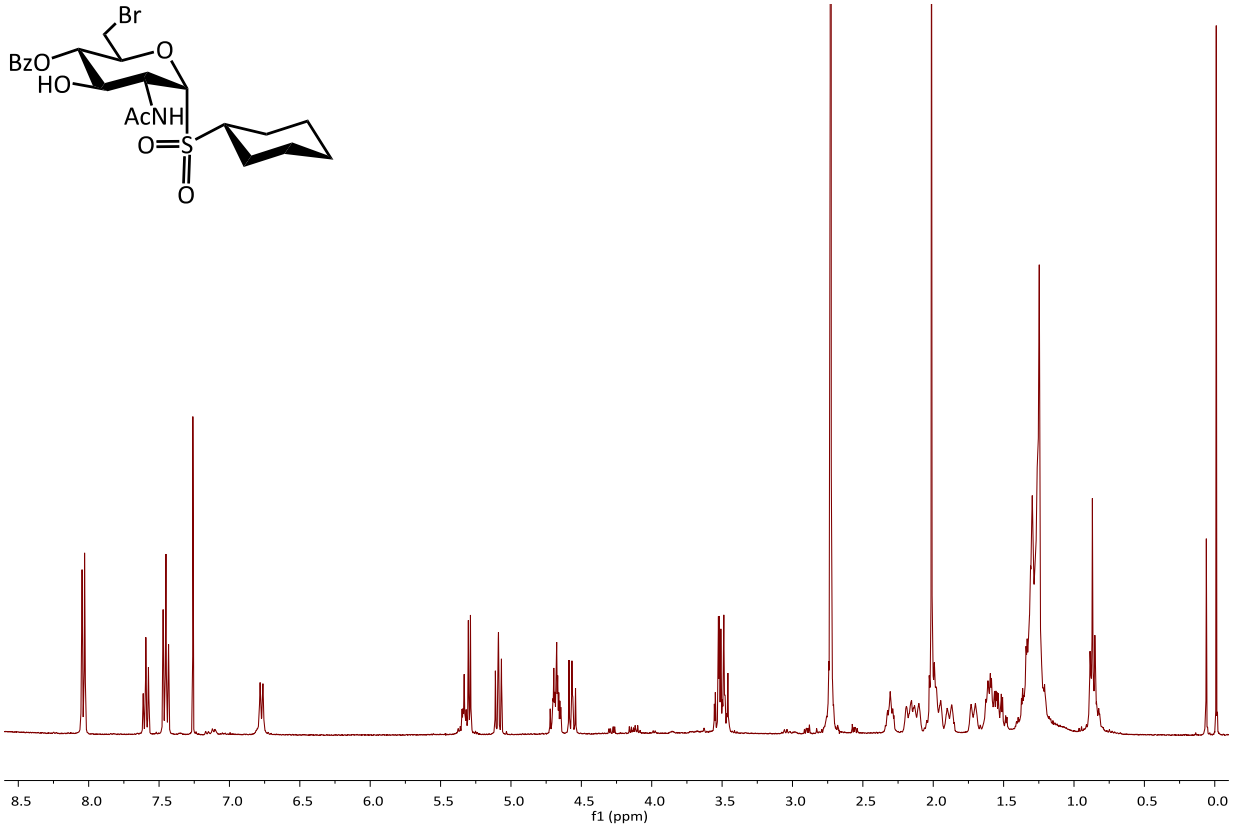


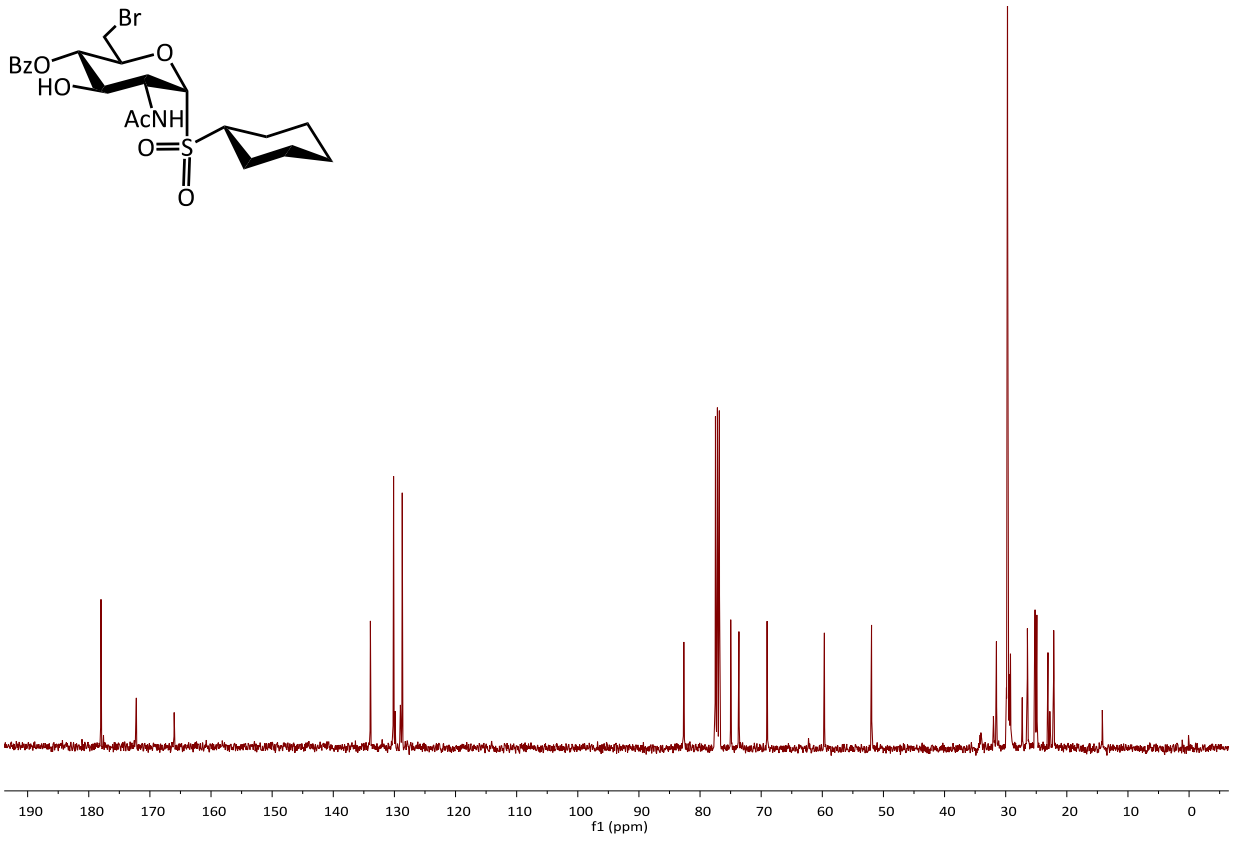


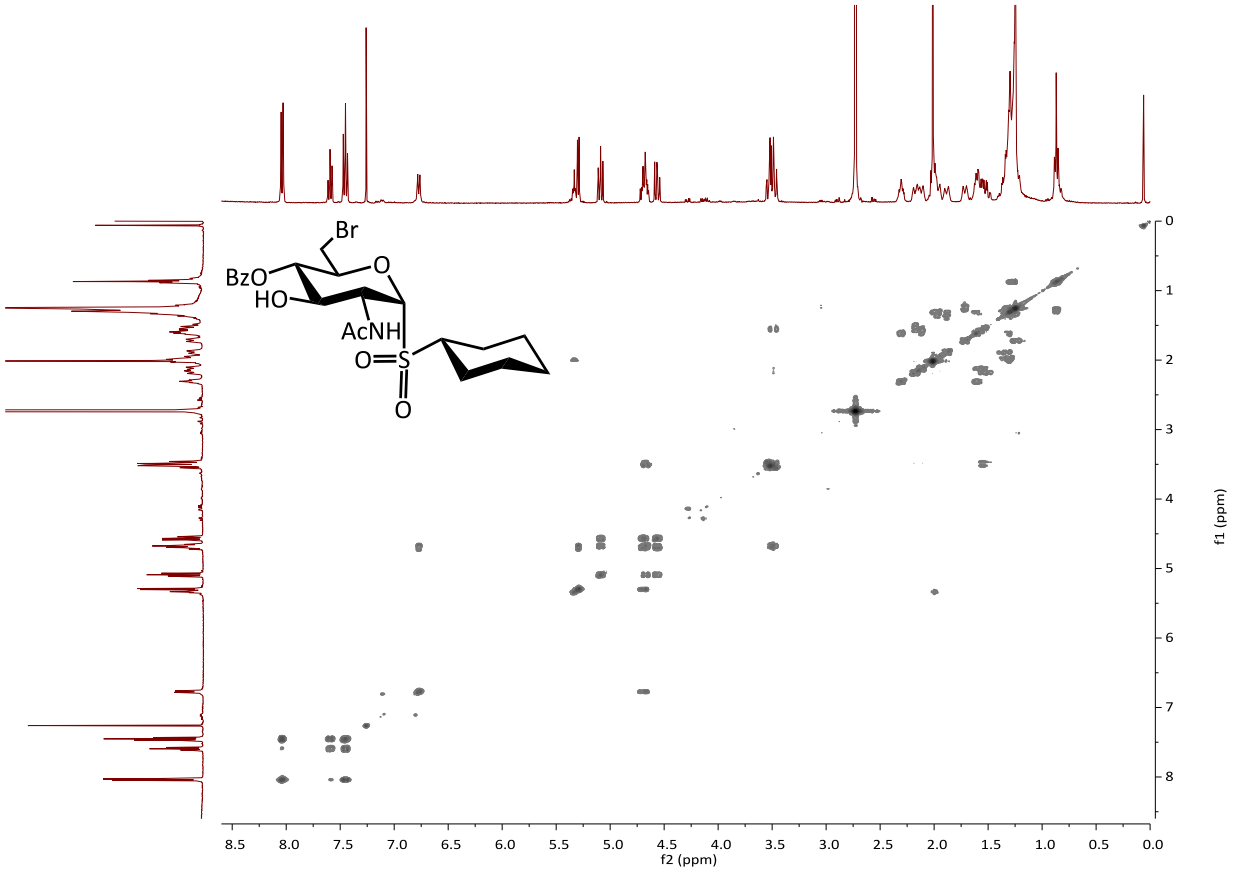

Ph
O
O
O
HO
AcNH
O=S
O
f2 (ppm)
f1 (ppm)

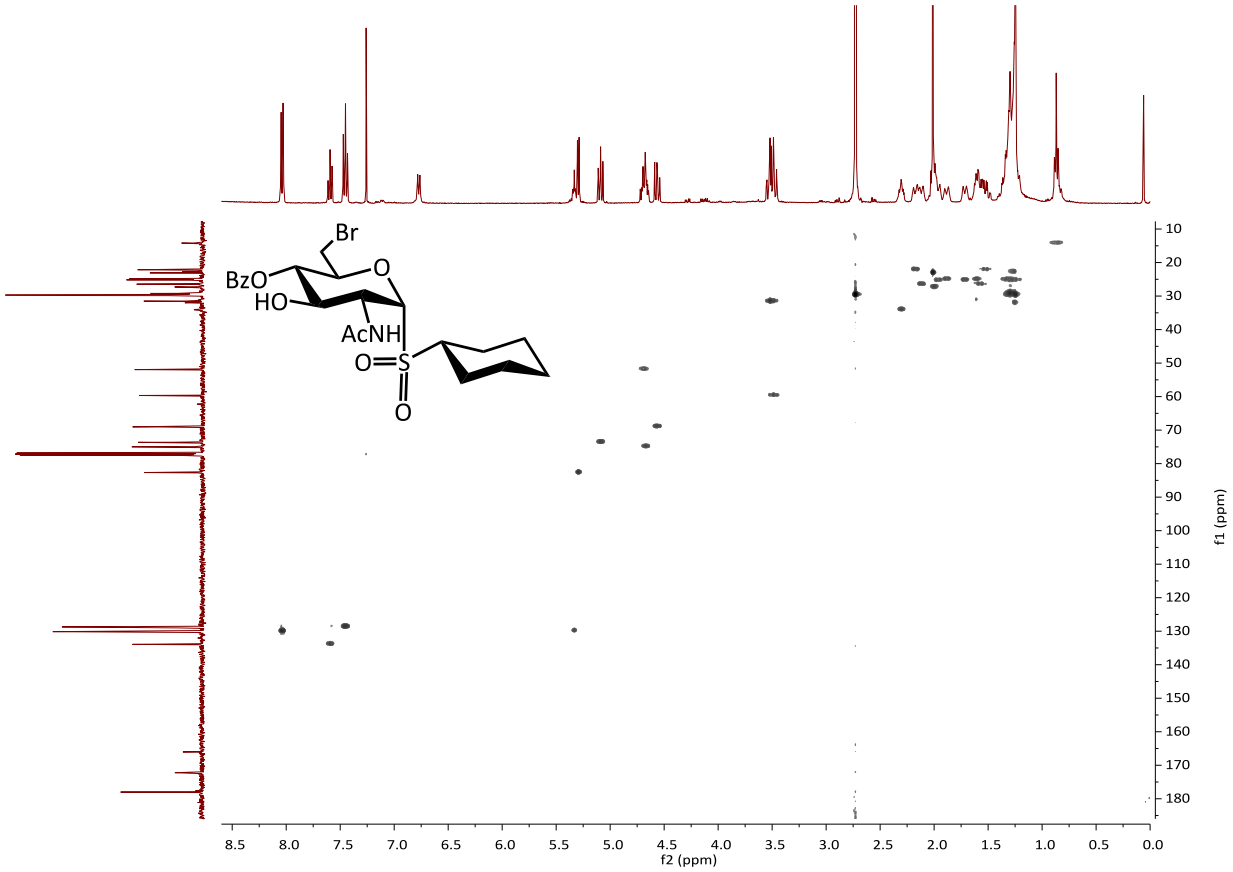

Ph
O
O
O
HO
AcNH
O=S
O
f2 (ppm)
f1 (ppm)

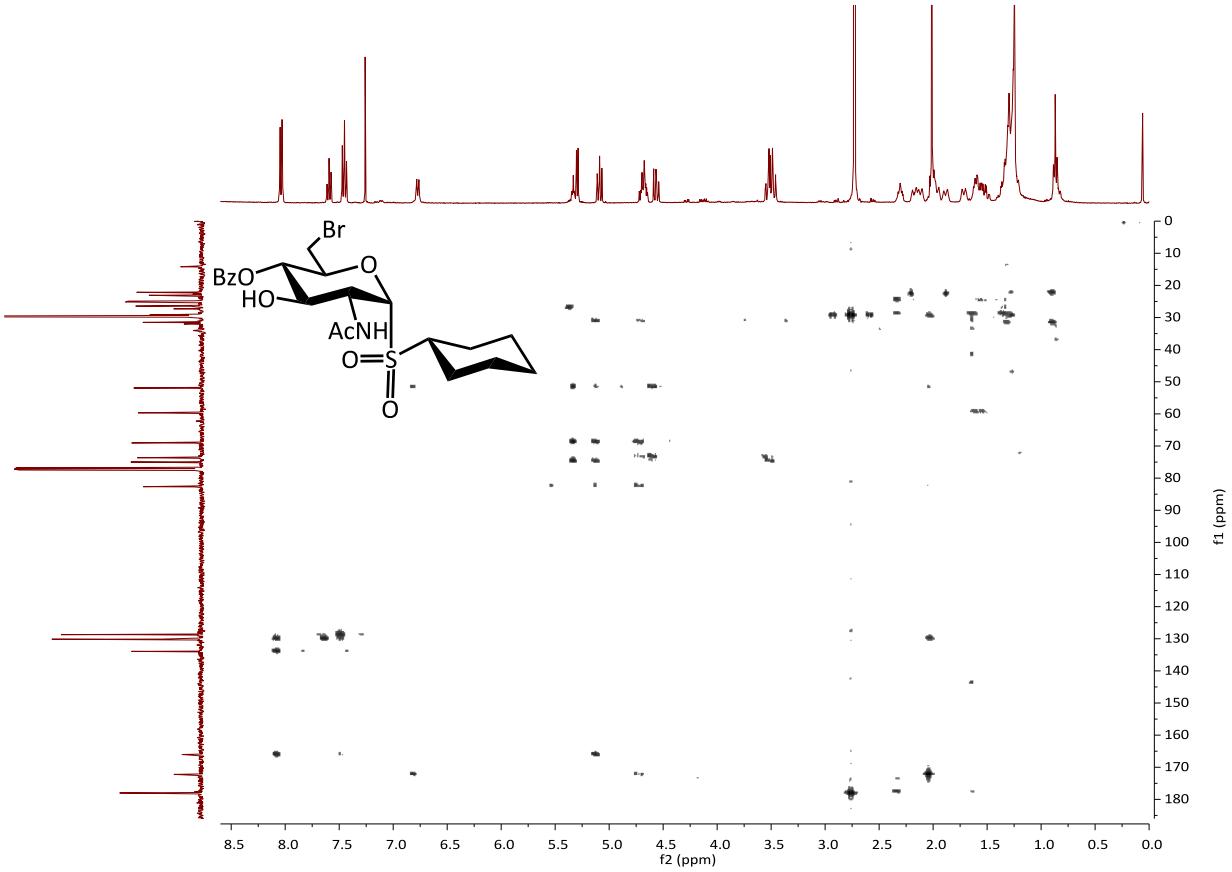
Cyclohexyl 2-acetamido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-1-sulfonyl-α-D-glucopyranoside (2.43).



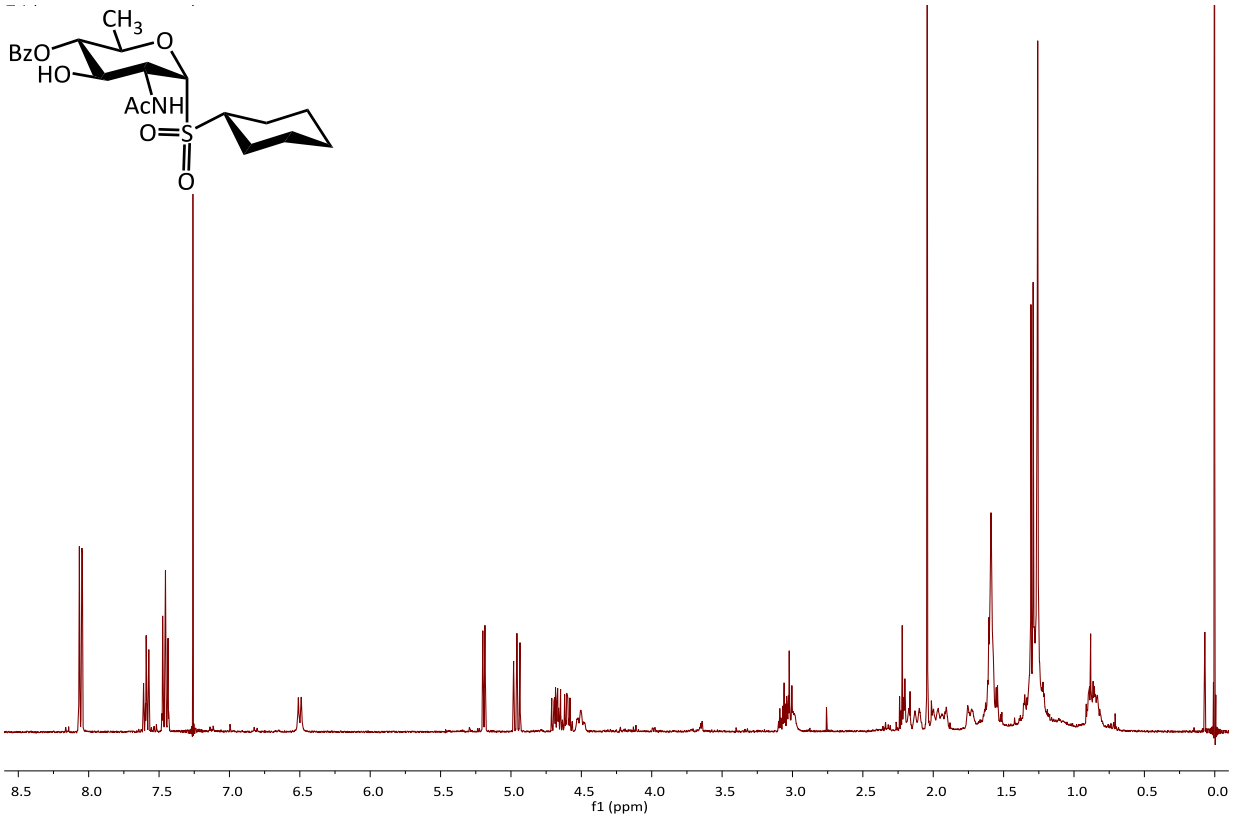


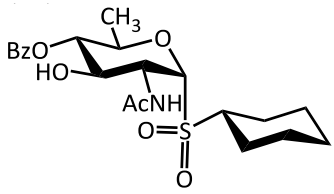

Br
BzO
HO
O
AcNH
O=S
O
f2 (ppm)
f1 (ppm)

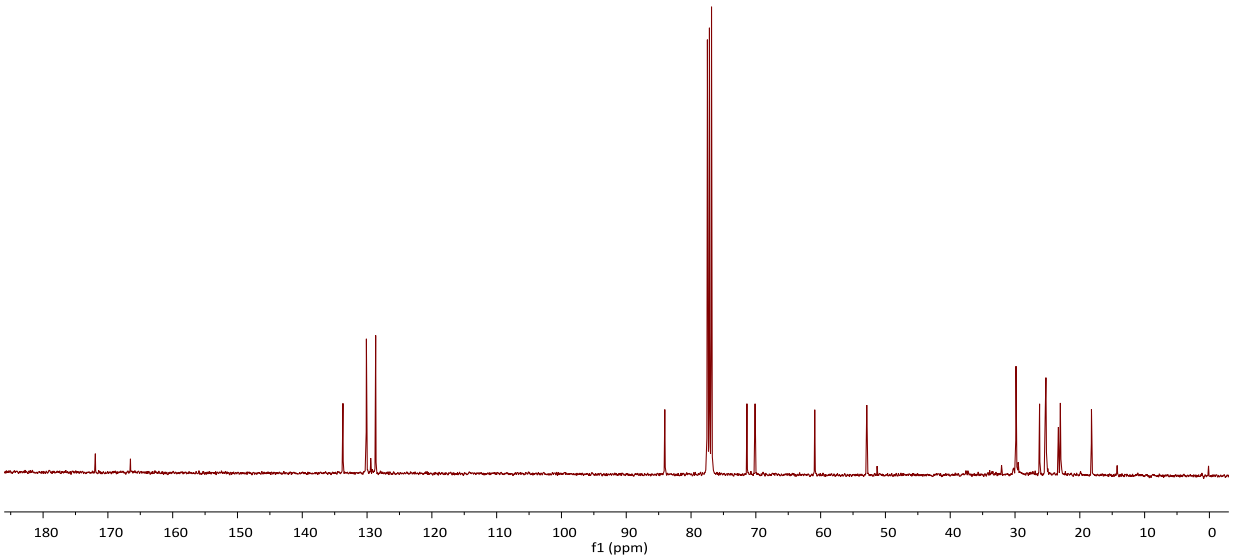

Br
BzO
HO
O
AcNH
O=S
O
f2 (ppm)
f1 (ppm)

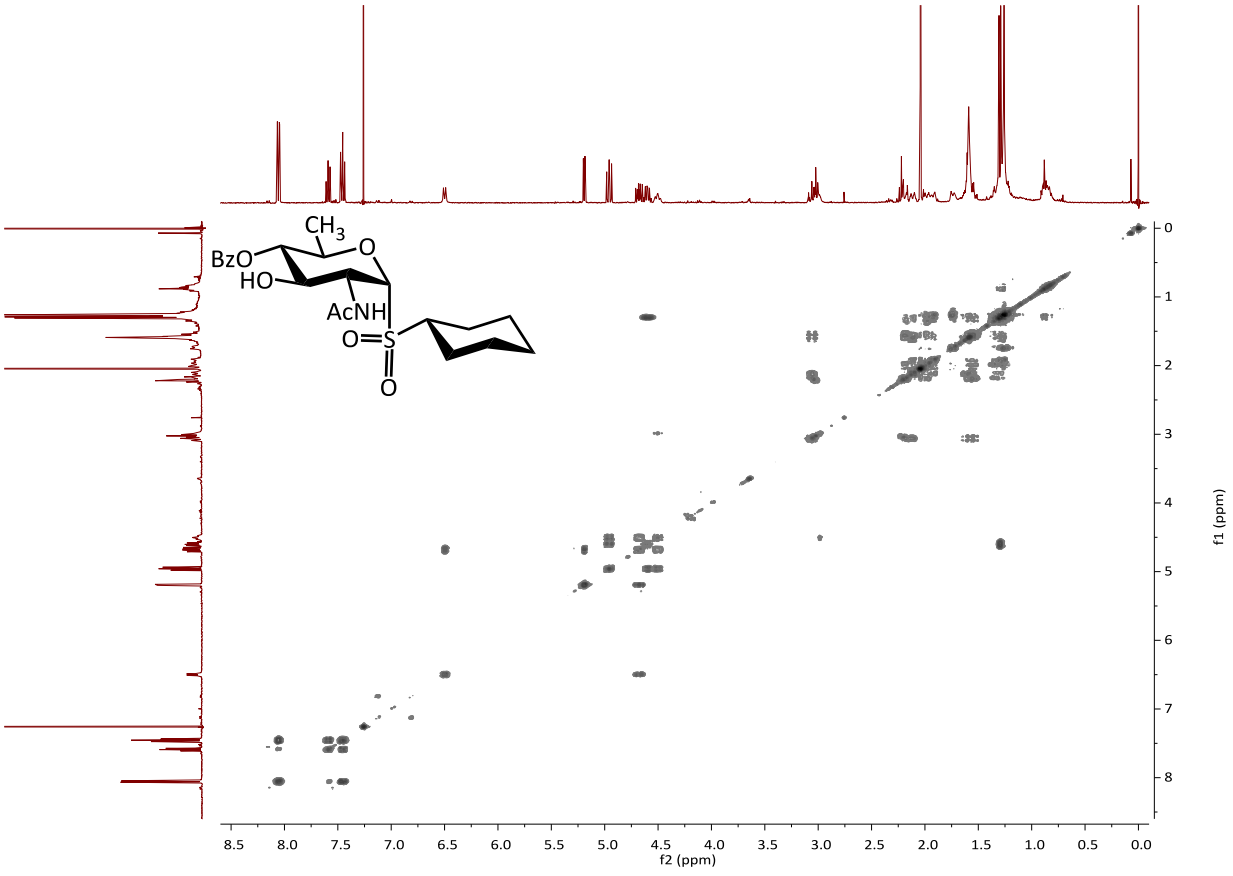


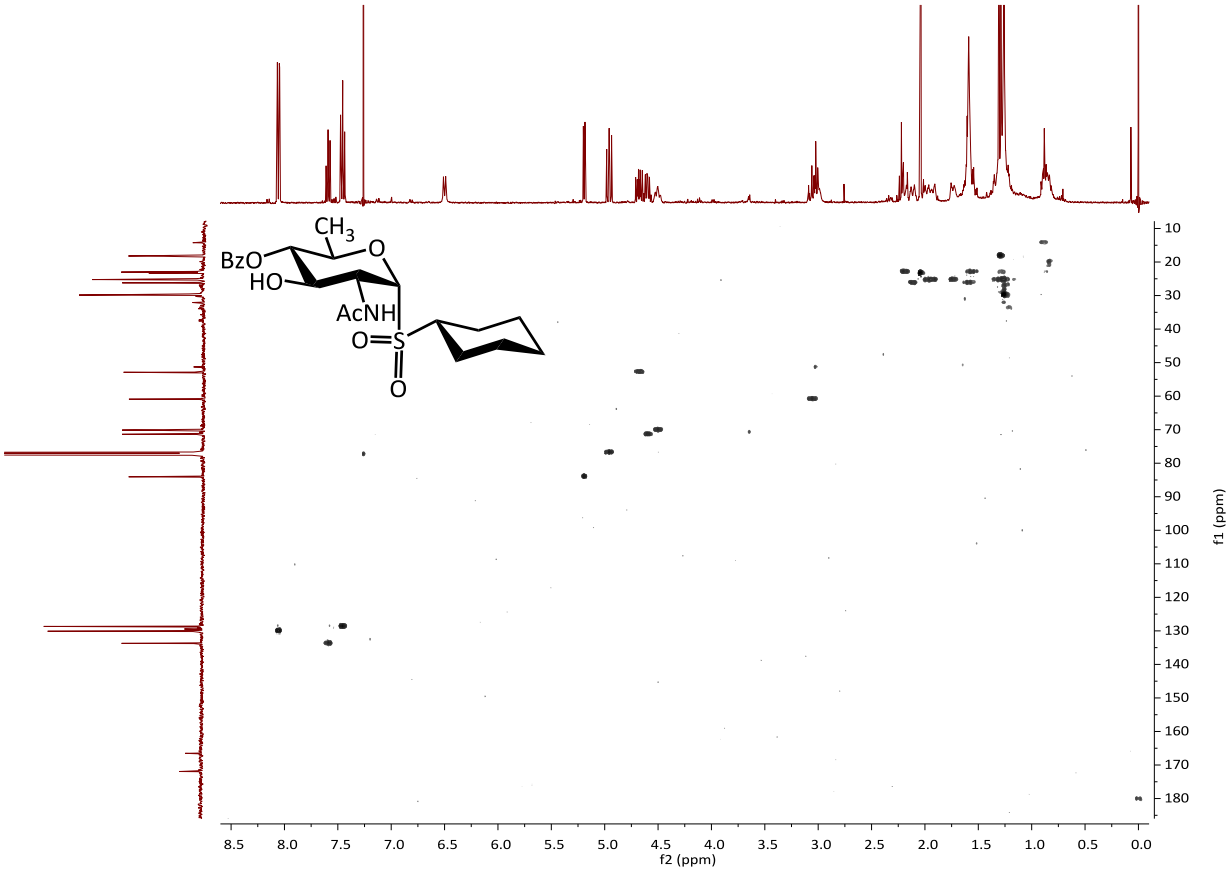
Cyclohexyl 2-acetamido-4-*O*-benzoyl-2,6-dideoxy-1-sulfonyl-α-D-glucopyranoside (2.44).



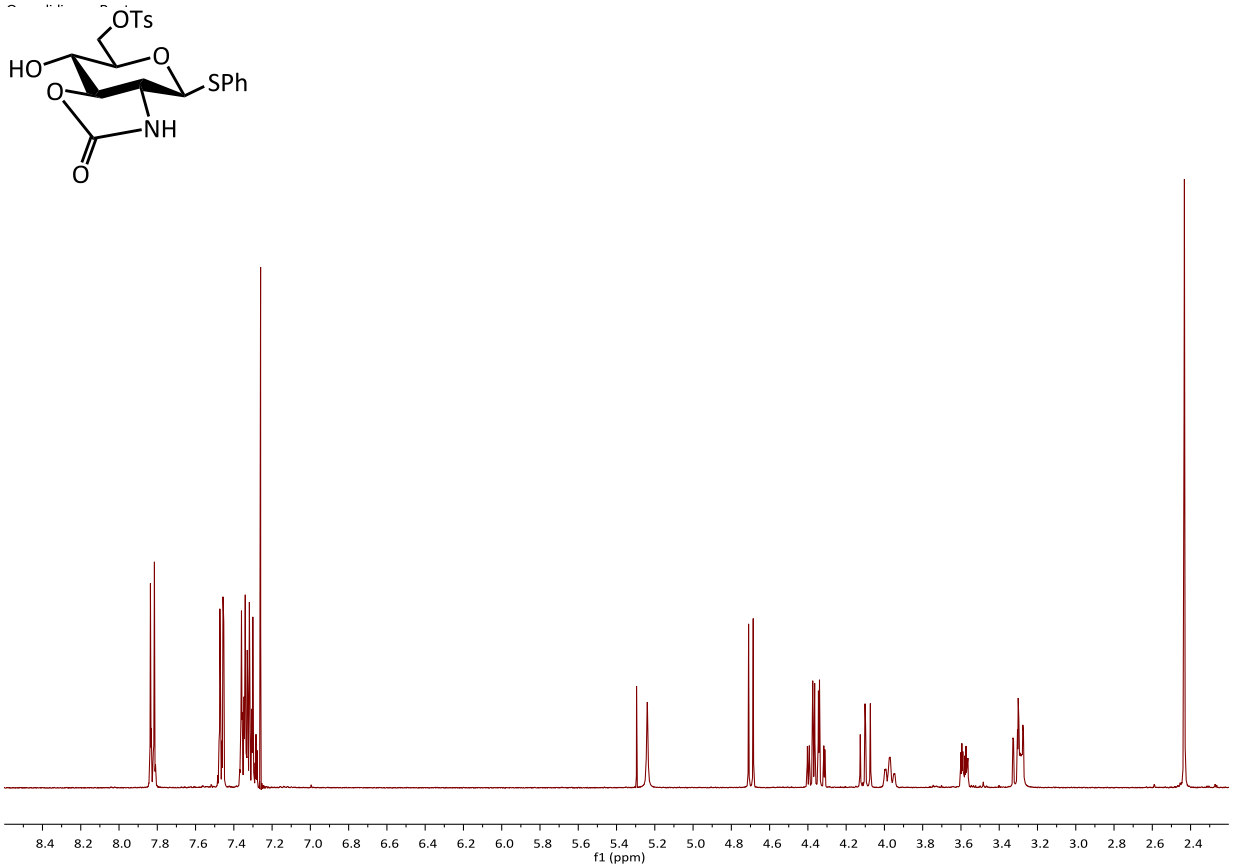

CH3
O
BzO
HO
AcNH
O=S
O

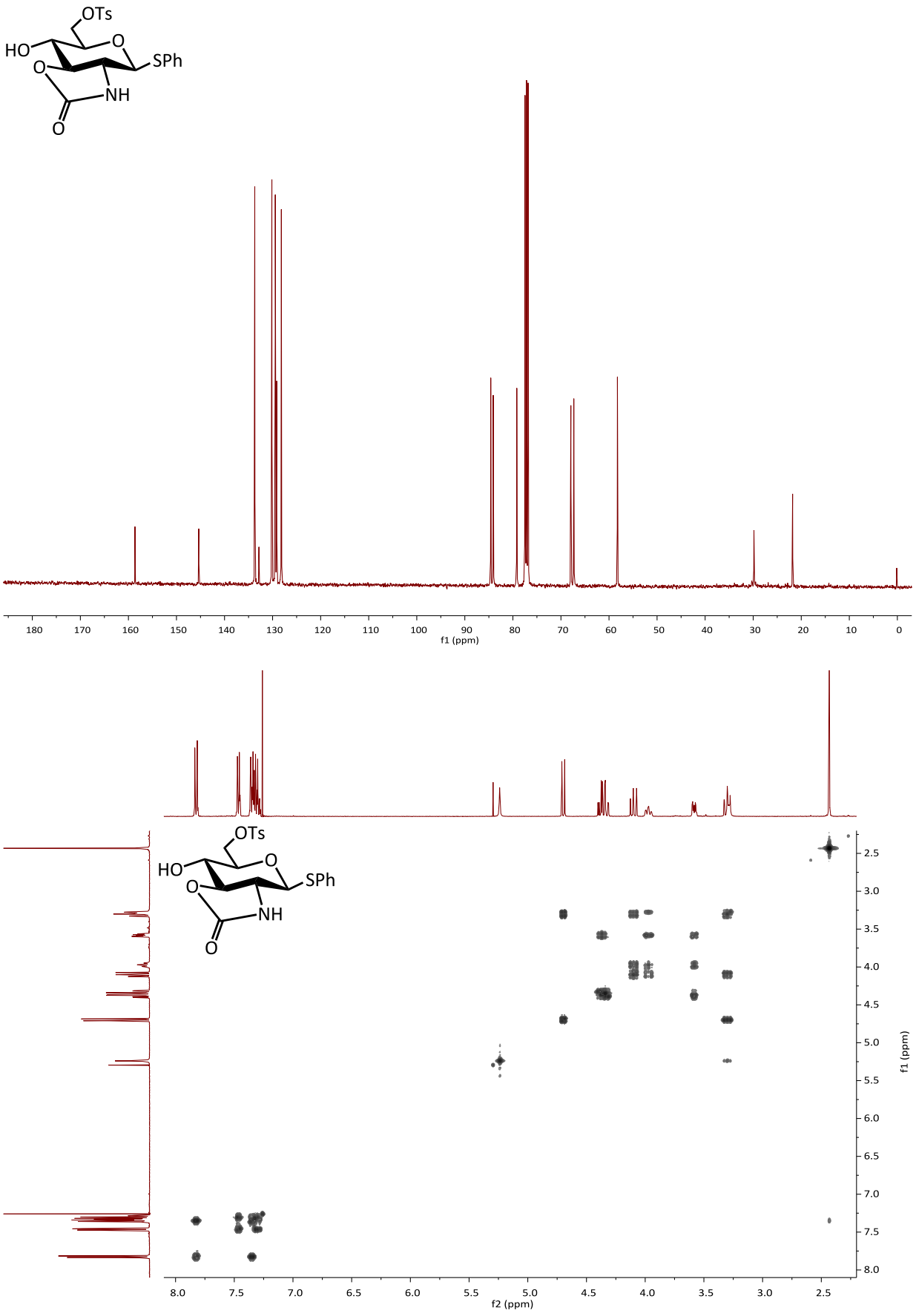

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

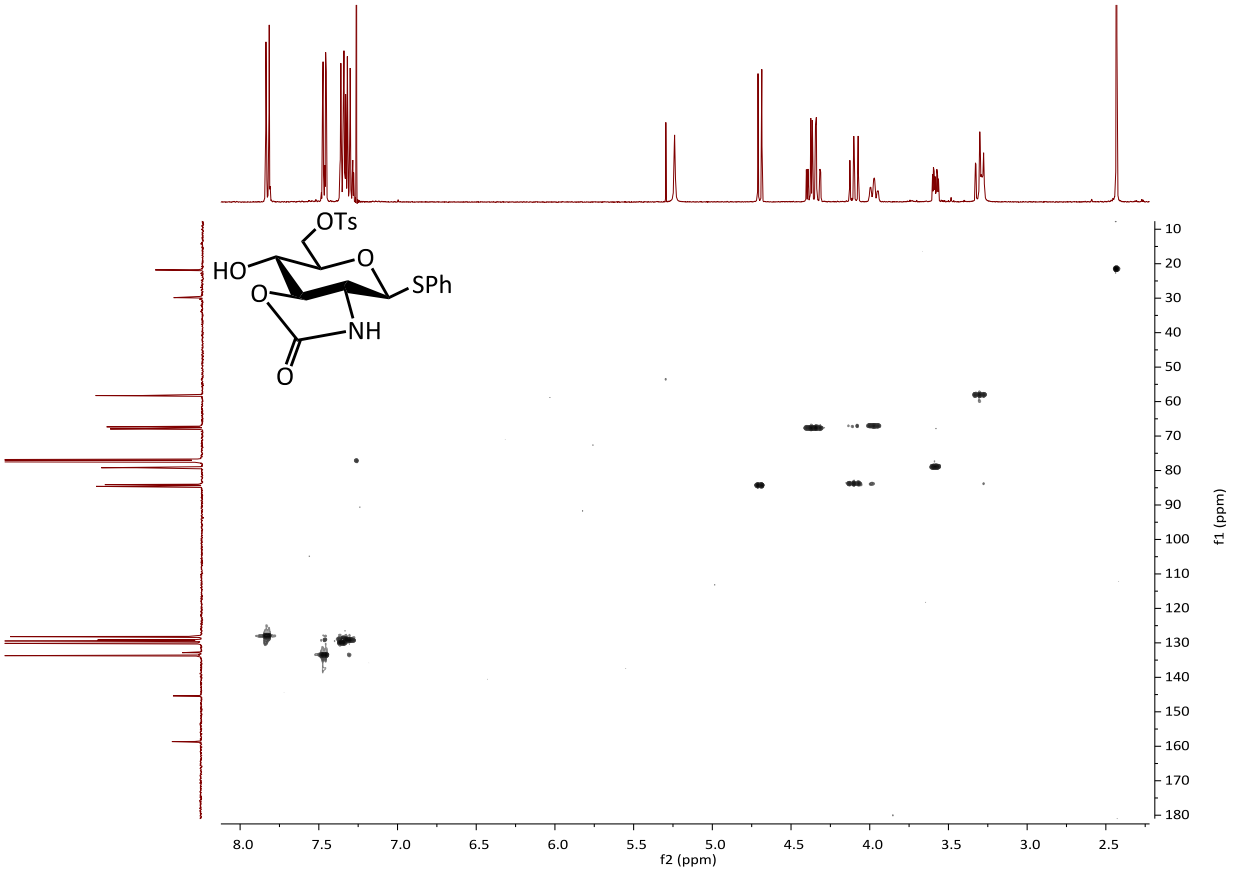

CH3
O
BzO
HO
AcNH
O=S
O
0 1 2 3 4 5 6 7 8
f1 (ppm)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)

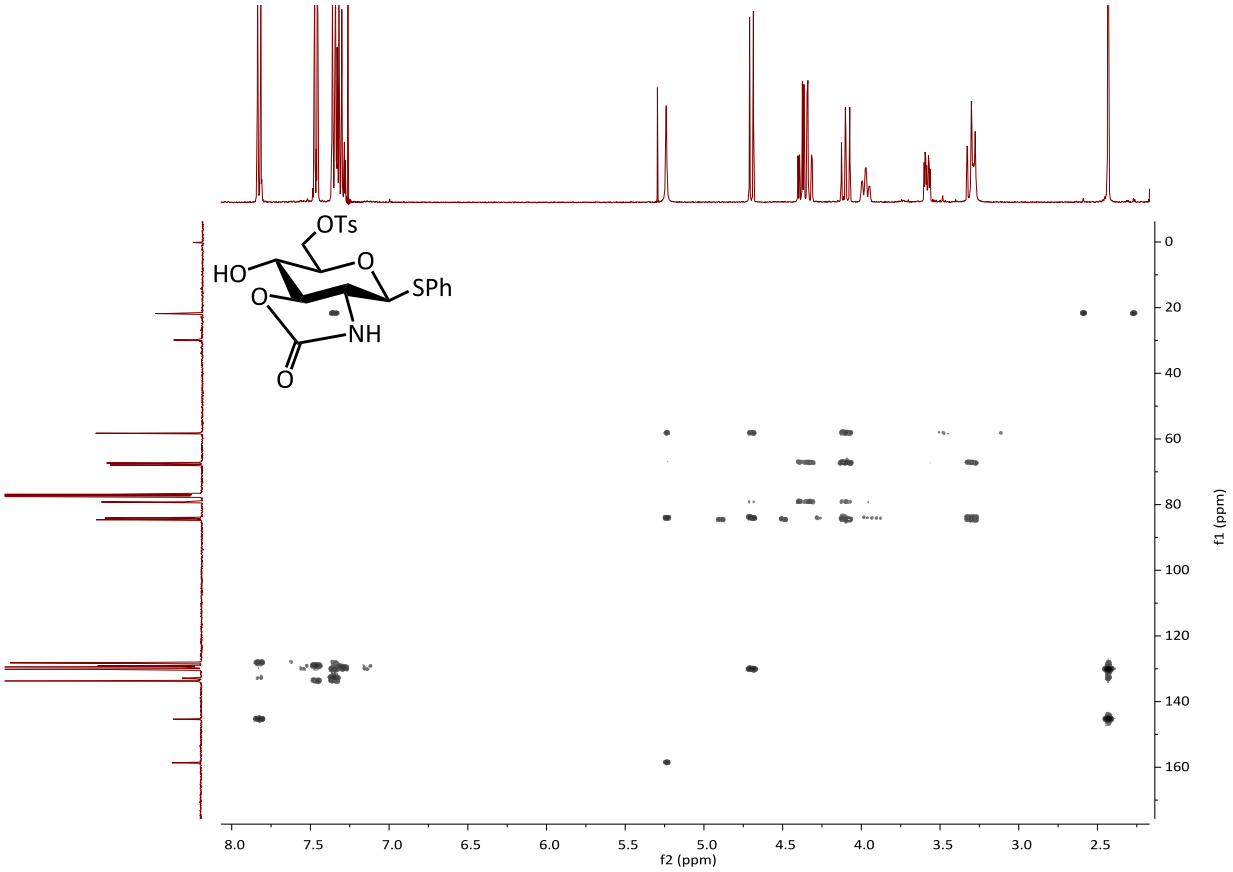


Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-6-*O*-tosyl-β-D-glucopyranoside (2.54).

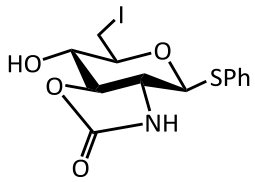


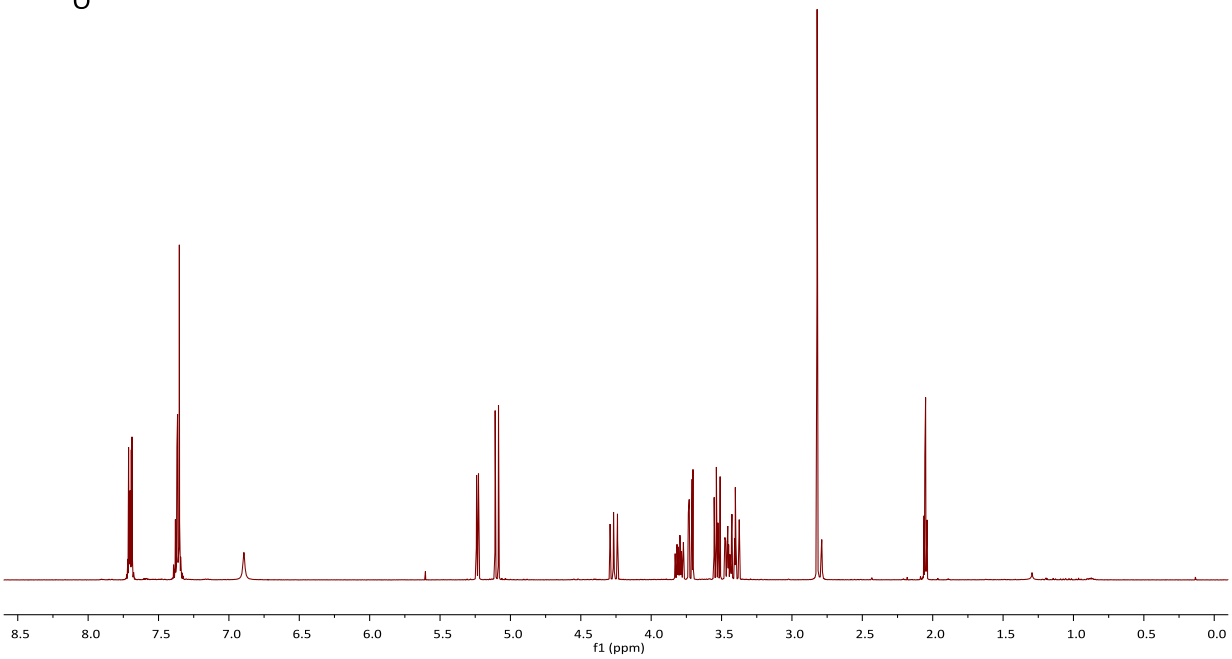

OTs
HO
O
O
SPh
NH
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)
OTs
HO
O
O
SPh
NH
O
2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
f1 (ppm)
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5
f2 (ppm)

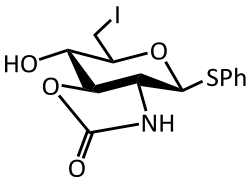

OTs
HO
O
O
SPh
NH
O
f2 (ppm)
f1 (ppm)

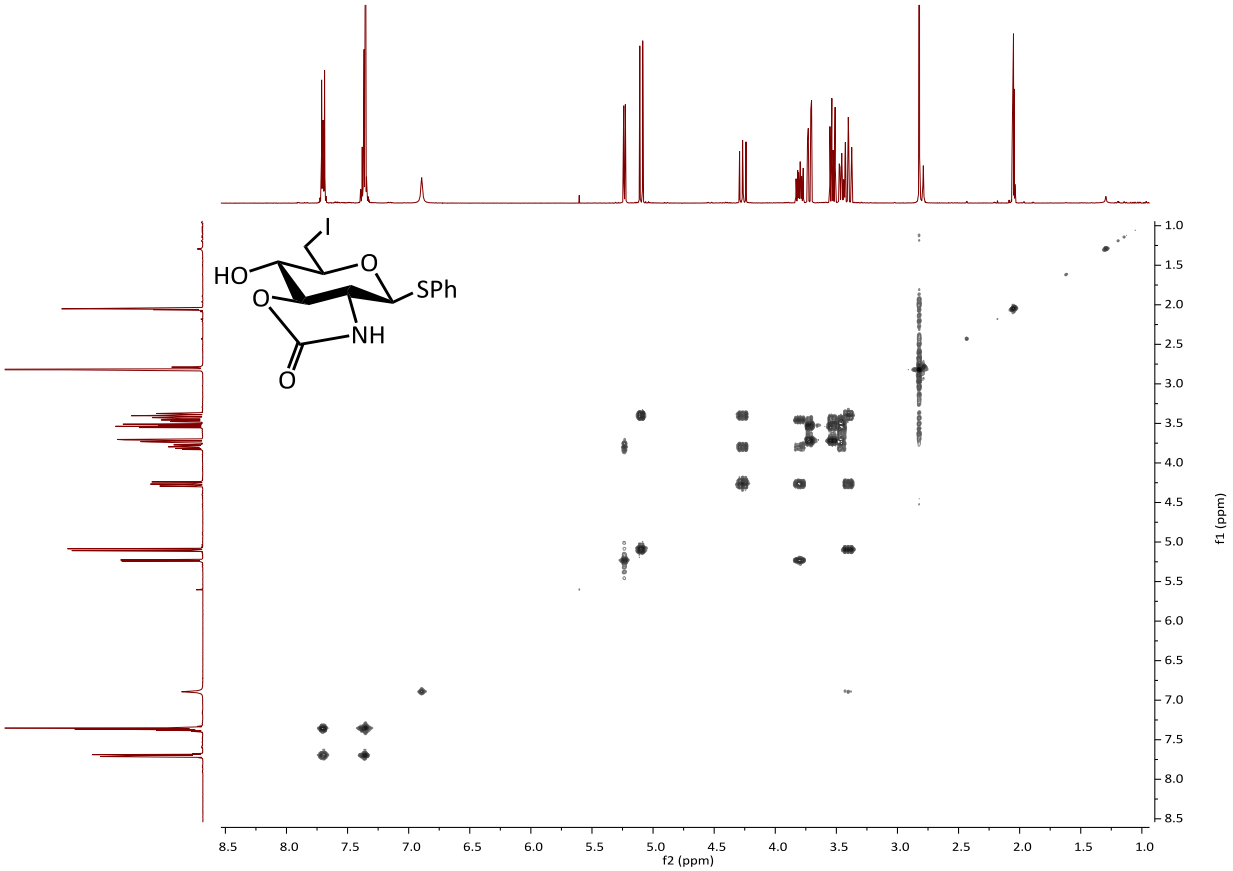

OTs
HO
O
O
SPh
NH
O
f2 (ppm)
f1 (ppm)

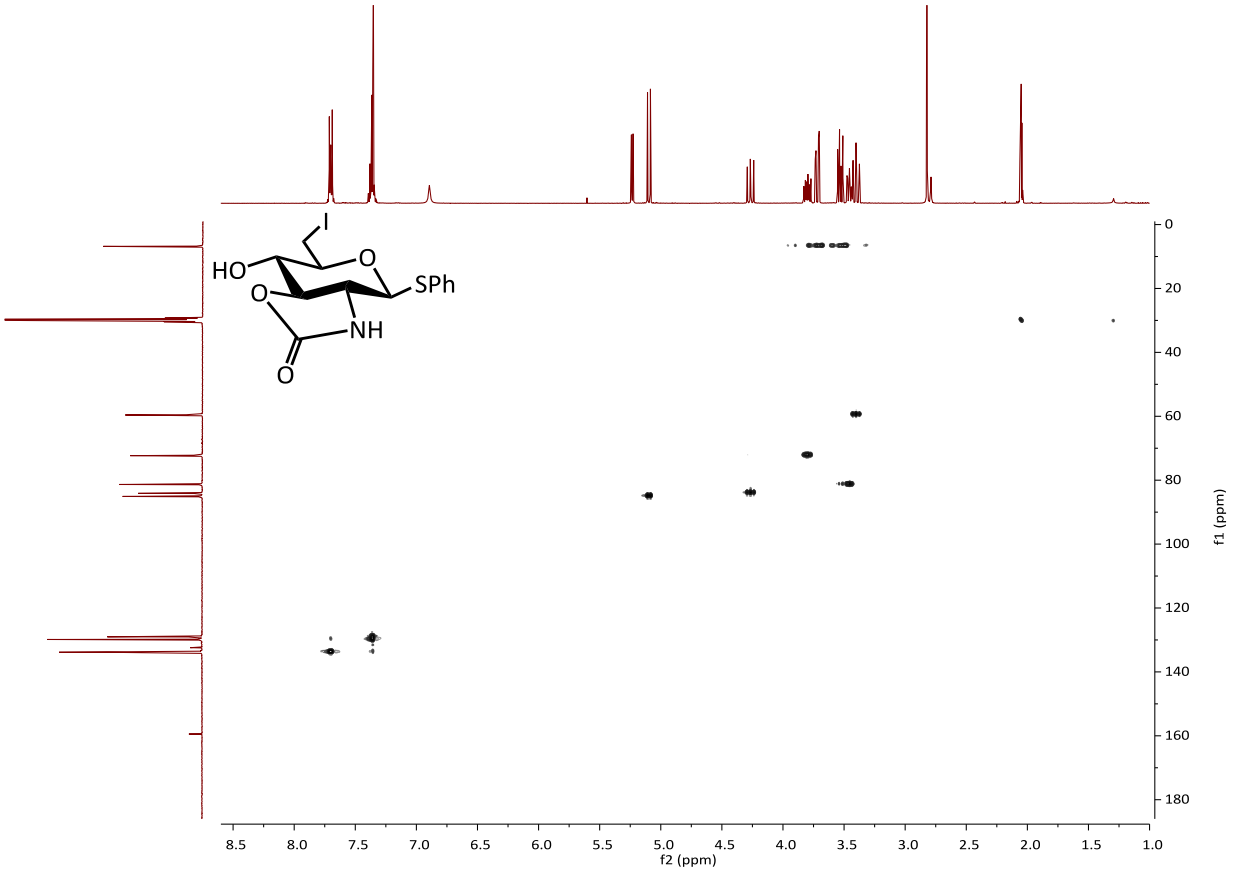
Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-β-D-glucopyranoside (2.55).



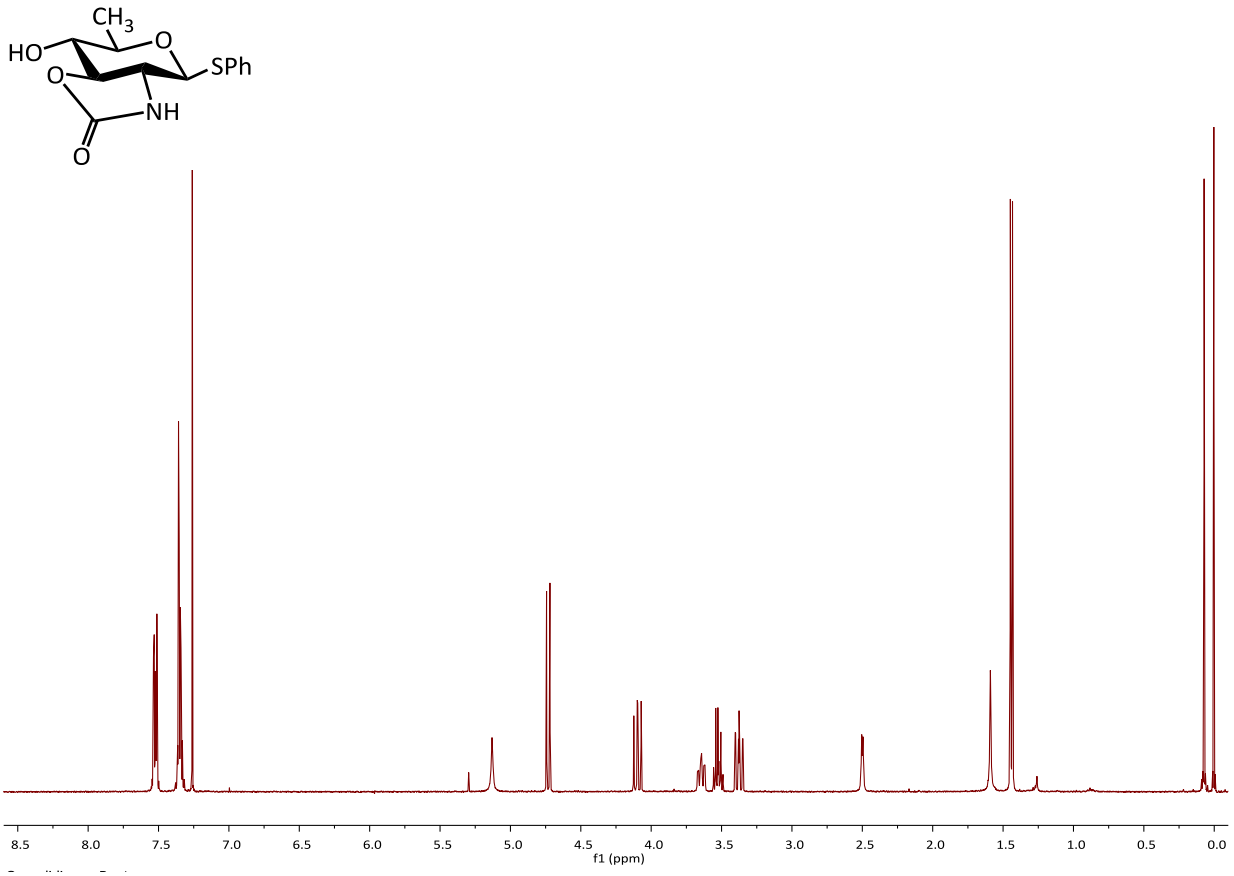


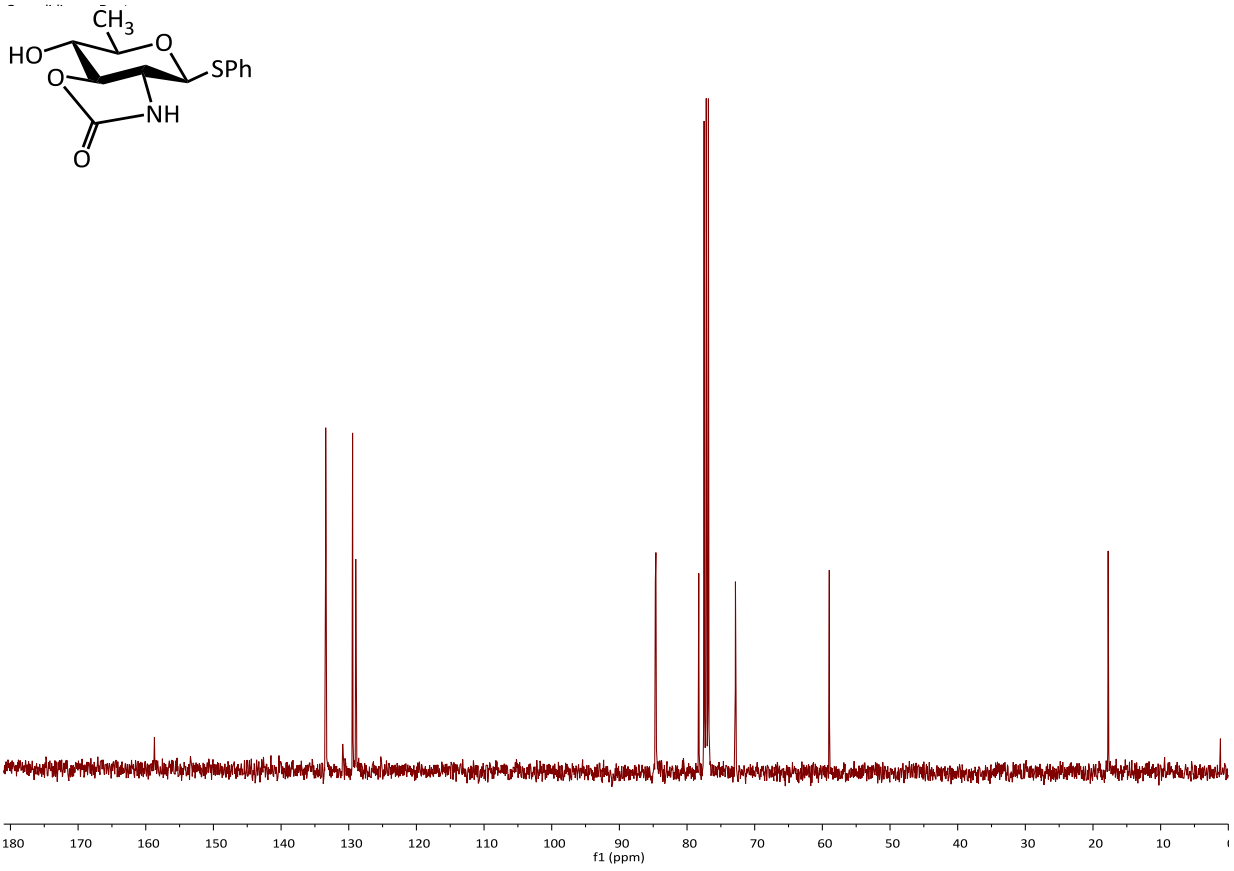


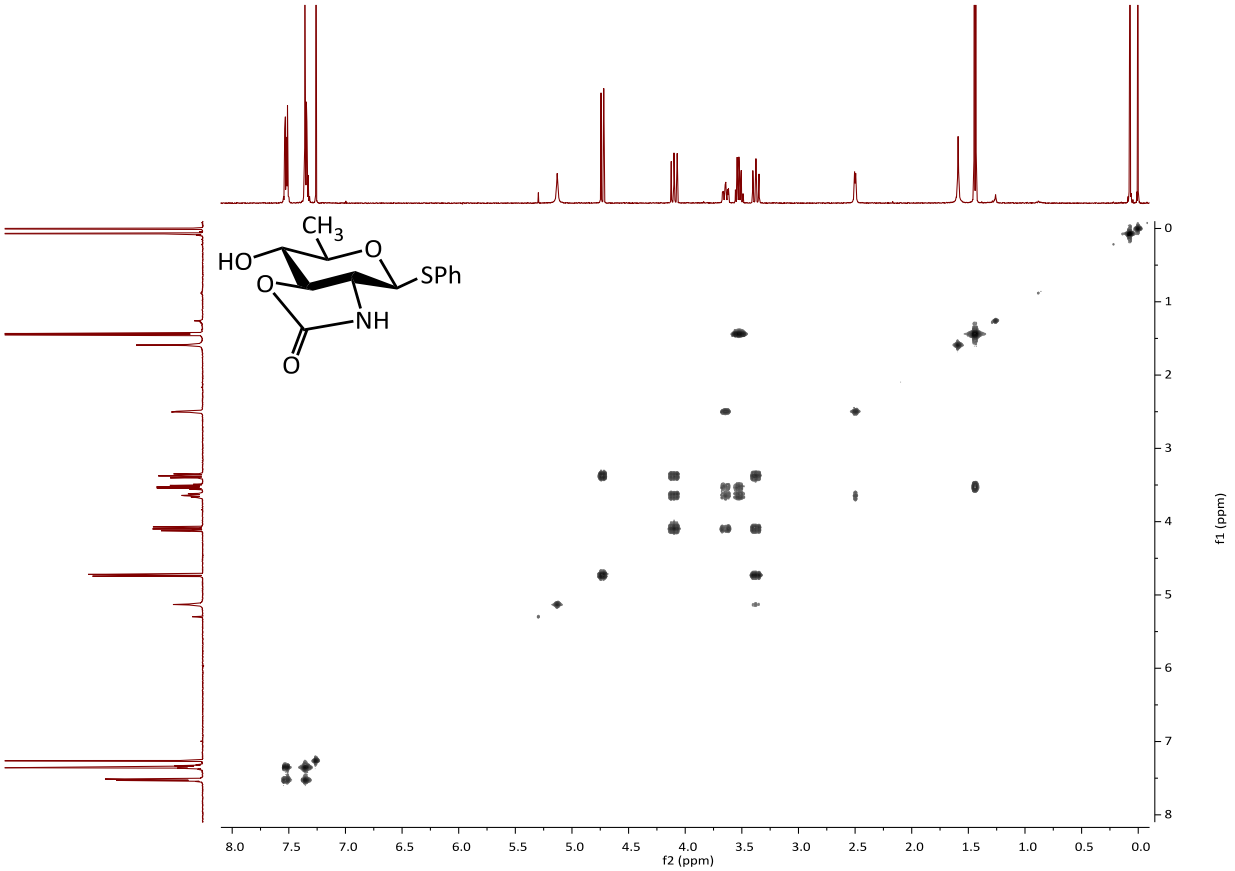

I
HO
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

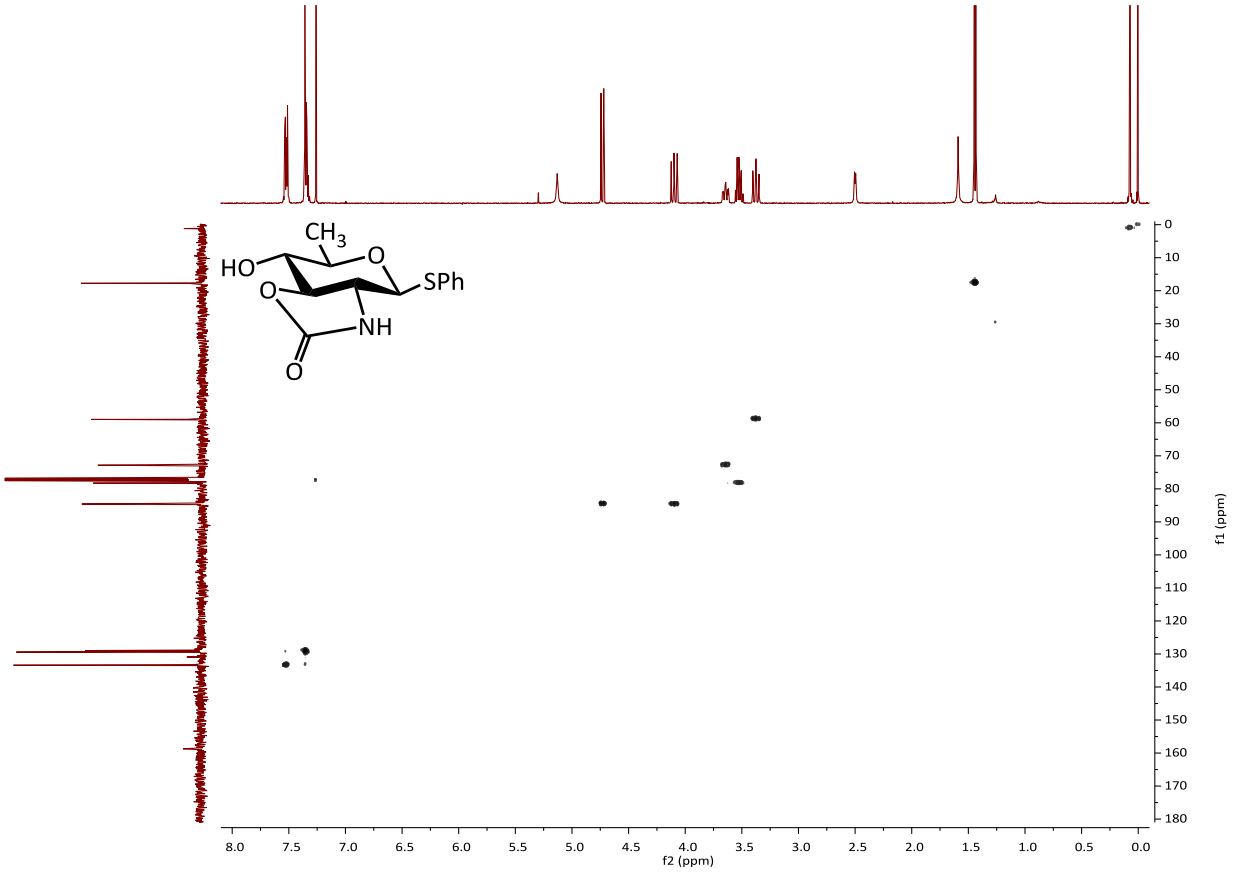

I
HO
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-β-D-glucopyranoside (2.48).

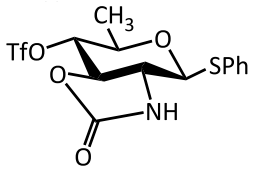


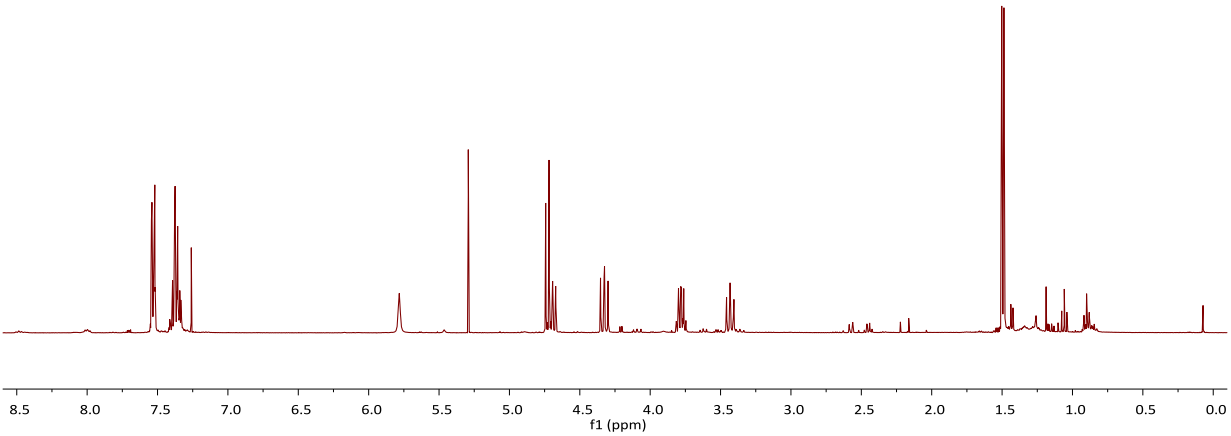


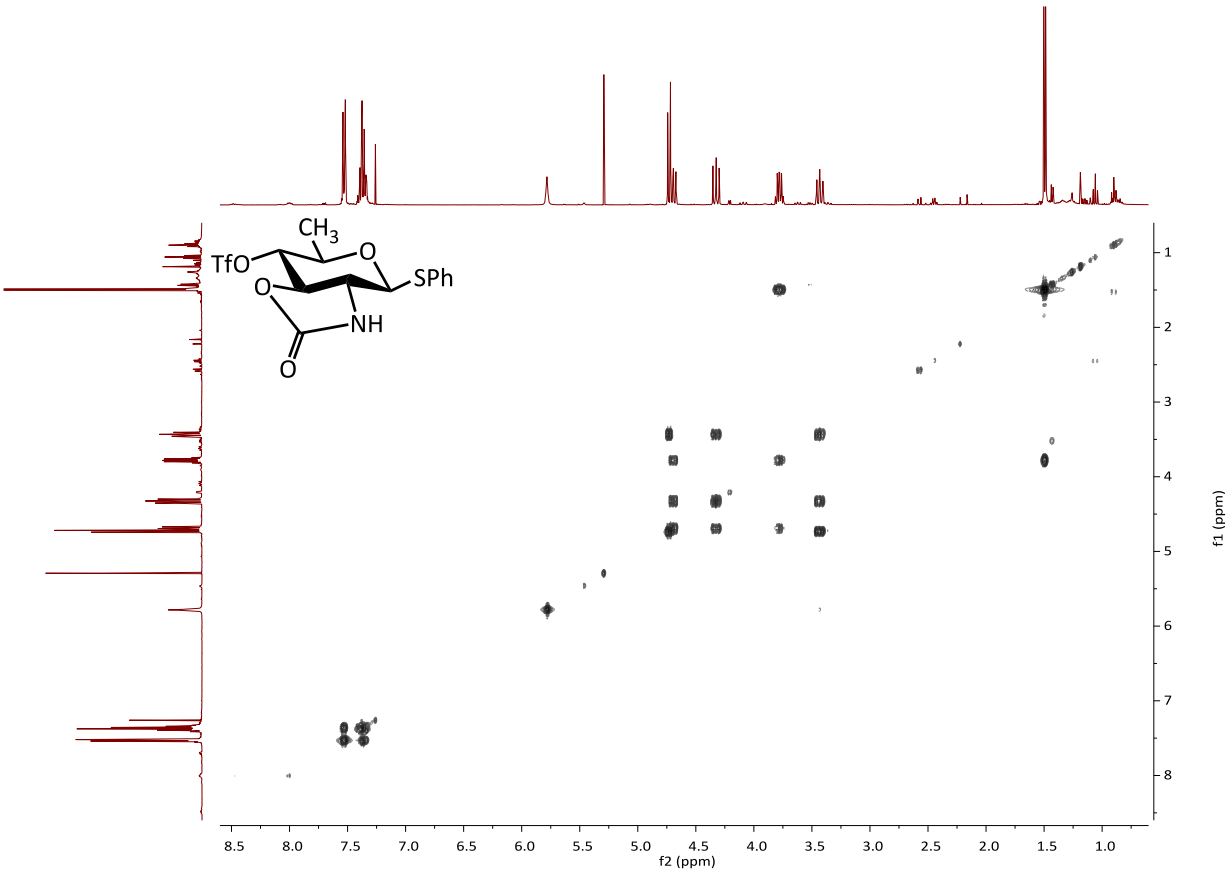

CH3
HO
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

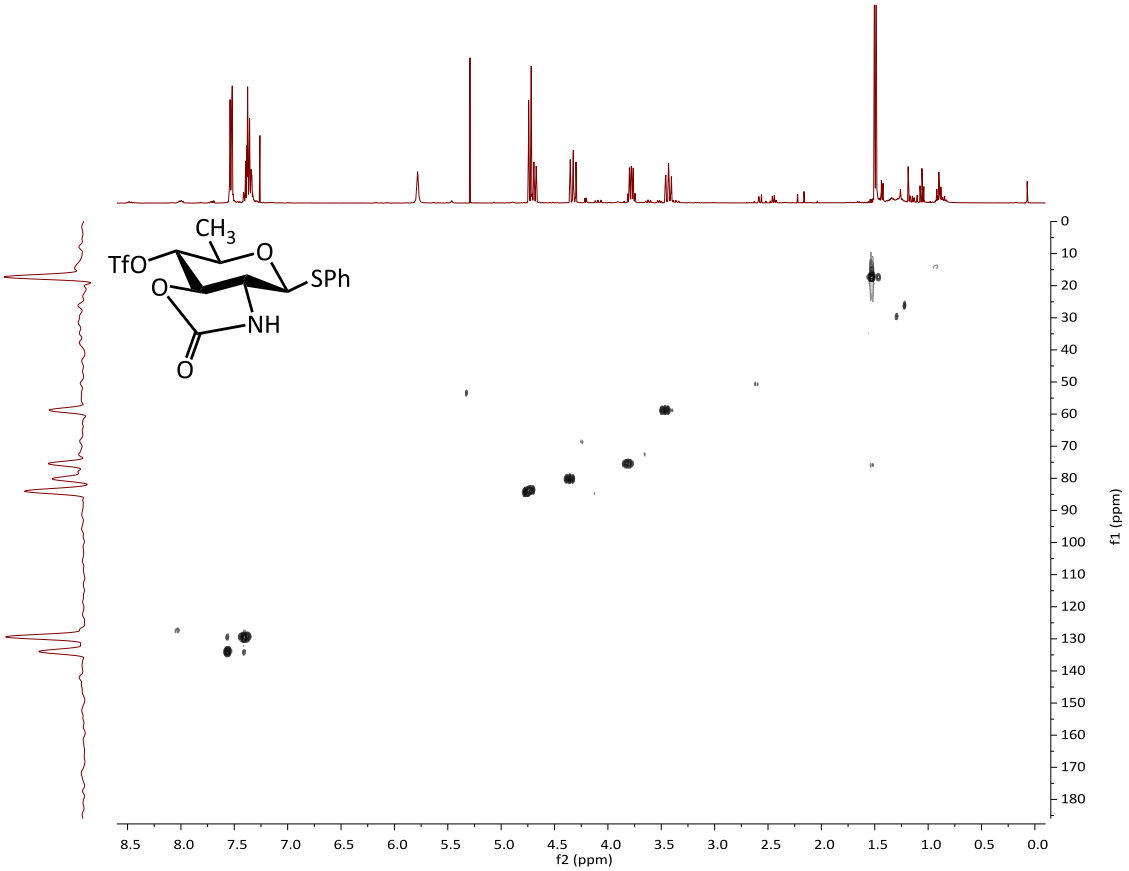

CH3
HO
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-4-*O*-triflyl-*β*-D-glucopyranoside.

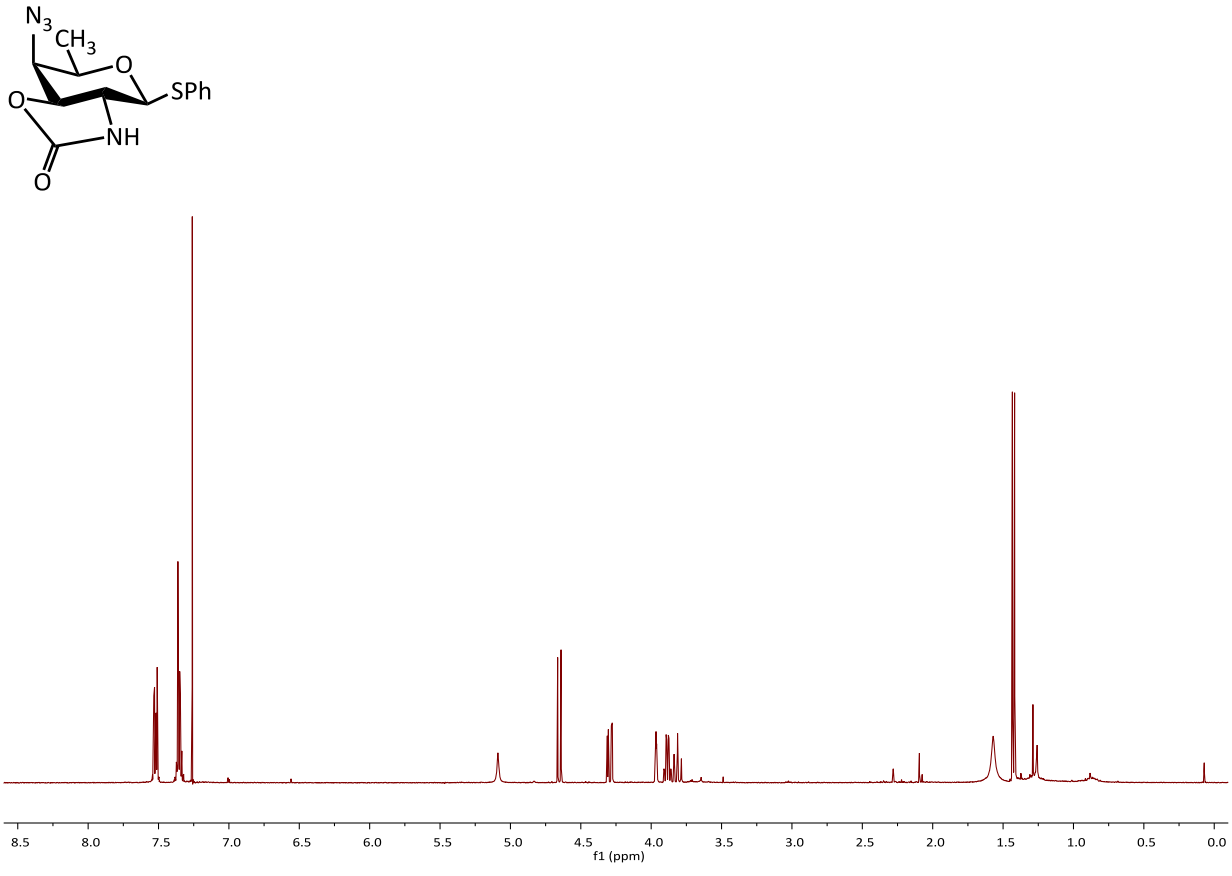


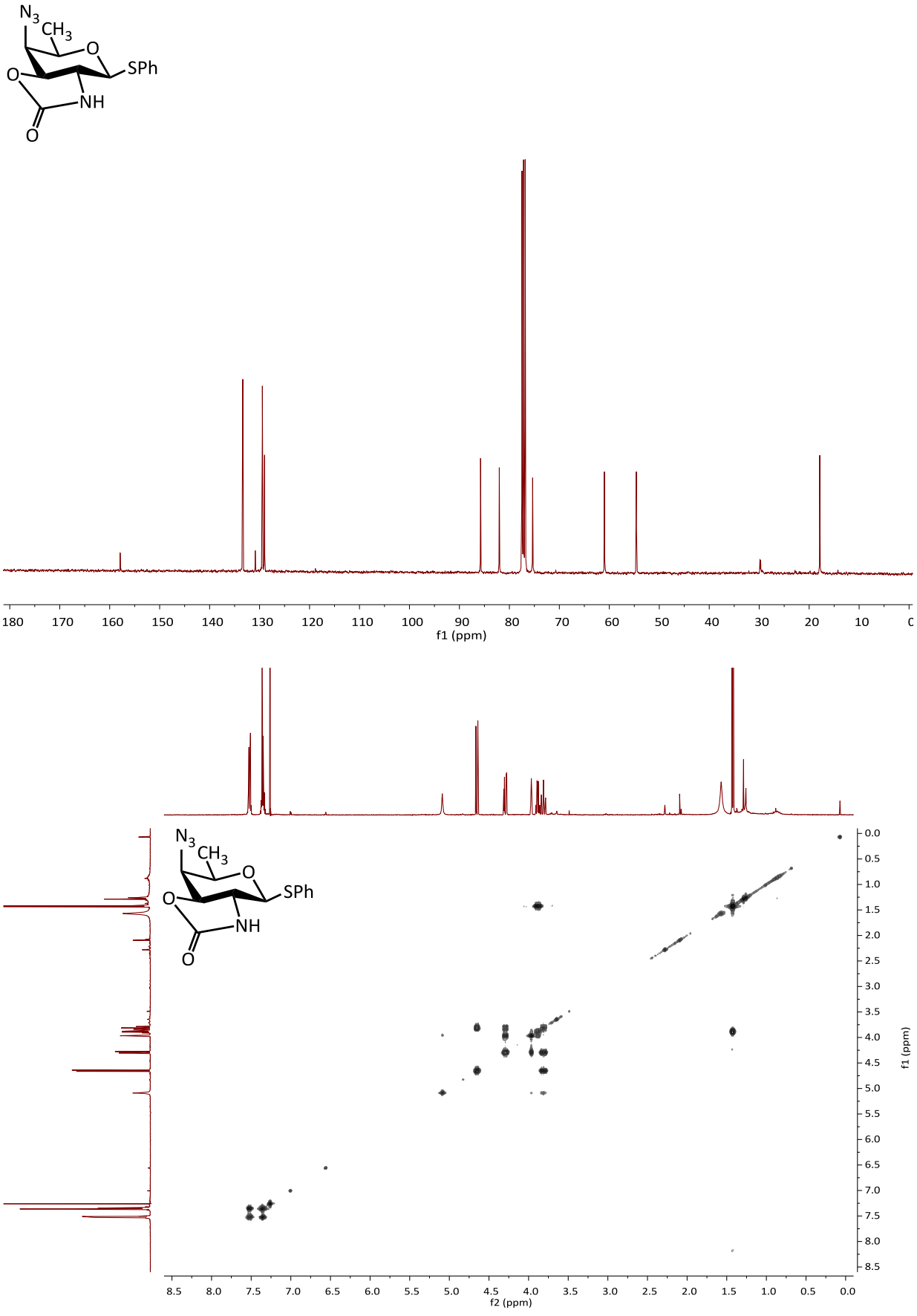


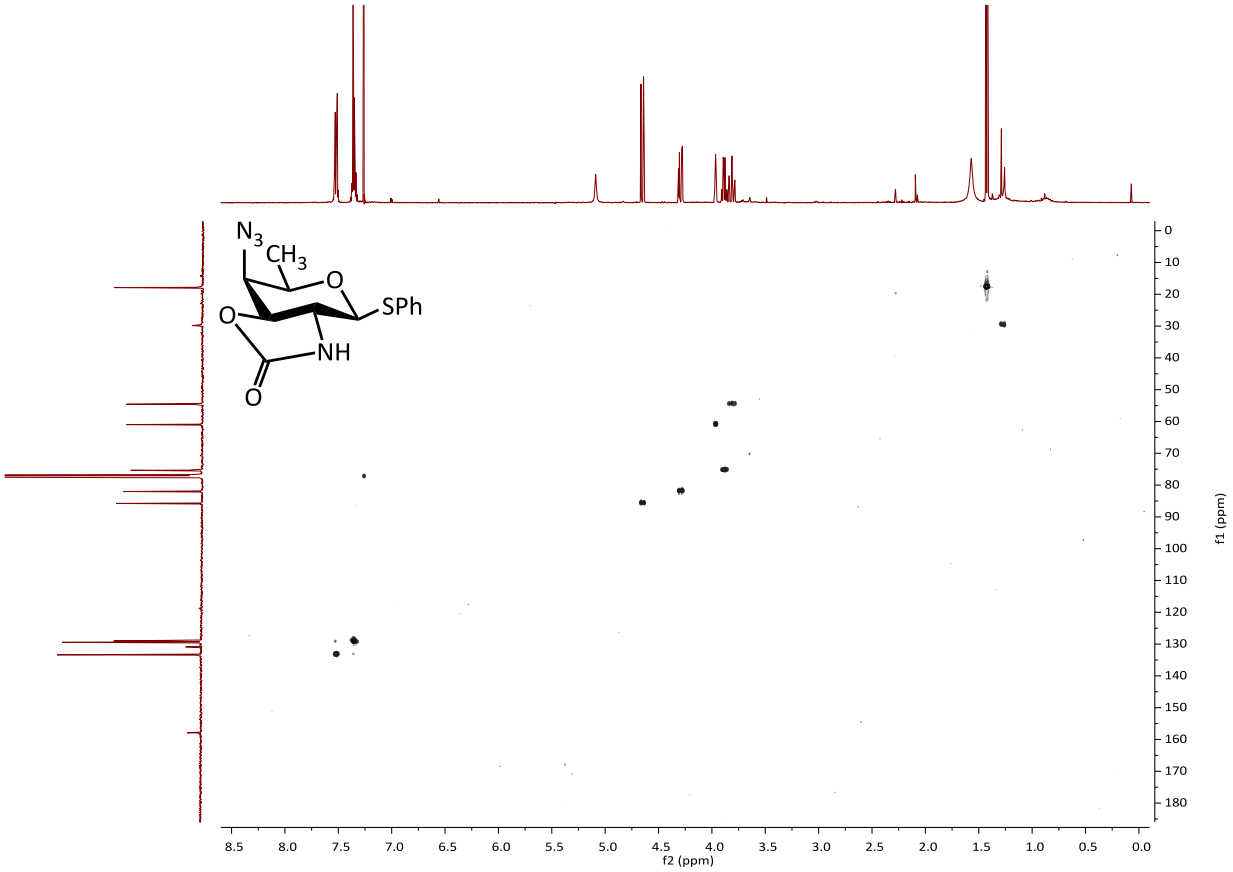


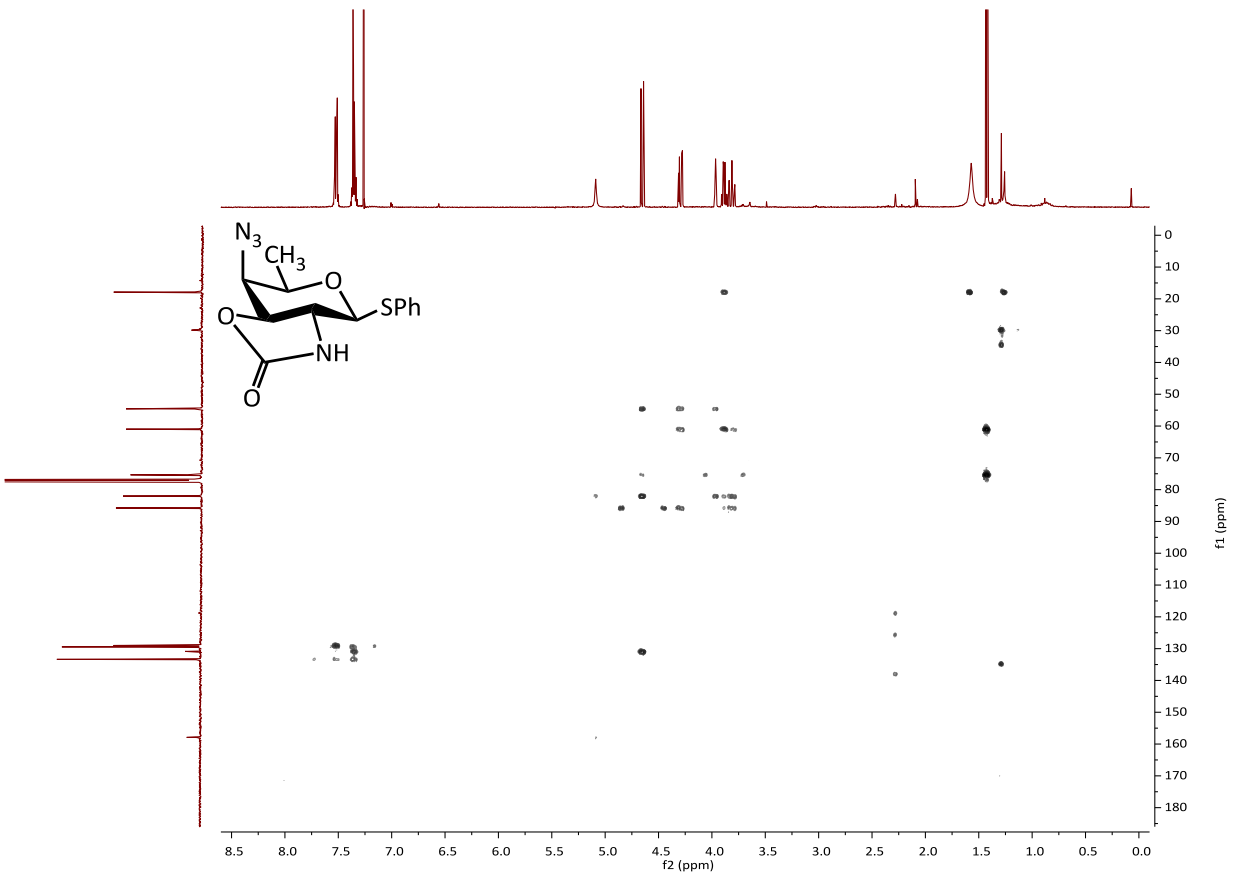


Phenyl 2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.49).

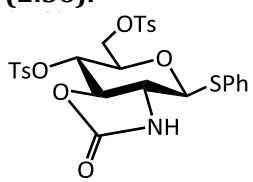


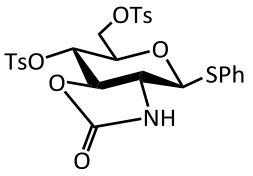

N3
CH3
O
SPh
O
NH
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5
f1 (ppm)

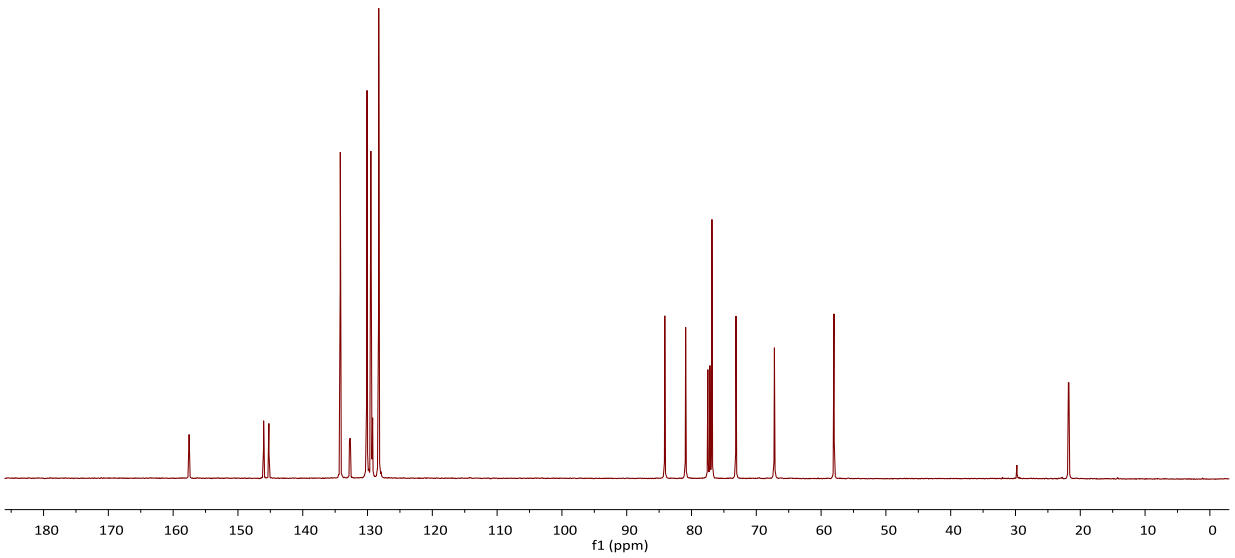

N3
CH3
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

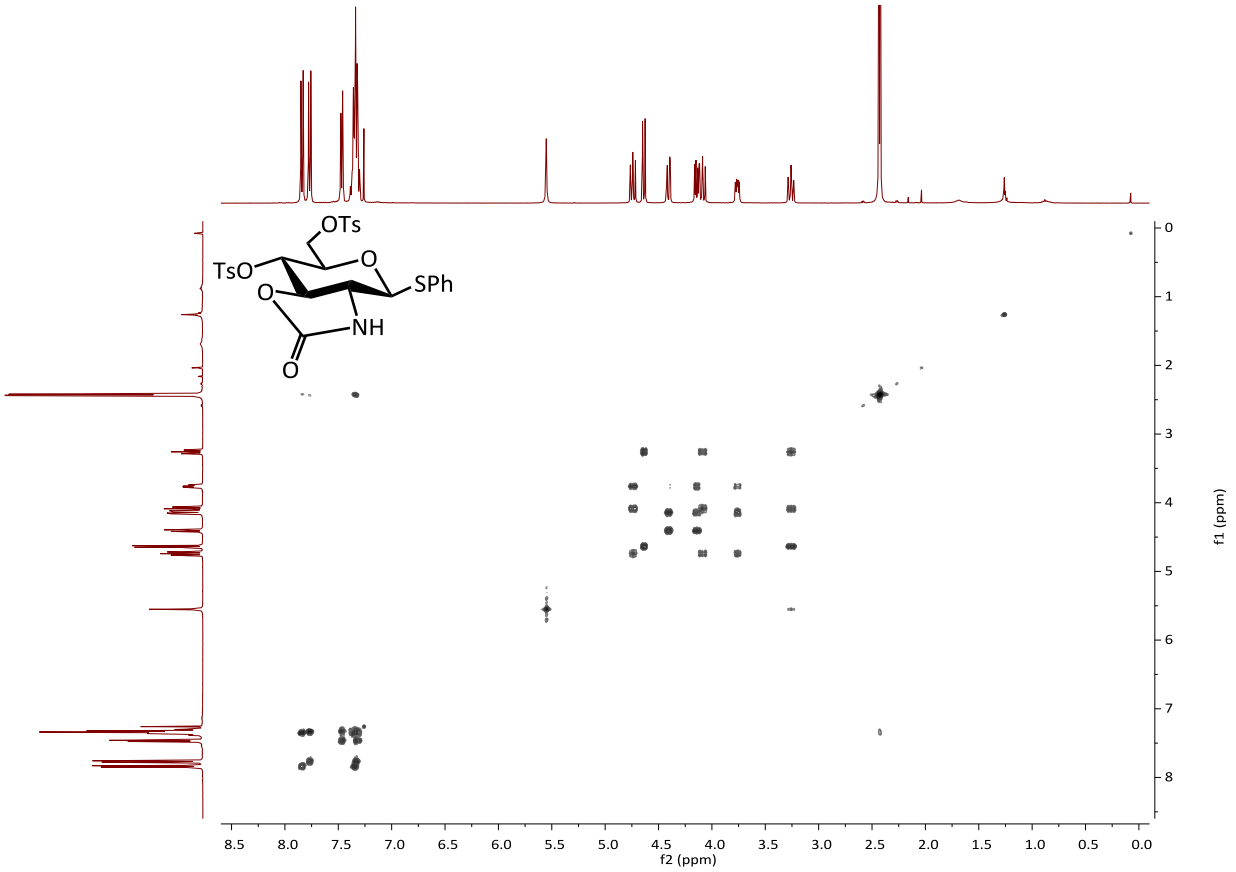

N3
CH3
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

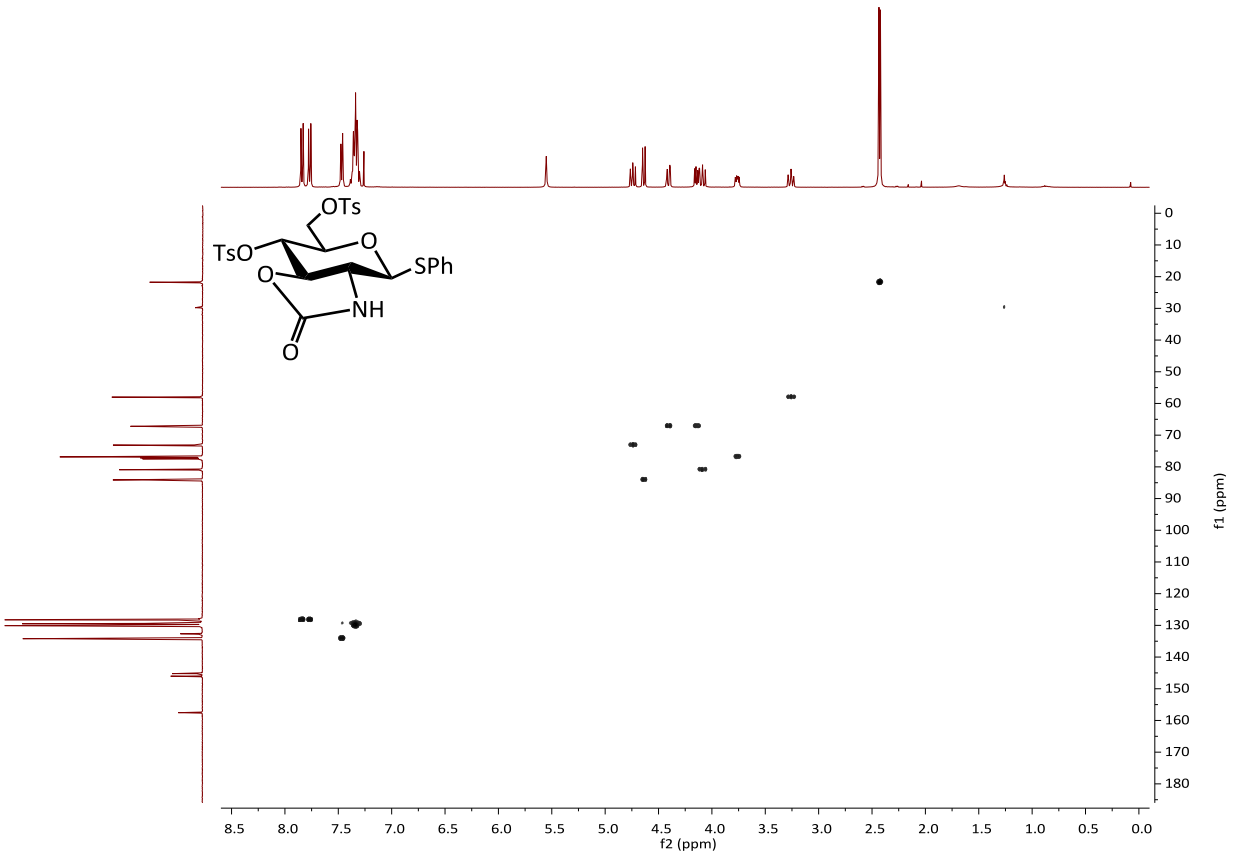
Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-4,6-di-*O*-tosyl-β-D-glucopyranoside (2.56).

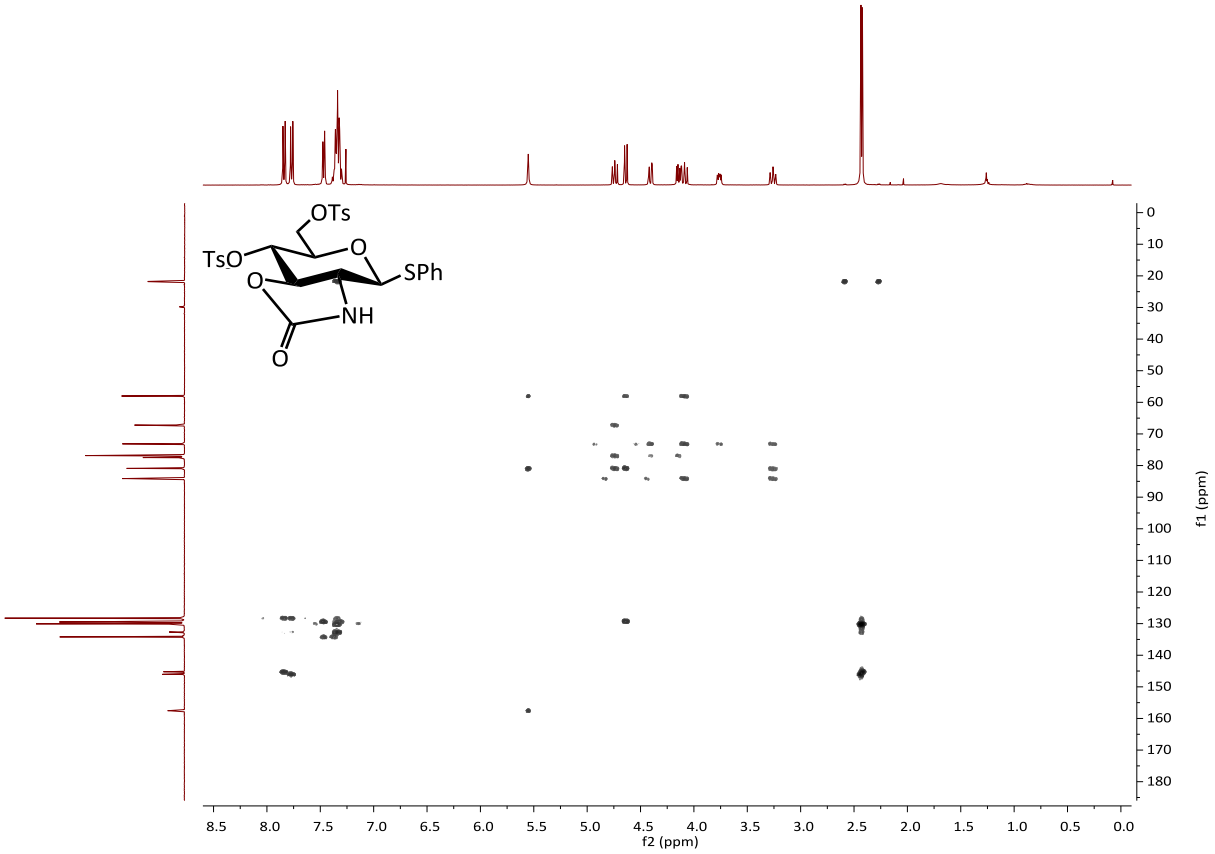




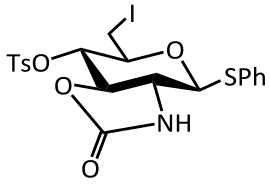


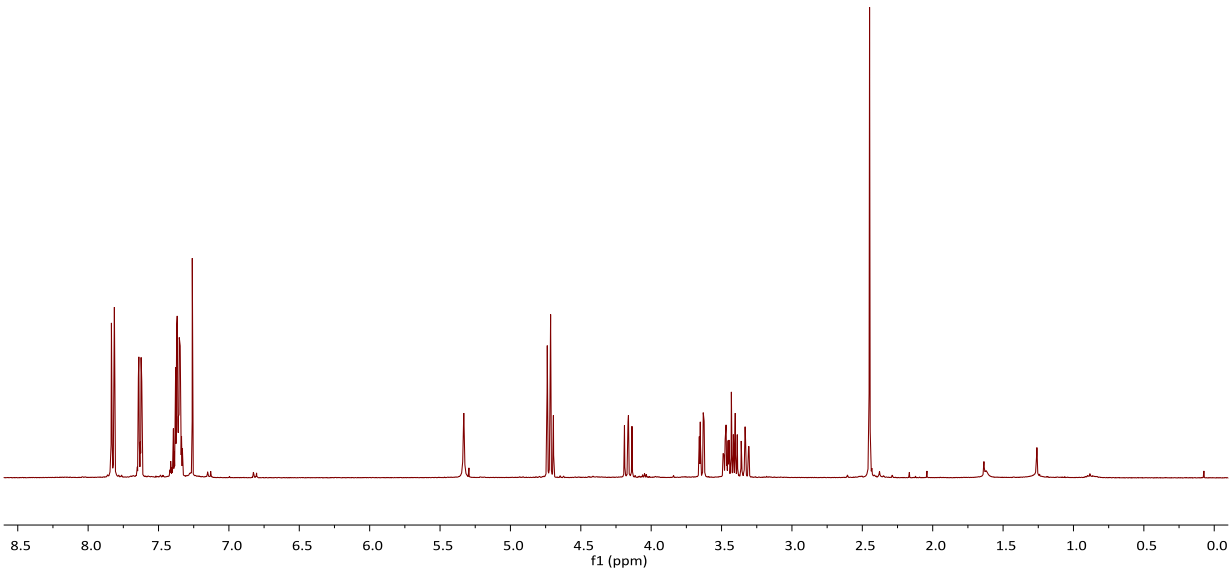

OTs
TsO
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

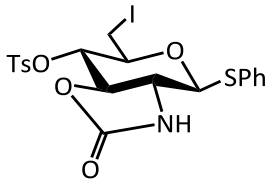

OTs
TsO
O
SPh
O
NH
O
f2 (ppm)
f1 (ppm)

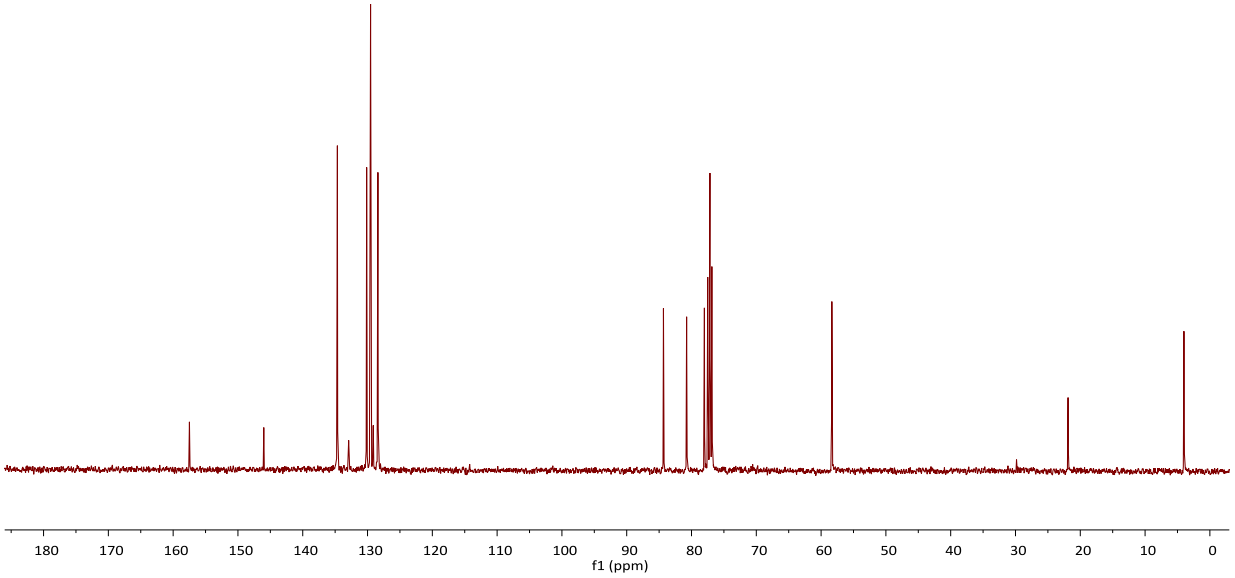


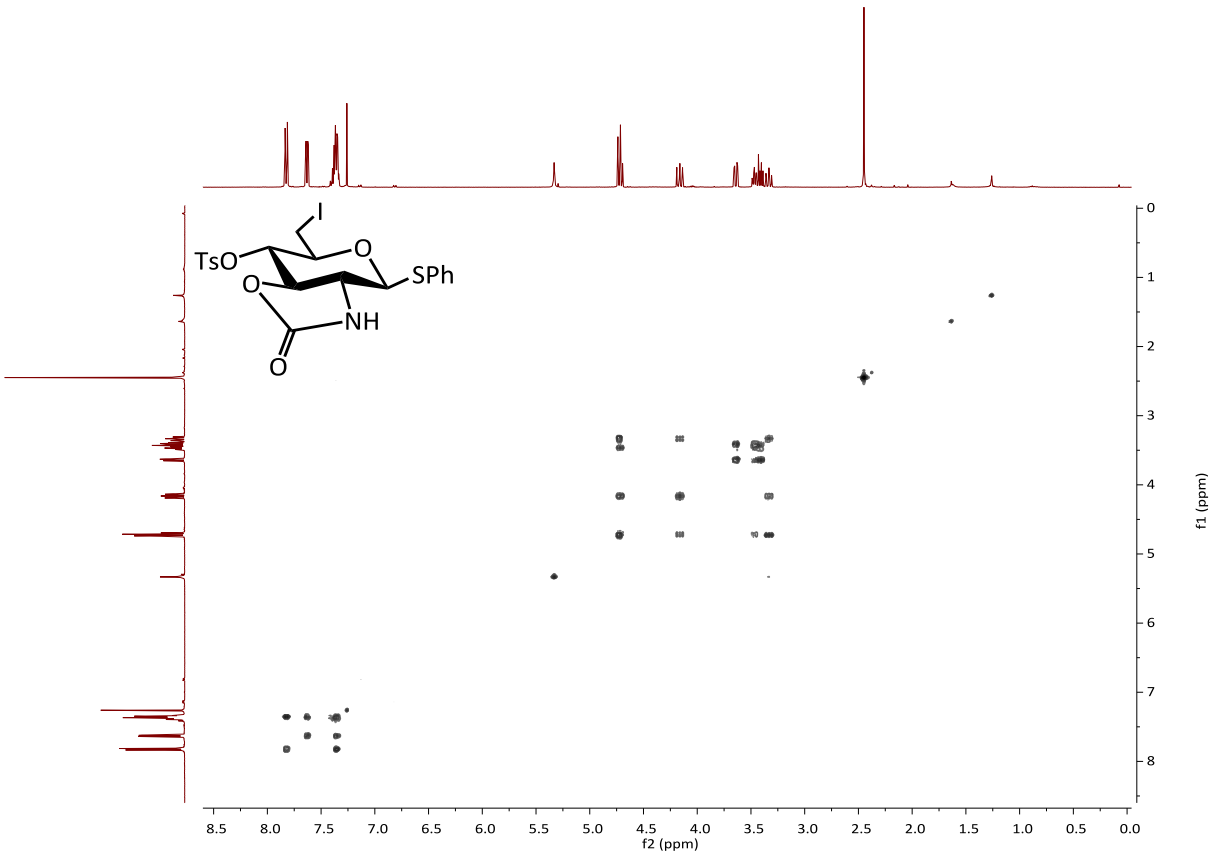
Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-4-*O*-tosyl-β-D-glucopyranoside (2.57).

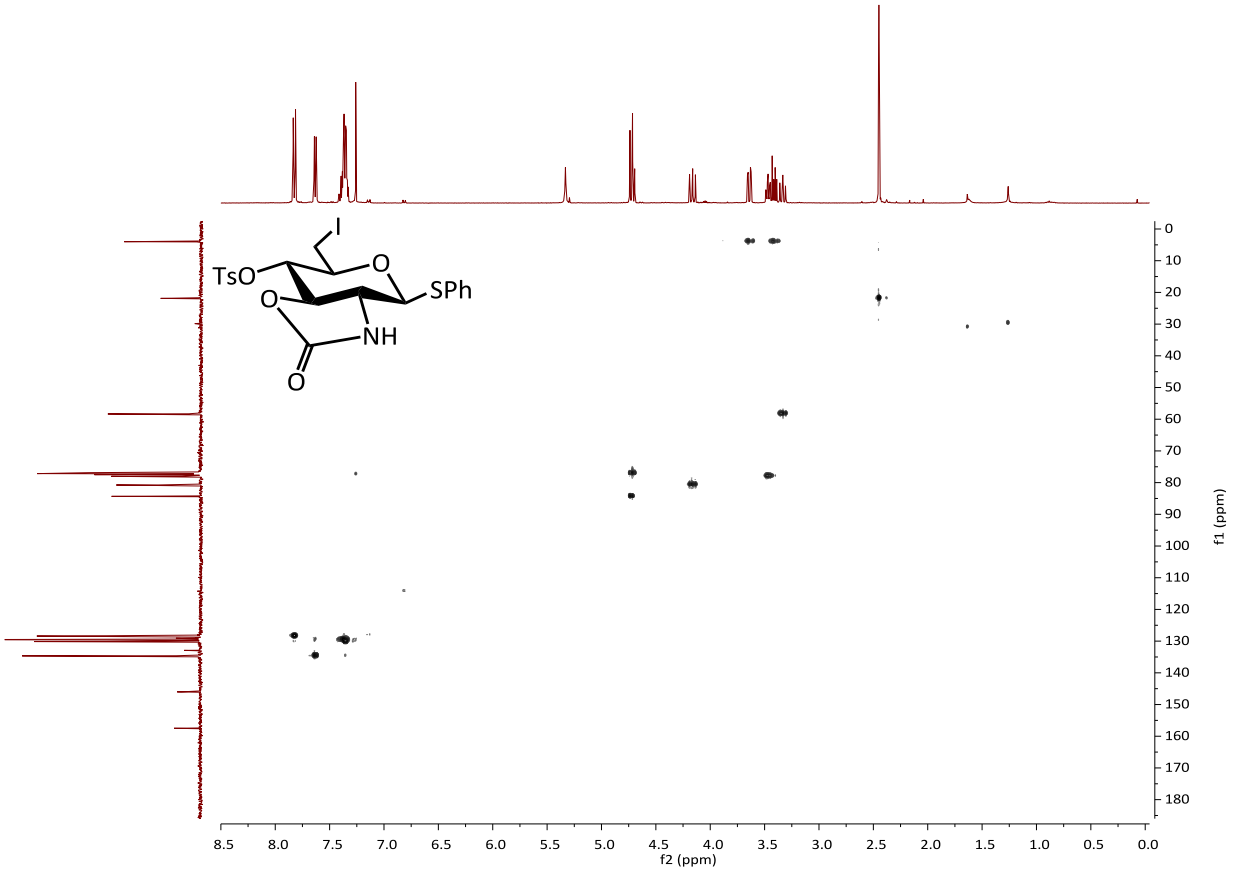


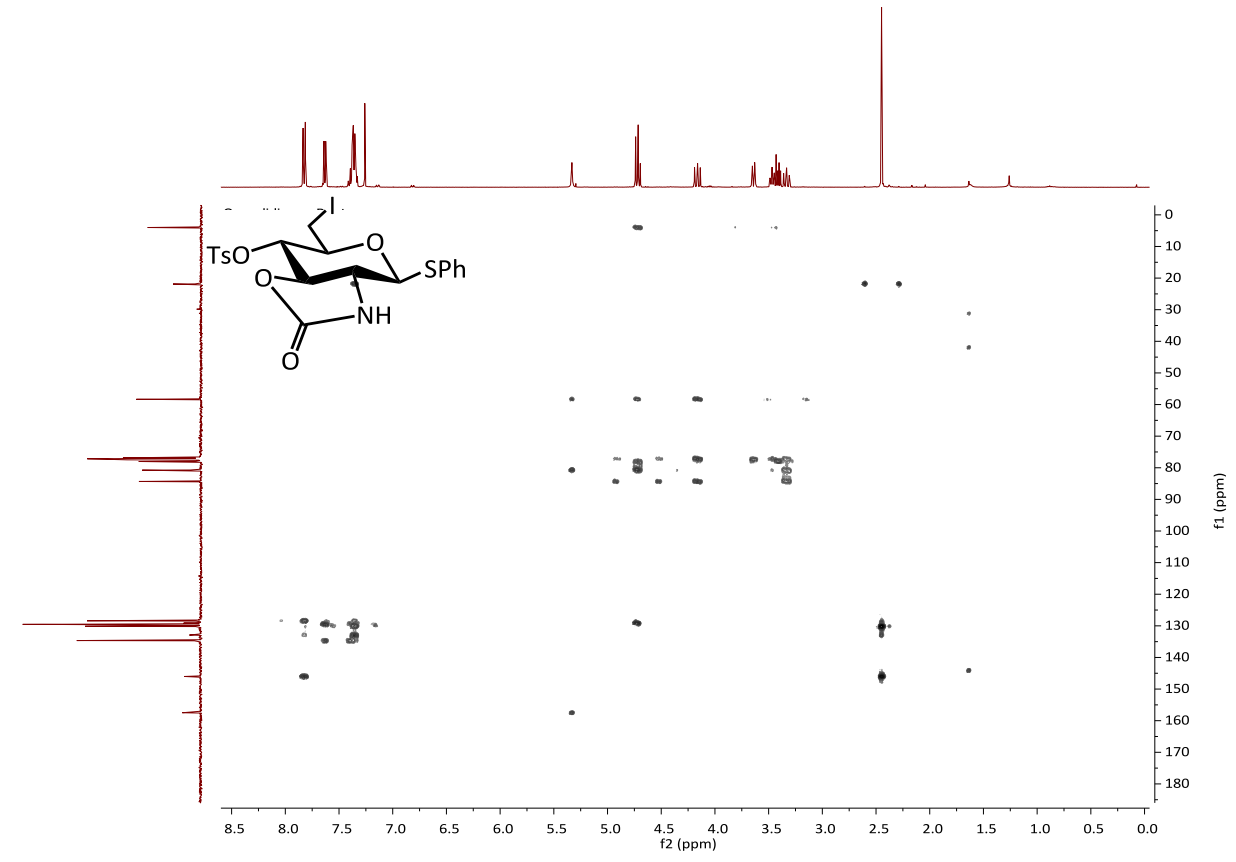



I
TsO
O
SPh
O
NH
O

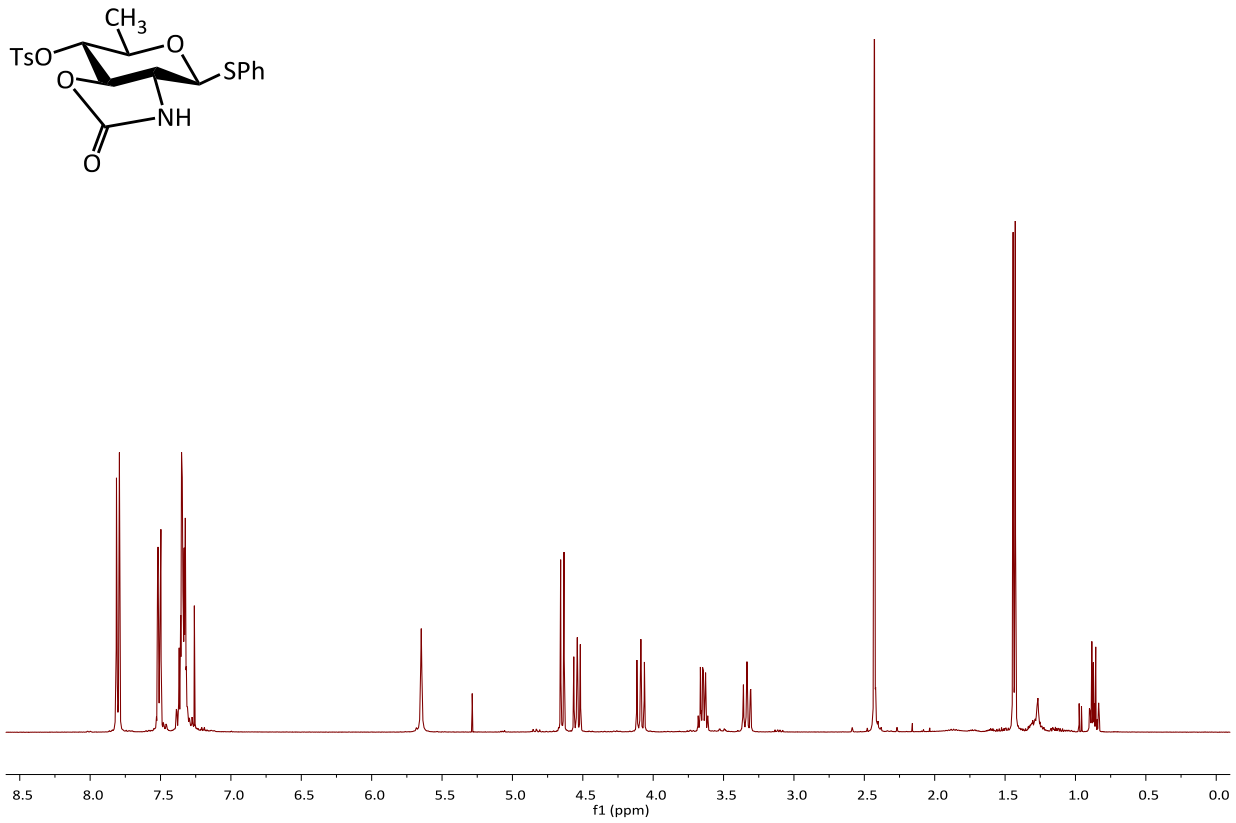

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

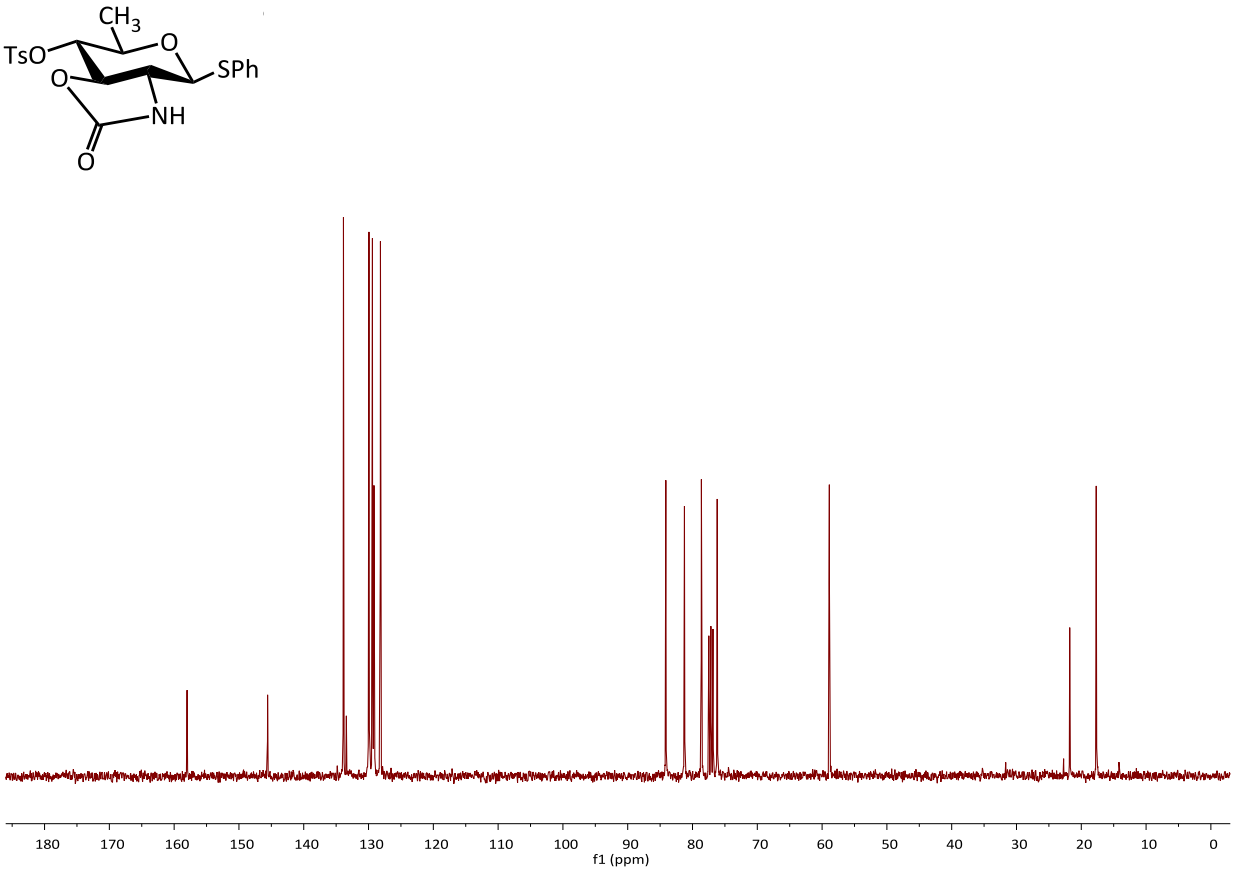

I
TsO
O
O
SPh
NH
O
0 1 2 3 4 5 6 7 8
f1 (ppm)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)

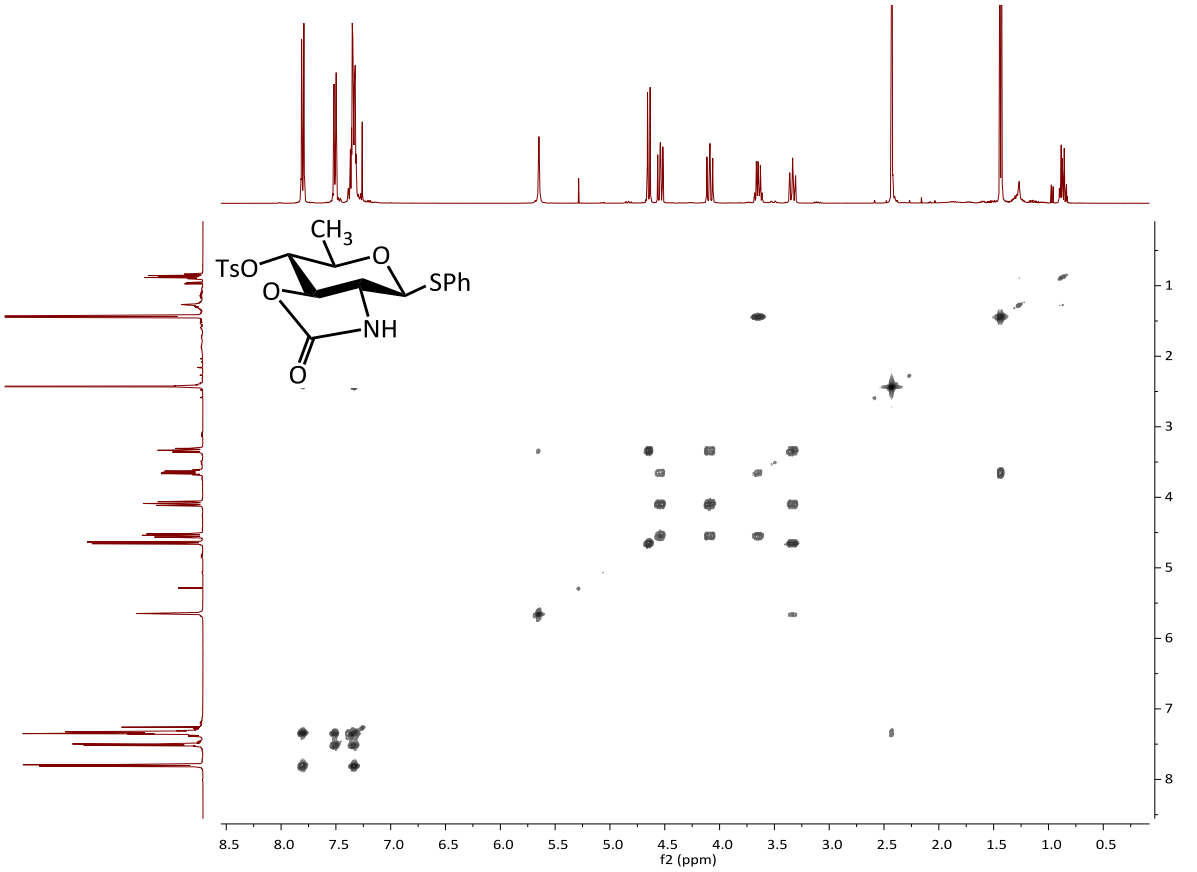

I
TsO
O
O
SPh
NH
O
f2 (ppm)
f1 (ppm)

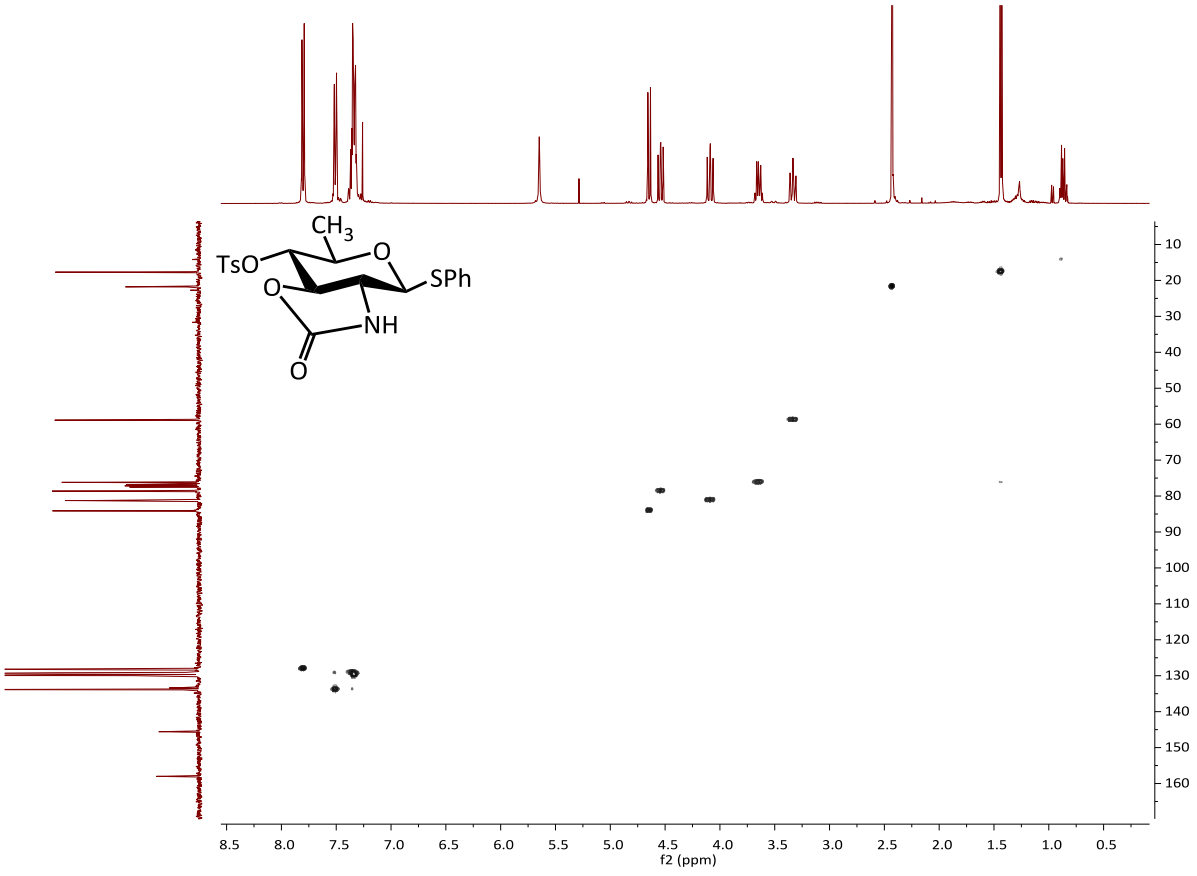

I
TsO
O
O
SPh
NH
O
f2 (ppm)
f1 (ppm)

Phenyl 2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-4-*O*-tosyl-β-D-glucopyranoside (2.58).

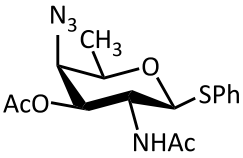


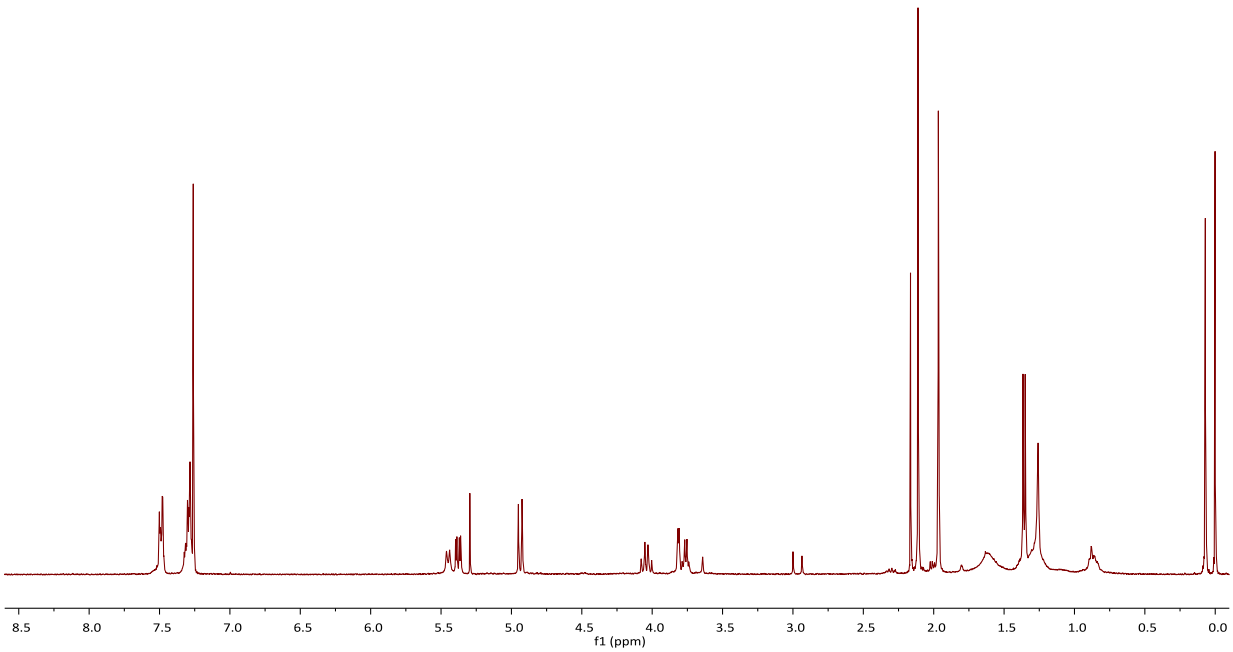


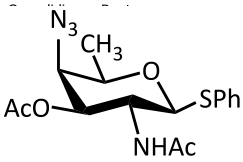

CH3
TsO
O
SPh
O
NH
O
f2 (ppm)

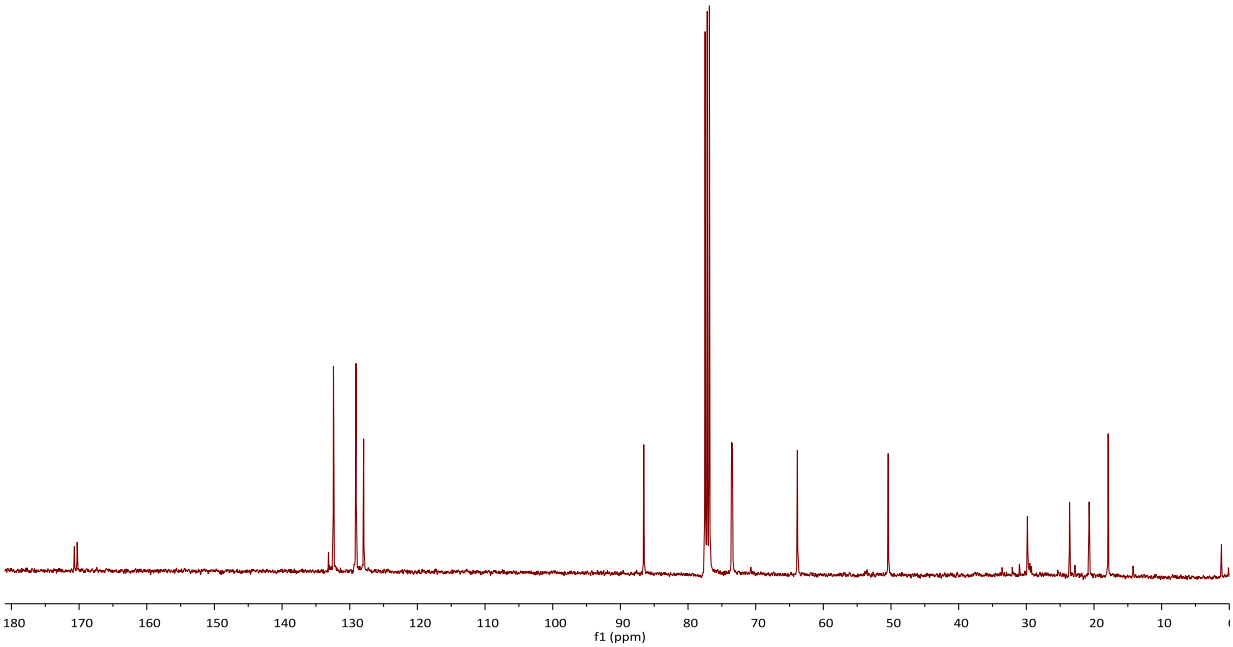

CH3
TsO
O
SPh
O
NH
O
f2 (ppm)

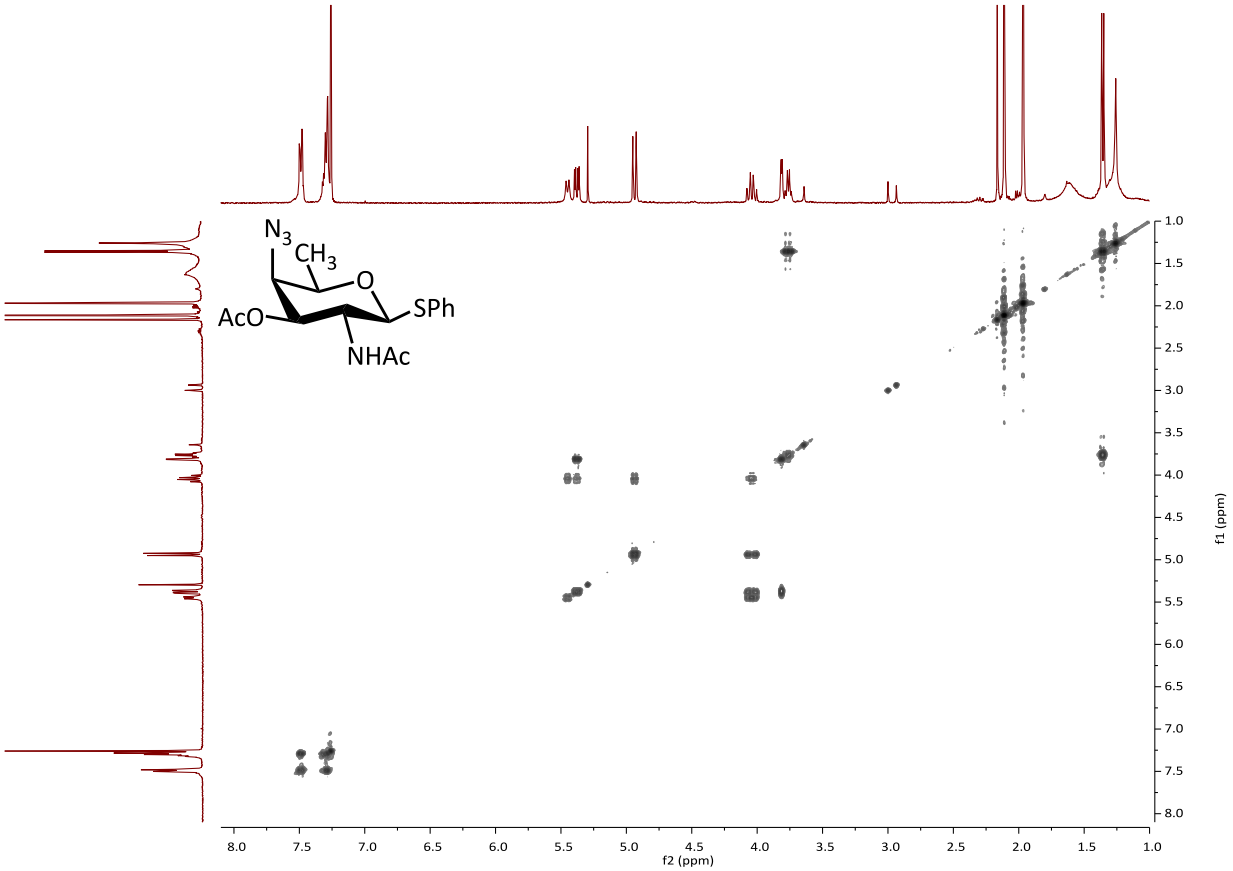
Phenyl 2-acetamido-3-*O*-acetyl-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.51).

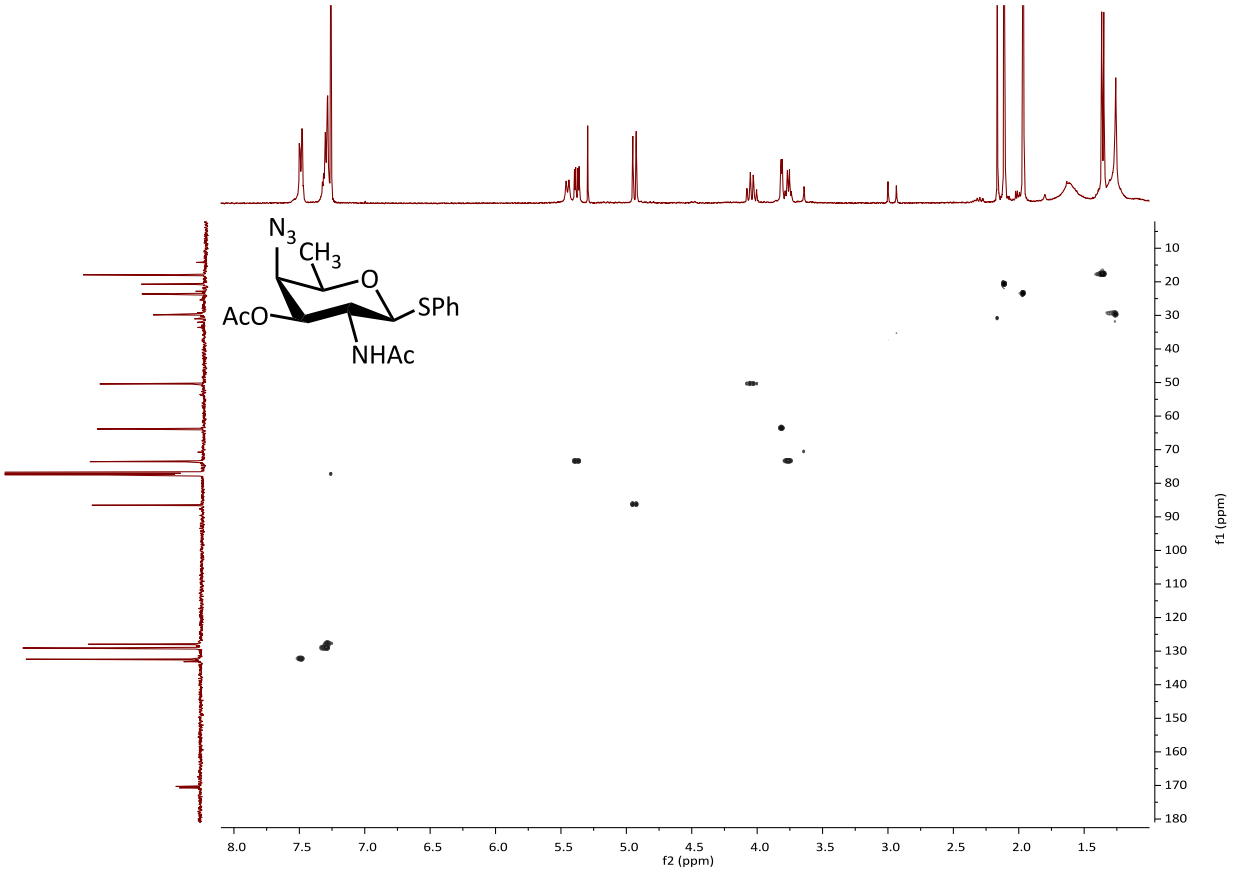


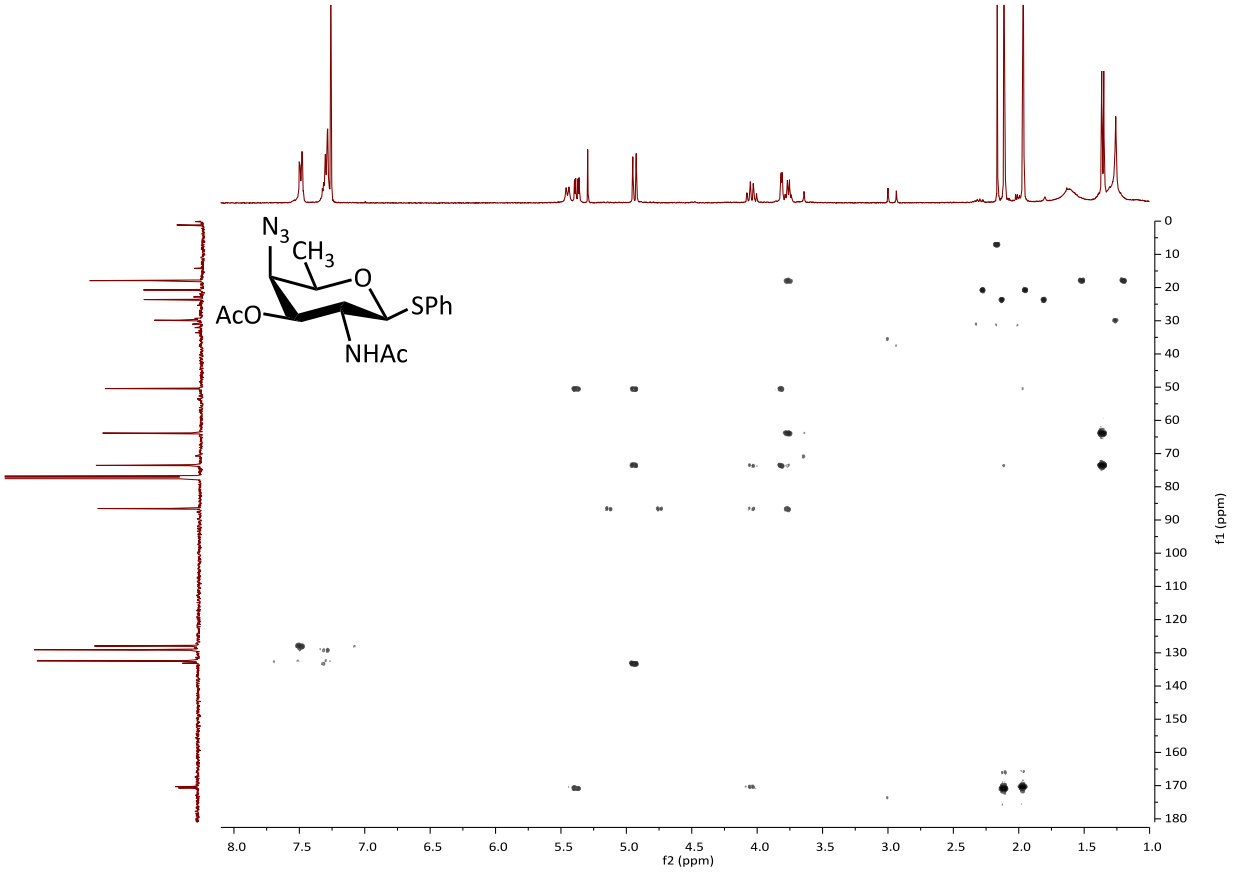




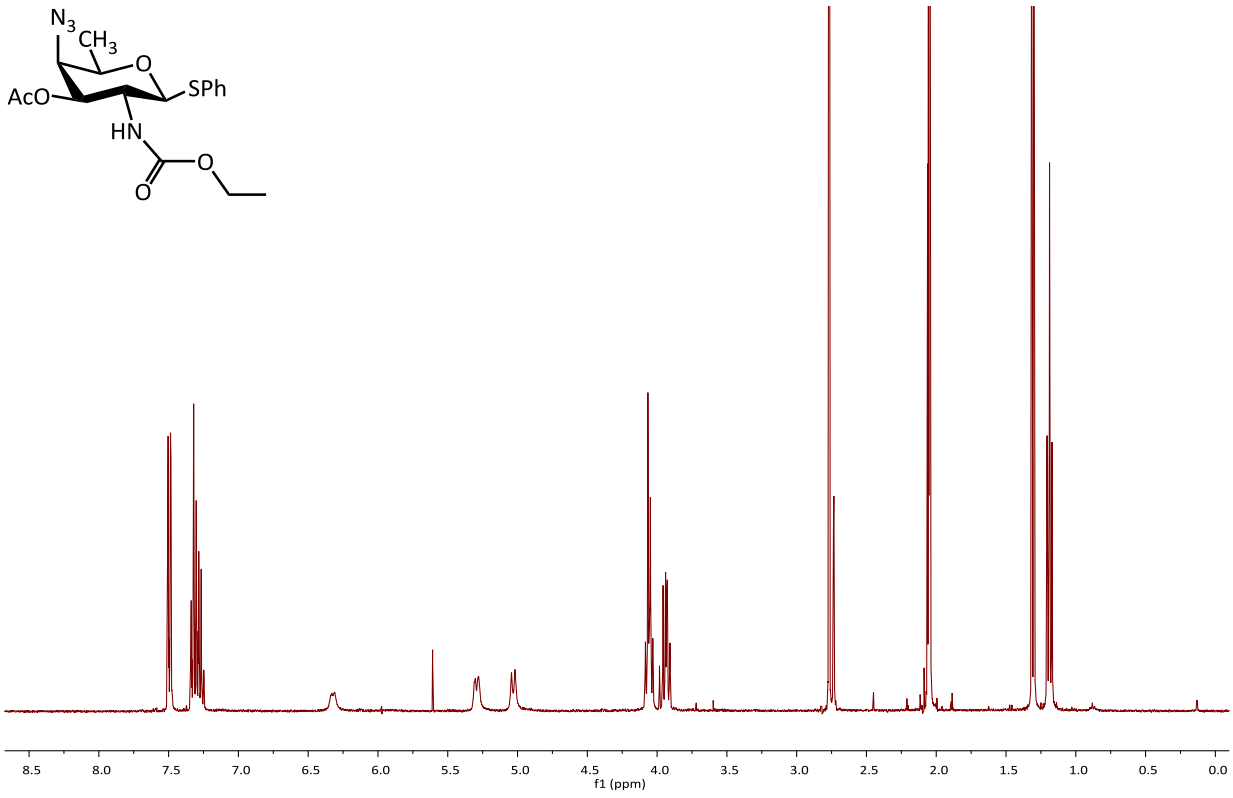


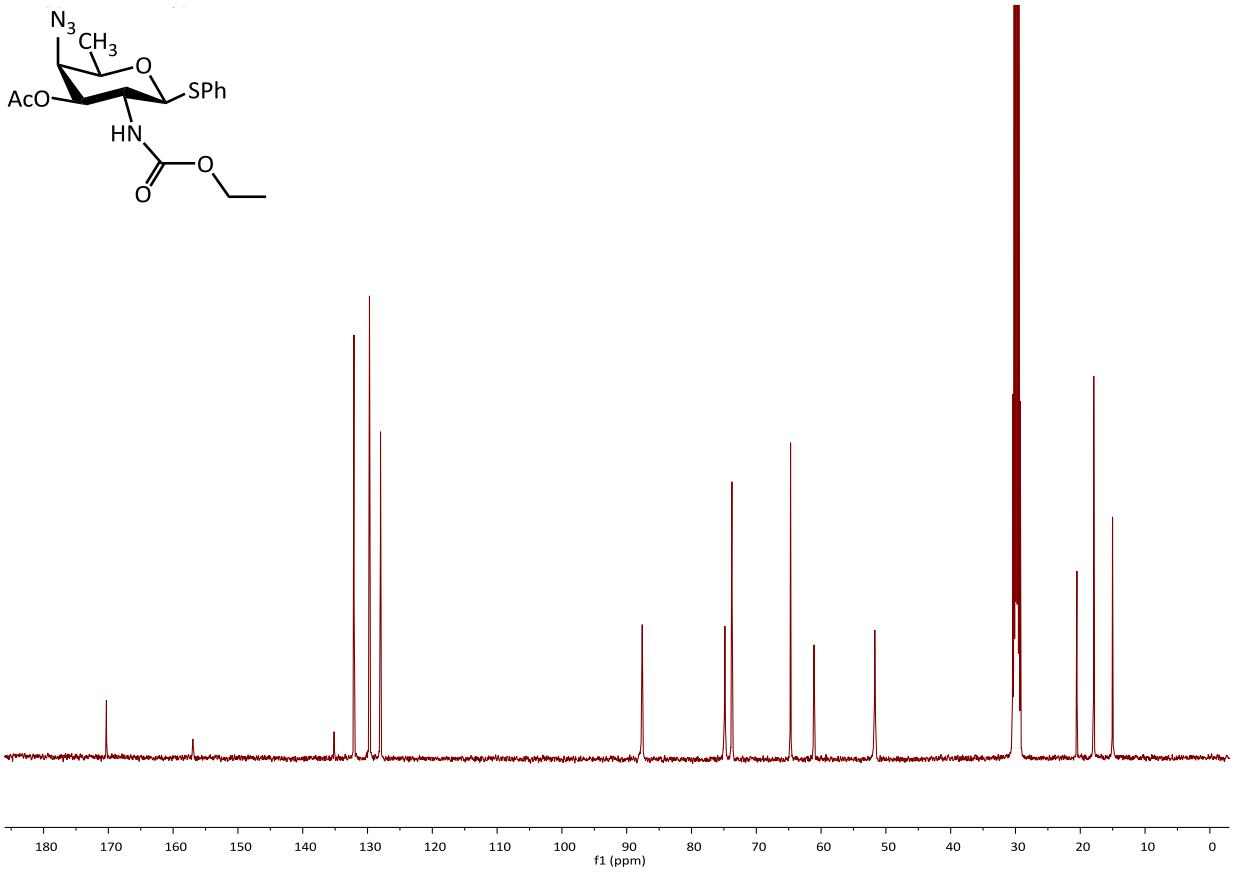

N3
CH3
O
AcO
SPh
NHAc
f2 (ppm)
f1 (ppm)

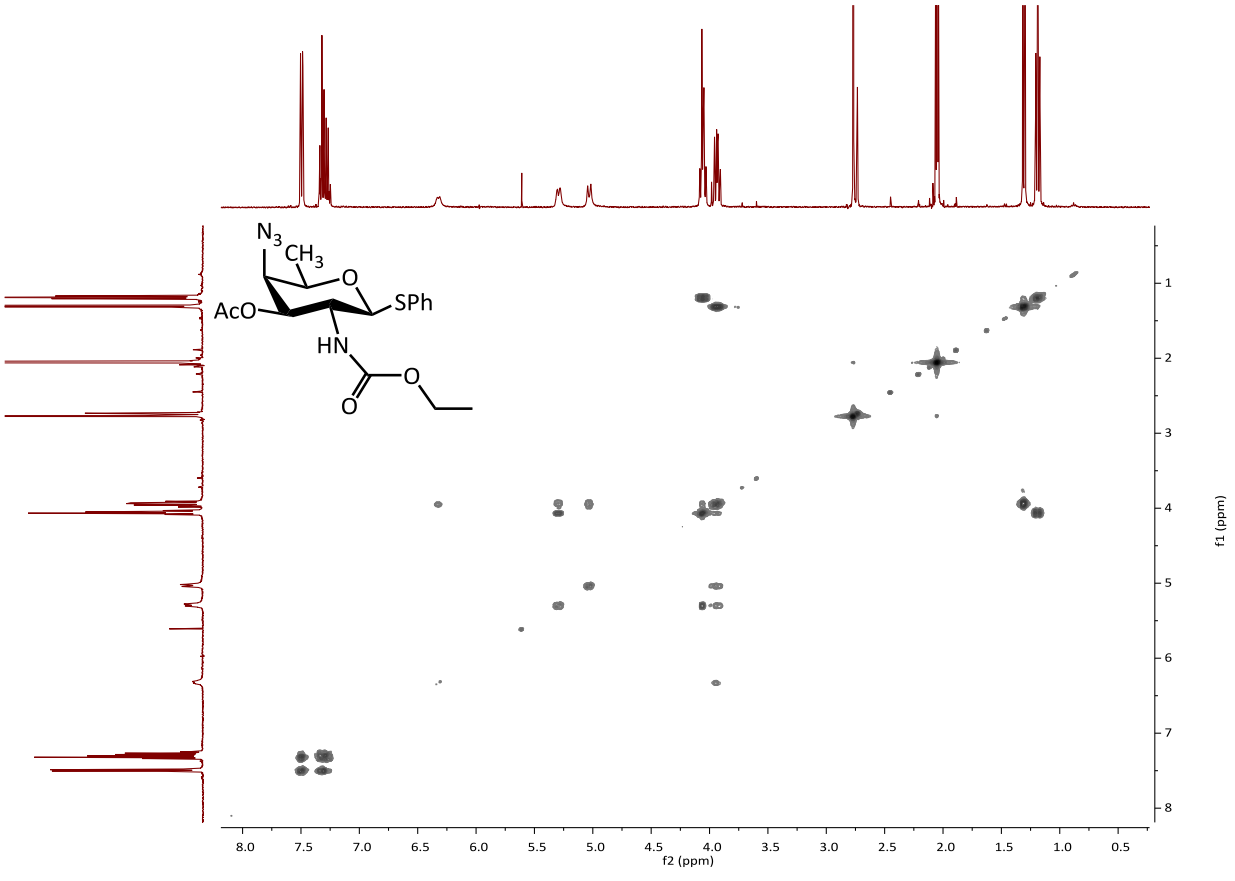

N3
CH3
O
AcO
SPh
NHAc
f2 (ppm)
f1 (ppm)

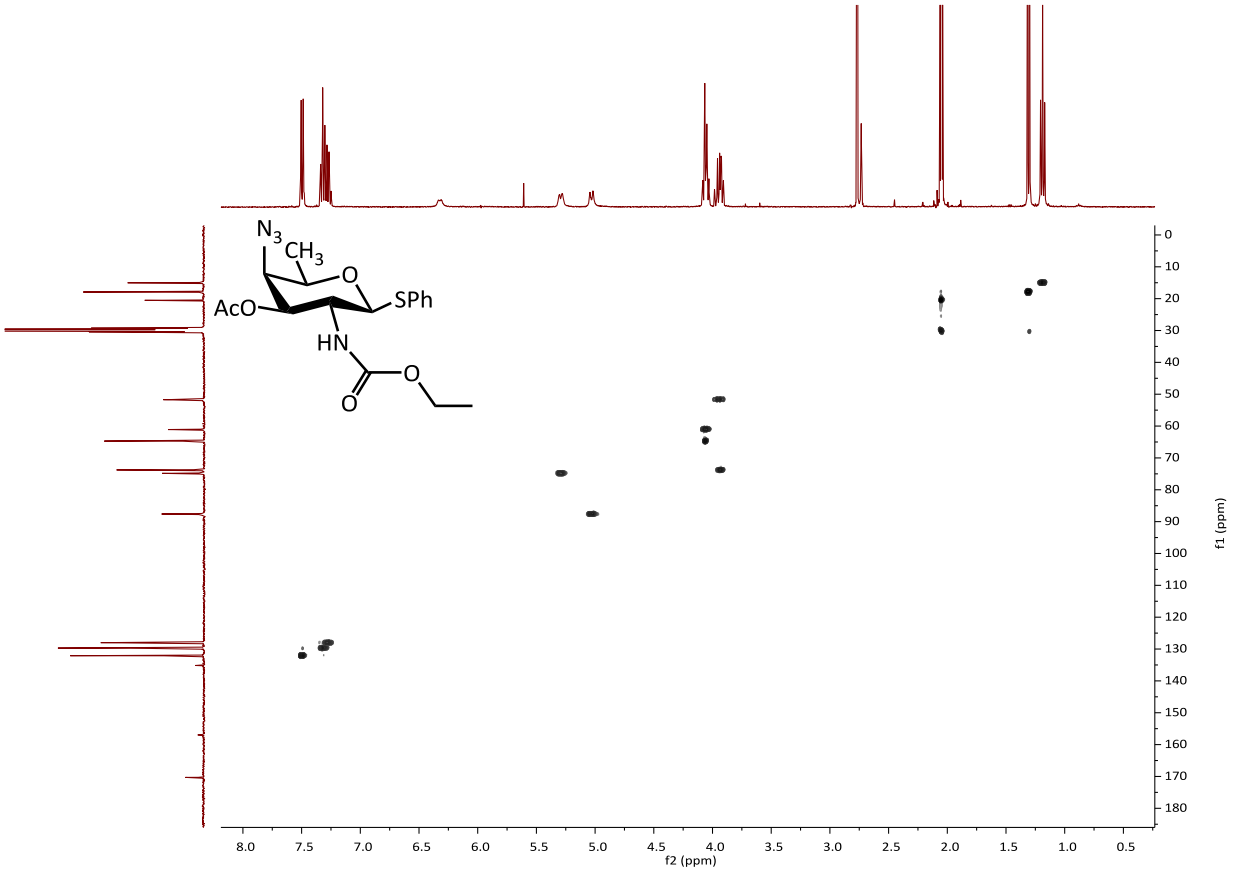


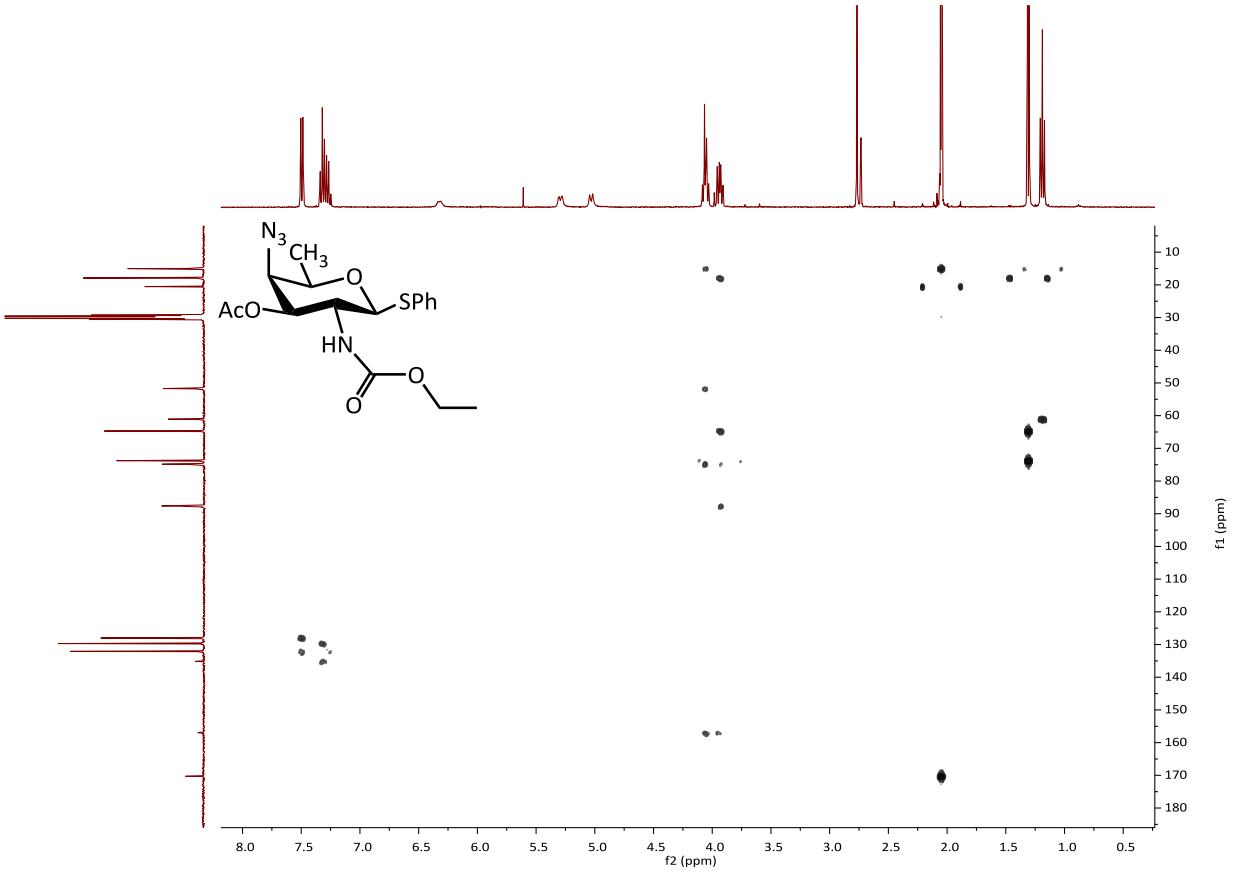
Phenyl 3-*O*-acetyl-4-azido-2,4,6-trideoxy-2-ethoxycarbonylamino-1-thio-β-D-galactopyranoside (2.59).



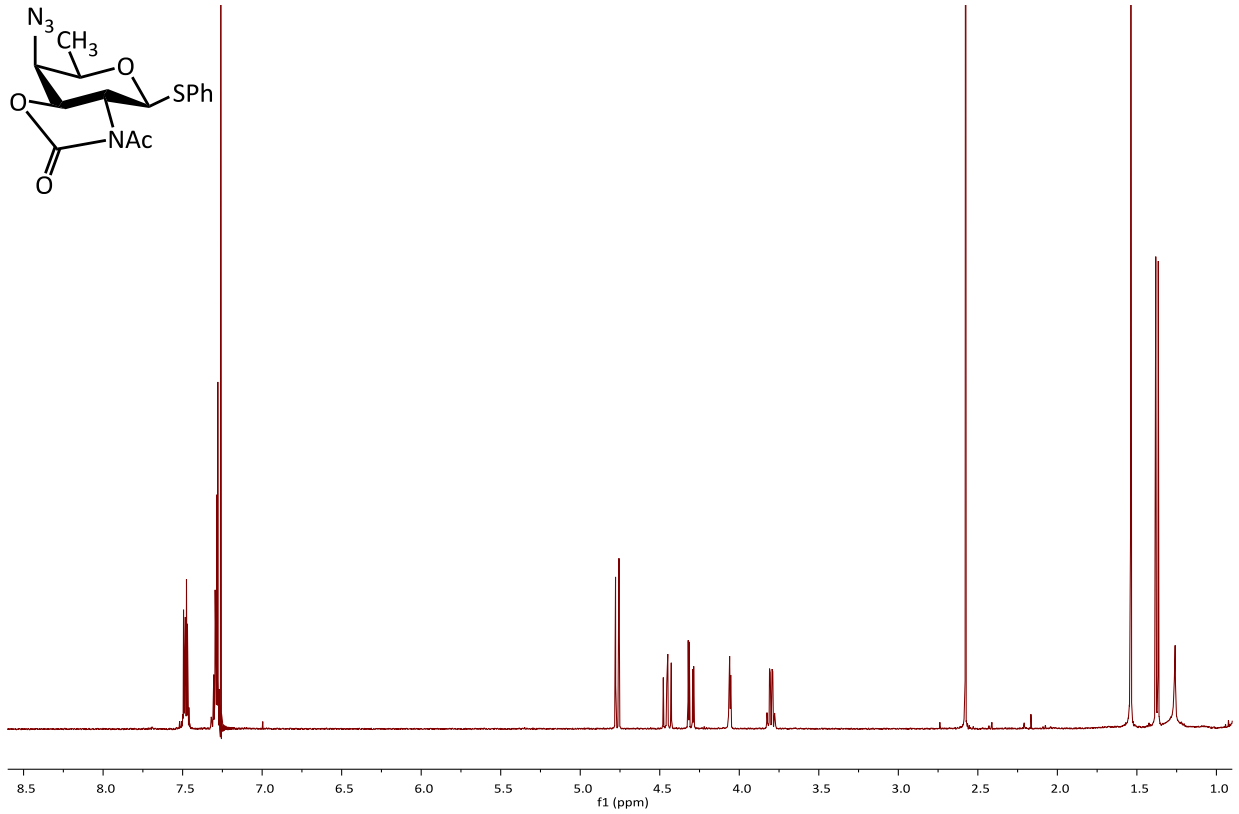

N3
CH3
O
AcO
SPh
HN
O
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

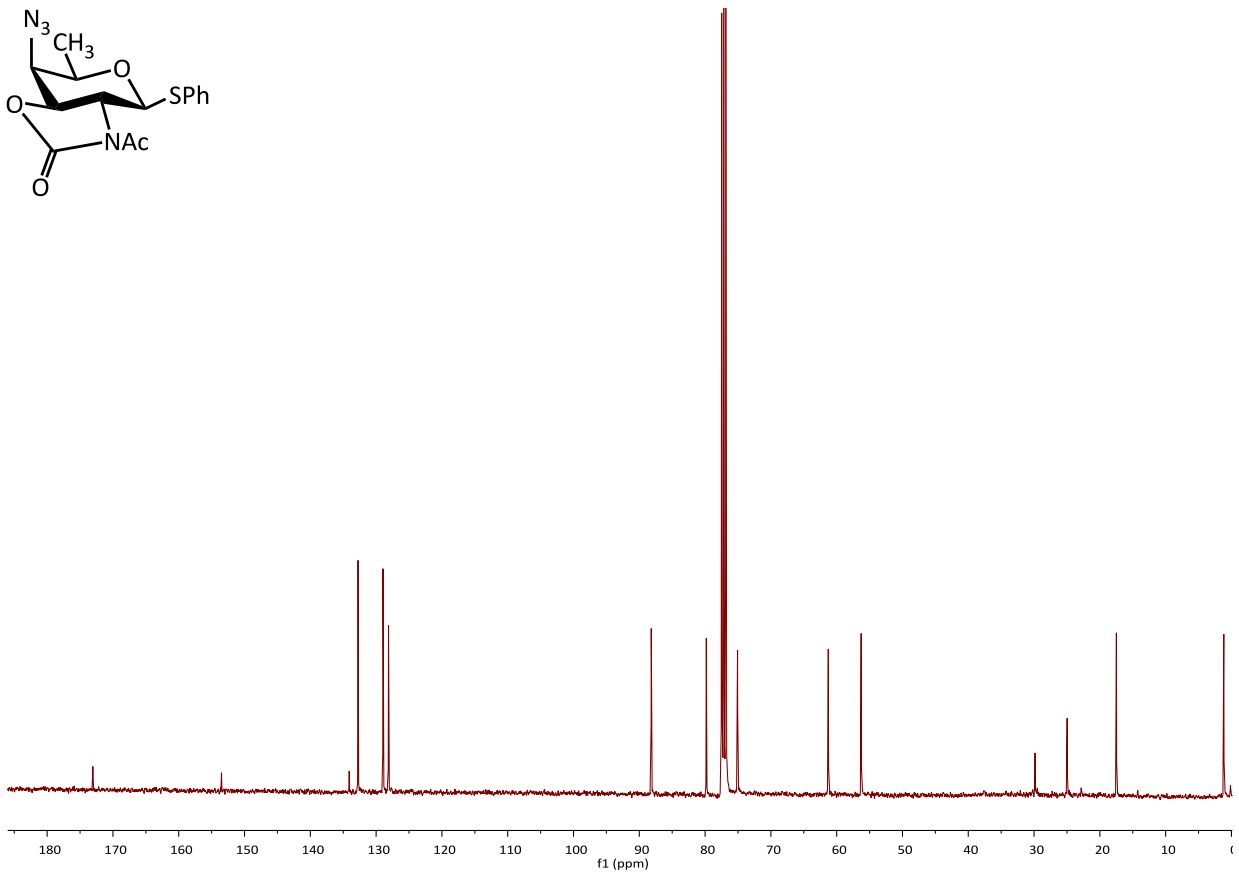

N3
CH3
O
AcO
SPh
HN
O
O
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)
1 2 3 4 5 6 7 8
f1 (ppm)

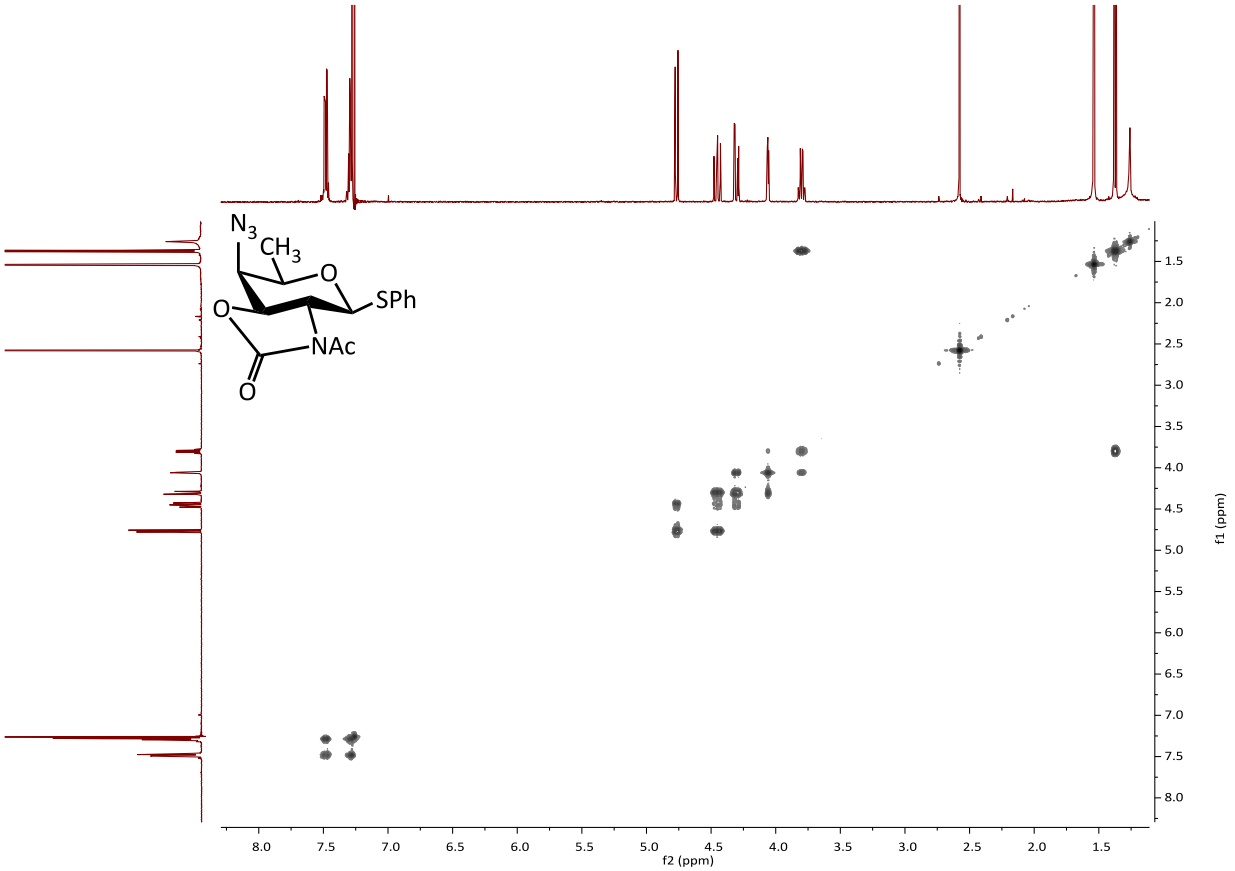

N3
CH3
O
AcO
SPh
HN
O
O
f2 (ppm)
f1 (ppm)

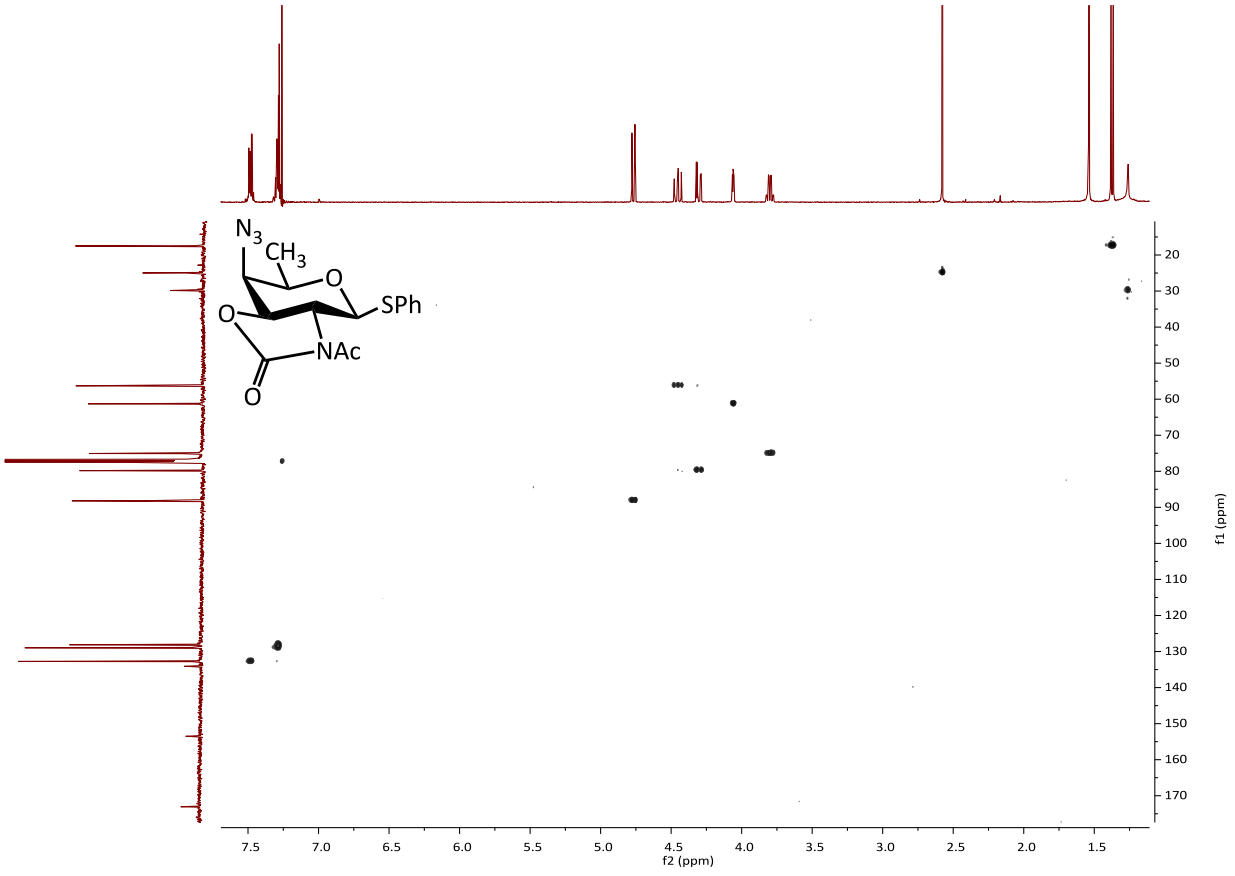

N3
CH3
O
AcO
SPh
HN
O
O
f2 (ppm)
f1 (ppm)

Phenyl 2-*N*-acetyl-2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.60).

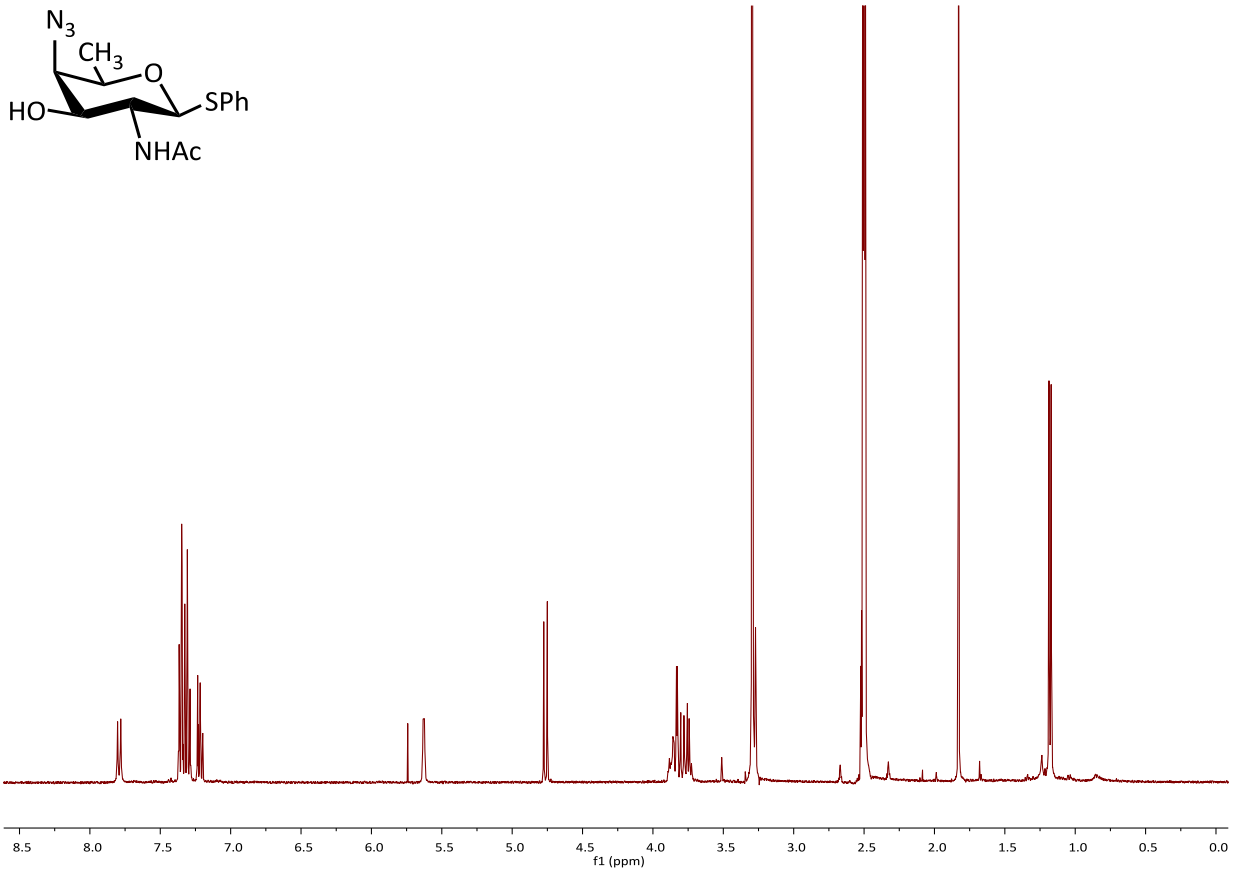


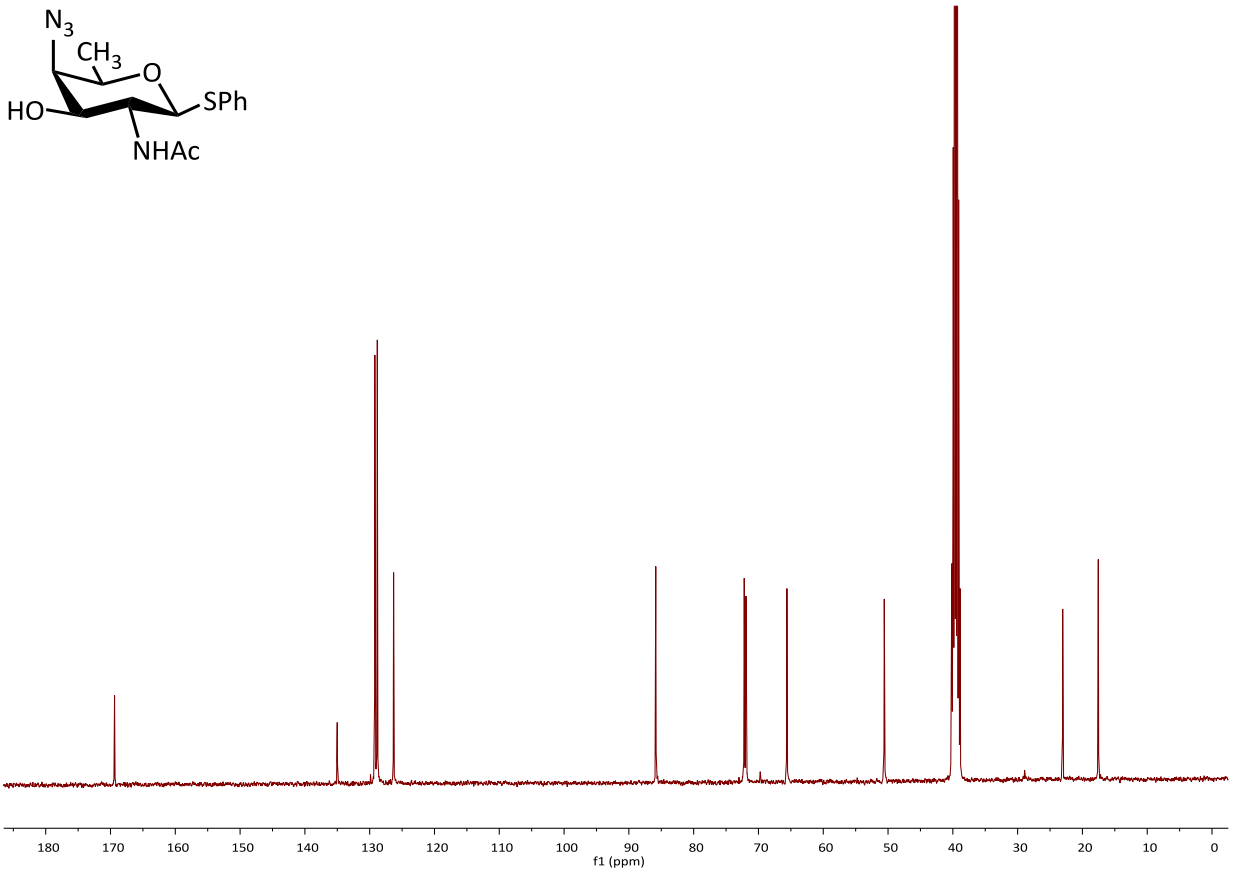


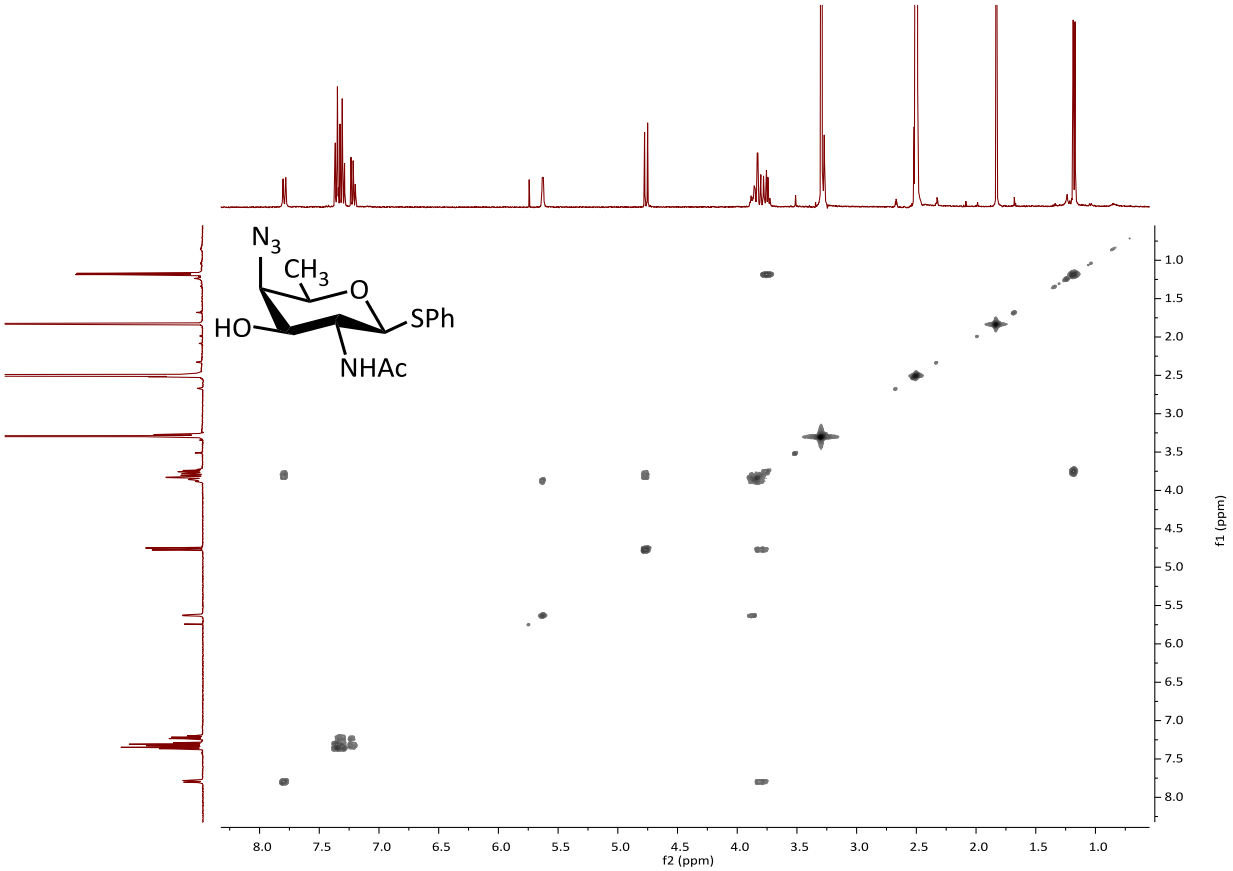

N3
CH3
O
SPh
O
NAc
O
f2 (ppm)
f1 (ppm)

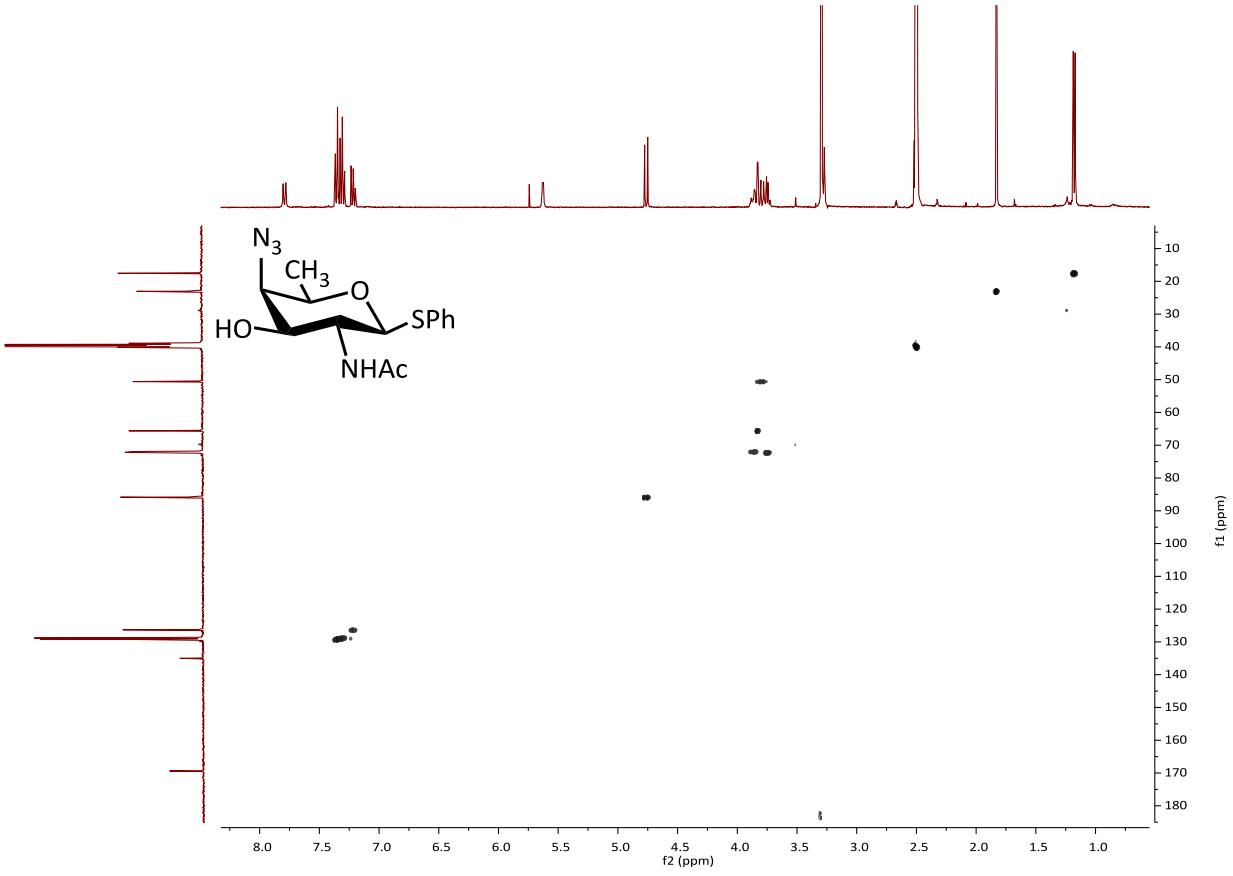

N3
CH3
O
SPh
O
NAc
O
f2 (ppm)
f1 (ppm)

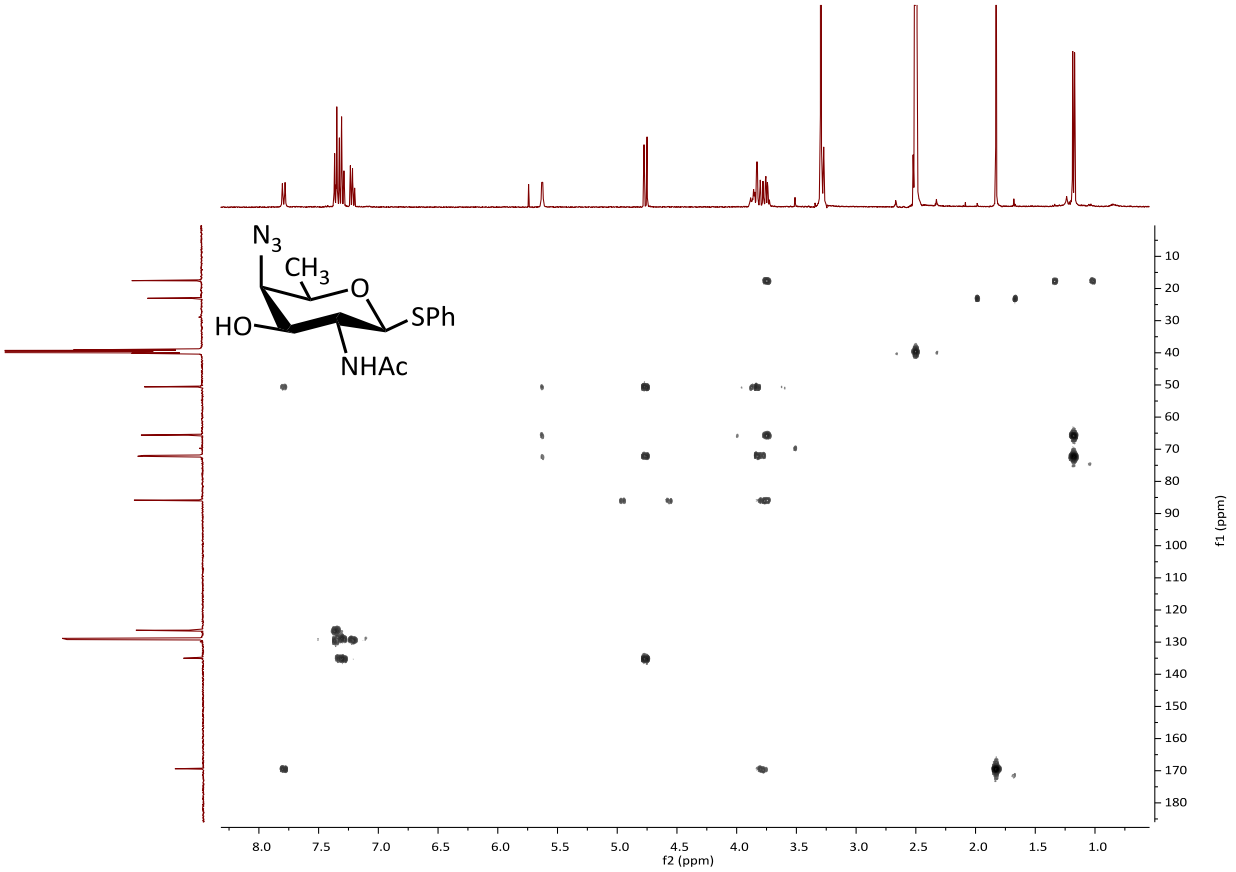
Phenyl 2-acetamido-4-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.50).



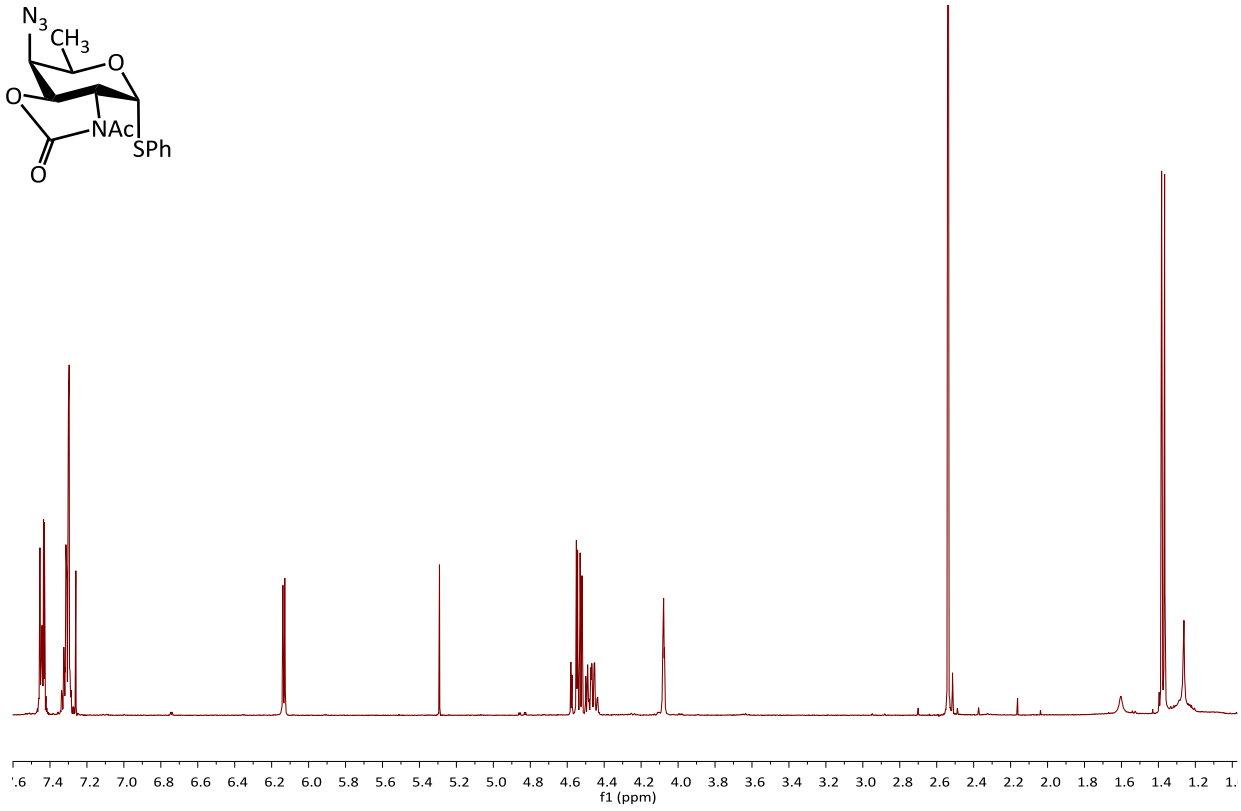


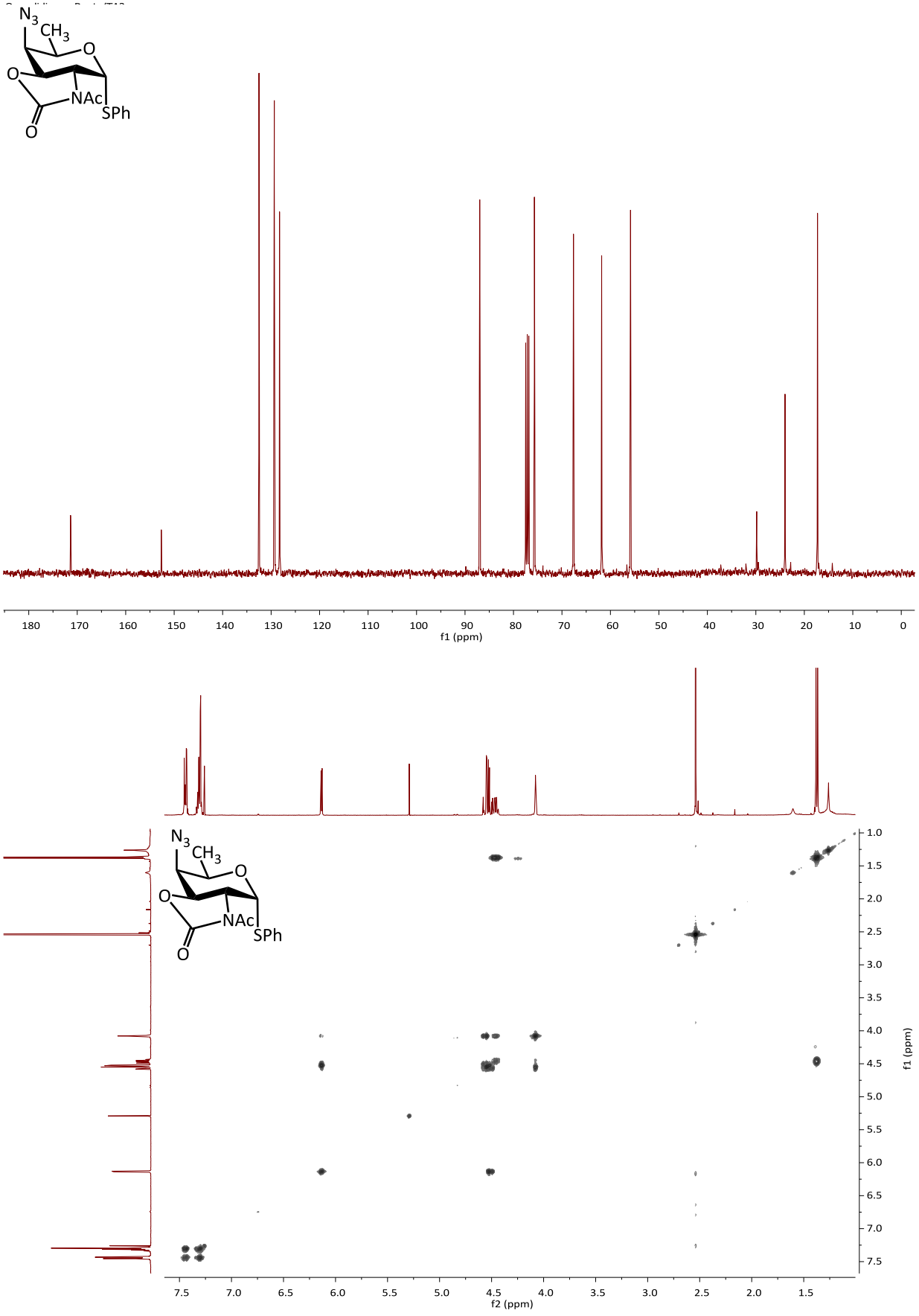

N3
CH3
O
HO
SPh
NHAc
f2 (ppm)
f1 (ppm)

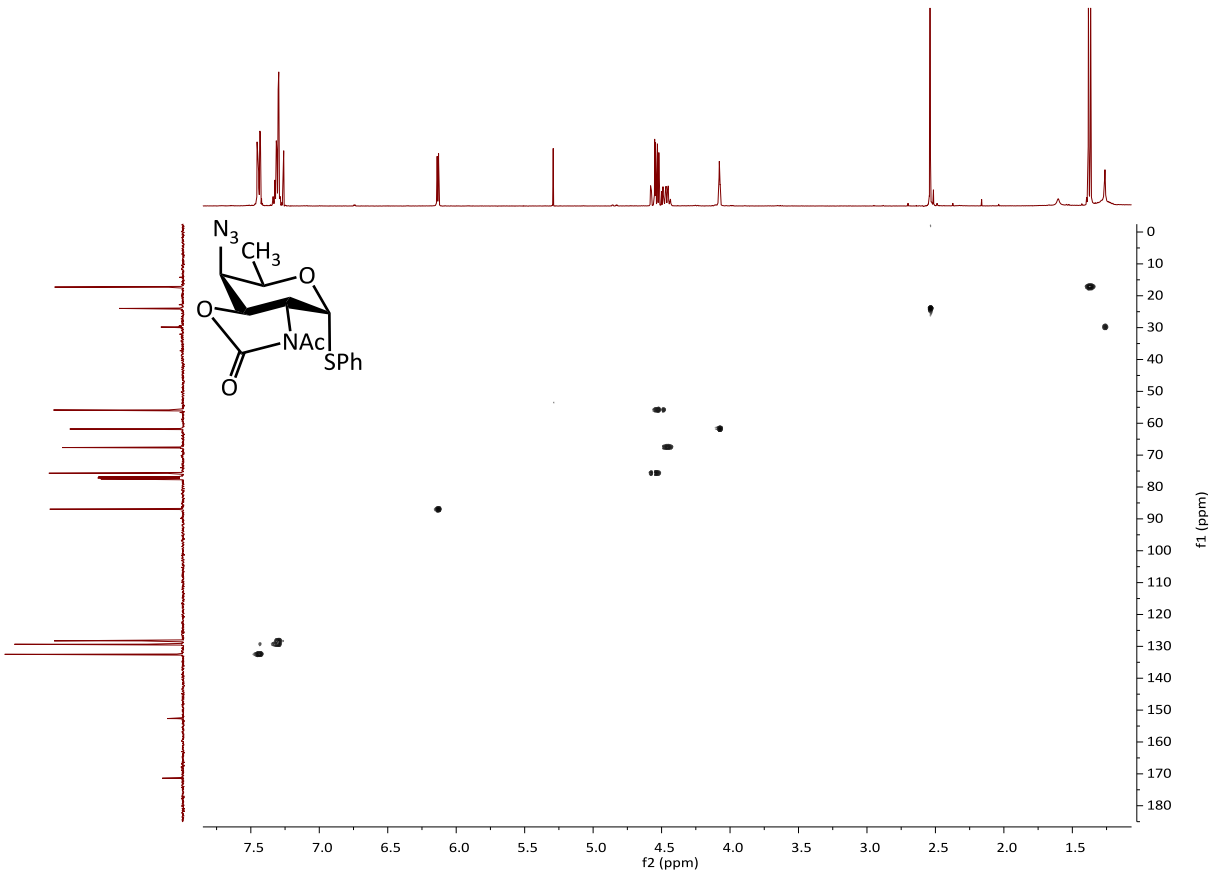

N3
CH3
O
HO
SPh
NHAc
f2 (ppm)
f1 (ppm)

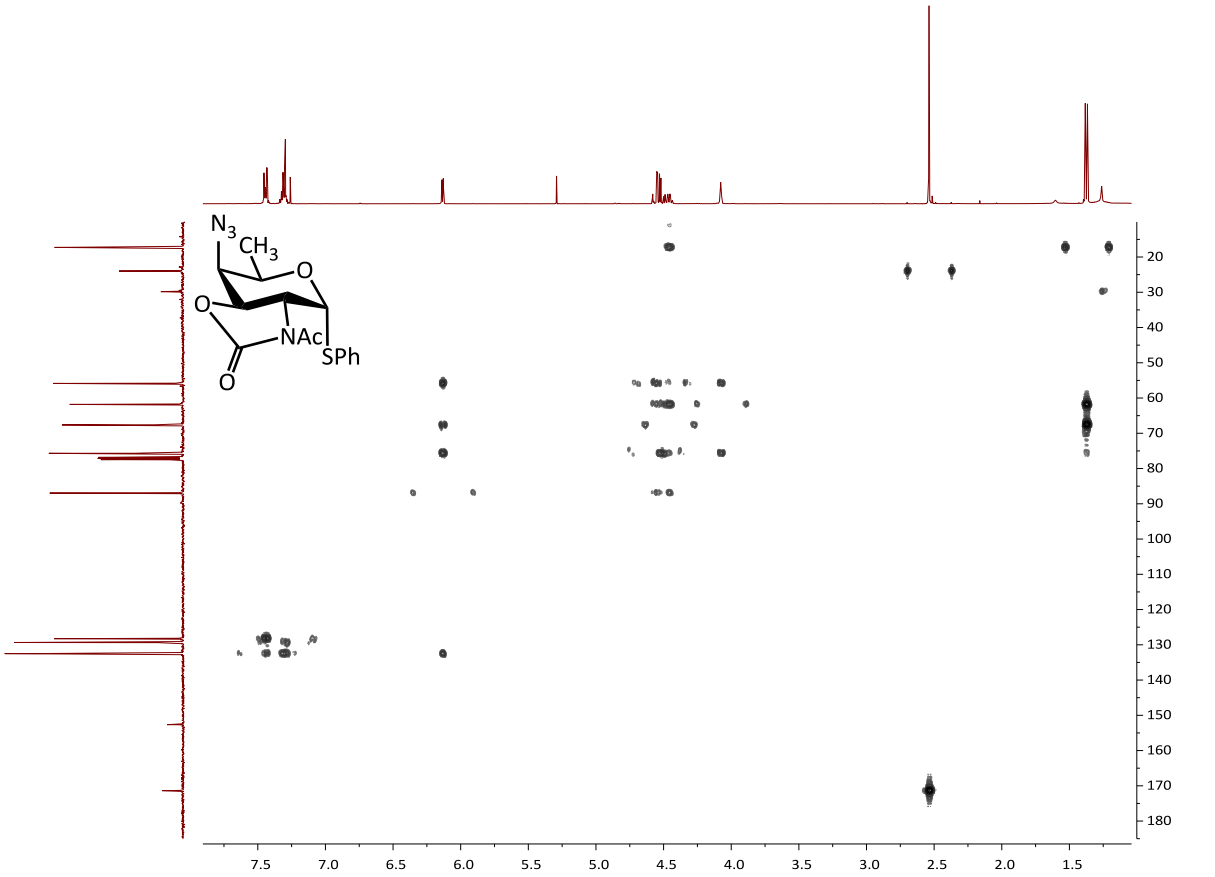


Phenyl 2-*N*-acetyl-2-amino-4-azido-2-*N*,3-*O*-carbonyl-2,4,6-trideoxy-1-thio-α-D-galactopyranoside (2.61).

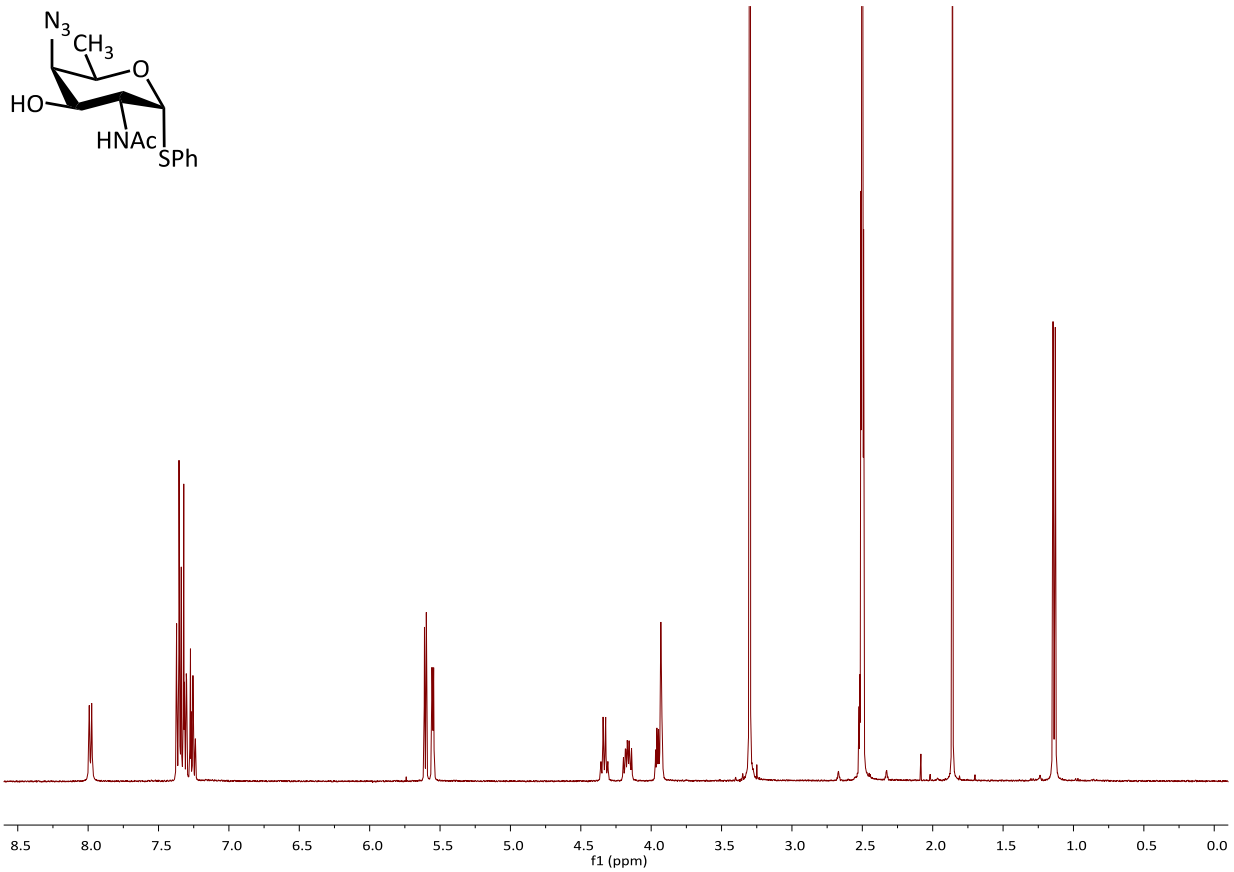


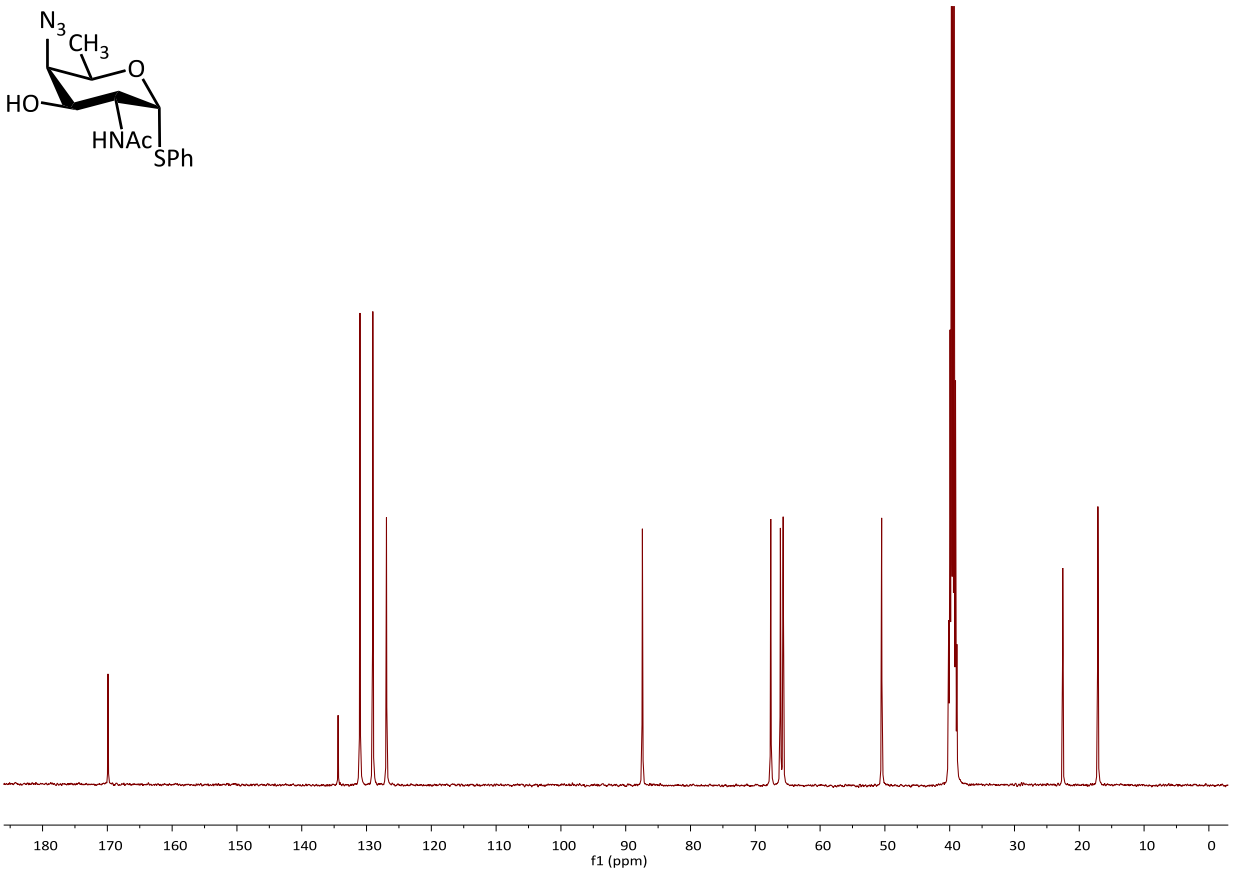

N3
CH3
O
O
NAc
SPh
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)
1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5
f1 (ppm)
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f2 (ppm)

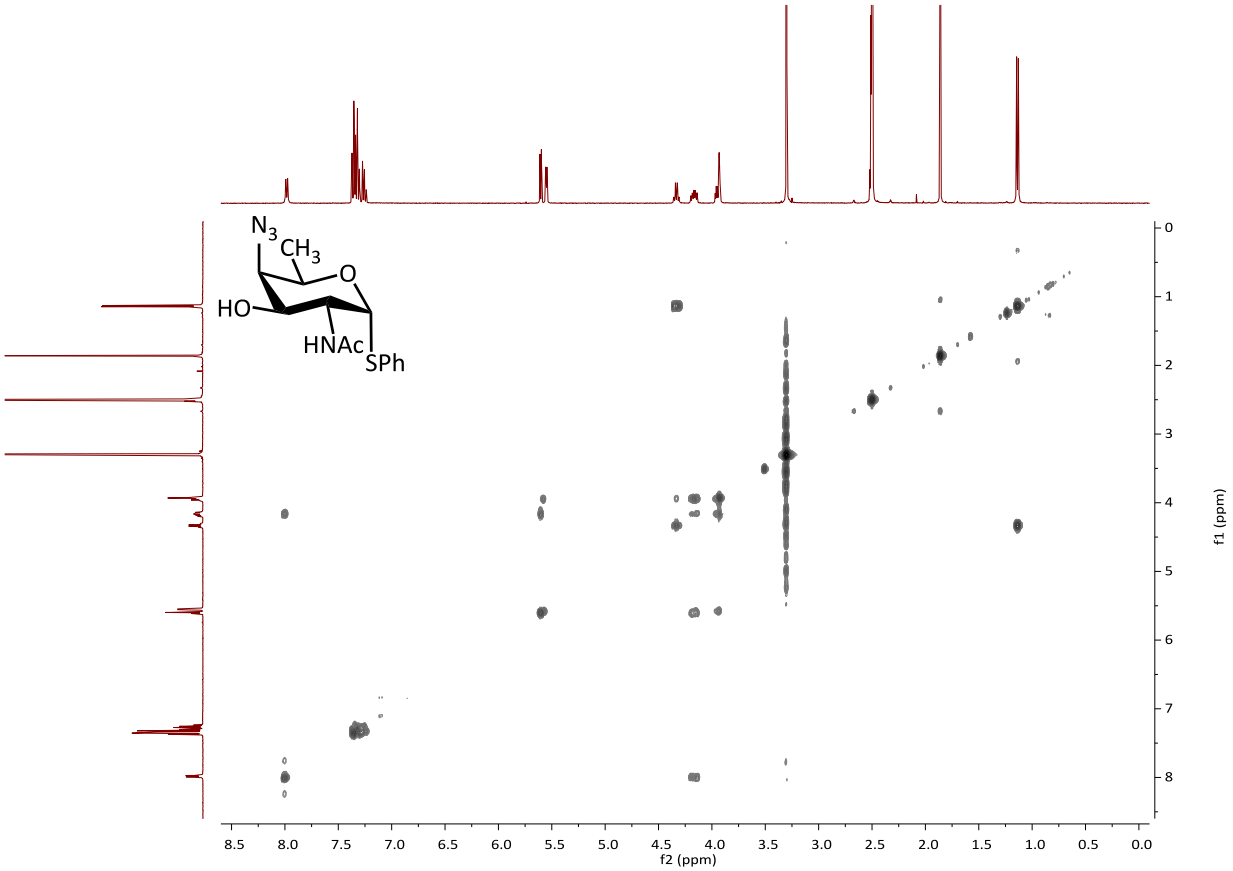

N3
CH3
O
O
NAc
SPh
O
f1 (ppm)
f2 (ppm)

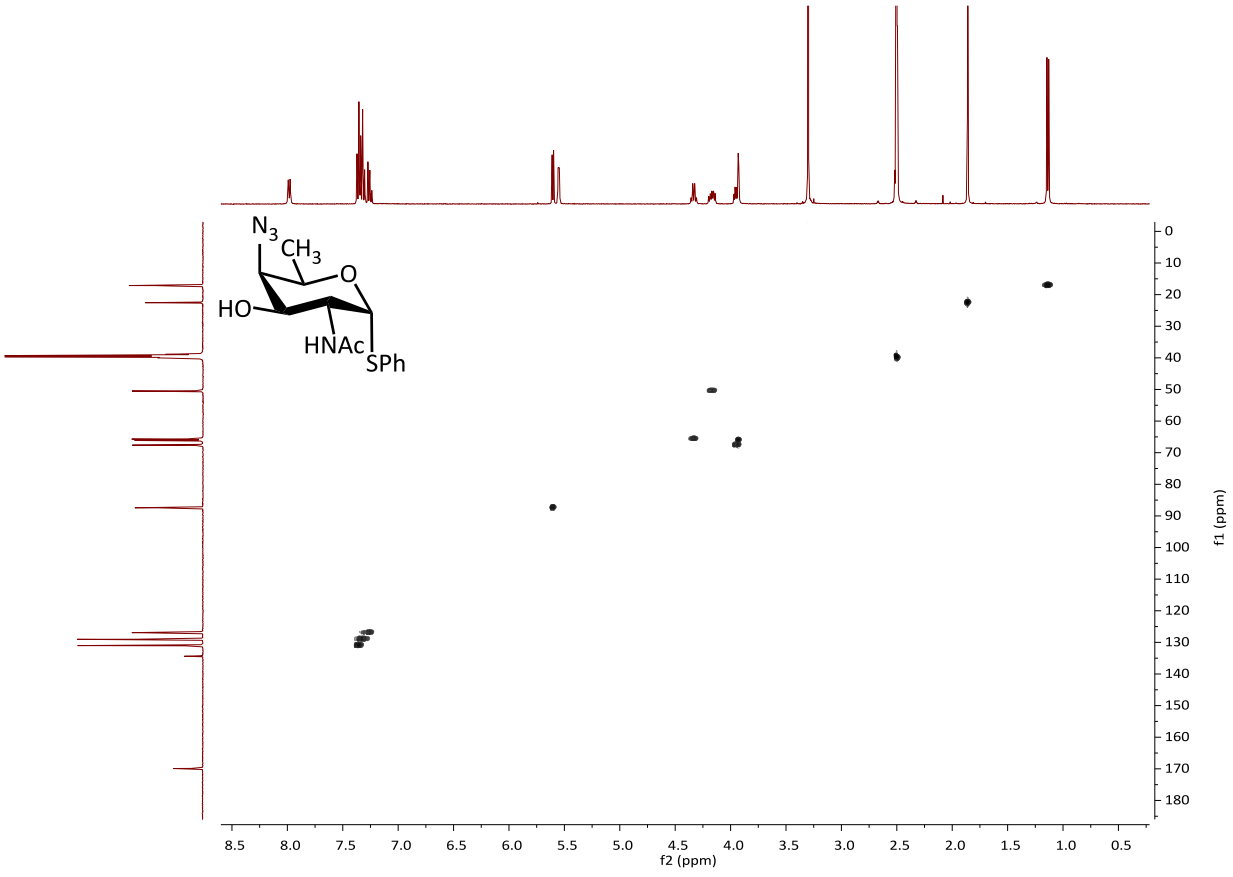

N3
CH3
O
O
NAc
SPh
O

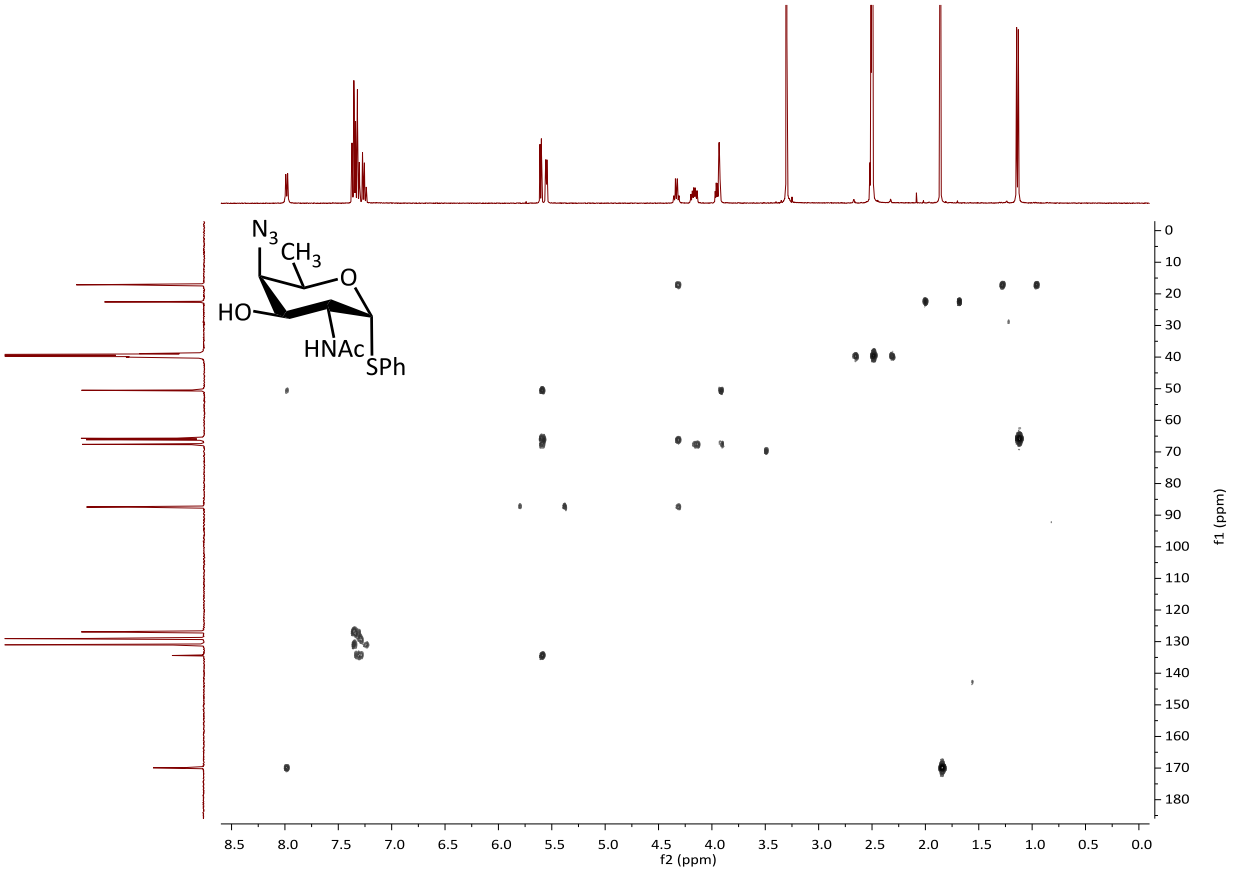
Phenyl 2-acetamido-4-azido-2,4,6-trideoxy-1-thio-α-D-galactopyranoside (2.62).



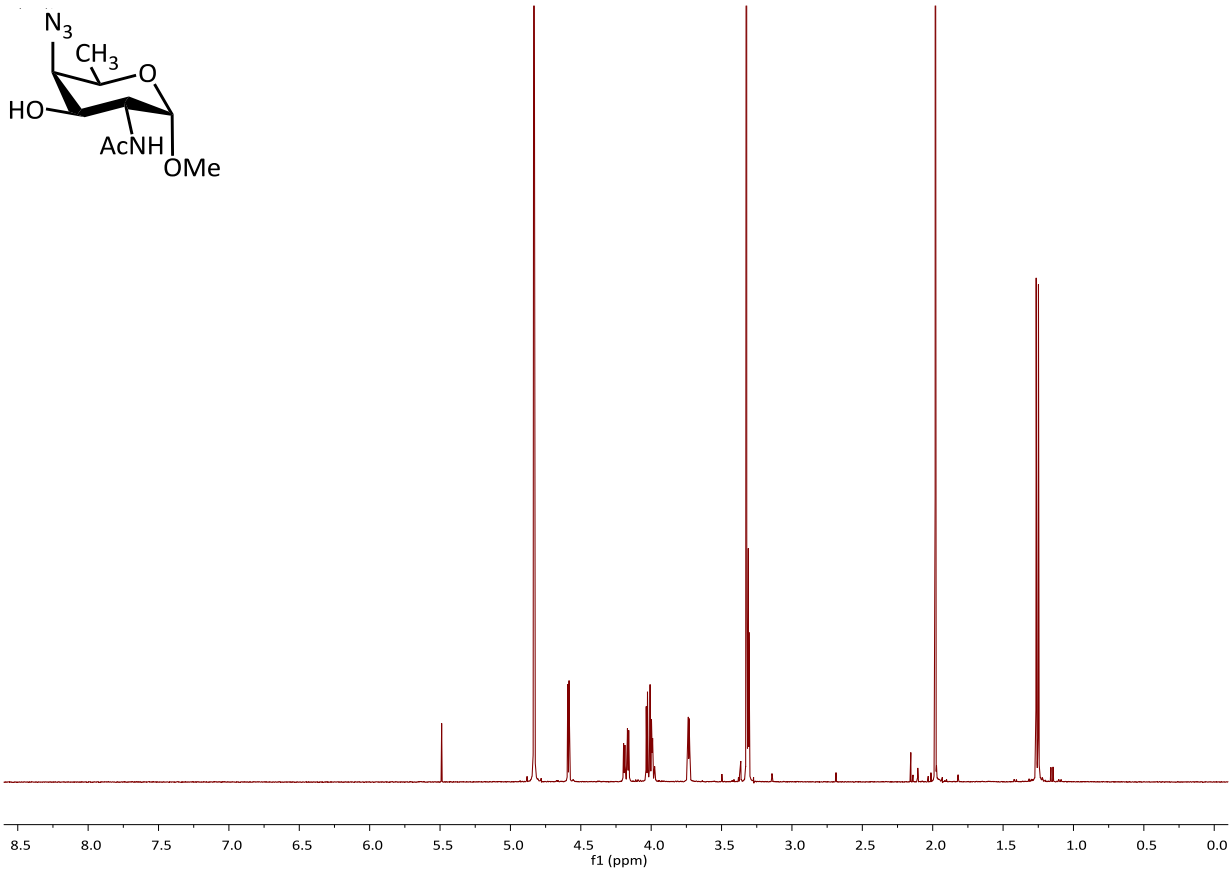


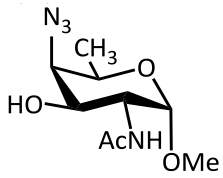

N3
CH3
O
HO
HNAc
SPh
f2 (ppm)
f1 (ppm)

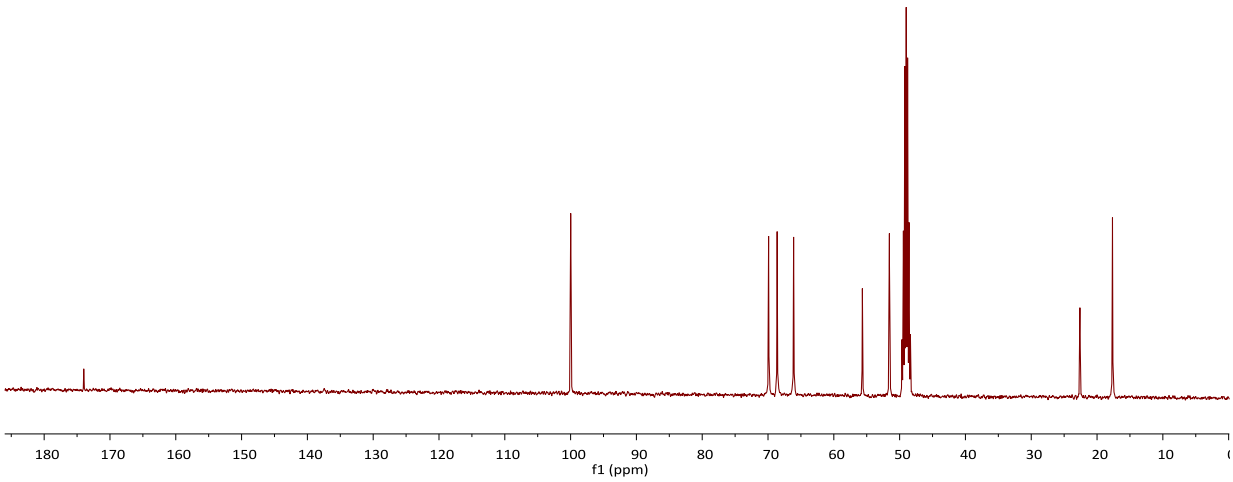

N3
CH3
O
HO
HNAc
SPh
f2 (ppm)
f1 (ppm)

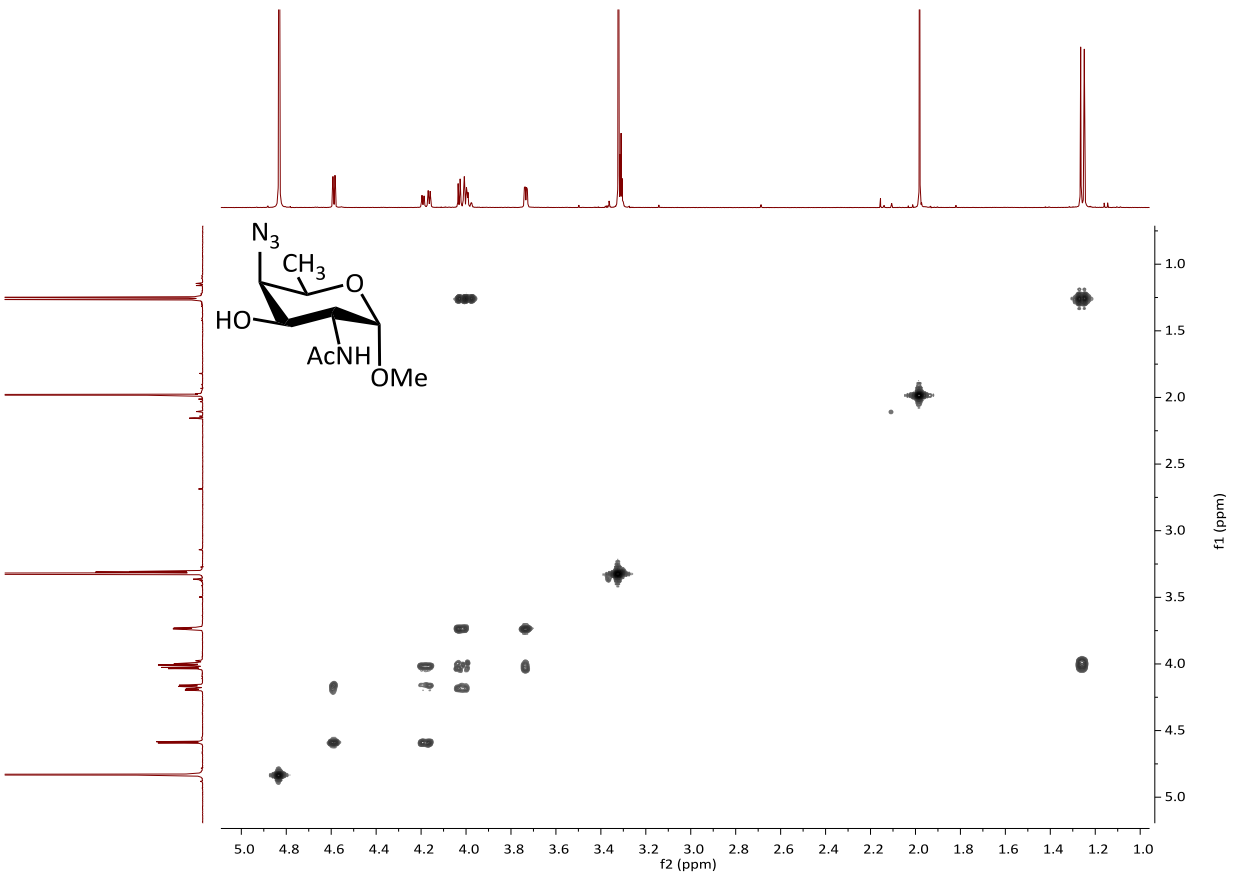


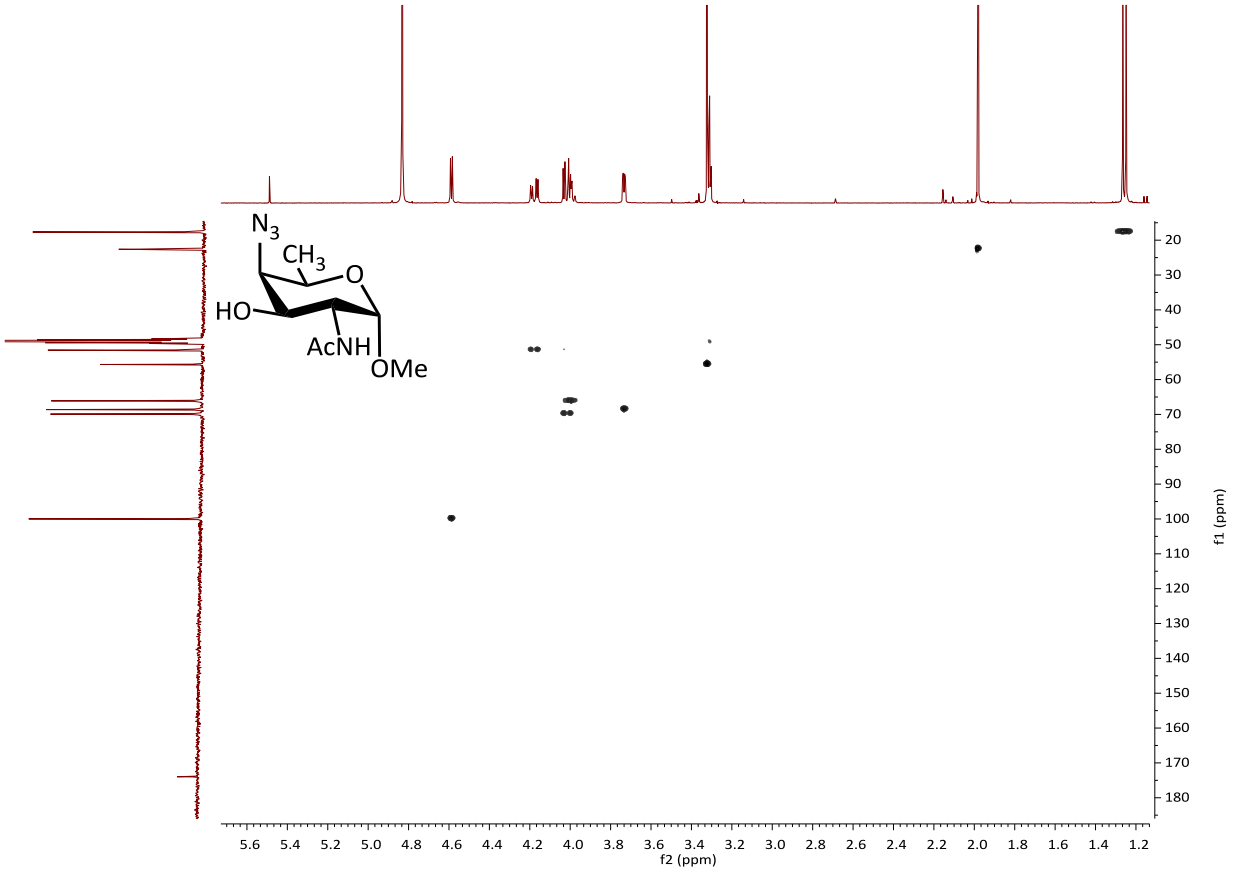
Methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-galactopyranoside (2.63).

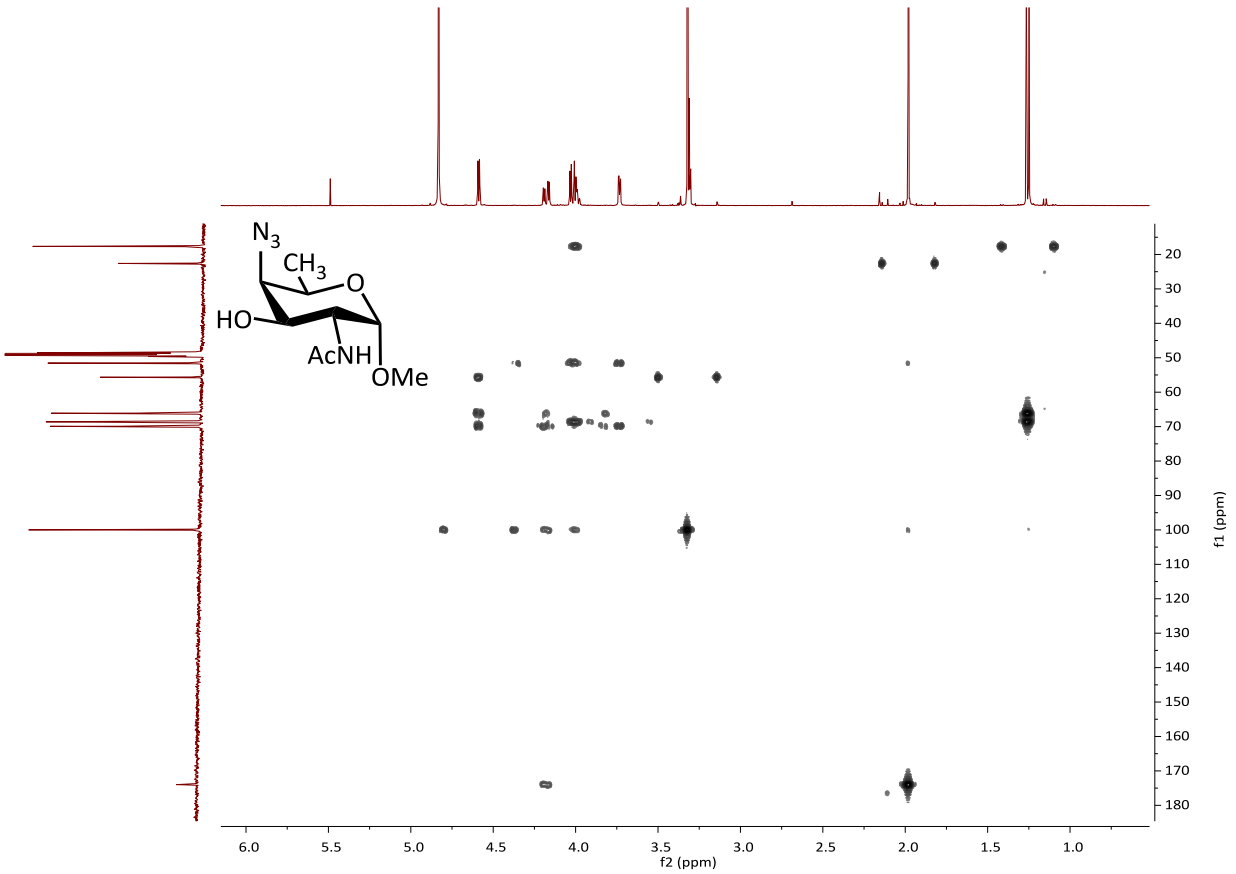



N3
CH3
O
HO
AcNH
OMe

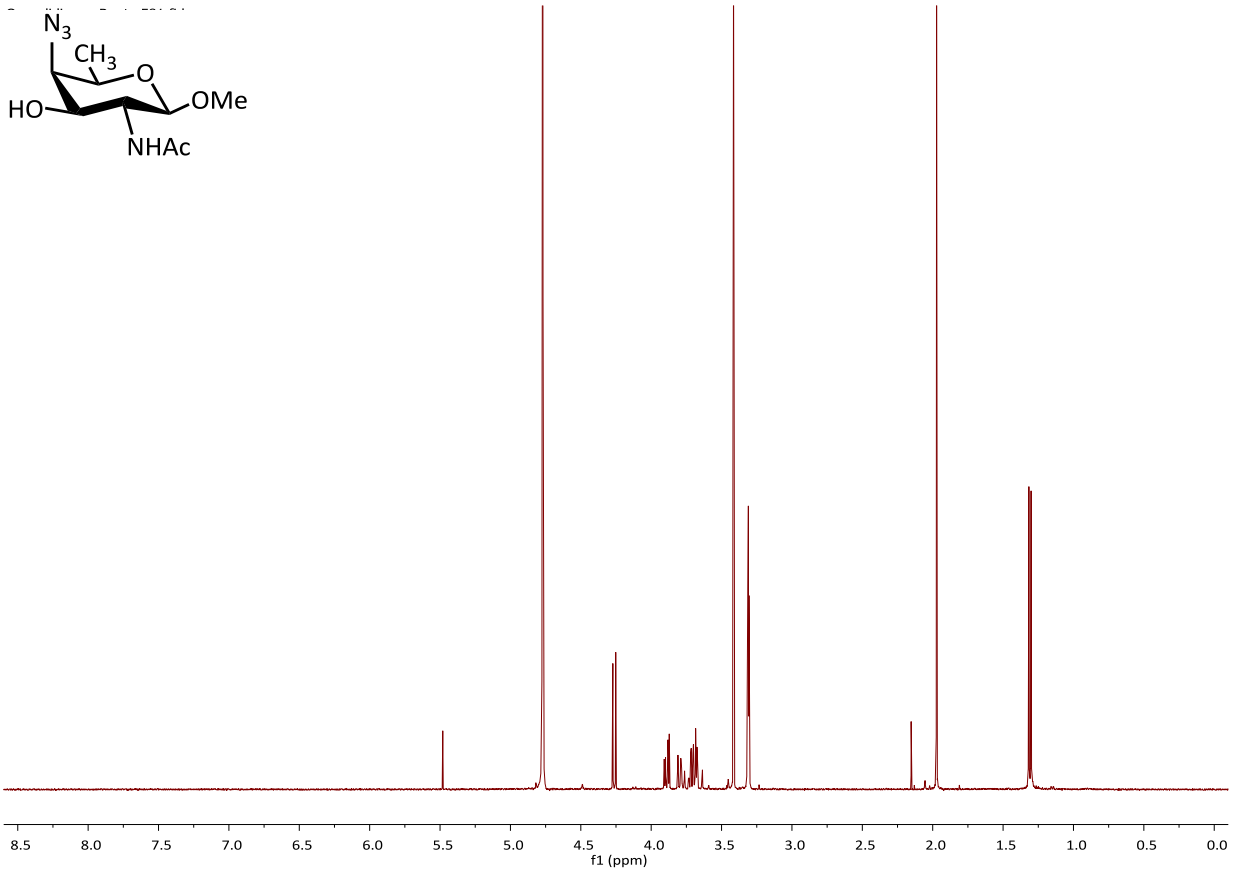

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

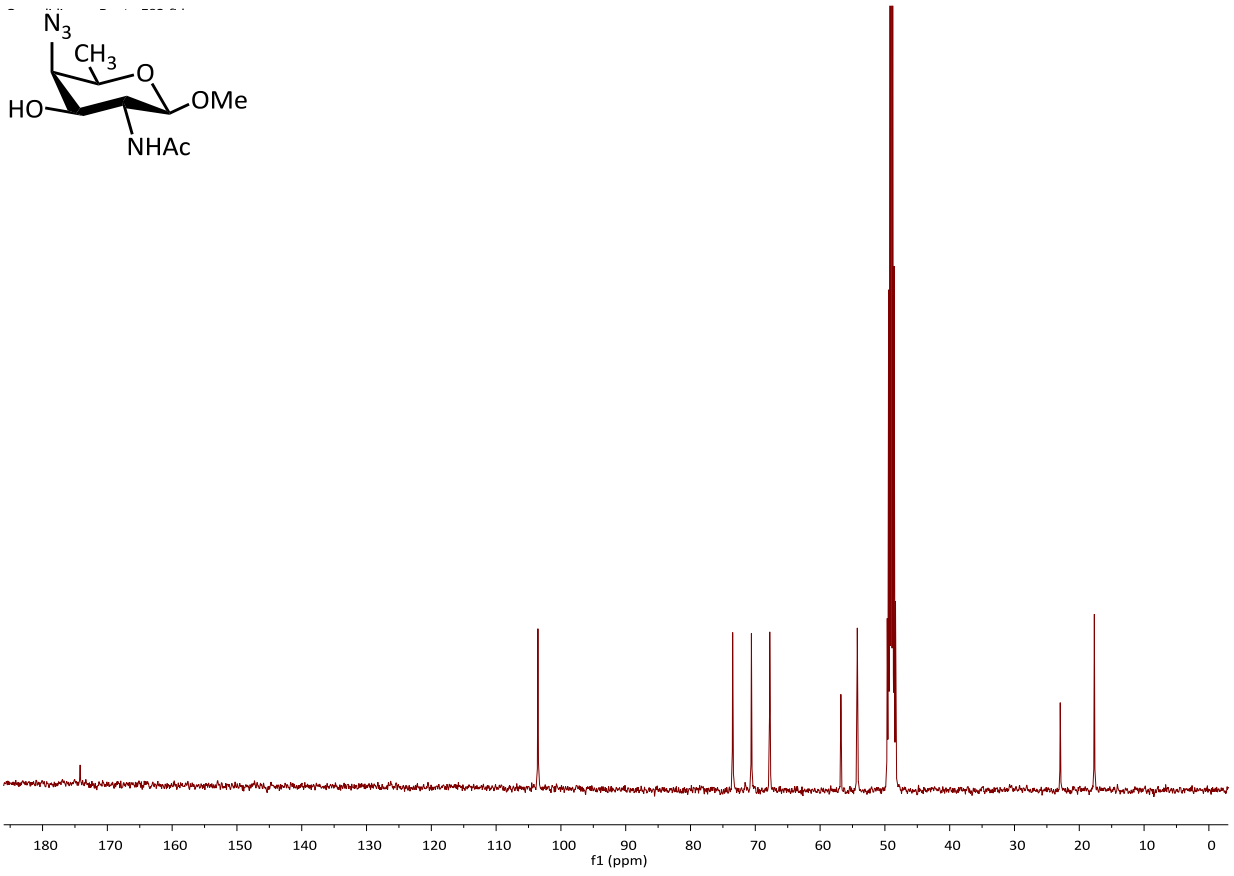

N3
CH3
O
HO
AcNH
OMe
1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
f1 (ppm)
5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f2 (ppm)

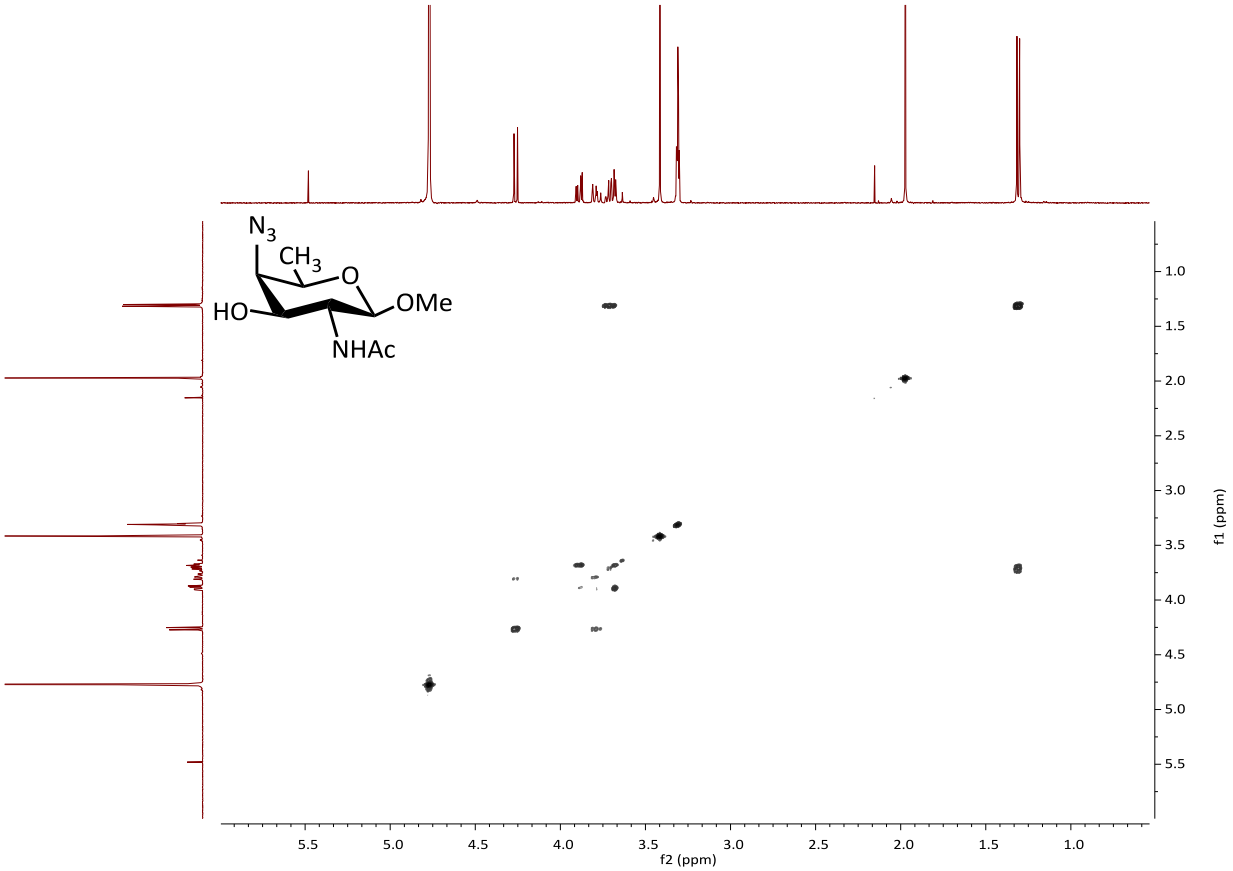

N3
CH3
O
HO
AcNH
OMe
f2 (ppm)
f1 (ppm)

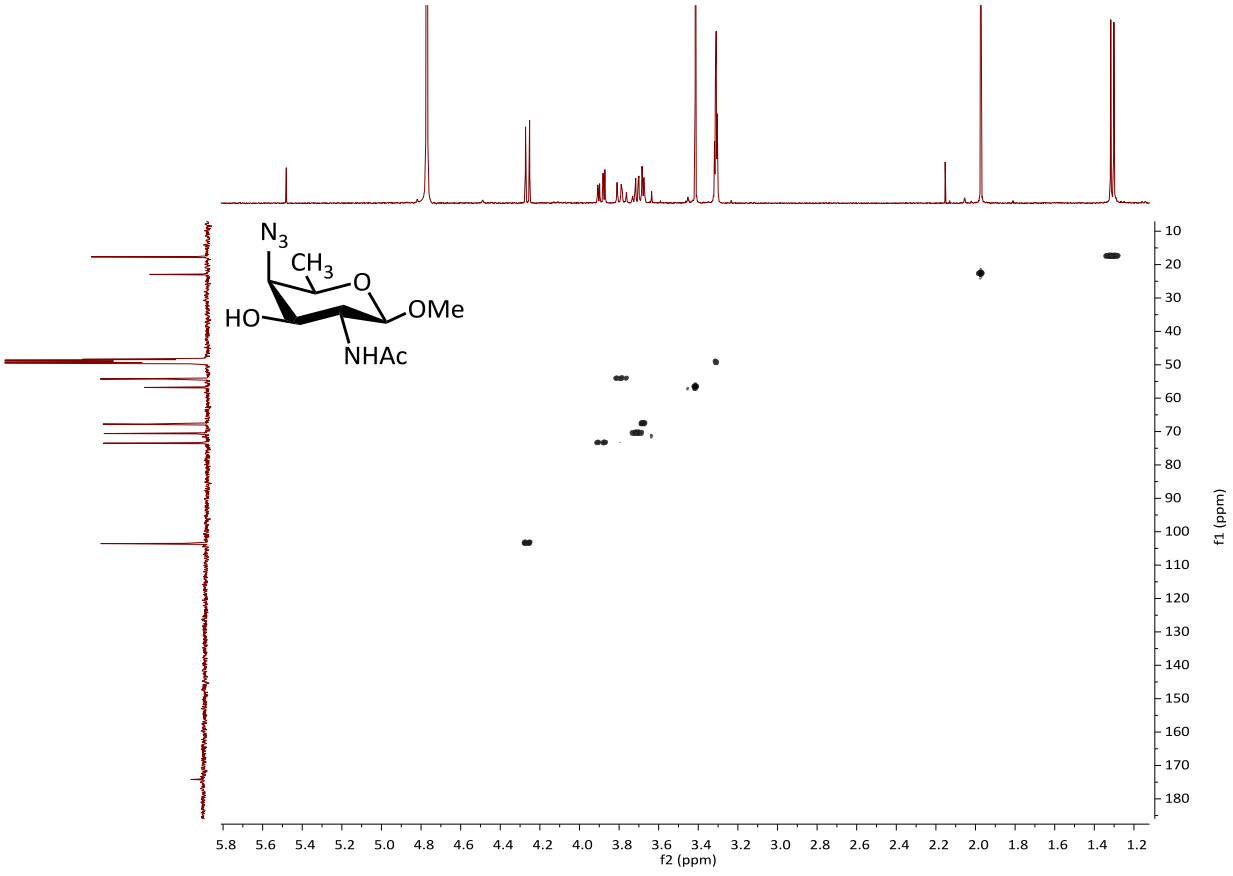

N3
CH3
O
HO
AcNH
OMe
f2 (ppm)
f1 (ppm)

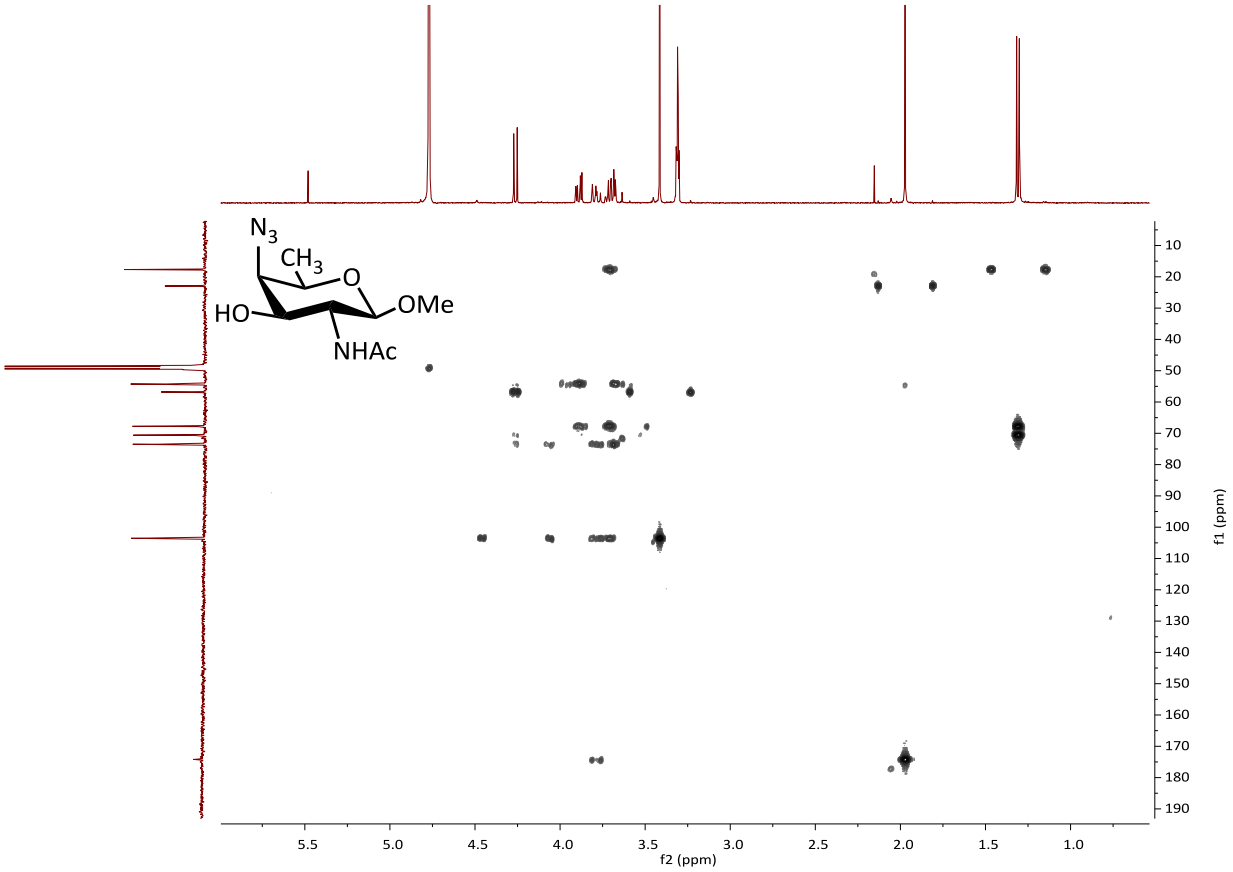
Methyl 2-acetamido-4-azido-2,4,6-trideoxy-β-D-galactopyranoside (2.63).



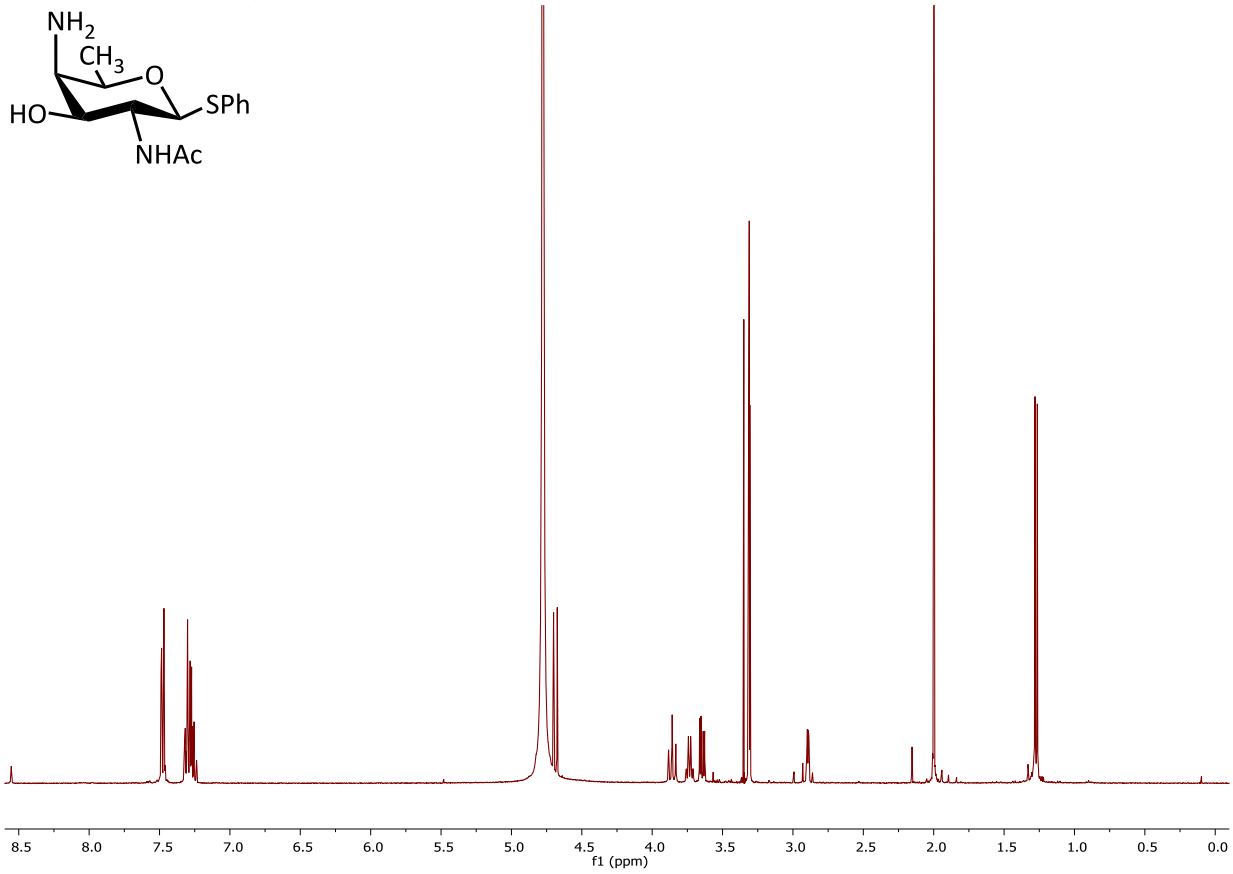


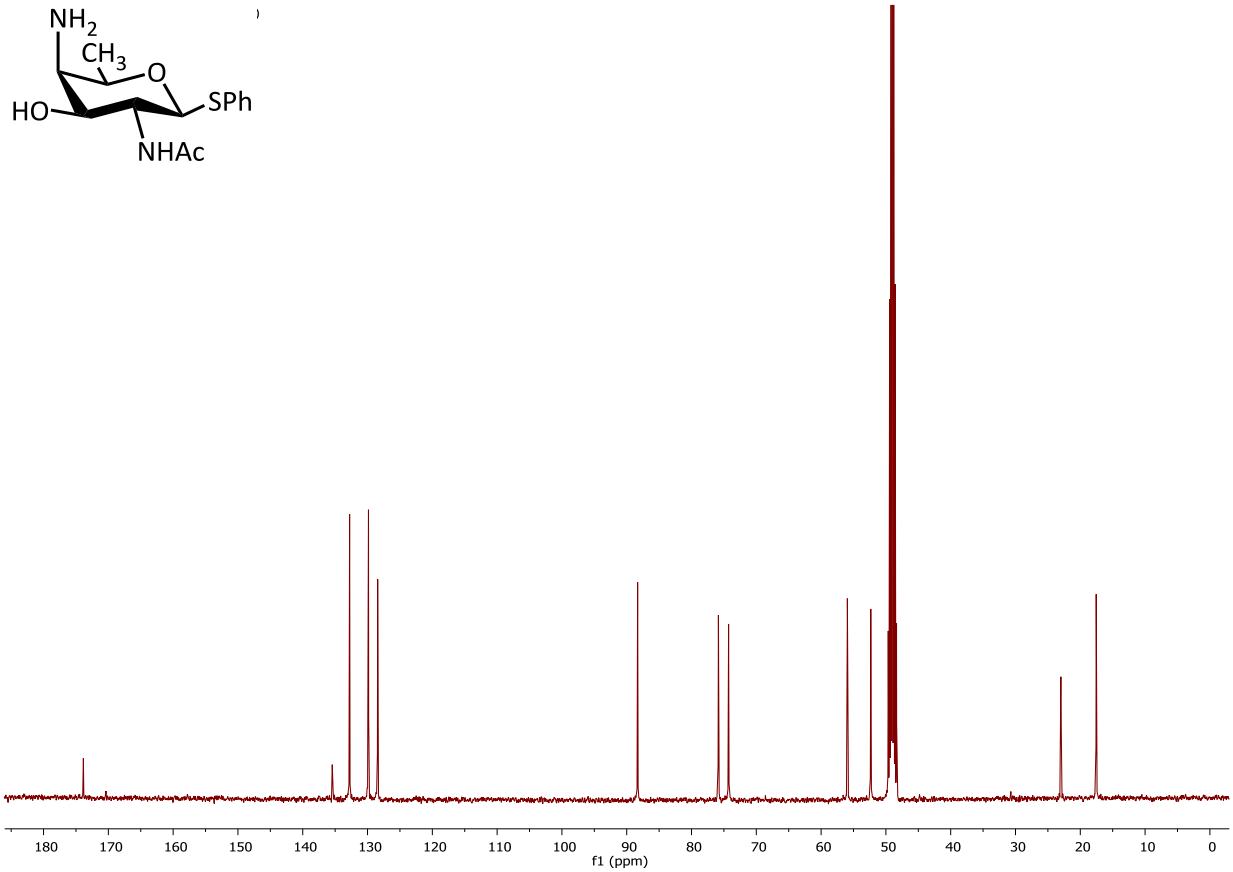

N3
CH3
O
HO
OMe
NHAc
f2 (ppm)
f1 (ppm)

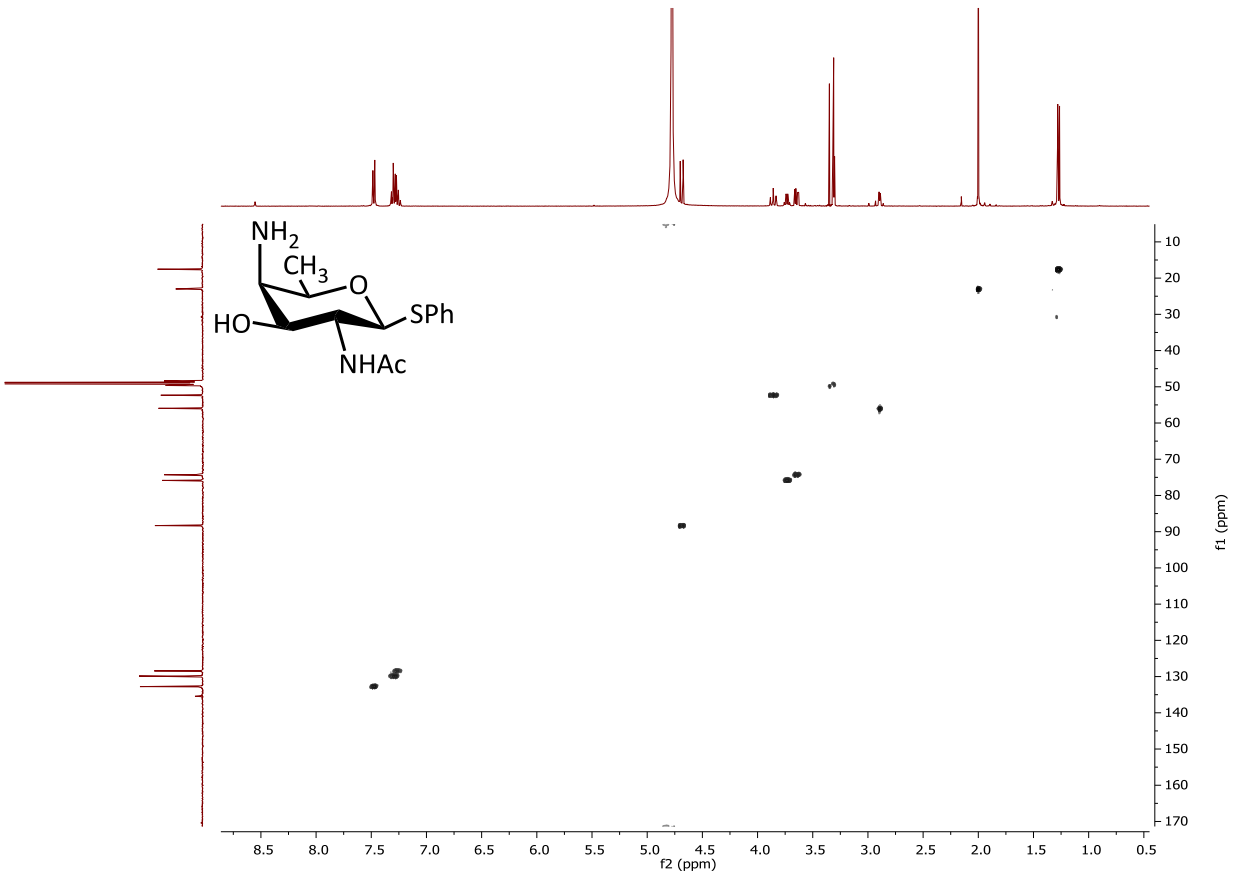

N3
CH3
O
HO
OMe
NHAc
f2 (ppm)
f1 (ppm)

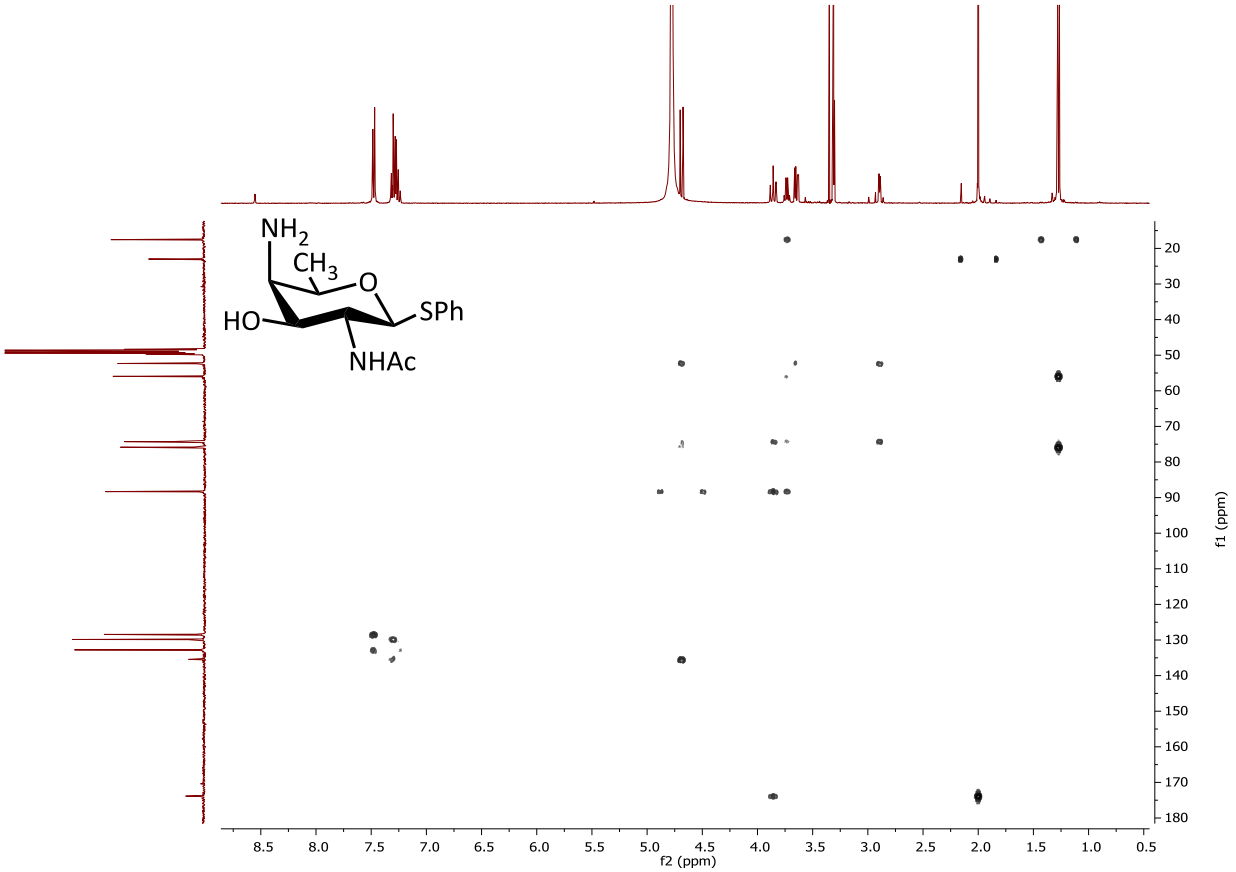


Phenyl 2-acetamido-4-amino-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (2.64).



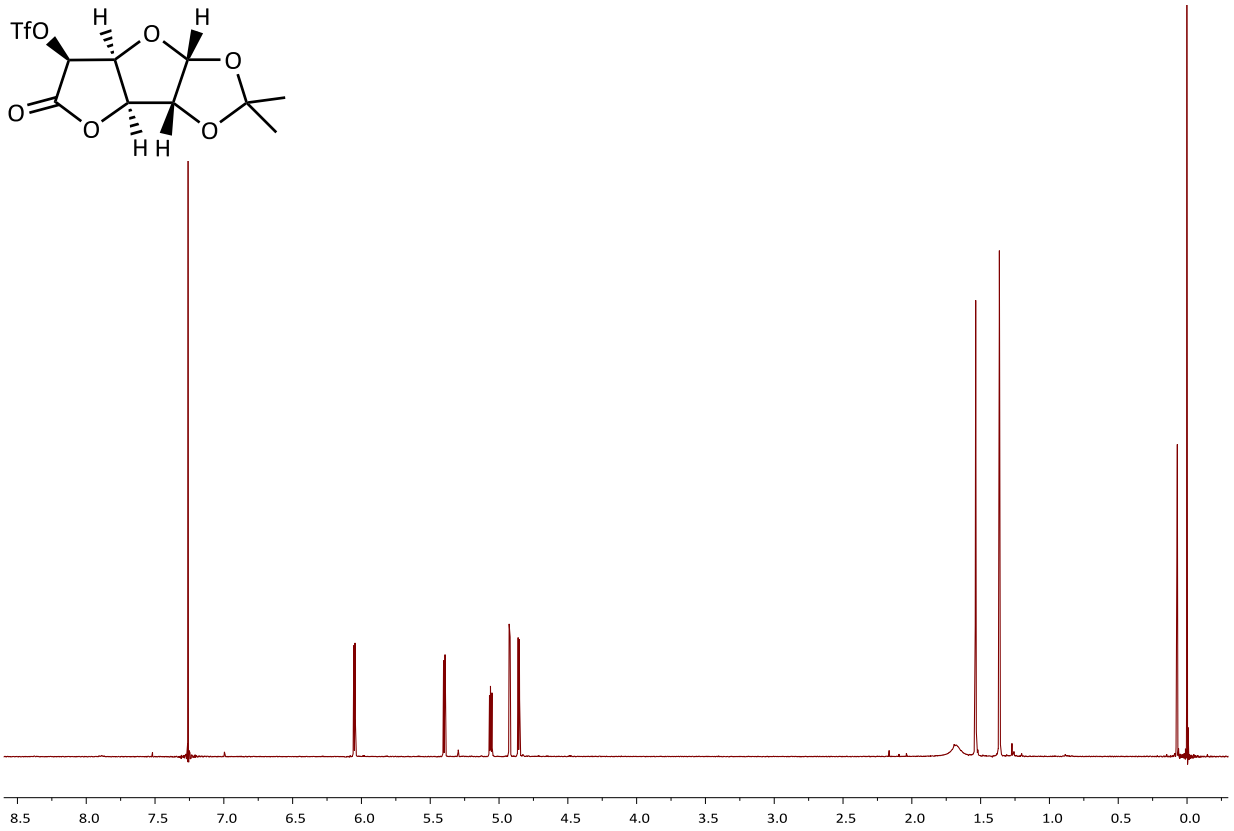

NH2
CH3
O
HO
SPh
NHAc
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

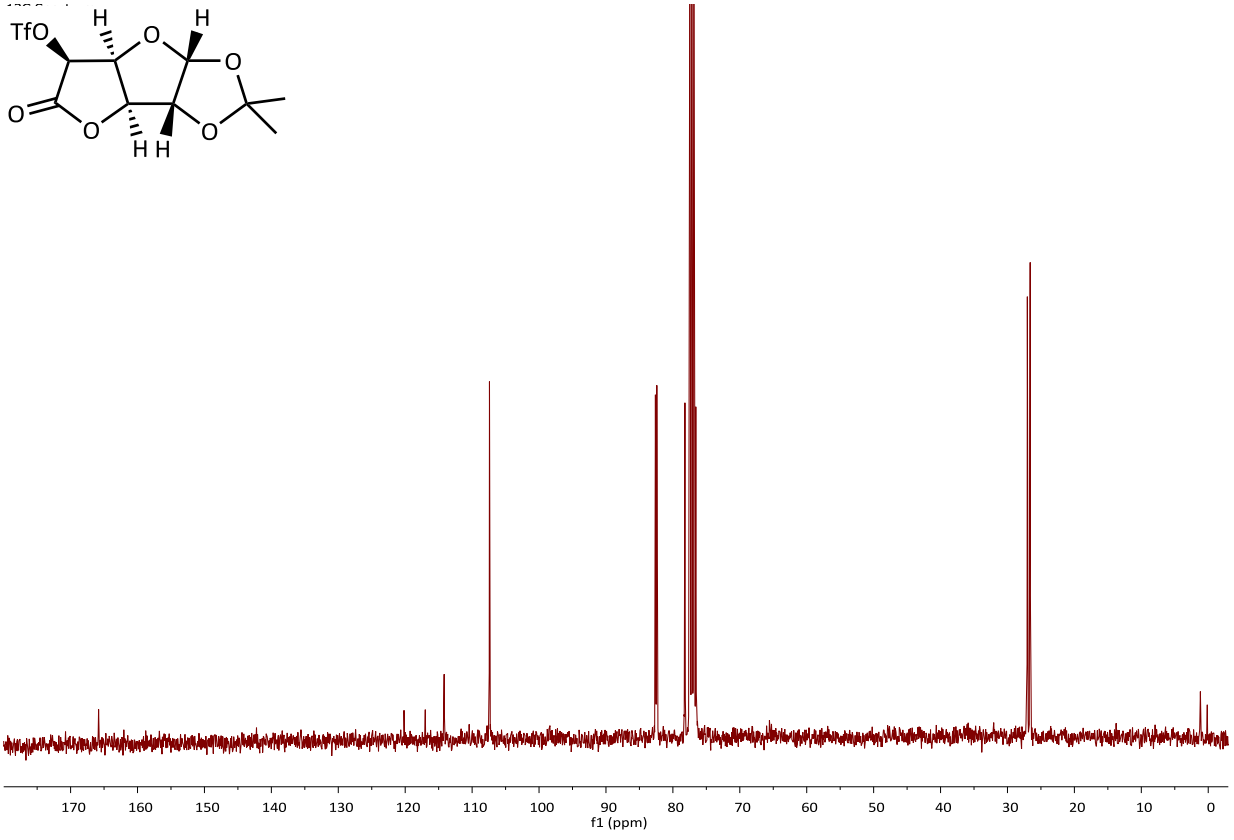

NH2
CH3
O
HO
SPh
NHAc
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
f1 (ppm)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)


NH2
CH3
O
HO
SPh
NHAc
f2 (ppm)
f1 (ppm)

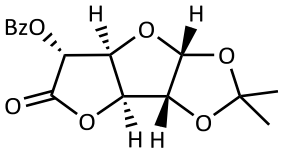
2.2 Chapter 3 NMR spectra

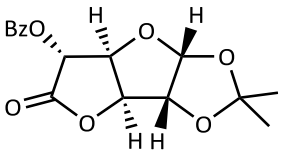
5-*O*-triflyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone.

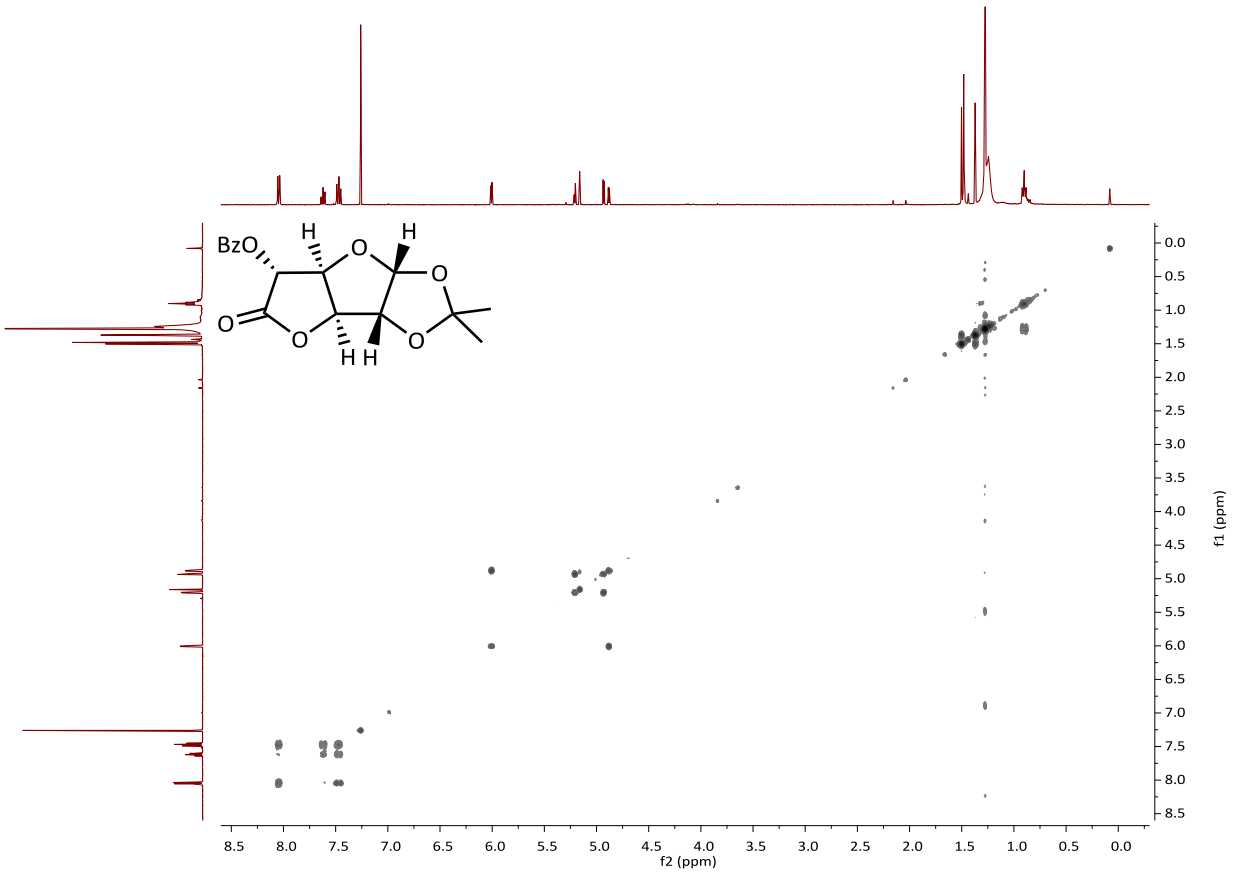


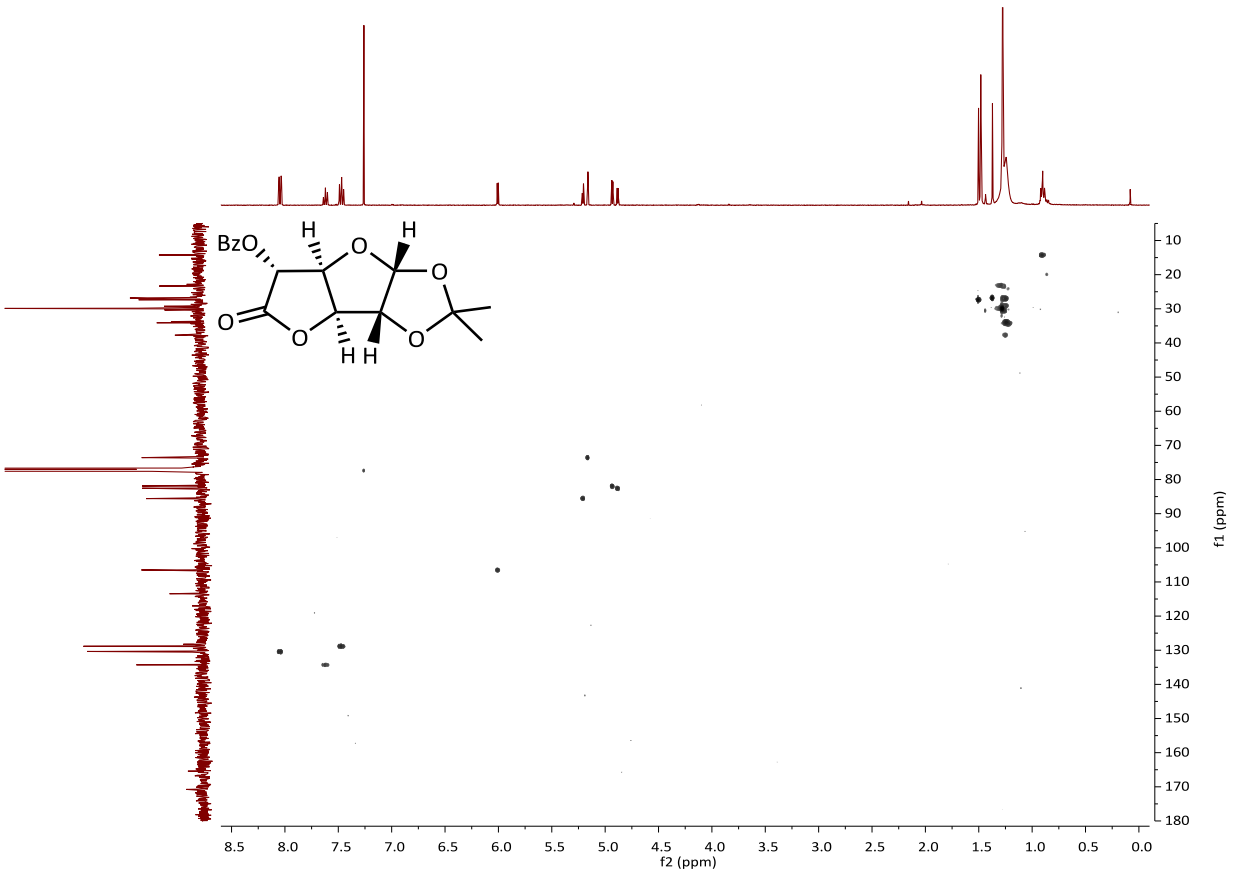


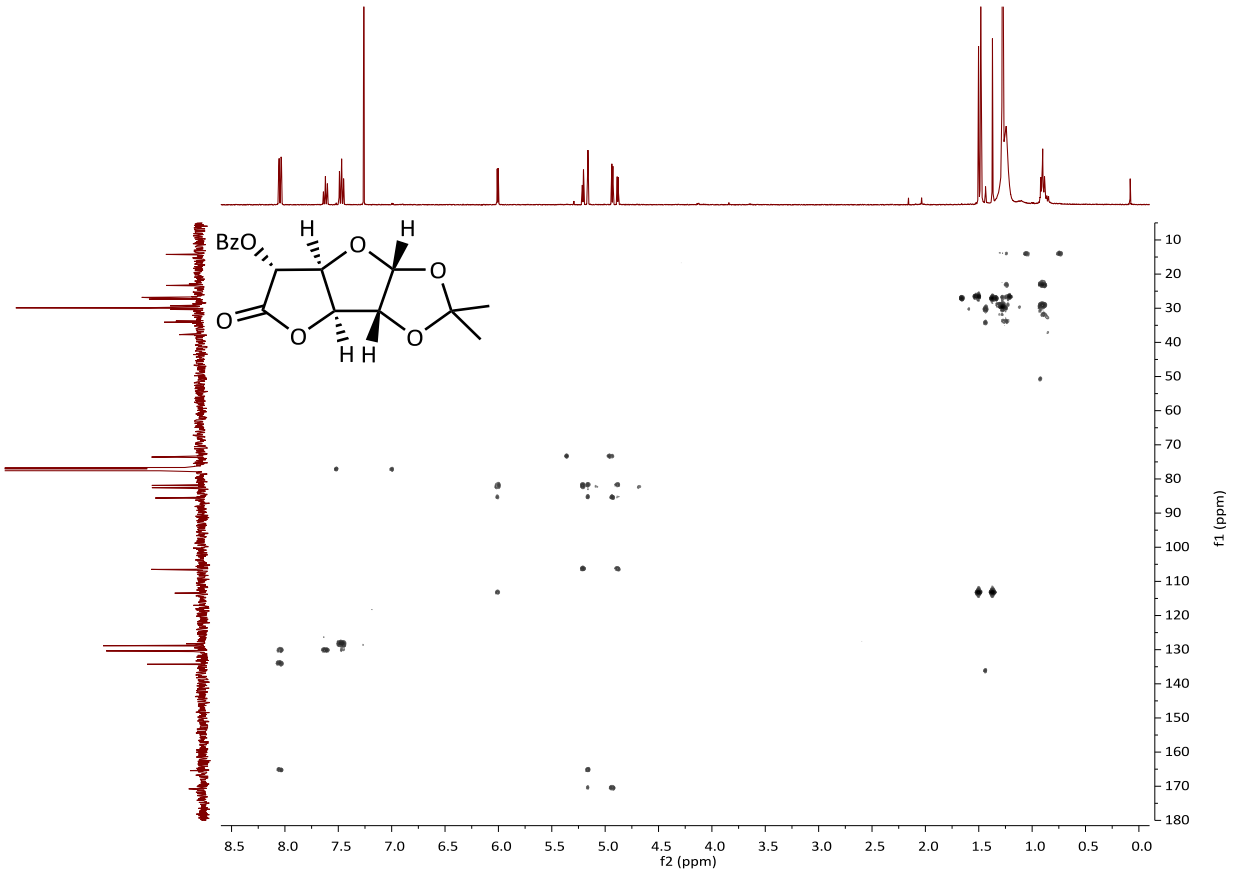
5-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone (3.1).



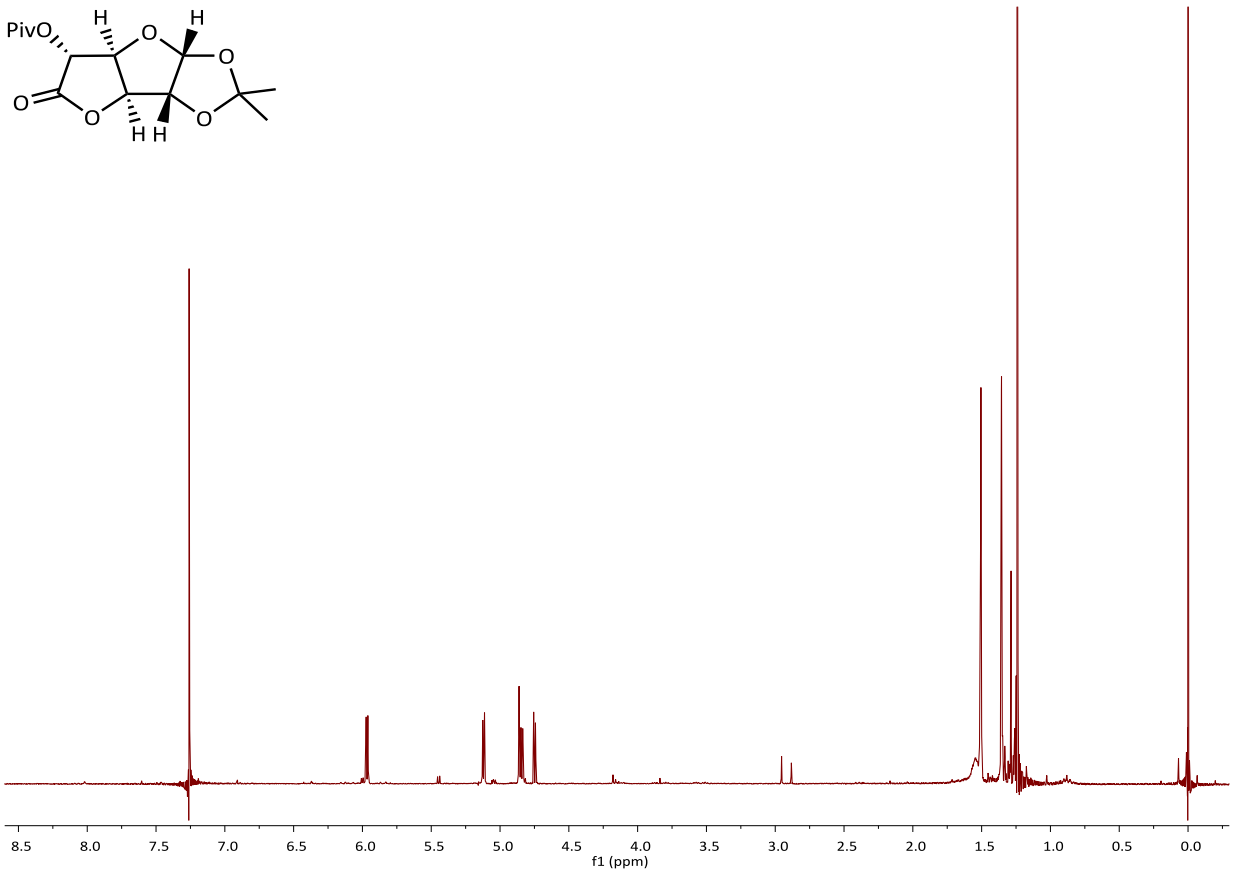


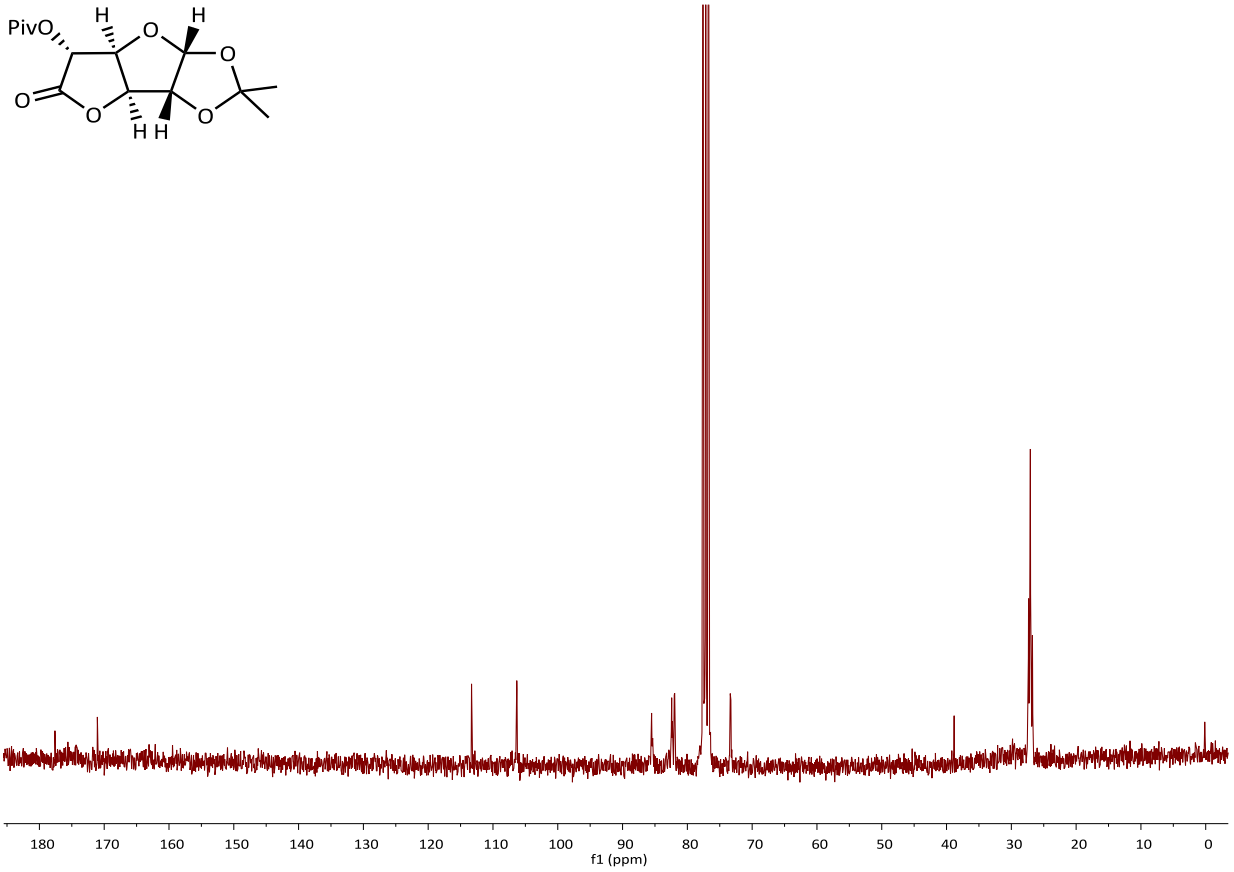

BzO
H
O
H
O
O
O
O
H H
f2 (ppm)
f1 (ppm)


BzO
H
O
H
O
O
O
O
H H
f2 (ppm)
f1 (ppm)

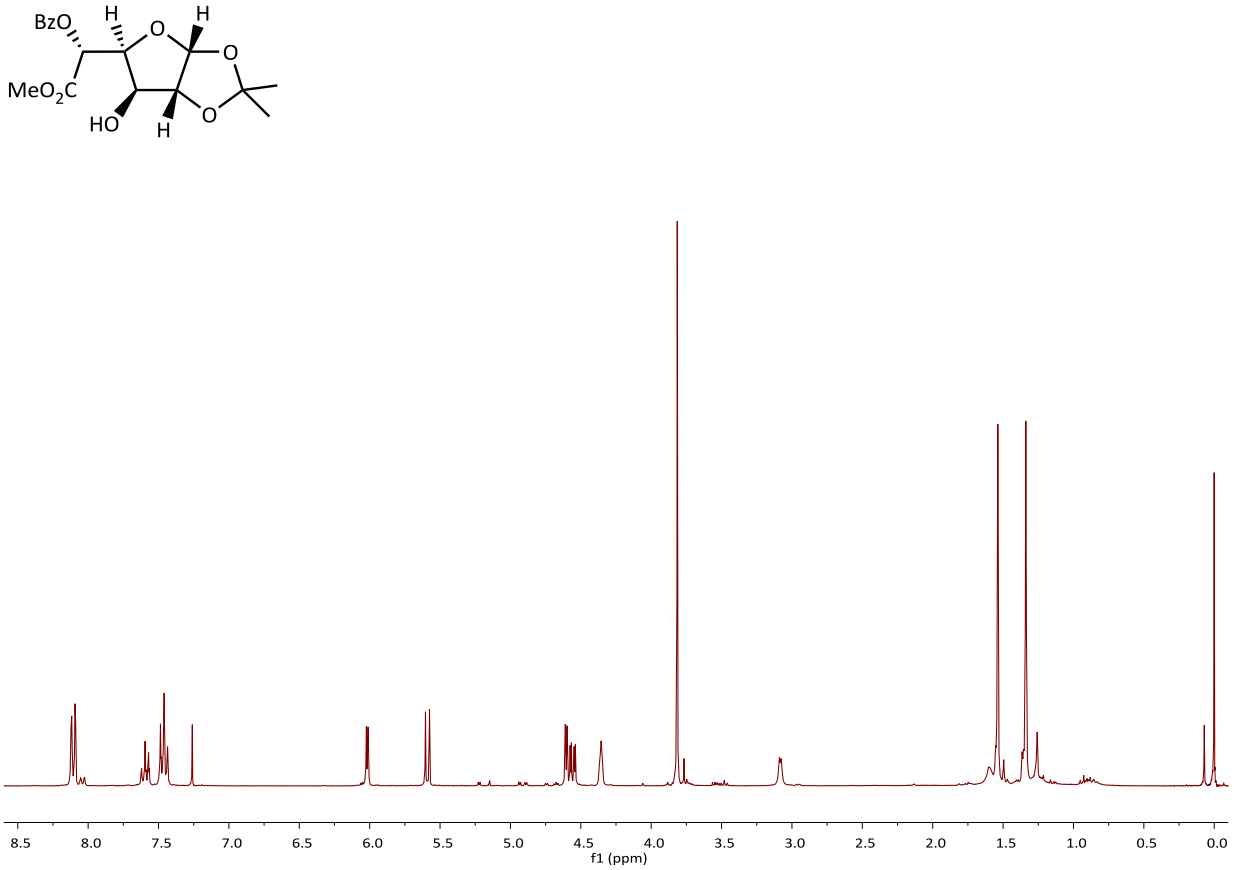


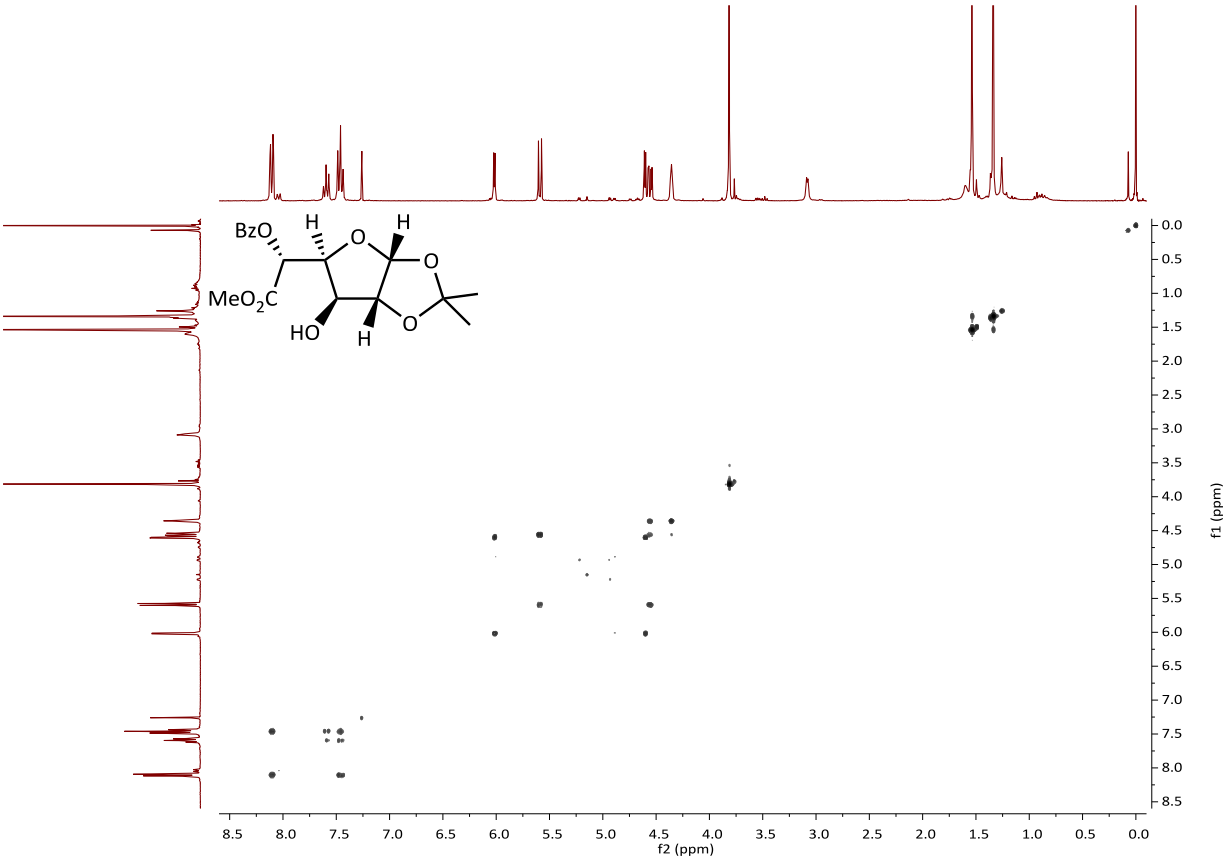
5-*O*-pivaloyl-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone.



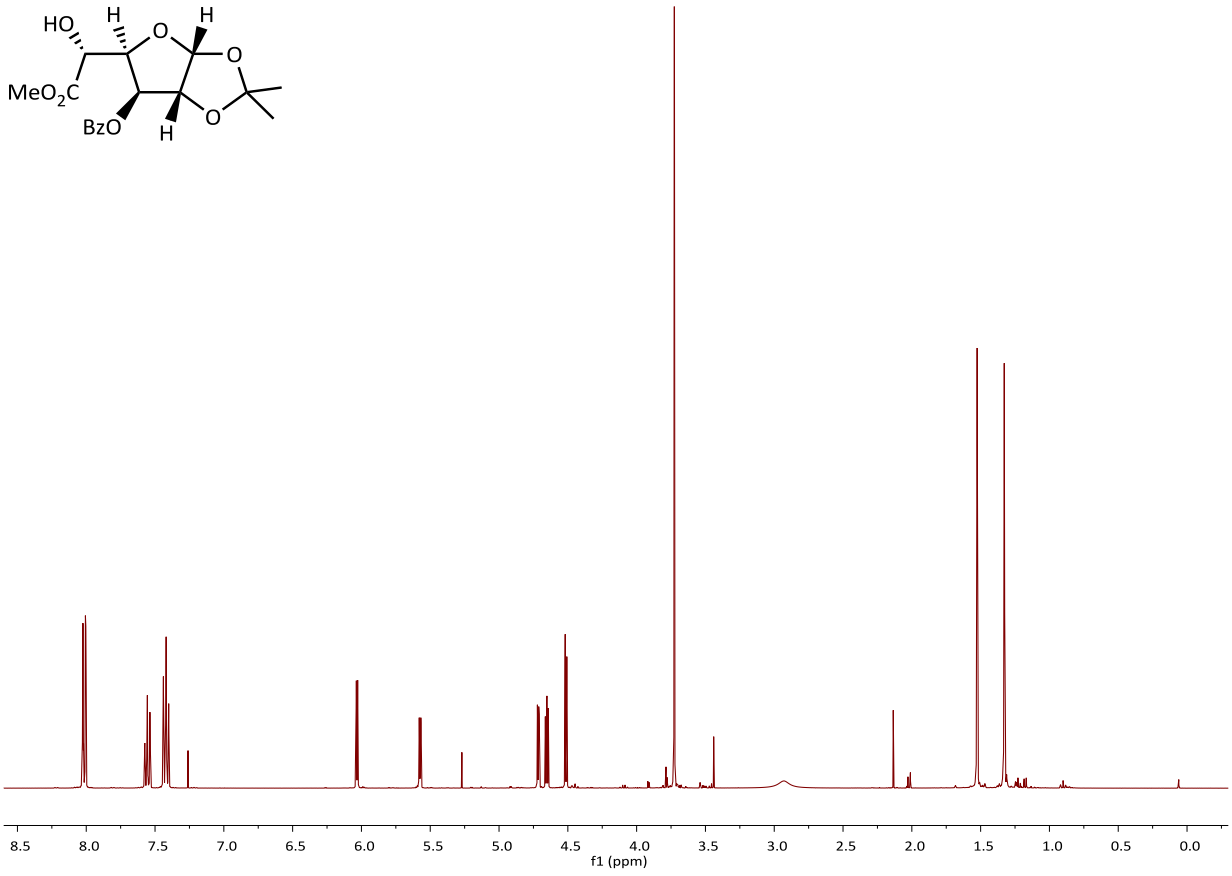


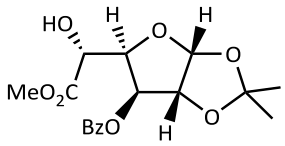
Methyl 5-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (3.8A)

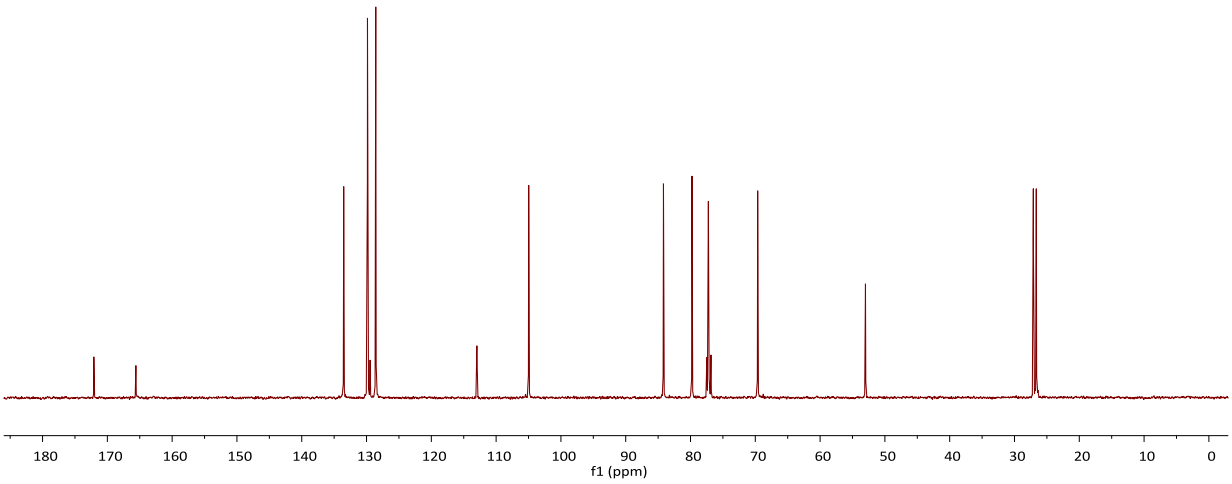


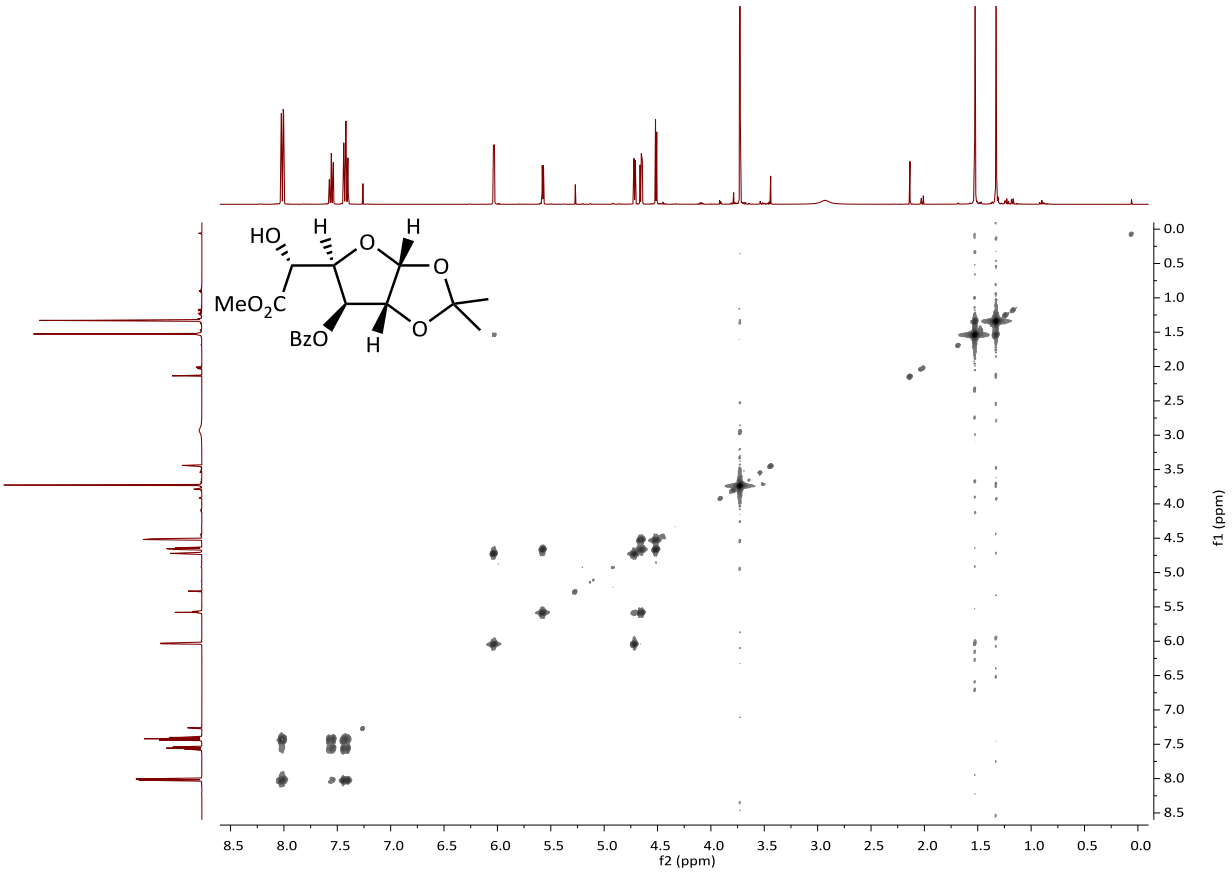


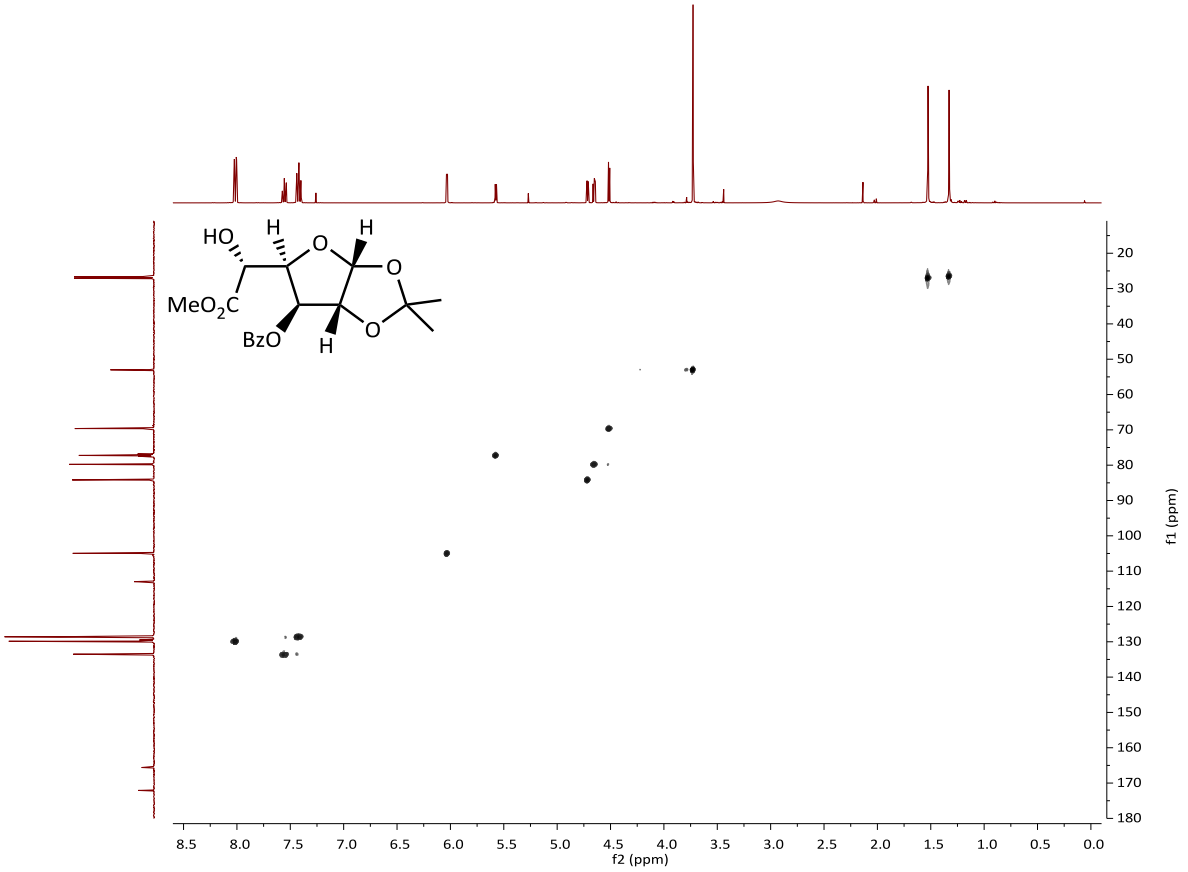
Methyl 3-*O*-benzoyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (3.8B).

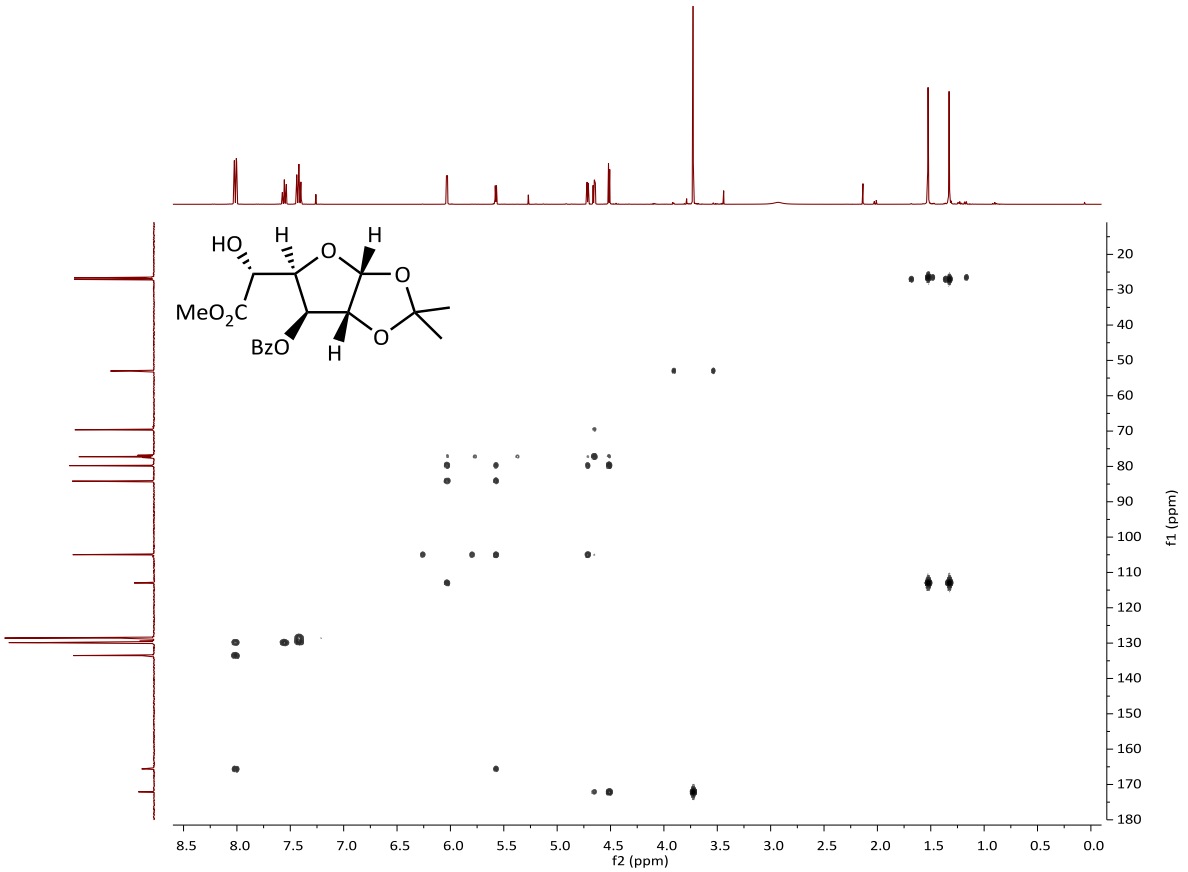



HO
H
O
H
O
MeO2C
BzO
O
H

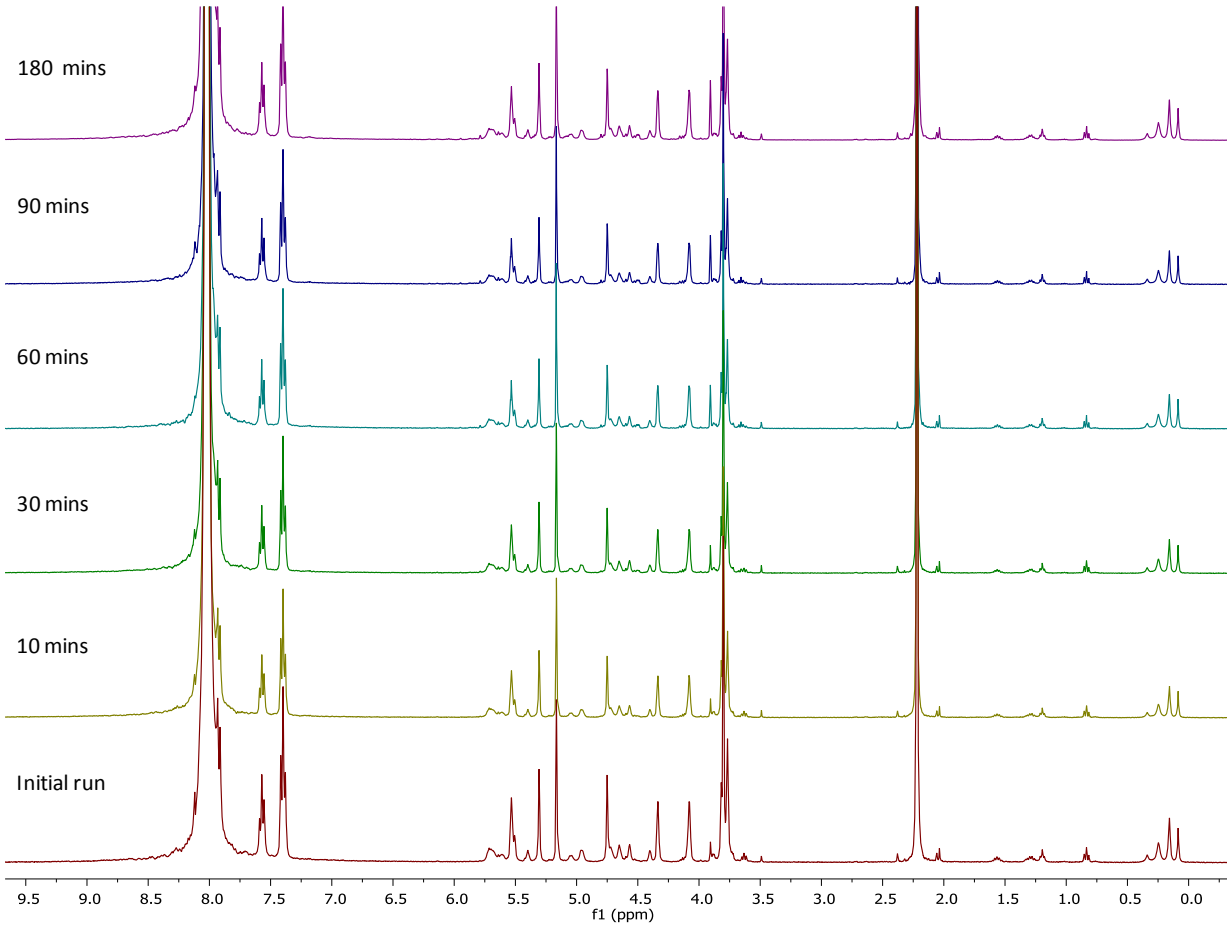

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


HO
H
O
H
O
MeO2C
BzO
O
H
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5
f1 (ppm)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)

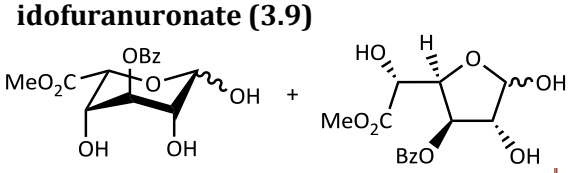

HO
H
O
H
O
MeO2C
BzO
O
H
f2 (ppm)
f1 (ppm)

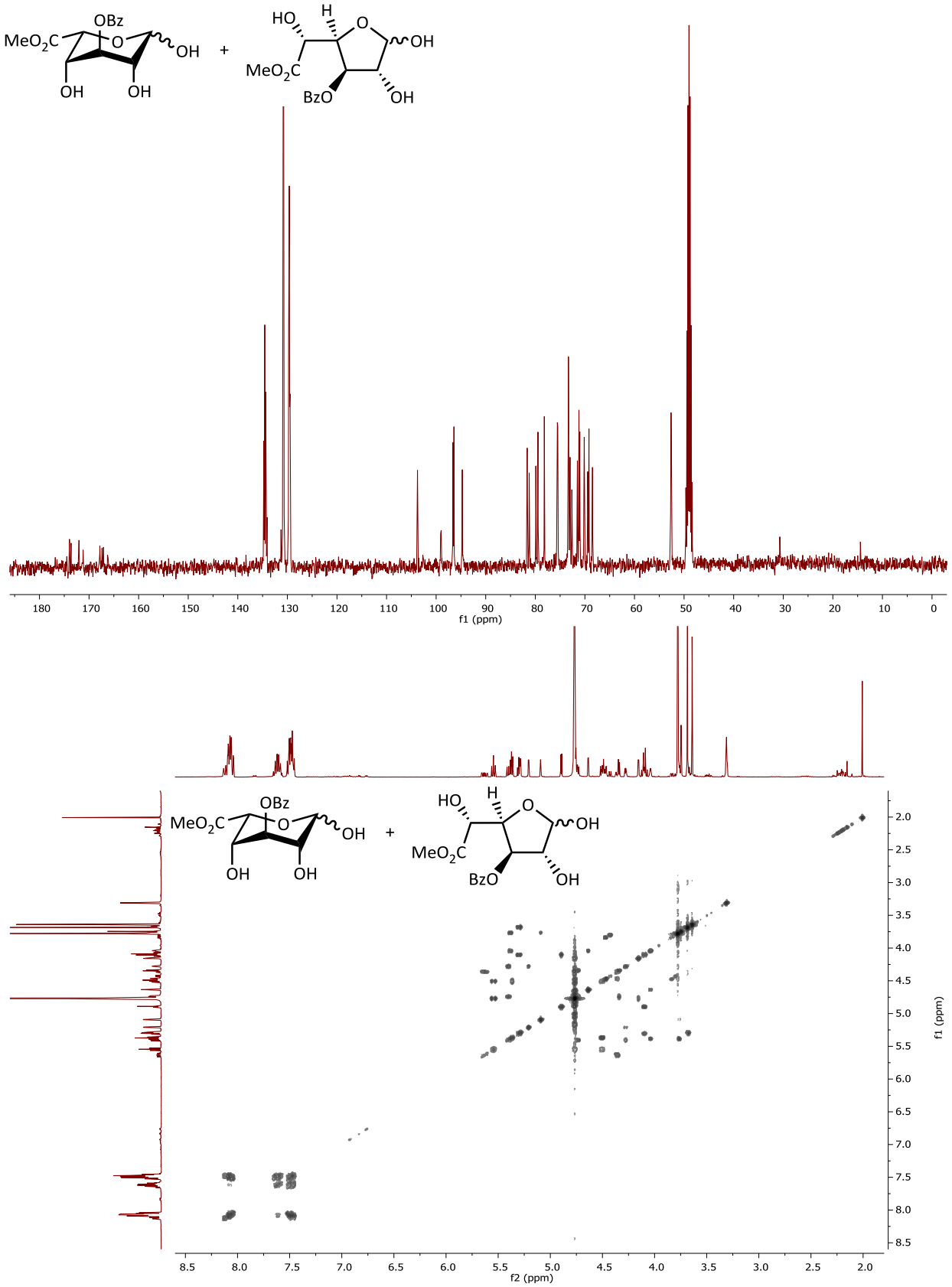

HO
H
O
H
O
MeO2C
BzO
O
H
f2 (ppm)
f1 (ppm)

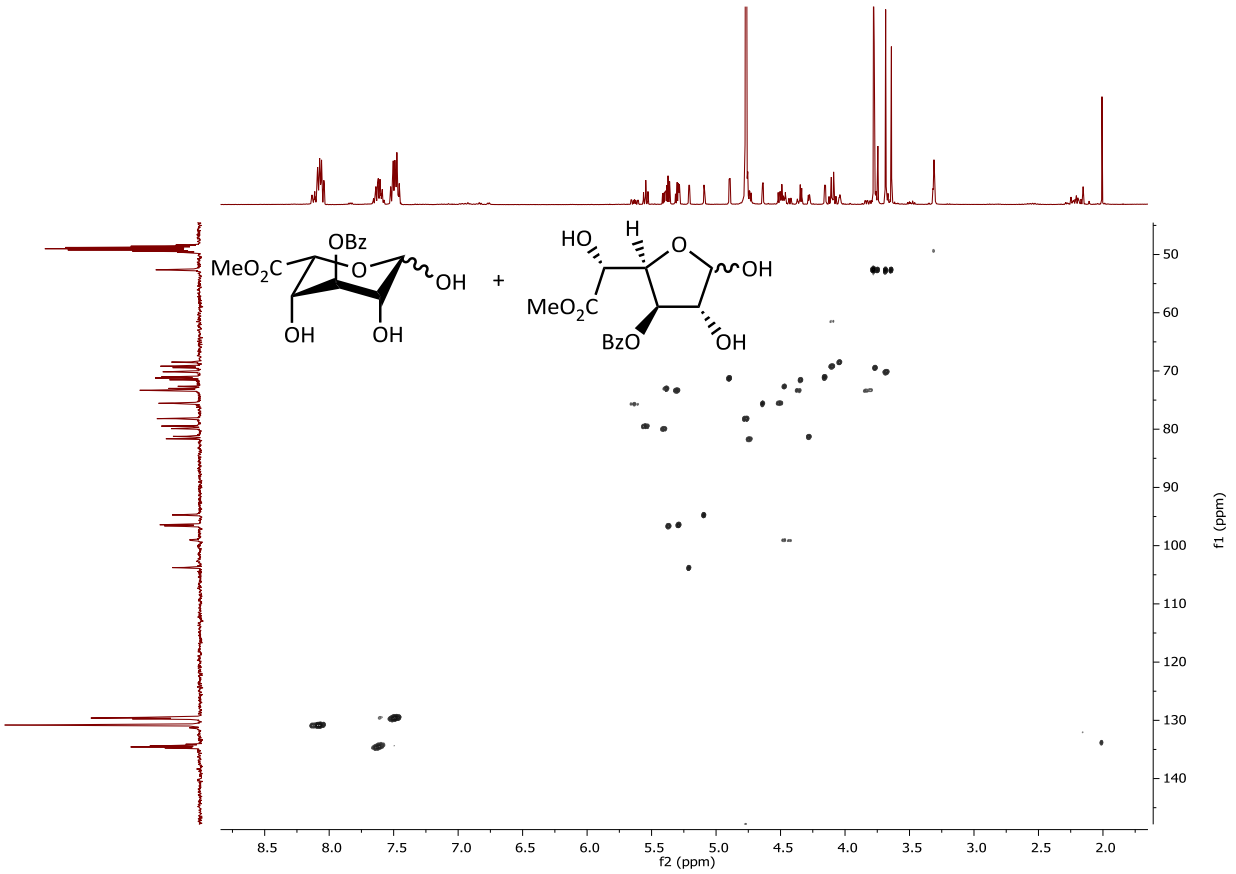
NMR experiment for conversion of 3.8B to 3.2

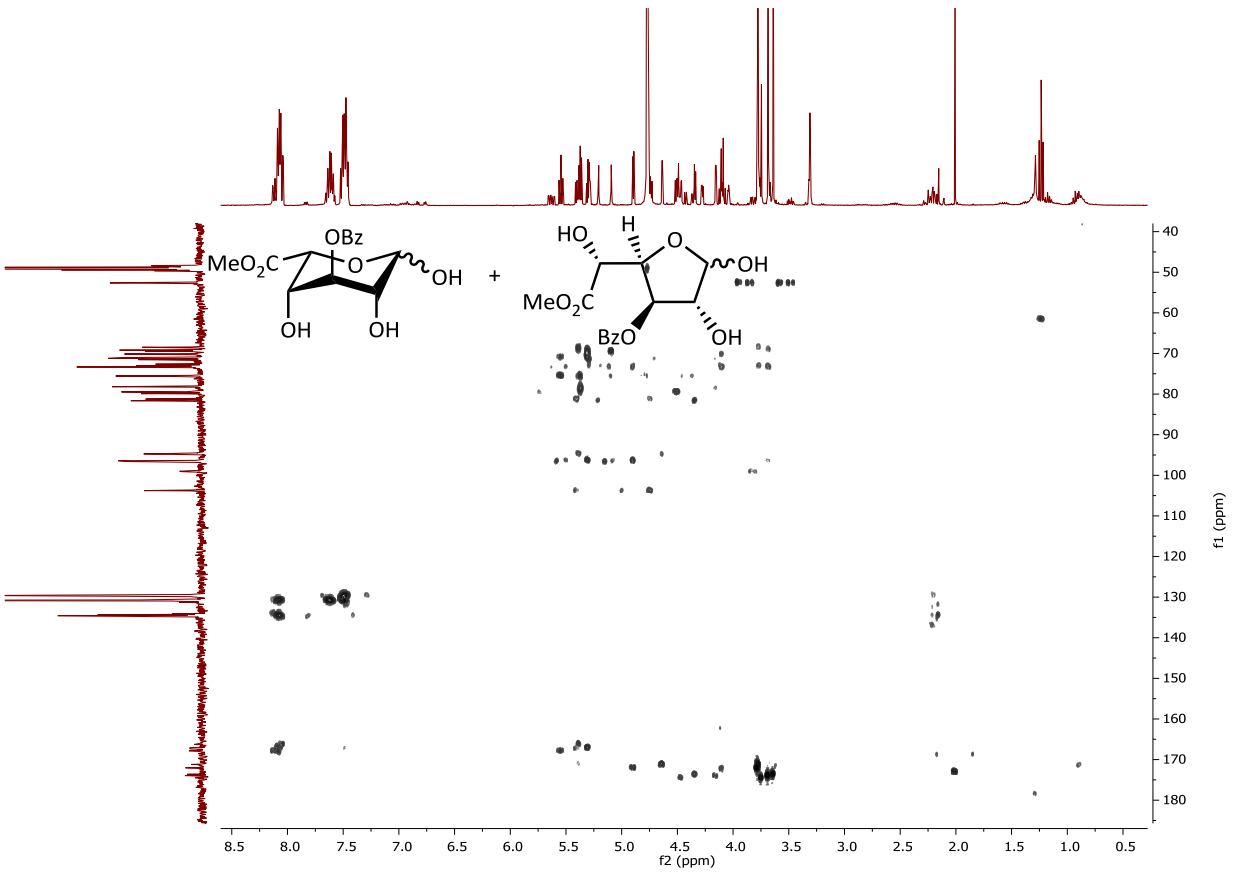


Methyl 5-*O*-benzoyl-α/β-L-idopyranuronate (3.2) and Methyl 5-*O*-benzoyl-α/β-L-idofuranuronate (3.9)

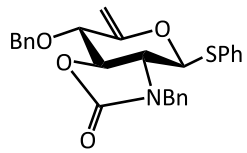


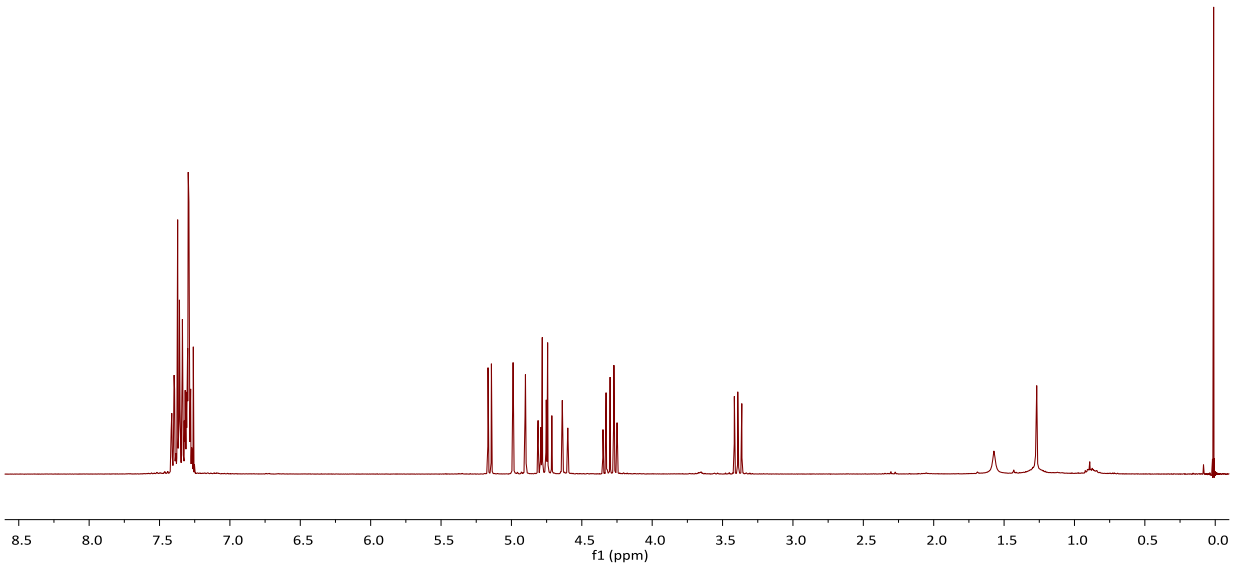

OBz
MeO2C
O
OH
OH
OH
+
HO
H
O
OH
MeO2C
BzO
OH
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)
OBz
MeO2C
O
OH
OH
OH
+
HO
H
O
OH
MeO2C
BzO
OH
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
f2 (ppm)
2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5
f1 (ppm)

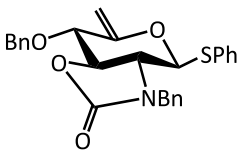

OBz
MeO2C
O
OH
OH
OH
+
HO
H
O
OH
MeO2C
BzO
OH
f2 (ppm)
f1 (ppm)

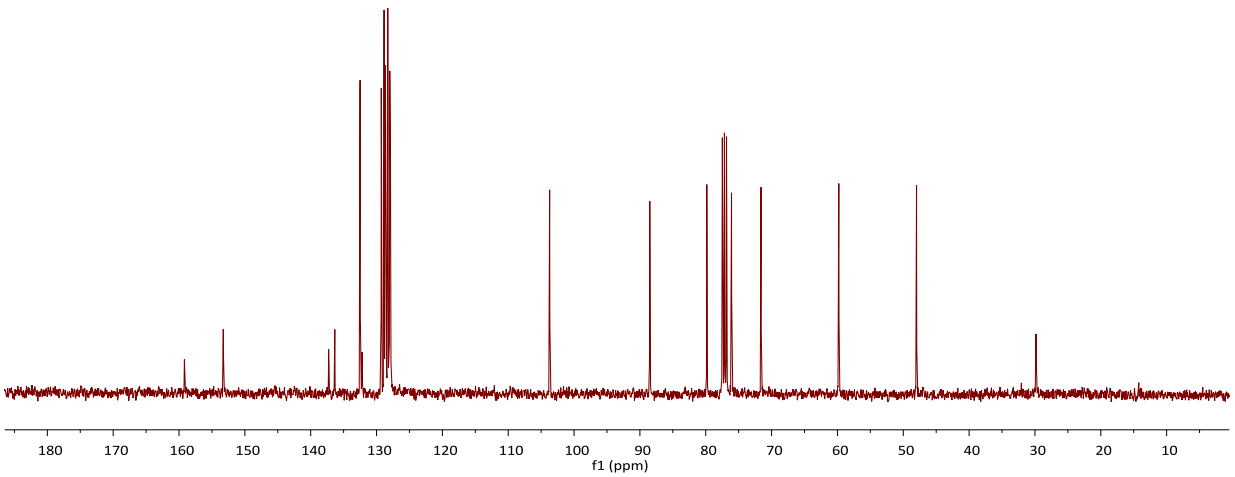

OBz
MeO2C
O
OH
OH
OH
+
HO
H
O
OH
MeO2C
BzO
OH
f2 (ppm)
f1 (ppm)

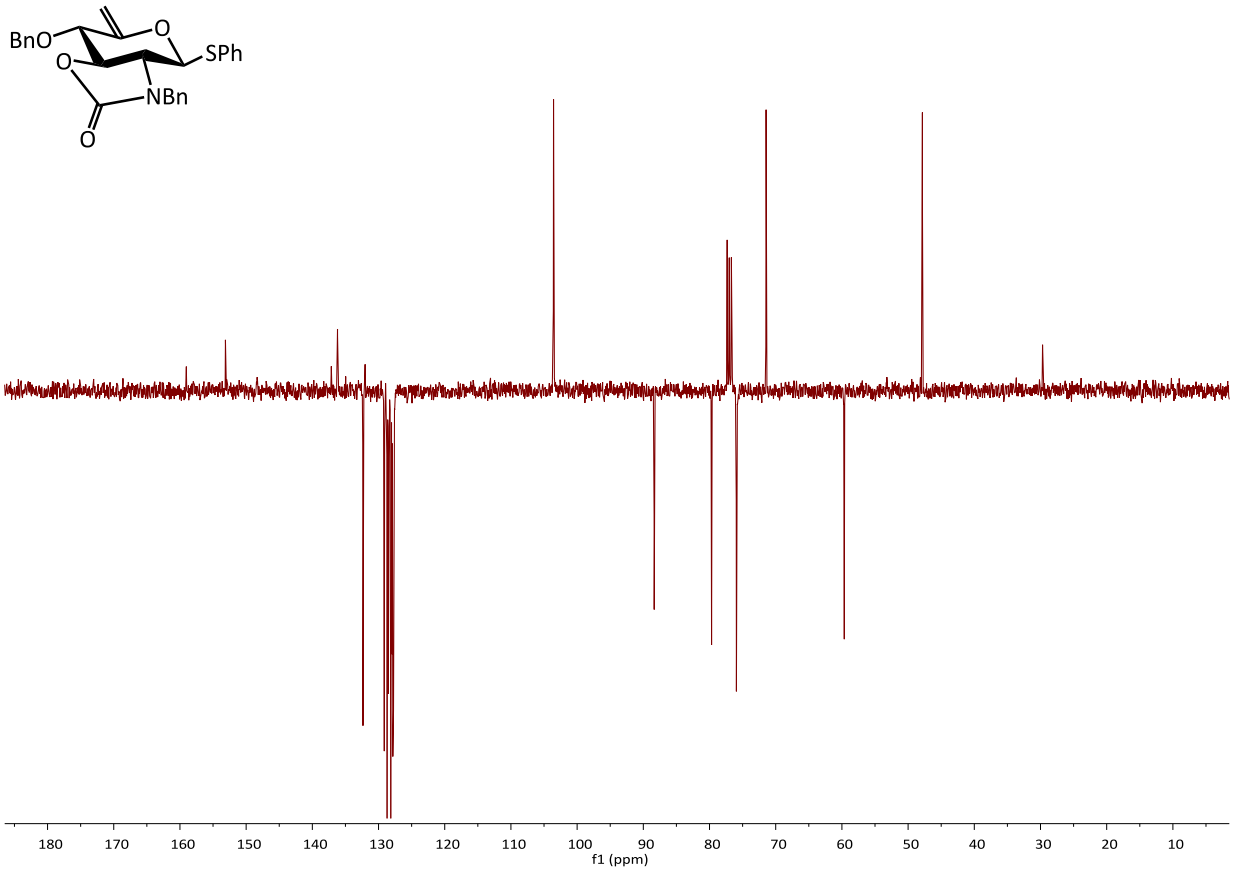
Phenyl 2-*N*-benzyl-4-*O*-benzyl-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-β-D-xylo-hex-5-enopyranoside (3.14).

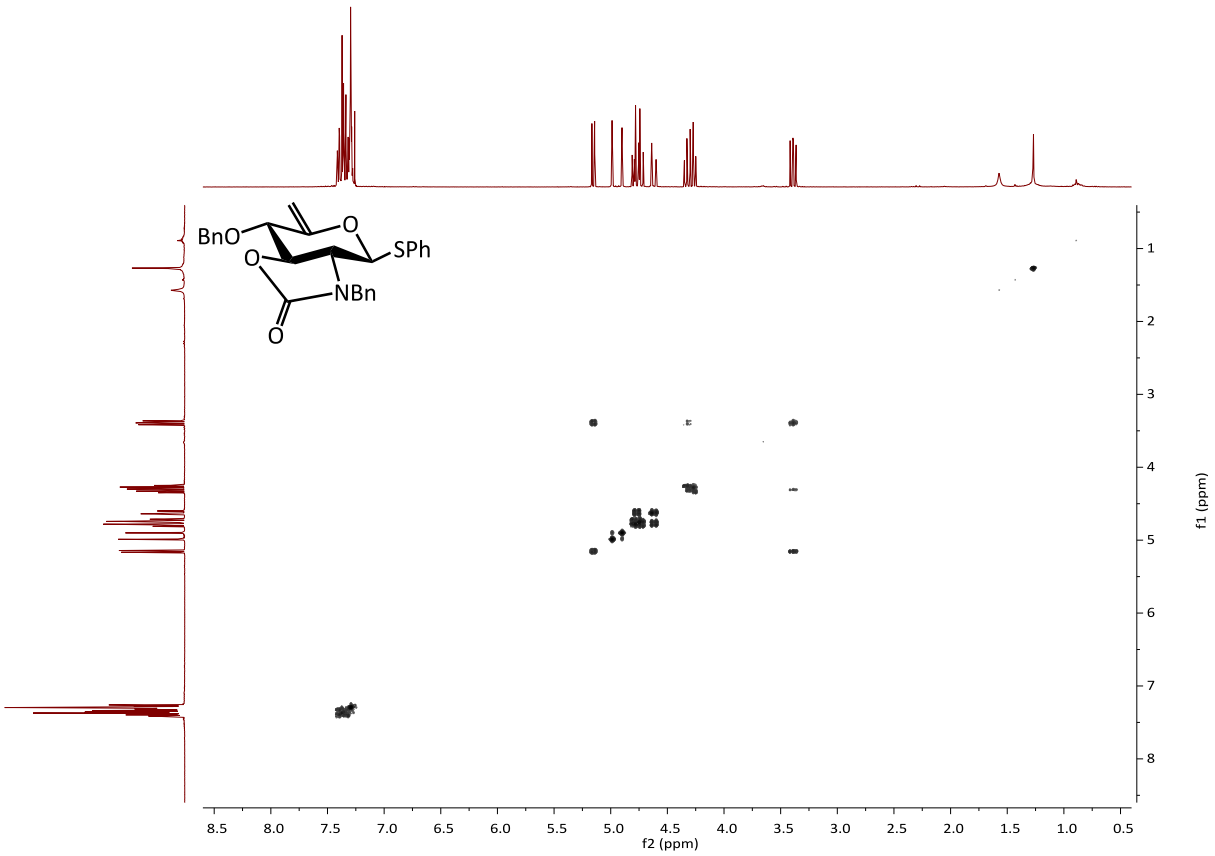


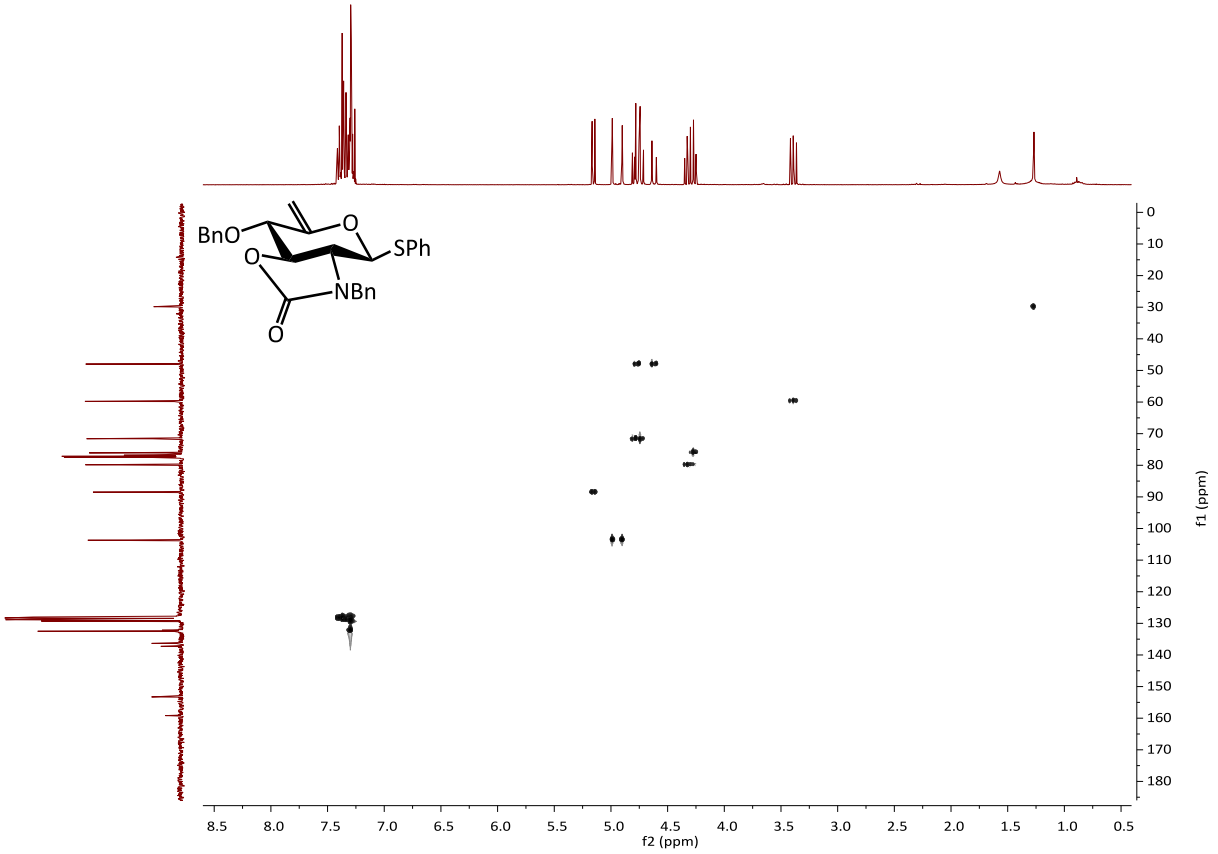




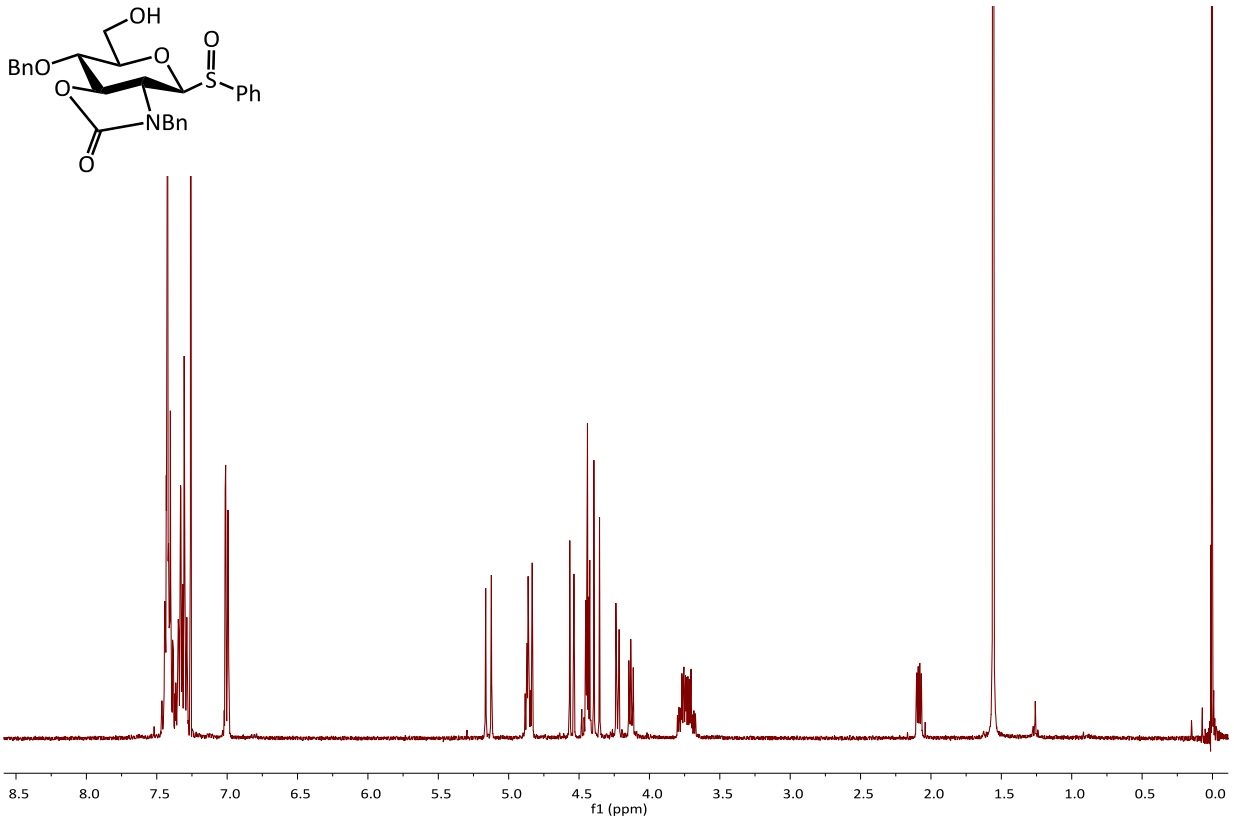


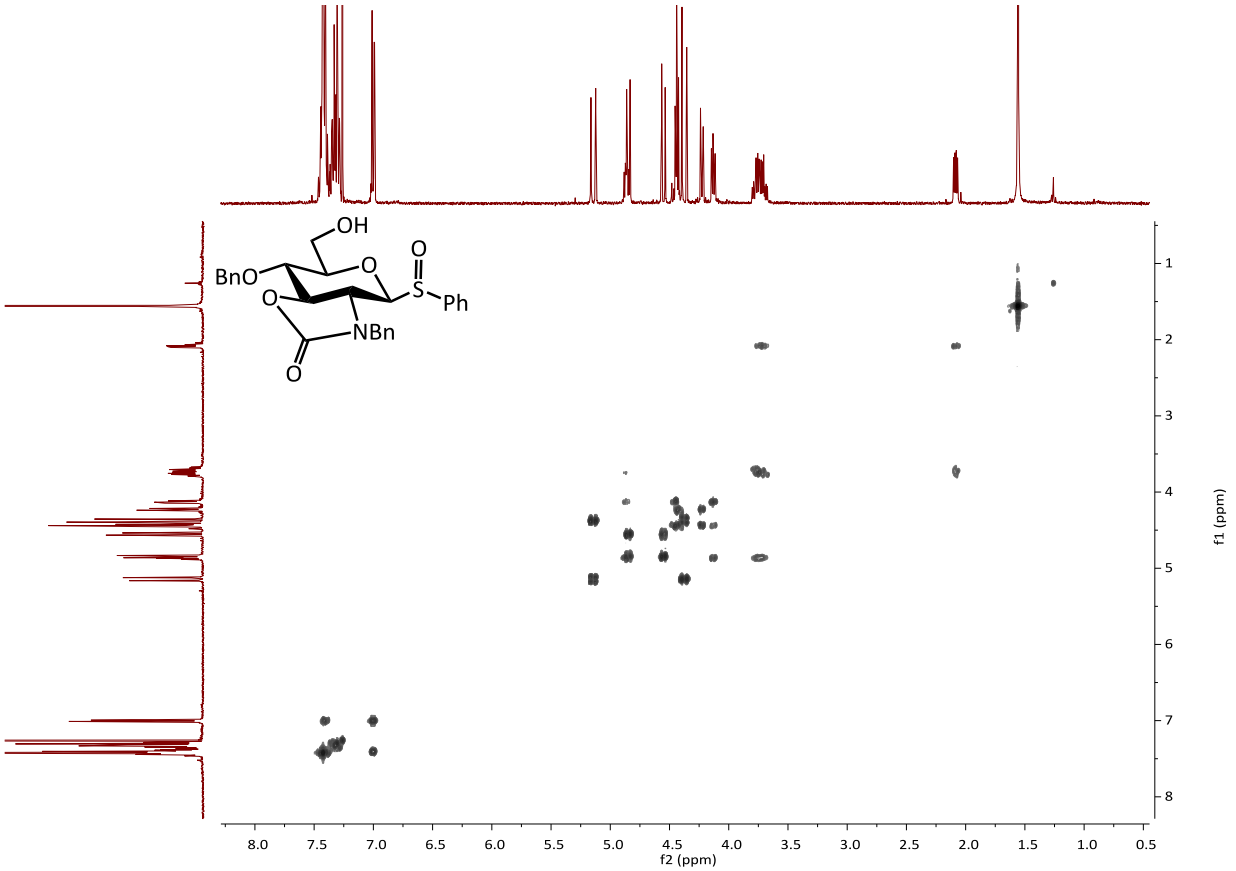

BnO
O
O
SPh
NBn
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

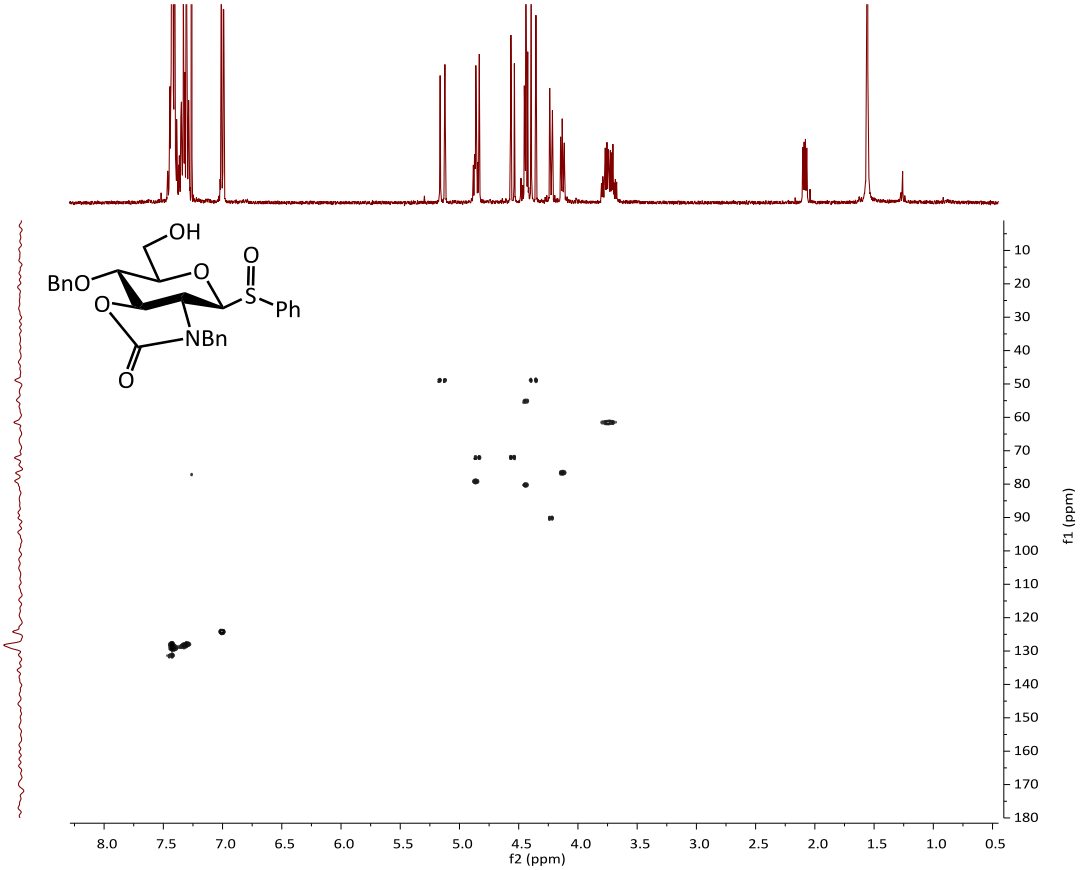

BnO
O
O
SPh
NBn
O
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)
1 2 3 4 5 6 7 8
f1 (ppm)

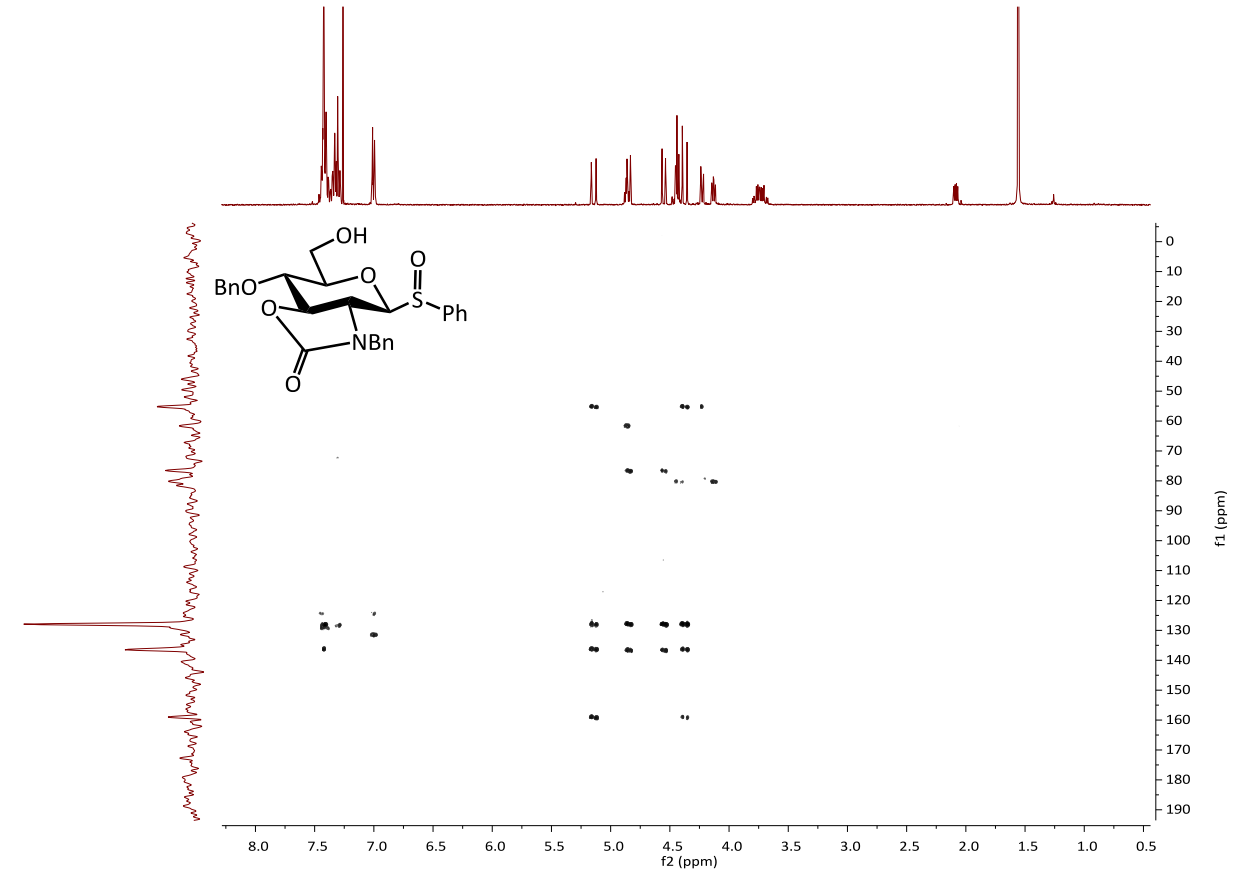


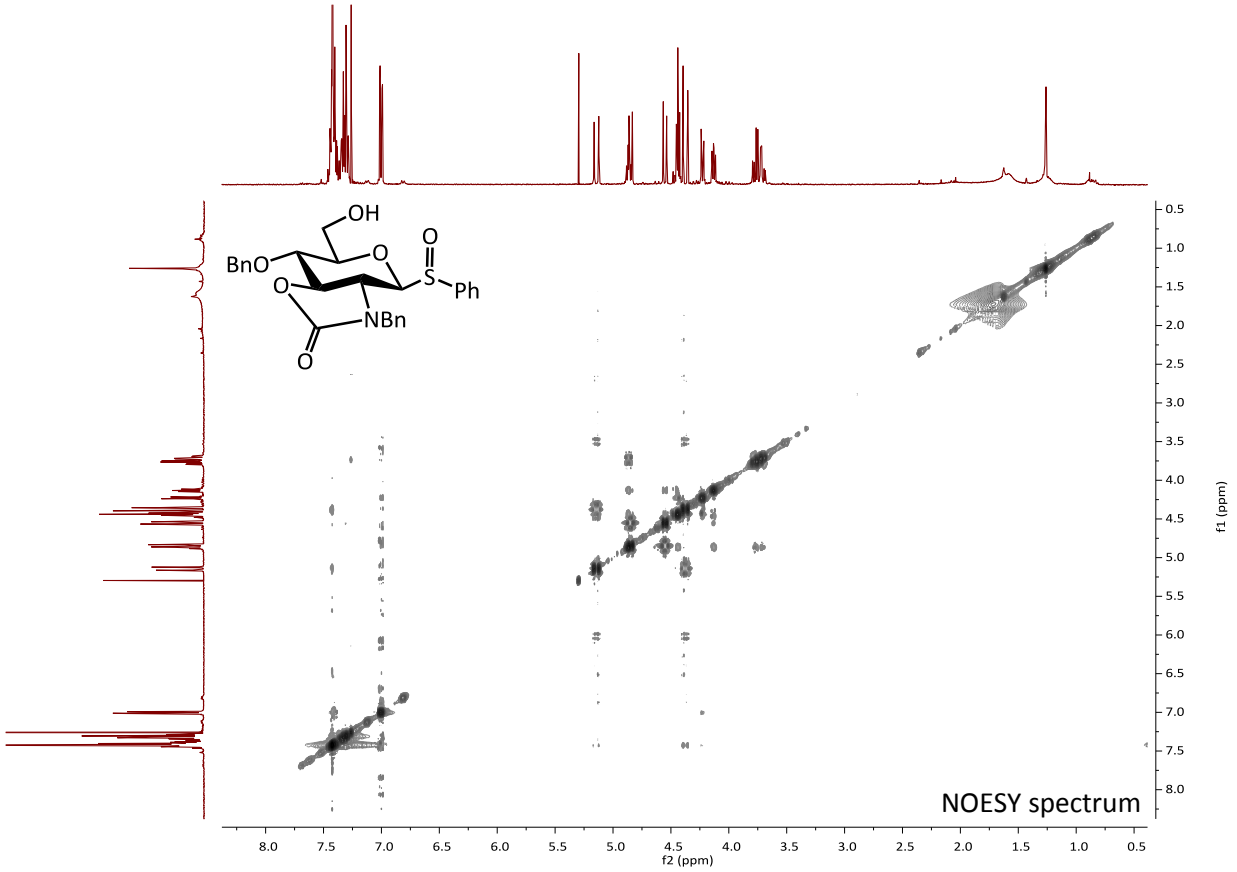
Phenyl 2-*N*-benzyl-4-*O*-benzyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-sulfinyl-β-D-glucopyranoside (3.16).



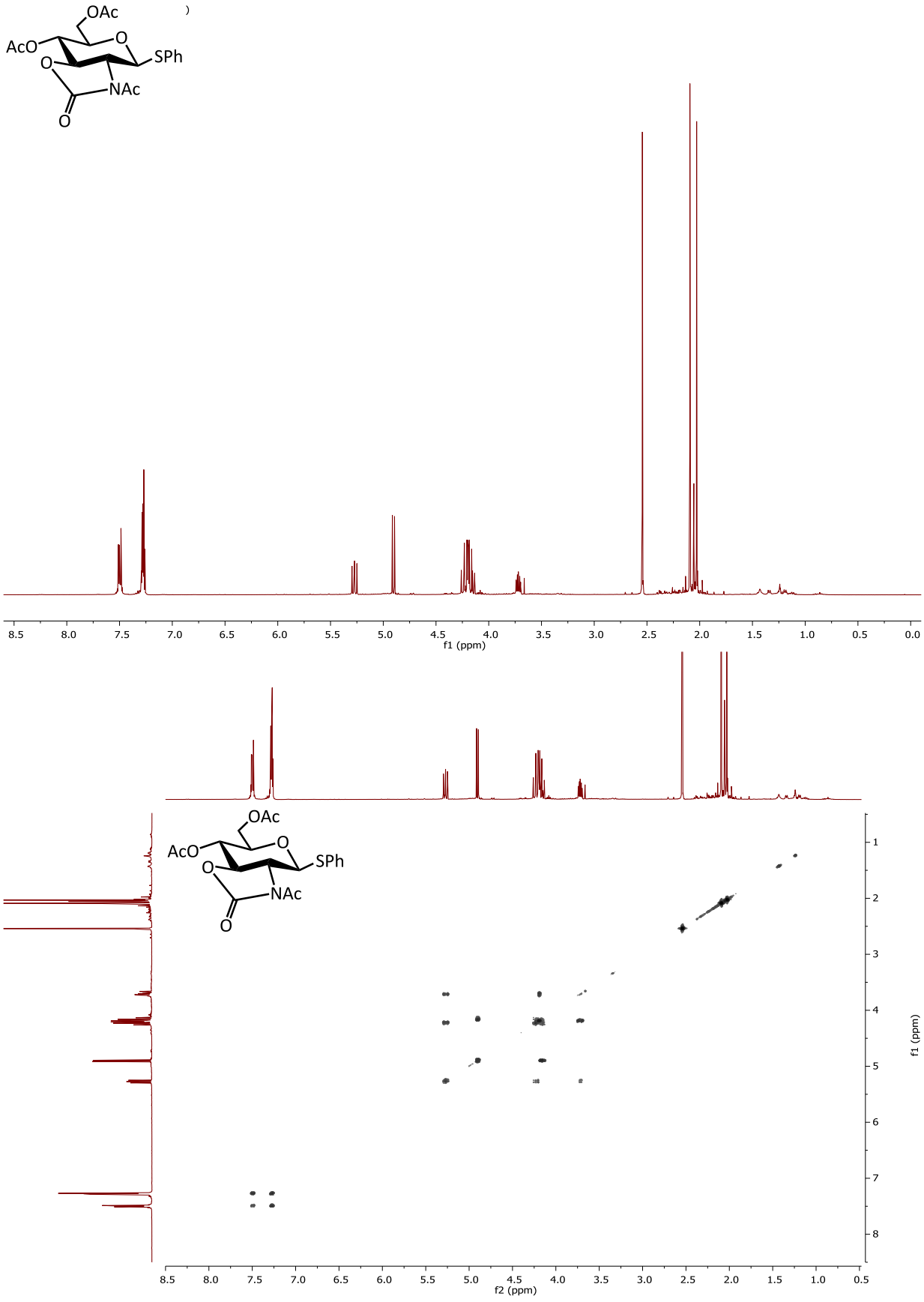

OH
BnO
O
O
S
Ph
NBn
O
f2 (ppm)
f1 (ppm)


OH
BnO
O
O
S
Ph
NBn
O
f2 (ppm)
f1 (ppm)

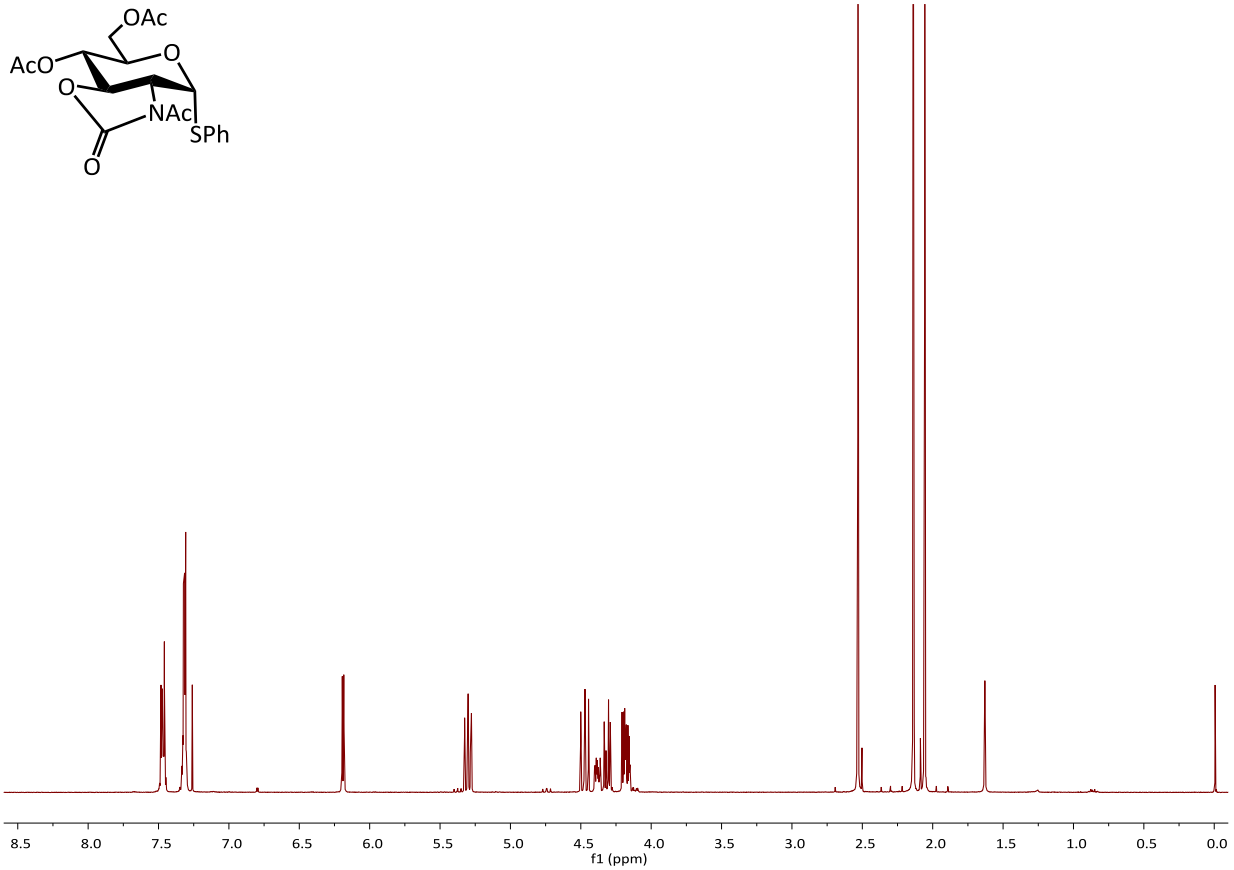

OH
BnO
O
O
S
Ph
NBn
O
f2 (ppm)
f1 (ppm)

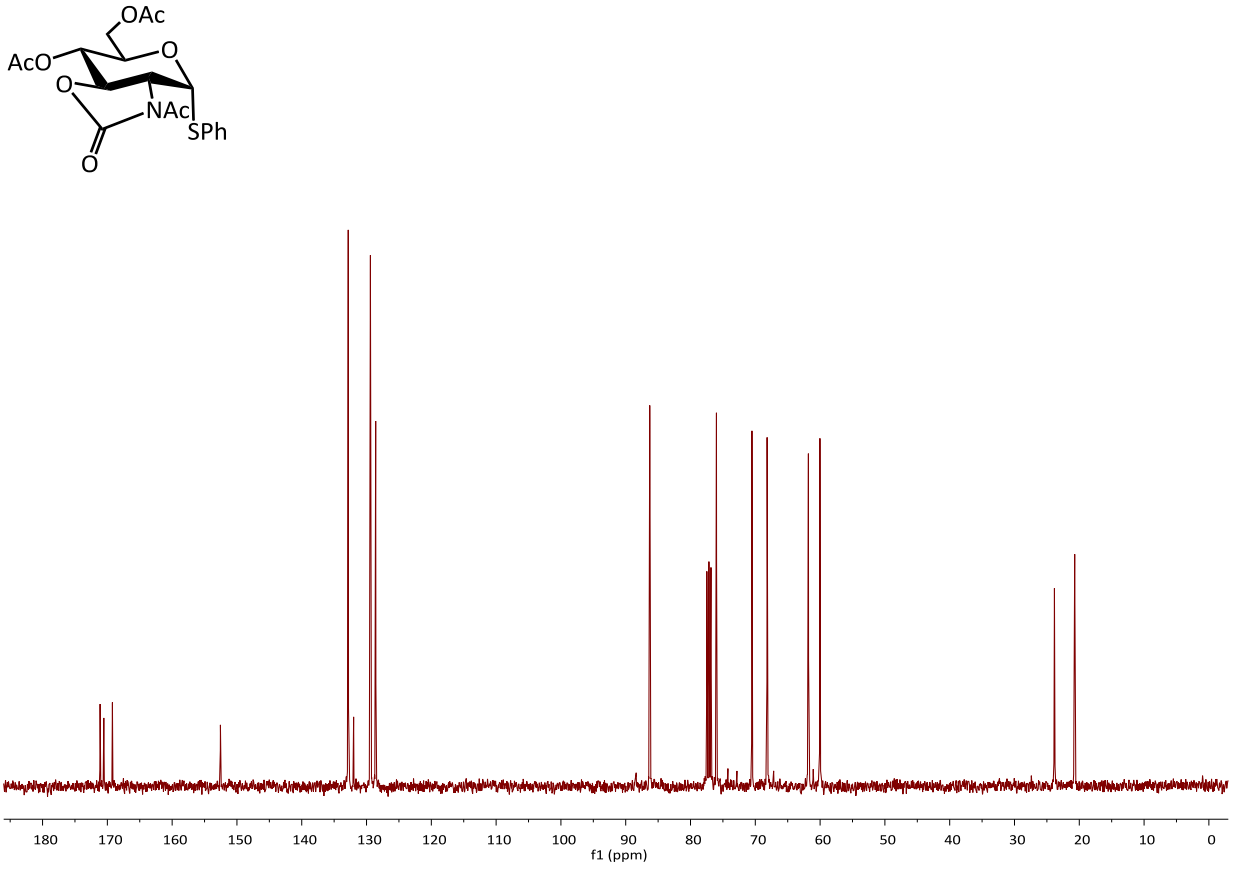

OH
BnO
O
O
S
Ph
NBn
O
f2 (ppm)
f1 (ppm)
NOESY spectrum

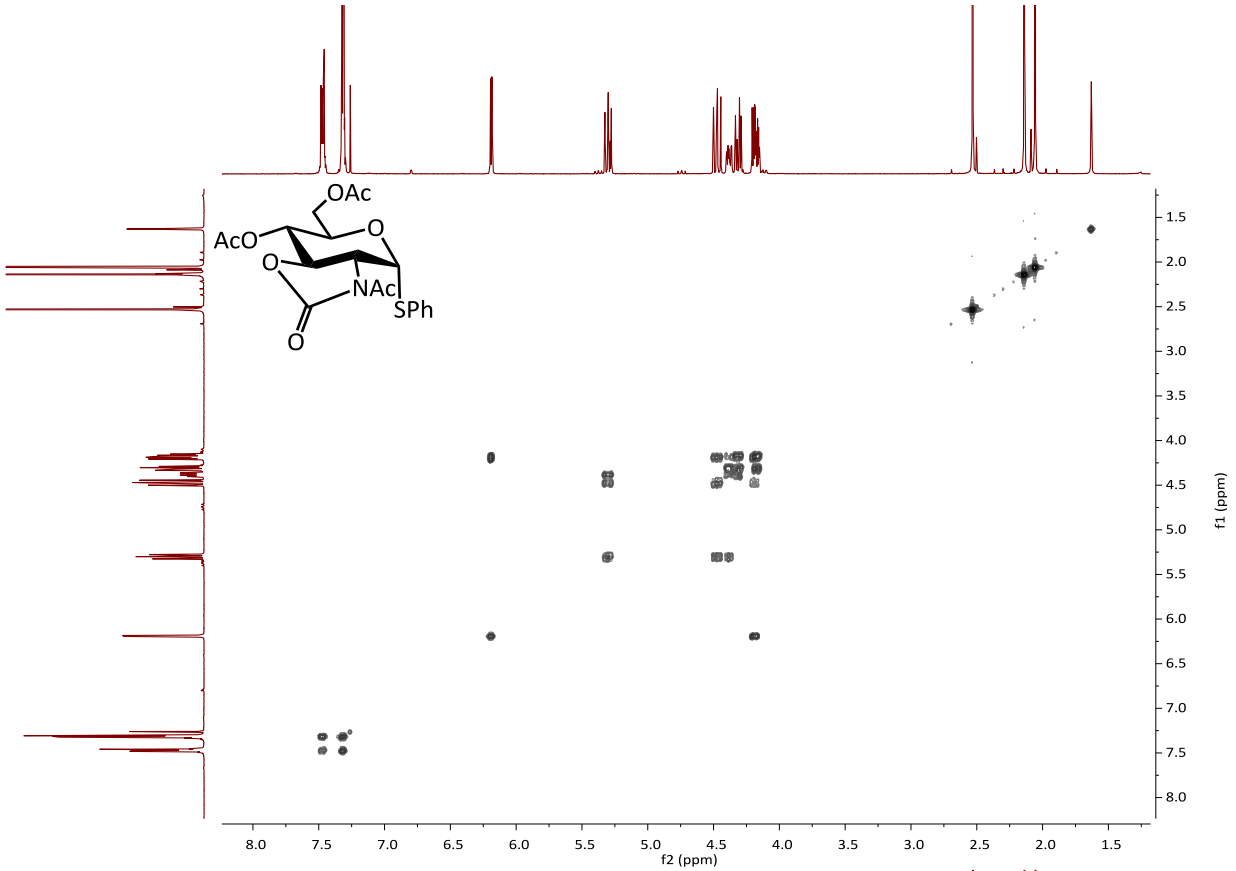
Phenyl 2-*N*-acetyl-2-amino-di-4,6-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.18).

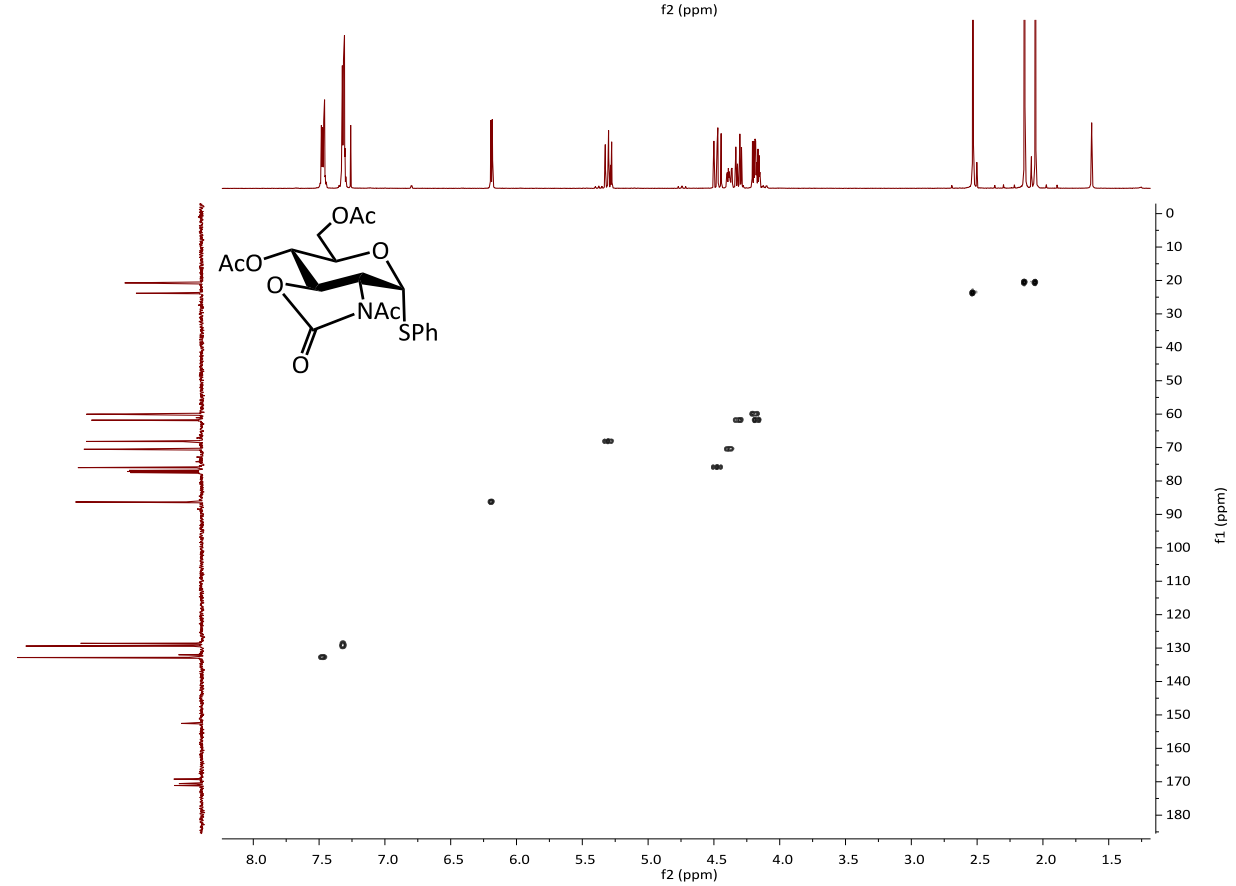


Phenyl 2-*N*-acetyl-2-amino-di-4,6-O-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.19).

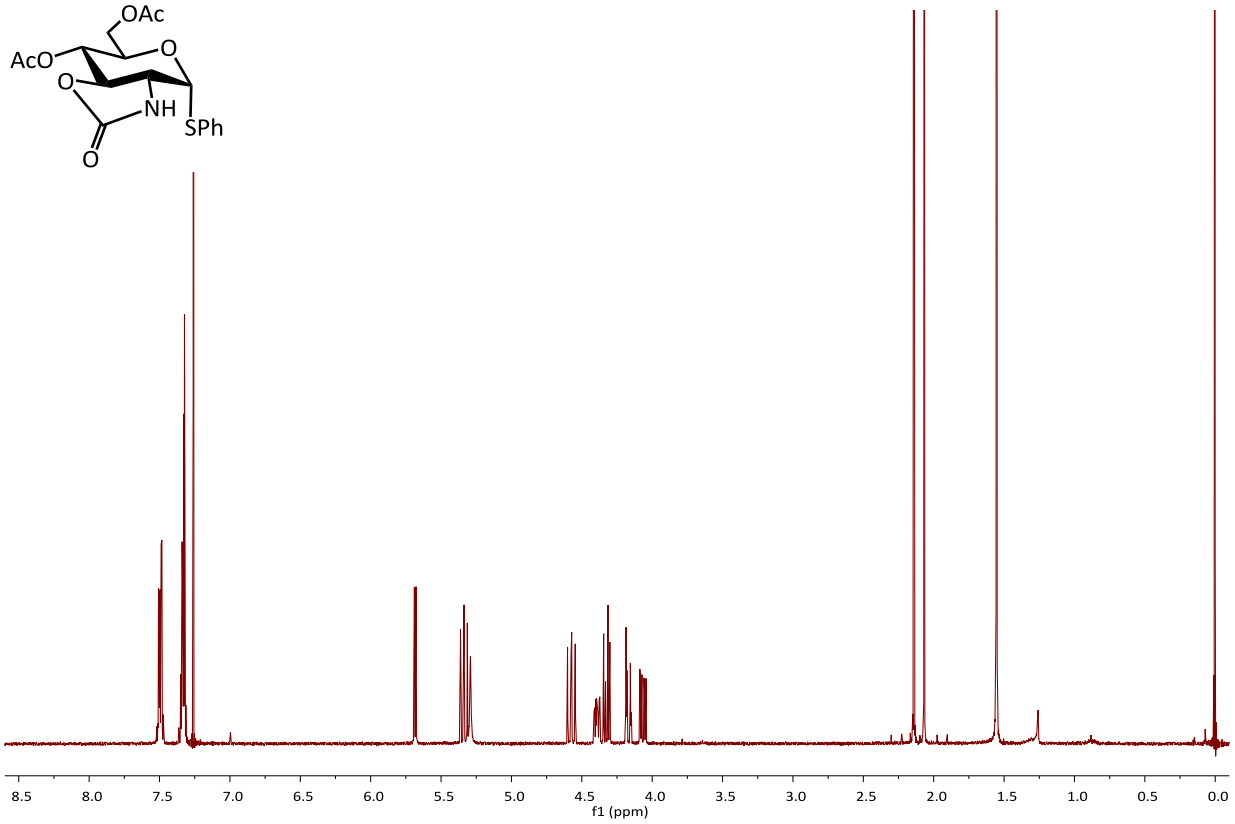


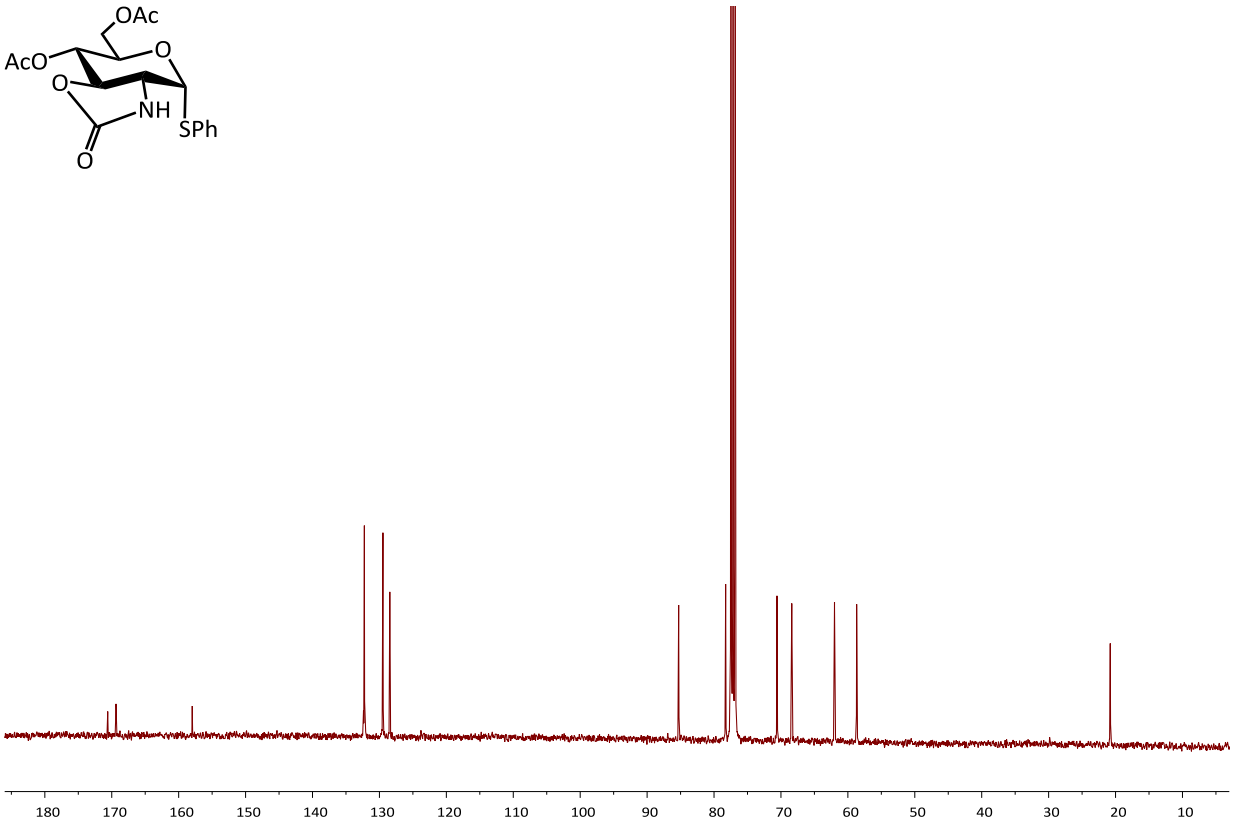


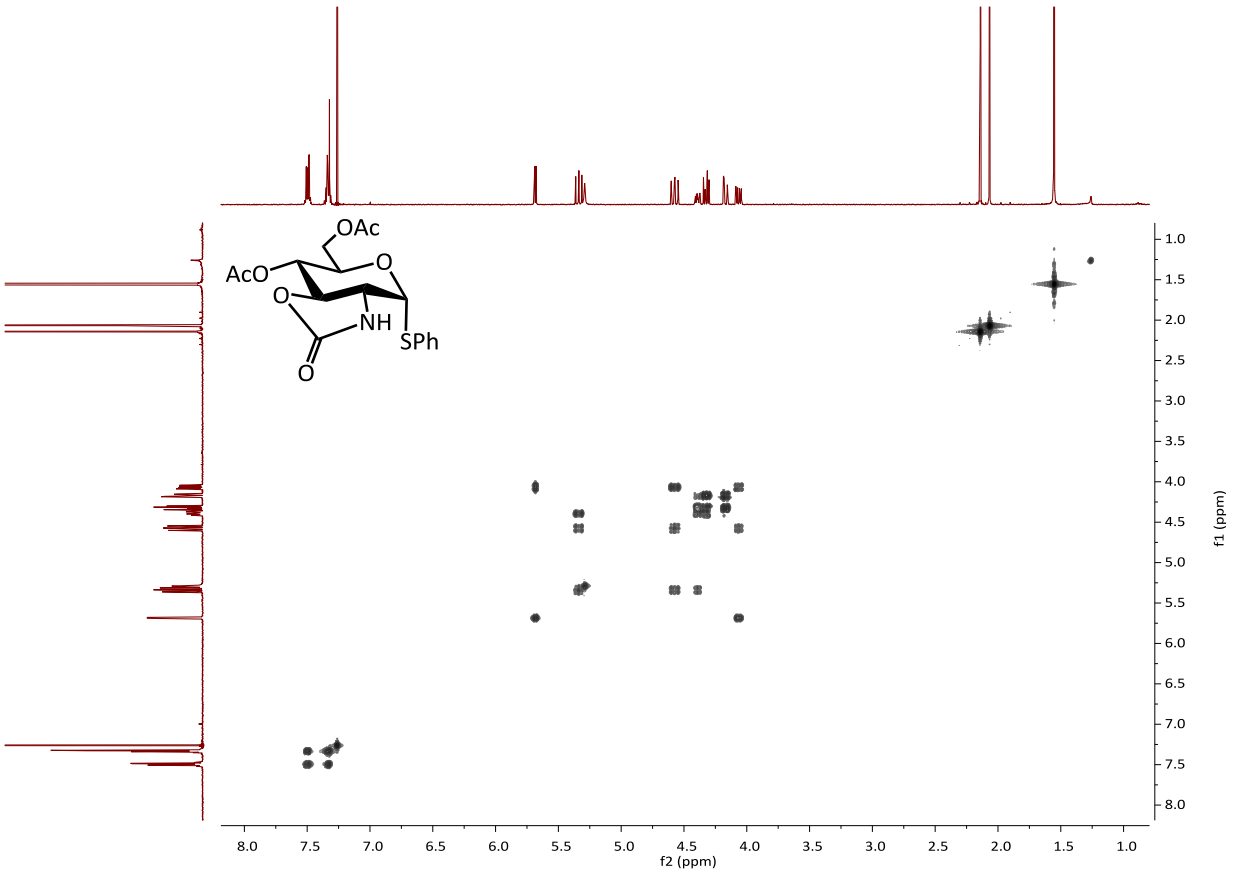

OAc
AcO
O
O
NAc
SPh
O
f1 (ppm)
f2 (ppm)

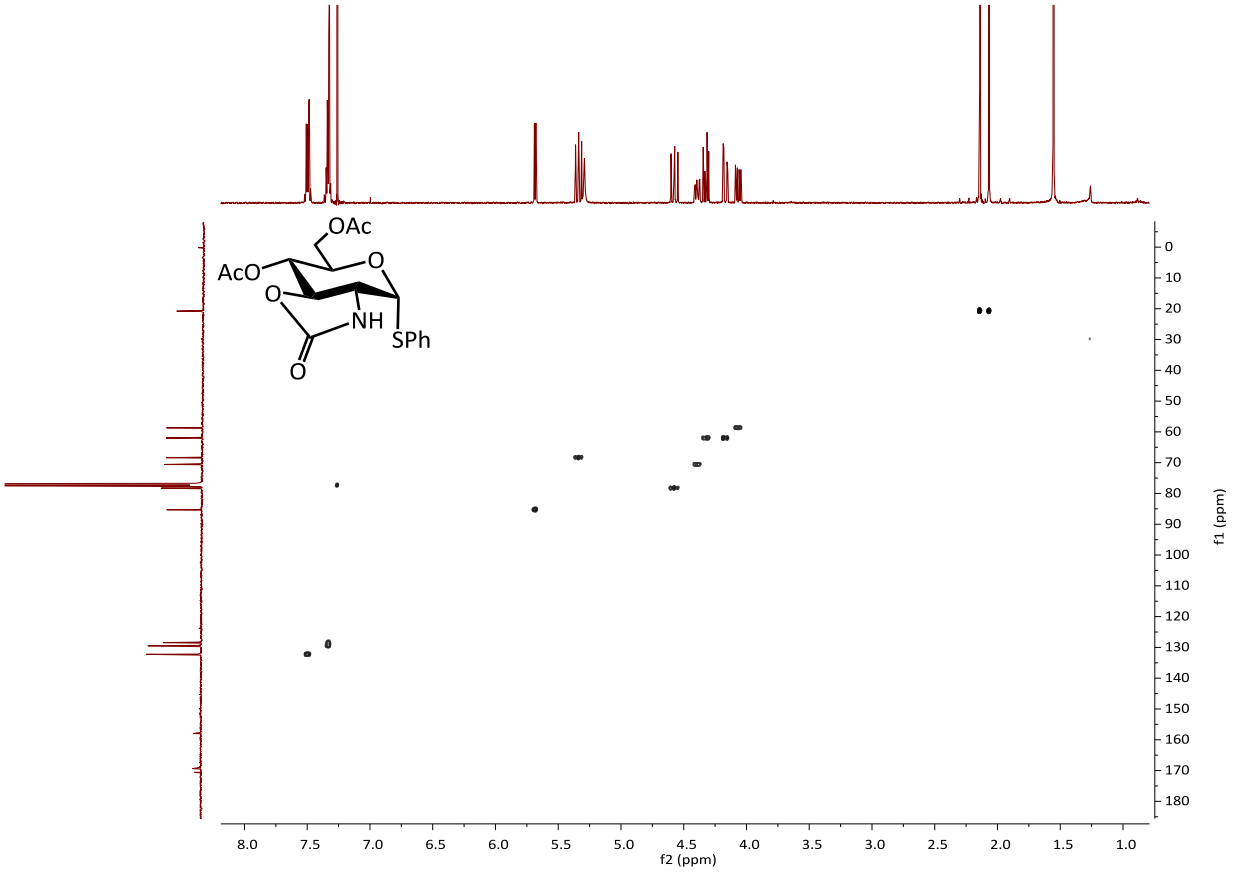

OAc
AcO
O
O
NAc
SPh
O
f1 (ppm)
f2 (ppm)

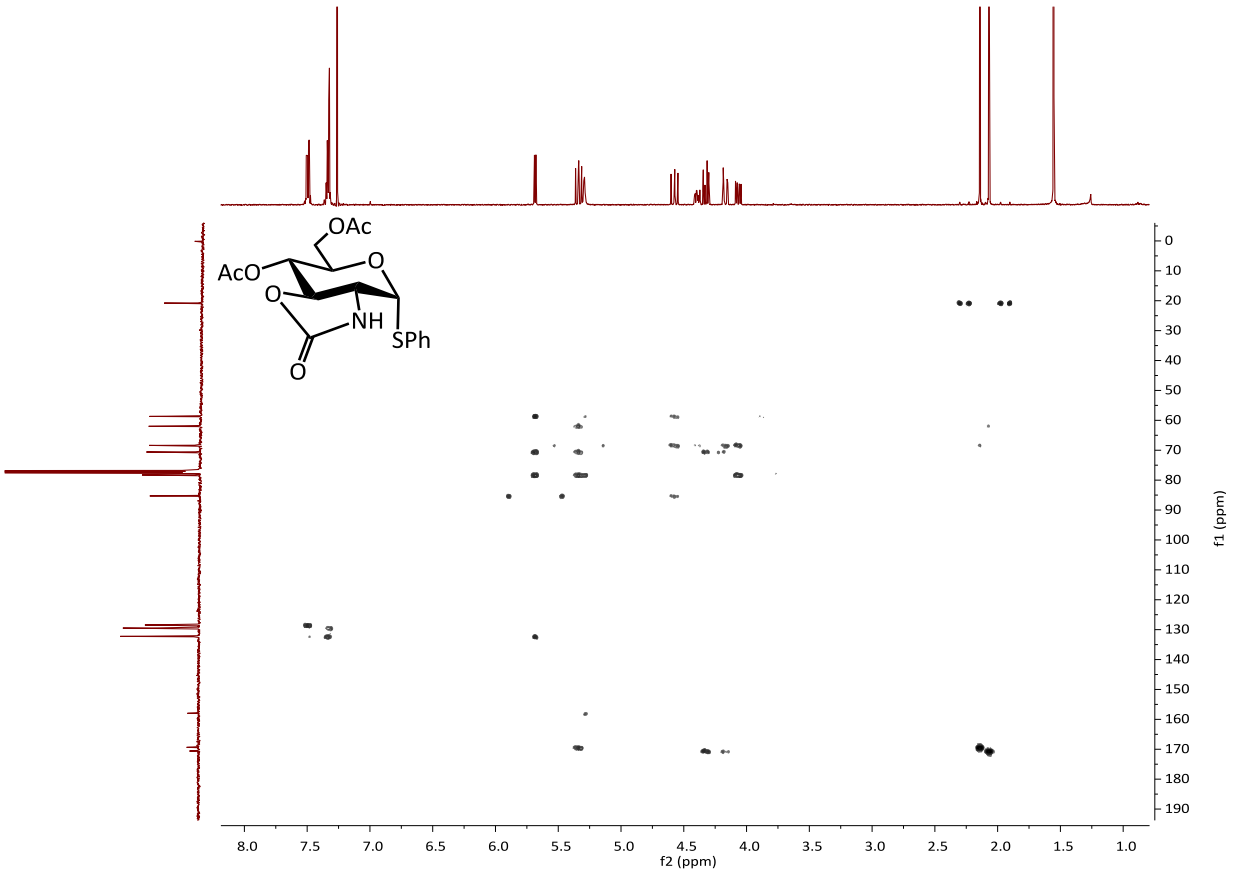
Phenyl 2-amino-4,6-di-*O*-acetyl-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.21).



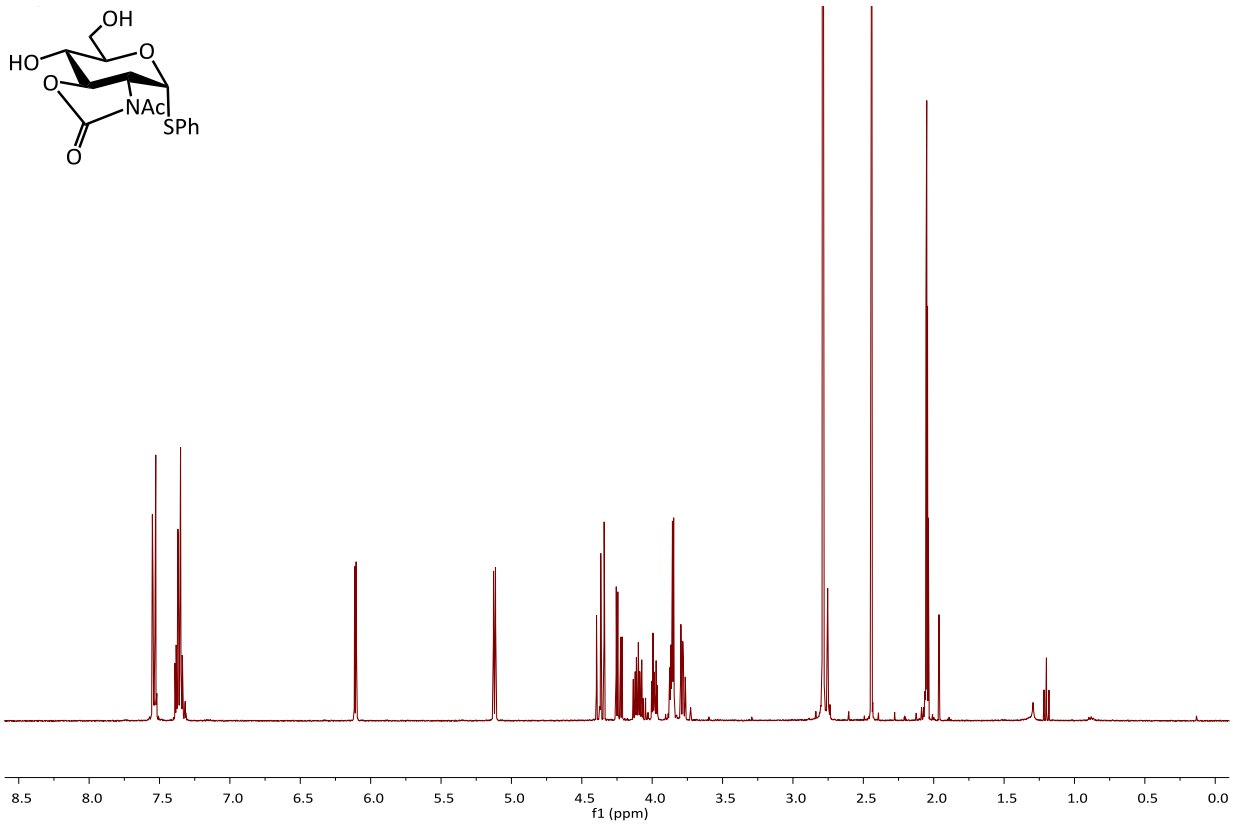


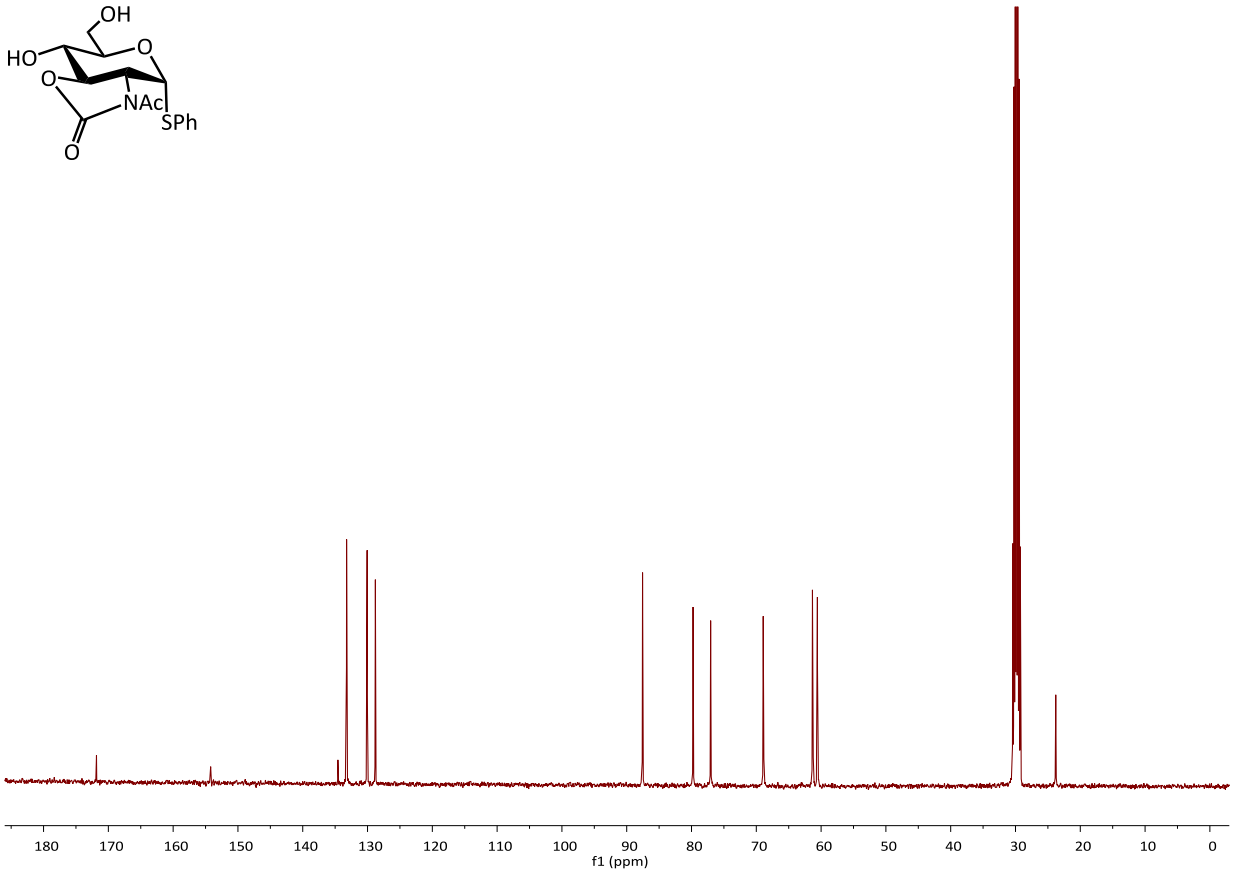

OAc
AcO
O
O
NH
SPh
O
f1 (ppm)
f2 (ppm)

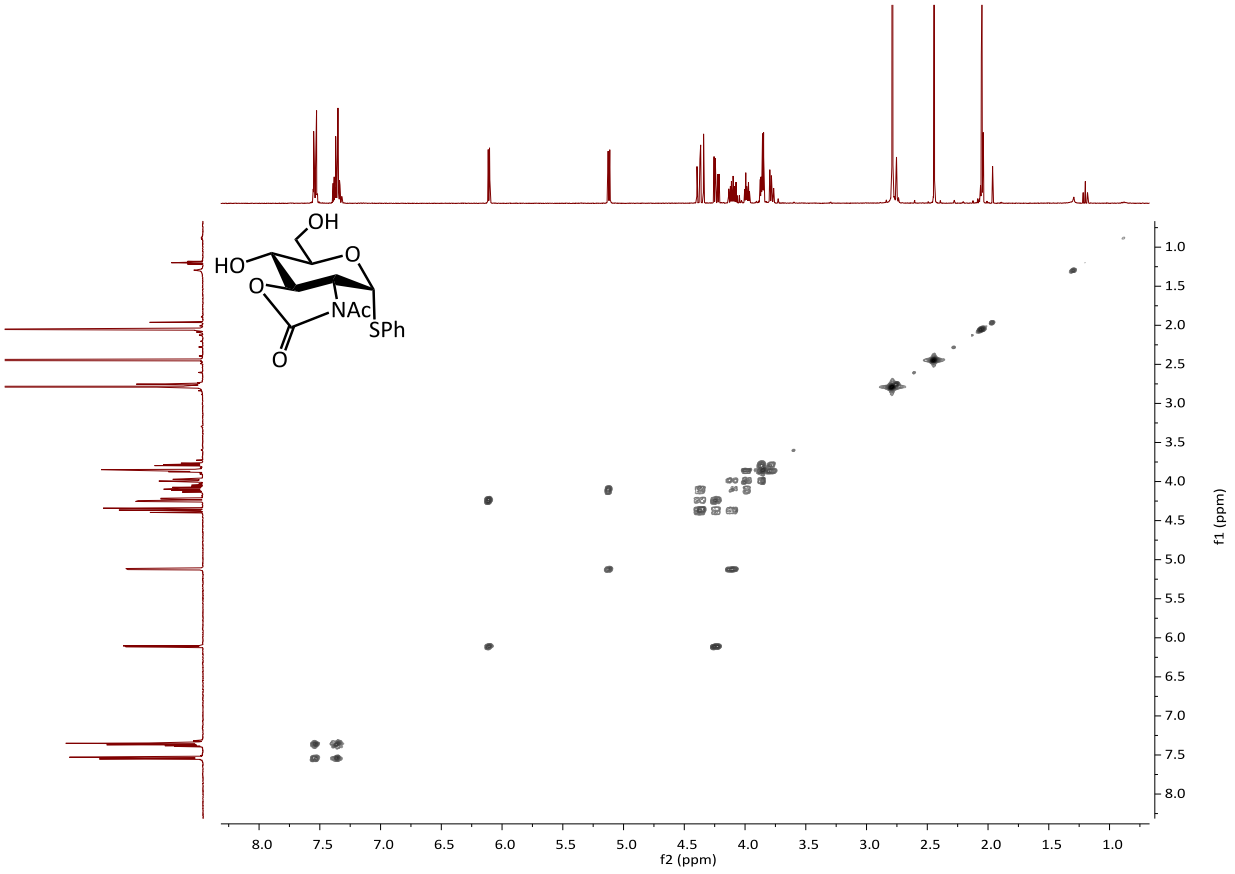

OAc
AcO
O
O
NH
SPh
O
f1 (ppm)
f2 (ppm)



Phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-1-thio-α-D-glucopyranoside (3.20).




OH
HO
O
O
NAc
SPh
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


OH
HO
O
O
NAc
SPh
O
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f2 (ppm)
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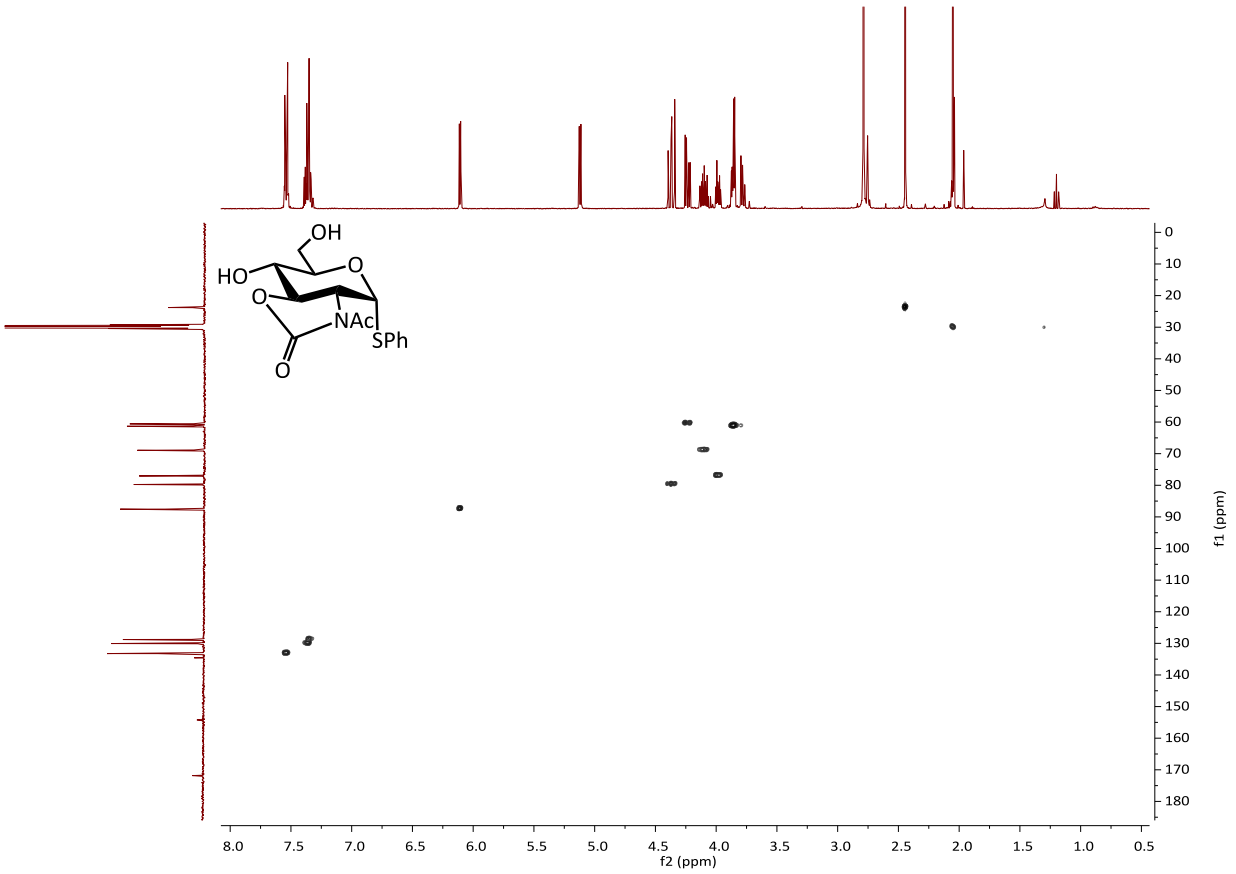
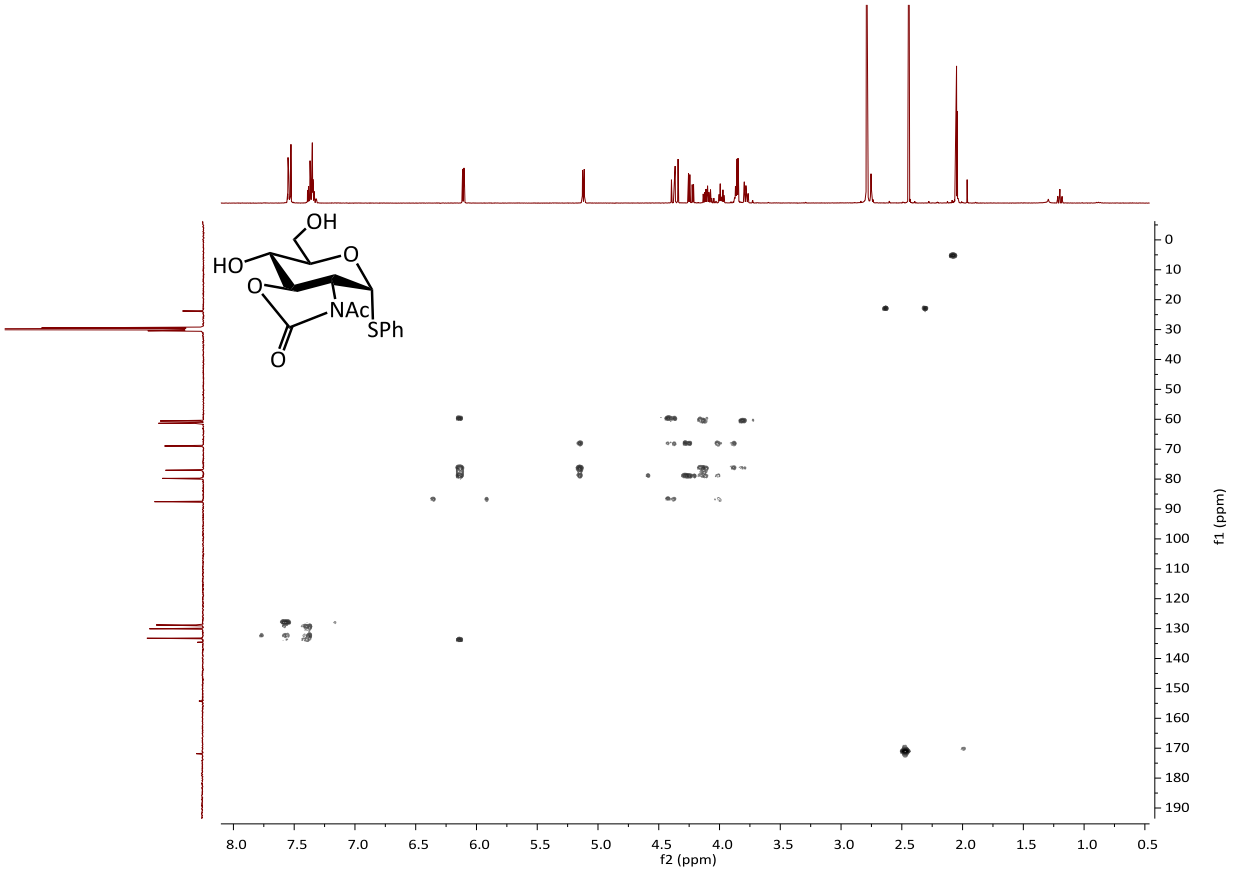
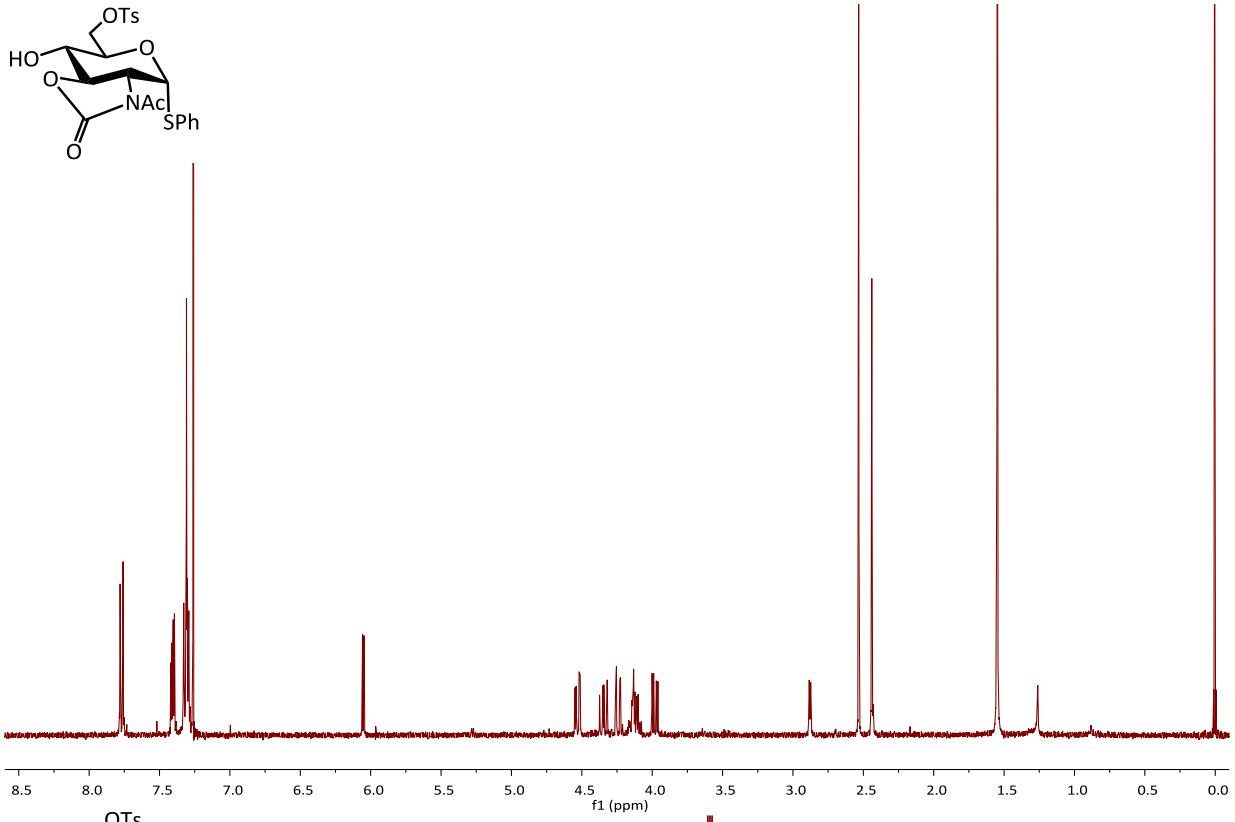
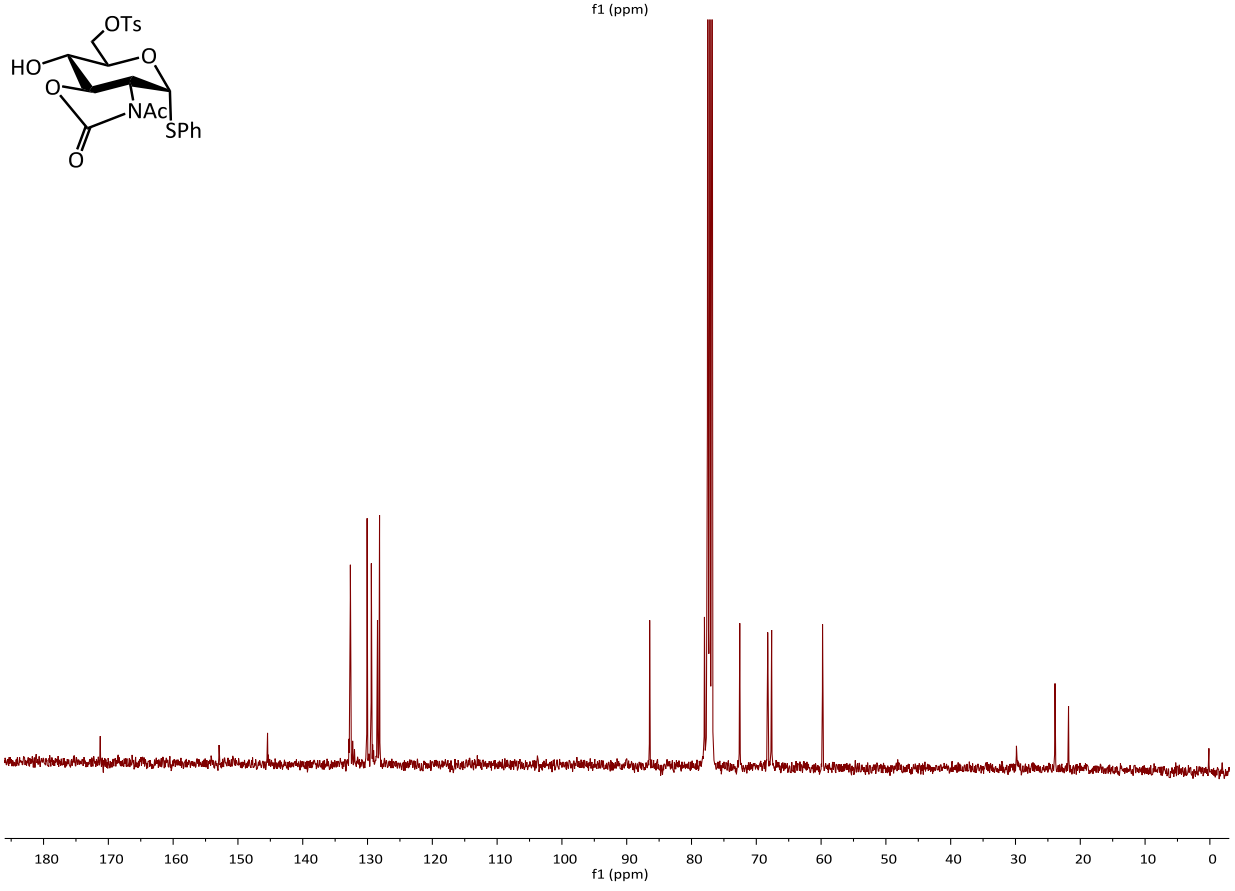
f1 (ppm)

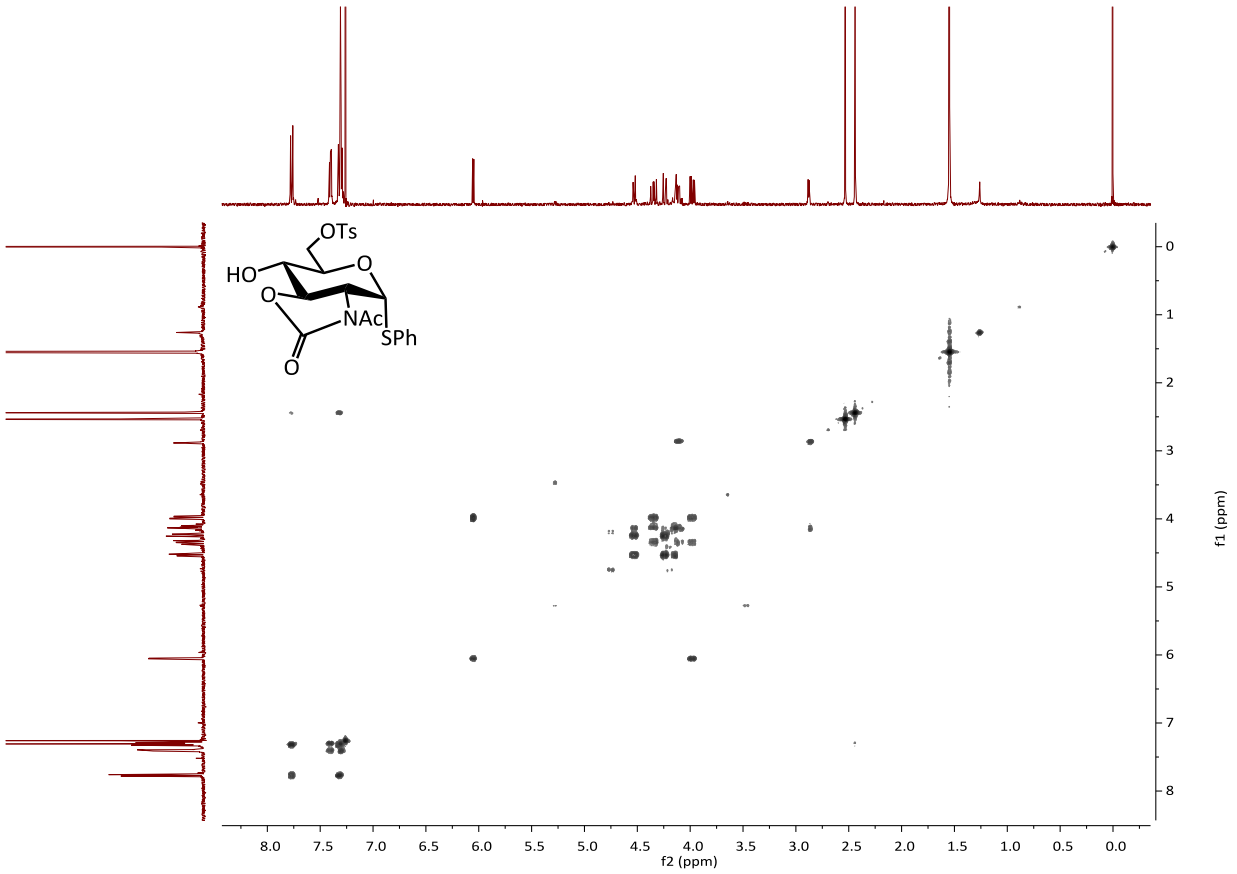

OH
HO
O
O
NAc
SPh
O
f1 (ppm)
f2 (ppm)

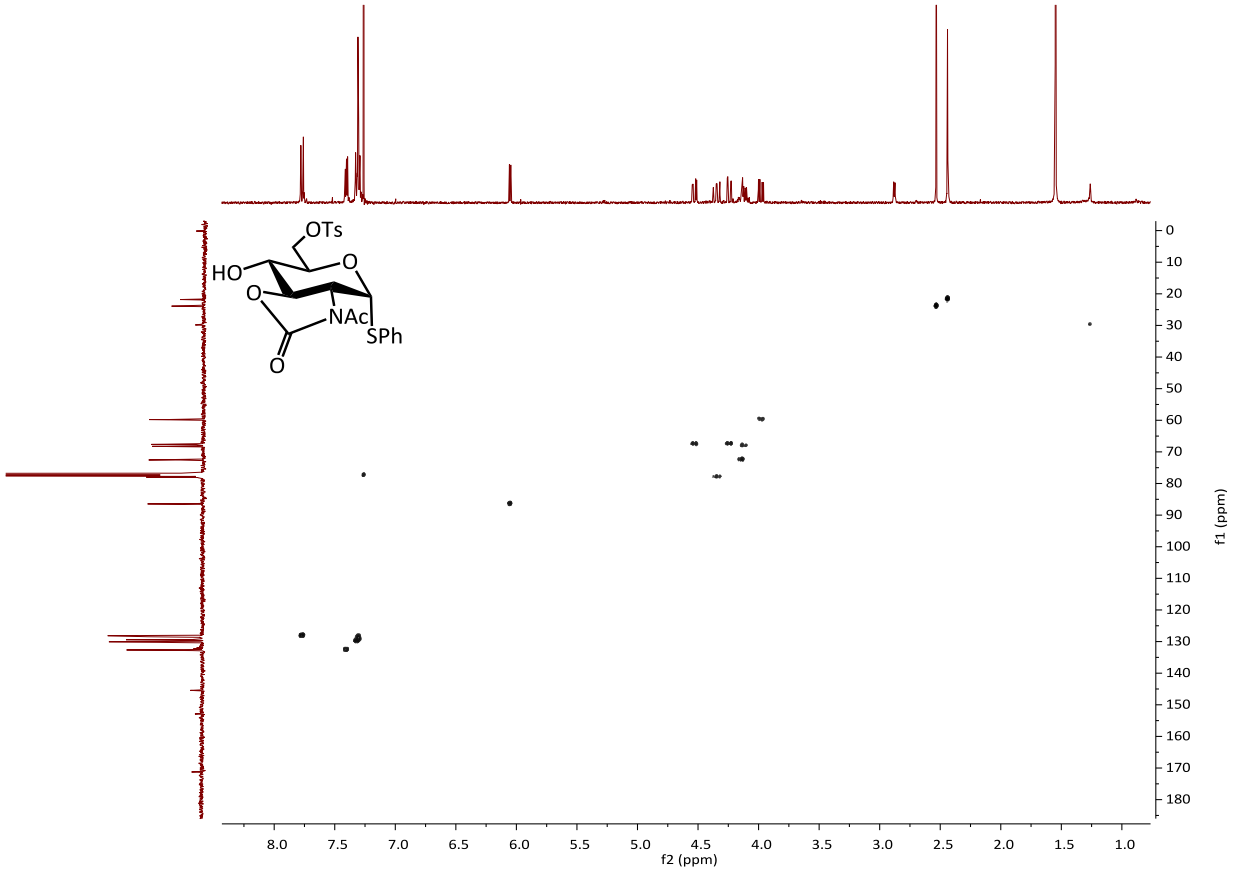

OH
HO
O
O
NAc
SPh
O
f1 (ppm)
f2 (ppm)

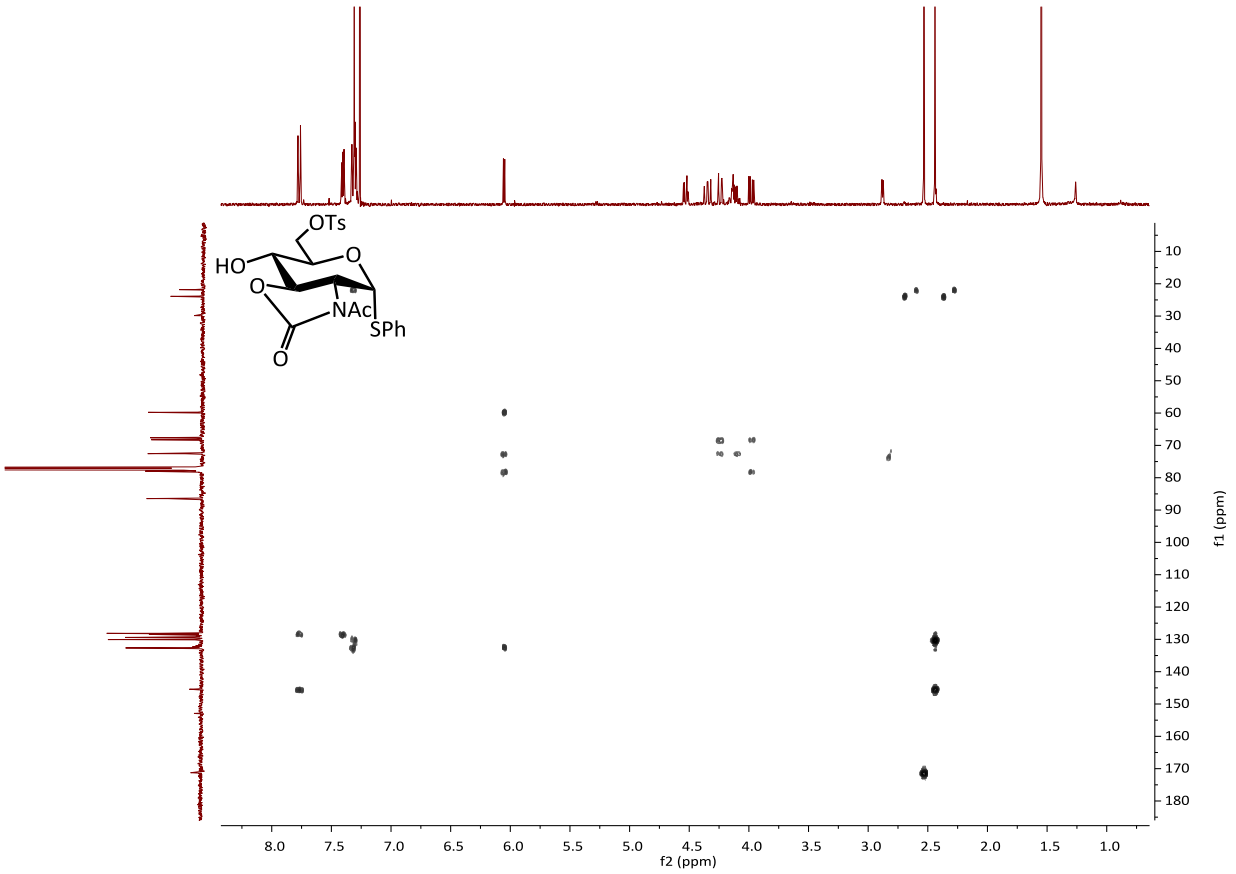
Phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-6-*O*-tosyl-1-thio-α-D-glucopyranoside (3.25).



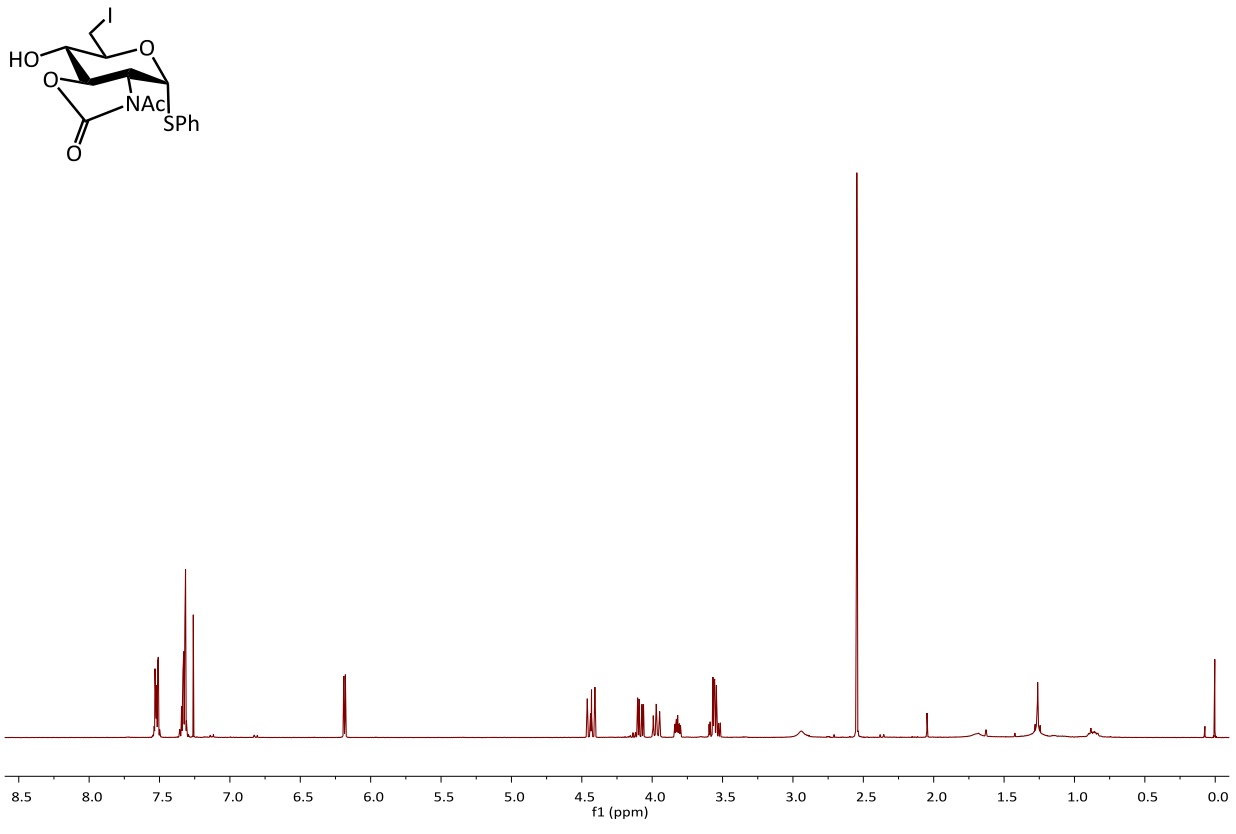


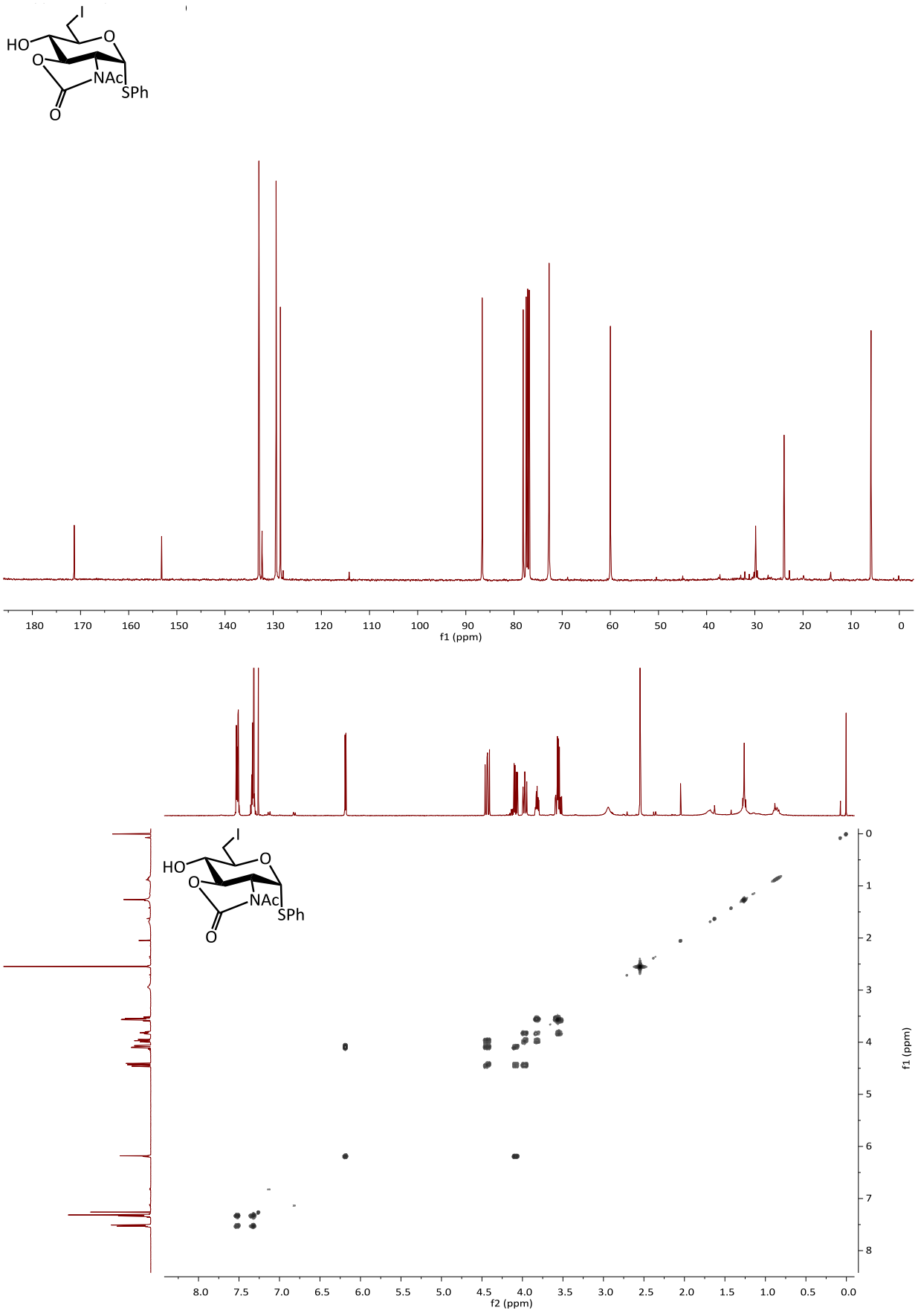

OTs
HO
O
O
NAc
SPh
O
f2 (ppm)
f1 (ppm)

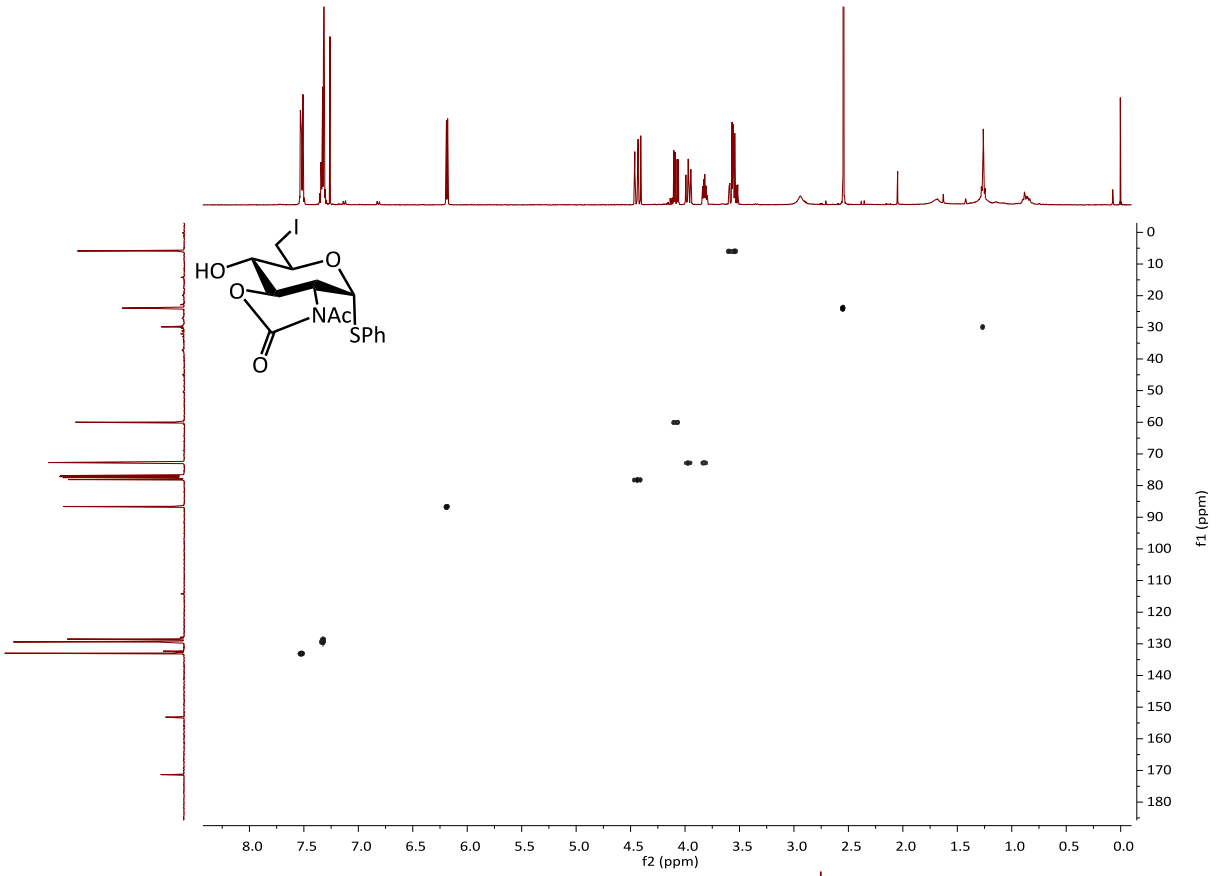

OTs
HO
O
O
NAc
SPh
O
f2 (ppm)
f1 (ppm)

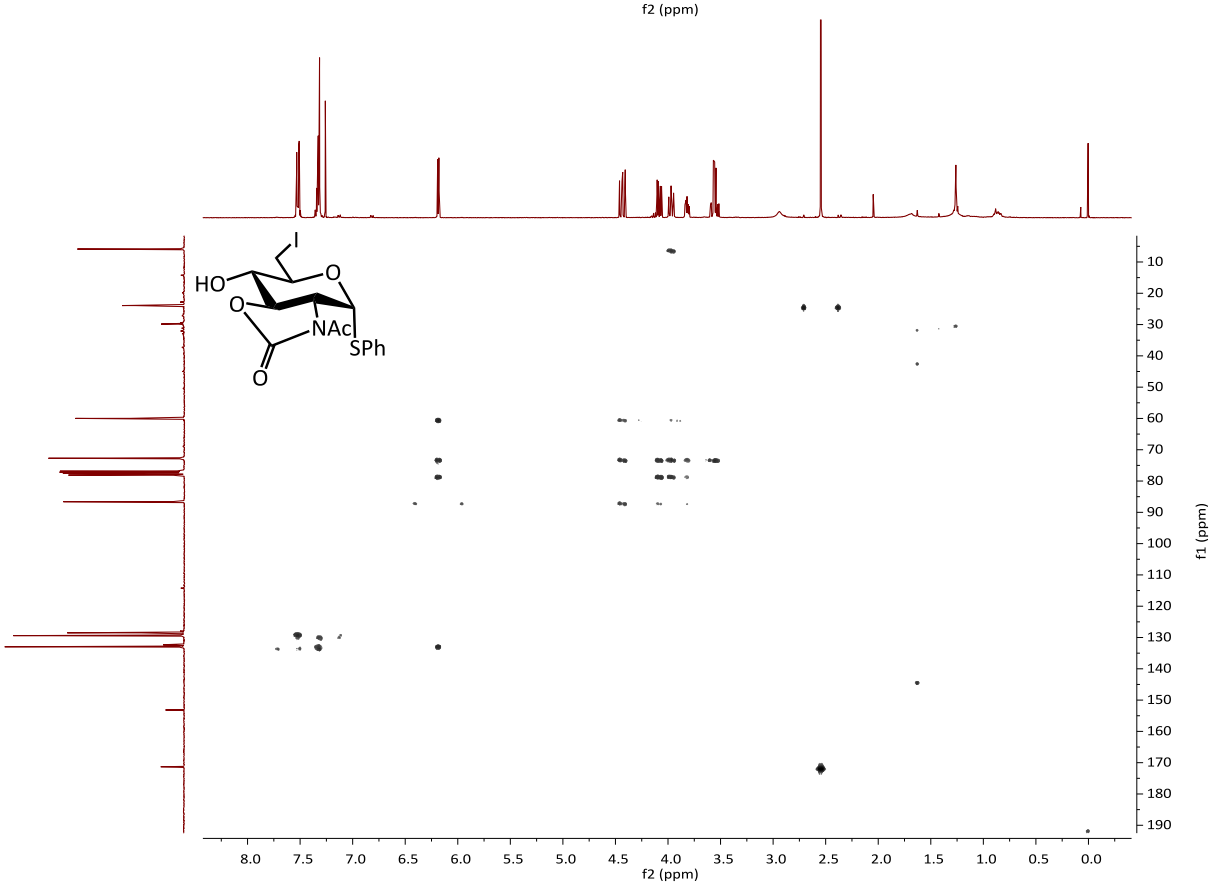


Phenyl 2-*N*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2,6-dideoxy-6-iodo-1-thio-α-D-glucopyranoside (3.26).

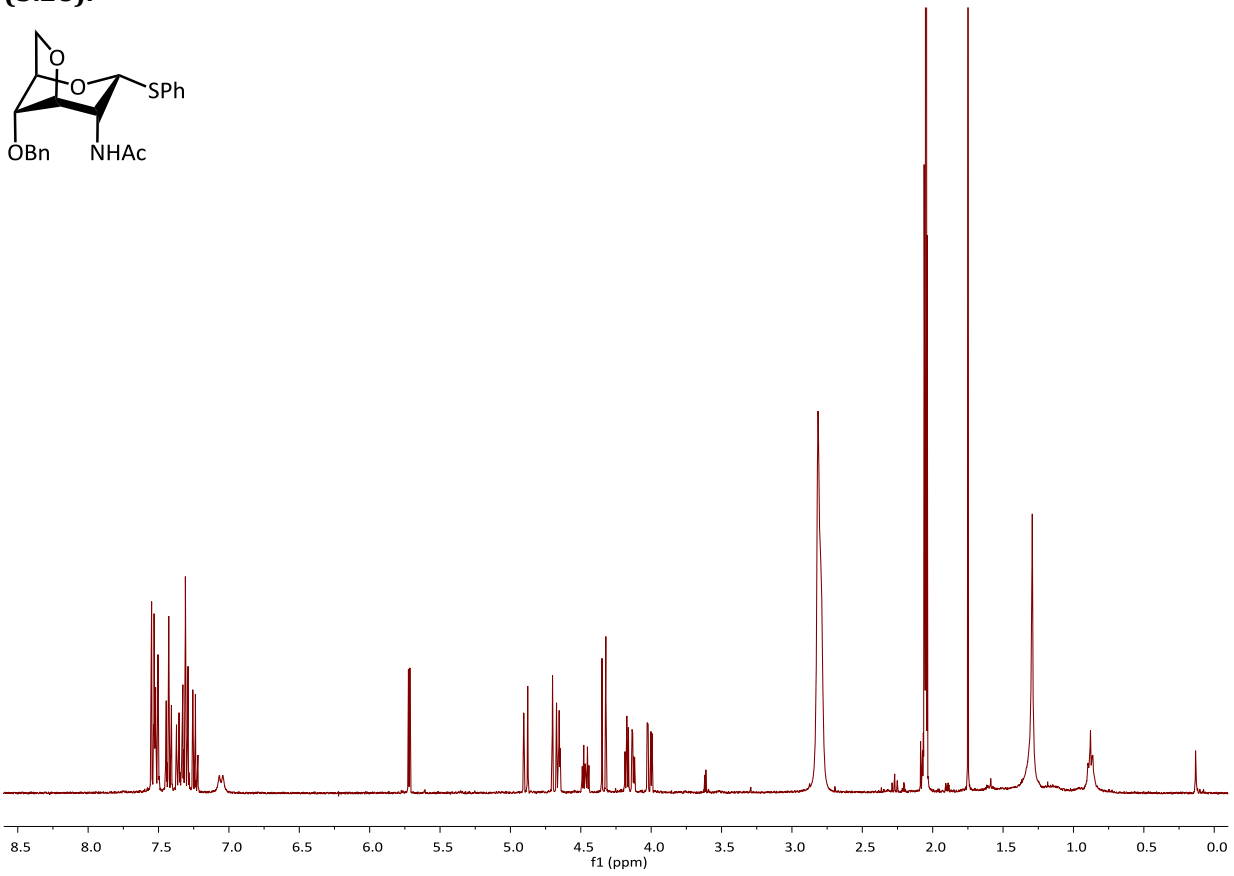


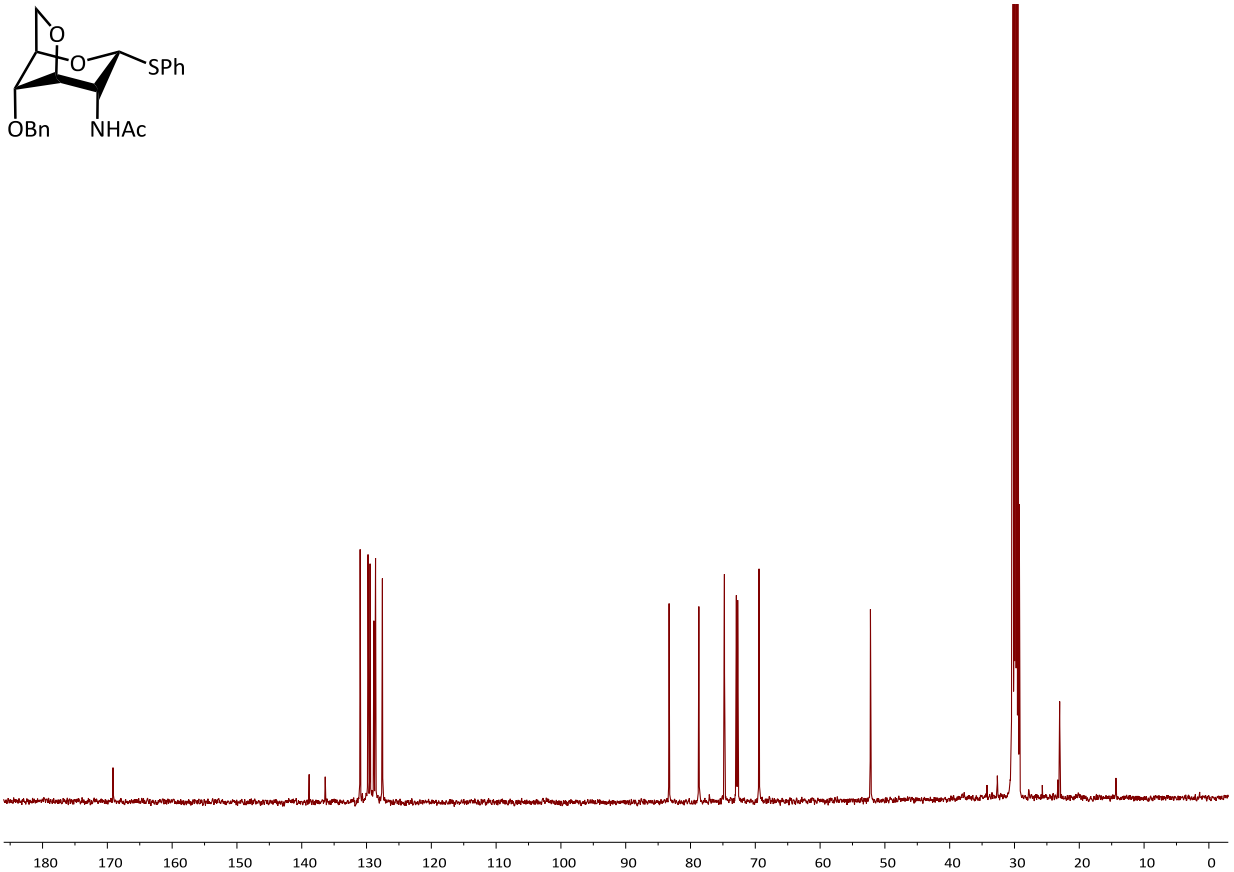

HO
O
O
NAc
SPh
I
f1 (ppm)
f2 (ppm)

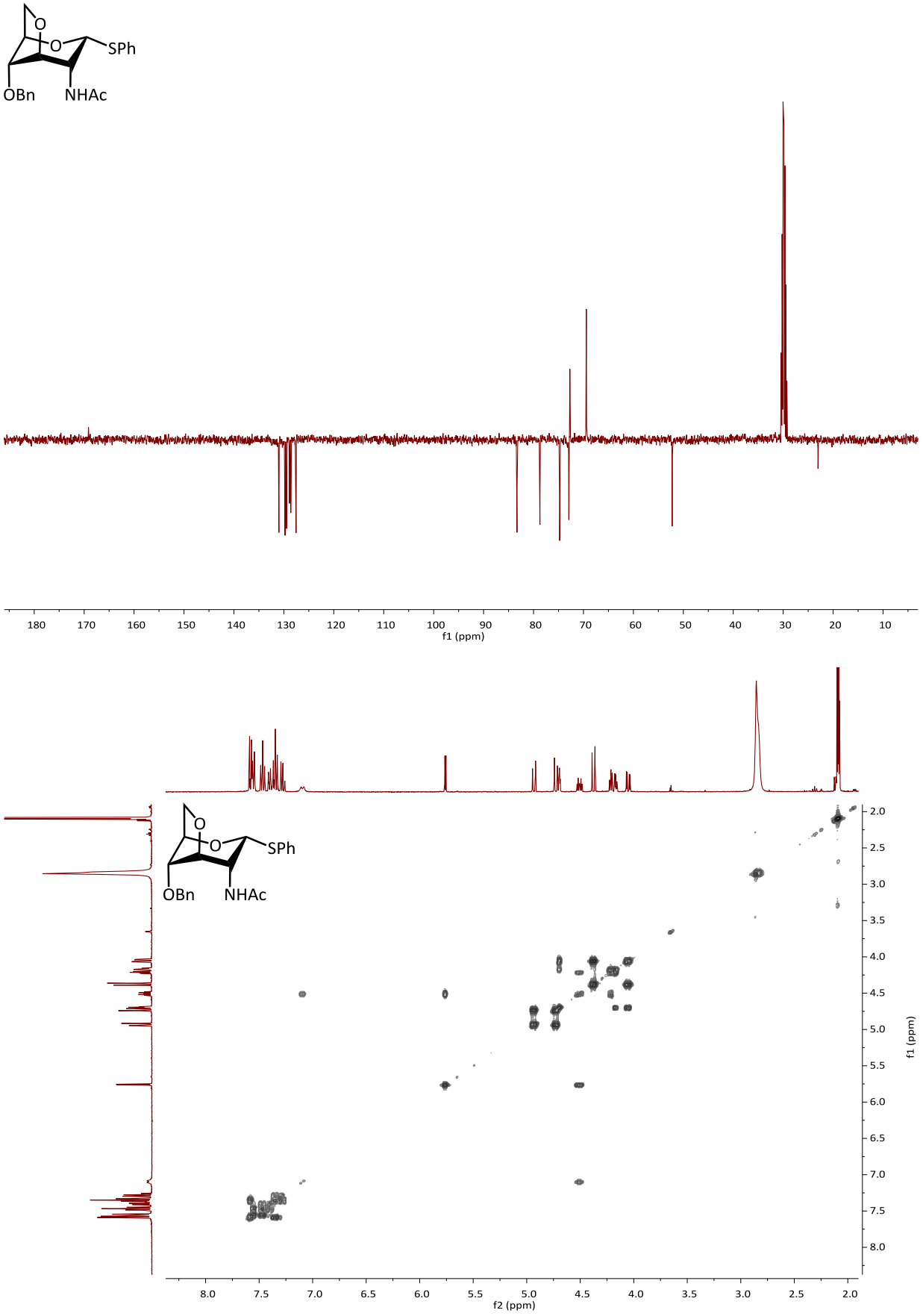

I
HO
O
O
NAc
SPh
O
f2 (ppm)
f1 (ppm)

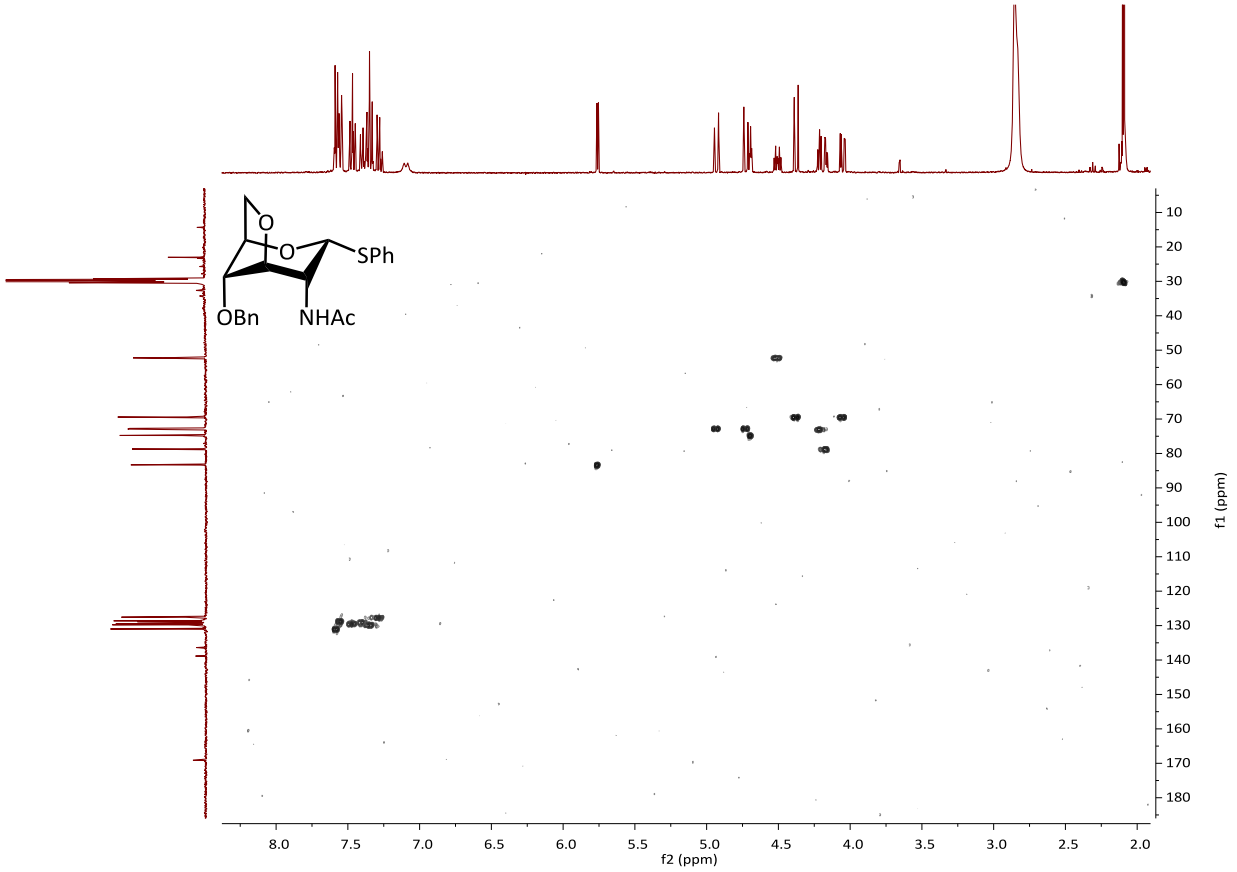

I
HO
O
O
NAc
SPh
O
f2 (ppm)
f1 (ppm)

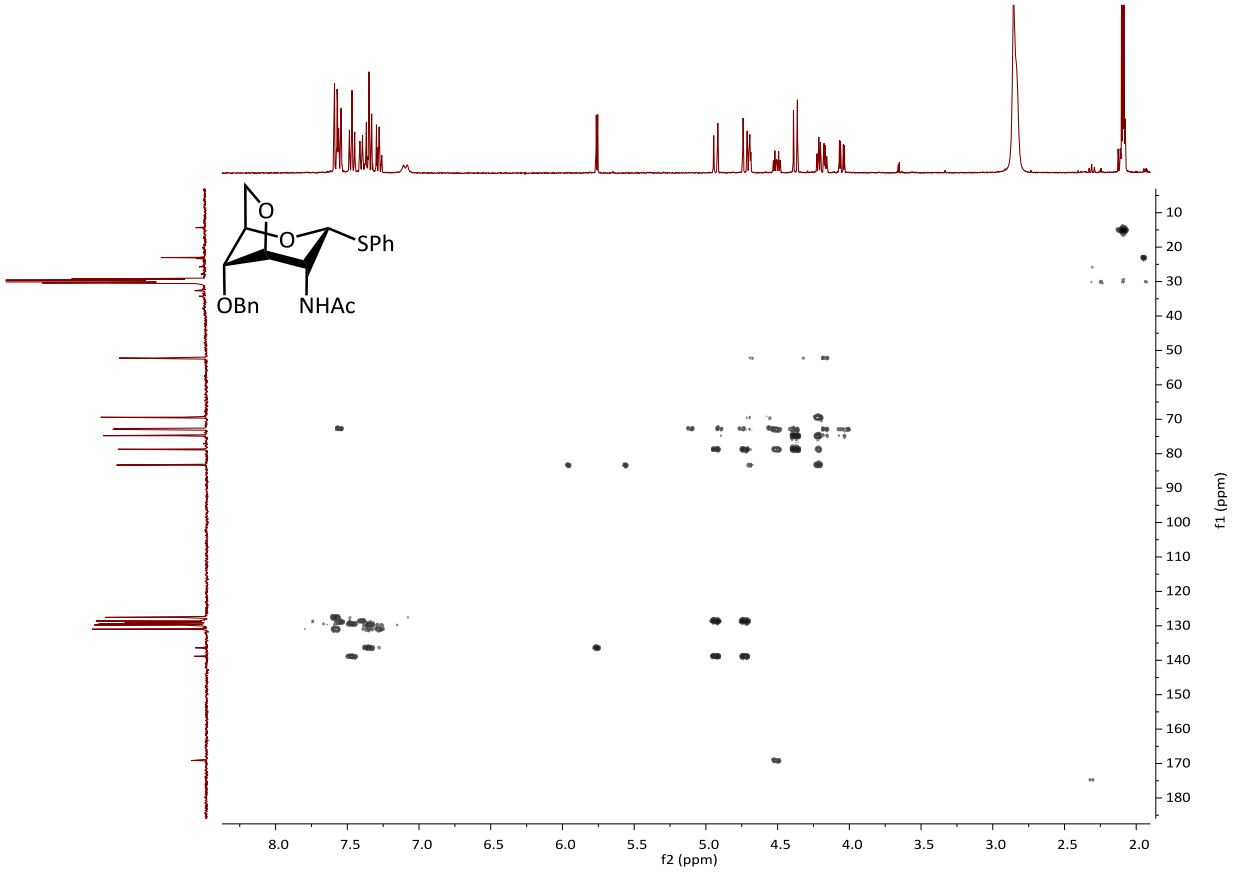
Phenyl 2-acetamido-3,6-anhydro-4-*O*-benzyl-2.6-dideoxy-1-thio-α-D-glucopyranoside (3.28).



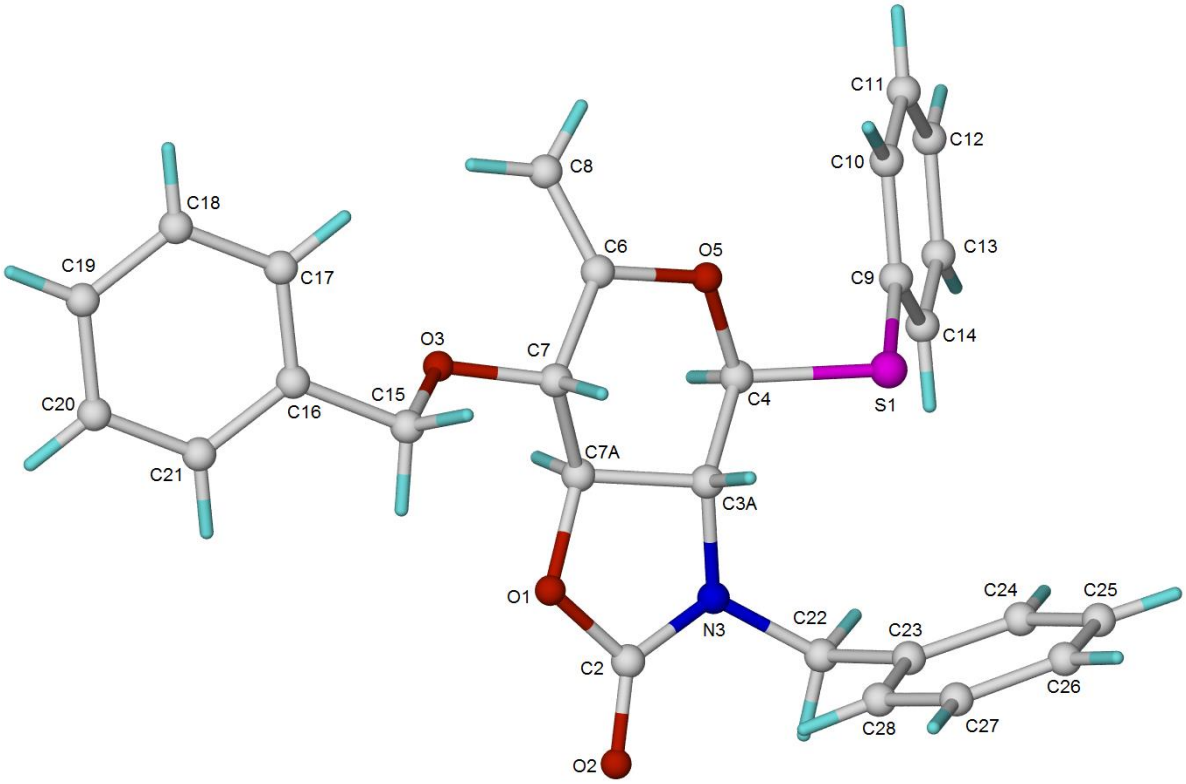



O
O
SPh
OBn
NHAc
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)
O
O
SPh
OBn
NHAc
2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
f1 (ppm)
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
f2 (ppm)


O
O
SPh
OBn
NHAc
f2 (ppm)
f1 (ppm)


O
O
SPh
OBn
NHAc
f2 (ppm)
f1 (ppm)

Appendix 3: Crystal structure information for phenyl 2-*N*-benzyl-4-*O*-benzyl-2-*N*,3-*O*-carbonyl-2,6-dideoxy-1-thio-β-D-xylo-hex-5-enopyranoside (3.14).



Single-crystal X-ray diffraction data were collected on a Bruker KAPPA APEX II DUO diffractometer using graphite-monochromated Mo-Kα radiation (χ = 0.71073 Å). Data collection was carried out at 173(2)K. Temperature was controlled by an Oxford Cryostream cooling system (Oxford Cryostat). Cell refinement and data reduction were performed using the program SAINT.[1] The data were scaled and absorption correction performed using SADABS[2].

The structure was solved by direct methods using SHELXS-97[2] and refined by full-matrix least-squares methods based on F^2 using SHELXL-97[2] and using the graphics interface program X-Seed[3]. The programs X-Seed[3] and POV-Ray[4] were both used to prepare molecular graphic images. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in idealised positions and refined in riding models with U_{iso} assigned the values to be 1.2 times those of their parent atoms and the constraint distances of C-H ranging from 0.95 Å to 1.00 Å. The structure was refined to R factor of 0.0347. The Flack x parameter is equal to 0.0123 with esd 0.0612.

Appendix 3, Table 1: Data collection and refinement parameters for **3.14**

Formula:	$C_{27}H_{25}NO_4S$
Formula Weight	459.55
Crystal System	Orthorhombic
Space Group	P212121 (No. 19)
Unit cell constants	
a (Å)	5.4082(8)
b (Å)	11.4356(15)
c (Å)	37.712(5)
α (°)	90
β (°)	90
γ (°)	90
Volume ((Å^3)	2332.3(6)
Z	4
Density$_{calc}$ (g.cm^{-3})	1.309
F (000)	968
Temperature of Data collection (K)	173
Crystal size (mm)	0.15 x 0.19 x 0.27
μ (mm^{-1})	0.173
Range scanned Θ (°)	1.9 - 28.2
Index ranges	h: -7: 7 k: -13: 15 l; -50: 42
Total no. reflections collected	20881
Unique reflections	5724
R (int)	0.036
No. of reflections with l > 2σ (l)	4885
No. of parameters	298
S	1.02
R_1 [l > 2σ (l)]	0.0374
wR_2	0.0851
Max. and Av. Shift/Error	0.00, 0.00
Min and Max. Resd. Dens. [e/Å^3]	-0.22, 0.24

References

1. SAINT 7.60a, Bruker AXS Inc., Madison, Wisconsin, 2006.
2. G. M. Sheldrick, SHELXS-97, SHELXL-97 and SADABS 2.05, University of Göttingen, Germany, 1997.
3. L. J. Barbour, *J. Supramol. Chem*., 2001, **1**, 189-191.
4. POV-Ray 3.6, Persistence of Vision Pty. Ltd., Williamstown, Victoria, 2004.