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Synthesis of biologically active carbohydrate derivatives including the synthesis of selectively 13C-labebelled Lewis b Hexasaccharides for protein binding studies and the incorporation of Chitosan into a Polyurethane foam

Long, Mark Bryan

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Synthesis of Biologically Active Carbohydrate Derivatives including the Synthesis of Selectively ^{13}C-labelled Lewis b Hexasaccharides for Protein Binding Studies and the Incorporation of Chitosan into a Polyurethane Foam

Mark Bryan Long

A thesis submitted for the degree of

Doctor of Philosophy

in the School of Chemistry

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To my friends, both here in North Wales and further afield, for your encouragement, support and, most importantly, your companionship.

And to my family. Without you I would be so little, you mean the world to me.

Abstract

Carbohydrates have a diverse range of structures and biological roles. The synthesis of a range of carbohydrate tools for studying biological processes are presented in this work.

A range of ^{13}C-labelled Lewis b hexasaccharides have been synthesised, for use in NMR binding studies with BabA, a surface protein from the bacterium *Helicobacter pylori*. During this synthetic sequence, an efficient synthesis of fucose from galactose was developed, for the purpose of providing the required ^{13}C-labelled fucosyl donor in sufficient yield from the ^{13}C galactose starting material. Glycosylation reactions were performed with this and other building blocks to produce the required ^{13}C-labelled hexasaccharides.

A highly neuroactive polybioside from the venom of the social wasp *Polybia paulista* has been reported. An analogue of the reported structure was synthesised with the aim of comparing its NMR spectra with that of the reported polybioside. Synthesis of the analogue was achieved through peptide coupling between a glucosyl amine and a derivative of urocanic acid, with NMR spectra from the target molecule showing similarities to the spectra of the reported polybioside.

Chitosan, the deacetylated form of the naturally occurring biopolymer chitin, has attracted interest from researchers due to its antimicrobial properties. The aim of the study was to investigate the possibility of incorporating chitosan into polyurethane foams, with the potential application for use in wound dressings. Chitosan was added to a prepolymer during a foaming reaction and the structure of the foam analysed with solid state NMR.

Abbreviations

Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
BabA	blood group antigen binding adhesin
Boc	*tert*-butyloxycarbonyl
Bn	benzyl
BnBr	benzyl bromide
Bz	benzoyl
CLIP-HSQC	Clean Inphase HSQC
COSY	Correlated Spectroscopy
DCM	dichloromethane
DD	degree of deacetylation
DEPT	Distortionless Enhancement by Polarization Transfer
DEPTQ	DEPT including quaternary carbons
DMF	*N*,*N*-dimethylformamide
EDA	ethylene diamine
Fuc	fucose
Gal	galactose
GalNAc	*N*-acetylgalactosamine
Glc	glucose
GlcNAc	*N*-acetylglucosamine
HSQC	Heteronuclear Single-Quantum Coherence
H. pylori	*Helicobacter pylori*
Lac	lactose
MS	Mass Spectrometry
$NaBH_3CN$	sodium cyanoborohydride
NIS	*N*-iodosuccinimide
NMR	Nuclear Magnetic Resonance
PU	polyurethane
R_f	retention factor
TBDMSCl	*tert*-butyldimethylsilyl chloride
THAP	2’,4’,6’-trihydroxyacetophenone

TLC	thin layer chromatography
TOCSY	Total Correlation Spectroscopy
TsCl	*p*-toluenesulfonyl chloride

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1 Introduction

1.1 Carbohydrate Chemistry

1.1.1 Carbohydrates and Their Roles

Carbohydrates are found throughout the natural world and, along with proteins, lipids and nucleic acids, are amongst the most important classes of organic compounds to life on Earth. Their role as structural compounds, such as the biopolymers cellulose and chitin in the cell walls of plants and fungi respectively, as well as energy stores and metabolic intermediates, make carbohydrates the most abundant class of organic compounds on our planet. With ribose and deoxyribose making up part of the structure of RNA and DNA, as well as the presence of glycolipids and glycoproteins, carbohydrates have a huge variety of structures and biological roles.[1,2]

1.1.2 Structure of Carbohydrates

The name was originally derived from the belief that they were, quite literally, the 'hydrate of carbon', owing to their empirical formula $C_n(H_2O)_n$. With a better understanding of their structure, carbohydrates, sugars and saccharides are now considered as polyhydroxylated organic compounds, most frequently in the form of aldose and ketoses (**Figure 1**). This broad classification includes substituted compounds, oligosaccharides and polysaccharides, sugars with multiple carbonyls, deoxy sugars and also the inclusion of additional functional groups.

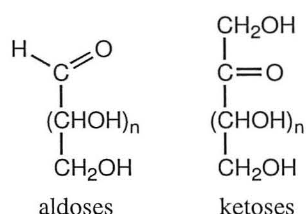


Figure 1 The two most common classes of carbohydrate; aldose and ketoses.

In the case of the more common aldoses, the triose glyceraldehyde is the simplest structure, with three carbons and just one stereogenic centre. By increasing the chain length, larger sugars with more stereogenic centres are formed.

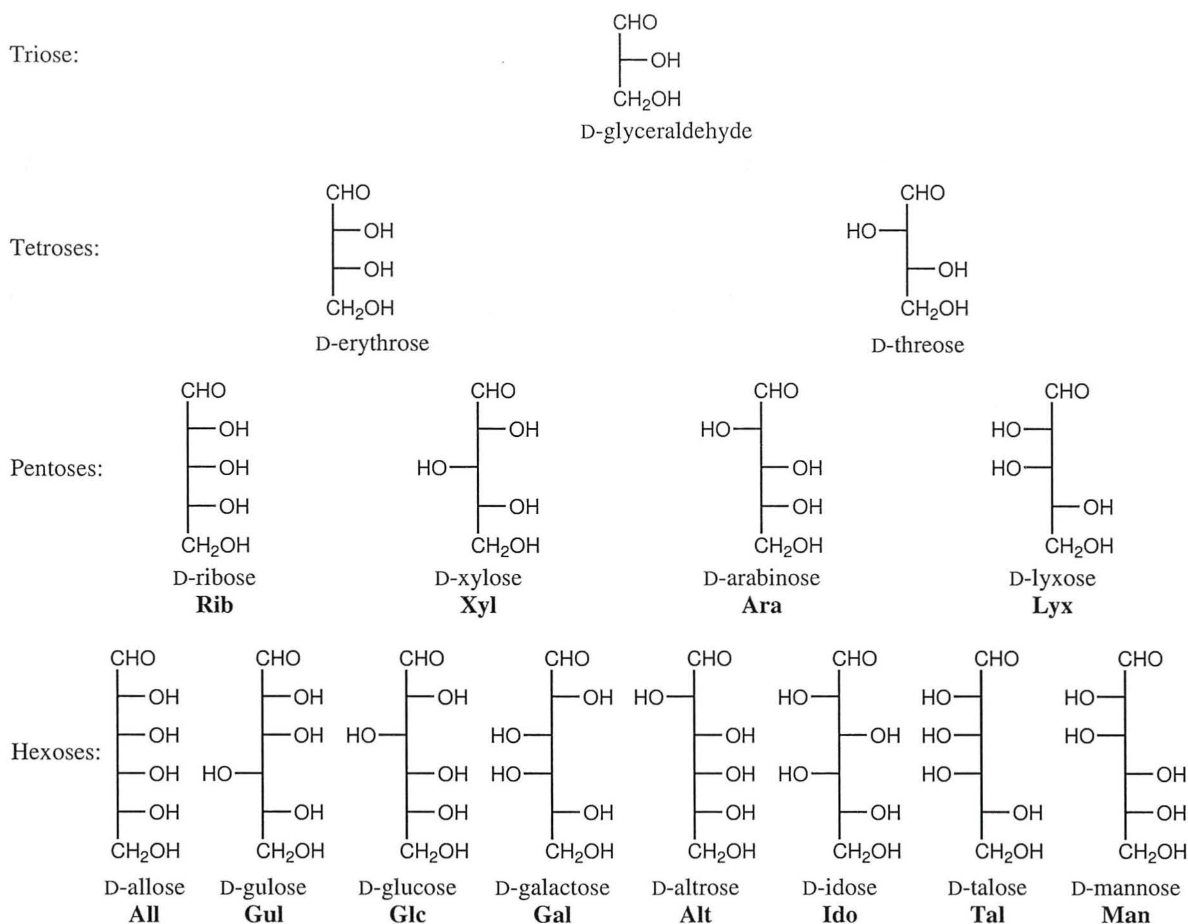


Figure 2 Fischer projections of the D-aldose sugars.

Emil Fischer described the chirality of many of these monosaccharides[3], with their stereochemistry being displayed in what has come to be known as 'Fischer Projections'. **Figure 2** shows the Fischer projections of the aldoses up to the aldohexoses, which have six carbons and four stereogenic centres in their open chain form. Of these, the configurational atom is defined as the highest order locant which is a stereogenic centre (**Figure 3**). The absolute configuration of this point is used to define the sugar as the D or L enantiomer, with the orientation of the remaining stereogenic centres relative to this giving the sugar its trivial name.

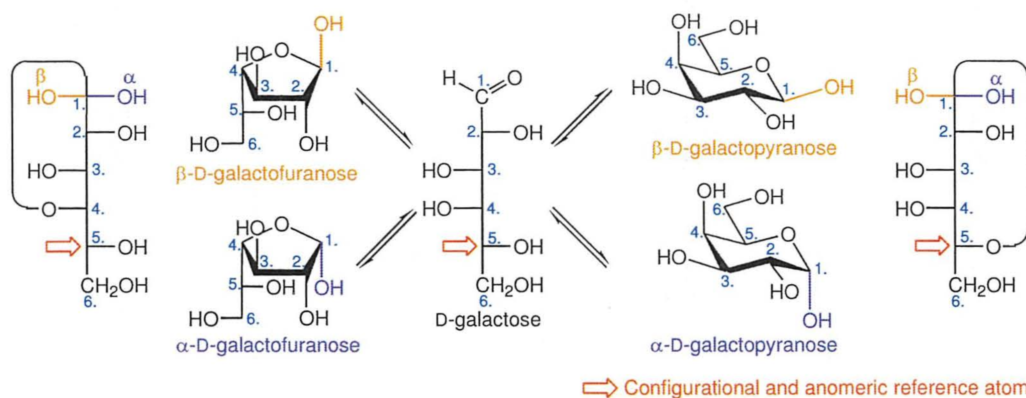


Figure 3 The equilibrium structures of galactose. In solution the structures shift between the furanose and pyranose forms, as well as their α and β-anomers, via the open chain sugar.

Open chain sugars cyclise to give hemiacetals, creating a new stereogenic centre and thus two epimers (or anomers) of the parent sugar. These are described as the α and β-anomers, defined as being *cis* and *trans* respectively to the anomeric reference atom in the Fischer projection of the cyclic sugar (**Figure 3**). The anomeric reference atom is the same as the configurational atom in hexoses and smaller monosaccharides, or in larger sugars the highest order stereogenic locant in the parent sugar in which the ring has formed.[4] In solution, mixtures of α/β-anomers are in equilibrium via the open chain form, in a process known as mutarotation.

Different size rings can also form and will be in equilibrium with each other while in solution. For the aldohexoses, pyranose rings are more favourable than furanose rings due to increased ring strain in the latter.

1.1.3 The Anomeric Effect

The anomeric effect originates from the observation that electron withdrawing substituents at the anomeric position in D-pyranosides are frequently found in an α-orientation, despite steric implications which would normally favour an equatorial position.[5]

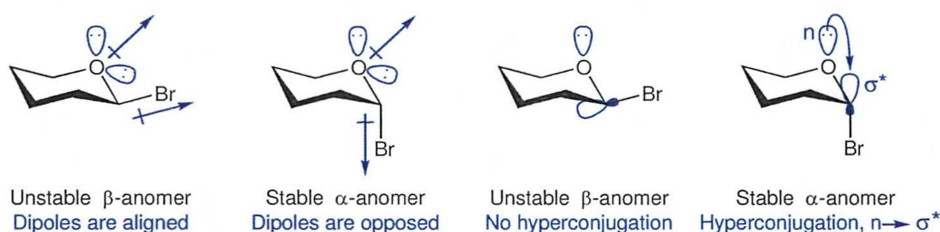


Figure 4 Two explanations for the anomeric effect. In the dipole model the α-anomer is more stable due to the dipoles opposing each other, compared to the β-anomer where the dipoles are aligned and will therefore repel each other. Hyperconjugation is also used to explain the α-anomer stability by n-σ* donation lowering the energy of this system.

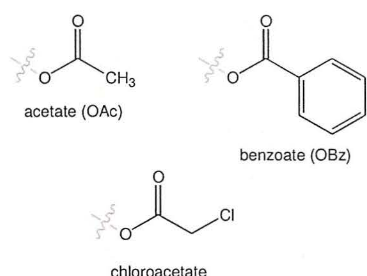
Explanations for this effect include stabilisation through the balancing of dipole interactions and the more recent interpretation that hyperconjugation occurs from the heteroatom lone pair to the anomeric antibonding orbital (σ*), which is able to stabilise the α-anomer but not the β (**Figure 4**).[6] Therefore, the anomeric effect can influence the outcome of reactions taking place at this position.

1.1.4 Protecting Groups

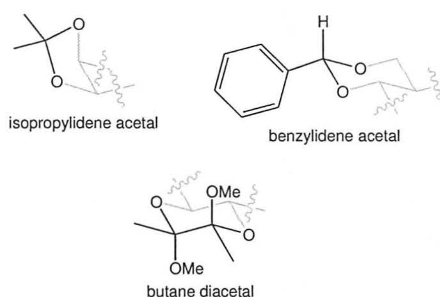
Protecting groups are employed when the reactivity of a functional group needs to be blocked so that transformations can be carried out elsewhere on the molecule.[7] Therefore, a protecting group should be able to be introduced in high yield, easy to characterise, stable to chromatography, be resilient to the conditions in subsequent reactions, and be easily removed and separated from the substrate when required.[8]

Protecting group strategies can be devised around groups which are orthogonal to each other, where one group can be removed while the other remains intact. This can include the use of temporary protecting groups, which may only be in place for a few reaction steps, and permanent protecting groups which remain on the molecule through many manipulations until a final deprotection to give the desired product (**Figure 5**). As carbohydrates contain multiple hydroxyls and other functional groups, the regioselectivity and chemoselectivity that protecting group strategies can provide are of great importance. Protecting groups also influence the reactivity of the sugars, due to their ability to withdraw or donate electrons and also by introducing steric strain to the molecule. Protecting group chemistry therefore plays a central role in carbohydrate and oligosaccharide synthesis.[6]

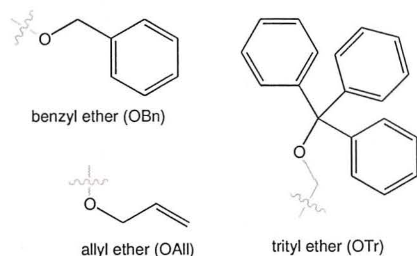
Protection of hydroxyls as esters

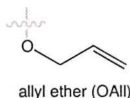


Protection of diols as acetals



Protection of hydroxyls as ethers





Protection of hydroxyls as silyl ethers

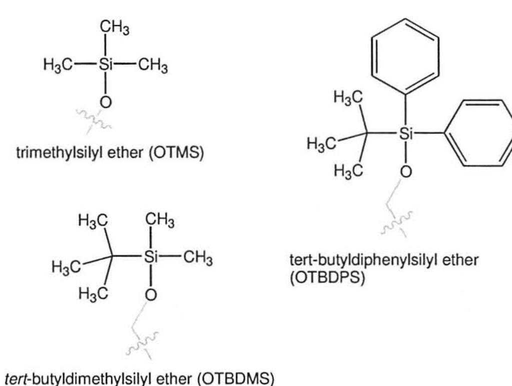


Figure 5 Common hydroxyl protecting groups used in carbohydrate synthesis. These include permanent protecting groups such as benzyl ethers, and temporary protecting groups such as silyl ethers.

1.1.5 Oligosaccharides and Glycosylation

Glycosidic bonds exist between sugar units and non-sugar aglycones in substances such as glycoproteins and glycolipids, as well as with other sugar units in oligosaccharides and polysaccharides (**Figure 6**).

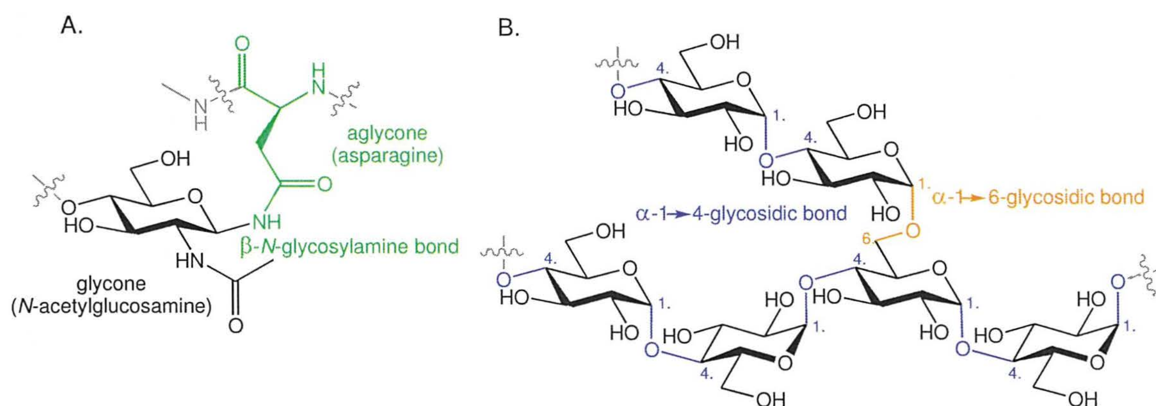


Figure 6 Examples of naturally occurring glycosylamines and oligosaccharides. A) A portion of a glycoprotein with part of the aglycone (asparagine) shown covalently bound to the glycone (*N*-acetylglucosamine) through an *N*-glycosylamine bond. B) The structure of the energy storage polysaccharide glycogen. Glucose units are linked by α-1→4 glycosidic bonds to form a chain, with branching occurring through α-1→6 glycosidic bonds approximately ten units apart. Adapted from Berg, Tymoczko and Stryer.[2]

Glycosylation reactions take place when a suitable acceptor undergoes a nucleophilic attack on a glycosyl donor to give the corresponding glycoside. These reactions therefore play a pivotal role in oligosaccharide synthesis. Glycosylations producing glycosylamines, carbon-glycosyl compounds, thioglycosides and selenoglycosides can also be achieved, with the latter two also providing a source of glycosyl donors.[9,10]

A key factor in glycosylation reactions is the stereochemistry of the newly formed bond at the anomeric position. This is of importance for optimising the yield of the glycosylation as well as avoiding the production of unwanted stereoisomers which may be difficult to separate. Many factors, such as leaving groups, protecting groups, steric hindrance, conformation, temperature, solvent and choice of promoter can affect the result of the glycosylation.[11]

1.1.6 1,2-*trans* Glycosylation

Glycosylation to give the 1,2-trans product (β-glucosides, β-galactosides, α-mannosides etc.) can be accomplished with good stereoselectivity through participation with a neighbouring group.

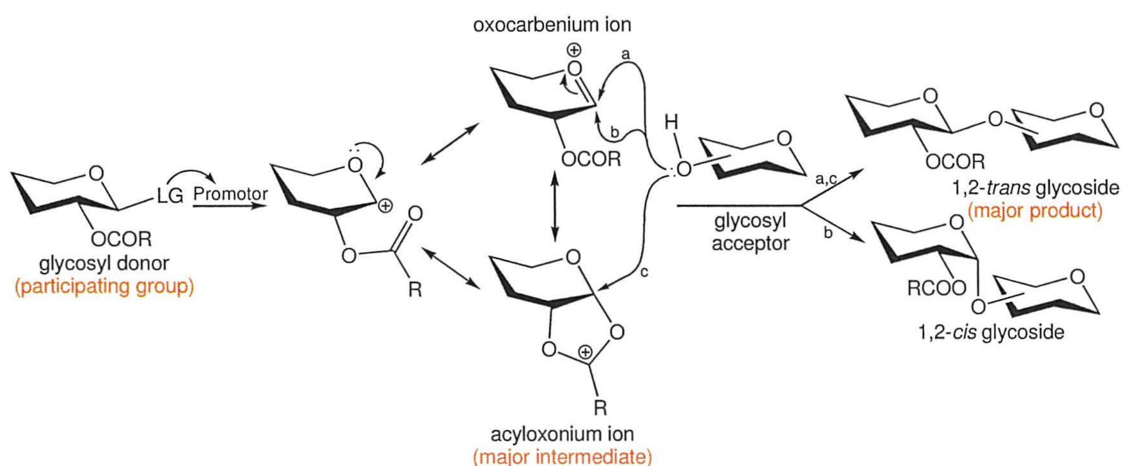


Figure 7 Glycosylation with neighbouring group participation, favouring the 1,2-*trans* product. Adapted from A. Demchenko.[11]

Acyl groups such as *O*-acetyl, *O*-benzoyl and *N*-phthalimido at the *C*-2 stabilize the glycosyl cation after the departure of the leaving group, giving an acyloxonium intermediate (**Figure 7**). The acceptor can therefore only attack from the face *trans* to the acyloxonium, giving the 1,2-*trans* glycoside as the major product.

1.1.7 1,2-*cis* Glycosylation

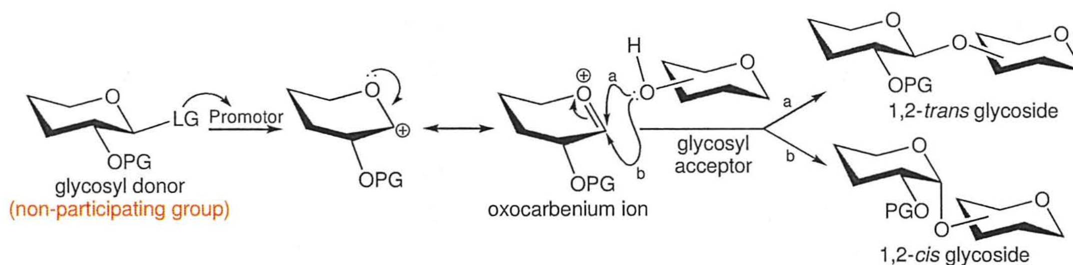


Figure 8 Glycosylation without neighbouring group participation often gives a mixture of 1,2-*trans* and 1,2-*cis* glycosides. Adapted from A. Demchenko.[11]

Glycosylations in the absence of a participating group at the *C*-2 often give an anomeric mixture of products (**Figure 8**). Though the α-anomers are more thermodynamically favourable, standard conditions for glycosylations are irreversible and so 'kinetic anomeric effects' appear unlikely to influence stereoselectivity.[12]

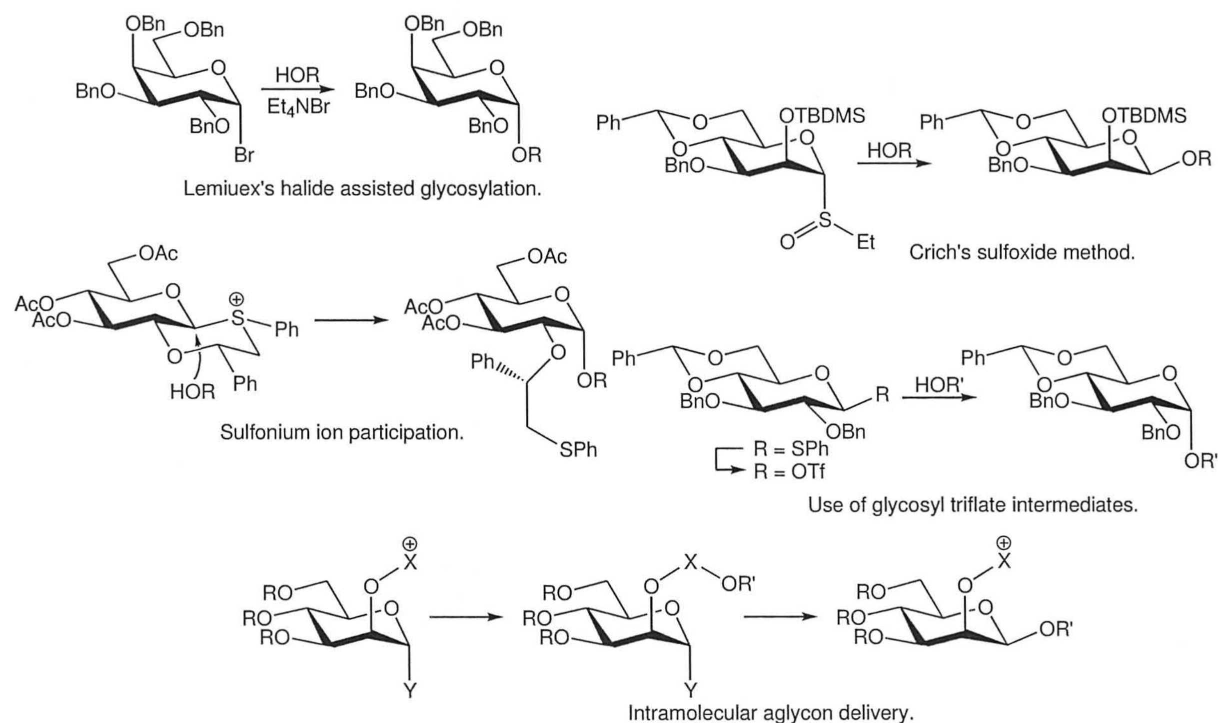


Figure 9 Different approaches to creating 1,2-*cis* glycosidic linkages.

To improve stereoselectivity towards 1,2-cis glycosides, several strategies have been devised (**Figure 9**). Formation of α-linkages has been shown to be achievable using bromide donors by Lemieux[13], while Crich's approaches of using sulfoxides[14] and glycosyl triflates[15] favour β, 1,2-*cis* glycoside formation for mannosides and α-linkages for glucosides. Intramolecular aglycon delivery[16] also provides a route to β-mannosides, while sulfonium ion intermediates from neighbouring groups have also been shown to

be α-directing for glucosides and galactosides,[17] though the exact mechanism is not yet fully understood.[18]

1.1.8 Glycosyl Donors

The choice of glycosyl donor has implications on the stereochemistry of the new bond (as shown in the previous section) and needs to be chosen while considering its stability during other manipulations of the donor and, potentially, with its orthogonality with other donors present. Though a plethora of different donors have been reported, many glycosylations can be achieved by the use of three main groups; glycosyl halides, trichloroacetimidates, and thioglycosides.

Glycosyl halide donors have been significant throughout the course of oligosaccharide synthesis, with early examples such as Michael's synthesis of phenyl glucosides,[19] later work by Zemplén and Gerecs,[20,21] and what has become known as the Koenigs-Knorr glycosylation.[22] Here, a glycosyl bromide or chloride is activated using silver salts (such as Ag_2O, Ag_2CO_3, AgOTf and $AgClO_4$) or mercury (II) compounds ($HgBr_2$, $Hg(CN)_2$) and coupled with an acceptor (**Figure 10**)

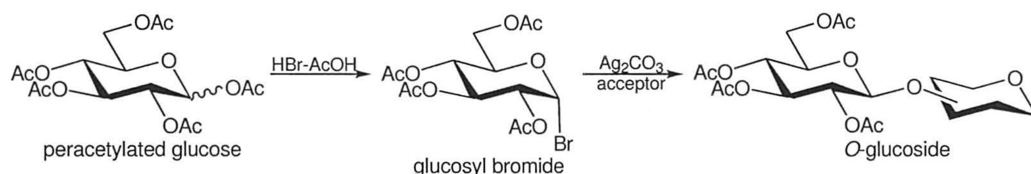


Figure 10 Glycosyl bromides can be prepared from peracetylated aldohexoses, which can then go on to form β-glycosides when reacted with an acceptor and a halide scavenger.

Glycosyl trichloroacetimidates were first described by Schmidt and Michel,[23] building on previous work on glycosyl imidates by Sinaÿ *et al.*[24,25] These are produced with good stereoselectivity between the α and β-anomers by changing the base used.[26] Strong bases such as sodium hydride (NaH) favour the formation of the thermodynamic α-product, with potassium carbonate (K_2CO_3) favouring the kinetic β-anomer (**Figure 11**).[26]

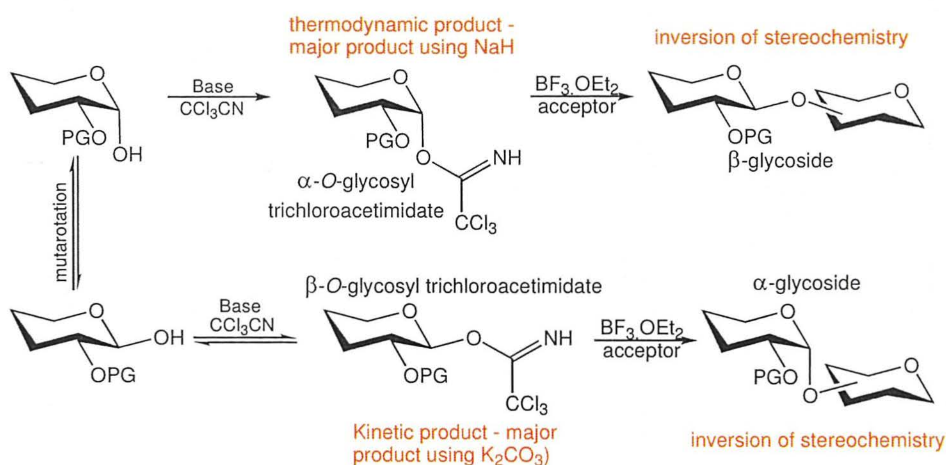


Figure 11 Synthesis of glycosyl trichloroacetimidates donors and their coupling with a glycosyl acceptor. Adapted from Schmidt and Michel[23,26]

For glycosylations with a suitable acceptor, glycosyl trichloroacetimidates can be activated with a Lewis acid such as $BF_3.OEt_2$. In the absence of participating groups, an inversion of stereochemistry at the anomeric position is seen, favouring α-glycosides from β-*O*-glycosyl trichloroacetimidates and vice versa.[26]

Thioglycosides have become a popular choice of glycosyl donor due to their stability and versatility.[9] They are stable in a large range of reaction conditions, providing the option of carrying out manipulations at other positions on the thioglycoside, including glycosylations with other glycosyl donors.[27] They are also able to be converted into a range of other functionalities, including halides and trichloroacetimidates (**Figure 12**).

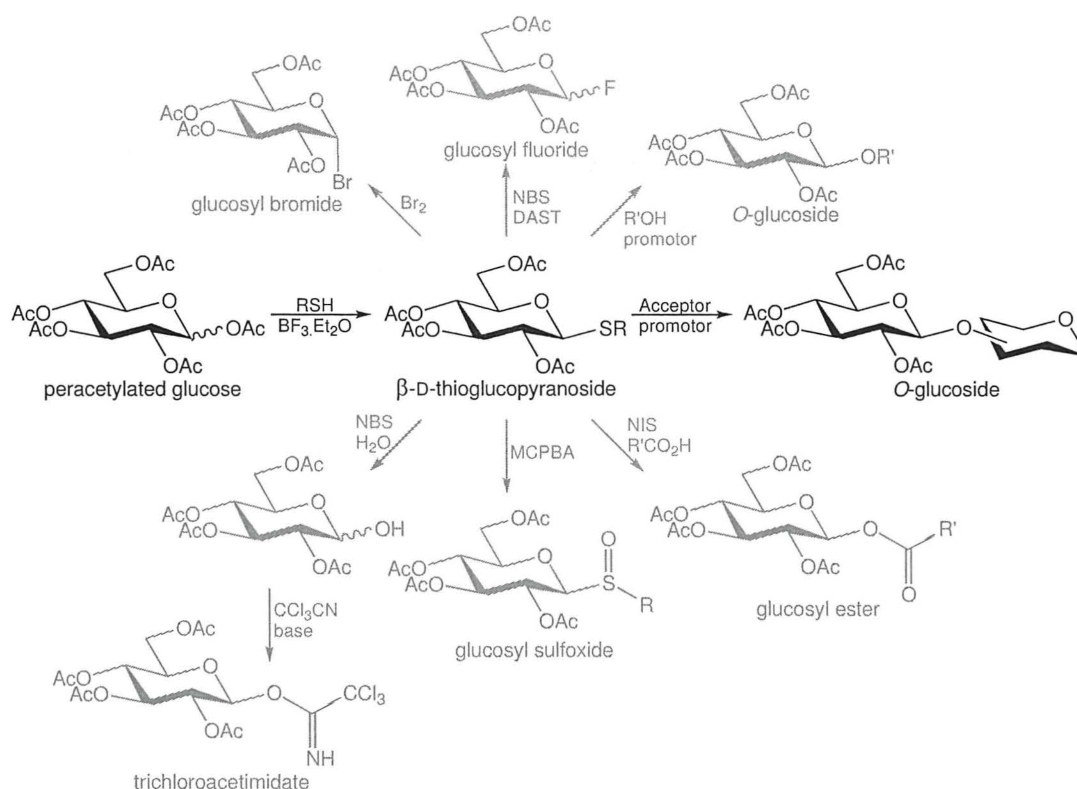


Figure 12 Thioglycosides can be made from acetylated sugars and are used as glycosyl donors in oligosaccharide synthesis, or can be converted to a variety of other groups. Adapted from T.K. Lindhorst.[6]

A range of thiophilic promoters can be used to activate thioglycosides to act as donors, such as sources of iodonium ions like *N*-iodosuccinimide (NIS), iodonium dicollidine perchlorate (IDCP),[28] and dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST).[29]

1.1.9 NMR Coupling Constants

Coupling constants obtained from NMR can be used to assign the configuration of glycosidic linkages and other groups at the anomeric position. This is described by the Karplus equation and its variants, where approximate values for the coupling constants of vicinal hydrogens ($^3J_{H,H}$) can be predicted based on their dihedral angle (**Figure 13**).[30,31]

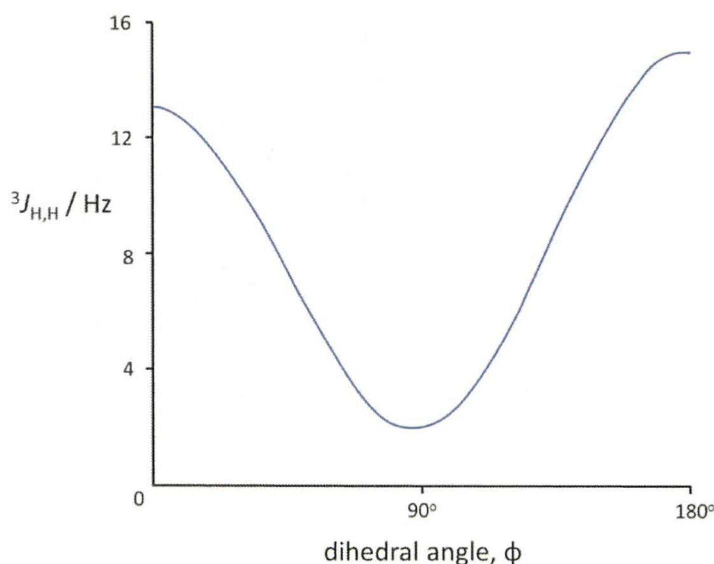


Figure 13 A plot demonstrating how the vicinal coupling constant, $^{3}J_{H,H}$, between two protons varies with their dihedral angle, ϕ, according to the Karplus equation.[31]

In a hexopyranose where the *H*-2 proton is axial, such as glucose and galactose, the β-anomer will have a larger vicinal coupling constant ($^{3}J_{H,H}$) between the *H*-1 and *H*-2 protons than the corresponding α-anomer (8-10 Hz and 3-4 Hz respectively). This is due to the lower dihedral angle between these protons for the α-anomer (**Figure 14**).

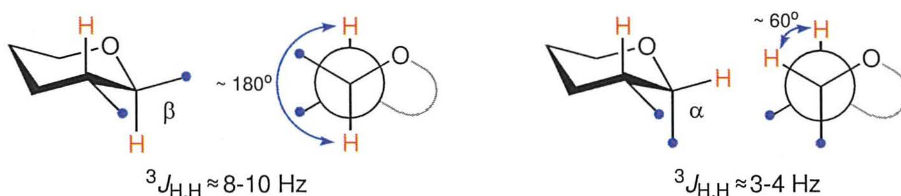


Figure 14 $^{3}J_{H,H}$ coupling constants between an axial *H*-2 and the *H*-1 for α and β anomers.

However, this approach cannot be applied to sugars such as mannose or talose, where the *H*-2 proton is equatorial and therefore has similar values for the dihedral angle with *H*-1 for both the α and β-anomers (**Figure 15**).

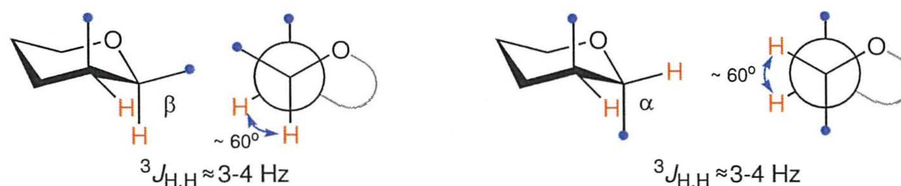


Figure 15 $^{3}J_{H,H}$ coupling constants between an equatorial *H*-2 and the *H*-1 for α and β anomers will be indistinguishable from each other due to their similar dihedral angles.

The dihedral angle will be close to 60° for both the α and β-anomers, with coupling constants of approximately 3-4 Hz. The geminal $^{1}J_{C,H}$ coupling can also be used to assign conformation at the anomeric position.[32] Typical values of 160 Hz for axial and

170 Hz for equatorial anomeric protons can be observed, with the α-anomer routinely 10 Hz higher than the for the corresponding β-anomer (**Figure 16**).[33]

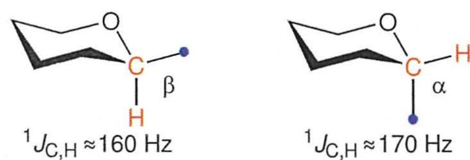


Figure 16 $^1J_{C,H}$ coupling constants for α and β anomers.

2 Synthesis of ^{13}C-Labelled Lewis^b Hexasaccharide Structures

2.1 *Helicobacter pylori*

Helicobacter pylori (*H. pylori*) is a Gram negative bacterium which inhabits the gastrointestinal tract of 50% of the world's population. For the majority of people carrying *H. pylori* the infection is asymptomatic, however the infection can lead to chronic gastritis, peptic ulcers, and gastric cancer and adenocarcinoma.[34,35,36,37] During infection, the bacterium attaches itself to gastric epithelial cells in the gastric mucosa by recognising and adhering to structures on these cells. This is achieved through interactions between a protein on the surface of the bacterium known as the blood group antigen binding adhesin (BabA), and Lewis blood group antigens, such as Lewis b (Le^b), on the host cells.[38,39,40]

2.1.1 Lewis Blood Group Antigens

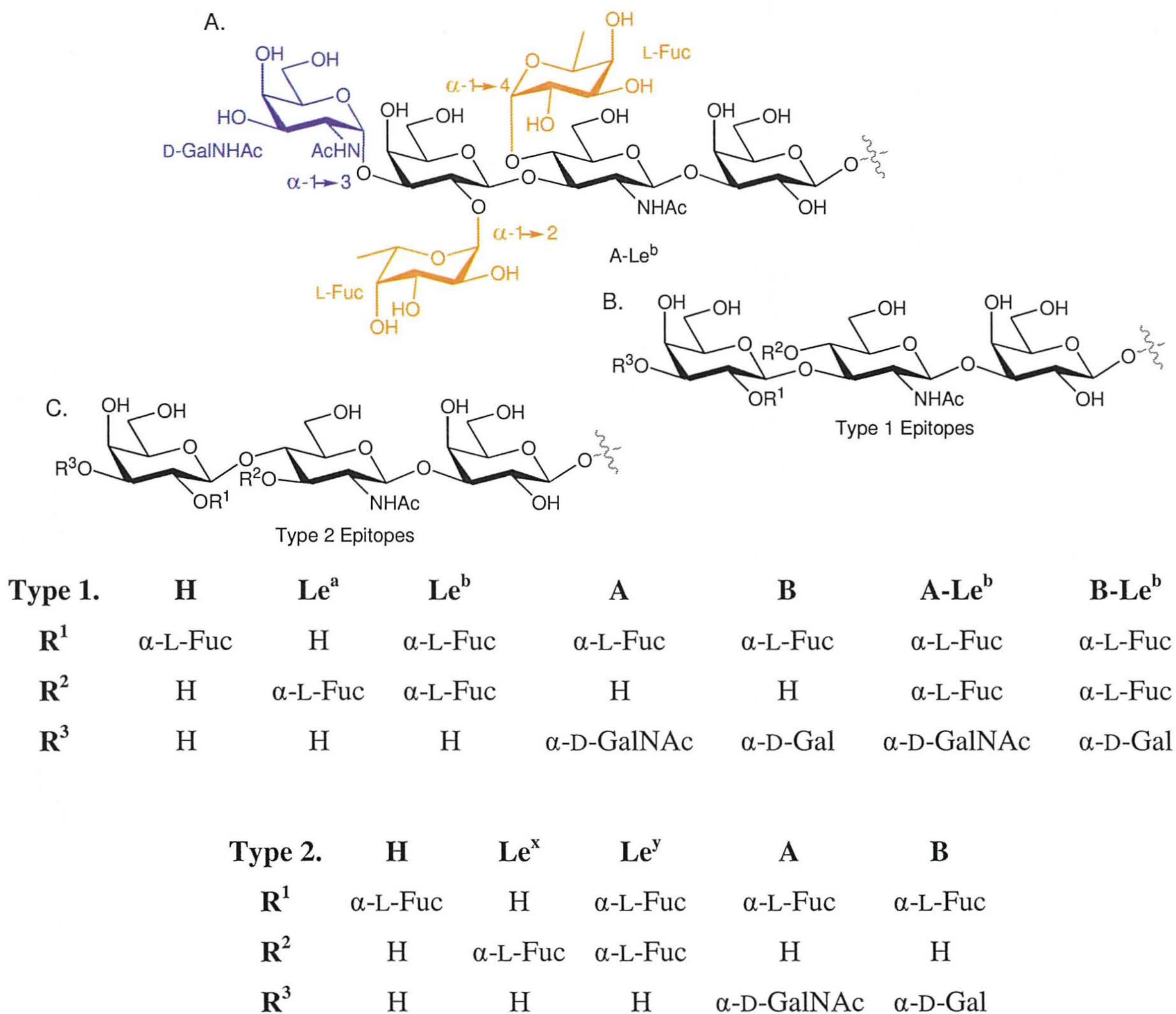
The Lewis antigens are one of many classes of blood group antigen, being closely linked to the ABO blood group system.[41] These antigens are formed by the presence of specific sugar moieties attached to an oligosaccharide chain precursor,[41] with the backbones of the Type 1 and Type 2 epitopes forming the basis of the A, B, H and Lewis determinants (**Figure 17**).[42]

Terminal *N*-acetylgalactosamine (GalNAc) residues are associated with A blood phenotypes, as seen for the A-Leb epitope (**Figure 17**), while a galactose moiety is present in B blood types. The absence of a residue at this position represents O blood groups.

Le^a and Le^b determinants are found in secretions and on the surface of a range of cells, including red blood cells.[43] While they have not yet been found on blood cells, Le^x and Le^y antigens (Type 2 epitopes) are expressed in epithelial cells and are considered to be associated with tumours, therefore being a target for research in cancer treatments.[44,45]

H. pylori has been shown to bind to the fucosylated Le^b determinant, though binding to A-Le^b or B-Le^b epitopes was not observed, indicating that there may be decreased binding in A or B blood groups compared to persons with an O blood type.[39] However,

a more recent study investigating strains of *H. pylori* from different global regions showed binding with A, A-Le^{b}, B-Le^{b}, H, and Le^{b}, suggesting that *H. pylori* strains have adapted to their host populations through modifications of BabA.[40]



Type 1.	H	Le^{a}	Le^{b}	A	B	A-Le^{b}	B-Le^{b}
R^{1}	α-L-Fuc	H	α-L-Fuc	α-L-Fuc	α-L-Fuc	α-L-Fuc	α-L-Fuc
R^{2}	H	α-L-Fuc	α-L-Fuc	H	H	α-L-Fuc	α-L-Fuc
R^{3}	H	H	H	α-D-GalNAc	α-D-Gal	α-D-GalNAc	α-D-Gal

Type 2.	H	Le^{x}	Le^{y}	A	B
R^{1}	α-L-Fuc	H	α-L-Fuc	α-L-Fuc	α-L-Fuc
R^{2}	H	α-L-Fuc	α-L-Fuc	H	H
R^{3}	H	H	H	α-D-GalNAc	α-D-Gal

Figure 17 Structures of blood group A, B, H and Lewis epitopes. A) The structure of the A-Le^{b} epitope, a difucosylated member of the Type 1 series. B) The backbone of the Type 1 epitopes, with substituted groups given in the table shown. C) The backbone of the Type 2 epitopes, with substituted groups given in the table shown. Adapted from K. Lloyd.[42]

2.1.2 Carbohydrate-Lectin Interactions

As discussed in the previous section, *H. pylori* utilises the protein BabA to act as a lectin in binding to Le^{b} structures present on epithelial cells.

Lectins are defined as sugar-binding proteins which are able to precipitate or agglutinate glycoconjugates,[46] with their name being derived from the Latin *lego*, meaning ‘I choose’.[47] This reversible, non-covalent binding takes place at carbohydrate

recognition domains (CRDs) on the lectin, which are widely varied in size and structure.[6]

Lectins also vary in their biological roles. Plant lectins are potentially employed as potent defence mechanisms, such as the lectins ricin and abrin from the plants *Ricinus communis* and *Abrus precatorius* respectively,[48,49] while lectins in animals are utilised in recognition processes in cell to cell interactions, for example between glycoproteins on blood cells and liver lectins during regulation.[50,51]

As well as some bacterial lectins acting as toxins, such as the cholera toxin choleragen,[52] carbohydrate-lectin interactions are also utilised by bacteria and viruses during infection. For example, HIV infection is mediated between the gp120 glycoprotein (containing mannose and sialic acid units) located on the surface membrane of the virus and the DC-SIGN protein on the host cell.[53]

Carbohydrate-lectin interactions have therefore attracted interest in research into new vaccines and treatments for infections.

A popular method for studying the interaction of proteins with ligands is saturation transfer difference (STD) NMR. STD is based upon the nuclear Overhauser effect and works by subtracting a spectrum in which the protein is selectively saturated from a spectrum with no protein saturation.[54] The result will be a spectrum in which only the areas of the ligand which received saturation transfer from the protein will be visible.

Heteronuclear saturation transfer difference (HSTD) was first conducted by observing ^{13}C signals from the ligand rather than those from protons, in an attempt to solve the problem of overcrowding of ^{1}H spectra (a particular problem in carbohydrate ^{1}H spectra) and to investigate binding sites with an insufficient number of hydrogens.[55] However, the low natural abundance of ^{13}C and its gyromagnetic ratio being roughly one quarter of that of ^{1}H are limiting factors in this approach, but can be overcome by ^{13}C labelling in the ligand.[56]

Isotopically enriching individual carbons in monosaccharides has been utilised in confirming the assignment of ^{13}C chemical shifts, as well as providing information on ^{13}C-^{13}C and ^{13}C-^{1}H coupling constants and their relationships with conformation and configuration of sugars.[57,58,59,60,61] Isotopic labelling has also been employed in studying the mechanisms of reactions involving carbohydrates, such as in the case of pyrolysis

reactions for conversion of biomass to energy.[62] The Lewis antigens have also been targets for 13C labelling, with sialyl-Le^x and Le^x structures synthesised for the use in NMR binding studies with the cell adhesion molecule E-selectin.[63,64]

Our aim was therefore to synthesis a Le^b hexasaccharide with a ^{13}C label incorporated into its structure, for the purpose of conducting HSTD experiments with the Le^b structure acting as a ligand to the lectin BabA.

2.2 Incorporating a ^{13}C label – The L-Fucose Residue

The L-fucosyl residue on the Le^b hexasaccharide (**Figure 18**) was chosen as a suitable candidate for carrying the ^{13}C labels required. This was largely due to the presence of its methyl group at the 6-position, which was expected to appear in an area of the ^{13}C spectrum unoccupied by other shifts from the hexasaccharide.[65,66] Previous studies had also suggested the importance of these individual residues on the Le^b hexasaccharide during binding with BabA.[39,40]

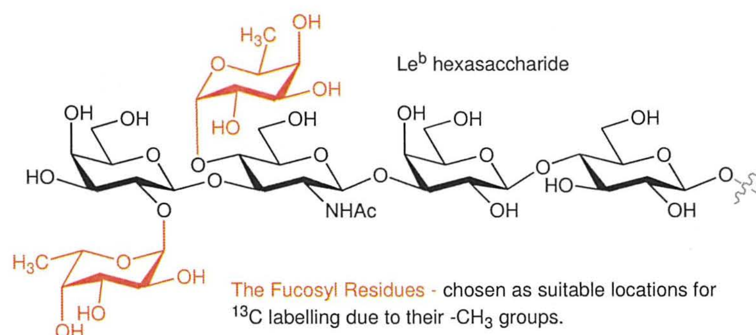
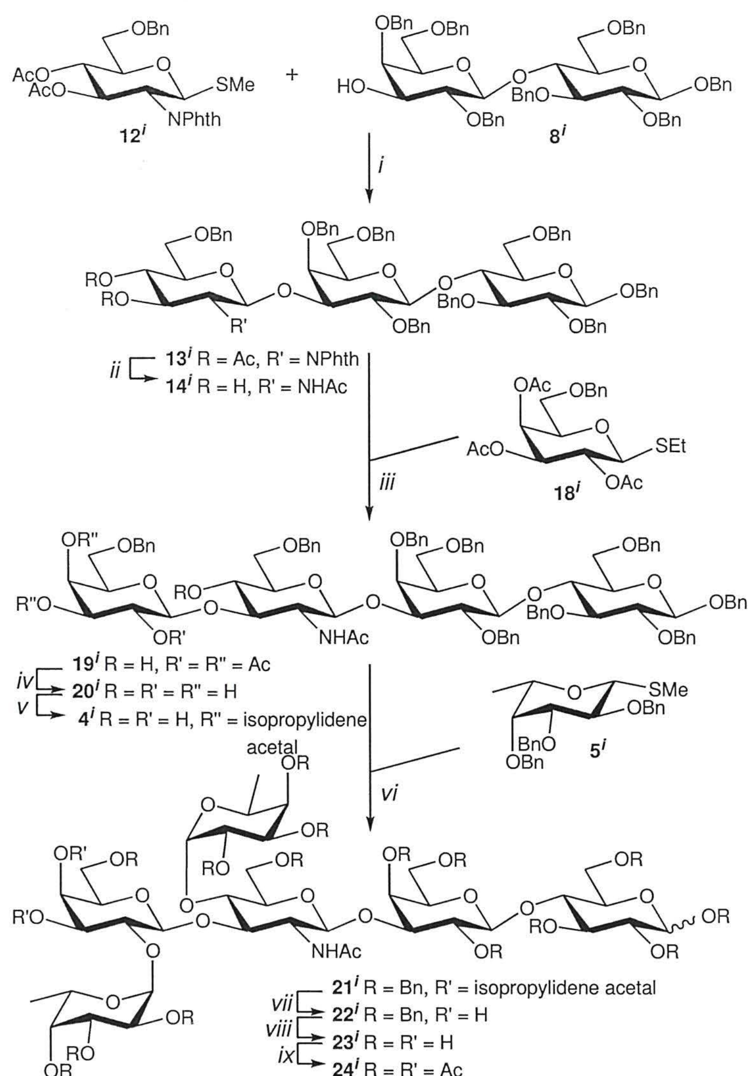


Figure 18 The Le^b hexasaccharide with L-fucosyl residues highlighted.

Due to the high cost and low availability of ^{13}C-labelled starting materials, a synthetic approach in which the fucosyl moieties could be incorporated into the molecule late on in the synthesis would be favourable.

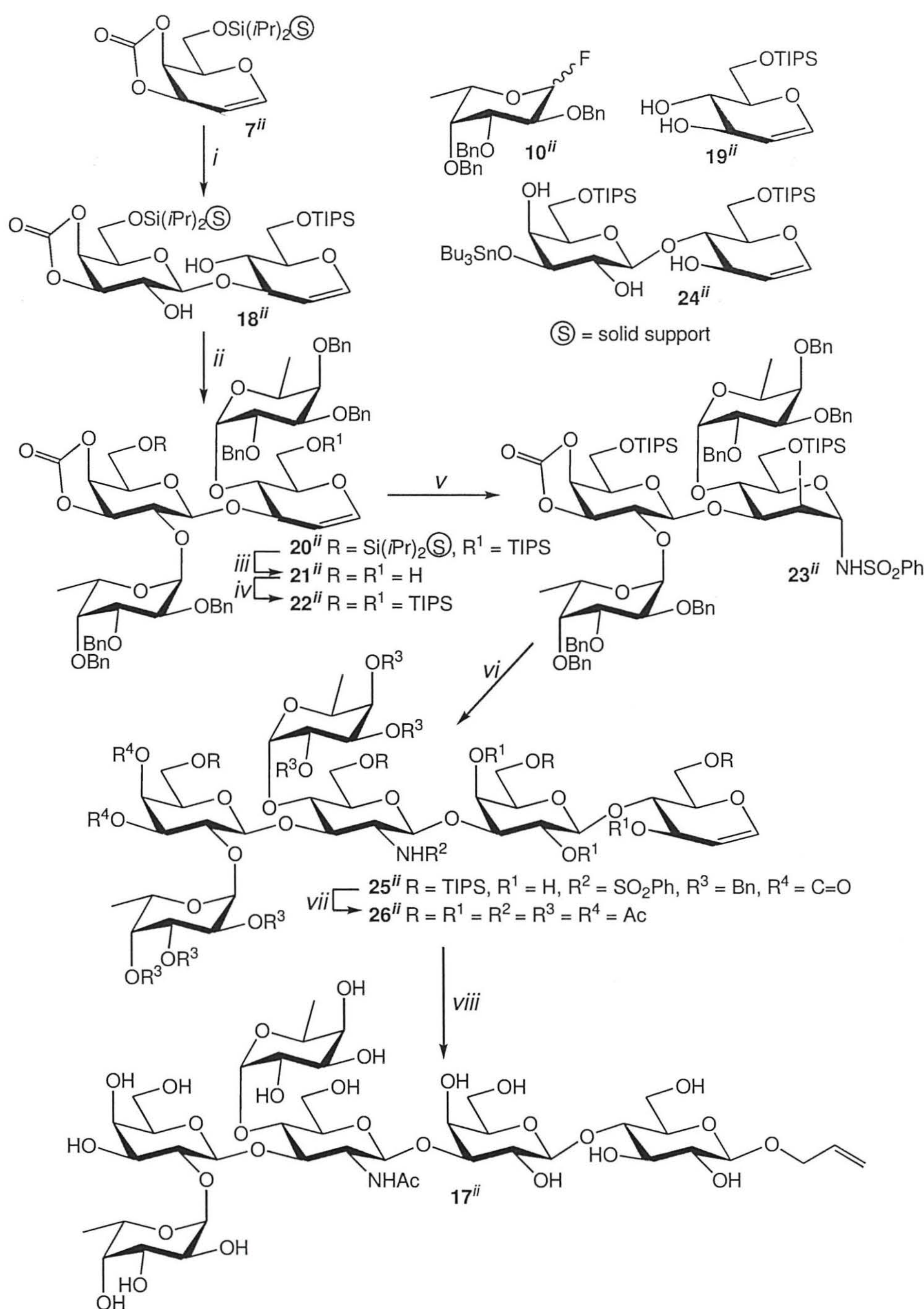
2.3 Previous Syntheses of Le^b Hexasaccharides

There are many aspects to take into account when devising synthetic routes towards oligosaccharides. These include the stereochemistry of glycosidic linkages which are formed, orthogonality of the protecting group and glycosylation strategies, the nucleophilicity and electrophilicity of the acceptors and donors respectively, the overall efficiency of the synthesis, and also if a linear or convergent approach is preferable. Le^b hexasaccharides have been prepared previously using a range of different strategies.



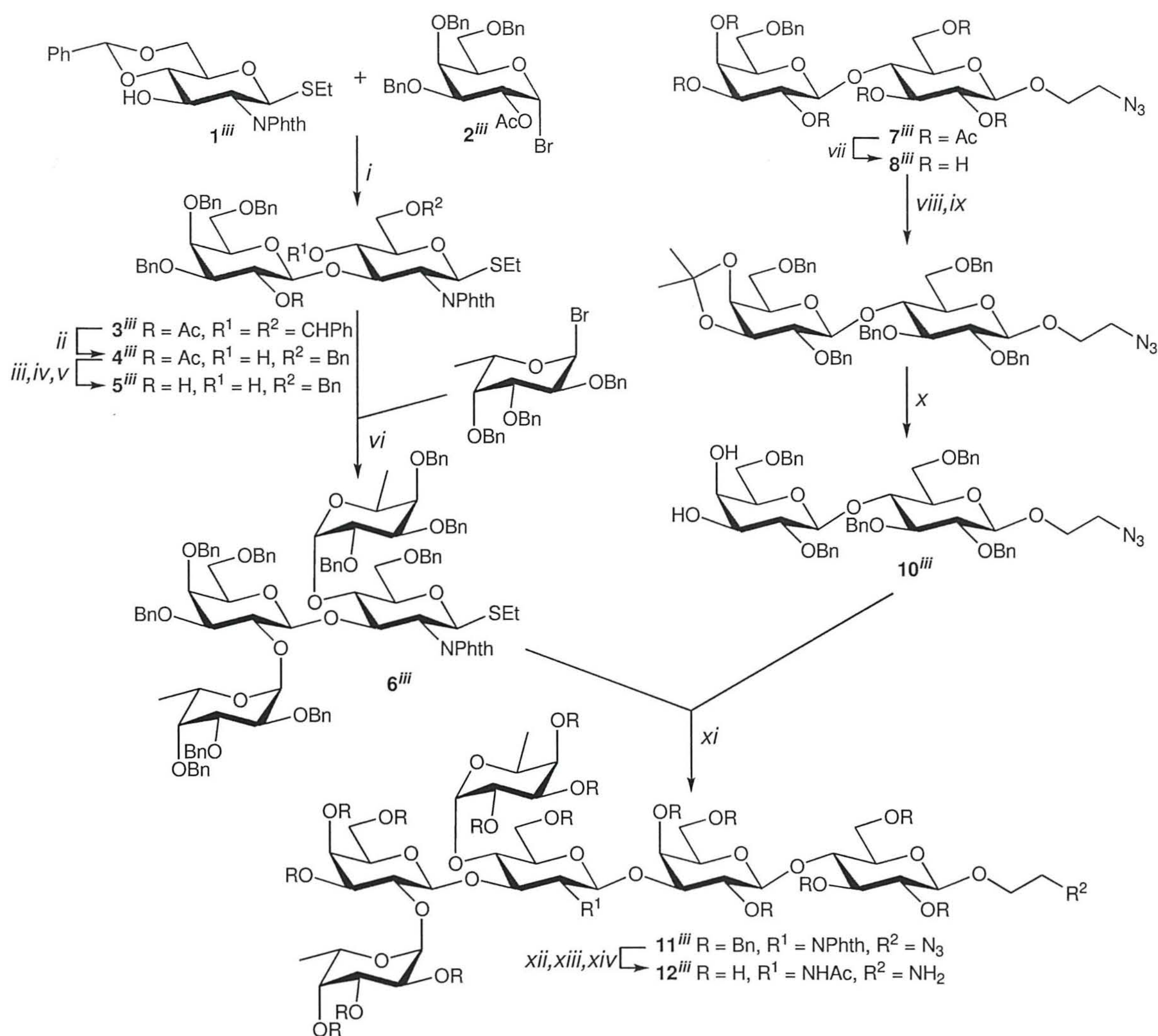
Scheme 1 Synthetic route used by Sato *et al*, adapted from the 1986 publication.[67] i. $MeOSO_2CF_3$, CH_3NO_2 ii. a. NaOMe, MeOH b. $H_2NNH_2.H_2O$, EtOH c. Ac_2O, MeOH (58% yield from ***8ⁱ***) iii. $CuBr_2$, Bu_4NBr, $HgBr_2$ (77%) iv. NaOMe, MeOH v. 2,2-DMP, *p*TsOH (71% yield from **19ⁱ**) vi $CuBr_2$, Bu_4NBr, $HgBr_2$, $MeNO_2$ (68%) vii. CF_3CO_2H, THF/H_2O viii. 10% Pd/C, H_2, MeOH/AcOH 1:1 ix. Ac_2O, pyridine, DMAP (91% yield from **22ⁱ**)

Sato *et al* first synthesised a Le^b hexasaccharide using a linear approach, building a tetrasaccharide backbone before incorporating the fucosyl moieties in a final glycosidation step.[67] This route starts with the coupling of a benzylated lactoside glycosyl acceptor and an *N*-phthalimido-glucosyl donor (**Scheme 1**), before further manipulation and glycosylation with galactosyl and fucosyl donors. A disadvantage of this route is that several protecting group manipulation steps needed to be conducted on the tetrasaccharide, including the incorporation of an isopropylidene acetal, before the final glycosidation step could be undertaken. Also, further steps from the acetylated hexasaccharide were required to introduce a linker to the molecule, which could instead have been carried out on the lactoside acceptor prior to the glycosidation steps.



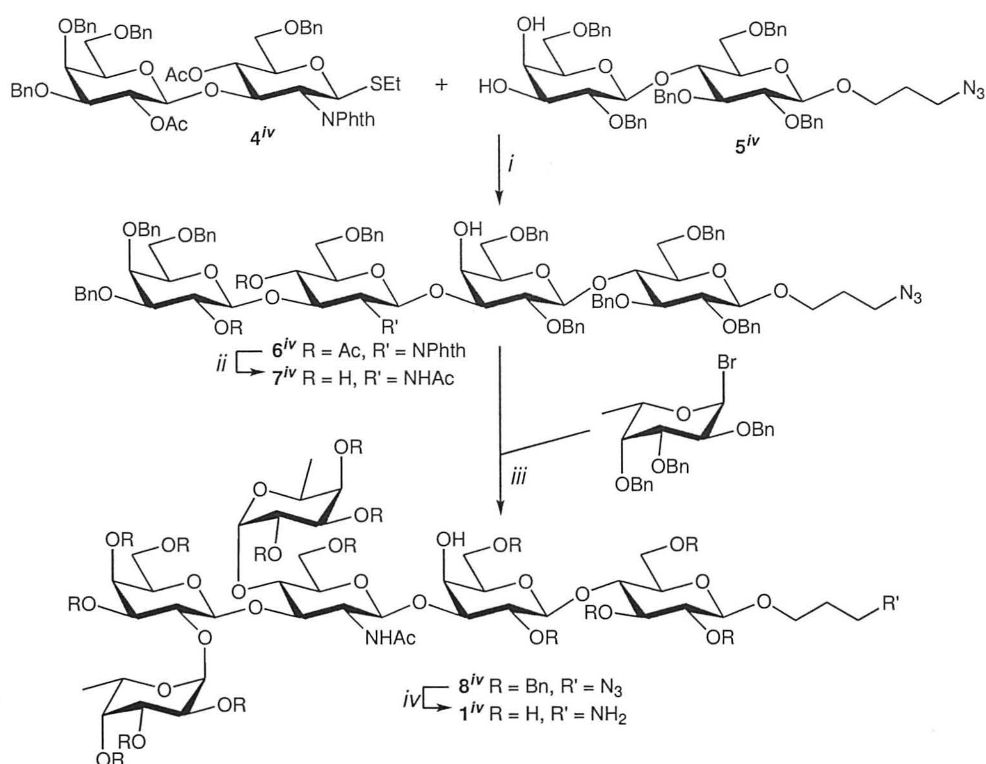
Scheme 2 Glycal assembly method used by Danishefsky *et al* for the synthesis of a Le^b hexasaccharide. Adapted from the 1994 publication.[68] i. a. 3,3-dimethyldioxirane, DCM b. **19**^{ii}, $ZnCl_2$, THF ii. **10**^{ii}, $Sn(OTf)_2$, di-*tert*-butylpyridine, THF iii. TBAF, AcOH, THF (40% from **7**) iv. TIPSCl, imidazole, DMF v. $I(coll)_2ClO_4.PhSO_2NH_2$, DCM vi. **24**^{ii}, $AgBF_4$, THF vii. a. TBAF, AcOH, THF (55% yield) b. Na/NH_3 c. Ac_2O, pyridine viii a. 3,3-dimethyldioxirane, DCM b. allyl alcohol, $ZnCl_2$ c. NaOMe, MeOH.

Danishefsky *et al* later synthesised a Le^b hexasaccharide using a glycal assembly method,[68] which followed on from their previous work with Le^y blood group determinants.[69] This approach utilised a protected galactal held on a polymer scaffold, before coupling with other glycal units and fucose donors to give a Le^b hexasaccharide (**Scheme 2**). A similar approach was later adopted without the use of the polymer scaffold, instead using a triisopropylsilyl ether (TIPS) protecting group in its place.[70]



Scheme 3 A convergent approach towards the synthesis of a Le^b hexasaccharide utilised by Chernyak *et al*, adapted from a publication in 2000.[65] i. AgOTf, 2,6-di-*tert*-butyl pyridine, DCM, toluene (-74 °C, 84%) ii. $NaBH_3CN$, HCl/Et_2O, THF (77%) iii. NaOMe, MeOH/DCM iv. TFAA, pyridine v. $Mg(OMe)_2$, MeOH/DCM (91% yield over 3 steps) vi. Et_4NBr, DCM (94%) vii. NaOMe/MeOH (95%) viii. acetone, PTS ix. BnBr, NaH, DMF (55% yield over 2 steps) x. 70% $TFA_{(aq)}$, DCM (92%) xi. DMTST, DCM (-78 °C – rt, 72%) xii. NH_2NH_2/H_2O, EtOH/dioxane (heat, 59%) xiii. Ac_2O – MeOH/DCM (73%) xiv. 10% Pd/C, H_2 (120 psi), EtOH/EtOAc, 60% $AcOH_{(aq)}$ (67 %).

The synthesis of a Le^b hexasaccharide by Chernyak *et al* was achieved by the synthesis of a Le^b tetrasaccharide donor, before coupling with a lactoside acceptor with free 3' and 4' hydroxyl groups (**Scheme 3**). However, difficulties were reported for this final coupling step, with large equivalencies of the promoter dimethylsulfonium triflate (DMTST) required to avoid ethanethiol 1,2-elimination from donor **6** and to achieve a 72 % yield. Subsequent conversion of the *N*-phthalimido group to the *N*-acetate and complete removal of the benzyl ethers also proved difficult, with a 29 % yield over the final three deprotection steps reported.



Scheme 4 A convergent approach used by Lahmann *et al*, adapted from a publication in 2004.[66] i. NIS, AgOTf, DCM (78%) ii. a. EDA, EtOH, (70 °C) b. NaOMe c. Ac_2O, MeOH (74% yield from **6**) iii. Et_4NBr, DMF/DCM (79%) iv. Pd/C, THF; Pd/C, 1M HCl (1 eq), THF/EtOH/H_2O, 1 atm (rt, 71%).

To overcome the problem with elimination and also to allow transformation of the phthalimido group at an earlier stage in the synthetic sequence, Lahmann *et al* reported an approach where the tetrasaccharide backbone was produced from two disaccharides, before further glycosidation to give the hexasaccharide.[66] Initial work showed poor regioselectivity during the glycosylation with the lactoside diol, with equal amounts of β-1→3 and β-1→4 product being formed. Protecting group manipulation of the glycosyl donor alleviated this problem, giving the desired β-1→3 product in a 78% yield as represented in (**Scheme 4**).

2.4 Retrosynthetic Pathway

For the synthesis if the Le^b hexasaccharide, knowledge acquired from previous work in this area was used and a similar synthetic approach adopted.[66,71] Due to the cost of the labelled starting material, and as the intention was to have the ^{13}C label present on the fucosyl moieties, a route in which the glycosylation with the fucosyl donors was close to the end of the synthetic pathway was desirable.

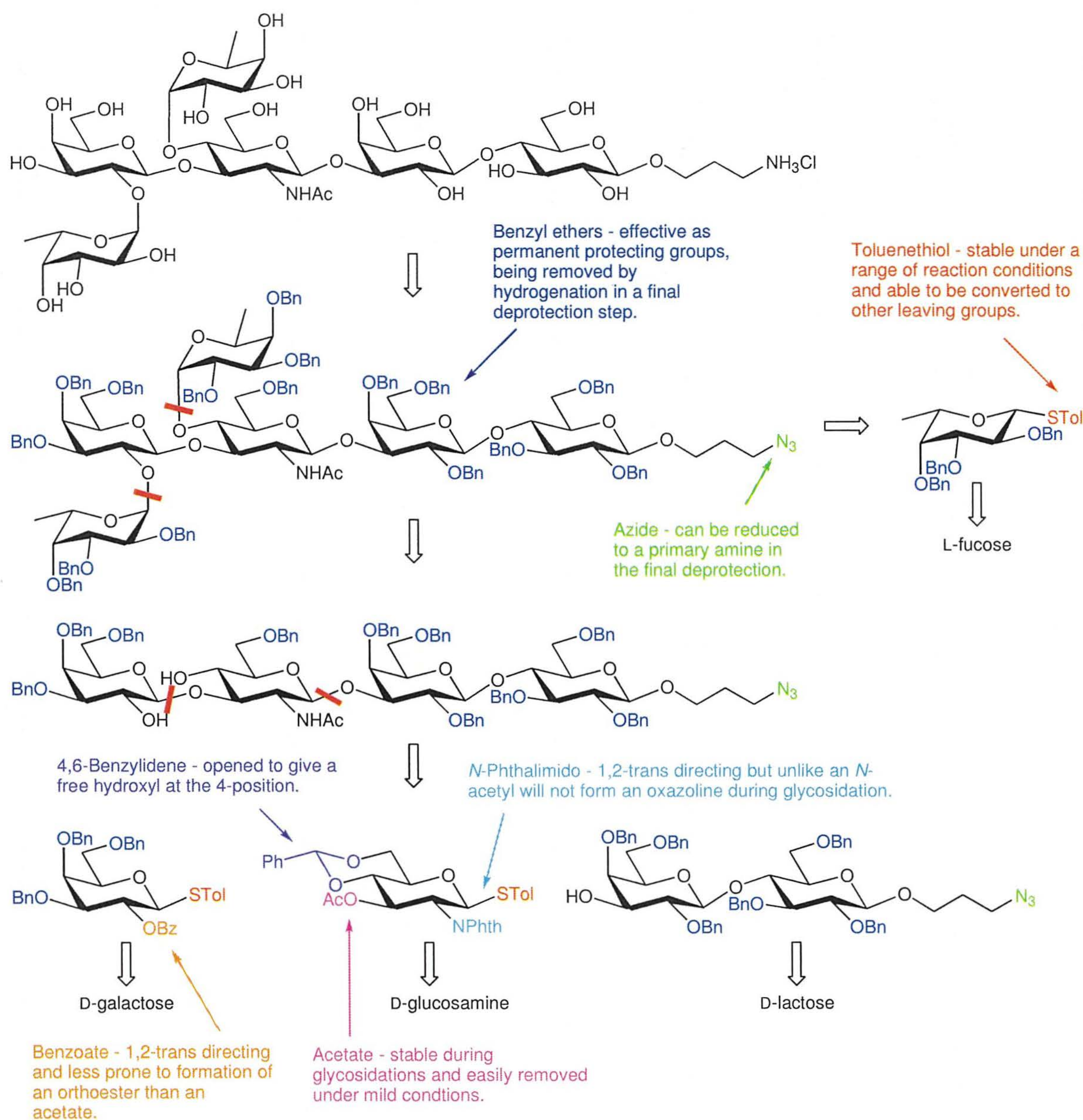


Figure 19 A retrosynthetic pathway for the Le^b hexasaccharide target molecule. This strategy utilises a lactoside acceptor equipped with a linker, and *N*-acetylglucosamine, galactosyl and fucosyl donors.

The building blocks required for the oligosaccharide include a lactoside acceptor equipped with a linker for possible glycoconjugation, an *N*-phthalimido-glucosyl donor and a galactosyl donor to produce a tetrasaccharide backbone. This tetrasaccharide could then be deprotected to remove its temporary protecting groups before coupling with a ^{13}C-labelled fucosyl donor (**Figure 19**).

2.5 Synthesis of the L-[$^{13}C_6$]Fucosyl Donor

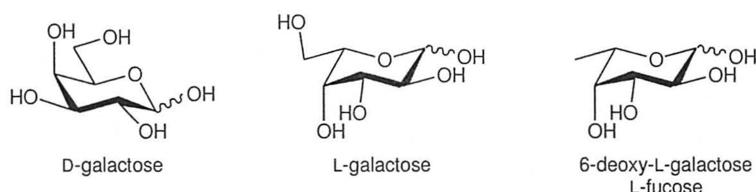


Figure 20 L-fucose, the 6-deoxy form of L-galactose.

Fucose can also be described as 6-deoxy-galactose (**Figure 20**) though fucose is more naturally abundant as the L-enantiomer, in contrast to the more common D-galactose. As per-*O*-benzylated fucosyl donors had previously been used successfully in the synthesis of Le^{b} hexasaccharide structures, the analogous ^{13}C-labelled version was intended as the target molecule for the ^{13}C-labelled fucosyl donor (**22**, **Figure 21**).

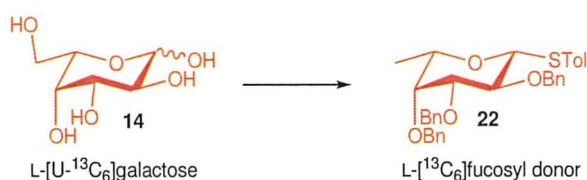


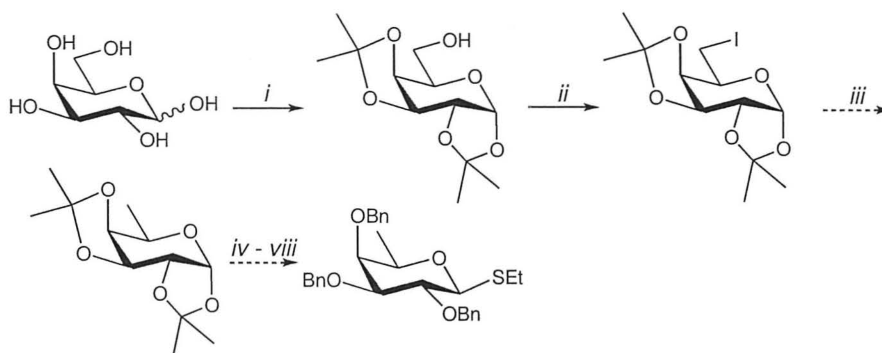
Figure 21 The ^{13}C-labelled L-galactose starting material and target molecule. The structures in red are derived from L-[U-$^{13}C_6$]galactose **14**, in which the six sugar carbons are uniformly ^{13}C-labelled.

The 2,3 and 4-positions were to be protected with benzyl ether protecting groups and a thiol leaving group was decided upon, due to their interchangeability with other possible leaving groups and their resilience to a range of reaction conditions. The simplest synthetic route would be to start with ^{13}C labelled L-fucose and perform the necessary steps to give the donor. However, this material was not readily available and was prohibitively expensive. The alternative was to use the more easily available L-[U-$^{13}C_6$]galactose **14** as a starting material and incorporating additional steps into the synthesis to remove the 6-hydroxyl group.

Due to high cost of L-[U-$^{13}C_6$]galactose, it was deemed prudent to develop an efficient synthesis for the conversion of galactose to the corresponding fucosyl donor before applying to the labelled galactose starting material. For this purpose, readily available D-galactose was used.

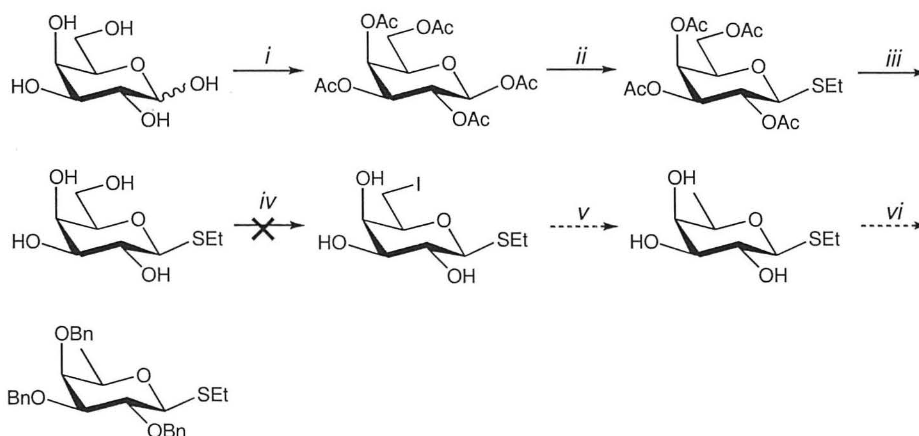
As it had been reported that D-fucosides were synthesised from D-galactosyl compounds via 6-deoxy-6-iodo-galactosyl intermediates,[72] previous experiments conducted within the group set about protecting the 1,2,3 and 4-positions using isopropylidene protecting groups before carrying out a Mitsunobu reaction to introduce

an iodine at the 6-position.[73] The intention was to then reduce the compound with lithium aluminium hydride ($LiAlH_4$) or sodium borohydride ($NaBH_4$) to give the 6-deoxy sugar, before the introduction of a thiol leaving group and protection of the remaining hydroxyl groups with benzyl ethers (**Scheme 5**).



Scheme 5 A previous attempt towards the synthesis of a fucosyl donor from D-galactose, utilising isopropylidene acetal protecting groups and an iodine leaving group. This route was abandoned after the first two reaction steps provided a poor yield.

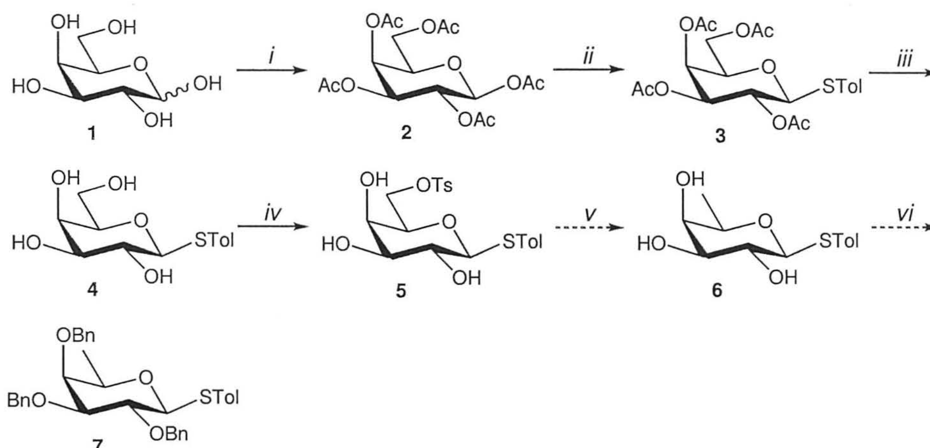
The first two steps to reach the 6-deoxy-6-iodo-1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranoside were low yielding, giving a combined yield of only 36%, far lower the reported yield of 95% for this compound.[74] This reaction sequence was abandoned at this point.



Scheme 6 A previous attempt towards the synthesis of a fucosyl donor from D-galactose. Though the first 3 reaction steps were high yielding, the required 6-deoxy-6-iodo-galactoside was not produced.

An alternative approach was trialled in which D-galactose was acetylated a thiol introduced at the anomeric position. These two steps were reported to be high yielding, as was the deacetylation step which followed. However, no generation of the 6-deoxy-6-iodo-galactoside was observed during the Mitsunobu reaction (**Scheme 6**).

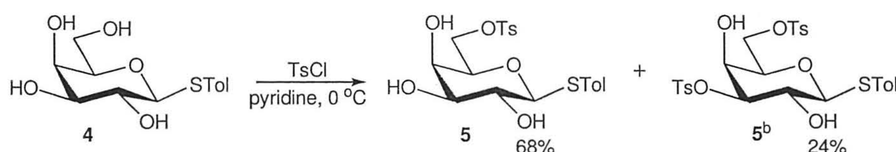
Based on the results of these previous studies, the proposed synthetic route for the trials with D-galactose was to start with the synthesis of the thio-galactoside before installing a tosyl leaving group at the 6-position with *p*-toluenesulfonyl chloride (TsCl). A reduction with $LiAlH_4$ or $NaBH_4$ could then be carried out to give the 6-deoxy galactoside before benzylation of the remaining hydroxyl groups (**Scheme 7**)



Scheme 7 First route attempted in developing a synthesis for the fucosyl donor. The reduction and benzylation steps were not carried out as this route was abandoned due to low yields during the tosylation. i. NaOAc, Ac_2O (140 °C, 30 min, 100%) ii. MePhSH, $BF_3.OEt_2$, DCM (rt, 2 h, 92%) iii. NaOMe, MeOH (rt, 30 min, 100%) iv. TsCl, pyridine (rt, 3 h, 68%).

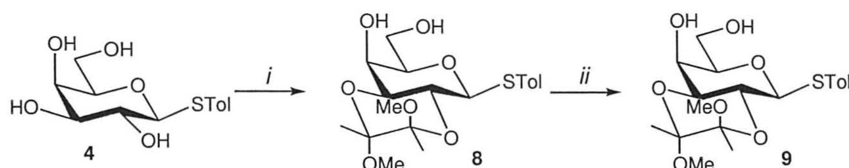
The first step of this six step synthesis was the acetylation of D-galactose **1** to give per-*O*-acetyl-β-D-galactopyranoside **2**. This was carried out with sodium acetate (NaOAc) in acetic anhydride (Ac_2O) and produced an α/β 1:6 mixture in quantitative yields.[75,76] Reaction of **2** with *p*-toluenethiol (MePhSH) in the presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$)[77] gave the thiogalactoside **3**, exclusively as the β anomer due to neighbouring group participation with the adjacent acetate group. The removal of the acetyl protecting groups to give *p*-tolyl 1-thio-β-D-galactopyranoside **4** was carried out as a transesterification under Zemplén conditions with sodium methoxide (NaOMe) in methanol (MeOH).[78]

The next step was to then regioselectively introduce a tosyl leaving group at the 6-position which could then be reduced to give the deoxy sugar. This was attempted using TsCl in pyridine, owing to its selectivity towards primary alcohols.[79] This approach did show some regioselectivity towards the 6-position, but a di-substituted product **5**[b] also containing a tosyl group at the 3-position was found in every temperature range (-40 °C – rt) and stoichiometry attempted, with the highest yield obtained for compound **5** being 68% (**Scheme 8**).



Scheme 8 Attempts to introduce a tosyl leaving group at the 6-position with secondary hydroxyls also available gave the desired compound **5** as well as the di-tosylated by-product **5**^{b}.

Due to this loss of regioselectivity, additional protecting group steps were attempted in the hope that they might improve the overall yield. Protection of the 2,3-position using a butane diacetal (BDA) protecting group was therefore undertaken to prevent the 3-position from taking part in the subsequent tosylation (**Scheme 9**). The thiogalactoside **4** was heated to reflux in methanol with 2,3-butanedione, trimethyl orthoformate ($CH(OCH_3)_3$), and a catalytic amount of camphorsulfonic acid (CSA).[80] A yield of 50% was recorded, with multiple reaction by-products visible by TLC. Tosylation of the BDA protected thiogalactoside went to completion, selectively producing the *C*-6 tosylated compound **9** only. However, this approach was abandoned at this stage due to the low yielding BDA protection step.

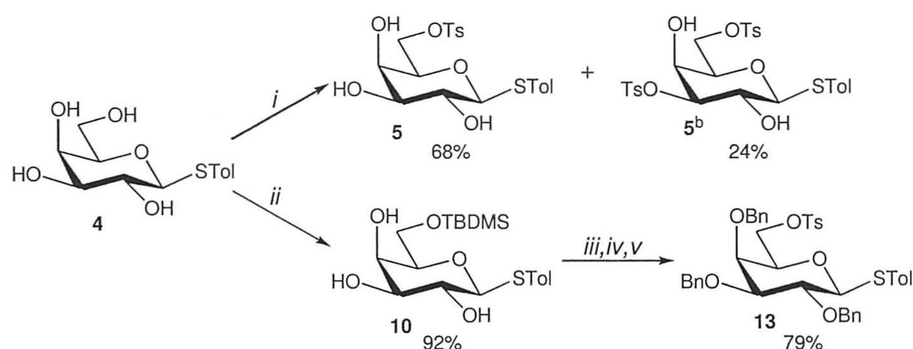


Scheme 9 Incorporation of a BDA protecting group prior to tosylation. While the regioselectivity of the tosylation was greatly improved, the BDA protection step proved too low yielding to make this a viable route. i. 1,2-butanedione, $CH(OCH_3)_3$, CSA, MeOH (reflux, 16 h, 50%) ii. TsCl, pyridine (0 °C, 3 h, crude product not purified)

The next approach was to use a bulky silyl ether protecting group to temporarily protect the 6-position while the remaining hydroxyl groups were benzylated. The silyl ether could then be removed before creating the tosyl leaving group at the 6-position.

The *tert*-butyldimethylsilyl ether **10** was produced in a yield of 92% from *tert*-butyldimethylsilyl chloride (TBDMSCl) in pyridine,[81] before introduction of benzyl ethers with benzyl bromide (BnBr) and sodium hydride (NaH).[82,83] This step also gave a good yield of 88%. The silyl ether was then cleaved using tetrabutylammonium fluoride (Bu_4NF)[81] to give compound **12** before creating the tosyl leaving group at the 6-position, giving **13** in a 94% yield. When compared to our original route, this approach appeared to be favourable with a higher overall yield despite the additional steps (73%

over 4 steps, compared to 68%, **Scheme 10**) and also includes the introduction of the required benzyl ethers, which would otherwise have to be installed later in the synthesis.



Scheme 10 Attempts to introduce a tosyl leaving group at the 6-position with secondary hydroxyls also available gave a mixture of products. By using a TBDMS protecting group before protecting the remaining hydroxyls improved the yield for the subsequent tosylation step. i. TsCl, pyridine (0 °C - rt, 2 h, 68 %) ii. TBDMSCl, pyridine (0 °C - rt, 4 h, 92 %) iii. BnBr, NaH, DMF (0 °C - rt, 2 h, 88 %) iv. Bu_4NF, THF (rt, 16 h, 96 %) v. TsCl, pyridine (rt, 3 h, 94 %).

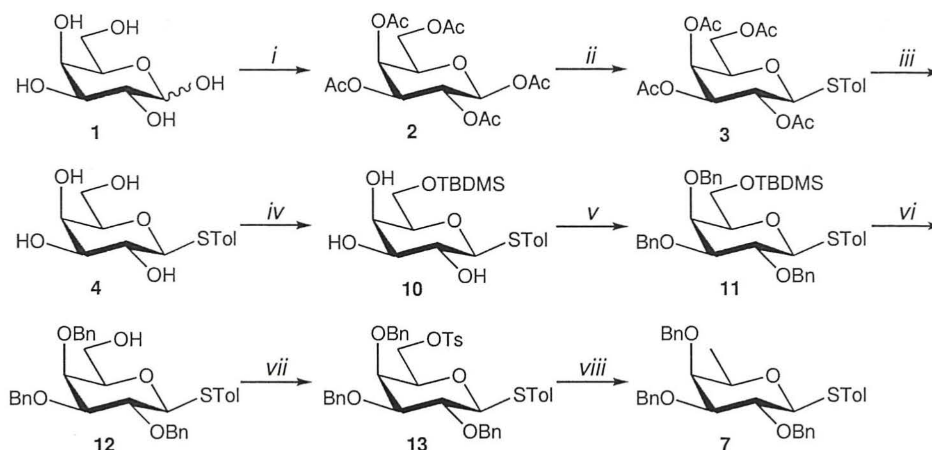
The reduction of the p-tolyl 2,3,4-tri-*O*-benzyl-6-tosyl-1-thio-β-D-galactopyranoside **13** to give the thiofucoside **7** was attempted with different reducing agents and number of equivalences to the starting material **13**.[84] THF and DMF were both used as solvents and the reaction conditions altered (**Table 1**).

Table 1 Reduction of compound under differing conditions. $LiAlH_4$ was the only reducing agent that proved successful in producing the deoxy sugar.

Reducing agent	equivalents	Solvent	Conditions	Yield (%)
$NaBH_4$	5	THF	rt	-
	15	THF	60 °C	-
	15	DMF	60 °C	-
	30	DMF	60 °C	-
$LiAlH_4$	5	THF	rt	-
	10	DMF	50 °C	<10%
	18	THF	rt	<10%
	26	THF	reflux	58%
NaH	15	DMF	60 °C	-

The results indicated that $NaBH_4$ was not strong enough to effectively reduce compound **13** to the deoxy sugar **7**. $LiAlH_4$ appears to be more effective at reducing the

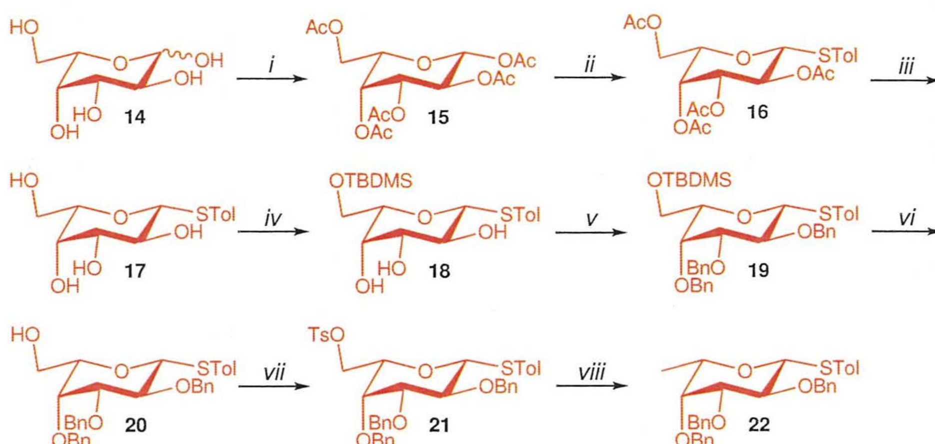
compound, but hydrolysis back to compound **12** was also observed. The best yield obtained was 58%, achieved with a large excess of $LiAlH_4$ (26 eq) under reflux in THF.



Scheme 11 An efficient synthetic route from D-galactose to produce the D-fucosyl donor in a yield of 39% over 8 steps. i. NaOAc, Ac_2O (140 °C, 30 min, 100%) ii. MePhSH, $BF_3.OEt_2$, DCM (rt, 2 h, 92%) iii. NaOMe, MeOH (rt, 30 min, 100%) iv. TBDMSCl, pyridine (0 °C - rt, 4 h, 92%) v. BnBr, NaH, DMF (rt, 2 h, 88%) vi. Bu_4NF, THF (rt, 16 h, 96%) vii. TsCl, pyridine (rt, 3 h, 94%) viii. $LiAlH_4$, THF (reflux, 2 h, 58%).

In summary, the D-fucosyl donor was produced from D-galactose **1** in an 8 step synthesis with an overall yield of 39% (**Scheme 11**). As this yield was considered acceptable, this synthetic pathway could now be applied to the L-[U-$^{13}C_6$]galactose to produce the required L-[$^{13}C_6$]fucosyl donor.

As described for the trials with D-galactose, an acetylation was performed by refluxing the starting material **14** (0.35 g) in Ac_2O with NaOAc to give the acetylated product **15** in a quantitative yield.[75,76] A toluenethiol group was then introduced at the anomeric position using MePhSH in the presence of $BF_3.OEt_2$.[77] The remaining acetates were then cleaved under Zemplén conditions to give **17**.[78]



Scheme 12 Synthesis of the L-[$^{13}C_6$]fucosyl donor **22**, with a yield of 41% over 8 steps. These structures in red are derived from L-[U-$^{13}C_6$]galactose **14**, in which the six sugar carbons are uniformly ^{13}C-labelled. i. NaOAc, Ac_2O (140 °C, 30 min, 99 %) ii. MePhSH, $BF_3.OEt_2$, DCM (rt, 2 h, 98 %) iii. NaOMe, MeOH (rt, 30 min, 100 %) iv. TBDMSCl, pyridine (0 °C - rt, 4 h, 95 %) v. BnBr, NaH, DMF (rt, 2 h, 79 %) vi. Bu_4NF, THF (rt, 16 h, 96 %) vii. TsCl, pyridine (rt, 3 h, 94 %) viii. $LiAlH_4$, THF (reflux, 2 h, 61 %).

As before, these initial steps proved to be high yielding. The subsequent introduction of the TBDMS silyl ether protecting group was then achieved, giving **18** in a yield of 95%.[81] Benzylation was carried out with BnBr and NaH[82,83] before removal of the TBDMS group with Bu_4NF,[81] with yields of 79% and 96% for compounds **19** and **20** respectively. The tosyl leaving group was then introduced using TsCl in pyridine[79] to give **21**, before reduction with $LiAlH_4$ in THF to give the deoxy sugar **22**.[84] These last 2 steps were also similar in yield as in our previous trials, at 94% and 61% respectively. This furnished us with 0.42 g of the L-[$^{13}C_6$]fucosyl donor **22** in an overall yield of 41% from the L-[U-$^{13}C_6$]galactose starting material **14**.

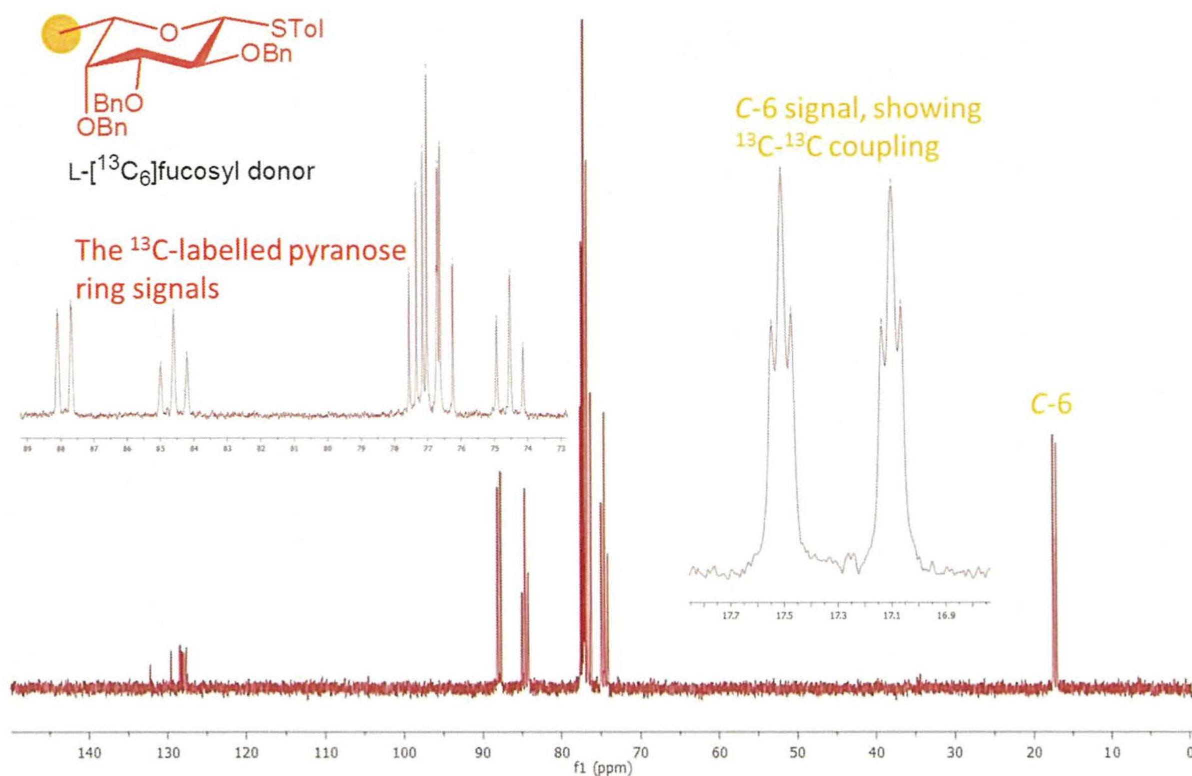


Figure 22 ^{13}C Spectrum of L-[$^{13}C_6$]fucosyl donor **22**. ^{13}C-^{13}C coupling is visible for the ^{13}C-labelled positions, whose signals appear strongly in the spectrum.

NMR of the ^{13}C-labelled compounds contained some interesting complications due to the emergence of ^{13}C-^{1}H and ^{13}C-^{13}C coupling in the ^{1}H and ^{13}C spectra respectively. In the ^{13}C spectrum (**Figure 22**), the methyl signal from the *C*-6 is clearly visible as a doublet due to the ^{13}C-^{13}C coupling, which would not be seen in a ^{13}C spectrum of an unlabelled compound due to the low natural abundance of ^{13}C. These experiments were run with a low number of scans (64) and showed strong sugar signals compared to weaker signals from unlabelled, aromatic signals from the protecting groups and toluenethiol. While the experiments were ^{13}C-^{1}H decoupled, ^{13}C-^{13}C coupling was clearly visible in the spectrum.

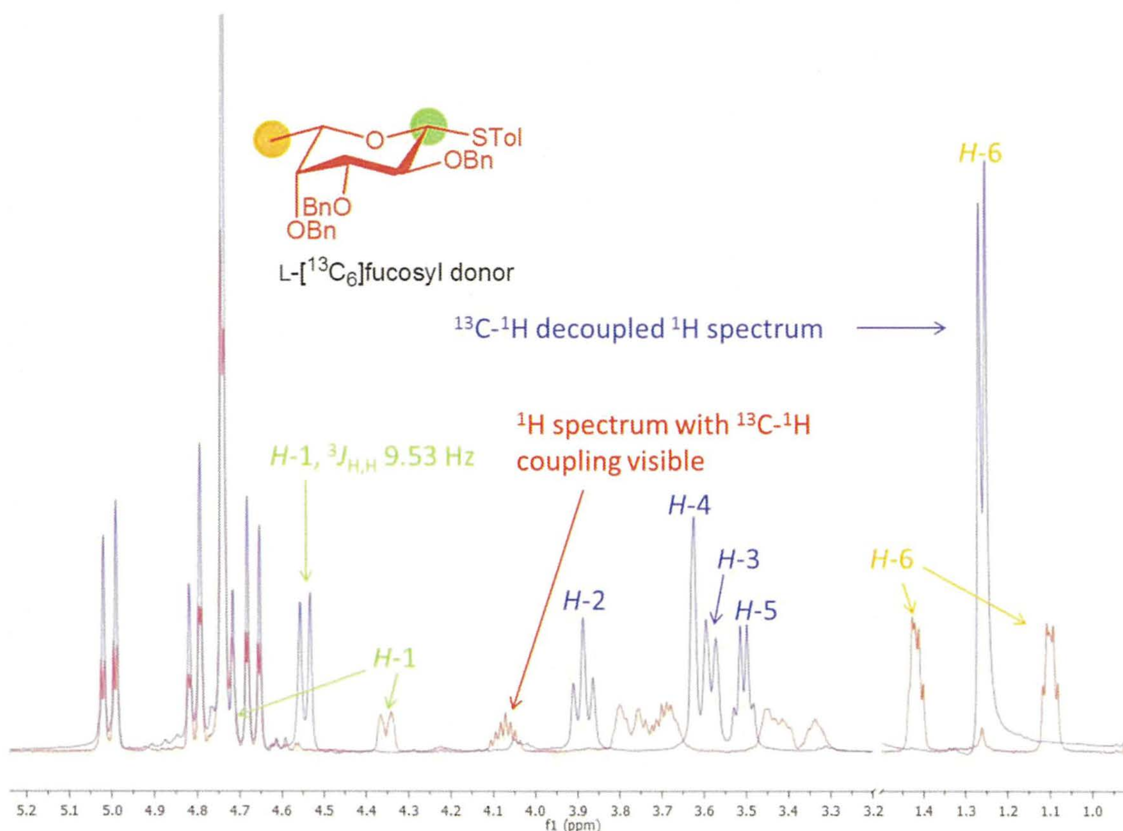


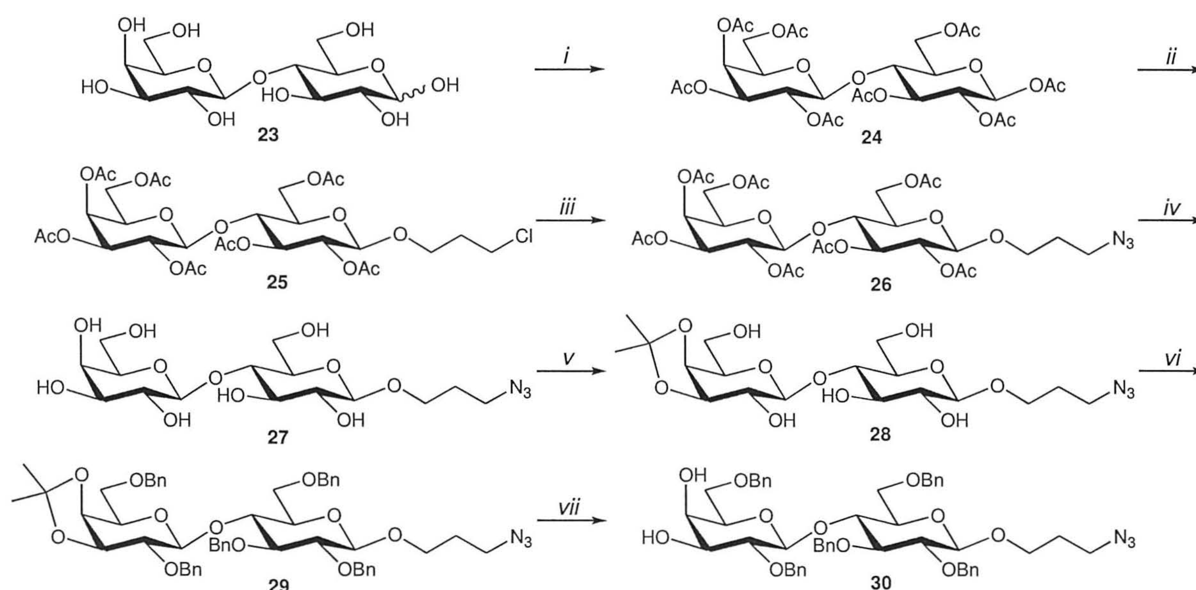
Figure 23 ^{1}H spectra of L-[$^{13}C_6$]fucosyl donor **22.** ^{13}C-^{1}H coupling is visible at the ^{13}C-labelled positions in the standard ^{1}H experiment, while a ^{13}C-^{1}H decoupled experiment was run and compared to the spectrum for the unlabelled analogue of donor **22**.

In the ^{1}H spectrum of the labelled material, ^{13}C-^{1}H couplings could be seen, with the signals originating from hydrogens attached to the ^{13}C isotopes exhibiting complex coupling patterns (**Figure 23**). Signals from the protecting groups remained unchanged. A ^{13}C-^{1}H decoupled experiment was also run to obtain ^{1}H spectra without the ^{13}C-^{1}H coupling present. By comparing the decoupled spectrum of the L-[$^{13}C_6$]fucosyl donor **22** to the ^{1}H spectrum with ^{13}C-^{1}H coupling, the decoupled signals can be seen in between the broader signals from the ^{1}H spectrum without decoupling and are in agreement with spectra obtained for the unlabelled analogue of donor **22** as well as reported spectra.[85] The $^3J_{H,H}$ value of 9.5 Hz indicates that this is the β-anomer.

2.6 Synthesis of the Lactoside Acceptor

A lactoside acceptor was required, with a linker which could be used for conjugation after a final deprotection. An azide was chosen for this purpose as it could then be converted to an amine during the hydrogenation required to remove the benzyl ether protecting groups, which were employed in the protection of all available hydroxyl groups except the 3'-position which was to act as the acceptor position.

The synthesis of the lactoside acceptor had been established earlier,[86] so the main objective was the synthesis to be able to produce the lactoside acceptor quickly and in a large amount, with minimal purification steps between them to limit time and solvent waste (**Scheme 13**). Lactoside acceptors **30** and **32** were purified by silica gel column chromatography to ensure they were of suitable purity as not to hamper the efficiency of any glycosylation steps to follow.



Scheme 13 Synthetic route towards a lactoside acceptor. These steps were conducted on a large scale in 2 batches from compound **23** (50 g, 0.15 mol per batch) to give the diol acceptor **30** in a 25% overall yield (65g, 0.074 mol combined) i. NaOAc, Ac_2O (140 °C, 30 min) ii. $Cl(CH_2)_3OH$, $BF_3.OEt_2$, DCM (rt, 2 h) iii. NaN_3, KI, DMF (50 – 140 °C, 2h) iv. NaOMe, MeOH (rt, 3 h) v. 2,2-DMP, *p*TSA, acetone/DMF 4.7:1 (rt, 6 days) vi. BnBr, NaH, DMF (0 °C – rt, 135 min) vii. TFA/H_2O 9:1, DCM (rt, 15 min).

The D-lactose starting material **23** (50 g, 0.15 mol) was acetylated in Ac_2O with NaOAc.[75,76] This gave the expected β-anomer **24** as the major product, which was purified by recrystallization in ethanol. A BF_3 – promoted glycosidation using boron trifluoride diethyl etherate ($BF_3.OEt_2$) was performed with the peracetylated compound **24** and 3-chloro-1-propanol to install the linker.[87] Substitution to give the terminal azide

as compound **26** occurred readily by heating **25** in DMF with potassium iodide (KI) to give the iodide intermediate, before adding sodium azide (NaN_3).[88] Some partial deacetylation during this sequence accounted for the appearance of deprotected material on TLC, though this material could also be taken to the next step which was a Zemplén deacetylation with NaOMe in MeOH to remove the remaining acetate protecting groups to give compound **27**.[78] More efficient conditions for the introduction of azide groups have been reported by using biphasic conditions[89] or silicate intermediates,[90] but the performed reaction was straightforward and provided sufficient material to proceed.

It was then necessary to temporarily protect the 3' and 4'-positions before adding benzyl ether protecting groups onto the remaining positions. Protection of these positions has been reported in galactose[91] and lactose[92,82] using isopropylidene acetal protecting groups. Acetone or 2,2-dimethoxypropane (2,2-DMP) can be used as reagents for the acetal formation, with an acid catalyst such as *p*-toluenesulfonic acid (*p*TSA).[93] Under these conditions the formation of the acetal is reversible, with the thermodynamic product being formed preferentially. Lactoside **27** was dissolvent in a mixture of acetone/DMF (4.7:1) with *p*TSA as an acid catalyst. As this reaction proved to be slow, 2,2-DMP was added every 24 h to act as a water scavenger and to push the equilibrium towards the isopropylidene protected lactoside **28**. Subsequent benzylation was performed in DMF using NaH as a base in the presence of benzyl bromide.[82,83] The isopropylidene protecting groups were then removed by hydrolysis in acidic conditions using trifluoroacetic acid and water (TFA/H_2O 9:1)[92] to give the lactoside acceptor **30**. These steps were repeated with the same amount of D-lactose starting material **23** to provide 65 g of compound **30**, at an overall yield of 25%.

The resulting lactoside acceptor **30** had free hydroxyl groups on both the 3' and 4'-positions. It has be shown that glycosylation reactions between this acceptor and glycosyl donors regioselectively favours the 3'-position,[65,66,86,94] though glycosylation at the 4'-position can also occur.[94] This was an issue in a previous synthesis of a Le^b hexasaccharide where glycosylation between this lactoside acceptor and a donor produced equal amounts of the β-1→3 and β-1→4 products (**Figure 24**).[66]

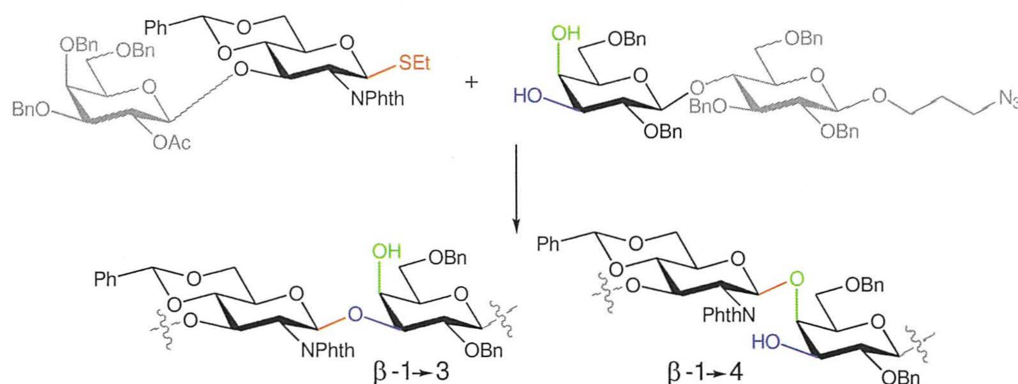
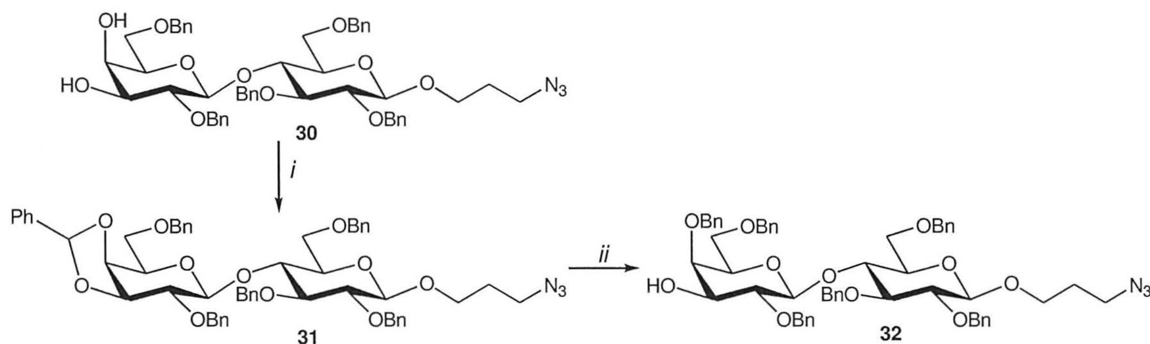


Figure 24 Glycosylation with a diol lactoside acceptor, adapted from a study by Lahmann *et al.*[66] The β-1→3 and β-1→4 products were produced in equal amounts from the glycosylation between this combination of donor and acceptor.

It was assumed that conformational strain caused by the benzylidene acetal may have been the source of the loss of regioselectivity. Thus, reductive ring opening of the acteal and subsequent acetylation of the free hydroxyl group in the donor was undertaken to improve the regioselectivity of the following glycosylation. An alternative is to form a benzylidene acetal at the 3' and 4'-positions, as regioselective reductive ring opening has been shown open the ring to give a benzyl ether at the 4'-position and the required 3'-hydroxyl group (**Scheme 14**).[67,82,95]



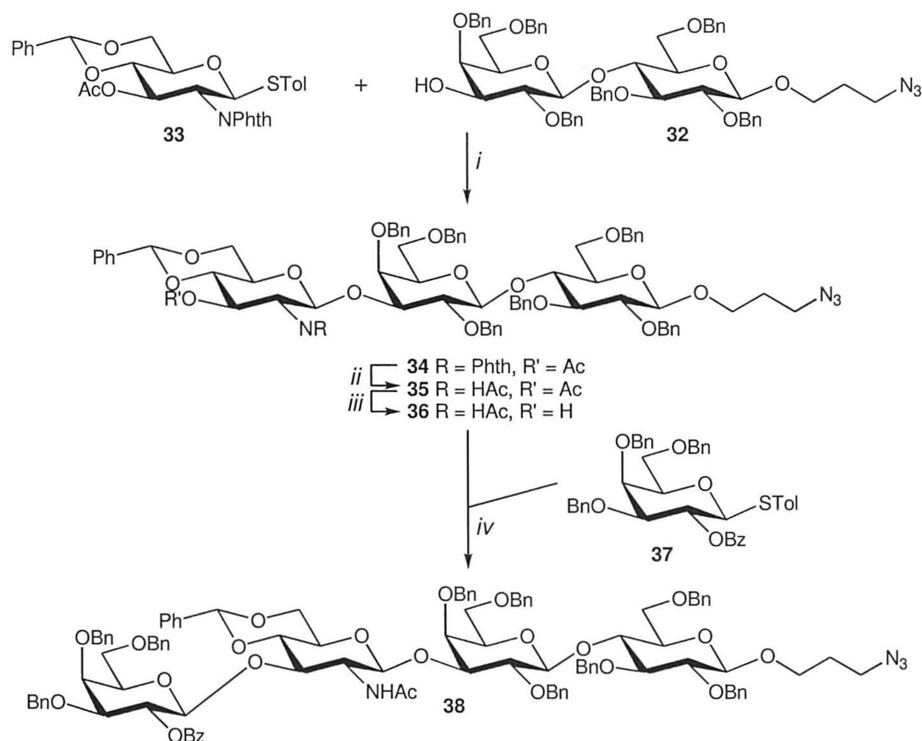
Scheme 14 Addition and reductive ring opening of a benzylidene acetal to give the lactoside acceptor **32**. i. $PhCH(OMe)_2$, *p*TSA, THF (rt, 2 h, 94%) ii. $NaBH_3CN$, HCl/Et_2O, THF (rt, 70 min, 95%)

This approach was chosen for the lactoside donor. A benzylidene acetal was first introduced into the molecule using benzaldehyde dimethyl acetal ($PhCH(OMe)_2$) with *p*TSA to give the protected lactoside **31** in a 94% yield. A system of sodium cyanoborohydride/hydrogen chloride ($NaBH_3CN/HCl$) has been reported to be compatible with benzyl ethers[96,97], and was selected the reductive ring opening, giving the benzylated lactoside acceptor **32** with a free hydroxyl only at the 3'-position in a 95% yield. The position of the free hydroxyl was confirmed by performing a

benzoylation on acceptor **32** in pyridine[98] and comparing the ^{1}H NMR with that of **32**, with a clear change in the position of the 3'-signal.

These last two steps were repeated to provide a sufficient amount of the acceptor **32** to begin performing glycosylation reactions with the glycosyl donors.

2.6.1 Synthesis of Tetrasaccharide Backbone



Scheme 15 Synthesis of the fully protected tetrasaccharide backbone **38**. i. NIS, AgOTf, DCM (rt, 20 min, 58%) ii. a. EDA, EtOH (reflux, 5 h) b. Ac_2O, pyridine (rt, 16 h, 72%) iii. NaOMe, MeOH/DCM 7:1 (rt, 2 h, 98%) iv. NIS, AgOTf, DCM (rt, 40 min, 81%).

The glycosidation between the lactoside acceptor **32** and *N*-phthalimido-glucosyl donor **33** (**Scheme 15**) was performed using *N*-iodosuccinimide/silver triflate (NIS/AgOTf)[99,28,100] as a promoter. Only the β-product was observed due to the directing effect of the *N*-phthalimido group. At 83%, the yield was encouragingly high when performed on the small scale with 0.44 mmol of lactoside **32**. However, the yield remained modest at 58% when the reaction was scaled up to 2.28 mmol of lactoside **32**, with hydrolysed donor being visible by TLC. It was suspected that this may have been due to the quality of the AgOTf used.

The next step was to remove the *N*-phthalimido protecting group before acetylating to give an acetamido moiety. This was achieved through the use of a diamine to attack the

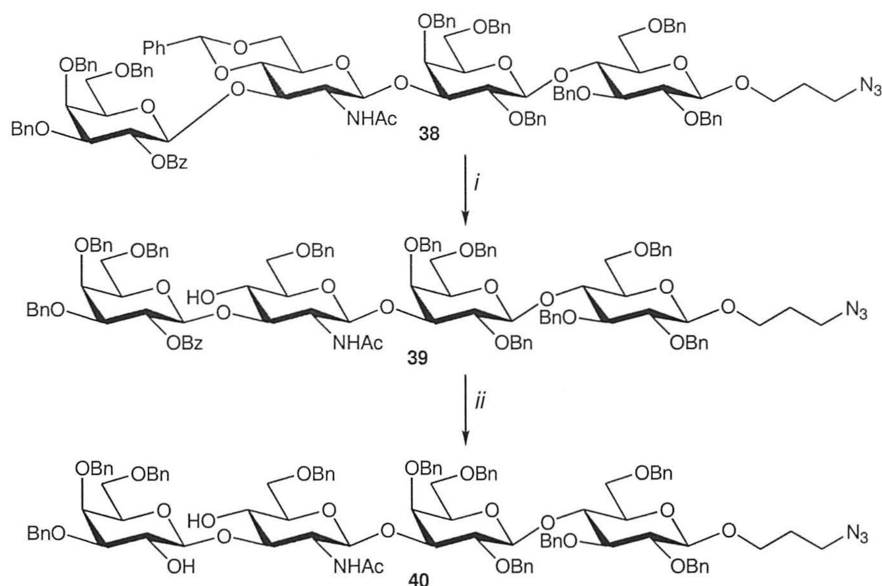
phthalimido carbonyls, opening up the ring to leave the desired glycosyl amine. Hydrazine can be used for this process[101], though due its toxicity and instability, the use of other reagents, such as ethylenediamine (EDA)[102], has been reported. The *N*-phthalimido group on trisaccharide **34** was removed by refluxing in ethanol (absolute) and EDA. TLC indicated a successful deprotection and the resulting amine was then acetylated in a pyridine/acetic anhydride (pyridine/Ac_2O)[98] mixture before purification by column chromatography gave the acetylated product **35** in a 72% yield over these two steps. The 3-*O*-acetyl protecting group was then be removed by a Zemplén deacetylation[78] to give the trisaccharide **36** required for the next coupling at a 98% yield.

The coupling between the galactosyl donor **37** and the trisaccharide acceptor **36** was performed, as before, using an NIS/AgOTf system as the promoter, affording the tetrasaccharide **38** in a yield of 81%. These steps were repeated to give a comfortable amount of tetrasaccharide **38** to work with (3.85 g, 2.15 mmol).

2.6.2 Synthesis of the Le^b Hexasaccharide

Before producing ^{13}C-labelled hexasaccharides using the valuable [$^{13}C_6$]fucosyl donor **22**, it was decided to produce unlabelled material to assess the efficiency of the approach and to acquire spectroscopic information for the molecule.

With the tetrasaccharide **38** synthesised, it was possible to remove the temporary protecting groups ready for the final coupling reactions with the fucosyl donors (**Scheme 16**). The chosen approach was to open the benzylidene acetal ring by reductive ring opening to give **39**, followed by debenzoylation to give tetrasaccharide **40** with both positions unprotected and available for coupling.



Scheme 16 Deprotection to give the tetrasaccharide acceptor **40**. i. $NaBH_3CN$, HCl/Et_2O, THF (rt, 100 min, 85%) ii. NaOMe, MeOH/DCM 7:1 (rt-40 °C, 64 h, 88%)

Reductive ring opening of 4,6-benzylidene acetals has proven to be a useful reaction in carbohydrate protection, as changing the reagents and reaction conditions changes the regioselectivity of the opening[103], yielding either the free 4-OH or 6-OH group, with the other position protected by a benzyl ether. Early findings demonstrated that systems of lithium aluminium hydride with aluminium trichloride ($LiAlH_4$-$AlCl_3$) were effective in opening benzylidene acetals, with the regioselectivity varying when changing other substituents.[104,105] Since then, a plethora of reagents have been developed for this purpose. A more recent publication demonstrated that by using cyanuric chloride as a catalyst, using sodium borohydride ($NaBH_4$) gives the 6-position free[106] while using sodium cyanoborohydride ($NaBH_3CN$) as a reducing agent yields the free 4-position.[107] This regioselective control negates the need for inclusion of other protecting group steps. Systems of $NaBH_3CN$/HCl applied to 4,6-*O*-benzylidene protected hexopyanosides have been shown to give the free 4-OH in high yields and to be compatible with benzyl ether and N-acetyl groups[96,97]. Therefore, tetrasaccharide **38** was dissolved in tetrahydrofuran (THF), while using molecular sieves to ensure water was excluded from the reaction. $NaBH_3CN$ and a hydrogen chloride solution (2M in Et_2O) were then used to open up the benzylidene acetal ring to give product **39** with a free hydroxyl at the 4''-position with an 85% yield. Debenzoylation was undertaken using Zemplén conditions, as described earlier for the deacetylation.[78] This reaction did however take a substantially longer time and required heating to 40 °C. This gave the 2''',4''-deprotected tetrasaccharide acceptor **40** in a 88% yield.

The remaining coupling reaction in this approach was between tetrasaccharide acceptor **40** and the fucosyl donor **41** to give two new α-linkages. This was attempted using conditions described by Lemieux,[13] in which *O*-benzylated-glycosyl bromide donors and suitably protected acceptors proved to be highly stereoselective towards the formation of α-*O*-glycosidic linkages.

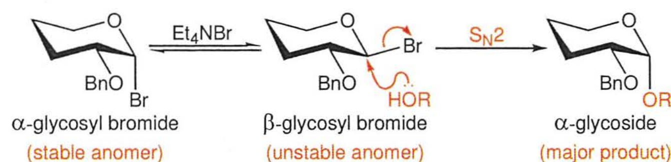
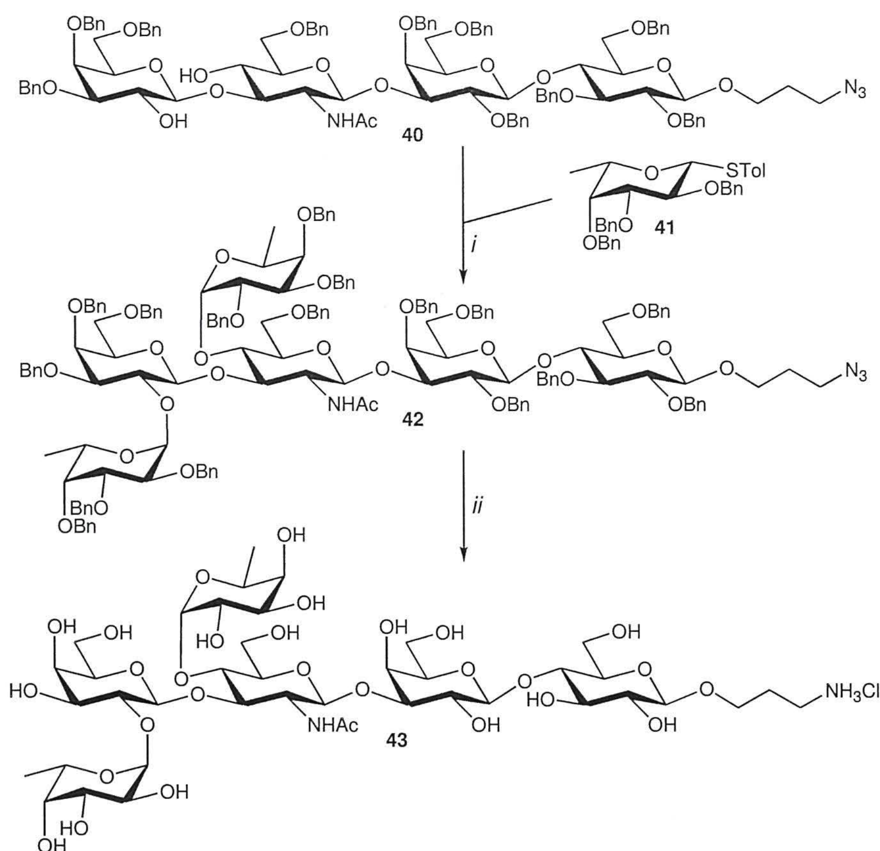


Figure 25 Halide assisted coupling in the absence of a participating group. The more reactive β-anomer undergoes nucleophilic attack by an S_N2 mechanism to give the α-glycoside. Adapted from Lemieux[13] and Kaeothip.[108]

The proposed mechanism for this reaction is that, by using a tetraethylammonium bromide (Et_4NBr) as a source of bromide ions, the glycosyl bromide undergoes a rapid equilibrium between the α-glycosyl bromide and the more thermodynamically unstable β-anomer (**Figure 25**). The glycosyl acceptor will then favour reacting with the β-anomer following an S_N2 type process, giving an inversion of stereochemistry and so yielding an α-*O*-glycosidic linkage[108].

Due to the versatility of thioglycosides as donors, it is straightforward transforming them to the corresponding glycosyl bromide by treating with bromine prior to glycosidation[109,9]. This also presents the opportunity for orthogonal glycosylation with other thioglycosides, provided that the glycosyl bromide is isolated and all residual bromine is removed, either by evaporation or the use of an alkene, before using alongside thioglycosides[109,110]. In this synthesis, this kind of orthogonal strategy was not necessary. Also, it was desirable to limit the risk of hydrolysis of our labelled L-[$^{13}C_6$]fucosyl donor **22** prior to the glycosylation reaction, so instead a one-pot procedure was followed.[71] Here the unlabelled fucosyl donor **41** was transformed to the corresponding bromide in the presence of the tetrasaccharide acceptor **40**, with the glycosylation facilitated by Et_4NBr. This proved successful in giving the hexasaccharide **42** in a 80% yield.



Scheme 17 Coupling with fucosyl donors, followed by deprotection to give the Le[b] hexasaccharide. i. Et_4NBr, Br_2, DCM/DMF 9:1 (rt, 16 h, 80%) ii. Pd/C, H_2, $HCl_{(aq)}$, dioxane/H_20 3:1 (rt, 20 h, 82%)

Deprotection to remove the *O*-benzyl ether protecting groups and hydrogenation of the azide to the amine[88] was carried out with a palladium/carbon (Pd/C wt.10%) catalyst under an atmosphere of hydrogen. An equivalency of $HCl_{(aq)}$ was added to protonate the resulting amine to avoid poisoning the catalyst. Only one product spot was visible by TLC, however the material was run through a size exclusion column (Bio-Gel® P-2, 3.5 W x 75 H, butanol (1%) in ultra-pure water as the mobile phase) to desalt and ensure that the hexasaccharide was isolated. From this sequence, 8.5 mg of the deprotected hexasaccharide **43** was obtained in a 82% yield (**Scheme 17**).

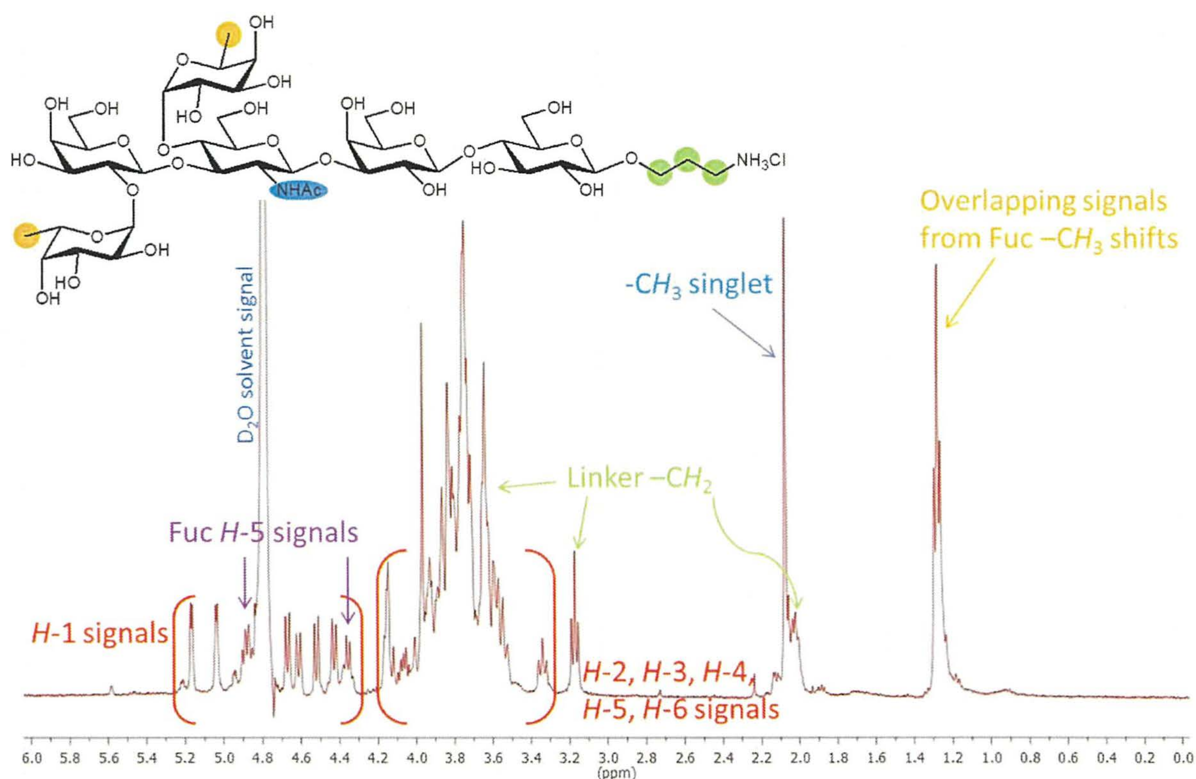


Figure 26 ^{1}H spectrum of the unlabelled Le^b hexasaccharide **43**. The $-CH_3$ signals from the fucosyl residues and GlcNAc can be seen, as can the $-CH_2$ signals from the linker. The majority of the H-2 to H-6 sugar signals are overlapping with shifts between 3.3 and 4.2 ppm, though the individual H-1 signals and the two fucosyl H-5s can be isolated.

^{1}H NMR of the hexasaccharide showed the presence of all six H-1 signals, the linker, and the methyl signals from the GlcNAc and fucosyl moieties (**Figure 26**). Though many of the other sugar signals were overlapping, these could be individually seen using a 2D HSQC technique and are in agreement with values reported in the literature.[66]

1D TOCSY was also employed, in the hope of gaining more information in the area of the spectrum with overlapping signals. **Figure 28** shows the 1D TOCSY spectra generated by focusing on the six H-1 signals individually. While we were unable to gather much more information from the crowded area of the spectra, we were able to see the H-6 $-CH_3$ signals from the fucosyl residues. Thus, we were able to confirm which H-1 signals belong to the fucosyl residues, as well as identifying both the fucosyl H-5 signals in the same region occupied by the anomeric signals.

A variant of INEPT NMR, CLIP-HSQC, was also run to confirm the stereochemistry of the glycosidic linkages (**Figure 27**). This provided the $^1J_{C,H}$ coupling constants for the anomeric positions and, together with the $^3J_{H,H}$ values obtained from the ^{1}H spectrum,

confirmed the presence of α-Fuc linkages, with β-linkages for the other four glycosidic bonds.

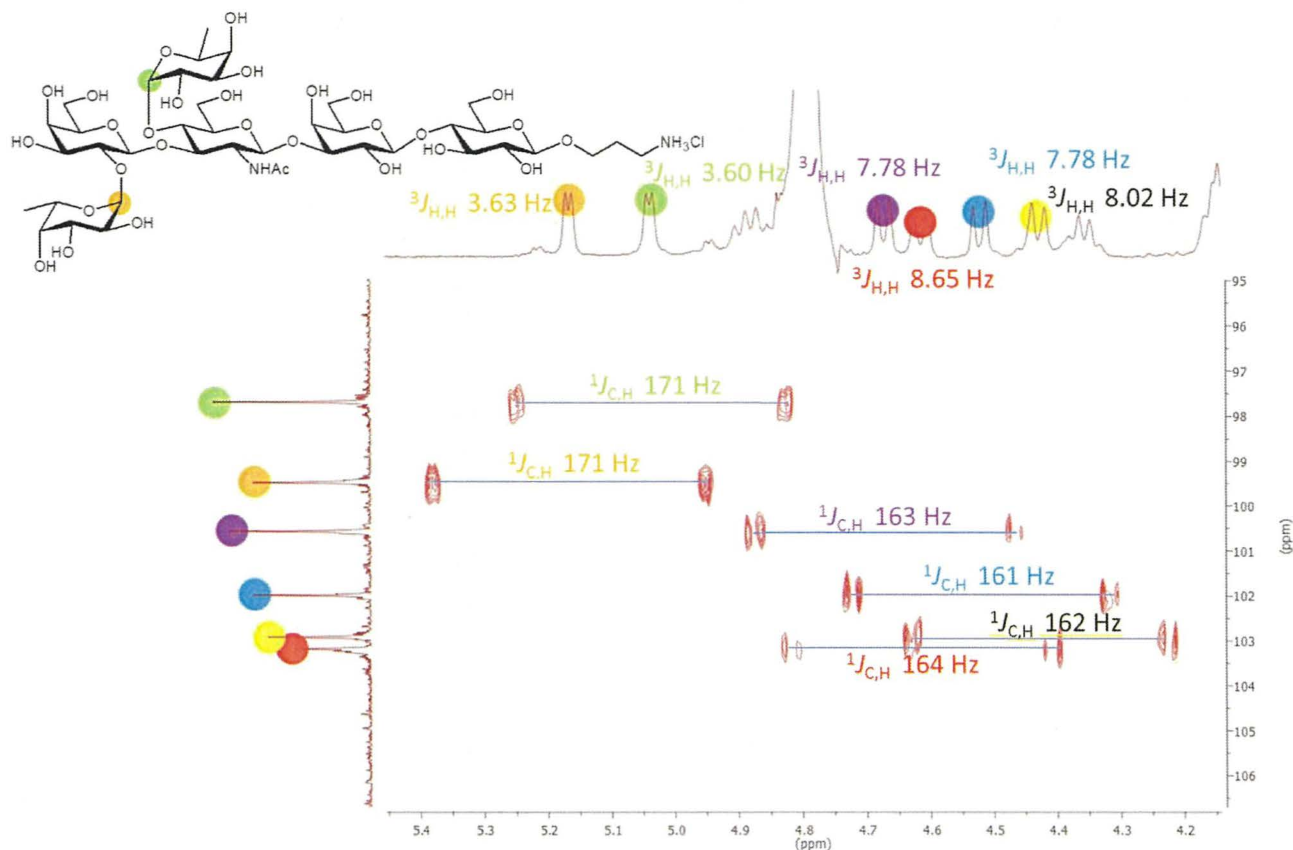


Figure 27 CLIP-HSQC of the unlabelled Le^b hexasaccharide **43** showing the signals from the anomeric *H*-1 positions. The values of the $^1J_{C,H}$ and $^3J_{H,H}$ coupling constants can be used to assign the stereochemistry at these positions, with the values of $^1J_{C,H}$ 171 Hz and $^3J_{H,H}$ 3.63 and 3.60 for the fucose residues confirming that these are α linkages. The four other *H*-1 signals are the result of β linkages, with lower $^1J_{C,H}$ and higher $^3J_{H,H}$ values.

The successful performance of the reaction sequence from the tetrasaccharide **40** to the deprotected hexasaccharide **43** showed the efficacy of these synthetic steps, and could then be repeated using the ^{13}C-labelled fucosyl donor **22** to give the labelled hexasaccharides.

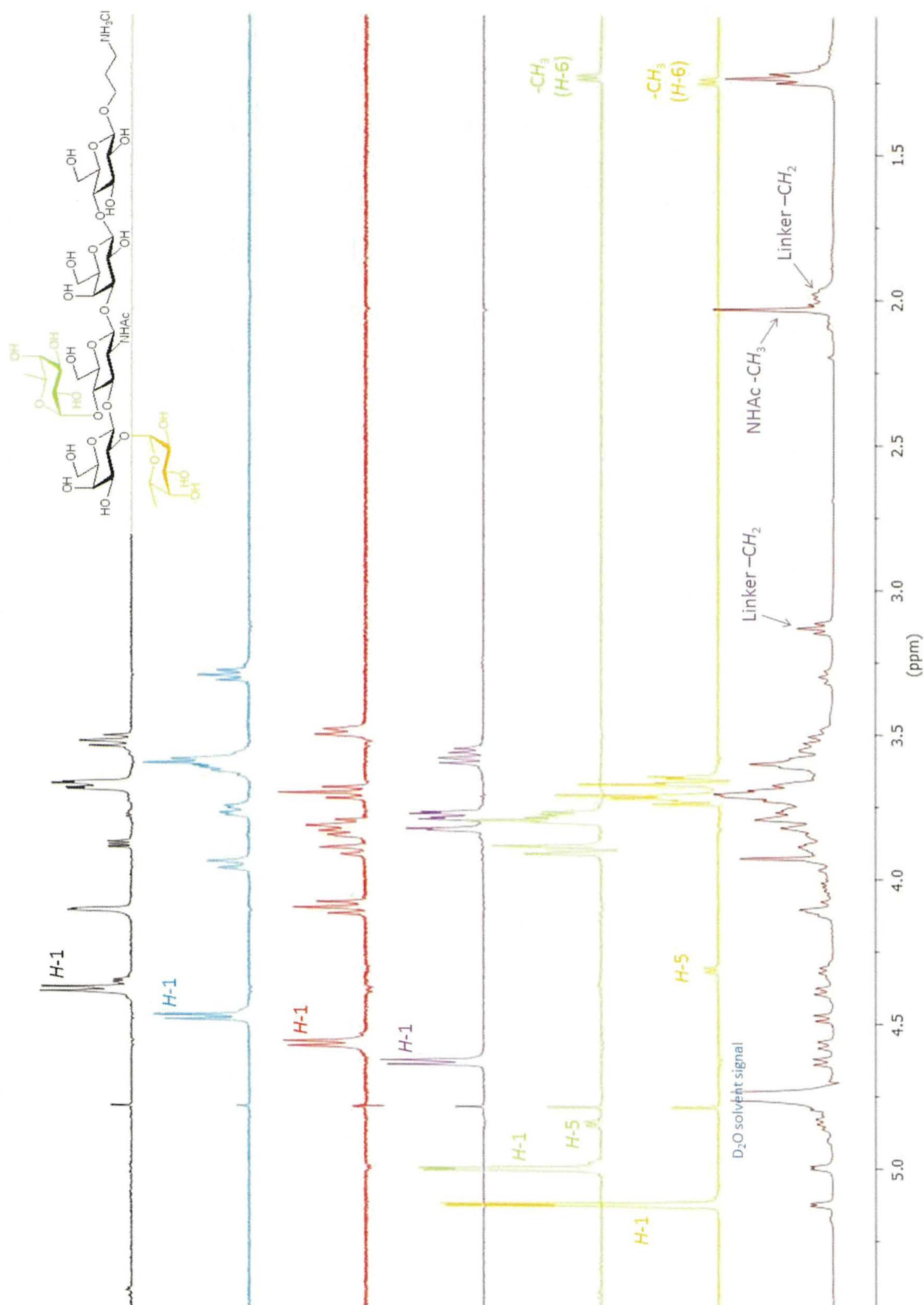
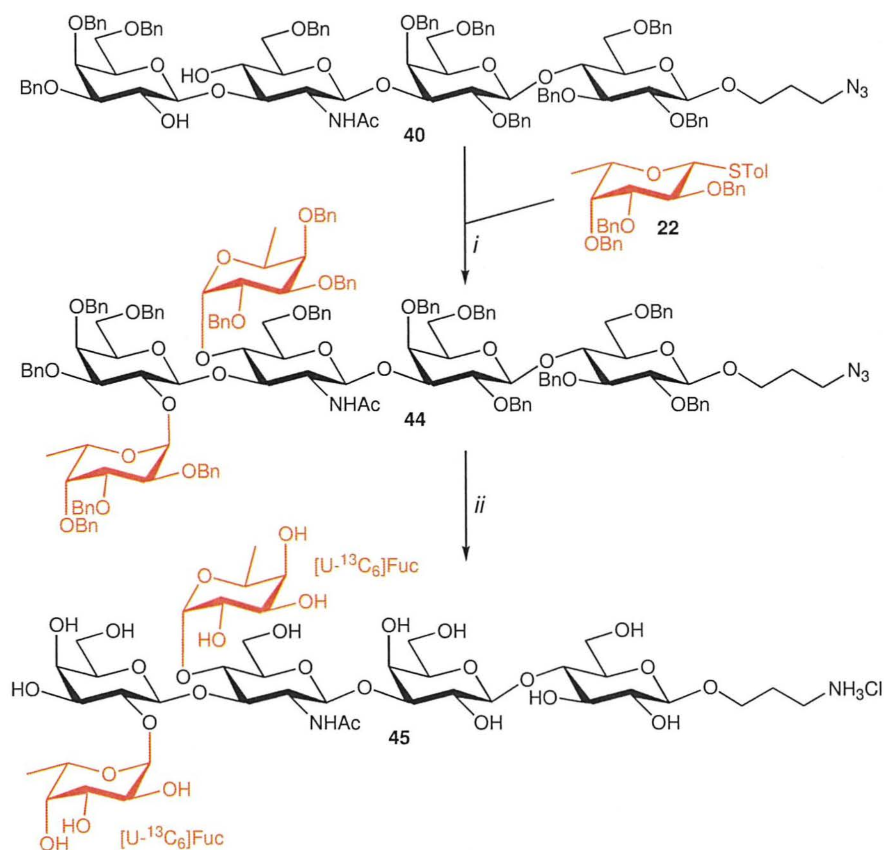


Figure 28 1D TOCSY experiments with the Le[b] hexasaccharide **43**. The anomeric signals were targeted to generate spectra for each individual sugar moiety and then compared to the overall ^{1}H spectrum (bottom spectrum). The *H*-1 signals for the two fucosyl residues are seen in the same spin-systems as their *H*-6 –CH_3 signals.

2.6.3 Synthesis of a ^{13}C-Labelled Le^b Hexasaccharide

The tetrasaccharide acceptor **40** was then used to produce the Le^b hexasaccharide containing two ^{13}C-labelled fucosyl moieties, by performing a glycosylation with the [$^{13}C_6$]fucosyl donor **22** and subsequent deprotection, using the same approach described in the previous section.



Scheme 18 Glycosylation between the L-[$^{13}C_6$]fucosyl donor **22** and tetrasaccharide acceptor **40** to give the ^{13}C-labelled Le^b hexasaccharide. i. Bu_4NBr, Br_2, DCM/DMF 9:1 (rt, 16 h, 52%) ii. Pd/C, H_2, $HCl_{(aq)}$, dioxane/$H_2$0 3:1 (rt, 40 h, 26%)

The coupling to give the benzyl ether protected hexasaccharide **44** by a halide-assisted coupling conditions was carried out by adding bromine to a mixture of the diol tetrasaccharide acceptor **40**, [$^{13}C_6$]fucosyl donor **22** and Bu_4NBr in an inert atmosphere. Unfortunately this gave a disappointing yield of 52% for hexasaccharide **44**. The deprotection step was also more problematic than for the un-labelled material, with loss of some compound due to degradation to a pentasaccharide. The hydrogenolysis was carried out by dissolving the protected material **44** in a solution of 1,4-dioxane and water and hydrogenating in the presence of a Pd/C catalyst at atmospheric pressure. A P-2 size exclusion column (Bio-Gel® P-2, 3.5 W x 75 H) was run with a solution of

butanol (1%) in ultra-pure water as the mobile phase. The solvent was eluted at a rate of 36 ml/h and collected using a Gilson fraction collector (5.5 ml per test tube). TLC and MALDI TOF of the sugar containing fractions showed the presence of a pentasaccharide by-product in many of the fractions that also contained the hexasaccharide **45**. An additional purification step was therefore carried out using a reversed phase column (RP-C18). The fractions were checked using TLC and MALDI TOF, and the fractions containing the hexasaccharide **45** without impurities were combined and lyophilized to give the product in a 26% yield.

^{1}H NMR of the target molecule **45** showed the emergence of ^{13}C-^{1}H couplings, with splitting visible for the signals from the labelled fucosyl residues (**Figure 29**). The position of these signals were also in agreement with the signals observed in the CLIP-HSQC experiments performed on the unlabelled hexasaccharide **43**, used to obtain $^{1}J_{C,H}$ values for the anomeric protons.

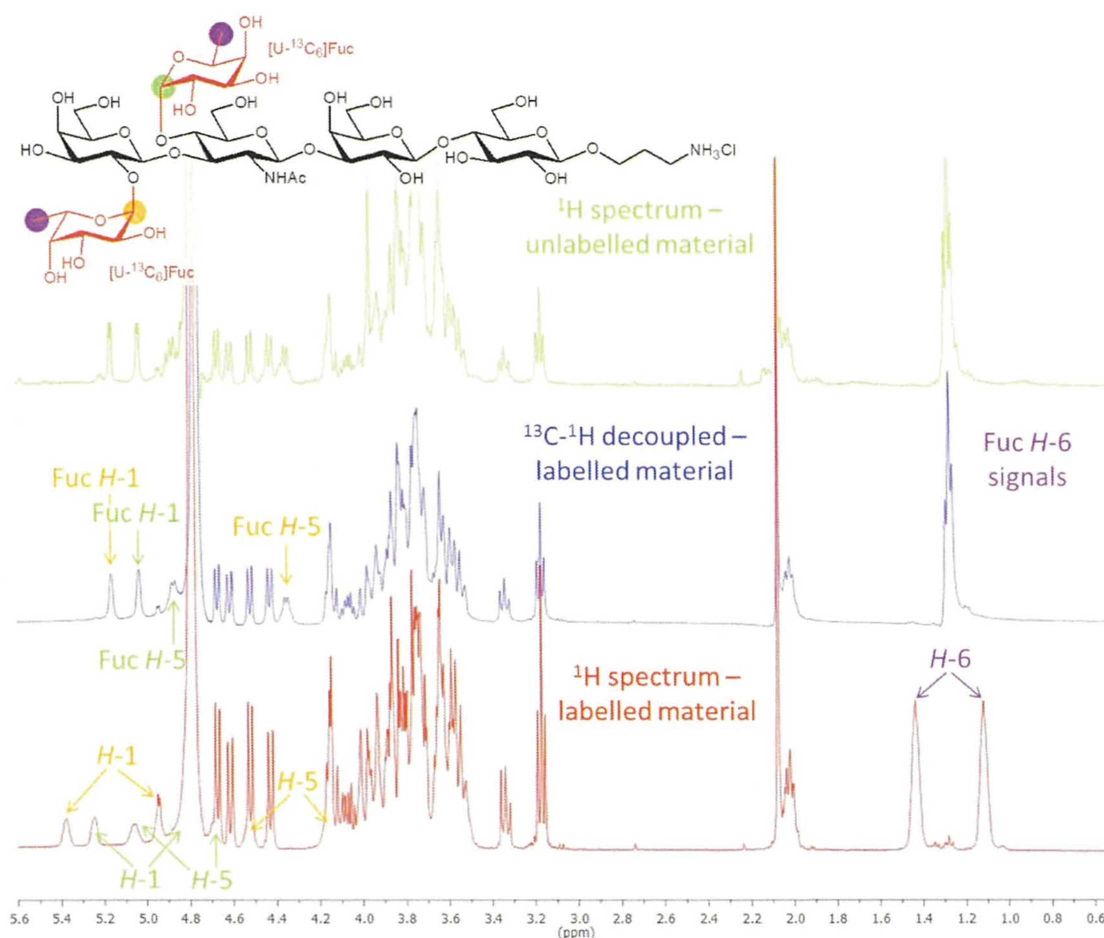


Figure 29 ^{1}H spectra of the ^{13}C-labelled Le^{b} hexasaccharide **45** with ^{13}C-^{1}H coupling visible (bottom). A ^{13}C-^{1}H decoupled experiment was also run (middle) and was in agreement with the ^{1}H spectrum from the unlabelled material **43** previously described (top).

^{13}C-^{1}H Decoupled experiments were also run, producing spectra in good agreement with the spectra obtained from the unlabelled Le^{b} hexasaccharide **43**.

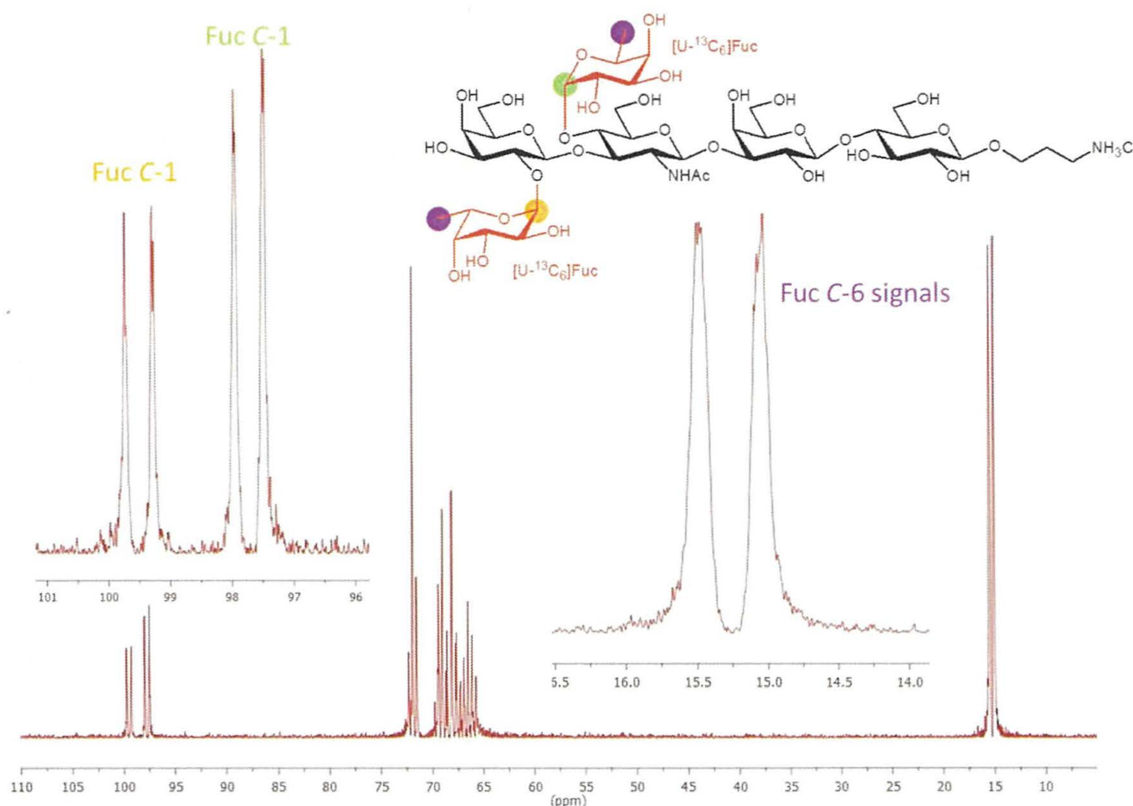
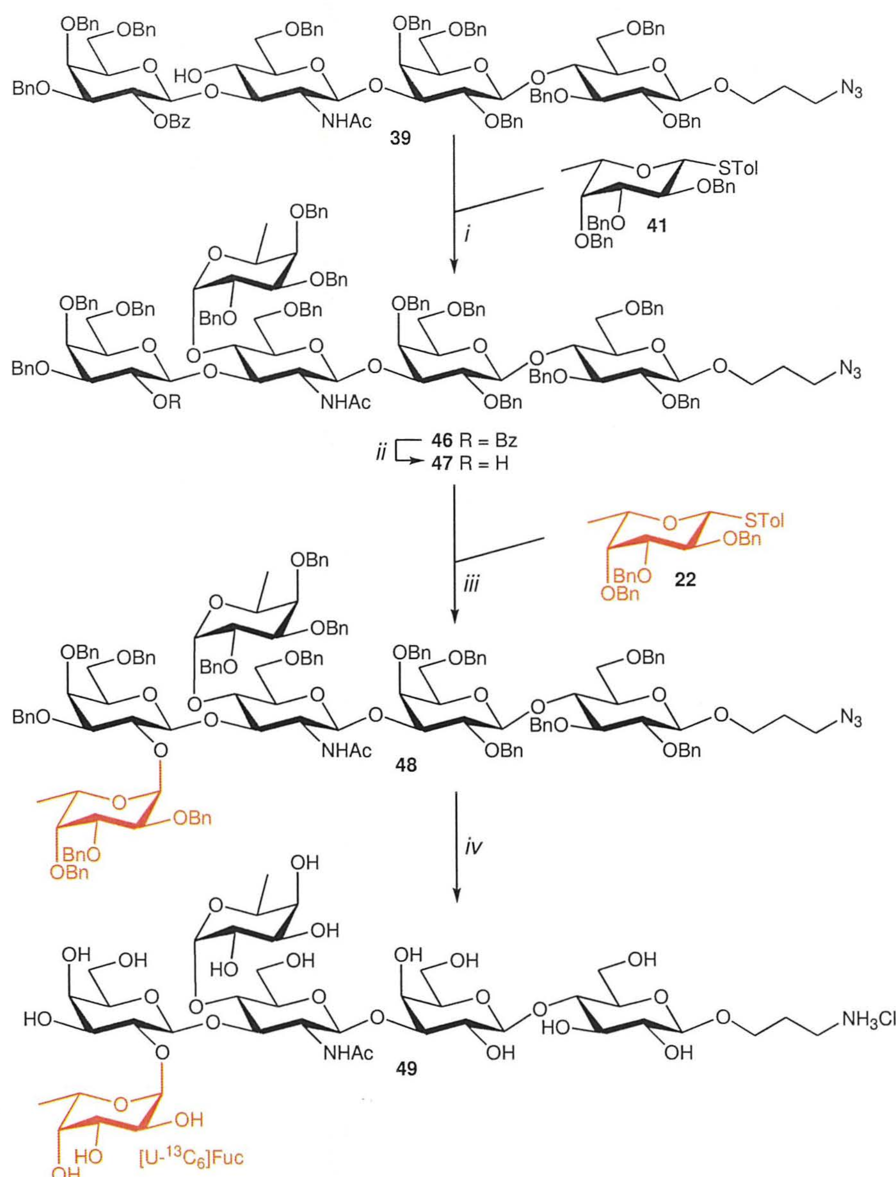


Figure 30 ^{13}C spectrum of the labelled Leb hexasaccharide **45**. Only the ^{13}C-labelled portions of the molecule are visible. A pair of doublets is seen for the anomeric carbons, while the two *C*-6 –*C*H_3 signals overlap each other to give a broad doublet.

2.6.4 Synthesis of α–L-[U-$^{13}C_6$]Fuc-(1→2)-β-D-Gal Le^{b} Hexasaccharide

We were also interested in material where only one of the fucosyl moieties was ^{13}C-labelled, in the hope that subsequent NMR binding studies may provide information about the role of the individual fucosyl moieties in the binding. As before, tetrasaccharide **38** with its 4,6-*O*-benzylidene acetal and 2-*O*-benzoyl ester still in place would be the starting point. Our aim was to perform one deprotection step followed by a halide assisted glycosylation (as described above) to give pentasaccharide structures without any ^{13}C-labelling. By completing the second temporary protecting group deprotection step it was then be possible to perform another halide assisted coupling, this time with the ^{13}C-labelled donor **22**, to give Le^{b} hexasaccharides with only one of the fucosyl residues labelled.



Scheme 19 Synthesis of a protected Le^{a} pentasaccharide en route to the α-L-[U-$^{13}C_6$]Fuc-(1→2)-β-D-Gal Le^{b} hexasaccharide. i. Et_4NBr, Br_2, DCM/DMF 9:1 (rt, 16 h, 45 %) ii. NaOMe, MeOH/DCM 7:1 (rt—40 °C, 26 h, 93 %) iii. Bu_4NBr, Br_2, DCM/DMF 9:1 (rt, 16 h, 79 %) iv. Pd/C, H_2, $HCl_{(aq)}$, Dioxane/H_2O (rt, 48 h, 97 %)

The first route taken was to produce pentasaccharide **46** with a fucosyl moiety on the *N*-acetylglucosamine residue, by performing a glycosylation with tetrasaccharide **39** with the unlabelled fucosyl donor **41**. This was done by a halide assisted coupling by converting the thioglycoside donor to halide in situ with the tetrasaccharide acceptor using bromine and Et_4NBr, to give the pentasaccharide **46** with an α-linkage at the fucosyl moiety in a 45% yield. Debenzoylation under Zemplén conditions then gave the pentasaccharide acceptor **47** in a 93% yield.

The remaining coupling with the labelled [$^{13}C_6$]fucosyl donor **22** was then carried out, again by a halide assisted coupling to give the benzylated hexasaccharide **48** with an L-

[$^{13}C_6$]fucosyl moiety at the terminal galactose in a 79% yield. The final deprotection step to remove the benzyl ether protecting groups and convert the azide to an amine was carried out by hydrogenation in the presence of a Pd/C catalyst. A solvent system of 1,4-dioxane and water was used to facilitate the change in solubility of the hexasaccharide during deprotection, with one equivalent of $HCl_{(aq)}$ added to the reaction after 15 min along with additional Pd/C to give a chloride salt with the amine to avoid poisoning the catalyst. After 2 days only one compound was visible by TLC, and MALDI TOF mass spectroscopy was run on a sample of the reaction mixture, confirming that the hexasaccharide **48** had indeed been fully deprotected to give compound **49**. Additionally, no other compounds were visible in the mass spectra obtained. The hexasaccharide was then run through a P-2 size exclusion column (Bio-Gel® P-2, 3.5 W x 75 H) as a desalting step, using a solution of butanol (1%) in ultra-pure water as the mobile phase (36 ml/h, 5.5 ml per test tube). TLC and MALDI TOF were used to ascertain which fractions contained hexasaccharide **49**, with no other sugar compounds visible in any of the fractions. The combined fractions were then lyophilised to give the deprotected hexasaccharide **49** in a 97% yield from the deprotection and purification (**Scheme 19**).

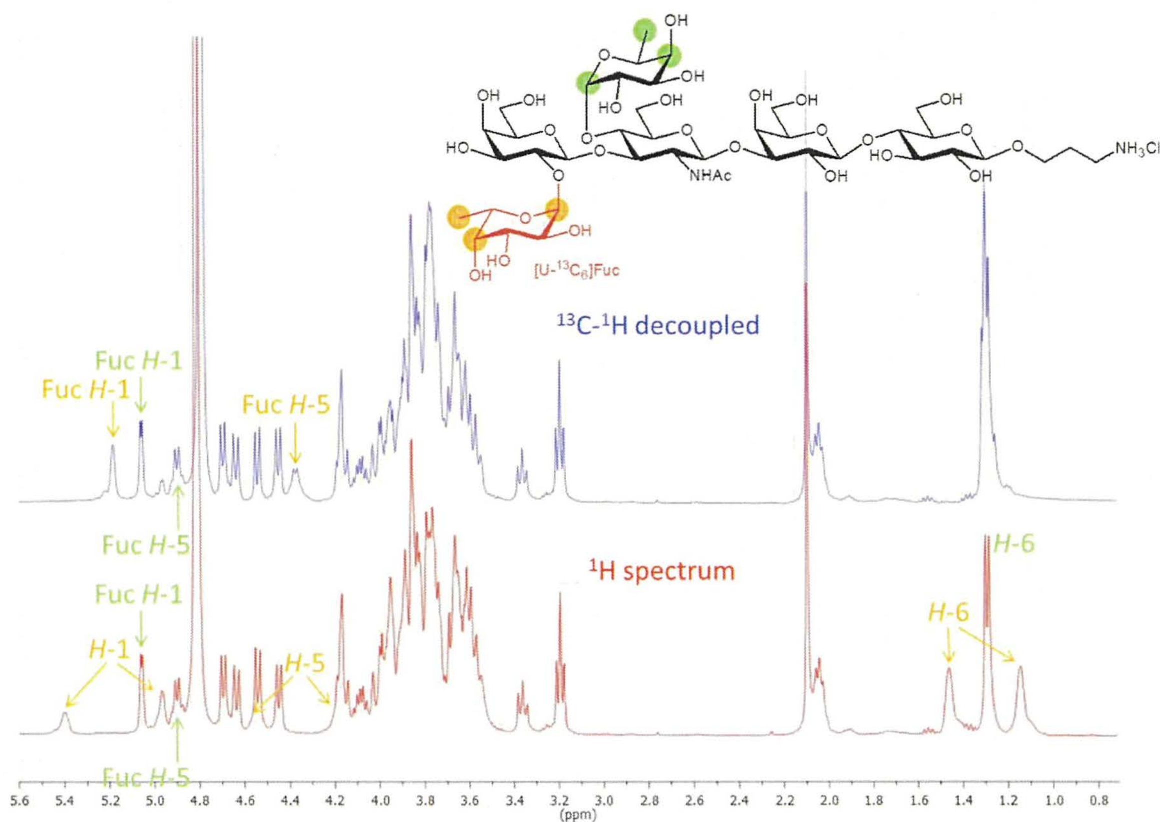


Figure 31 Proton NMR of α–L-[U-$^{13}C_6$]Fuc-(1→2)-β-D-Gal Le^b hexasaccharide **49**. The ^{13}C-^{1}H decoupled spectrum is in agreement with the spectra obtained for unlabelled material. The ^{1}H spectrum shows ^{13}C-^{1}H coupling in the signals from the ^{13}C-labelled fucosyl moiety.

^{1}H NMR is shown in **Figure 31**, with ^{13}C-^{1}H coupling visible in the signals from the α–L-[U-$^{13}C_6$]Fuc residue. ^{13}C-^{1}H Decoupled NMR was also run to compare with the spectra obtained for the unlabelled material **43**.

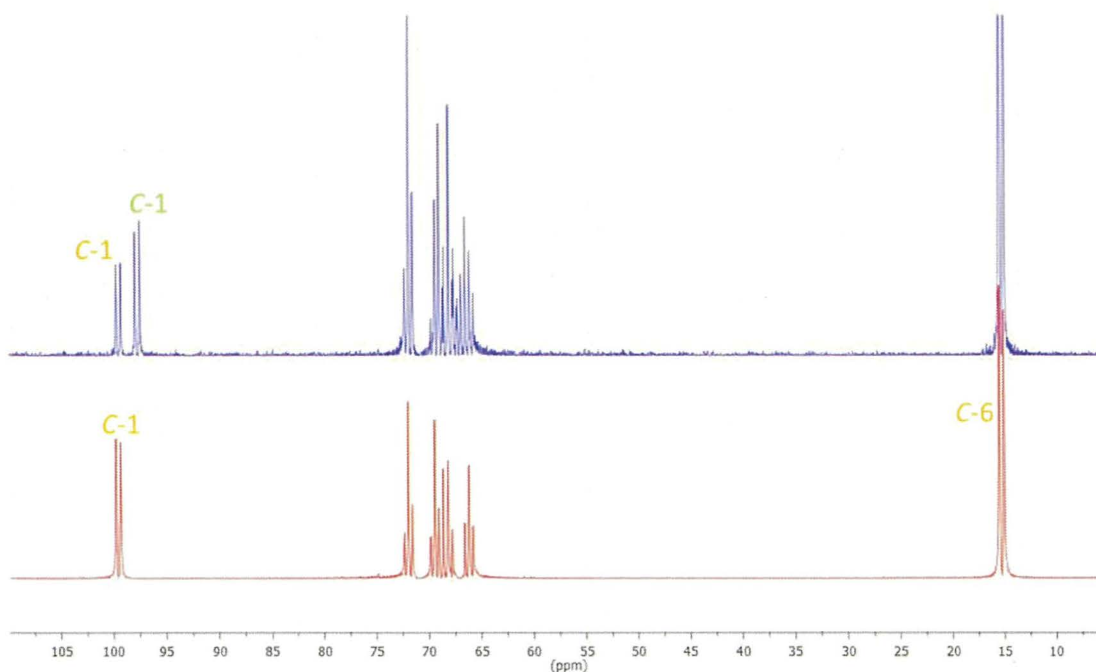


Figure 32 A ^{13}C spectrum from the α–L-[U-$^{13}C_6$]Fuc-(1→2)-β-D-Gal Le^b hexasaccharide **49** is shown (bottom) with doublets visible for the C-1 and C-6 signals. This can be compared to a spectrum from the Le^b compound **45** (top), where additional signals are seen and the *C*-6 signals overlap.

^{13}C Spectra of compound is shown in **Figure 32** and compared to the spectrum from Le^b compound. The *C*-1 and *C*-6 signals are easily identifiable, with the *C*-6 signals being shown to overlap in the Le^b hexasaccharide with two α–L-[U-$^{13}C_6$]Fuc residues.

2.6.5 Towards a Synthesis of α–L-[U-$^{13}C_6$]Fuc-(1→4)-β-D-GlcNAc Le^b Hexasaccharide

A synthetic route towards the Le^b hexasaccharide **50** (**Figure 33**), with a [U-$^{13}C_6$]fucosyl residue present on the GlcNAc moiety was also investigated.

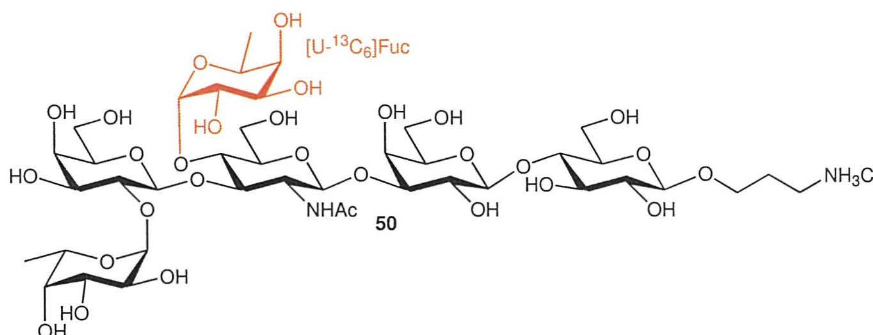
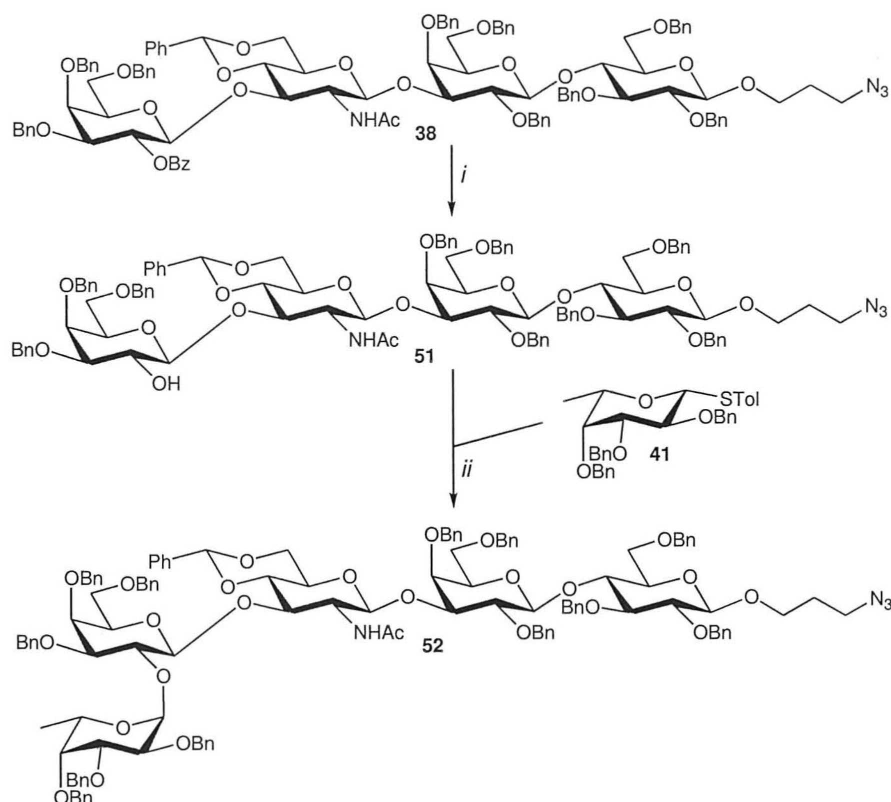


Figure 33 Le^b hexasaccharide **50**, with a ^{13}C-labelled fucosyl residue attached to the GlcNAc moiety via an α-(1→4) glycosidic linkage.

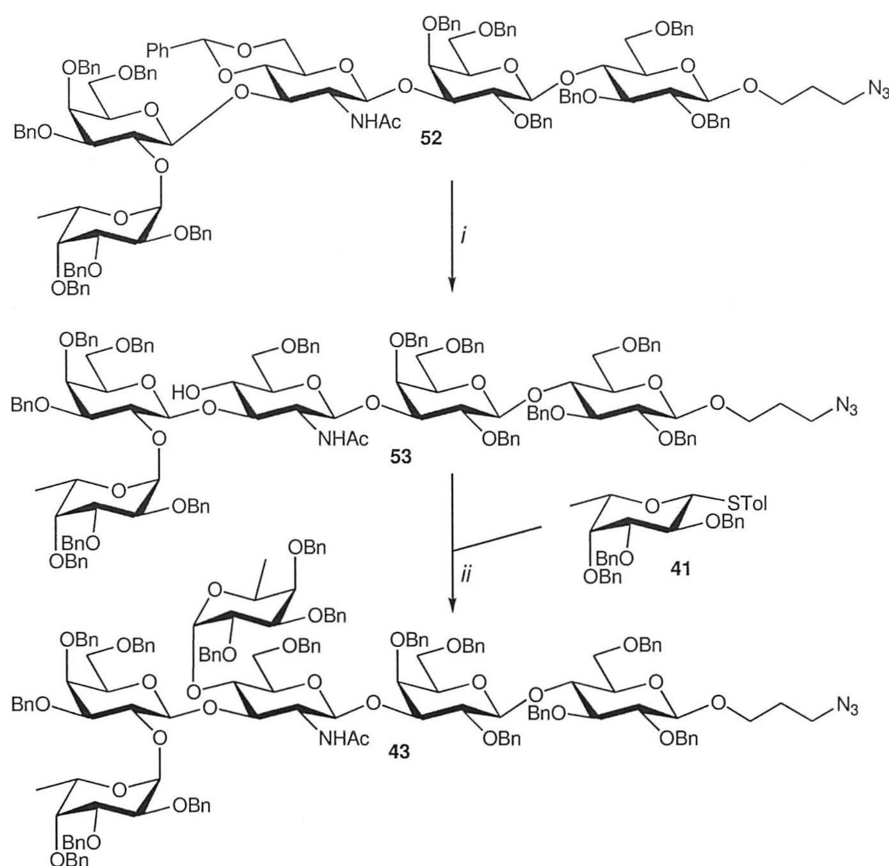
A debenzoylation was carried out on compound **38** with NaOMe in MeOH/DCM, to give the tetrasaccharide acceptor **51** in a 73% yield (**Scheme 20**). This reaction

progressed at a slow rate and heating was required to take the reaction to completion. A stereoselective glycosylation with the unlabelled fucosyl donor **41** was then performed under conditions described in the previous section to give the pentasaccharide **52** in a 74% yield.



Scheme 20 i. NaOMe, MeOH/DCM 7:1 (40 °C, 48 h, 73 %) ii. Et_4NBr, Br_2, DCM/DMF 9:1 (rt, 16 h, 74%).

The benzylidene acetal on pentasaccharide **52** was then opened to give the hydroxyl at the 4-position, giving pentasaccharide acceptor **53** in a 66% yield for the final glycosylation step (**Scheme 21**). A halide assisted coupling was performed with the unlabelled donor **41** to confirm that this would work with the more valuable ^{13}C-labelled donor **22**. This step was successful and gave the protected hexasaccharide **43** in a 81% yield.



Scheme 21 i. $NaBH_3CN$, HCl/Et_2O, THF (rt, 80 min, 66%) ii. Et_4NBr, Br_2, DCM/DMF 9:1 (rt, 16 h, 81%).

Though the opening of the benzylidene acetal on compound **52** to give **53** was only achieved at a reasonable yield of 66%, the efficacy of this sequence has been shown and can now be utilised to produce the ^{13}C-labelled material **50**.

2.7 Conclusion

Two ^{13}C-labelled variants of the Le^{b} hexasaccharide have been successfully synthesised, along with unlabelled material for comparison of NMR spectra (**Figure 34**). A route towards the labelled hexasaccharide **50**, with one [U-$^{13}C_6$]fucosyl residue present on the GlcNAc moiety has also been explored and has been shown to be successful in producing the non-labelled Le^{b} hexasaccharide.

During this synthesis, an efficient route for the conversion of galactose to a fucosyl donor was developed and applied to L-[U-$^{13}C_6$]galactose to give the L-[$^{13}C_6$]fucosyl donor **22**, required for the synthesis of the Le^{b} hexasaccharides.

The Le^{b} structures are now awaiting NMR binding studies with the bacterial lectin BabA.

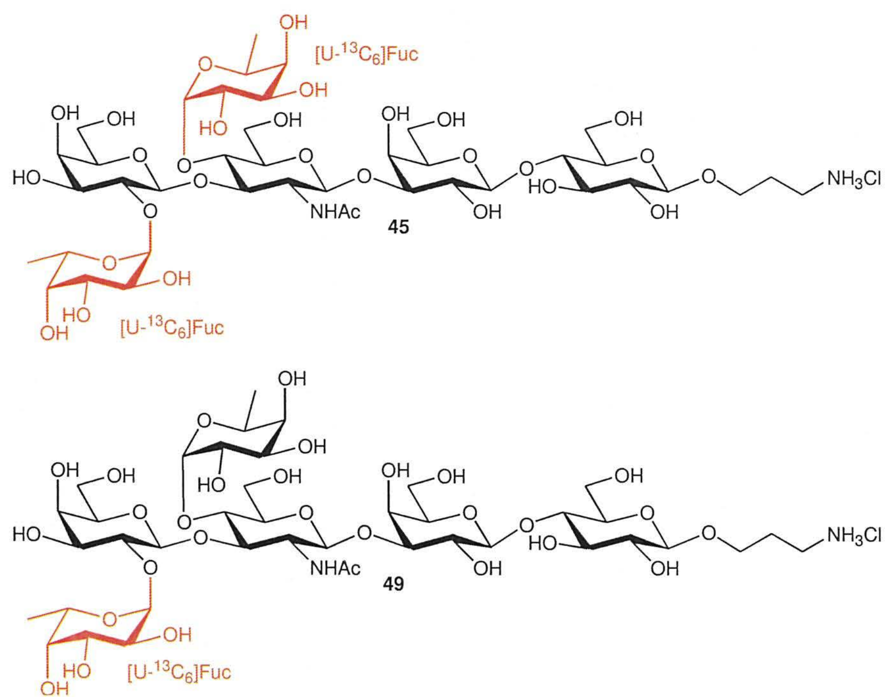


Figure 34 The ^{13}C-labelled Le^{b} hexasaccharide structures **45** and **49** synthesised for NMR binding studies with BabA.

3 Towards a Synthesis of a Neuroactive Polybioside from the Venom of the Social Wasp *Polybia paulista*

3.1 Introduction

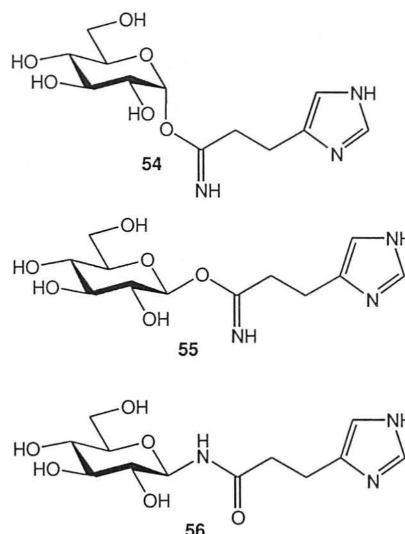


Figure 35 Social wasps belonging to the genus *Polybia* (left). Image courtesy of Sean McCann.[111] The reported structures[112] of the polybioside, 3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl 3-(1*H*-imidazol-4-yl)propanimidate **54** and **55** (α and β respectively) and our target molecule 3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide **56** (right).

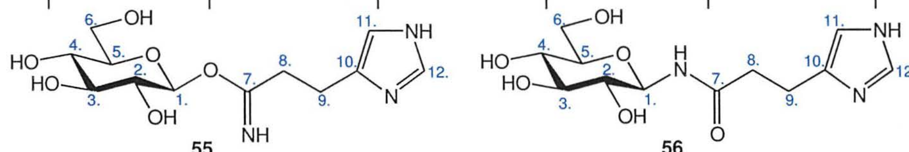
Polybia paulista (*P. paulista*) is a social wasp endemic to parts of Brazil. A polybioside has been isolated from its venom and its structure reported by Saidemberg *et al* as a glucose derivative with an imidate group at the anomeric position linking it to an imidazole.[112] This was based on the ^{1}H, ^{13}C and DEPT NMR obtained, as well as the fragmentation observed during MS. By comparing ^{13}C and DEPT NMR, they attributed the signals observed at δ_C 163.5 and δ_C 128.7 to quaternary carbons belonging to an imidate group and an imidazole ring respectively. The structure was therefore reported as 3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl 3-(1*H*-imidazol-4-yl)propanimidate as an α/β mixture (compounds **54** and **55**, **Figure 35**).[112]

However, we would expect to see similar shifts in NMR for an amide as with an imidate. Predictive NMR (MestReNova NMR processing software) was run for the reported structure **55** and its amide analogue 3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide **56** and compared (**Table 2**). The predictive NMR spectra were very similar to each other (though both differing from the NMR reported for the

polybioside), suggesting that the imidate and amide isomers could exhibit similar chemical shifts for their respective quaternary carbons.

Table 2 NMR data reported by Saidemberg *et al* for their reported β anomer, compound **55**.[112] This is shown in comparison to shifts from predictive NMR for compound **55** and its amide analogue compound **56** (structures shown below). The δ_H of 8.54 Hz reported for the *H*-6 in **55** is uncharacteristically high and is perhaps from the imidazole *H*-11 or *H*-12 instead.

	δ_H			δ_C		
	55	$\mathbf{55}_{predicted}$	$\mathbf{56}_{predicted}$	**55**	$\mathbf{55}_{predicted}$	$\mathbf{56}_{predicted}$
1	4.52	5.70	5.01	96.3	93.5	81.3
2	3.1	3.46	3.44	74.5	73.6	71.4
3	3 - 4	3.42	3.70	71.9	74.8	70.7
4	3 - 4	3.42	3.48	69.9	70.47	70.5
5	3.36	3.19	3.23	76.3	76.7	79.1
6	3 - 4	3.71-3.46	3.71-3.46	61.1	62.3	62.3
7	-	-	-	163.5	173.2	176.4
8	3.03	2.63	2.59	38.3	32.9	34.0
9	3.22	2.95	3.01	22.5	23.1	20.3
10	-	-	-	128.7	138.5	138.5
11	7.26	7.04	7.08	117.3	121.7	121.7
12	8.54	7.70	7.43	134.2	136.0	136.0

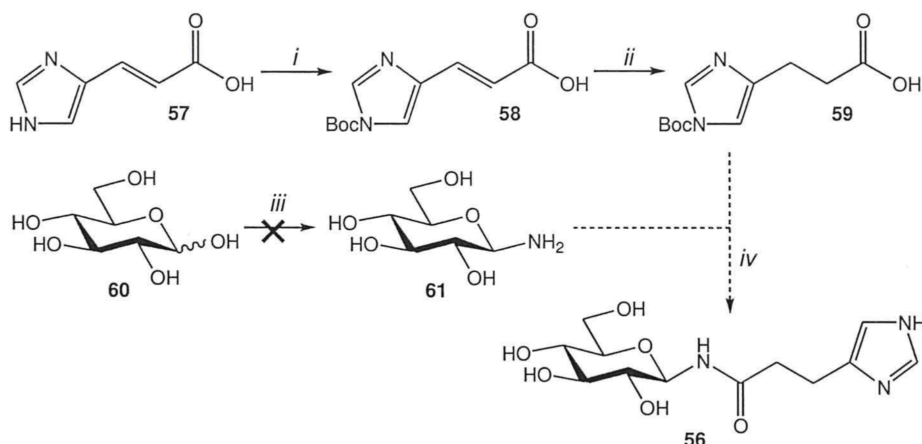


The stability of the reported structures **54** and **55** is also of interest, as imidates at the anomeric position are usually labile. In fact glycosyl imidates, such as trichloroacetimidates[26] and trifluoroacetimidates,[113] are frequently used as glycosyl donors, activated under acidic conditions.[114,113] In contrast glycosyl amides are more stable and would be more likely to have survived intact during the purification processes described for isolation of the polybioside.

Here we attempt the synthesis of the amide, 3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide **56**, to compare its NMR and MS spectra with that observed for the Polybioside isolated from *P. paulista*.

3.2 Synthesis of 3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide

Retrosynthesis for compound **3** suggested D-glucose **60** and urocanic acid **57** as possible starting materials. The synthesis of the polybioside would therefore involve production of a glucosyl amine **61** and performing a peptide coupling with the urocanic acid derivative **59** (**Scheme 22**).



Scheme 22 The first approach towards synthesis of compound **56**. i. Boc_2O, Et_3N, DMF (rt, 16 h, 90%) (ii) Pd/C, H_2, MeOH (rt, 24 h, 100%) iii. $(NH_4)_2CO_{3(aq)}$ (rt, 72 h) and $NH_4OH_{(aq)}$ (rt, 72 h) - the glucosyl amine was not successfully isolated from these reactions.

Peptide coupling of urocanic acid **57** and *N*-Boc-protected urocanic acid **58** has been described using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as coupling agents with moderate yields (45% and 68% for compounds **57** and **58** respectively) suggesting that an unprotected imidazole function could take part in a peptide coupling.[115] Combinations of *N,N'*-dicyclohexylcarbodiimide (DCC) and NHS were also trialled with lower yields, though a coupling of *N-tert*-butyloxycarbonyl-2,3-dihydrourocanic acid **59** is reported with high yields using DCC and 1-hydroxybenzotriazole (HOBt) as coupling agents.[116] Synthesis of *N-tert*-butyloxycarbonyl-2,3-dihydrourocanic acid **59** was therefore attempted for coupling using DCC with HOBt or NHS.

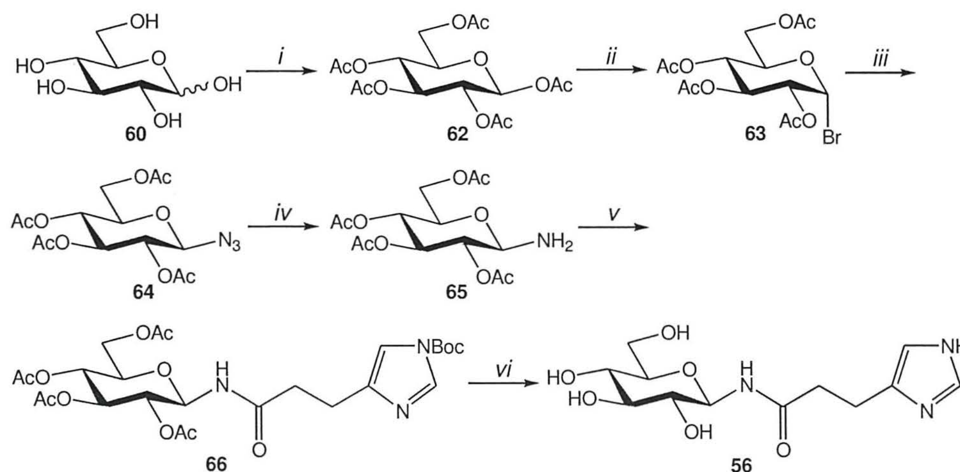
Urocanic acid **57** and di-*tert*-butyl dicarbonate (Boc_2O) were dissolved in DMF and Et_3N and left stirring overnight to give us *N-tert*-butyloxycarbonyl-urocanic acid **58** in a 90% yield. Hydrogenation of the double bond with Pd/C (10% wt.) under H_2 then gave *N-tert*-butyloxycarbonyl-2,3-dihydrourocanic acid **59** in a quantitative yield.

A glucosyl amine needed to be produced for coupling with the activated ester. Saturated $(NH_4)_2CO_3$ solutions have been used to produce glycosyl amines from 2-acetamido-2-

deoxy-D-glucose and D-glucose,[117,118] but the glycosyl amines formed have proven to be labile and easily hydrolysed under acidic conditions. Glycosyl amines with an electron withdrawing group at the 2-position, such as an *N*-acetate, appear to be less labile, perhaps due to a reduction of the basicity of the amine. Efforts have been made to limit hydrolysis by trapping as an amide and also to decrease the need of removal of aqueous solutions of volatile salts required for this synthesis,[118] such as a procedure developed using concentrated ammonium hydroxide solution.[119] This still however presents difficulty purifying and handling due to the instability of the product.

The synthesis of glucosyl amine **61** was attempted by the procedures described using saturated $(NH_4)_2CO_3$ solutions and concentrated ammonium hydroxide, where ammonia attacks the reducing sugar carbonyl to give the glucosyl amine, followed by removal of solvent by freeze drying. However, the glucosyl amine **61** was not successfully isolated from these reactions.

Another approach towards the synthesis of a glycosyl amine is by the synthesis and subsequent reduction of glycosyl azides. This approach has been used to produce unprotected glycosyl amines[120] and protected sugars from acetylated starting material,[121,122,123] with the presence of the deactivating acetate groups reducing the basicity of the glycosyl amines and therefore improving their stability.



Scheme 23 The second approach towards the synthesis of compound **56**. i. Ac_2O, NaOAc (reflux, 30 min, 99%) ii. HBr/AcOH (0 °C - rt, 1 h, 100%) iii. NaN_3, DMF (60 °C, 1 h, 67%) iv. Pd/C, H2, Et_3N, EtOAc/MeOH 1:1 (rt, 30 min) v. **59**, DCC, HOBt, DIPEA, DMF (0 °C – rt, 16 h, 35% over 2 steps from **64**) vi. a. NaOMe, MeOH (rt, 30 min) b. TFA (rt, 30 min, 100%).

Per-*O*-acetyl-β-D-glucopyranose **62** was produced in high yields by refluxing D-glucose **60** in a suspension of Ac_2O and NaOAc. The glucosyl bromide **63** was then produced in quantitative yield from **62** by reacting with a solution of hydrobromic acid in acetic acid

(HBr/AcOH). Substitution occurred readily by heating the glucosyl bromide **63** in DMF with sodium azide to give compound **64** in a 67% yield.[88] This step to give the glucosyl azide **64** was also attempted using KI as a catalyst, though no improvement in yield was observed and the amount of material already obtained was more than sufficient to proceed.

Reduction of the glucosyl azide **64** to 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl amine **65** was attempted using a nickel (II) boride complex formed in situ from sodium $NaBH_4$ and nickel (II) chloride ($NiCl_2$), as described for the reduction of nitrile groups.[124] This simple procedure successfully reduced the glucosyl azide to the corresponding amine. Peptide couplings were attempted using DCC[125] and NHS[126] to create an activated ester from **59**, as well as reacting **65** in situ with **59** with DCC and HOBt,[127] though unfortunately these were unsuccessful in producing compound **66**.'[128] It was suspected that perhaps some residual boron complexes may be inhibiting the coupling, so another approach to reducing the azide was sought. This reduction has been reported using Raney® nickel,[129] with Staudinger ligations using triphenylphosphine (PPh_3) also providing a viable option.[130,131,132] A route employing Pd/C with H_2 was chosen due to the simple workup procedure.[88]

Glucosyl azide **64** was dissolved in EtOAc/MeOH 1:1 with Et_3N. Hydrogenation in the presence of Pd/C (10% wt.) was carried out, and after 30 min TLC indicated that all of the starting material had been converted to one product, later confirmed to be glucosyl amine **65** by NMR. The Pd/C was removed by filtration and the solvent removed under vacuum. Compound **65** was used directly for the next step without further purification to limit hydrolysis. *N-tert*-butyloxycarbonyl-2,3-dihydrourocanic acid **59**, DCC and HOBt were dissolved in DMF and stirred for 30 min. Glucosyl amine **65** and *N,N'*-diisopropylethylamine (DIPEA) were added and the reaction left overnight. This gave the product **66** in a 35% yield, with its structure confirmed by MS and NMR. Deprotection was carried out under Zemplén conditions with a catalytic amount of NaOMe to remove the acetate groups.[78] The Boc groups was then removed using TFA[8] and the crude product purified through a RP-C18 column to give the target molecule 3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide **56** in a quantitative yield from **66**.

3.3 NMR Analysis of 3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide

NMR experiments were run to compare the spectra from compound **56** with those reported for the polybioside.[112] ^{1}H NMR indicated the presence of a small amount of impurity, potentially from the α-anomer of **56**, with the signal at 5.57 Hz being attributed its anomeric proton.

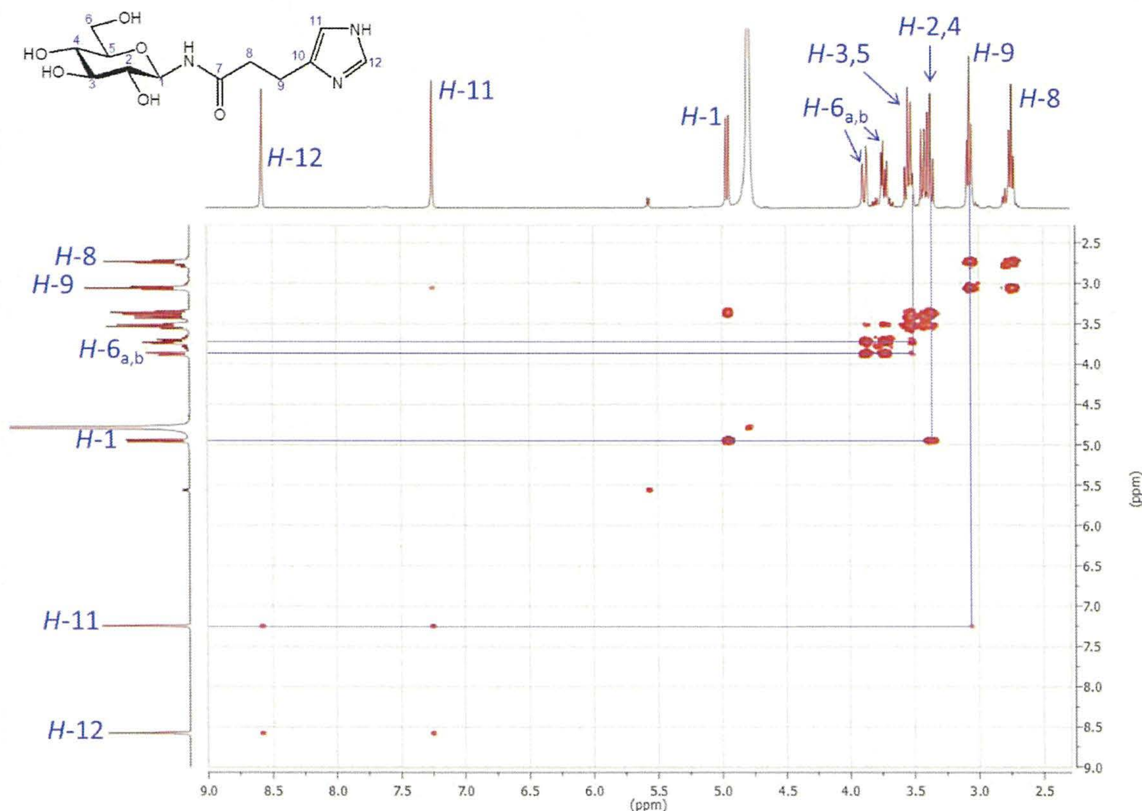


Figure 36 COSY spectrum of compound **56**. This technique is useful in assigning the individual signals to the structure.

COSY NMR was run to assist in assigning the NMR data. The *H*-1 and *H*-6 signals can be used to assign the *H*-2 and *H*-5 positions, and then in turn the *H*-3 and *H*-4 (**Figure 36**). The imidazole *H*-11 showed correlation with *H*-12 and also the aliphatic *H*-9. HSQC NMR was used to gather more information for the carbon signals, as well as providing more information on regions in the ^{1}H spectra where signals overlap (**Figure 37**). The ^{13}C data was collected using DEPTQ, which also gave information on the whether signals were attributable to CH_1, CH_2, or quaternary carbons. **Figure 38** shows *C*-7 and *C*-10 signals, with *C*-10 being distinguishable from the nearby *C*-12 signal by its phase. Though very weak, the *C*-12 signal was confirmed in the HSQC.

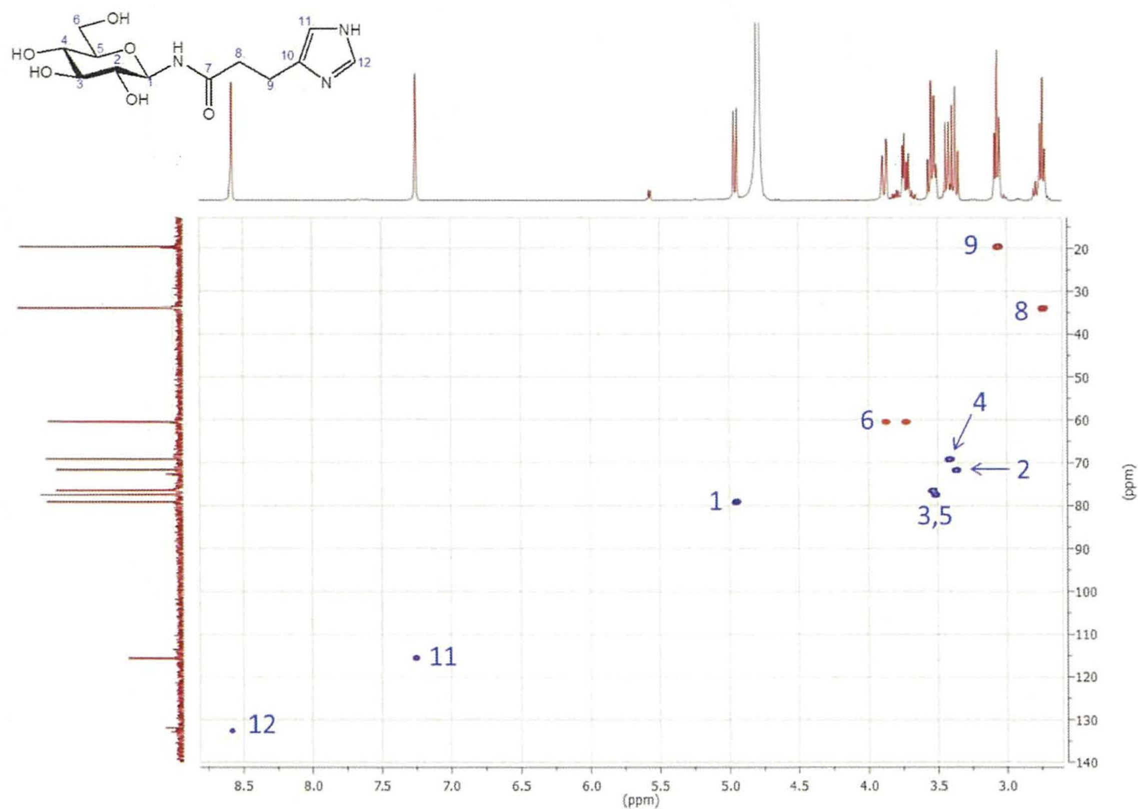


Figure 37 HSQC of compound **56**, showing the correlation between the ^{1}H and DEPTQ spectra.

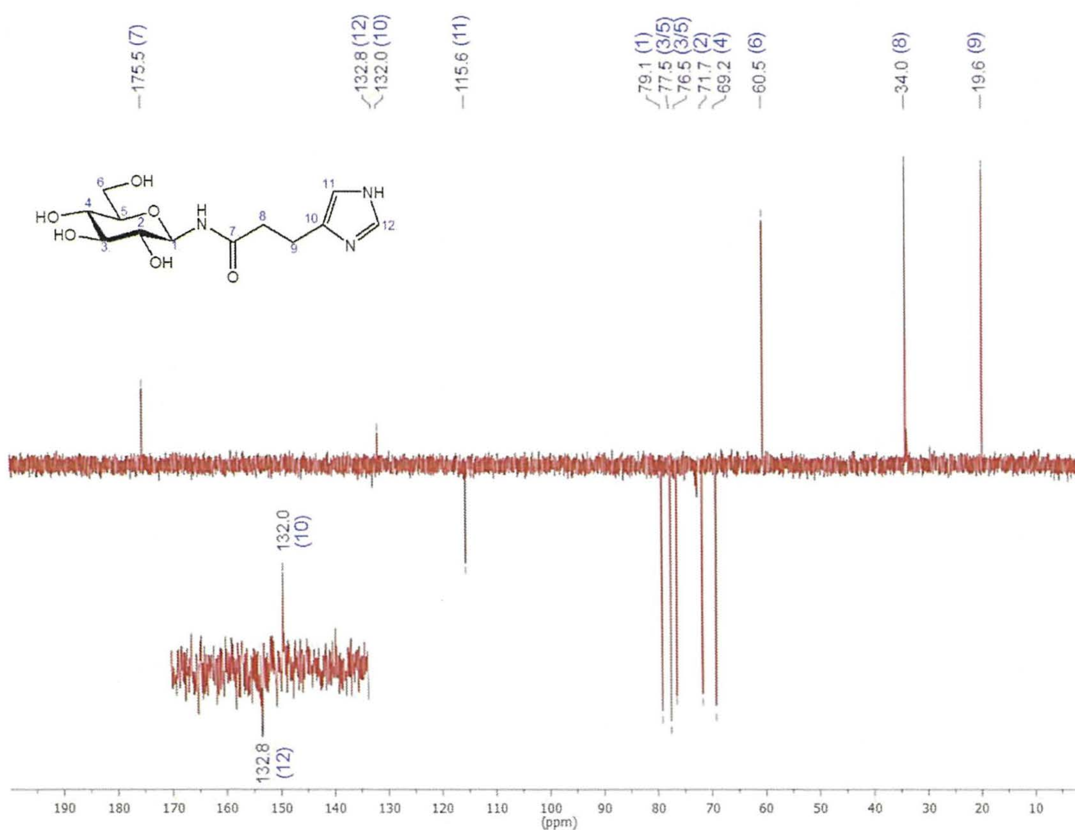
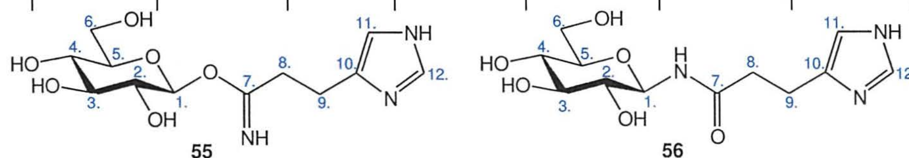


Figure 38 DEPTQ spectrum of compound **56**. The quaternary carbons *C*-7 and *C*-10 exhibited chemical shifts of 175.5 Hz and 132.0 Hz respectively.

The data obtained was compared to the NMR data reported by Saidemberg *et al*[112] (**Table 3**). This demonstrated that the polybioside described was not the same as our synthesised structure **56**, with significant differences particularly for values of the quaternary *C*-7 suggesting that this may indeed be from an imidate rather than an amine. The *H*-1 signal reported for **55** does however appear to be lower than we might expect when compared to the amide analogue **56** and the predicted NMR. The signals from the imidazole ring were in similar positions to those described for the polybioside.

Table 3 NMR data reported by Saidemberg *et al* for the reported β-anomer, compound **55**,[112] shown alongside NMR data obtained from compound **56** and shifts from predictive NMR.

	δ_H				δ_C			
	55	**55**$_{pred}$	**56**	**56**$_{pred}$	**55**	**55**$_{pred}$	**56**	**56**$_{pred}$
1	4.52	5.70	4.95	5.01	96.3	93.5	79.1	81.3
2	3.1	3.46	3.39	3.44	74.5	73.6	71.7	71.4
3	3 - 4	3.42	3.53	3.70	71.9	74.8	-	70.7
4	3 - 4	3.42	3.39	3.48	69.9	70.47	69.2	70.5
5	3.36	3.19	3.53	3.23	76.3	76.7	-	79.1
6	3 - 4	3.71-3.46	3.88 – 3.73	3.71-3.46	61.1	62.3	60.5	62.3
7	-	-	-	-	163.5	173.2	175.5	176.4
8	3.03	2.63	2.74	2.59	38.3	32.9	34.0	34.0
9	3.22	2.95	3.07	3.01	22.5	23.1	19.6	20.3
10	-	-	-	-	128.7	138.5	132.0	138.5
11	7.26	7.04	7.25	7.08	117.3	121.7	115.6	121.7
12	8.54	7.70	8.58	7.43	134.2	136.0	132.8	136.0



3.4 Conclusions

3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide **56** has been successfully synthesised and its NMR data compared to the data reported for the polybioside.[112] This has confirmed that the polybioside is not compound **56** and may be the glucosyl imidate **55**, with the NMR for the imidazole ring and glucose portion of the molecule for the

reported polybioside and **56** closely resembling each other. The presence of an anomeric mixture of the polybioside does however suggest the sugar may be present as a hemiacetal, with substitution elsewhere on the sugar residue.

4 Incorporation of Chitosan into a Polyurethane Foam

4.1 Introduction

Behind cellulose, chitin is the second most naturally abundant biopolymer on the planet, being found in the exoskeletons of insects and crustaceans as well as in the cell walls of fungi and yeasts.[133] Its structure is made up primarily of repeating units of *N*-acetylglucosamine (GlcNAc), linked together by β-1→4-glycosidic bonds (**Figure 39**). These are found naturally in three crystalline forms, α-, β-, and γ-chitin. The α-form, for example, can be obtained from the exoskeletons of crab, shrimp and lobster species, β-chitin from cuttlefish and squid pens, and γ-chitin from fungi.[134,135,136]

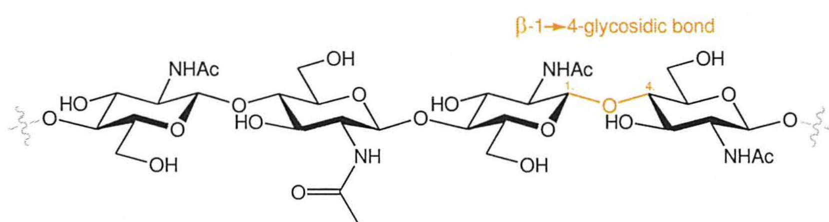


Figure 39 Structure of the naturally occurring biopolymer chitin. The polymer chain is made of GlcNAc repeating units, joined by β-1→4-glycosidic linkages, dependent on the source of the chitin.

Chitosan is the predominantly deacetylated form of chitin, with glucosamine repeating units in place of GlcNAc residues (**Figure 40**). Some GlcNAc residues may remain in chitosan and, conversely, a number of glucosamine residues may be present in naturally occurring chitin.[133] Therefore for chitin and chitosan, the proportion of glucosamine residues present is termed the degree of deacetylation (DD). This can also be expressed in terms of remaining GlcNAc units as the degree of acetylation (DA).

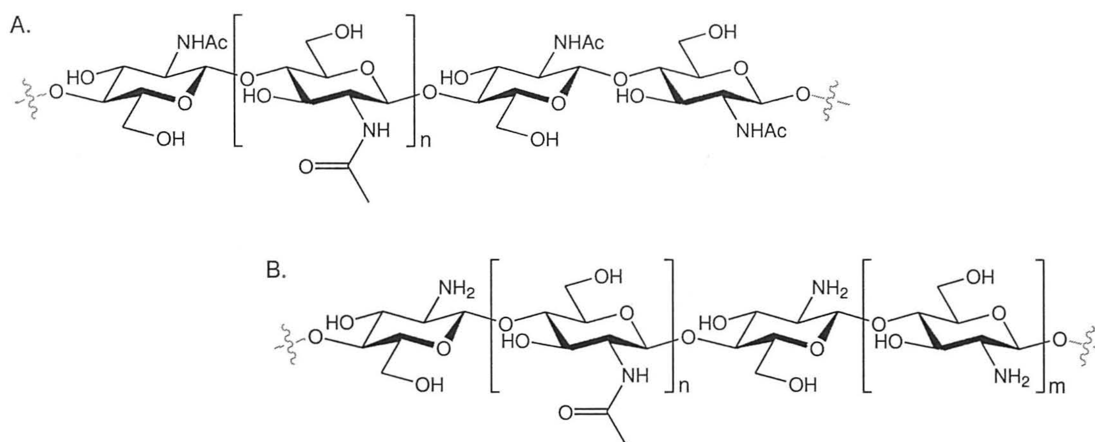


Figure 40 Structures chitin and its deacetylated form chitosan. A) Chitin consisting primarily of GlcNAc repeating units. B) Chitosan is the largely deacetylated form of chitin, containing glucosamine units as well as residual GlcNAc units. The degree of deacetylation (DD) can be expressed as the percentage of sugar residues which are glucosamine, or $\frac{m}{n+m} \times 100 = DD$.

Due to the high natural abundance of chitin and it's straightforward conversion to chitosan by hydrolysis under basic conditions,[137] applications of chitin and chitosan have attracted much interest, with potential uses in metal chelation and waste water treatment, textiles, pharmaceutical and medical applications.[133,138]

4.2 Properties of Chitosan

4.2.1 Degree of Deacetylation

As the properties of chitosan are closely linked to its degree of deacetylation (DD), it is therefore important to be able determine this with a simple and reliable procedure.

A range of techniques utilising IR spectroscopy have been described,[139] using the ratios of the absorbance bands, such as for the amide (1653 cm^{-1}) and hydroxyl groups (3450 cm^{-1}), and comparing with standards of known DD from IR or another technique such as tritration.[140] These procedures are relatively quick to perform, though carry complications as moisture must be excluded to avoid unwanted hydroxyl signals.

Other techniques such as first derivative UV-spectrophotometry have been shown to be effective in determining DD,[141,142] as well as more recent publications showing potentiometric and conductometric titrations to be relatively cost effective and to give reliable results, validated by elemental analysis.[143,144]

^{1}H NMR can also be used for this purpose by comparing the integrals from the acetate CH_3 and carbohydrate H-1 signals.[145,146] This has advantages over many of the other

methods described in that there is there is no need to compare with standards or data from other experimental methods. An additional problem associated with other techniques is the necessity to ensure that the sample is completely dry, largely to avoid inaccuracy during weighing of samples and also of unwanted hydroxyl signals in IR spectra. DD can also be estimated in solid state NMR, resolving issues of poor solubility, with solid state ^{13}C and ^{15}N experiments shown to give values in good agreement with those obtained by other techniques.[147,148]

4.2.2 Solubility

The solubility of chitosan in aqueous media is influenced by factors such as pH and temperature of solution, as well as DD and MW of the chitosan.[149,150] Chitosan with DD of 28% and 49 % reported to be soluble in solutions of acetic acid and water respectively.[151] Fully deacetylated chitosan is insoluble in water, with procedures being reported to partially acetylate chitosan to improve its solubility.[152]

While solubility of chitosan in organic solvents remains poor, efforts have been made to improve this by inclusion of protecting groups onto the polymer. Holappa *et al* reported the synthesis of 6-*O*-triphenylmethylchitosan, showing solubility in pyridine, as a step towards chitosan-*N*-betainates.[153]

The solubility of chitin and chitosan in ionic liquids has also been assessed,[154] with a solubility for chitosan of 12 wt.% reported with [bmim][CH_3COO].[155]

4.2.3 Molecular weight

Molecular weight of chitosan can be determined as a viscosity-average molecular weight, by measuring the viscosity of a solution of chitosan using a viscosimeter and applying the Mark-Houwink-Sakurada equation.[156]

Molecular mass of chitosan can be adjusted by depolymerisation of longer chains with nitrous acid,[157] incubating in acetic acid over different timescales,[158] as well as more recent publications where acid catalysed hydrolysis is performed in ionic liquids[159] or achieved using microwave irradiation.[160]

4.2.4 Antimicrobial Action of Chitosan and Modified Chitosan

Chitosan has a broad spectrum of antimicrobial action, being effective against Gram negative and Gram positive bacteria, as well as suppressing spore germination in fungi.[161] Though the mode of action is not fully understood, there are numerous studies linking changes in positive charge density with the antibacterial efficiency of chitosan.[161] This has been demonstrated by varying the DD, with higher values, and therefore higher density of amino functions, displayed stronger inhibitory effects against *Staphylococcus aureus* (*S. aureus*).[162] Conversely, decreasing the positive charge density by testing under basic conditions, or substitution with anionic groups such as in *N*-carboxymethyl chitosan, results in poor inhibition.[163] Therefore, extensive efforts have been made in modifying the structure of chitosan to improve upon antimicrobial action, as well as other properties such as solubility in neutral and basic solutions (**Figure 41**).[164]

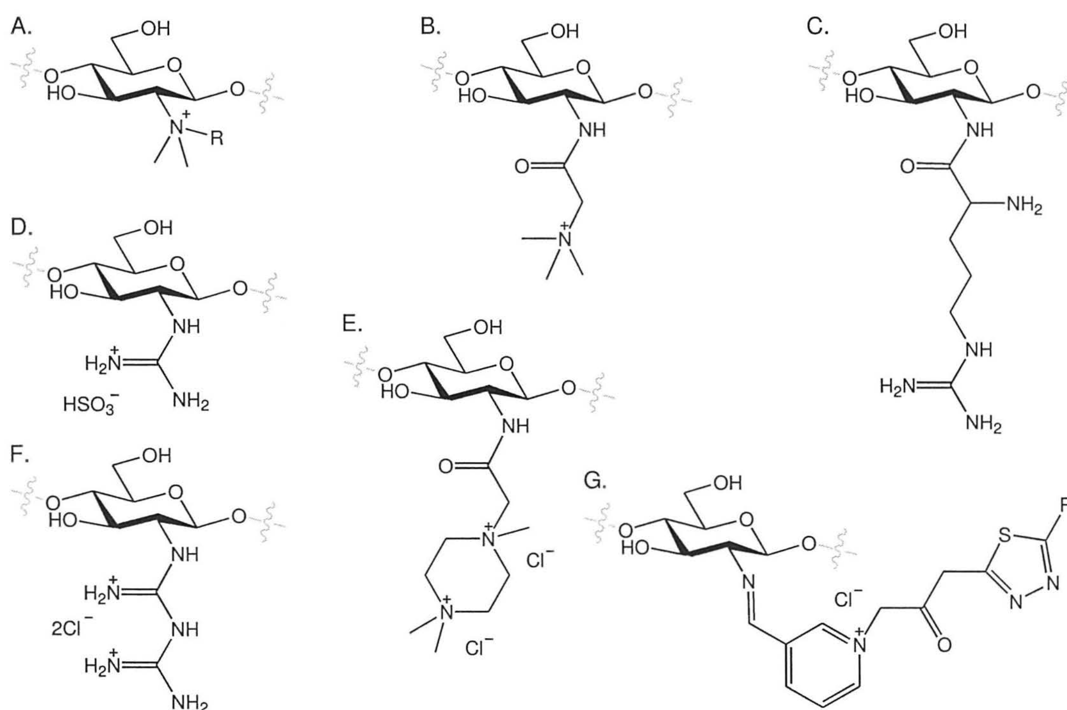


Figure 41 Examples of reported modified chitosan structures prepared with the aim of improving the antimicrobial properties of chitosan. A) Quaternized *N*-alkyl chitosan[165] B) Chitosan *N*-betainates[153] C) Chitosan *N*-arginine[166] D) Guanidinylated chitosan[167] E) Piperazine functionalized chitosans[168] F) Chitosan biguanide[169] G) Thiadiazole functionalized chitosans[170].

Many of these modifications involve substitution at the chitosan amine groups with guanidine or quaternary ammonium functions. Interestingly, the position of the cation in relation to the polymer backbone may also be of importance, as it has been reported

that chitosan *N*-betainates have reduced antimicrobial activity, despite the presence of a quaternary ammonium group.[153]

4.3 Polyurethane Foams

Polyurethane (PU) foams can be produced by the reaction between isocyanates and alcohols to give urethane linkages. Polyethylene glycol (PEG) can therefore be reacted with diisocyanates to produce prepolymer mixtures with isocyanate groups remaining (**Figure 42**). Addition of water then initiates the foaming reaction, by reacting with isocyanate groups to form carbamic acid intermediates. These decompose, liberating $CO_{2(g)}$ and producing primary amines, which can attack remaining isocyanate groups to give urea linkages.[171]

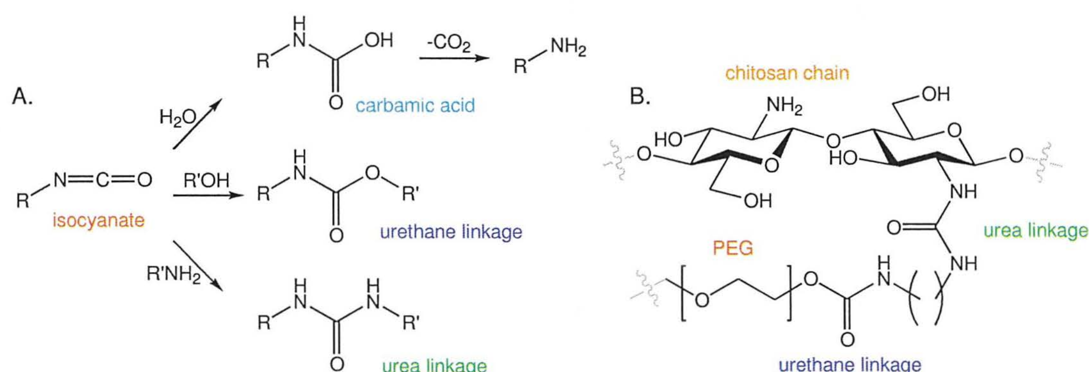


Figure 42 Reactions with isocyanates. A) Isocyanates react with amines and alcohols to give urea and urethane linkages respectively. Reactions with water generate unstable carbamic acids, which decompose to the amine with evolution of $CO_{2(g)}$. B) The aim was to incorporate chitosan chains into the structure of a polyurethane foam by urea linkages to the amine groups.

Hydrophilic PU foams are used in wound dressings, due to their ability to hold large quantities of wound exudate.[172] The aim of the project was to investigate the possibility of incorporating chitosan into the structure of a PU foam during the foaming process, for the purpose of inhibiting the growth of bacteria in the dressing, thus reducing the frequency at which dressings need to be changed and therefore reducing discomfort for the patient.

4.4 Incorporation of Chitosan into a PU Foam

The DD of a sample of chitosan was run as described by Lavertu *et al*,[146] with a presaturation for solvent signal suppression. Chitosan (5 mg) was dissolved in a D_2O (0.98 ml) and DCl (20% wt. in D_2O, 0.02 ml). Running the experiment at 70 °C ensured that there was no overlap of the solvent signal with the chitosan signals. Though

hydrolysis of the chitosan amide can occur under these conditions, this can be quantified by the appearance of the resulting acetic acid -CH_3 signal in the spectra.

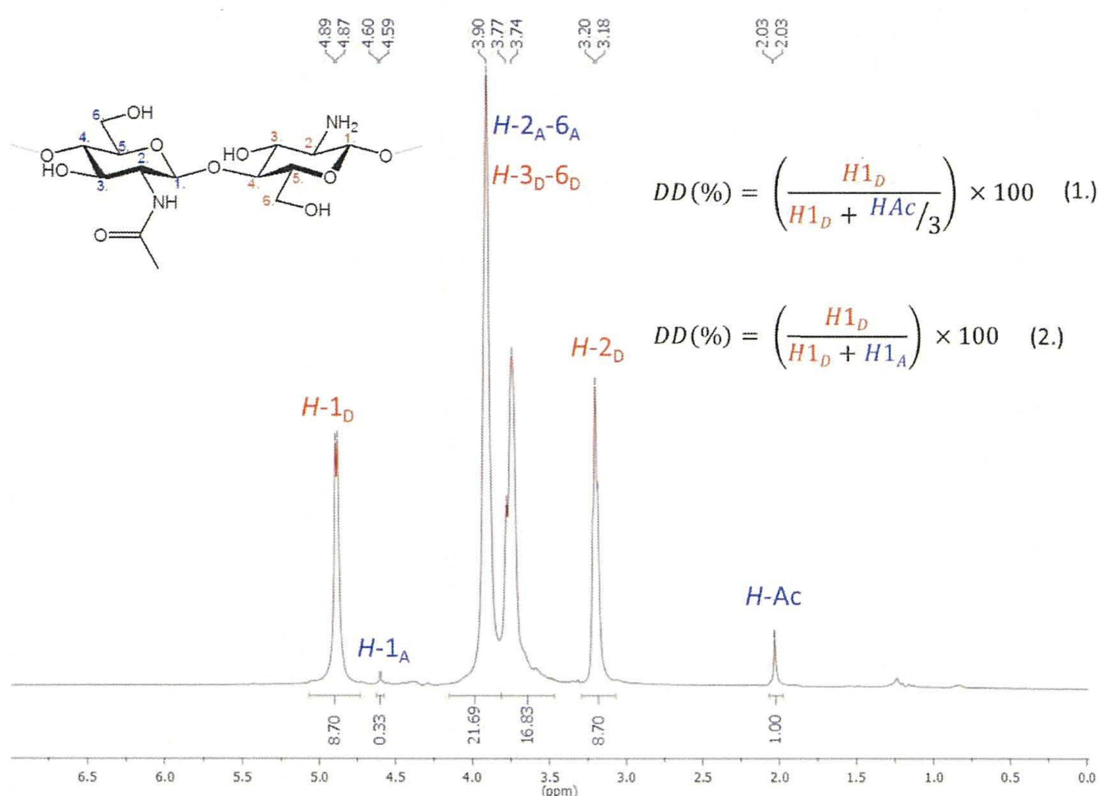


Figure 43 ^{1}H NMR spectrum of chitosan. The integrals obtained for the signals from the acetylated and deacetylated portions of the polymer can be applied to equations (1.) and (2.) to estimate a value for DD. The values shown suggest a DD of 96%.

By integrating the signals in the ^{1}H NMR spectrum of chitosan (**Figure 43**) the DD was estimated to be 96%. This value is within the range of which chitosan is reported to exhibit antimicrobial effects.[162]

Chitosan was solubilised by stirring in $HCl_{(aq)}$ (0.15 M) and filtering off any remaining solid before removing the solvent by lyophilisation. The protonated chitosan was then redissolved in water and mixed with a prepolymer and $NaHCO_{3(aq)}$ to produce a PU foam. The foam was then washed with water, with no sugar visible in the washings by TLC.

Solid state NMR was performed on the resulting foam and compared to PU foam without a chitosan additive, as well as native chitosan.

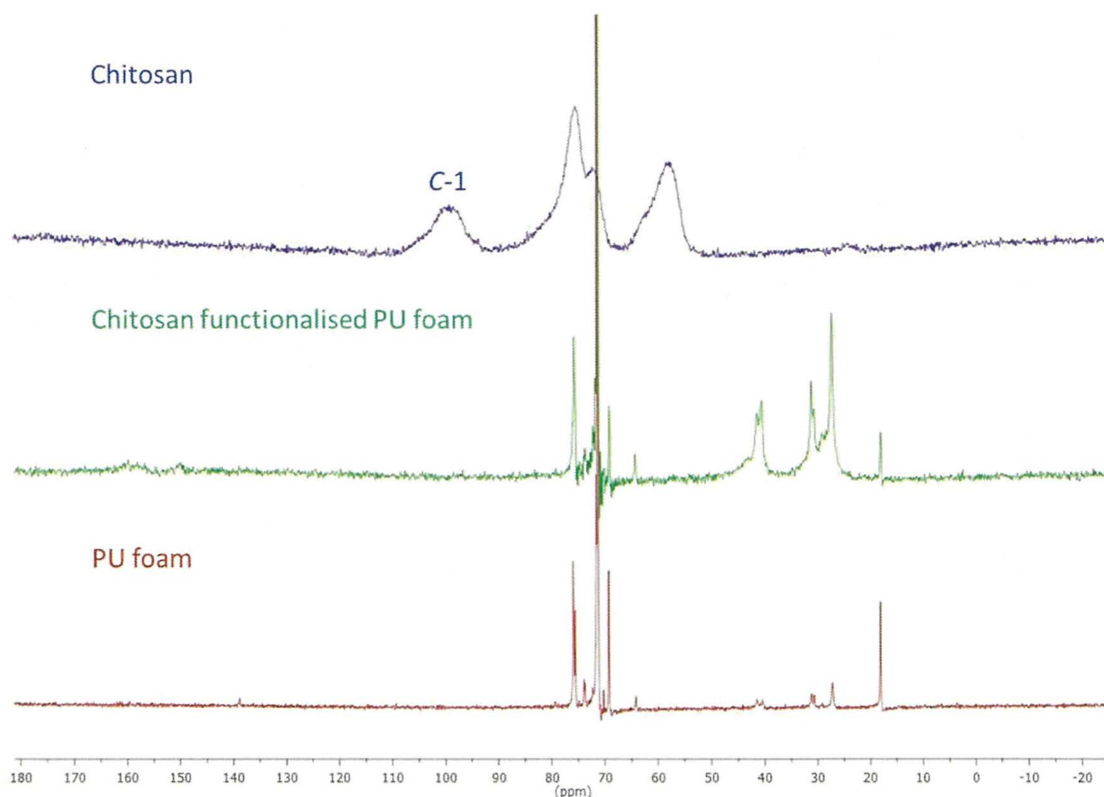


Figure 44 ^{13}C Solid state NMR of PU foam, chitosan functionalised PU foam and chitosan.

^{13}C Spectra for the PU foam and chitosan functionalised PU foam show the same signals (**Figure 44**). The chitosan *C*-1 signal is shown to be distinguishable from the signals from the PEG sections of the polymer (75.82 – 64.15 ppm) but is not visible in the chitosan functionalised PU foam. This suggests either the absence of chitosan from the foam or is due to the low quantity of chitosan in the structure.

4.5 Conclusion

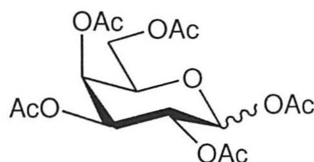
Chitosan was solubilised and added to a prepolymer during a foaming reaction to produce chitosan functionalised PU foam. Observations during the reaction suggested that the chitosan had been incorporated into the foam structure, though the relatively low amount of chitosan within the foam has made it difficult to detect through ^{13}C solid state NMR.

5 Experimental

5.1 General Procedures

Solvents were supplied by Fisher Scientific. L-[U-$^{13}C_6$]Galactose was supplied by Omicron Biochemicals. Prepolymer (9-IC) was manufactured by Bayer. All other reagents were purchased from Alfa Aesar, Fisher Scientific and Sigma Aldrich. Silica gel column chromatography was carried out using Fluorochem 6A silica gel, LiChroprep® RP-C18 (40 – 63 μm), and BIO-RAD Bio-Gel® P-2 Gel (45 – 90 μm). TLC was performed using Merck silica gel $60F_{254}$ glass plates and visualised under UV, sulfuric acid ($H_2SO_{4(aq)}$ 10%), ninhydrin (0.3 g in n-butanol (100 ml) an AcOH (3 ml)), iodine (absorbed onto silica powder), and Dragendorff's reagent (A. bismuth nitrate (0.17 g) dissolved in H_2O/AcOH (4:1, 10 ml) B. KI (4 g) dissolved in H_2O/AcOH (2:1, 30 ml). Solutions A and B mixed together and diluted to 100 ml with H_2O). NMR experiments were run on Bruker 400 MHz and 500 MHz spectrometers. Solid state ^{13}C and ^{15}N experiments were run by the EPSRC National Solid-State NMR Service at Durham. Spectra were calibrated using TMS, $CDCl_3$, CD_3OD and D_2O as internal standards. Mass spectrometry was carried out on a Bruker MICRO-TOF and MALDI-TOF mass spectrometers, as well as by the EPSRC National Mass Spectroscopy Facility, Swansea. MALDI-TOF was run using 2',4',6'-trihydroxyacetophenone (THAP) in acetonitrile/water ($MeCN/H_2O$ 1:1) as a matrix.

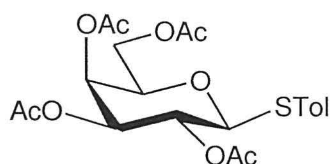
Per-*O*-acetyl-D-galactopyranose **2**[76]



A suspension of NaOAc (1 eq, 0.46 g, 5.52 mmol) in Ac_2O (10 eq, 5.25 ml, 55.2 mmol) was stirred at 140 °C. D-Galactose **1** (1eq, 0.994 g, 5.52 mmol) was added in small portions over a period of 6 minutes, and the reaction refluxed for a further 25 minutes after the final addition. The reaction mixture was poured onto crushed ice (50 ml) and stirred for 2 h. EtOAc (50 ml) was added to the mixture and the organic layer washed with water (2 x 30 ml) and $NaHCO_3$(aq) (30 ml) before drying over $MgSO_4$ and concentrating under reduced pressure. The product was purified by silica gel column

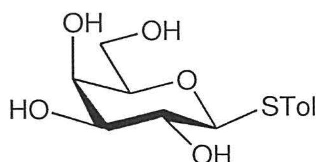
chromatography (toluene/EtOAc at 6:1→1:1) to give the white solid **2** as an α/β mixture (2.14g, 5.48 mmol, 99%). R_f = 0.44 (toluene/EtOAc 1:1). δ $_H$ (500 MHz, $CDCl_3$) 5.70 (1 H, d, *J* 8.2, *H*-1), 5.42 (1 H, d, *J* 3.5, *H*-4), 5.33 (1 H, t, *J* 8.2, *H*-2), 5.08 (1 H, dd, *J* 10.4, 3.1, *H*-3), 4.11 - 4.20 (2 H, m, H_2-6), 4.05 (1 H, t, *J* 6.7, *H*-5), 2.16 (3 H, s, $COCH_3$), 2.12 (3 H, s, $COCH_3$), 2.04 (6 H, s, $COCH_3$), 1.99 (3 H, s, $COCH_3$). δ $_C$ (126 MHz, $CDCl_3$) 170.3, 170.1, 170.0, 169.4, 169.0, 92.2, 71.7, 70.8, 67.8, 66.8, 61.0, 20.8, 20.6, 20.5.

p-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **3**[173]



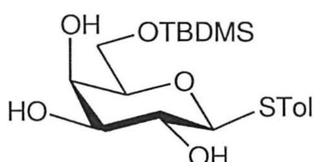
Per-*O*-acetyl-β-D-galactopyranoside **2** (1 eq, 1.10 g, 2.56 mmol) was dissolved in DCM (10 ml) before adding *p*-Thiocresol (1.5 eq, 0.48 g, 3.8 mmol) and $BF_3.OEt_2$ (1.8 eq, 0.57 ml, 4.6 mmol) and the reaction left to stir under N_2 for 110 min at rt. The reaction was monitored with TLC (toluene/EtOAc 3:2). After completion, the reaction was quenched with triethylamine (1.3eq, 0.45 ml) in an ice bath and stirred for a further 30 min. DCM (30 ml) was added to the reaction mixture and was washed with saturated NaCl(aq) solution (2 x 30 ml). The organic layer was dried with $MgSO_4$ and concentrated under vacuum, before being purified by silica gel column chromatography (toluene/EtOAc 1:0 (600 ml), 6:1 (630 ml), 3:1 (200 ml)) to give the β-anomer **3** as a white, crystalline solid (1.05 g, 2.31 mmol, 90%). Rf = 0.53 (toluene/EtOAc 3:2). δ $_H$ (500 MHz, $CDCl_3$) 7.41 (2 H, d, *J* 7.9, Ph*H*), 7.12 (2 H, d, *J* 7.9, Ph*H*), 5.40 (1 H, d, *J* 2.5, *H*-4), 5.21 (1 H, t, *J* 10.1, *H*-2), 5.03 (1 H, dd, *J* 3.2, 9.8, *H*-3), 4.64 (1 H, d, *J* 9.8, *H*-1), 4.09 – 4.20 (2 H, m, H_2-6), 3.90 (1 H, t, *J* 6.3, *H*-5), 2.34 (3 H, s, $PhCH_3$), 2.11 (3 H, s, $COCH_3$), 2.09 (3 H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 1.97 (3 H, s, $COCH_3$). δ $_C$ (126 MHz, $CDCl_3$) 170.3, 170.2, 170.0, 169.4, 138.5, 133.2, 129.6, 128.6, 87.0, 74.4, 72.1, 67.3, 61.6, 21.1, 20.8, 20.6.

p-Tolyl 1-thio-β-D-galactopyranoside **4**[174]



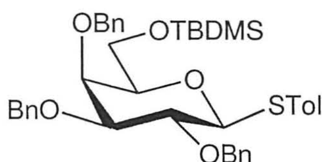
A solution of NaOMe/MeOH (excess, 7.5 ml, 1 M) and MeOH (100 ml) was made and compound **3** (1 eq, 5.01 g, 11.0 mmol) added to it. The reaction was stoppered and stirred at rt for 30 min. The pH of the solution was tested periodically to ensure it remained basic and progress monitored by TLC (DCM/MeOH 3:1). After completion, Amberlite IR-120 H-form resin was added and stirring continued until the solution no longer appeared to be basic. The Amberlite was then filtered off, washed with MeOH, and solvent removed under vacuum to give the crystalline product **4** (3.21 g, 11.2 mmol, 100%). $R_f = 0.63$ (DCM/methanol 3:1). δ_H (500 MHz, D_2O) 7.47 (2 H, d, *J* 7.9, Ph*H*), 7.24 (2 H, d, *J* 7.6, Ph*H*), 4.67 (1 H, d, *J* 9.8, *H*-1), 3.97 (1 H, s, *H*-4), 3.59 – 3.75 (5 H, m, *H*-2, *H*-3, *H*-5, H_2-6), 2.32 (3 H, s, PhCH_3).

p-Tolyl 6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside **10**[175]



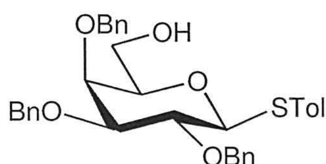
Compound **4** (1 eq, 0.492 g, 1.72 mmol) was dried under vacuum before chilling the reaction vessel in an ice bath. TBDMSCl (1.1 eq, 0.286 g, 1.90 mmol) and pyridine (2 ml) were added and the reaction stirred under N_2 for 4 h, during which time the reaction mixture was allowed to reach rt. Upon completion, the pyridine was removed by co-evaporation with toluene (3 x 5 ml) before the crude product was purified by silica gel column chromatography (toluene/EtOAc 1:0→1:3) in a column with buffered silica gel (Et_3N 5% in toluene).The product **10** was present as a viscous oil (0.635 g, 1.59 mmol, 92%). $R_f = 0.62$ (DCM/MeOH 6:1). δ_H (500MHz, $CDCl_3$) 7.45 (2 H, d, *J* 8.2, Ph*H*), 7.10 (2 H, d, *J* 8.2, Ph*H*), 4.45 (1 H, d, *J* 9.5, *H*-1), 4.07 (1 H, s, *H*-4), 3.87 – 3.95 (2 H, m, H_2-6), 3.65 (1 H, t, *J* 9.5, *H*-2), 3.58 (1 H, d, *J* 3.5, *H*-3), 3.50 (1 H, t, *J* 4.8, *H*-5), 0.90 (9 H, s, SiC(CH_3)$_3$), 0.10 (6 H, d, *J* 7.3, Si(CH_3)$_2$). δ_C (126 MHz, $CDCl_3$) 138.2, 133.0, 129.8, 128.4, 88.8, 78.1, 75.0, 69.9, 69.4, 63.2, 25.8, 21.2, 18.2.

p-Tolyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside **11**



A mixture of NaH (3.9eq, 0.14 g, 5.9 mmol) and BnBr (3.6 eq, 0.65 ml, 5.49 mmol) in DMF (2 ml) were stirred at 0 °C. Compound **11** (1 eq, 0.611g, 1.52 mmol) was dissolved in DMF (5 ml) and added to the reaction vessel in portions. The reaction was allowed to reach rt and stirred for 2 h under N_2. The solvent was removed by co-evaporation with toluene (2 x 5 ml) before dissolving in DCM (50 ml) and washing with NaCl(aq) solution (saturated, 25 ml) and water (25 ml). The organic phase was dried with $MgSO_4$ and the solvent removed under reduced pressure. TLC indicated a minor product, potentially from removal of the silyl ether group and a benzyl ether protecting group being present in the 6-position as well. The product was then purified with silica gel column chromatography (toluene→toluene/EtOAc 2%) in buffered silica (Et_3N 0.5% in toluene) to give compound **11** as a clear, colourless, syrup (0.898 g, 1.34 mmol, 88%). R_f = 0.46 (toluene/EtOAc 20:1). δ_H (500MHz, $CDCl_3$) 7.48 (2 H, d, *J* 8.2, Ph*H*) 7.18 – 7.42 (15 H, m, Ph*H*), 7.01 (2 H, d, *J* 7.9, Ph*H*), 5.00 (1 H, d, *J* 11.7, $PhCH_2$), 3.92 (1 H, t, *J* 9.5, *H*-2), 4.73 – 4.81 (4 H, m, $PhCH_2$), 4.64 (1 H, d, *J* 11.4, $PhCH_2$) 4.60 (1 H, d, *J* 9.8, *H*-1), 3.96 (1 H, m, *H*-4), 3.73 – 3.79 (2 H, m, H_2-6), 3.61 (1 H, dd, *J* 2.8, 9.5, *H*-3), 3.44 (1 H, t, *J* 6.8, *H*-5), 2.31 (3 H, s, $PhCH_3$), 0.90 (9 H, s, $SiC(CH_3)_3$), 0.05 (6 H, s, $Si(CH_3)_2$). δ_C (126 MHz, $CDCl_3$) 136.6, 136.1, 136.0, 134.7, 129.6, 128.0, 127.1, 126.4, 126.0, 125.9, 125.8, 125.7, 125.4, 125.3, 125.2, 125.2, 124.9, 122.9, 85.6, 81.9, 76.4, 73.2, 72.1, 71.1, 70.4, 69.7, 59.1, 23.6, 23.5, 19.1, 18.7, 15.8.

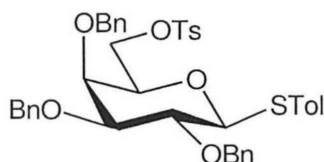
p-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside **12**[176]



Compound **11** (1 eq, 0.859 g, 1.28 mmol) and Bu_4NF (1.2 eq, 0.41 g, 1.54 mmol) were dissolved in THF (1.6 ml) and the reaction stirred under N_2 for 16 h at rt. The reaction was monitored with TLC (toluene/EtOAc 9:1), before the solvent was removed by co-evaporation with toluene (2 x 3 ml) to give a yellow oil. This was applied to the top of a silica gel column with a little toluene for purification by chromatography (toluene/EtOAc 1:0→6:1) to give the product **13** (0.682 g, 1.23 mmol, 96%). R_f = 0.15 (toluene/EtOAc 9:1). δ_H (500MHz, $CDCl_3$) 7.42 (2 H, d, *J* 8.2, Ph*H*) 7.14 – 7.38 (15 H, m, Ph*H*) 7.00 (2 H, d, *J* 7.9, Ph*H*), 4.94 (1 H, d, *J* 12.0, $PhCH_2$) 4.72 – 4.81 (4 H, m,

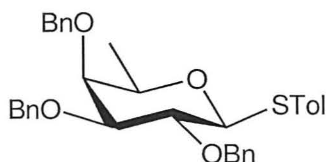
PhCH_2), 4.60 (1 H, d, J 11.7, PhCH_2), 4.56 (1 H, d, J 9.8, H-1), 3.89 (1 H, t, J 9.5, H-2), 3.78 – 3.82 (2 H, m, H_2-6), 3.57 (1 H, dd, J 2.8, 9.1, H-3), 3.48 (1 H, dd, J 5.0, 9.3, H-4), 3.38 (1 H, t, J 6.2, H-5), 2.27 (3 H, s, PhCH_3). δ_C (126 MHz, $CDCl_3$) 138.4, 138.3, 138.2, 137.9, 137.4, 132.3, 130.1, 129.6, 129.1, 128.5, 128.4, 128.4, 128.3, 128.2, 127.8, 127.8, 127.7, 125.3, 88.1, 84.3, 78.7, 75.7, 74.2, 73.3, 73.1, 62.3, 21.1.

p-Tolyl 2,3,4-tri-*O*-benzyl-6-tosyl-1-thio-β-D-galactopyranoside **13**[177]



Compound **12** (1 eq, 0.655 g, 1.18 mmol) and TsCl (1.5 eq, 0.34 g, 1.8 mmol) were dried together under vacuum. Pyridine (2.5 ml) was added and the clear, colourless solution stirred under N_2 at rt for 3 h. Upon completion, the pyridine was removed by co-evaporation with toluene (2 x 5 ml) and the mixture diluted with EtOAc (40 ml) and washed with water (2 x 40 ml) before drying with MgSO4 and concentrating under reduced pressure. The compound was then purified by silica gel column chromatography (toluene/EtOAc 1:0→3:1) to give compound **13** as a white crystalline solid (0.783 g, 1.10 mmol, 94%). $R_f = 0.51$ (toluene/EtOAc 9:1). δ_H (500MHz, $CDCl_3$) 7.74 (2 H, d, J 8.2, PhH). 7.22 – 7.39 (19 H, m, PhH) 6.99 (2 H, d, J 7.9, PhH), 4.93 (1 H, d, J 11.4, PhCH_2), 4.68 – 4.77 (4 H, m, PhCH_2), 4.51 (1 H, d, J 9.8, H-1), 4.47 (1 H, d, J 11.3, PhCH_2), 4.03 – 4.13 (2 H, m, H_2-6), 3.89 (1 H, s, H-4), 3.82 (1 H, t, J 9.5, H-2), 3.62 (1 H, t, J 6.5, H-5), 3.56 (1 H, dd, J 2.5, 9.2, H-3), 2.39 (3 H, s, PhCH_3), 2.30 (3 H, s, PhCH_3). δ_C (126 MHz, $CDCl_3$) 145.1, 138.2, 138.2, 138.0, 137.5, 132.4, 132.2, 129.9, 129.8, 129.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.8, 127.6, 88.0, 83.0, 75.6, 75.4, 74.4, 73.0, 73.0, 67.9, 21.7, 21.1.

p-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-fucopyranoside **7**[85] (L-enantiomer)



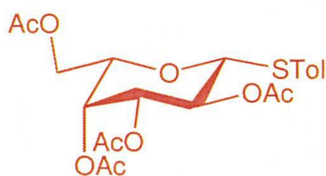
Compound **14** (1eq, 0.115 g, 0.162 mmol) and $LiAlH_4$ (26 eq, 0.157g, 4.15 mmol) were dried together under vacuum for 2 h. Dry THF (2 ml) was added and the reaction

mixture refluxed under N_2 for 2 h. After completion the reaction mixture was diluted by adding THF (10ml) and added dropwise to a solution of THF (10 ml) and glacial acetic acid (5 ml) being chilled in an ice bath. Water (30 ml) was added and a small amount of hydrochloric acid (2 M) was added dropwise until the precipitate dissolved. An extraction with EtOAc (3 x 40 ml) was then carried out. The organic layers were combined, dried with MgSO4 and concentrated under reduced pressure. The product was then purified by silica gel chromatography (toluene→toluene/EtOAc 2%) to give compound **7** as a white solid (0.051 g, 0.094 mmol, 58%). R_f = 0.42 (toluene/EtOAc 20:1). δ_H (500 MHz, $CDCl_3$) 7.50 (2 H, d, *J* 7.9, Ph*H*), 7.29 – 7.41 (15 H, m, Ph*H*), 7.02 (2 H, d, *J* 7.9, Ph*H*), 5.01 (1 H, d, *J* 11.4, $PhCH_2$), 4.72 - 4.82 (4 H, m, $PhCH_2$), 4.67 (1 H, d, *J* 11.7, $PhCH_2$), 4.55 (1 H, d, *J* 9.8, *H*-1), 3.90 (1 H, t, *J* 9.5, *H*-2), 3.63 (1 H, d, *J* 2.6, *H*-4), 3.59 (1 H, dd, *J* 2.9, 9.2, *H*-3), 3.51 (1 H, q, *J* 6.3, *H*-5), 2.3 (3 H, s, $PhCH_3$), 1.27 (3 H, d, *J* 6.3, H_3-6). δ_C (126 MHz, $CDCl_3$) 138.8, 138.5, 138.4, 137.1, 132.2, 130.5, 130.1, 129.8, 129.5, 129.1, 128.6, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.0, 127.8, 127.7, 127.6, 127.4, 87.9, 84.6, 75.5, 74.6, 72.9, 30.9, 21.1, 17.3.

Per-*O*-acetyl-L-[$^{13}C_6$]galactopyranoside **15**[178]

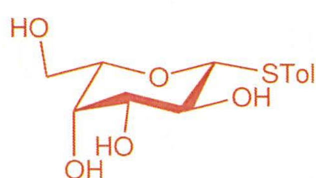
A suspension of NaOAc (1 eq, 0.16 g, 1.93 mmol) in Ac_2O (10 eq, 1.95 ml, 19.5 mmol) was stirred at 140 °C. L-[U-$^{13}C_6$]Galactose **14** (1eq, 0.349 g, 1.92 mmol) was added in small portions over a period of 6 minutes, and the reaction refluxed for a further 25 minutes after the final addition. The reaction mixture was poured onto crushed ice (50 ml) and stirred for 2 h. EtOAc (50 ml) was added to the mixture and the organic layer washed with water (2 x 30 ml) and $NaHCO_3$(aq) (30 ml) before drying over $MgSO_4$ and concentrating under reduced pressure. The product was purified by silica gel column chromatography (toluene/EtOAc at 6:1 (420 ml), 3:1 (400 ml), 1:1 (200 ml)) to give the white solid **15** as an α/β mixture (0.757 g, 1.91 mmol, 99%). R_f = 0.40 (toluene/EtOAc 1:1). δ_H (500 MHz, $CDCl_3$) 5.72 (1 H, d, *J* 8.5, *H*-1), 5.44 (1 H, d, *J* 3.4, *H*-4), 5.34 (1 H, t, *J* 8.2, *H*-2), 5.09 (1 H, dd, *J* 2.9, 10.7, *H*-3), 4.12 - 4.22 (2 H, m, H_2-6), 4.07 (1 H, t, *J* 6.4, *H*-5), 2.18 (3 H, s, $COCH_3$), 2.13 (3 H, s, $COCH_3$), 2.06 (6 H, s, $COCH_3$), 2.01 (3 H, s, $COCH_3$).

p-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-L-[$^{13}C_6$]galactopyranoside **16**[173] (D-enantiomer)



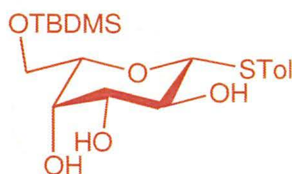
Per-*O*-acetyl-β- L-[$^{13}C_6$]galactopyranoside **15** (1 eq, 0.757 g, 1.91 mmol) was dissolved in DCM (6 ml) before adding *p*-Thiocresol (1.5 eq, 0.356 g, 2.87 mmol) and $BF_3.OEt_2$ (1.8 eq, 0.57 ml, 3.44 mmol) and the reaction left to stir under N_2 for 110 min at rt. The reaction was monitored with TLC (toluene/EtOAc 3:2). After completion, the reaction was quenched with triethylamine (1.3eq, 0.35 ml) in an ice bath and stirred for a further 30 min. DCM (20 ml) was added to the reaction mixture and was washed with saturated NaCl(aq) solution (2 x 20 ml). The organic layer was dried with MgSO4 and concentrated under vacuum, before being purified by silica gel column chromatography (toluene/EtOAc 1:0→3:1) to give the β-anomer **16** as a white, crystalline solid (0.880 g, 1.91 mmol, 98%). R_f = 0.58 (toluene/EtOAc 3:2). δ_H (500 MHz, $CDCl_3$) 7.40 (2 H, d, *J* 7.9, Ph*H*), 7.12 (2 H, d, *J* 7.9, Ph*H*), 5.41 (1 H, d, *J* 2.5, *H*-4), 5.20 (1 H, t, *J* 10.1, *H*-2), 5.03 (1 H, dd, *J* 3.0, 9.8, *H*-3), 4.63 (1 H, d, *J* 9.8, *H*-1), 4.09 – 4.20 (2 H, m, H_2-6), 3.90 (1 H, t, *J* 6.3, *H*-5), 2.34 (3 H, s, PhCH_3), 2.11 (3 H, s, COCH_3), 2.09 (3 H, s, COCH_3), 2.04 (3 H, s, COCH_3), 1.97 (3 H, s, COCH_3).

p-Tolyl 1-thio-β-L-[$^{13}C_6$]galactopyranoside **17**[174] (D-enantiomer)



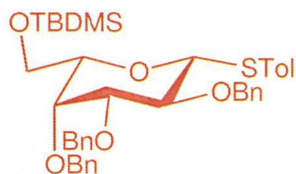
A solution of NaOMe/MeOH (excess, 7.5 ml, 1 M) and MeOH (100 ml) was made and compound **16** (1 eq, 0.880 g, 1.91 mmol) added to it. The reaction was stoppered and stirred at rt for 30 min. The pH of the solution was tested periodically to ensure it remained basic and progress monitored by TLC (DCM/MeOH 3:1). After completion, Amberlite IR-120 H-form resin was added and stirring continued until the solution no longer appeared to be basic. The Amberlite was then filtered off, washed with MeOH, and solvent removed under vacuum to give the crystalline product **17** (0.567 g, 1.91 mmol, 100%). R_f = 0.65 (DCM/methanol 3:1).

p-Tolyl 6-*O*-*tert*-butyldimethylsilyl-1-thio-β-L-[$^{13}C_6$]galactopyranoside **18**[175] (D-enantiomer)



Compound **17** (1 eq, 0.557 g, 1.91 mmol) was dried under vacuum before chilling the reaction vessel in an ice bath. TBDMSCl (1.1 eq, 0.320 g, 2.10 mmol) and pyridine (3 ml) were added and the reaction stirred under N_2 for 4 h, during which time the reaction mixture was allowed to reach rt. Upon completion, the pyridine was removed by co-evaporation with toluene (3 x 5 ml) before the crude product was purified by silica gel column chromatography (toluene/EtOAc 1:0→1:3) in a column with buffered silica gel (Et_3N 5% in toluene) to give the product **18** (0.736 g, 1.81 mmol, 95%). R_f = 0.69 (DCM/MeOH 6:1). δ_H (500MHz, $CDCl_3$) 7.45 (2 H, d, *J* 8.1, Ph*H*), 7.10 (2 H, d, *J* 8.2, Ph*H*), 4.44 (1 H, d, *J* 9.7, *H*-1), 4.07 (1 H, s, *H*-4), 3.87 – 3.95 (2 H, m, H_2-6), 3.65 (1 H, t, *J* 9.5, *H*-2), 3.58 (1 H, d, *J* 9.5, *H*-3), 3.50 (1 H, t, *J* 4.8, *H*-5), 0.90 (9 H, s, $SiC(CH_3)_3$), 0.10 (6 H, d, *J* 7.3, $Si(CH_3)_2$).

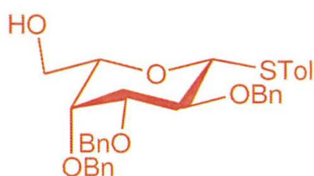
p-Tolyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-L-[$^{13}C_6$]galactopyranoside **19**



A mixture of NaH (3.9 eq, 0.17 g, 7.1 mmol) and BnBr (3.6 eq, 0.65 ml, 6.5 mmol) in DMF (2 ml) were stirred at 0 °C. Compound **18** (1 eq, 0.736 g, 1.81 mmol) was dissolved in DMF (5 ml) and added to the reaction vessel in portions. The reaction was allowed to reach rt and stirred for 2 h under N_2. The solvent was removed by co-evaporation with toluene (2 x 5 ml) before dissolving in DCM (50 ml) and washing with NaCl(aq) solution (saturated, 50 ml) and water (50 ml). The organic phase was dried with $MgSO_4$ and the solvent removed under reduced pressure. The product was then purified with silica gel column chromatography (toluene→toluene/EtOAc 2%) in buffered silica (Et_3N 0.5% in toluene) to give compound **19** (0.97 g, 1.43 mmol, 79%). R_f = 0.47 (toluene/EtOAc 20:1). δ_H (500MHz, $CDCl_3$) 7.48 (2 H, d, *J* 8.2, Ph*H*) 7.18 – 7.42 (15 H, m, Ph*H*), 7.01 (2 H, d, *J* 7.9, Ph*H*), 5.00 (1 H, d, *J* 11.7, PhCH_2), 3.92 (1 H,

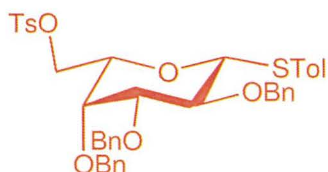
t, J 9.5, H-2), 4.73 – 4.81 (4 H, m, PhCH_2), 4.64 (1 H, d, J 11.4, PhCH_2) 4.60 (1 H, d, J 9.8, H-1), 3.96 (1 H, m, H-4), 3.73 – 3.79 (2 H, m, H_2-6), 3.61 (1 H, dd, J 2.8, 9.5, H-3), 3.44 (1 H, t, J 6.8, H-5), 2.31 (3 H, s, PhCH_3), 0.90 (9 H, s, SiC(CH_3)$_3$), 0.05 (6 H, s, Si(CH_3)$_2$).

p-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-[$^{13}C_6$]galactopyranoside **20**[176] (D-enantiomer)



Compound **19** (1 eq, 0.97 g, 1.43 mmol) and Bu_4NF (1.2 eq, 0.45 g, 1.7 mmol) were dissolved in THF (2 ml) and the reaction stirred under N_2 at rt for 16 h. The solvent was removed under reduced pressure before the crude product was purified by silica gel column chromatography (toluene/EtOAc 1:0→6:1) to give the product **20** (0.77 g, 1.37 mmol, 96%). R_f = 0.21 (toluene/EtOAc 9:1). δ_H (500MHz, $CDCl_3$) 7.40 (2 H, d, J 8.3, PhH) 7.14 – 7.38 (15 H, m, PhH) 7.00 (2 H, d, J 7.9, PhH), 4.94 (1 H, d, J 12.0, PhCH_2) 4.72 – 4.81 (4 H, m, PhCH_2), 4.61 (1 H, d, J 11.7, PhCH_2), 4.56 (1 H, d, J 10.0, H-1), 3.88 (1 H, t, J 9.5, H-2), 3.77 – 3.81 (2 H, m, H_2-6), 3.57 (1 H, dd, J 2.8, 9.1, H-3), 3.48 (1 H, d, J 5.0, H-4), 3.38 (1 H, t, J 6.2, H-5), 2.27 (3 H, s, PhCH_3).

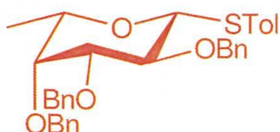
p-Tolyl 2,3,4-tri-*O*-benzyl-6-tosyl-1-thio-β-L-[$^{13}C_6$]galactopyranoside **21**[177] (D-enantiomer)



Compound **20** (1 eq, 0.77 g, 1.37 mmol) and TsCl (1.3 eq, 0.34 g, 1.8 mmol) were dried together under vacuum. Pyridine (3 ml) was added and the clear, colourless solution stirred under N_2 at rt for 3 h. Upon completion, the pyridine was removed by co-evaporation with toluene (2 x 5 ml) and the mixture diluted with EtOAc (40 ml) and washed with water (2 x 40 ml) before drying with MgSO4 and concentrating under reduced pressure. The compound was then purified by silica gel column chromatography (toluene/EtOAc 1:0→3:1) to give compound **21** as a white crystalline solid (0.92 g, 1.29 mmol, 94%). R_f = 0.48 (toluene/EtOAc 9:1). δ_H (500MHz, $CDCl_3$) 7.75 (2 H, d, J 8.2, PhH). 7.23 – 7.39 (19 H, m, PhH) 6.99 (2 H, d, J 7.9, PhH), 4.94 (1

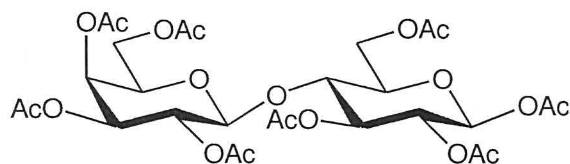
H, d, J 11.4, PhCH_2), 4.68 – 4.78 (4 H, m, PhCH_2), 4.51 (1 H, d, J 9.8, H-1), 4.47 (1 H, d, J 11.3, PhCH_2), 4.04 – 4.13 (2 H, m, H_2-6), 3.89 (1 H, s, H-4), 3.82 (1 H, t, J 9.5, H-2), 3.64 (1 H, t, J 6.5, H-5), 3.56 (1 H, dd, J 2.5, 9.2, H-3), 2.39 (3 H, s, PhCH_3), 2.30 (3 H, s, PhCH_3).

p-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-[$^{13}C_6$]fucopyranoside **22**[85]



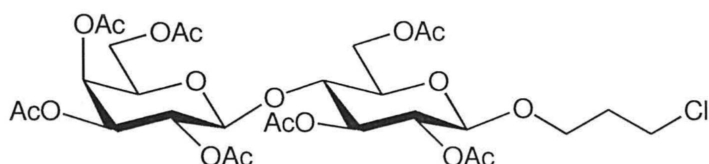
Compound **21** (1eq, 0.923 g, 1.29 mmol) and $LiAlH_4$ (30 eq, 0.147g, 38.7 mmol) were dried together under vacuum for 2 h. Dry THF (2 ml) was added and the reaction mixture refluxed under N_2 for 2 h. After completion the reaction mixture was diluted by adding THF (10ml) and added dropwise to a solution of THF (10 ml) and glacial acetic acid (5 ml) being chilled in an ice bath. Water (30 ml) was added and a small amount of hydrochloric acid (2 M) was added dropwise until the precipitate dissolved. An extraction with EtOAc (3 x 40 ml) was then carried out. The organic layers were combined, dried with MgSO4 and concentrated under reduced pressure. The product was then purified by silica gel chromatography (toluene→toluene/EtOAc 2%) to give compound **22** as a white solid (0.427 g, 0.781 mmol, 61 %). R_f = 0.40 (toluene/EtOAc 20:1). δ_H (400 MHz, $CDCl_3$) 7.49 (3 H, d, J 8.0, PhH), 7.35 (17 H, m, PhH), 7.02 (2 H, d, J 7.9, PhH), 5.00 (1 H, d, J 11.6, PhCH_2), 4.81 (1 H, d, J 10.1, PhCH_2), 4.73 (3 H, d, J 9.1, PhCH_2), 4.67 (1 H, d, J 11.6, PhCH_2), 4.54 (1 H, d, J 9.5, H-1), 3.89 (1 H, t, J 9.2, H-2), 3.65 – 3.55 (2 H, m, H-3, H-4), 3.51 (1 H, q, J 6.1, H-5), 2.30 (3 H, s, PhCH_3), 1.26 (3 H, d, J 6.2, H_3-6). δ_C (101 MHz, $CDCl_3$) 132.2, 129.5, 128.4, 128.4, 128.3, 128.2, 128.0, 127.7, 127.6, 88.1, 87.7, 85.0, 84.6, 84.2, 77.6, 77.3, 77.1, 77.0, 76.7, 76.63, 76.2, 74.9, 74.5, 74.1, 17.6, 17.5, 17.5, 17.1.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranoside **24**[179]



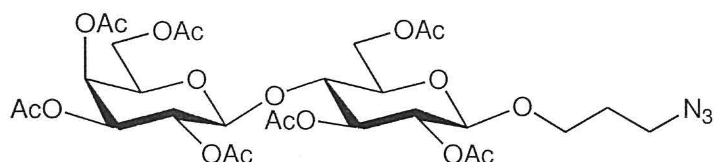
A suspension of anhydrous sodium acetate (2 eq, 24.0 g, 0.292 mol) in acetic anhydride (20 eq, 275 ml, 2.92 mol) was heated to 140 °C and D-lactose **23** (1 eq, 50 g, 0.146 mol) added in portions over a period of ten minutes. The clear, straw-yellow mixture was refluxed for a further 30 min before quenching over crushed ice (1 L) and left to stir overnight. The beige solid was isolated by suction filtration and washed with water and cold ethanol. The solid was then recrystallized in ethanol to give the product **24** as a white, crystalline solid. R_f = 0.56 (toluene/EtOAc 1:2) δ_H (400 MHz, $CDCl_3$) 5.66 (1 H, d, *J* 8.3, *H*-1), 5.33 (1 H, d, *J* 2.2, *H*-4'), 5.23 (1 H, t, *J* 9.1, *H*-3), 5.09 (1 H, dd, *J* 10.2, 8.1, *H*-2'), 5.04 (1 H, dd, *J* 9.8, 5.5, *H*-2), 4.94 (1 H, dd, *J* 10.4, 3.4, *H*-3'), 4.45 (2 H, m, H-1', *H*-6_a), 4.16 – 4.02 (3 H, m, *H*-6_b, *H*-6'), 3.90 – 3.79 (2 H, m, *H*-4, *H*-5'), 3.75 (1 H, dd, *J* 9.9, 3.0, *H*-5), 2.14 (3 H, s, COC*H*3), 2.11 (3 H, s, COC*H*3), 2.08 (3 H, s, COC*H*3), 2.05 (3 H, s, COC*H*3), 2.04 (3 H, s. COC*H*3), 2.03 (3 H, s, COC*H*3), 2.02 (3 H, s, COC*H*3), 1.95 (3 H, s, COC*H*3). δ_C (101 MHz, $CDCl_3$) 170.5 (170.5 – 169.0 *C*OCH3), 170.5, 170.3, 170.2, 169.8, 169.7, 169.2, 169.0, 101.1 (*C*-1'), 91.7 (*C*-1), 75.8 (*C*-4), 73.6 (*C*-5), 72.7 (*C*-3), 71.1 (*C*-3'), 70.9 (*C*-5'), 70.6 (*C*-2), 69.1 (*C*-2'), 66.7 (*C*-4'), 61.9 (*C*-6), 60.9 (*C*-6'), 21.0 (CO*C*H3), 20.9 (CO*C*H3), 20.9 (CO*C*H3), 20.8 (CO*C*H3), 20.7 (CO*C*H3), 20.7 (CO*C*H3), 20.6 (CO*C*H3).

3-Chloropropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **25**[180]



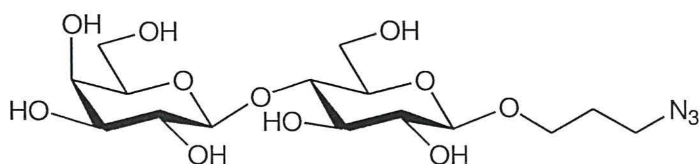
Lactoside **24** (1 eq, 110 g, 0.162 mol) was dried by co-evaporation in toluene and under reduced pressure before being dissolved in dry DCM (150 ml) and stirred under nitrogen. 3-Chloro-1-propanol (2 eq, 27.0 ml, 0.324 mol) and $BF_3.O(Et)_2$ (2 eq, 40.0 ml, 0.324 mol) and stirred for a 2 h at rt. After completion the reaction was quenched by addition of ice and stirred for 2 h. The organic layer was then washed with saturated $NaHCO_{3\ (aq)}$ and water before being dried with $MgSO_4$ and concentrated under reduced pressure. The yellow solid was taken for the next step without further purification. R_f = 0.66 (toluene/EtOAc 1:2).

3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **26**[86]



Crude compound **25** and KI (2 eq, 53.8 g, 0.324 mol) were dissolved in dry DMF (400 ml) and stirred for 1 h at 50 °C. A suspension of KCl appeared. Sodium azide (6 eq, 63.2, 0.972 mol) was added in small portions and the temperature raised to 140 °C. After the final addition on sodium azide the mixture was refluxed for 1 h before quenching in ice (2 L) and stirred for 2 h. The aqueous layer was then decanted off and kept aside, leaving a dark syrup. This was dissolved in EtOAc (800 ml) and washed with water (600 ml) and brine (600 ml). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The dark solid was taken for the next step without further purification. R_f = 0.61 (toluene/EtOAc 1:3). δ_H (400 MHz, $CDCl_3$) 5.32 (1 H, d, *J* 2.1, *H*-4), 5.17 (1 H, t, *J* 9.3, *H*-3), 5.08 (1 H, dd, *J* 10.2, 8.1, *H*-2), 4.93 (1 H, dd, *J* 10.4, 3.3), 4.87 (1 H, t, *J* 8.7, *H*-2), 4.51 – 4.42 (3 H, m, *H*-1,*H*-1, *H*-6_a), 4.18 – 4.00 (3 H, m, *H*-6, *H*-6_b), 3.92 – 3.82 (2 H, m, $OCH_{2a}CH_2CH_2N_3$, *H*-5), 3.76 (1 H, m, *H*-4), 3.62 – 3.52 (2 H, m, $OCH_{2b}CH_2CH_2N_3$, *H*-5), 3.41 – 3.29 (2 H, m, $OCH_2CH_2CH_2N_3$), 2.13 (3 H, s, $COCH_3$), 2.10 (3 H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 2.02 (6 H, s, $COCH_3$), 1.94 (3 H, s, $COCH_3$), 1.85 – 1.74 (2 H, m, $OCH_2CH_2CH_2N_3$). δ_C (101 MHz, $CDCl_3$) 170.5 ($COCH_3$), 170.5 ($COCH_3$), 170.3 ($COCH_3$), 170.2 ($COCH_3$), 169.9 ($COCH_3$), 169.7 ($COCH_3$), 169.2 ($COCH_3$), 101.2 (*C*-1), 100.7 (*C*-1), 76.3 (*C*-4), 72.9 (*C*-3), 72.8 (*C*-5), 71.7 (*C*-2), 71.1 (*C*-3), 70.8 (*C*-5), 69.2 (*C*-2), 66.7 (*C*-4), 66.6 ($OCH_2CH_2CH_2N_3$), 62.0 (*C*-6), 60.9 (*C*-6), 48.0 ($OCH_2CH_2CH_2N_3$), 29.1 ($OCH_2CH_2CH_2N_3$), 21.0 ($COCH_3$), 20.9 ($COCH_3$), 20.9 ($COCH_3$), 20.8 ($COCH_3$), 20.7 ($COCH_3$), 20.6 ($COCH_3$).

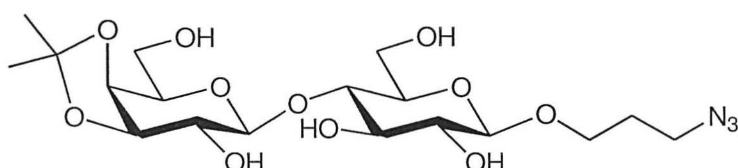
3-Azidopropyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **27**[86]



The crude product **26** was dissolved in MeOH (250 ml) and a solution of NaOMe in MeOH (1 M, 100 ml). The mixture was stirred at rt and monitored with TLC, whilst checking the mixture remained basic. After completion Amberlite® IR-120 H-form

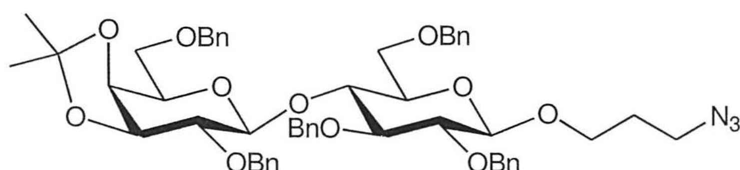
resin was added and stirring continued until indicator paper showed the mixture was acidic. The resin was removed by suction filtration and the product concentrated under reduced pressure. R_f = 0.66 (EtOAc/MeOH/H_2O 3:2:1). δ_H (400 MHz, D_2O) 4.50 (1 H, d, *J* 8.0), 4.46 (1 H, d, *J* 7.8), 4.05 – 3.96 (2 H, m, O$CH_{2a}CH_2CH_2N_3$, *H*-6_a), 3.94 (1 H, d, *J* 2.8, *H*-4), 3.85 – 3.74 (4 H, m, O$CH_{2b}CH_2CH_2N_3$, *H*-6_b), 3.72 (1 H, d, *J* 6.8), 3.67 (2 H, m, *H*-3, *H*-3'), 3.63 (1 H, d, *J* 7.8, *H*-5), 3.56 (1 H, t, *J* 9.4), 3.48 (2 H, t, *J* 6.7, $OCH_2CH_2CH_2N_3$), 3.33 (1 H, t, *J* 7.8), 1.97 – 1.88 (2 H, m, $OCH_2CH_2CH_2N_3$). δ_C (101 MHz, D_2O) 102.9, 102.1, 78.4 , 75.3, 74.8, 74.4, 72.8, 72.5, 70.9, 68.5, 67.4 ($OCH_2CH_2CH_2N_3$), 61.0, 60.0, 47.9 ($OCH_2CH_2CH_2N_3$), 28.2 ($OCH_2CH_2CH_2N_3$).

3-Azidopropyl 3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **28**[86]



The crude compound **27** (0.162 mol) was dissolved in mixture of DMF (150 ml) and acetone (700 ml). p-TSA.1H_2O (0.3 eq, 9.24 g, 0.049 mol) was added and the mixture stirred under nitrogen and monitored with TLC (DCM/MeOH 6:1, samples were quenched in Et_3N before being applied to TLC plate). After 24 h 2,2-dimethoxypropane (3 ml) was added and stirring continued for a further 24 h. This last step was repeated 4 times, until no starting material remained visible. The reaction was quenched with Et_3N (10 ml) and concentrated under reduced pressure. The crude product was used directly in the next step without further purification. R_f = 0.58 (DCM/MeOH 6:1)

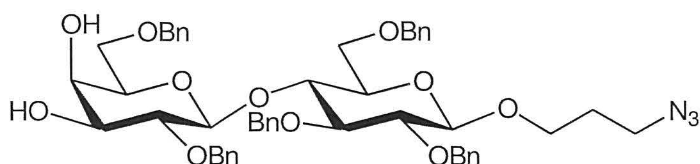
3-Azidopropyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **29**[86]



The crude product **28** was co-evaporated in dry toluene to give a solution of the lactoside in DMF. A solution of benzyl bromide (6 eq, 116 ml, 0.972 mol) in dry DMF (325 ml) was stirred under nitrogen and NaH (6.5 eq, 42.1 g as a 60% dispersion in

mineral oil, 1.05 mol) added. The reaction vessel was cooled in an ice bath and the lactoside in DMF added cautiously using a pressure equalising dropping funnel over a period of 30 min. Care was taken to limit the build-up of hydrogen gas. The reaction was allowed to reach room temperature and was monitored with TLC (toluene/EtOAc 6:1). The reaction was quenched 105 min after the final addition of lactoside by careful addition of MeOH (80 ml) while stirring in an ice bath. EtOAc (200 ml) was added to stop the mixture solidifying. When evolution of gas had ceased a few drops of water were added to ensure all sodium hydride had been destroyed. The reaction mixture was extracted with EtOAc (300 ml) and washed with brine (2 x400 ml) and water (400 ml). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. δ_H (400 MHz, $CDCl_3$) 7.42 – 7.17 (25 H, m, Ph*H*), 4.96 (1 H, d, *J* 10.5, PhCH_2), 4.87 – 4.66 (5 H, m, PhCH_2), 4.59 (1 H, d, *J* 12.1, PhCH_2), 4.53 (1 H, d, *J* 12.1, PhCH_2), 4.46 – 4.40 (2 H, m, *H*-1, PhCH_2), 4.38 (1 H, d, *J* 7.7, *H*-1), 4.33 (1 H, d, *J* 12.1, PhCH_2), 4.12 (1 H, d, *J* 5.4), 4.04 (1 H, t, *J* 6.0), 3.97 (2 H, t, *J* 9.2, $OCH_{2a}CH_2CH_2N_3$), 3.82 (1 H, dd, *J* 10.8, 3.4), 3.71 (2 H, m), 3.60 (3 H, m, $OCH_{2b}CH_2CH_2N_3$), 3.44 – 3.33 (5 H, m, $OCH_2CH_2CH_2N_3$, *H*-2, *H*-2'), 1.90 (2 H, m, $OCH_2CH_2CH_2N_3$), 1.42 (3 H, s, $C(CH_3)_2$), 1.37 (3 H, s, $C(CH_3)_2$). δ_C (101 MHz, $CDCl_3$) 139.1 (139.1 - 127.5 Ph*C*), 138.7, 138.7, 138.5, 138.3, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 109.9 ($C(CH_3)_2$), 103.7, 101.9, 83.1, 81.9, 80.8, 79.5, 76.4, 75.6 (PhCH_2), 75.2, 75.2 (PhCH_2), 73.7, 73.5 (PhCH_2), 73.4 (PhCH_2), 73.3 (PhCH_2), 72.1, 69.1, 68.3, 66.6 ($OCH_2CH_2CH_2N_3$), 48.5 ($OCH_2CH_2CH_2N_3$), 29.4 ($OCH_2CH_2CH_2N_3$), 28.1 ($C(CH_3)_2$), 26.5 ($C(CH_3)_2$).

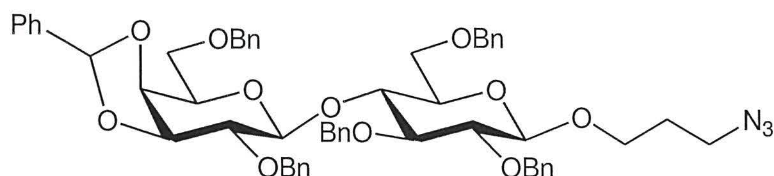
3-Azidopropyl 2,6-di-*O*-benzyl-3,4-hydroxyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **30**[86]



The crude mix of lactoside **29** in DMF (300 ml in total) was dissolved in dry DCM (150 ml) and a solution of TFA/H_2O (9:1, 120 ML) added while stirring. The reaction was monitored with TLC (toluene/EtOAc 3:1). After 15 min the reaction mixture was co-evaporated in toluene (150 ml) before cautiously adding Et_3N dropwise and further co-evaporations conducted until the mixture remained basic. The mixture was left stirring

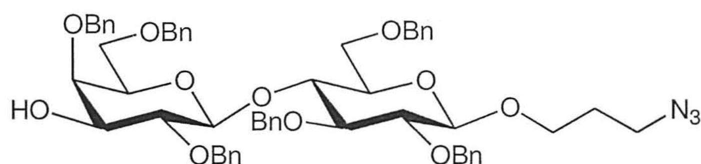
in water (1.5 L) for 1 h before being left to settle and the aqueous layer decanted. The oily product was co-evaporated in toluene and concentrated under reduced pressure. The crude product was then purified by silica gel column chromatography (toluene → toluene/EtOAc 2:1). δ_H (400 MHz, $CDCl_3$) 7.48 – 7.20 (25 H, m, Ph*H*), 5.01 (1 H, d, *J* 10.8, $PhCH_2$), 4.88 – 4.73 (3 H, m, $PhCH_2$), 4.69 (1 H, d, *J* 11.6, $PhCH_2$), 4.61 (1 H, d, *J* 12.1, $PhCH_2$), 4.45 (4 H, m, $PhCH_2$), 4.39 (1 H, d, *J* 7.5), 4.07 – 3.91 (3 H, m, $OCH_{2a}CH_2CH_2N_3$), 3.89 – 3.71 (2 H, m), 3.68 – 3.56 (3 H, m, $OCH_{2b}CH_2CH_2N_3$), 3.52 (1 H, dd, *J* 9.8, 5.0), 3.48 – 3.34 (2 H, m, $OCH_2CH_2CH_2N_3$, *H*-2, *H*-2'), 1.96 – 1.85 (2 H, m, $OCH_2CH_2CH_2N_3$). δ_C (101 MHz, $CDCl_3$) 139.2 (139.2 – 127.3 Ph*C*), 138.6, 138.5, 138.3, 138.1, 128.6, 128.6, 128.5, 128.5, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.4, 103.7, 102.7, 82.9, 81.9, 80.1, 76.6, 75.4 ($PhCH_2$), 75.2, 75.1 ($PhCH_2$), 75.0 ($PhCH_2$), 73.6, 73.6 ($PhCH_2$), 73.3 ($PhCH_2$), 73.0, 68.9, 68.80, 68.3, 66.6 ($OCH_2CH_2CH_2N_3$), 48.4 ($OCH_2CH_2CH_2N_3$), 29.4 ($OCH_2CH_2CH_2N_3$).

3-Azidopropyl 2,6-di-*O*-benzyl-3,4-*O*-*endo*-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **31**[95]



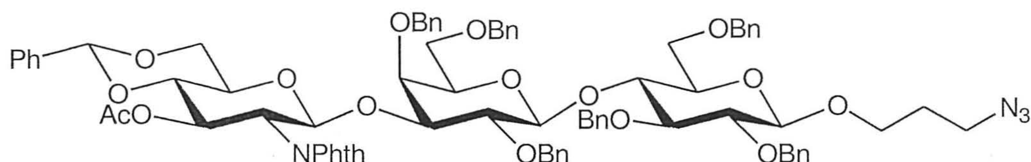
Compound **30** (1eq, 12.9 g, 14.7 mmol) was dissolved in dry THF (250 ml) and stirred under nitrogen at rt. Benzaldehyde dimethyl acetal (5 eq, 11.1 ml, 73.7 mmol) and p-TSA.1H_2O (0.3 eq, 0.84 g, 4.42 mmol) were added and the reaction monitored by TLC (toluene/EtOAc 6:1). After completion (150 min) the reaction was quenched with Et_3N (1 ml) and concentrated under reduced pressure. The mixture was co-evaporated with toluene and purified by silica gel column chromatography using buffered gel prepared from a solution of Et_3N (0.5%) in toluene (toluene → toluene/EtOAc 12%). Compound **31** was present a yellow solid (13.3 g, 13.8 mmol, 94%). R_f = 0.47 (toluene/EtOAc 6:1). δ_H (400 MHz, $CDCl_3$) 7.31-7.52 (30 H, m). 6.00 (1 H, s), 5.01 (1 H, d, *J* 10.7), 4.90 (1 H, d, *J* 10.7), 4.81 (1 H, d, *J* 11.1), 4.78 (1 H, d, *J* 11.7), 4.64 (2 H, t, *J* 12.2), 4.59 (1 H, d, *J* 12.0), 4.53 (1 H, d, *J* 8.2), 4.48 (1 H, d, *J* 12.0), 4.44 (1 H, s), 4.42 (1 H, d, *J* 4.1), 4.24-4.28 (2 H, m), 4.05 (1 H, dd, *J* 3.8, 9.5), 3.90 (1 H, dd, *J* 4.1, 11.1), 3.78-3.86 (3 H, m), 3.67-3.72 (2 H, m), 3.67 (1 H, t, *J* 9.0), 3.44-3.48 (5 H, m), 1.93-1.99 (2 H, m).

3-Azidopropyl 2,4,6-tri-*O*-benzyl-3-hydroxyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **32**[95]



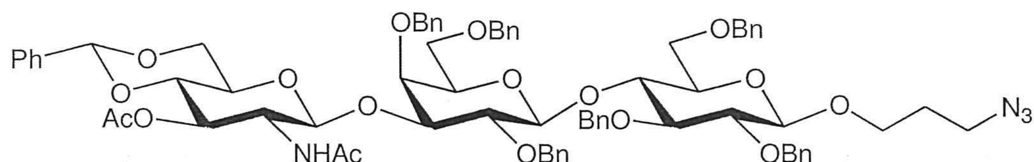
Compound **31** (1 eq, 7.25 g, 7.52 mmol) was dissolved in dry THF (72.5 ml) and stirred under N_2 with 3 Å powdered molecular sieves for 2 h. $NaBH_3CN$ (10 eq, 4.72 g, 75.2 mmol) was introduced into the reaction flask along with more molecular sieves and the solution stirred under N_2 for a further 2 h. A solution of HCl (2 M in Et_2O, 38 ml) was added until gas was no longer evolved. The reaction was monitored by TLC (toluene/EtOAc 3:1) and quenched after 15 min by addition of Et_3N (10 ml). The solvent was removed under reduced pressure and toluene (200 ml) added to the reaction mixture before washing with brine (200 ml), $NaHCO_{3(aq)}$ (200 ml) and water (200 ml). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (toluene → toluene/EtOAc 4:1) to give compound **32** as a clear, amber syrup (6.90 g, 7.14 mmol, 95%). R_f = 0.61 (toluene/EtOAc 3:1). δ_H (400 MHz, $CDCl_3$) 7.39 – 7.13 (30 H, m, Ph*H*), 5.03 (1 H, d, *J* 10.7, PhC*H*$_2$), 4.87 – 4.71 (5 H, m, PhC*H*$_2$), 4.69 (1 H, d, *J* 11.4, PhC*H*$_2$), 4.62 (1 H, d, *J* 11.6, PhC*H*$_2$), 4.58 (1 H, d, *J* 12.2, PhC*H*$_2$), 4.48 – 4.35 (4 H, m, *H*-1, *H*-1', PhC*H*$_2$), 4.28 (1 H, d, *J* 11.8, PhC*H*$_2$), 4.02 – 3.94 (2 H, m, OC*H*$_{2a}$CH$_2$CH$_2$N$_3$), 3.86 – 3.81 (1 H, m), 3.76 (2 H, m), 3.66 – 3.54 (3 H, m, OC*H*$_{2b}$CH$_2$CH$_2$N$_3$), 3.51 (2 H, d, *J* 5.5), 3.46 – 3.35 (6 H, m, OCH$_2$CH$_2$C*H*$_2$N$_3$), 1.89 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$). δ_C (101 MHz, $CDCl_3$) 139.1 (139.1 – 127.3 Ph*C*), 138.8, 138.7, 138.5, 138.3, 138.1, 128.5, 128.4, 128.4, 128.4, 128.4, 128.2, 128.1, 128.0, 127.8, 127.8, 127.8, 127.7, 127.6, 127.3, 103.6, 102.7, 83.0, 81.8, 80.7, 76.7, 76.0, 75.4 (Ph*C*H$_2$), 75.2, 75.2 (Ph*C*H$_2$), 75.1 (Ph*C*H$_2$), 75.0 (Ph*C*H$_2$), 74.2, 73.4 (Ph*C*H$_2$), 73.3, 73.2 (Ph*C*H$_2$), 68.3, 68.06, 66.55 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 48.39 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.32 (OCH$_2$*C*H$_2$CH$_2$N$_3$). m/z (MICRO-TOF) 988.43 $[M + Na]^+$ $C_{57}H_{63}N_3NaO_{11}$ requires 988.44.

3-Azidopropyl (3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(2,4,6-tri-*O*-benzyl-3-hydroxyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **34**[95]



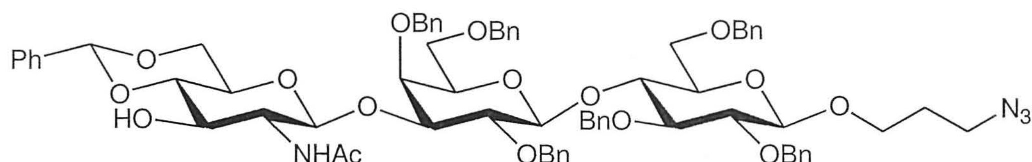
The lactoside acceptor **32** (1 eq, 2.20 g, 2.28 mmol) and *N*-phthalimido-glucosyl donor **33** (1.1 eq, 1.37 g, 2.51 mmol) were dissolved together in dry DCM (10 ml) and stirred under N_2 at rt with 4 Å powdered molecular sieves for 2 h. NIS (2 eq, 1.03 g, 4.56 mmol) and Ag(OTf) (cat) were added, turning the colourless mixture brick red in colour. The reaction was monitored with TLC (toluene/EtOAc 6:1) until the donor compound was no longer visible (20 min). The reaction was quenched with Et_3N (1 ml), turning the mixture muddy brown in colour. The reaction mixture was transferred to a separating funnel along with EtOAc (75 ml) and washed with $NaHCO_{3(aq)}$ (70 ml), $Na_2S_2O_{3(aq)}$ (10 %, 2 x 70 ml) and water (70 ml) before drying the organic layer with $MgSO_4$. The crude product was purified by silica gel column chromatography using buffered gel (toluene → toluene/EtOAc 9:1) to give the trisaccharide **34** as an amber solid (1.82 g, 1.31 mmol, 58 %). R_f = 0.22 (toluene/EtOAc 6:1). δ_H (500 MHz, $CDCl_3$) 7.49 (3 H, dd, *J* 4.9, 2.6, Ph*H*), 7.42 – 7.23 (38 H, m, Ph*H*), 7.22 – 7.14 (6 H, m, Ph*H*), 7.09 (2 H, d, J 7.5, Ph*H*), 6.93 – 6.89 (2 H, m, Ph*H*), 5.56 (1 H, s, PhC*H*), 4.98 (1 H, d, *J* 11.3), 4.90 (1 H, d, *J* 10.5), 4.77 (2 H, dd, *J* 10.6, 5.3), 4.71 (1 H, d, *J* 10.9), 4.56 (3 H, m), 4.47 (2 H, m), 4.36 (4 H, m), 4.31 – 4.23 (4 H, m), 4.19 (1 H, d, *J* 7.6), 4.06 (1 H, d, *J* 12.1), 3.95 – 3.83 (5 H, m), 3.78 (3 H, m), 1.87 (3 H, s, COC*H*$_3$), 1.83 (2 H, dd, *J* 12.5, 6.2). δ_C (101 MHz, $CDCl_3$) 170.1, 139.2, 139.1, 139.0, 138.6, 138.4, 138.4, 138.3, 138.2, 136.9, 129.2, 128.5, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.4, 127.1, 126.8, 126.3, 126.2, 126.2, 103.4, 102.4, 101.7, 99.9, 82.9, 81.9, 81.6, 79.3, 78.8, 76.5, 75.7, 75.4, 75.1, 75.0, 74.7, 74.0, 73.3, 73.0, 72.9, 69.5, 68.7, 68.1, 67.6, 66.4, 66.0, 64.7, 55.8, 48.3, 29.2, 20.6. m/z (MICRO-TOF) 1409.67 $[M + Na]^+$ $C_{80}H_{82}N_4NaO_{18}$ requires 1409.55.

3-Azidopropyl (3-*O*-acetyl-4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(2,4,6-tri-*O*-benzyl-3-hydroxyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **35**[95]



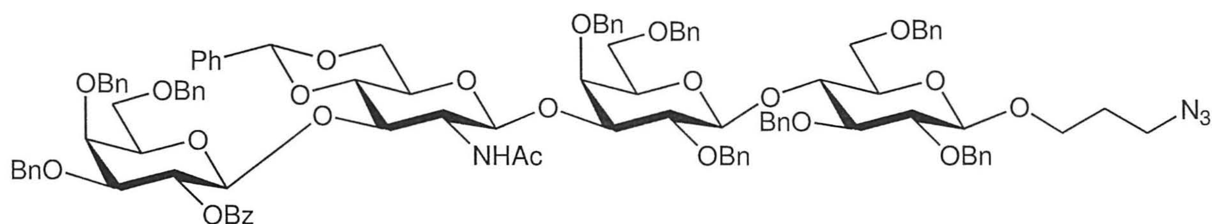
Trisaccharide **34** (1 eq, 1.65 g, 1.19 mmol) was dissolved in ethanol (absolute, 8 ml) and EDA (10 eq, 0.72 g, 11.9 mmol) and refluxed for 5 h. The reaction mixture was then co-evaporated with toluene to ensure removal of EDA and ethanol from the mixture, before drying under reduced pressure. The crude mixture was dissolved in pyridine (4 ml) before adding Ac_2O (4 eq, 0.49 g, 4.76 mmol) dropwise and leaving to stir overnight. The reaction was quenched with ethanol/water (1:1, 2 ml) whilst cooling in an ice bath, before co-evaporating the mixture with toluene under reduced pressure. The mixture was then redissolved in EtOAc (30 ml) before washing with $NaHCO_{3(aq)}$ (saturated, 2 x 30 ml). The organic layer was dried using $MgSO_4$, the solvent removed under reduced pressure and the product purified by silica gel column chromatography using buffered gel (toluene/EtOAc 5% → 40%) to give the trisaccharide **35** as an amber solid (1.12 g, 0.861 mmol, 72%). R_f = 0.40 (toluene/EtOAc 3:1). δ_H (500 MHz, $CDCl_3$) 7.52 – 7.14 (14 H, m), 5.01 (1 H, t, *J* 11.7), 4.95 (1 H, d, *J* 12.7), 4.82 (1 H, m), 4.71 (1 H, d, *J* 10.8), 4.64 (1 H, d, *J* 12.0), 4.59 (1 H, d, *J* 12.6), 4.53 (1 H, d, *J* 11.4), 4.48 (1 H, d, *J* 7.6), 4.45 (1 H, s), 4.41 (1 H, m), 4.34 (1 H, d, *J* 7.8), 4.28 (1 H, d, *J* 11.8), 4.20 (1 H, d, *J* 9.7), 4.04 – 3.93 (1 H, m), 3.90 – 3.82 (1 H, m), 3.79 (1 H, dd, *J* 10.9, 3.9), 3.75 – 3.70 (1 H, m), 3.69 – 3.64 (1 H, m), 3.58 (1 H, m), 3.51 – 3.46 (1 H, m), 3.44 – 3.33 (2 H, m), 3.31 (1 H, dd, *J* 9.5, 2.0), 2.03 (1 H, s), 1.90 (1 H, m), 1.56 (1 H, s). δ_C (126 MHz, $CDCl_3$) 171.1, 169.8, 139.3, 139.2, 139.1, 138.7, 138.5, 138.3, 137.0, 129.3, 128.9, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.5, 127.3, 126.3, 126.2, 103.7, 102.9, 102.8, 101.6, 83.0, 81.8, 80.9, 80.6, 78.7, 77.2, 76.5, 76.3, 75.6, 75.2, 75.2, 75.1, 74.5, 73.6, 73.5, 73.4, 72.2, 68.9, 68.3, 66.7, 66.6, 54.7, 48.5, 29.4, 23.0, 20.9. m/z (MICRO-TOF) 1321.55 $[M + Na]^+$ $C_{74}H_{82}N_4NaO_{17}$ requires 1321.56.

3-Azidopropyl (4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(2,4,6-tri-*O*-benzyl-3-hydroxyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **36**



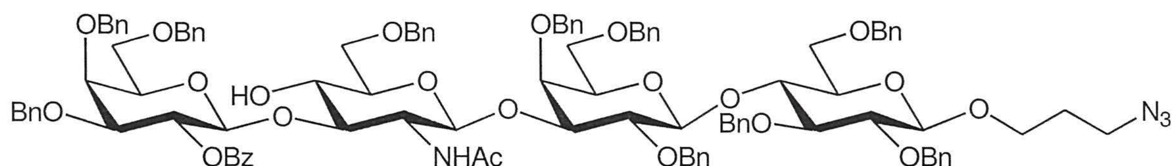
Trisaccharide **35** (1 eq, 1.63 g, 1.25 mmol) was dissolved in DCM (3 ml) and MeOH (18 ml) added dropwise with stirring while ensuring the starting material remained in solution. A solution of NaOMe in MeOH (3 ml, 1 moldm^{-3}) was added dropwise and the mixture left to stir at rt for 2 h. Solvent was removed under reduced pressure before adding EtOAc (60 ml) and washing with water (2 x 60 ml), before drying the organic layer over $MgSO_4$. The solvent was removed under vacuum and the product purified by silica gel column chromatography using buffered gel (toluene/EtOAc 10% → 40%) to give compound **36** as a colourless solid (1.54 g, 1.22 mmol, 98%). R_f = 0.60 (toluene/EtOAc 1:3). δ_H (400 MHz, $CDCl_3$) 7.54 (1 H, d, *J* 5.8), 7.47 – 7.12 (16 H, m), 5.58 (1 H, s), 5.51 (1 H, m), 5.03 (1 H, d, *J* 10.2), 4.93 (1 H, d, *J* 11.5), 4.85 (1 H, t, *J* 9.6), 4.79 (1 H, d, *J* 10.9), 4.74 (1 H, dd, *J* 9.1, 5.3), 4.65 (1 H, d, *J* 12.1), 4.56 (1 H, d, *J* 11.5), 4.50 (1 H, d, *J* 7.6), 4.45 – 4.33 (2 H, m), 4.29 (1 H, d, *J* 11.8), 4.05 (1 H, t, *J* 9.4), 3.97 (1 H, dd, *J* 10.6, 5.4), 3.93 (1 H, s), 3.85 – 3.74 (2 H, m, *J* 16.6, 9.0), 3.73 – 3.65 (1 H, m), 3.64 – 3.53 (2 H, m), 3.49 (1 H, dd, *J* 11.1, 5.0), 3.45 (1 H, d, *J* 7.7), 3.40 (1 H, t, *J* 6.8), 3.32 (1 H, d, *J* 9.5), 1.99 – 1.79 (1 H, m), 1.52 (1 H, s). δ_C (101 MHz, $CDCl_3$) 172.5, 139.1, 139.0, 138.6, 138.3, 138.1, 137.1, 130.1, 129.2, 128.9, 128.5, 128.4, 128.3, 128.3, 128.3, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.5, 127.3, 126.4, 126.1, 126.0, 103.6, 102.6, 101.9, 82.8, 81.7, 81.5, 80.0, 77.5, 77.2, 76.8, 76.3, 76.2, 75.5, 75.1, 75.1, 75.0, 74.3, 73.5, 73.4, 73.3, 72.7, 68.6, 68.1, 66.5, 66.5, 58.9, 48.3, 29.5, 29.3, 22.7. m/z (MICRO-TOF) 1279.54 $[M + Na]^+$ $C_{72}H_{80}N_4NaO_{16}$ requires 1279.55.

3-Azidopropyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **38**



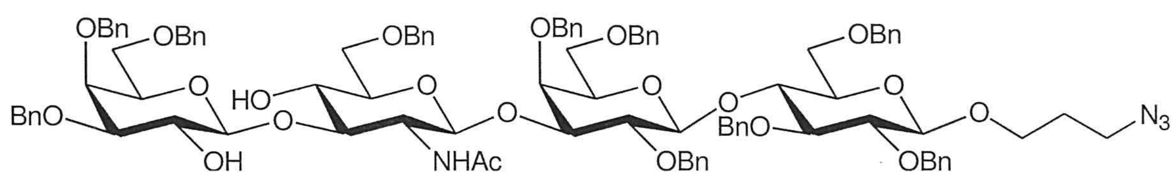
The trisaccharide acceptor **36** (1 eq, 1.54 g, 1.22 mmol) and galactoside donor **37** (1.2 eq, 0.971 g, 1.47 mmol) were dissolved together in dry DCM (10 ml) and stirred under $N_{2(g)}$ with 4 Å powdered molecular sieves for 2 h at rt. NIS (2 eq, 0.550 g, 2.44 mmol) and AgOTf (cat) were added, turning the colourless mixture brick red in colour. The reaction was quenched with Et_3N (0.5 ml) after 40 min, by which time compound was no longer visible with TLC (toluene/EtOAc 3:1). The reaction mixture was transferred to a separating funnel along with EtOAc (100 ml) and washed with $NaHCO_{3(aq)}$ (100 ml), $Na_2S_2O_{3(aq)}$ (10 %, 2 x 100 ml) and water (70 ml) before drying the organic layer with $MgSO_4$. The crude product was purified by silica gel column chromatography (toluene (0.5 % Et_3N) → toluene (30 % EtOAc)) to give the tetrasaccharide **38** as an amber solid (1.78 g, 0.991 mmol, 81 % yield). δ_H (400 MHz, $CDCl_3$) 7.99 (2 H, d, *J* 7.6), 7.59 – 7.07 (76 H, m), 5.63 – 5.56 (1 H, m), 5.51 (1 H, s, PhC*H*), 5.31 (1 H, d, *J* 8.1, *H*-1), 5.06 – 4.98 (1 H, m), 4.93 (2 H, t, *J* 12.7), 4.81 (2 H, m), 4.71 – 4.55 (9 H, m), 4.53 (1 H, d, *J* 12.1, PhCH_2), 4.51 (1 H, d, *J* 11.6, PhCH_2), 4.42 – 4.28 (8 H, m), 4.26 – 4.20 (3 H, m), 3.99 – 3.82 (5 H, m), 3.78 – 3.69 (4 H, m), 3.67 – 3.52 (10 H, m), 3.51 – 3.45 (4 H, m), 3.44 – 3.28 (10 H, m), 3.19 (1 H, d, *J* 9.5), 3.00 (1 H, m), 1.96 – 1.82 (2 H, m, $CH_2CH_2CH_2N_3$), 0.83 (3 H, s, $COCH_3$). δ_C (101 MHz, $CDCl_3$) 170.7, 165.3, 139.5, 139.1, 138.9, 138.7, 138.6, 138.4, 138.3, 137.8, 137.7, 133.2, 129.9, 129.2, 129.1, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 128.0, 127.9, 127.7, 127.6, 127.4, 127.2, 126.4, 126.3, 103.6, 102.5, 101.4 (PhCH), 101.0, 100.6, 83.0, 82.4, 81.7, 81.3, 80.0, 79.4, 77.2, 77.1, 76.4, 76.3, 75.4 (PhCH_2), 75.1 (PhCH_2), 75.1, 74.9 (PhCH_2), 74.8 (PhCH_2), 74.6 (PhCH_2), 73.5 (PhCH_2), 73.3 (PhCH_2), 73.2 (PhCH_2), 73.0, 72.9, 72.6, 72.1, 71.6 (PhCH_2), 69.0, 68.3, 68.3, 68.1, 66.5 ($CH_2CH_2CH_2N_3$), 65.5, 59.2, 48.4 ($CH_2CH_2CH_2N_3$), 29.3 ($CH_2CH_2CH_2N_3$), 22.4 ($COCH_3$). m/z (MICRO-TOF) 1815.73 $[M + Na]^+$ $C_{106}H_{112}N_4NaO_{22}$ requires 1815.77.

3-Azidopropyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **39**



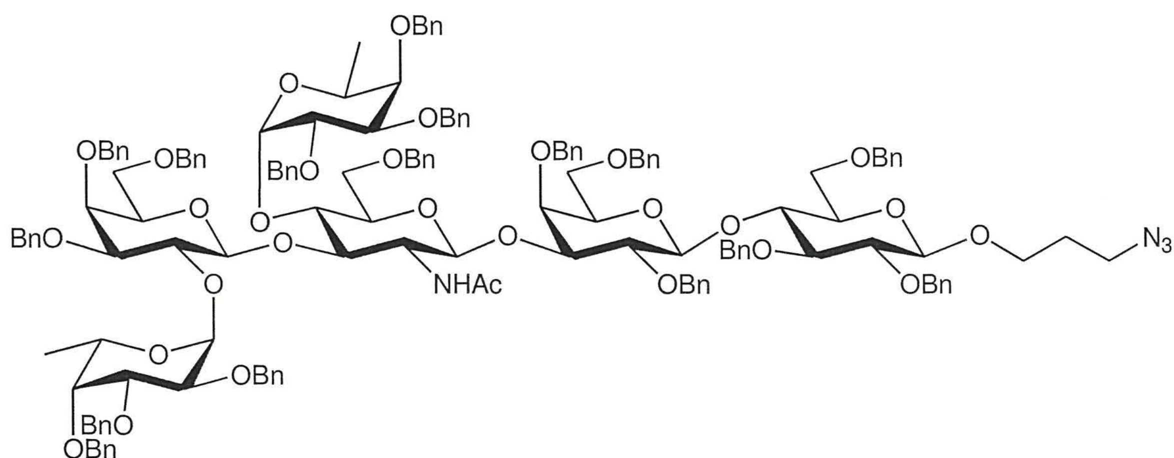
Tetrasaccharide **38** (1 eq, 0.569 g, 0.317 mmol) was dissolved in THF (5 ml) and stirred at rt with 3 Å molecular seives under a N_2 atmosphere for 3 h. $NaBH_3CN$ (10 eq, 0.199 g, 3.17 mmol) was added to the mixture along with some more seives, with stirring for a further 1 h. HCl in Et_2O (10 eq, 1.6 ml, 2 moldm^{-3}) was added dropwise until gas ceased to be evolved. The reaction was monitored by TLC (toluene/EtOAc 1:1) before quenching with Et_3N 40 min after the addition of HCl. The solvent was removed under vacuum and the mixture purified by silica gel column chromatography (toluene/EtOAc 10% → 50%) to give the product **39** as an off-white solid (0.481 g, 0.268 mmol, 85%). R_f = 0.66 (toluene/EtOAc 1:1) δ_H (400 MHz, $CDCl_3$) 7.97 (1 H, d, *J* 7.9), 7.56 (1 H, d, *J* 7.3), 7.42 (2 H, t, *J* 7.8), 7.35 – 7.06 (38 H, m), 5.62 (1 H, t, *J* 9.0), 5.10 (1 H, d, *J* 8.2), 5.00 (1 H, d, *J* 10.7), 4.94 (1 H, d, *J* 11.3), 4.92 (1 H, d, *J* 9.1), 4.81 (1 H, d, *J* 11.0), 4.73 (1 H, d, *J* 11.2), 4.66 (1 H, d, *J* 10.7), 4.62 (1 H, d, *J* 12.8), 4.58 (1 H, d, *J* 12.1), 4.54 (1 H, d, *J* 9.2), 4.51 – 4.43 (7 H, m), 4.42 (1 H, d, *J* 12.0), 4.36 – 4.27 (3 H, m), 4.29 (1 H, d, *J* 11.2), 4.26 (1 H, d, *J* 7.8), 4.18 (1 H, d, *J* 12.1), 3.98 (1 H, d, *J* 2.2), 3.96 – 3.89 (2 H, m), 3.92 (1 H, s), 3.91 – 3.85 (1 H, m), 3.85 (1 H, d, *J* 10.0), 3.73 – 3.69 (1 H, m), 3.68 – 3.62 (4 H, m), 3.61 – 3.54 (5 H, m), 3.53 – 3.42 (8 H, m), 3.40 – 3.31 (6 H, m), 3.21 – 3.15 (1 H, m), 2.78 (1 H, m), 1.86 (2 H, dd, *J* 11.0, 5.8), 0.87 (3 H, s). δ_C (101 MHz, $CDCl_3$) 170.7, 165.1, 139.8, 139.2, 138.9, 138.7, 138.6, 138.4, 138.2, 138.1, 137.6, 137.4, 133.4, 130.0, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 128.0, 128.0, 128.0, 127.3, 127.3, 127.1, 103.6, 102.6, 101.8, 99.9, 83.1, 83.0, 81.9, 81.7, 79.8, 79.5, 77.2, 77.2, 76.8, 75.4 (Ph*C*H_2), 75.2 (Ph*C*H_2), 75.1, 75.0, 74.8 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.6 (Ph*C*H_2), 73.9, 73.8 (Ph*C*H_2), 73.6 (Ph*C*H_2), 73.4 (Ph*C*H_2), 73.4, 73.2 (Ph*C*H_2), 72.3 (Ph*C*H_2), 72.2, 72.2, 70.3, 70.1, 68.8, 68.5, 68.0, 66.5 (*C*$H_2CH_2CH_2N_3$), 58.7, 48.4 (CH_2CH_2*C*H_2N_3), 29.4 (CH_2*C*$H_2CH_2N_3$), 22.8 (CO*C*H_3). m/z (MICRO-TOF) 1817.63 $[M + Na]^+$ $C_{106}H_{114}N_4NaO_{22}$ requires 1817.78.

3-Azidopropyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **40**



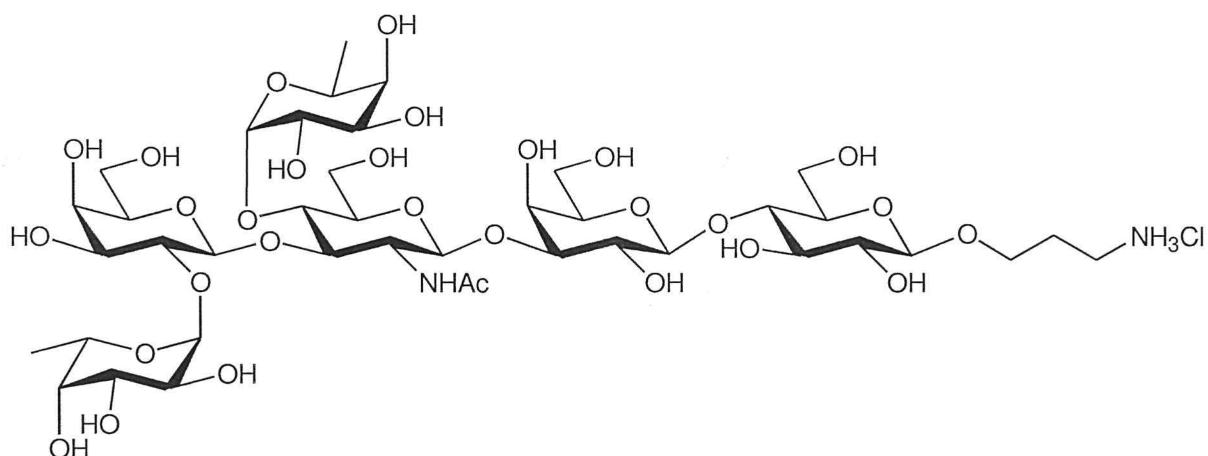
Compound **39** (1 eq, 0.557 g, 0.310 mmol) was dissolved in DCM (3 ml) and MeOH (18 ml) added dropwise with stirring while ensuring the starting material remained in solution. A solution of NaOMe in MeOH (3 ml, 1 moldm^{-3}) was added dropwise and the mixture left to stir at rt. After 16 h had elapsed, starting material was still observed by TLC (toluene/ EtOAc 1:1). The temperature was raised to 40 °C, with the reaction appearing to be complete after 2 days. Solvent was removed under reduced pressure before adding EtOAc (60 ml) and washing with water (2 x 60 ml), before drying the organic layer over $MgSO_4$. The solvent was removed under vacuum and the product purified by silica gel column chromatography (toluene/EtOAc 5% → 50%) to give compound **40** as a colourless solid (0.461 g, 0.273 mmol, 88%). R_f = 0.48 (toluene/EtOAc 1:1). δ_H (400 MHz, $CDCl_3$) 7.42 – 7.09 (1 H, m), 5.11 – 4.86 (1 H, m), 4.84 – 4.64 (1 H, m), 4.56 (1 H, m), 4.37 (1 H, m), 4.20 (1 H, d, *J* 12.0), 4.04 (1 H, d, *J* 7.5), 3.94 (1 H, m), 3.80 – 3.63 (1 H, m), 3.61 – 3.46 (1 H, m), 3.39 (1 H, m), 3.26 (1 H, d, *J* 8.8), 1.85 (2 H, d, *J* 4.4), 1.47 (3 H, s). δ_C (101 MHz, $CDCl_3$) 171.6, 139.2, 139.1, 139.0, 138.6, 138.5, 138.4, 138.3, 138.3, 138.2, 138.1, 137.5, 128.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.7, 127.7, 127.6, 127.5, 127.2, 127.2, 126.0, 104.8, 103.5, 102.5, 101.7, 87.4, 82.8, 81.6, 81.6, 81.4, 80.0, 76.3, 75.5, 75.4, 75.1, 75.0, 74.9, 74.6, 74.2, 74.1, 73.7, 73.6, 73.4, 73.3, 73.3, 72.9, 71.2, 70.1, 69.5, 68.9, 68.3, 68.0, 66.4, 55.7, 48.3, 29.2, 23.1. m/z (MICRO-TOF) 1713.76 $[M + Na]^+$ $C_{99}H_{110}N_4NaO_{21}$ requires 1713.76.

3-Azidopropyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **42**



Tetrasaccharide **40** (1 eq, 43.9 mg, 25.9 μmol), fucosyl donor **41** (3 eq, 42.1 mg, 77.8 μmol) and Et_4NBr (3 eq, 16.0 mg, 77.8 μmol) were dissolved in a solution of DCM/DMF (9:1, 1 ml) and stirred at rt with 4 Å molecular sieves under N_2. After 2 h, bromine (4.5 eq, 19 mg, 117 μmol) was added and the now yellow solution allowed to stir for 16 h. Upon completion, Et_3N was added and the reaction mixture was diluted in EtOAc (25 ml) and washed with $Na_2S_2O_{4(aq)}$ (10%, 2 x 25 ml) before drying the organic layer over $MgSO_4$ and removing the solvent under vacuum. The product was purified by silica gel column chromatography (toluene/EtOAc 2% → 20%) to give the product **42** as a colourless solid (52 mg, 20.6 μmol, 80%). R_f = 0.28 (toluene/EtOAc 3:1). δ_H (400 MHz, $CDCl_3$) 7.41 – 7.03 (99 H, m), 6.97 (2 H, d, *J* 7.3), 5.56 (1 H, d, *J* 3.2), 5.08 – 4.94 (4 H, m), 4.81 (9 H, m), 4.73 – 4.42 (23 H, m), 4.42 – 4.17 (12 H, m), 4.06 – 3.78 (16 H, m), 3.71 (6 H, m), 3.65 – 3.51 (8 H, m), 3.41 (10 H, m), 3.24 (2 H, d, *J* 11.3), 1.85 (2 H, dd, *J* 9.5, 6.0), 1.61 (3 H, s), 1.20 (6 H, d, *J* 5.8). δ_C (101 MHz, $CDCl_3$) 139.5, 139.4, 139.3, 139.1, 139.0, 138.8, 138.8, 138.6, 138.6, 138.4, 138.4, 138.3, 138.2, 138.2, 138.2, 138.1, 137.6, 128.7, 128.6, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.4, 127.3, 127.2, 127.1, 127.1, 127.0, 126.9, 126.3, 126.2, 103.5, 102.6, 102.1, 101.5, 98.4, 97.9, 83.8, 82.8, 81.6, 80.4, 79.2, 78.1, 76.4, 76.3, 75.6, 75.6, 75.4, 74.9, 74.9, 74.8, 74.4, 74.0, 73.6, 73.4, 73.3, 73.2, 73.1, 73.0, 72.9, 72.7, 71.8, 71.7, 71.2, 68.6, 68.3, 68.1, 67.4, 66.9, 66.6, 66.44, 60.4, 48.3, 29.2, 23.4, 16.3, 16.2.

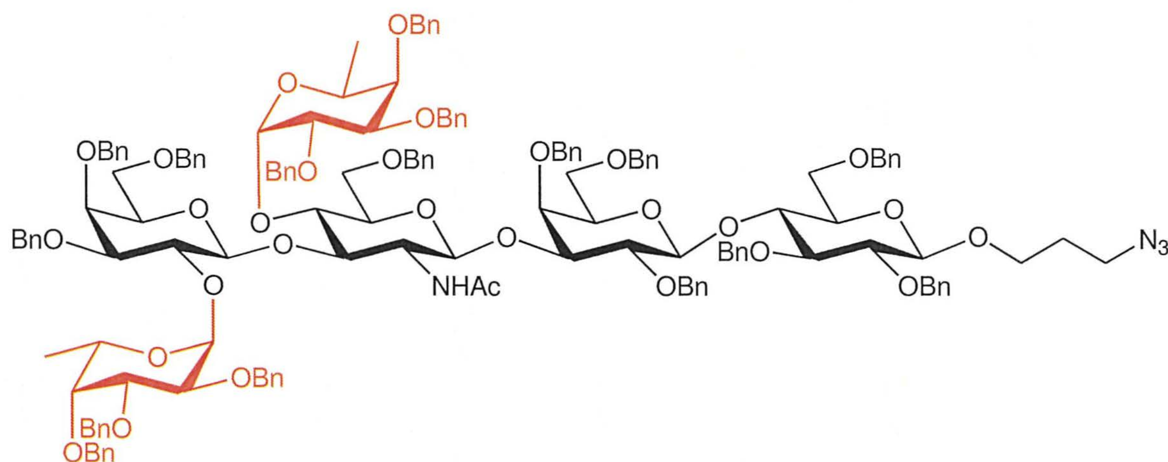
3-Aminopropyl (α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[α-L-fucopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl chloride **43**[66]



Hexasaccharide **42** (1 eq, 24.0 mg, 9.51 μmol) was dissolved in 1,4-dioxane (3 ml) and stirred while adding water (1 ml) dropwise. A precipitate appeared, which was redissolved by adding a little more 1,4-dioxane. The mixture was degassed and flushed through with $N_{2(g)}$ before adding Pd/C (10 wt. %, cat), $HCl_{(aq)}$ (1 eq, 0.1 moldm^{-3}, 95 μl) and stirring under $H_{2(g)}$. The reaction was monitored by TLC ($EtOH/H_2O/NH_4OH$ 1:1:1) until, after 20 h, only 1 product spot was visible. Additional water was added to the mixture which was then filtered through celite. The solvent was removed by lyophilisation before purification using a size exclusion column. Column was run with a solution of butanol (1%) in ultra-pure water as the mobile phase. The solvent was eluted at a rate of 36 ml/h and collected using a Gilson fraction collector (9 min, or ~5.4 ml per test tube). A solution of the hexasaccharide in the mobile phase was added to the top of the column before carefully adding more of the mobile phase via a reservoir. TLC showed the presence of the hexasaccharide in fractions 11-16. These were combined and lyophilised to give the deprotected hexasaccharide **43** as a fine, white solid (8.50 mg, 7.77 μmol, 82%). $R_f = 0.35$ ($EtOH/H_2O/NH_4OH$ 1:1:1). δ_H (500 MHz, D_2O) 5.12 (1 H, d, *J* 3.9, Fuc-*H*-1), 5.00 (1 H, d, *J* 3.8, Fuc-*H*-1), 4.63 (1 H, d, *J* 7.7, *H*-1), 4.57 (1 H, d, *J* 8.4, *H*-1), 4.48 (1 H, d, *J* 8.0, *H*-1), 4.38 (1 H, d, *J* 7.9, *H*-1), 4.11 (2 H, dd, *J* 11.6, 8.2), 4.05 – 4.00 (1 H, m), 3.96 (1 H, s), 3.94 (1 H, s), 3.93 (1 H, s), 3.89 (2 H, dd, *J* 10.4, 3.4), 3.85 (1 H, d, *J* 2.6), 3.78 (9 H, ddd, *J* 12.9, 10.4, 3.5), 3.74 – 3.65 (12 H, m), 3.65 – 3.57 (6 H, m), 3.56 – 3.47 (4 H, m), 3.30 (1 H, t, *J* 8.5), 3.13 (2 H, t, *J* 7.0), 2.03 (2 H, s), 1.98 (2 H, dd, *J* 9.1, 5.7), 1.24 (6 H, t, *J* 7.2). δ_C (101 MHz, D_2O) 103.1 (*C*-1), 102.9 (*C*-1), 102.0 (*C*-1), 100.6, 99.5 (*C*-1), 97.7 (*C*-1), 81.5, 78.1, 76.4, 75.1, 74.8, 74.4, 74.2, 73.6, 72.7, 71.9, 71.7, 70.1, 69.4, 69.0, 68.7, 68.5, 68.2, 67.7,

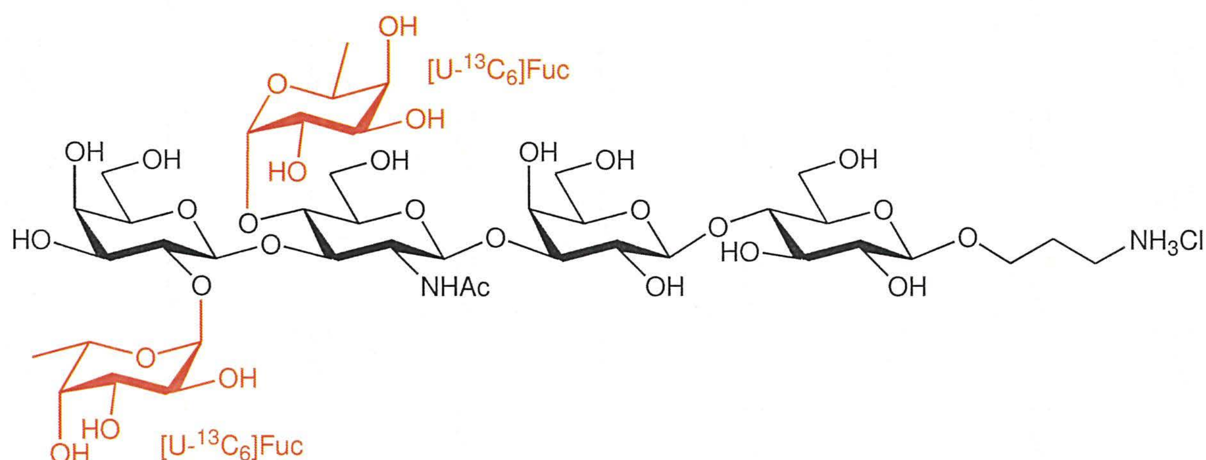
67.0, 66.2, 61.6, 60.9, 60.4, 59.9, 59.4, 55.7, 37.5, 26.6, 22.1 (CO*C*H_3), 15.3 (Fuc-*C*-6). m/z (MALDI-TOF) 1079.33 $[M + Na]^+$ $C_{41}H_{73}N_2NaO_{29}$ requires 1080.42.

3-Azidopropyl (2,3,4-tri-*O*-benzyl-α-L-[$^{13}C_6$]fucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-[$^{13}C_6$]fucopyranosyl)-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **44**



Tetrasaccharide **40** (1 eq, 50.3 mg, 29.7 μmol), $^{13}C_6$ labelled fucosyl donor **22** (3 eq, 48.3 mg, 89.2 μmol) and Bu_4NBr (3 eq, 28.7 mg, 89.2 μmol) were dissolved in a solution of DCM/DMF (9:1, 1 ml) and stirred at rt with 4 Å molecular sieves under N_2. After 2 h, bromine (4.5 eq, 21 mg, 130 μmol) was added and the now yellow solution allowed to stir under N_2 for 16 h. Upon completion, Et_3N was added and the reaction mixture was diluted in EtOAc (25 ml) and washed with $Na_2S_2O_{4(aq)}$ (10%, 2 x 25 ml) before drying the organic layer over $MgSO_4$ and removing the solvent under vacuum. The product was purified by silica gel column chromatography (toluene/EtOAc 2% → 20%) to give the product **44** as a colourless solid (39.2 mg, 15.5 μmol, 52%). $R_f = 0.30$ (toluene/EtOAc 3:1) m/z (MALDI-TOF) 2547.44 $[M + Na]^+$ $C_{153}H_{167}N_4NaO_{29}$ requires 2547.16.

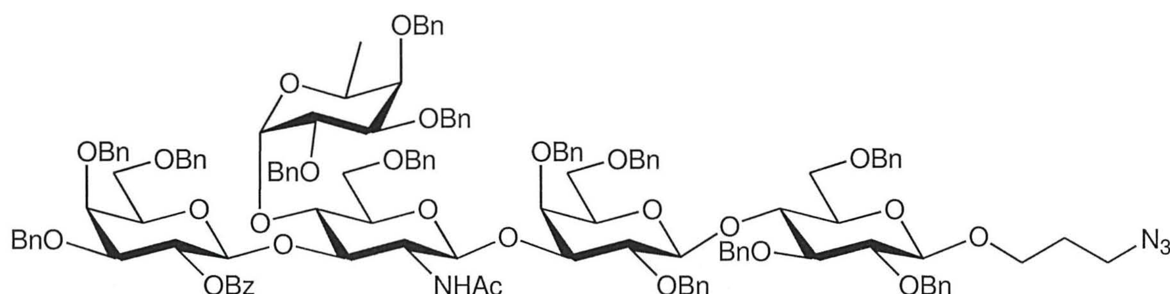
3-Aminopropyl (α-L-[U-$^{13}C_6$]fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[α-L-[U-$^{13}C_6$]fucopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl chloride **45**[66]



Hexasaccharide **44** (1 eq, 39.2 mg, 15.5 μmol) was dissolved in 1,4-dioxane (5 ml) and stirred while adding water (1.6 ml) dropwise. The mixture was degassed and flushed through with $N_{2(g)}$ before adding Pd/C (10 wt. %, cat), $HCl_{(aq)}$ (1 eq, 0.1 $moldm^{-3}$, 155 μl) and stirring under $H_{2(g)}$. The reaction was monitored by TLC ($EtOH/H_2O/NH_4OH$ 1:1:1) and after 16 h had not gone to completion. Fresh Pd/C was added and the mixture stirred for a further 24 h until only 1 product spot was visible. Additional water was added to the mixture which was then filtered through celite. The solvent was removed by lyophilisation before purification using a size exclusion column. Column was run with a solution of butanol (1%) in ultra-pure water as the mobile phase. The solvent was eluted at a rate of 36 ml/h and collected using a Gilson fraction collector (9 min, or ~5.4 ml per test tube). A solution of the hexasaccharide in the mobile phase was added to the top of the column before carefully adding more of the mobile phase via a reservoir. TLC and MALDI TOF showed the presence of an impurity in some of the fractions containing the product. These fractions were combined and a further purification performed using a RP-C18 column. This column had been prewashed with a step-gradient using 5 ml for each step (MeOH in H_20, 100%, 80%, 60%, 40%, 20%, 10%MeOH). The compound mixture was applied to the top of the column and eluted with a $MeOH/H_2O$ solvent mixture in a step-gradient with 5 ml for each step (MeOH in H2O, 10%, 20%, 30%, 40%). The fractions containing pure product were combined and lyophilised to give the deprotected hexasaccharide **45** as a fine, white solid (4.50 mg, 4.07 μmol, 26%). R_f = 0.35 ($EtOH/H_2O/NH_4OH$ 1:1:1). δ_H (400 MHz, D_2O) 5.19 (1 H, s, Fuc-*H*-1), 5.06 (1 H, s, Fuc-*H*-1), 4.89 (1 H, q, Fuc-*H*-5), 4.69 (1 H, d, *J* 7.7), 4.64 (1 H, d, *J* 8.4), 4.54 (1 H, d, *J* 8.0), 4.45 (1 H, d, *J* 7.8), 4.37 (1 H, d, *J* 6.2, Fuc-*H*-5), 4.20 – 4.11 (2 H, m), 4.12 – 4.05 (1 H, m), 4.03 – 3.81 (14 H, m), 3.75 (10 H, dd, *J* 19.4,

6.8), 3.69 – 3.46 (8 H, m), 3.36 (1 H, t, *J* 8.3), 3.19 (2 H, t, *J* 6.9), 2.10 (3 H, s, $COCH_3$), 2.07 – 1.99 (2 H, m), 1.30 (6 H, t, *J* 6.1, Fuc-*H*-6). δ *C* (101 MHz, D_2O) 99.73, 99.73, 99.28, 97.96, 97.49, 72.29, 71.93, 71.56, 69.77, 69.39, 69.04, 68.68, 68.58, 68.13, 67.73, 67.66, 67.34, 67.27, 66.97, 66.94, 66.56, 66.15, 65.76, 15.46, 15.07. m/z (MALDI-TOF) 1092.05 $[M + Na]^+$ $^{12}C_{29}{}^{13}C_{12}H_{72}N_2NaO_{29}$ requires 1091.88.

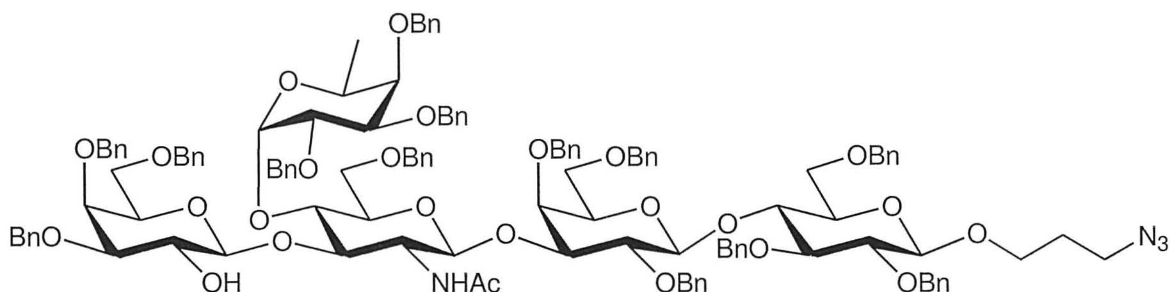
3-Azidopropyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **46**



Tetrasaccharide **39** (1 eq, 0.268 g, 0.149 mmol), fucosyl donor **41** (1.5 eq, 0.121 g, 0.224 mmol) and Et_4NBr (1.5 eq, 47.0 mg, 0.224 mmol) were dissolved in a solution of DCM/DMF (9:1, 2 ml) and stirred at rt with 4 Å molecular sieves under N_2. After 2 h, bromine (2.25 eq, 54 mg, 0.34 mmol) was added and the now yellow solution allowed to stir for 16 h. Upon completion, Et_3N was added and the reaction mixture was diluted in EtOAc (25 ml) and washed with $Na_2S_2O_{4(aq)}$ (10%, 2 x 25 ml) before drying the organic layer over $MgSO_4$ and removing the solvent under vacuum. The product was purified by silica gel column chromatography (toluene/EtOAc 5% → 40%) to give the product **46** as a colourless solid (0.147 g, 66.5 μmol, 45%). R_f = 0.73 (toluene/EtOAc 1:1) δ_H (400 MHz, $CDCl_3$) 7.99 (2 H, t, *J* 7.7), 7.54 (1 H, dd, *J* 12.9, 6.9), 7.46 – 7.07 (76 H, m), 5.53 (1 H, dd, *J* 15.9, 7.9), 5.19 (1 H, d, *J* 7.1), 5.05 – 4.98 (2 H, m), 4.96 (1 H, d, *J* 5.0), 4.94 (1 H, d, *J* 11.4), 4.88 (1 H, d, *J* 11.5), 4.85 (1 H, s), 4.81 (1 H, d, *J* 10.9), 4.80 (1 H, d, *J* 11.8), 4.75 (1 H, s), 4.75 (2 H, d, *J* 11.5), 4.72 (2 H, d, *J* 8.3), 4.68 (2 H, d, *J* 12.3), 4.66 (2 H, d, *J* 10.1), 4.64 (2 H, d, *J* 11.1), 4.62 (2 H, d, *J* 11.9), 4.61 – 4.56 (4 H, m), 4.53 (2 H, dd, *J* 7.8, 4.8), 4.49 (3 H, t, *J* 6.5), 4.45 (1 H, s), 4.44 (1 H, s), 4.40 (1 H, d, *J* 11.6), 4.35 – 4.27 (4 H, m), 4.30 (2 H, d, *J* 7.9), 4.26 – 4.16 (3 H, m), 4.12 (1 H, d, *J* 11.8), 4.06 (1 H, d, *J* 11.3), 4.06 – 4.04 (1 H, m), 4.01 (1 H, d, *J* 11.3),

4.02 – 3.90 (4 H, m), 3.89 (1 H, s), 3.87 (1 H, s), 3.84 – 3.81 (1 H, m), 3.80 – 3.74 (2 H, m), 3.73 – 3.68 (1 H, m), 3.67 – 3.58 (4 H, m), 3.62 – 3.55 (3 H, m), 3.58 – 3.47 (5 H, m), 3.46 (1 H, s), 3.43 (1 H, d, *J* 2.9), 3.42 – 3.27 (8 H, m), 3.29 – 3.21 (1 H, m), 3.20 – 3.11 (1 H, m), 1.91 – 1.82 (2 H, m), 1.54 (3 H, s). δ_C (101 MHz, $CDCl_3$) 139.8, 139.5, 139.2, 139.1, 139.0, 138.9, 138.8, 138.7, 138.6, 138.5, 138.2, 138.1, 137.7, 129.0, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.3, 127.3, 103.7, 102.6, 101.1, 100.4, 100.0, 97.8, 97.42, 83.2, 83.1, 81.7, 81.0, 80.9, 80.9, 80.8, 80.5, 80.5, 80.4, 79.8, 79.6, 78.6, 78.2, 77.4, 77.2, 76.5, 76.4, 76.3, 75.8, 75.6, 75.6, 75.4, 75.3, 75.2, 75.2, 75.0, 74.9, 74.7, 74.7, 74.6, 73.7, 73.6, 73.6, 73.6, 73.5, 73.5, 73.5, 73.4, 73.3, 73.1, 72.8, 72.4, 72.0, 72.0, 68.4, 68.1, 68.0, 68.0, 67.8, 67.0, 66.7, 66.6, 48.5, 29.4, 23.3. m/z (MICRO-TOF) 2234.19 $[M + Na]^+$ $C_{133}H_{142}N_4NaO_{26}$ requires 2233.98.

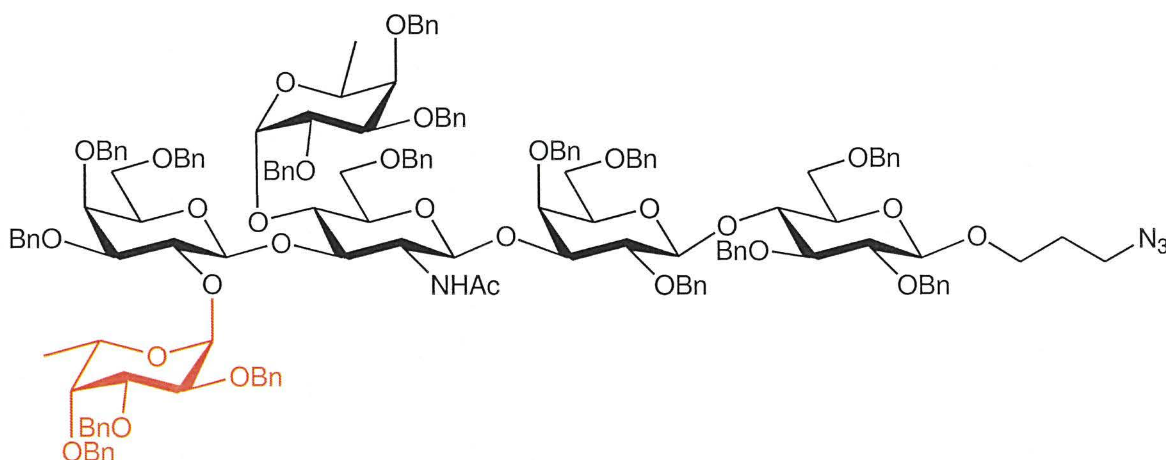
3-Azidopropyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **47**



Pentasaccharide **46** (1 eq, 125 mg, 56.5 μmol) was dissolved in DCM (1 ml) and MeOH (6 ml) added dropwise with stirring while ensuring the starting material remained in solution. A solution of NaOMe in MeOH (1 ml, 1 $moldm^{-3}$) was added dropwise and the mixture left to stir at rt. After 20 h had elapsed, starting material was still visible by TLC (toluene/ EtOAc 3:2). The temperature was raised to 40 °C and the reaction mixture stirred for a further 6 h until complete. The solvent was then removed under reduced pressure and purification by silica gel column chromatography (toluene/EtOAc 5% → 20%) carried out to give the product **47** as a white solid (110 mg, 52.4 μmol, 93%). R_f = 0.65 (toluene/EtOAc 3:2) δ_H (400 MHz, $CDCl_3$) 7.54 – 7.06 (28 H, m), 5.04 (2 H, t, *J* 9.4), 4.94 (1 H, t, *J* 11.2), 4.83 (3 H, m), 4.75 – 4.59 (4 H, m), 4.59 – 4.48 (2

H, m), 4.46 (1 H, s), 4.36 (3 H, m), 4.28 – 4.17 (3 H, m), 4.07 – 3.87 (5 H, m), 3.87 – 3.68 (3 H, m), 3.67 – 3.47 (5 H, m), 3.47 – 3.33 (4 H, m), 3.28 (1 H, d, *J* 8.9), 2.07 (3 H, s, $COCH_3$), 2.00 – 1.82 (2 H, m), 1.29 (3 H, td, *J* 7.1, 1.2, Fuc-*H*-6). δ_C (101 MHz, $CDCl_3$) 170.9, 139.3, 139.2, 139.2, 139.1, 138.9, 138.8, 138.7, 138.5, 138.3, 137.8, 129.1, 128.9, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 126.7, 103.6, 102.8, 102.5, 101.1, 98.0, 97.4, 82.9, 81.8, 81.7, 81.0, 80.4, 80.2, 78.2, 78.1, 77.2, 76.6, 76.3, 75.8, 75.5, 75.3, 75.1, 75.1, 74.6, 74.5, 74.1, 73.7, 73.6, 73.5, 73.4, 73.4, 73.3, 73.1, 72.9, 72.1, 71.2, 68.6, 68.3, 68.0, 67.9, 66.8, 66.5, 56.4, 48.4, 29.3, 21.1, 14.3. m/z (MICRO-TOF) 2129.80 $[M + Na]^+$ $C_{126}H_{138}N_4NaO_{25}$ requires 2129.95.

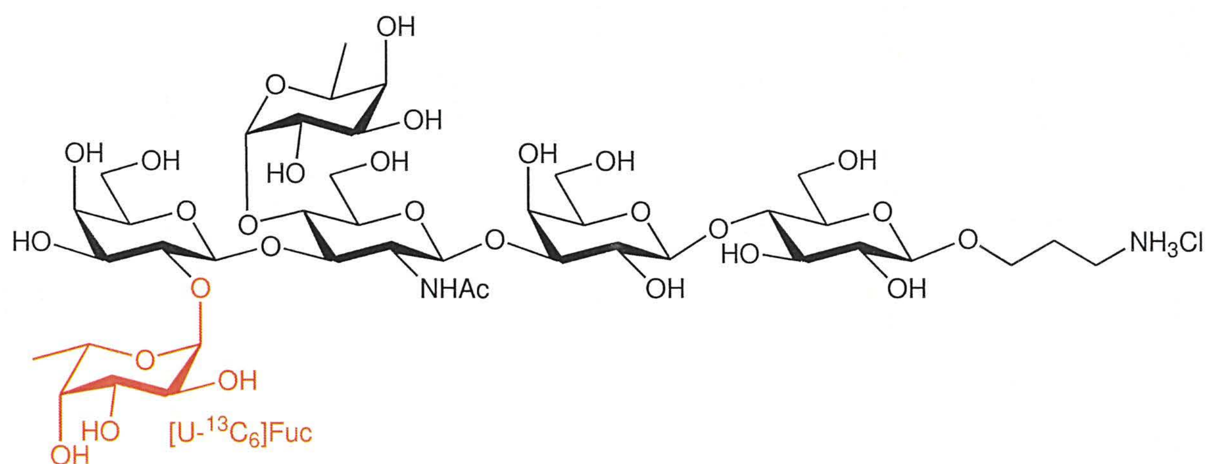
3-Azidopropyl (2,3,4-tri-*O*-benzyl-α-L-[$^{13}C_6$]fucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **48**



Pentasaccharide **47** (1 eq, 97.1 mg, 46.1 μmol), $^{13}C_6$ fucosyl donor **22** (1.5 eq, 37.8 mg, 69.1 μmol) and Bu_4NBr (1.5 eq, 22.3 mg, 69.1 μmol) were dissolved in a solution of DCM/DMF (9:1, 1.5 ml) and stirred at rt with 4 Å molecular sieves under an atmosphere of argon . After 2 h, bromine (2.25 eq, 16.6 mg, 104 μmol) was added and the yellow solution stirred under Ar for 16 h. Upon completion, Et_3N was added and the reaction mixture was diluted in EtOAc (25 ml) and washed with $Na_2S_2O_{4(aq)}$ (10%, 2 x 25 ml) before drying the organic layer over $MgSO_4$ and removing the solvent under vacuum. The product was purified by silica gel column chromatography

(toluene/EtOAc 5% → 20%) to give the product **48** as a colourless solid (91.5 mg, 36.2 μmol, 79%). R_f = 0.66 (toluene/EtOAc 2:1)

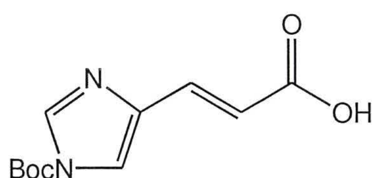
3-Aminopropyl (α-L-[U-$^{13}C_6$]fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[α-L-fucopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl chloride **49**[66]



Hexasaccharide **48** (1 eq, 89.1 mg, 35.0 μmol) was dissolved in 1,4-dioxane (9 ml) and stirred while adding water (3 ml) dropwise. The mixture was degassed and flushed through with $N_{2(g)}$ before adding Pd/C (10 wt. %, cat), degassing and flushing through with $H_{2(g)}$. After 15 min had elapsed, $HCl_{(aq)}$ (1 eq, 0.1 moldm^{-3}, 352 μl) and additional Pd/C was added and the reaction stirred under $H_{2(g)}$. The reaction was monitored by TLC ($EtOH/H_2O/NH_4OH$ 1:1:1) and after 16 h had not gone to completion. Fresh Pd/C was added and the mixture stirred for a further 24 h until complete. The reaction mixture was degassed and flushed through with $N_{2(g)}$ repeatedly before exposing to the air during TLC sampling or addition of reagents. Additional water was added to the mixture which was then filtered through celite. The solvent was removed by lyophilisation before purification using a size exclusion column. The column was run with a solution of butanol (1%) in ultra-pure water as the mobile phase. The solvent was eluted at a rate of 36 ml/h and collected using a Gilson fraction collector (9 min, or ~5.4 ml per test tube). A solution of the hexasaccharide in the mobile phase was added to the top of the column before carefully adding more of the mobile phase via a reservoir. The fractions were checked with TLC and MALDI TOF and the test tubes containing the product were combined and lyophilised to give the deprotected hexasaccharide **49** as a fine, white solid (37.3 mg, 33.9 μmol, 97%). R_f = 0.35 ($EtOH/H_2O/NH_4OH$ 1:1:1) δ_H

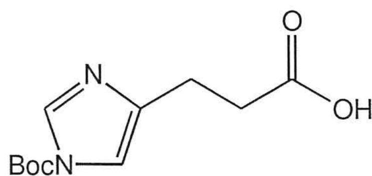
(400 MHz, D_2O) 5.18 (1 H, s, Fuc-*H*-1), 5.06 (1 H, d, *J* 3.6, Fuc-*H*-1), 4.90 (1 H, m), 4.69 (1 H, d, *J* 7.6, *H*-1), 4.64 (1 H, d, *J* 8.3, *H*-1), 4.54 (1 H, d, *J* 8.0, *H*-1), 4.45 (1 H, d, *J* 7.8, *H*-1), 4.37 (1 H, d, *J* 6.1), 4.17 (2 H, t, *J* 9.7), 4.08 (1 H, dd, *J* 10.9, 5.6), 4.03 – 3.74 (17 H, m), 3.70 – 3.53 (8 H, m), 3.36 (1 H, t, *J* 8.3), 3.19 (2 H, t, *J* 6.9), 2.10 (3 H, s, $COCH_3$), 2.07 – 1.99 (2 H, m), 1.30 (6 H, t, *J* 5.7, Fuc-*H*-6). δ_C (101 MHz, D_2O) 99.5 (d, *J* 44.3), 72.0 (t, *J* 37.2), 69.4 (t, *J* 37.8), 68.2 (t, *J* 41.8), 66.2 (t, *J* 39.5), 15.3 (d, *J* 41.6, Fuc-*C*-6). m/z (MALDI-TOF) 1085.78 $[M + Na]^+$ $^{12}C_{35}{}^{13}C_6H_{72}N_2Na\ O_{29}$ requires 1085.43.

N-tert-butyloxycarbonyl-urocanic acid **58**[116]



Urocanic acid **57** (1.05 g, 7.63 mmol) and Et_3N (2.13 ml, 15.3 mmol) were dissolved in DMF (25 ml) before adding Boc_2O (1.83 g, 8.39 mmol) and leaving the reaction stirring overnight at rt. Progress was monitored using TLC (DCM/MeOH 6:1). On completion, the solvent was removed under reduced pressure and the residue dissolved in EtOAc (50 ml) and washed with acetic acid (50 ml, 1 M) and brine (2 x 50 ml). The organic layer was then dried with MgSO4 and solvent removed under vacuum to give the crude product **58** as a waxy solid (1.66 g, 6.97 mmol, 91%). Rf = 0.56 (DCM/MeOH 6:1). δ_H (400 MHz, $CDCl_3$) 8.12 (1 H, s), 7.58 (2 H, dd, *J* 17.3, 12.5), 6.65 (1 H, d, *J* 15.6), 1.63 (9 H, s). δ_C (101 MHz, $CDCl_3$) 170.53, 148.12, 140.34, 140.13, 136.66, 120.82, 120.19, 88.13, 28.33.

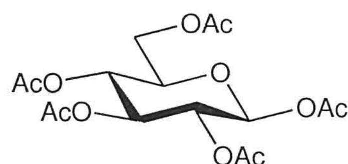
N-tert-butyloxycarbonyl-2,3-dihydrourocanic acid **59**[116]



The crude product **58** (1.44 g, 6.04 mmol) was dissolved in MeOH (20 ml) before adding Pd/C (10 wt. %, 0.09 g) and degassing the mixture while stirring under reduced pressure and replacing with an atmosphere of N_2. The mixture was degassed further while replacing with an atmosphere of H_2. The reaction mixture was left stirring for 24

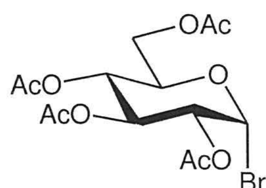
h and monitored by NMR. The catalyst was removed by filtration followed by removal of the solvent under reduced pressure to give the product **59** as a waxy solid (1.45 g, 6.04 mmol, 100%). δ_H (400 MHz, $CDCl_3$) 8.07 (1 H, s), 7.14 (1 H, s), 2.89 (2 H, t, *J* 7.2), 2.70 (2 H, t, *J* 7.3), 1.59 (9 H, s). δ_C (101 MHz, $CDCl_3$) 176.13, 146.88, 141.73, 136.71, 113.37, 85.89, 33.70, 27.93, 22.91.

Per-*O*-acetyl-D-glucopyranoside **62**[75]



A suspension of NaOAc (1 eq, 0.46 g, 5.52 mmol) in Ac_2O (10 eq, 5.25 ml, 55.2 mmol) was stirred at 140 °C before adding D-galactose **60** (0.994 g, 5.52 mmol) in small portions over a period of 5 min. The reaction was refluxed for a further 30 min before pouring onto crushed ice (50 ml) and stirring for 2 h. EtOAc (50 ml) was added to the mixture and the organic phase washed with water (2 x 40 ml) and $NaHCO_{3(aq)}$ (40 ml). The organic phase was then dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (toluene/EtOAc at 6:1 (420 ml), 3:1 (400 ml), 1:1 (200 ml)) to **62** as give a white solid as an α/β mixture (2.14g, 5.48 mmol, 99%). Rf = 0.53 (toluene/EtOAc 1:1). δ_H (400 MHz, $CDCl_3$) 5.71 (1 H, d, *J* 8.3, *H*-1), 5.25 (1 H, t, *J* 9.4, *H*-3), 5.17 – 5.06 (1 H, m, *H*-2, *H*-4), 4.27 (1 H, m, *H*-6a), 4.14 – 4.06 (1 H, m, *H*-6b), 3.83 (1 H, m, *J* 10.0, 4.5, 2.2, *H*-5), 2.11 (3 H, s, $COCH_3$), 2.08 (3 H, s, $COCH_3$), 2.03 (6 H, s, $COCH_3$), 2.01 (3 H, s, $COCH_3$). δ_C (101 MHz, $CDCl_3$) 170.74, 170.23, 169.52, 169.38, 169.09, 91.83, 77.16, 72.93, 72.86, 70.36, 67.88, 61.58, 20.95, 20.84, 20.70.

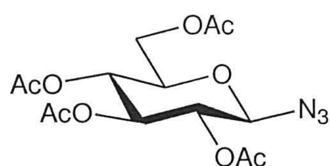
2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide **63**[22]



Per-*O*-acetyl-β-D-glucopyranose **62** (6.02 g, 15.4 mmol) was added to a solution of HBr (33% in acetic acid, 30 ml) at 0 °C. The reaction was allowed to reach rt and left stirring for 1 h. The reaction mixture was then coevaporated with toluene to remove the acid,

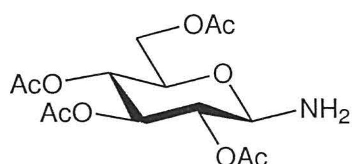
before dissolving the residue in EtOAc (50 ml) and washing with $NaHCO_{3(aq)}$ (2 x 50 ml) and water (50 ml). The organic layer was dried with $MgSO_4$ and concentrated under vacuum to give the product **63** as a pure , crystalline solid in quantitative yield. R_f = 0.64 (toluene/EtOAc 1:1). δ_H (400 MHz, $CDCl_3$) 6.59 (1 H, d, *J* 3.9, *H*-1), 5.54 (1 H, t, *J* 9.7, *H*-3), 5.14 (1 H, t, *J* 9.7, *H*-4), 4.82 (1 H, dd, *J* 10.0, 4.0, *H*-2), 4.36-4.27 (2 H, m, H_2-6), 4.11 (1 H, d, *J* 11.3, *H*-5), 2.08 (6 H, s, $COCH_3$), 2.03 (3 H, s, $COCH_3$), 2.02 (3 H, s, $COCH_3$). δ_C (101 MHz, CDCl3) 170.50, 169.84, 169.79, 169.46, 86.56, 72.12, 70.58, 70.14, 67.14, 60.93, 20.65, 20.62, 20.55.

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide **64**[88]



Compound **63** (6.30 g, 15.3 mmol) was dissolved in DMF (15 ml) at 60 °C and NaN_3 (5 eq, 5.01 g, 77.1 mmol) was added in portions to the reaction vessel. The mixture was stirred for a further 1 h before quenching by pouring onto crushed ice. The aqueous mixture was transferred to a separating funnel, EtOAc added (200 ml) and the organic layer washed with brine (2 x 200 ml) before drying with $MgSO_4$ and removing the solvent under vacuum. The crude product was then purified by silica gel column chromatography (toluene/EtOAc 3:1) to give the product **64** as a white, crystalline solid (3.83 g, 10.3 mmol, 67%). R_f = 0.69 (toluene/EtOAc 1:1). δ_H (400 MHz, $CDCl_3$) 5.20 (1 H, t, *J* 9.5, *H*-3), 5.09 (1 H, t, *J* 9.7, *H*-4), 4.94 (1 H, t, *J* 9.2, *H*-2), 4.64 (1 H, d, *J* 8.9, *H*-1), 4.33 – 4.08 (2 H, m, *H*-6), 3.78 (1 H, m, *H*-5), 2.09 (3 H, s, $COCH_3$), 2.06 (3 H, s, $COCH_3$), 2.01 (3 H, s, $COCH_3$), 1.99 (3 H, s, $COCH_3$). δ_C (101 MHz, CDCl3) 170.61, 170.12, 169.31, 169.21, 87.89, 73.99, 72.57, 70.60, 67.84, 61.63, 20.70, 20.56, 20.54.

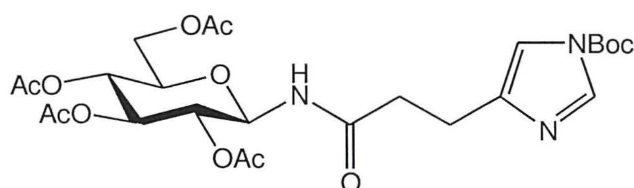
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl amine **65**[88]



$NaBH_4$ (2 eq, 0.04 g, 1.1 mmol) and a few drops of $NiCl_{2(aq)}$ (1 M) were added to a solution of EtOAc/EtOH 1:1 (10 ml) and compound **64** (0.20 g, 0.54 mmol). The

resulting black reaction mixture was stirred under N_2 at rt for 30 min before removing the solvent under vacuum, stirring the mixture in EtOAc and filtering off the black precipitate through celite. The filtrate was concentrated under reduced pressure to give the crude product as a crystalline solid. This was taken for the next step without further purification. R_f = 0.74 (toluene/EtOAc 1:3). δ_H (400 MHz, $CDCl_3$) 5.21 (1 H, t, *J* 9.3), 5.01 (1 H, t, *J* 9.5), 4.80 (1 H, t, *J* 9.1), 4.18 (2 H, s), 4.08 (1 H, d, *J* 11.8), 3.67 (1 H, d, *J* 5.7), 2.07 (3 H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 2.00 (3 H, s, $COCH_3$), 1.98 (3 H, s, $COCH_3$).). δ_C (101 MHz, $CDCl_3$) 170.65, 170.16, 169.52, 84.97, 73.14, 72.69, 72.03, 68.76, 62.27, 20.78, 20.75, 20.59. 16

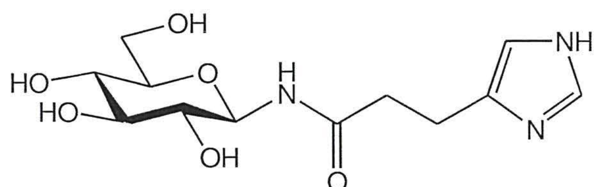
3-(1-*tert*-Butoxycarbonyl-imidazol-4-yl)-*N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanamide **66**



Glucosyl azide **64** (1.4 eq, 0.48 g, 1.29 mmol) was dissolved EtOAc/MeOH 1:1 (30 ml) under an N_2 atmosphere and $Pd/C_{(cat)}$ (10 wt. %) and Et_3N (0.5 ml, 3.6 mmol) added. The mixture was then repeatedly degased, exposed to a H_2 atmosphere and left to stir for 30 min, by which time no starting material was visible by TLC. The Pd/C was removed by filtration and the solvent removed under vacuum to give the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl amine **65**. DCC (0.19 g, 0.92 mmol), HOBt (0.14 g, 0.92 mmol) and *N*-*tert*-butyloxycarbonyl-2,3-dihydrourocanic acid **55** (0.22 g, 0.91 mmol) were dissolved in DMF (10 ml) at 0 °C under an atmosphere of N_2. A urea precipitate appeared and after 30 min, compound **65** and DIPEA (0.15 ml, 0.83 mmol) were added. The reaction mixture was left stirring at rt overnight. Upon completion, the reaction mixture was co-evaporated with toluene before diluting the residue in EtOAc (10 ml) and washing with brine (3 x 10 ml). The crude product was then purified using silica gel column chromatography (toluene/(EtOAc/MeOH 19:1) 2:1 → 1:2) to give the product **66** as a yellow solid (0.18 g, 0.32 mmol, 35 %). R_f = 0.12 (toluene/(EtOAc/MeOH 19:1) 2:1). δ_H (400 MHz, $CDCl_3$) 8.00 (1 H, d, *J* 1.1, $H_{aromatic}$), 7.11 (1 H, s, $H_{aromatic}$), 5.36 – 5.18 (2 H, m, *H*-1, *H*-3), 5.07 (1 H, t, *J* 9.7, *H*-4), 4.94 (1 H, t, *J* 9.6, *H*-2), 4.19 (2 H, m, H_2-6), 3.80 (1 H, m, *H*-5), 2.85 (2 H, t, *J* 7.2, $COCH_2CH_2$), 2.58 (1 H, td, *J* 7.3, 4.0, $COCH_2CH_2$), 2.08 (3 H, s, $COCH_3$), 2.02 (3 H, s,

$COCH_3$), 2.00 (3 H, s, $COCH_3$), 1.96 (3 H, s, $COCH_3$), 1.60 (9 H, s, CH_3). δ_C (101 MHz, $CDCl_3$) 172.89, 170.75, 170.07, 141.89, 136.87, 129.18, 128.36, 125.44, 113.45, 78.28, 77.16, 73.70, 73.14, 70.64, 68.29, 61.84, 35.53, 28.03, 23.22, 21.59, 20.88, 20.72, 20.66.

3-(1*H*-Imidazol-4-yl)-*N*-(β-D-glucopyranosyl)propanamide **56**



To a solution of compound **66** (0.086 g, 0.15 mmol) in MeOH (1 ml), NaOMe (0.4 eq, 3.2 mg, 0.06 mmol) was added. The reaction mixture was stirred at rt for 30 min and monitored by TLC. Upon completion, the solvent was removed under vacuum. TFA (6 ml) was added to the flask and left to stir for 30 min at rt. The reaction was monitored using TLC and when no starting material remained the solvent was removed by co-evaporation with toluene to give the crude product as a yellow oil. This was purified by column chromatography with a RP-C18 column. The column had been prewashed with a step-gradient using 5 ml for each step (MeOH in H_20, 100%, 80%, 60%, 40%, 20%, 10% MeOH). The compound mixture was applied to the top of the column and eluted with a MeOH/H_2O solvent mixture in a step-gradient with 5 ml for each step (MeOH in H_2O, 10%, 20%) to give the product **56** as a white solid (0.045 g, 0.15 mmol, 100 %). R_f = 0.79 (DCM/MeOH/NH_4OH 2:2:1). δ_H (400 MHz, D_2O) 8.58 (1 H, s, *J* 1.2, $H_{aromatic}$), 7.25 (1 H, s, $H_{aromatic}$), 4.95 (1 H, d, *J* 9.2, *H*-1), 3.88 (1 H, dd, *J* 12.3, 2.0, *H*-6a), 3.73 (1 H, dd, *J* 12.3, 5.3, *H*-6b), 3.58 – 3.49 (2 H, m, *H*-3, *H*-5), 3.45 – 3.34 (2 H, m, *H*-2, *H*-4), 3.07 (2 H, t, *J* 7.1, $COCH_2CH_2$), 2.74 (2 H, t, *J* 6.8, $COCH_2CH_2$). δ_C (101 MHz, D_2O) 175.52 ($COCH_2CH_2$), 132.86 ($CH_{aromatic}$), 131.98 ($C_{aromatic}$), 115.61 ($CH_{aromatic}$), 79.14 (*C*-1), 77.51 (*C*-5), 76.46 (*C*-3), 71.69 (*C*-2), 69.18 (*C*-4), 60.48 (*C*-6), 33.97 ($COCH_2CH_2$), 19.62 ($COCH_2CH_2$). m/z 302.1351 $[M + H]^+$ $C_{12}H_{20}N_3O_6$ requires 302.1347.

Incorporation of Chitosan into PU Foam

Chitosan was solubilised by stirring in HCl(aq) (0.15 M) for 2 h. Remaining solid was filtered off and the solvent removed by lyophilisation. The protonated chitosan (20 mg) was then redissolved in water (0.5 ml) before mixing with a prepolymer (16 g) and

$NaHCO_{3(aq)}$ (0.375 wt.%, 4 ml) at rt. The mixture was incubated at 40 °C in a water bath for 20 min. The resulting foam was allowed to cure at rt for 2 h, before washing with water and drying under a vacuum.

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