A Combinatorial Approach to Glycotherapeutics :

Template Synthesis

A Thesis Submitted for the Degree of Doctor of Philosophy

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University of St Andrews

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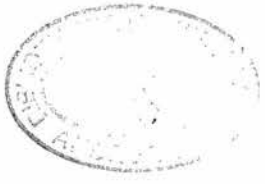

Abstract

In this thesis, general synthetic methods applicable to the development of carbohydrate mimetics were achieved. With such methodology in hand we turned to the development of inhibitor libraries for:

a) *Trypanosoma cruzi trans*-sialidase, an essential enzyme involved in the onset of South American Chagas' disease. Octyl galactoside is recognised by the enzyme so chemical modifications of this structure would be possible. The synthesis of octyl 6-azido-6-deoxy galactoside has been achieved by using two different chemical methods.

b) *E. coli* 0157 (verotoxin) is a food poisoning toxin (Wishaw, central Scotland). The minimum active component for interaction of sugar and toxin is galabiose [αGal-(1-4)-βGal-(1-4)-OMe]. In this thesis, the synthesis of galabiose and various galabiose template mimics are described. Modification of the galabiose structure at the 2 position (methoxycarbonylmethyl) and at the 6 position (amine) or both was successfully achieved. These structures are ready to be incorporated onto a solid-support or a dendrimer base for further evaluation. A small array of 6-amino functionalised galabiose compounds has been successfully achieved.

Declaration

(i) I, Darren Gibson, hereby certify that this thesis, which is approximately 36,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any other previous application for a higher degree.

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(ii) I was admitted as a research student in October 1997 and as a candidate for the degree of Doctor of Philosophy in October 1998; the higher study for which this is a record was carried out in the University of St. Andrews between 1997 and 2000.

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(iii) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St. Andrews and that the candidate is qualified to submit this thesis in application for that higher degree.

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I would like to dedicate my thesis to my grandfather Fox for giving me the drive and determination to succeed in everything I do…..

Abbreviations Used in Text

Ac	Acetyl
Bn	Benzyl
Bz	Benzoyl
Cer	Ceramide
CMCT	1-Cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-Toluenesulfonate
CI-MS	chemical ionisation mass spectrometry
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-Dimethylamino pyridine
DMF	*N*,*N*-Dimethylformamide
DTT	Dithiothreitol
E. coli	*Escherichia coli*
ELISA	Enzyme-Linked Immunosorbant Assay
ES-MS	Electrospray Mass Spectrometry
FAB-MS	Fast Atom Bombardment Mass Spectroscopy
Gal	Galactose
Gb_3	Globotriaosylceramide
Glc	Glucose
HUS	Hemolytic uremic syndrome
IR	Infra-red spectroscopy
LacNAc	*N*-Acetyllactosamine
lit.	Literature (reference)
MALDI-TOF	Matrix-Assisted Laser-Desorption-Ionisation Time-Of-Flight
Me	Methyl
Mol.S	Molecular sieves

Ms	Methanesulfonyl
N-CAM	Neural Cell Adhesion Molecule
NMR	Nuclear Magnetic Resonance
Oct	Octyl
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
sLex	Sialyl Lewis x
SLT	Shiga-Like Toxin
SPPS	Solid Phase Peptide Synthesis
T. cruzi	*Trypanosoma cruzi*
TBDMS	*t*-Butyldimethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TsCl	Toluenesulfonyl chloride (*p*)
VT	Verotoxin

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Chapter 1

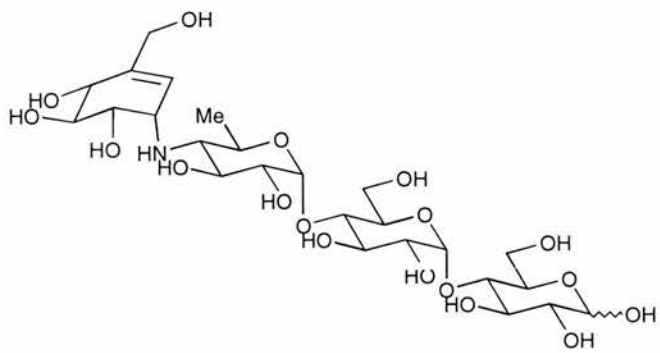
General Background

1.0 General Background

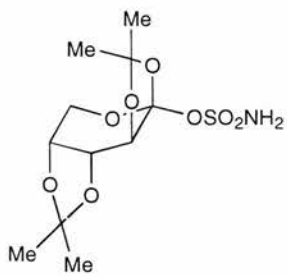
Oligosaccharides are widespread in nature and fulfil a number of biological roles, ranging from energy storage (starch, glycogen) and providing structural support (cellulose, chitin), to intricate recognition events associated with cell-cell interactions. Whilst the field of glycobiology is still in its infancy, there is much anticipation about the prospect of identifying new biochemical processes that offer scope for therapeutic intervention.[1,2,3]

1.1 Carbohydrate-Based Therapeutics

There has been a dramatic increase in the number of carbohydrate-based therapeutics over the past decade, with numerous compounds in drug development or undergoing clinical trails. The areas in which carbohydrate drugs are used vary widely, from diabetes to neurological disorders. In diabetes therapy the main class of carbohydrates of interest are aza-sugars and carba-sugars which inhibit the glycosidases that are responsible for the degradation of poly- and oligo-saccharides.[4] Inhibition of these enzymes reduces the amount of glucose entering the bloodstream. Such drugs can be used in conjunction with conventional agents, which aid either the release or effect of insulin. The carba-tetrasaccharide acarbose (**1**)[4] (**Figure 1**) is used in the treatment of hyperglycaemia. Another area which is benefiting from carbohydrate therapeutics is the neurological field. Topiramate (**2**) (**Figure 1**) is a novel anti-convulsant, which has shown promise in treatment of epilepsy.[5]



ACARBOSE
(Anti-hyperglycaemia)
(1)



TOPIRAMATE
(Anti-convulsant)
(2)

Figure 1: Carbohydrate –based therapeutics in current use.[5]

The main difficulty in the development of carbohydrate drugs is the complexity of

their synthesis. There are also pharmacological drawbacks, including low bioavailability of orally ingested carbohydrates.[6] Carbohydrate-based drugs do have some potential advantages, which include low toxicity and immunogenicity relative to their peptide counterparts.

Interactions between cell surface receptors and carbohydrates or peptides are some of the most important interactions in multicellular organisms.[7] There are numerous examples of protein-carbohydrate interactions in the body; a vast array of biological processes are involved. Integral membrane proteins often contain covalently-attached oligosaccharides, as do many secreted proteins such as antibodies and clotting factors.[8] Plants also contain carbohydrate-binding proteins called lectins (e.g., concanavalin A, from jack beans, which binds to internal and non-reducing terminal α-mannose residues).[8] In animals, carbohydrates are involved in cell-cell interactions. For example, in the adhesion of neurons in the development of the nervous system, which in part, is mediated by a neural cell adhesion molecule (N-CAM).[8]

One of the current major areas of interest in carbohydrate-binding proteins concerns selectin binding of the oligosaccharide antigen sialyl Lewis x (SLe^x) (**3**) (**Figure 2**), a key event in the inflammatory response.[9] The chemical synthesis of SLe^x (**3**) is complex, hence combinatorial methods have been explored in the development of simplified SLe^x mimetics.

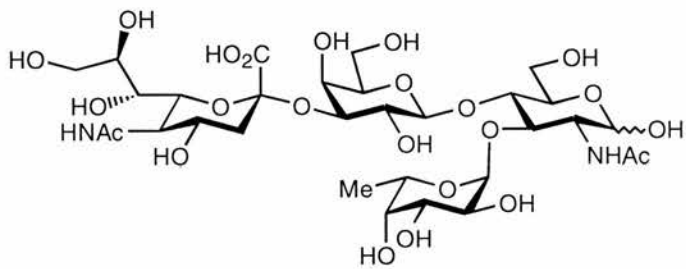


Figure 2 : The Sialyl Lewis X Antigen (3).[9]

1.2 Glycomimetics

The total synthesis of native oligosaccharide structures is a very complicated and time consuming process. Carbohydrate mimics have therefore attracted attention with a view to obtaining compounds with the same, if not better, binding interactions with cell surface receptors. It is also anticipated that the synthesis of carbohydrate mimics would simplify synthetic procedures and be less time consuming than conventional oligosaccharide chemistry. Synthetic approaches to the development of glycomimetics has recently been thoroughly reviewed by Wong[10,11] and Hindsgaul.[12]

The most well established glycomimetics are mimics of the anti-inflammatory sialyl Lewis X (SLe^x). SLe^x serves as a common ligand for all three types of selectins (E-, P-, and L-) and it is responsible for a variety of interactions with cell surface receptors.[9] E-selectin recognises SLe^x on the surface of neutrophils, P-selectin also binds SLe^x on neutrophils or leukocytes, but with a lower affinity, L-selectin weakly recognises SLe^x on endothelial cells.[9] The latter has a preference of binding SLe^x with galactose containing a sulphate at position 6.[9] A schematic representation of the key interactions between SLe^x and E-selectin is shown in **Figure 3**.[13]

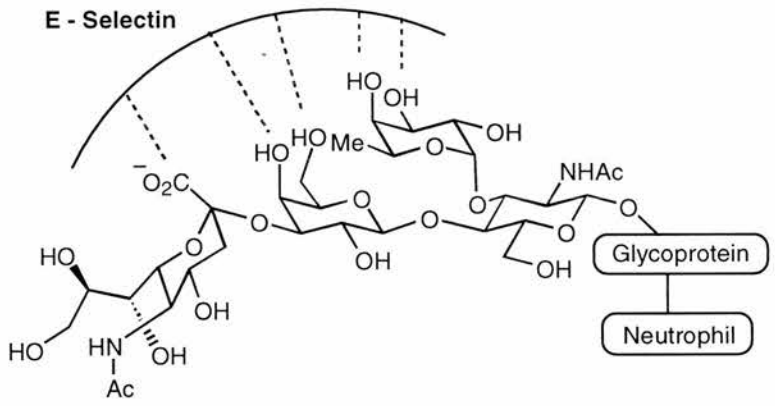


Figure 3: Interaction of the Sialyl Lewis X with E-selectin[13]

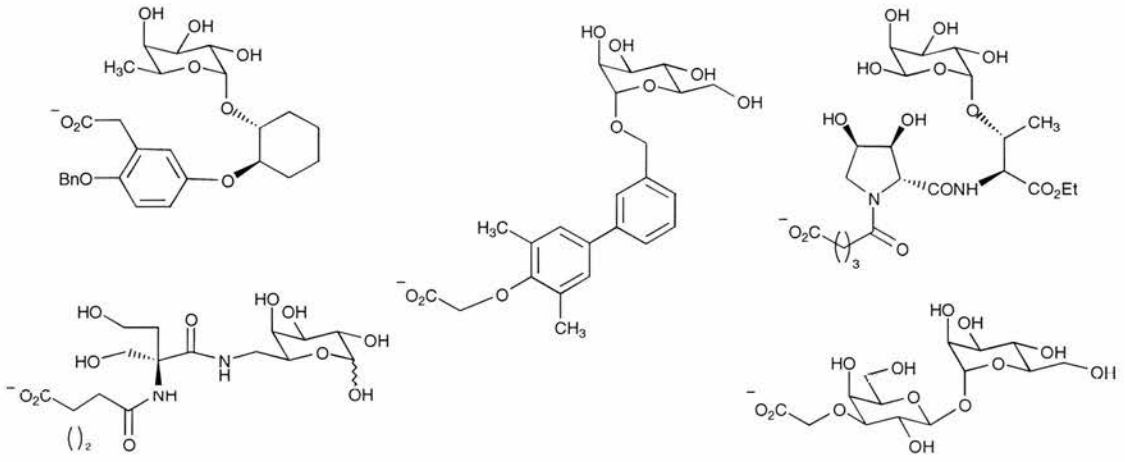


Figure 4 : Mimetics of Sialyl Lewis X.[9]

Many SLe^x mimetics have been developed to date and some show increased affinity for E-selectin. Representative examples are shown in **Figure 4**,[9] which clearly shows that many parts of the SLe^x structure can be replaced with non-carbohydrate material.

The synthesis of oligosaccharides can be a very time consuming and technically difficult, partially because of the numerous protection and deprotection steps involved and also because of the extreme moisture sensitivity and poor stereoselectivity of coupling procedures. Kahne[14, 15] and Hindsgaul,[12] in particular, have adopted novel approaches to overcome some of these problems.

Many different types of glycoside bond-forming procedure are available (reviewed in references 16 and 17) and typically the formation of the each different type of glycosidic linkage requires a different set of reaction conditions. Kahne has developed glycosyl sulfoxide chemistry such that a standard set of conditions can be used for most coupling procedures.[14] This methodology is expected to be compatible with automated, solid-phase synthesis of oligosaccharides in a combinatorial fashion, as outlined in **Figure 5**.[18, 19]

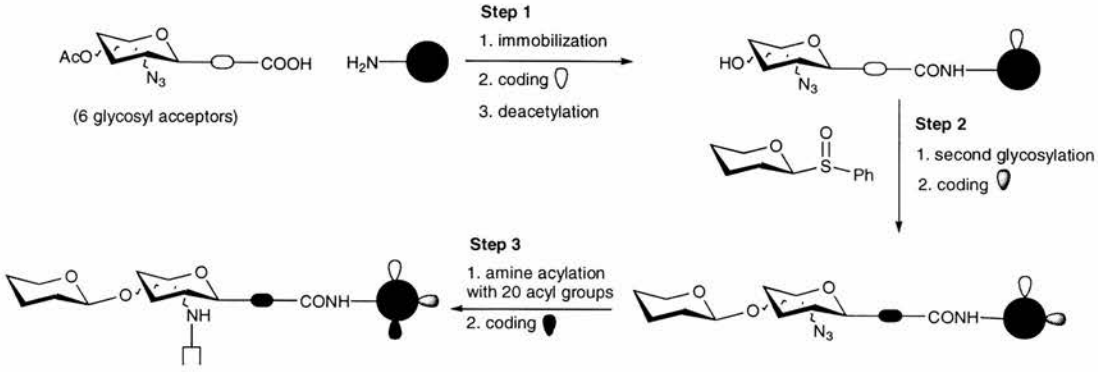


Figure 5: Kahnes solid-phase synthesis of acylated disaccharides.[19]

Using this approach, Kahne has identified unnatural carbohydrate derivatives (**4**) that effectively compete with the natural ligand (**5**) for the *Bauhinia purpurea* lectin (**Figure 6**).[19]

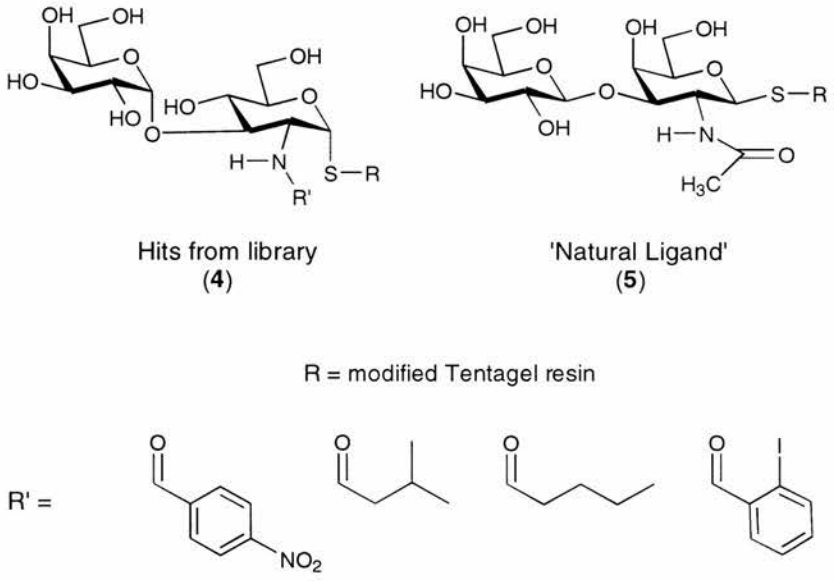


Figure 6: Di-saccharides synthesised from Kahnes solid phase synthesis.[19]

Hindsgaul has adopted a somewhat radical approach by choosing to conduct glycoside coupling chemistry in the absence of protecting groups on the acceptor sugar. That is, a random glycosylation strategy.[12] The aim of this strategy is to ensure that the number of protecting group manipulations and the number of chromatographic purification steps are kept to a minimum. A comparison of this approach with a conventional glycosylation strategy is outlined in **Figure 7.**[12]

An alternative approach to the synthesis of potentially biologically active oligosaccharides is to produce materials capable of mimicing oligosaccharide functionality. Ideally such an approach should use chemistry that is cheap, robust and compatible with automated solid-phase synthesis. A number of coupling chemistries can be envisaged, some of which are outlined in the following sections.

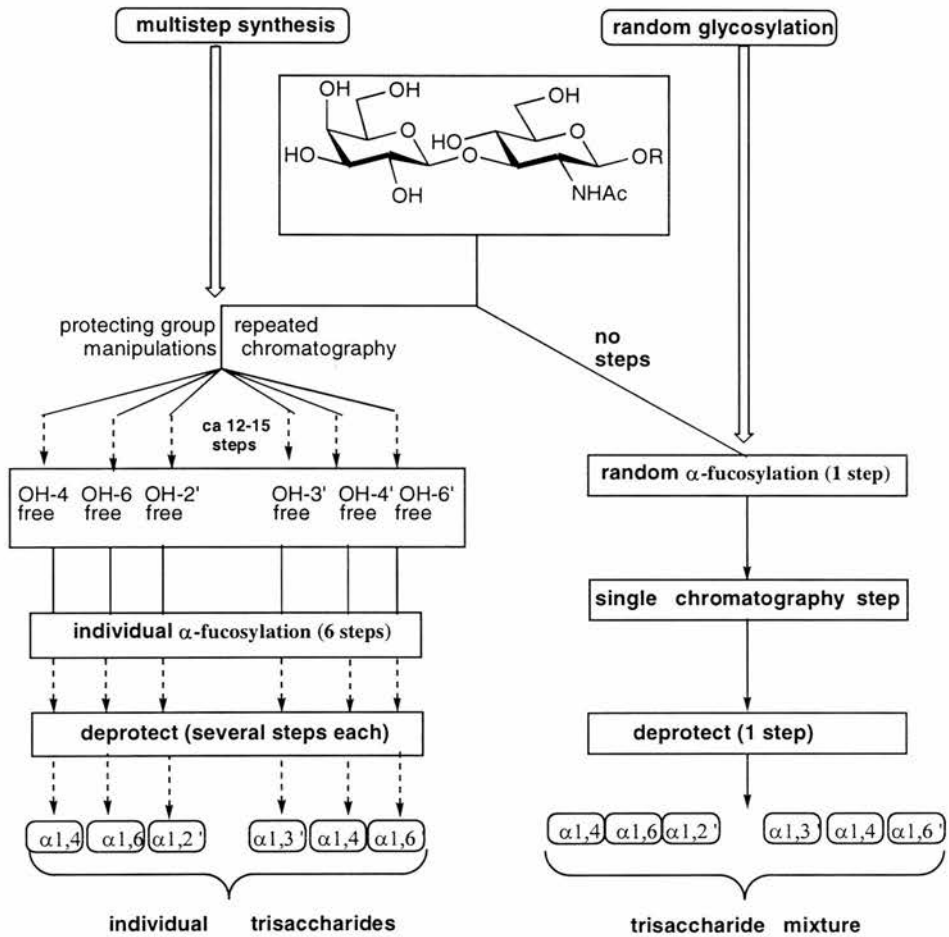


Figure 7: Comparison of conventional and random glycosylation strategies (adapted from Kanie and Hindsgaul).[12]

1.2.1 Peptide Chemistry

Automated solid-phase peptide synthesis was introduced by Merrifield over 30 years ago.[20] This type of coupling chemistry is widely used and the development of glycomimetics based on amide bond formation has received attention. A number of

carbohydrate-based amino acids have been prepared for incorporation into peptide libraries (**Figure 8**).[21-24]

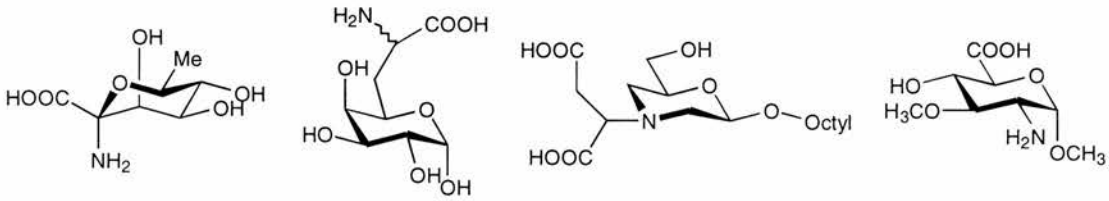


Figure 8: Representative examples of sugar amino acids that might find application in peptide library synthesis.[21-24]

The major drawback with peptide chemistry is that any mimetics made with peptide links will be peptidase-sensitive *in vivo*.

1.2.2 Michael Addition Chemistry

Figure 9 illustrates the preparation of an aglycon-functionalised β-galactoside library. The approach described utilises a 1-thio-β-D-galactose derivative (**6**) in a Michael-type addition reaction with various α,β-unsaturated carbonyl compounds, followed by reductive amination of the resulting ketone (**7**). The galactose residue was protected as its laurate ester, which facilitates the isolation of products by reverse-phase chromatography.[18]

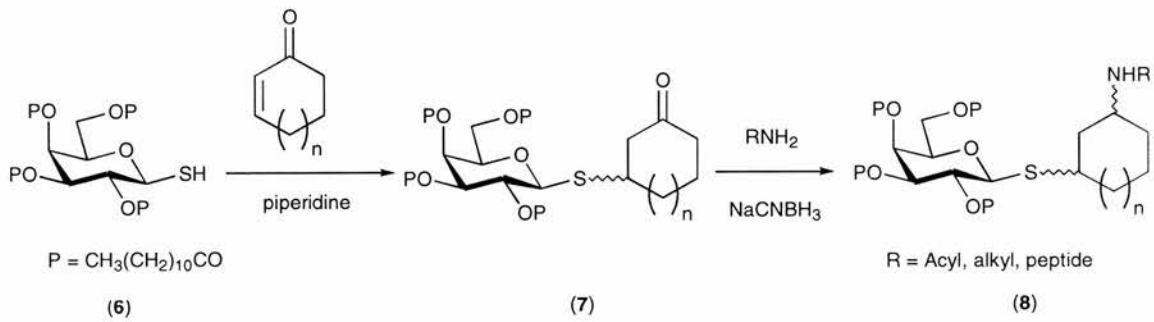


Figure 9: Library synthesis based on Michael-type addition chemistry.[18]

The approach outlined gives rise to a variety of mixed stereoisomeric products (**8**) but it is complicated by the generation of oxidizable thiol intermediates.

1.1.3 Ugi Reaction

The Ugi four-component condensation[25] (aldehyde, amine, isocynate, carboxylic acid) is a powerful approach for accessing a wide diversity of carbohydrate-based libraries, since each of the components can contain a carbohydrate moiety. The Wong group has recently reported the use of the use Ugi reaction in the combinatorial synthesis of mimetics of the aminoglycoside antibiotic neomycin (**Figure 10**).[26]

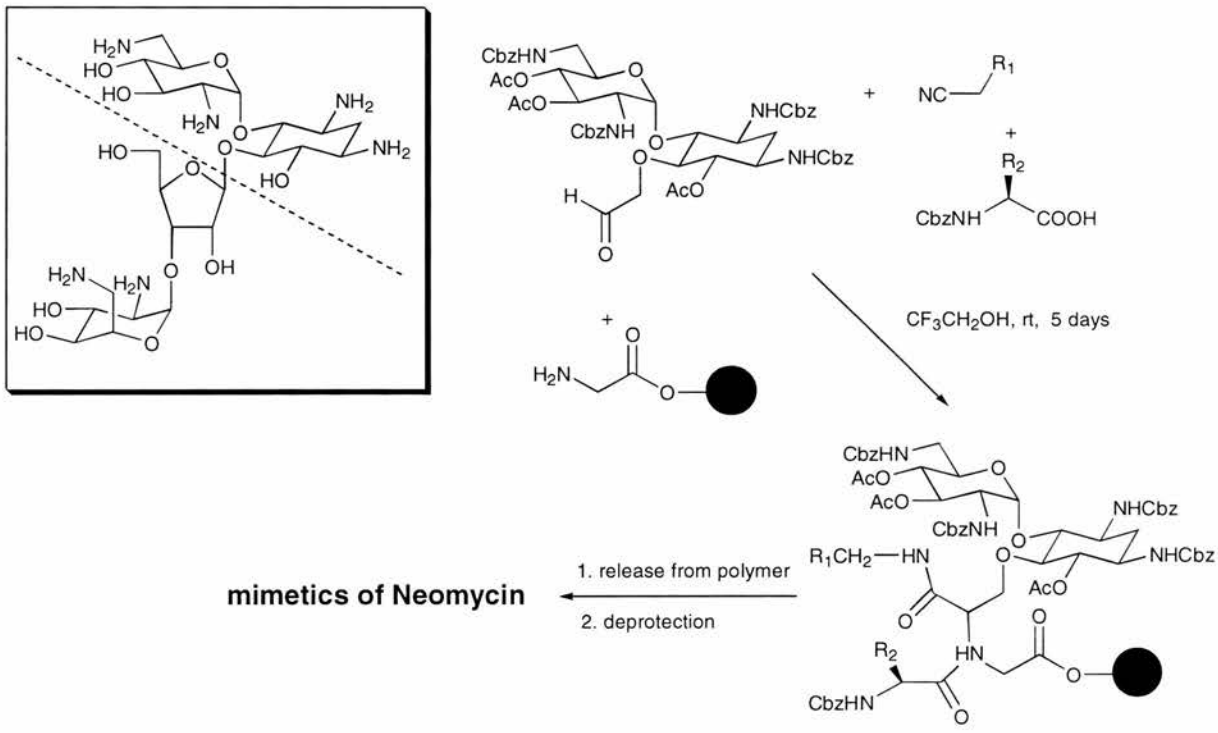


Figure 10: Generation of a library of neomycin B mimetics using the Ugi four- component condensation.[26]

1.1.4 1,3-Dipolar Cycloaddition Chemistry

1,3-Dipolar cycloaddition reactions have been used in both combinatorial synthesis and in conjunction with solid-phase supports (**Figure 11**).[27-29] This type of reaction has been developed by Paton for the synthesis of C-disaccharides.[30] However, to our knowledge, carbohydrate chemistry and 1,3-dipolar cycloaddition reactions have not been used together in a combinatorial fashion despite the clear scope for generation of much structural diversity in this fashion.

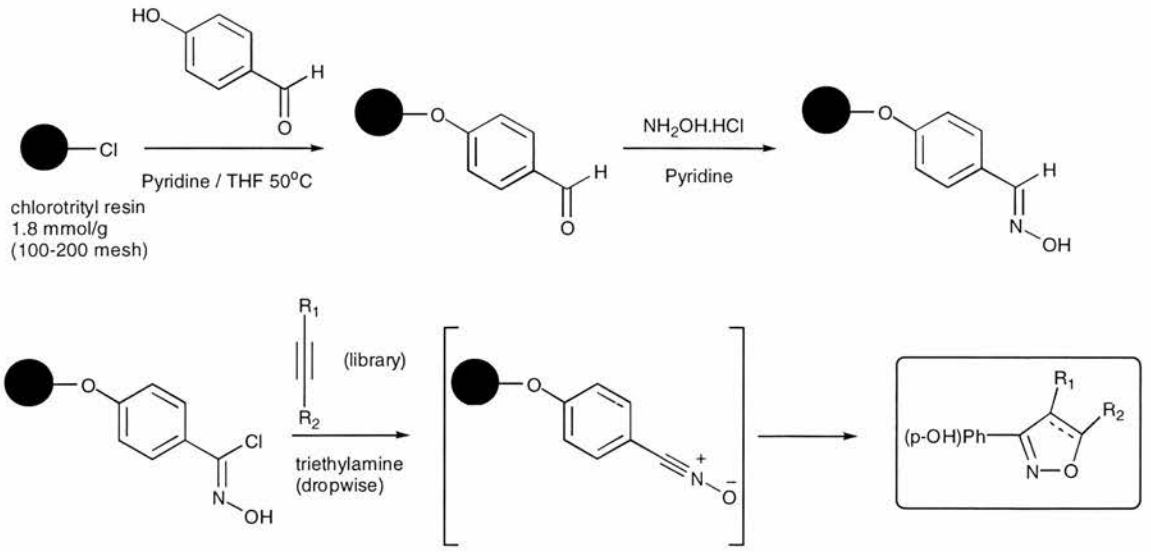


Figure 11: 1,3-Dipolar cycloaddition chemistry on a solid-phase support.[29]

1.1.5 Oxime Chemistry

Enzymatic oxidation of galactosides with galactose oxidase offers a simple route to a reactive aldehyde entity which can be chemoselectively derivatised even in the presence of other functionality. Bertozzi has exploited this type of chemistry in the synthesis of unnaturally glycosolated peptides and also for the derivitisation of glycoproteins on intact cell surfaces.[31-34]

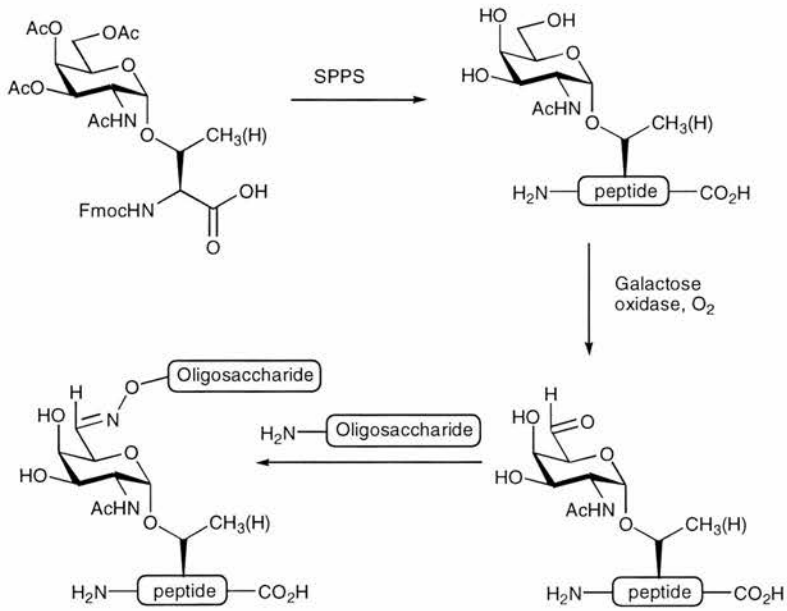


Figure 12: Synthesis of oxime-containing glycopeptide analogues.[31]

It appears that this type of chemistry has not yet been exploited in a combinatorial fashion either.

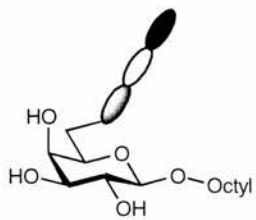
1.3 Aims and Objectives

An initial aim of this project was to establish general synthetic methods applicable to the development of carbohydrate mimetics. The objective was to identify simple, practical synthetic methods suitable for this purpose (i.e. few chemical steps). With such methodology in hand we would turn to the development of inhibitor libraries for:

a) *Trypanosoma cruzi trans*-sialidase, an essential enzyme involved in the onset of South American Chagas' disease.[35]

b) *E. coli* verotoxin, a food poisoning toxin.[36]

trans-Sialidase inhibitors might incorporate an octyl galactoside templated library. The 6-OH position of galactose (**9**) is not recognised by the enzyme therefore derivatisation can be performed at this position (**Figure 13**). We were therefore able to initiate this study with simple monosaccharide derivatisation.



(**9**)

Figure 13: The octyl galactoside template used for derivatisation.

Moving on to a more elaborate system *E.coli* verotoxin binds to galabiose (Gal-α-1,4-Gal). Hydroxyl groups at positions 2 and 6 of the reducing terminal sugar of galabiose are not essential for toxin binding therefore derivatisation, e.g. (**10**) should be possible in these positions. This would lead us into di-functionalsed disaccharide templates (**Figure 14**).

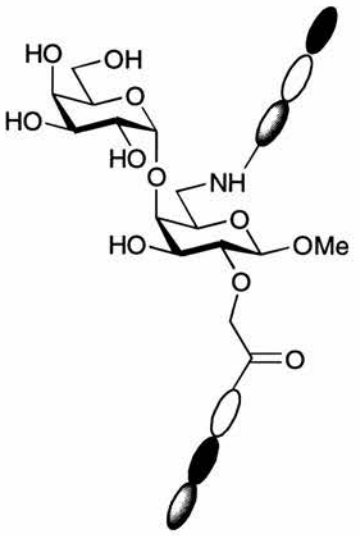


Figure 14: Galabiose backbone with possible derivatisation sites (**10**).

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Chapter 2

trans-Sialidase

2.1 Chagas' Disease

Chagas' disease was first discovered in 1909 in Brazil by Carlos Chagas.[1-3] This disease currently affects approximately 20 million people in South and Central America; a further 100 million are at risk of infection, including those in the southern United States. The protozoan-blood borne parasite *Trypanosoma cruzi* causes the disease and at present there is no effective treatment available. The parasite is transmitted by the excretion of faeces from the reduviid beetle, which irritates the host's skin, causing the host to scratch. This results in skin damage, so allowing the parasite to gain access to the bloodstream. To undergo replication the parasite must invade mammalian cells; the parasite must therefore initially adhere to cells.[4-5] Interaction of sialylated oligosaccharides on the parasite surface with protein receptors on the host is the proposed mechanism for this event.[5] However, the parasite does not actually produce sialic acid, it acquires it from mammalian glycocongugates by the action of a cell surface *trans*-sialidase enzyme.[6] *trans*-Sialidase removes and transfers a negatively charged α-(2,3)-linked sialic acid (**Figure 15**) from host glycoproteins and glycolipids to terminal β-galactoside acceptors on the parasite,[7] so generating parasite-host adhesion molecules.[7]

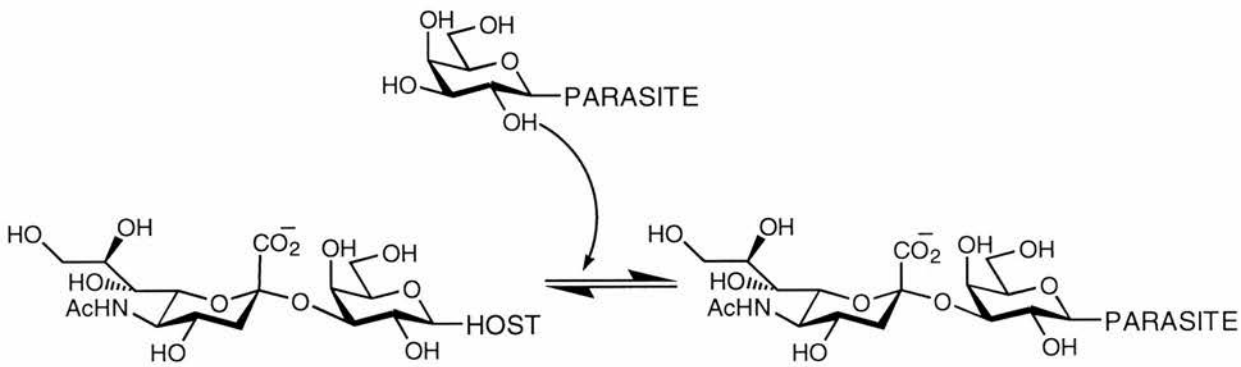


Figure 15: *trans*-Sialidase catalysed transfer of sialic acid from host to parasite.[8]

2.2 Documented Research

It has been well documented in the literature that a terminal β–galactose residue is all that is essential for sialyl transfer by *trans*-sialidase.[8] Ongoing studies have shown interactions between *trans*-sialidase and a derivatised LacNAc library in the Field group (**Figure 16**). It appears that the enzyme will only tolerate a wide variety of substitutions at the 3-, 6- and 6'-positions, (the 3'-position is the site of sialic acid attachment)[9]

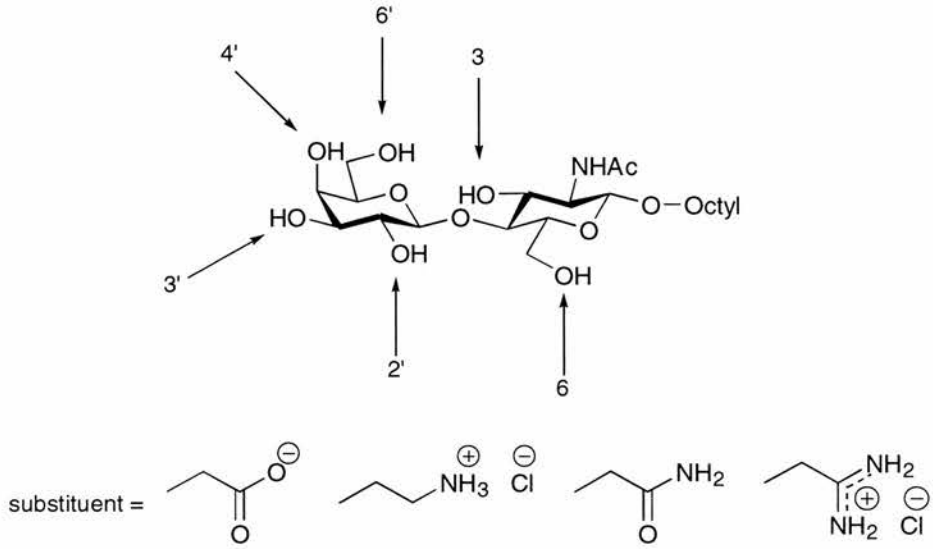


Figure 16: Acceptor substrate interactions with *trans*-Sialidase.[9]

Further studies with octyl galactoside derivatives (**Figure 17**) demonstrate that small modifications at the 4- position of galactose can be tolerated to some degree.[9]

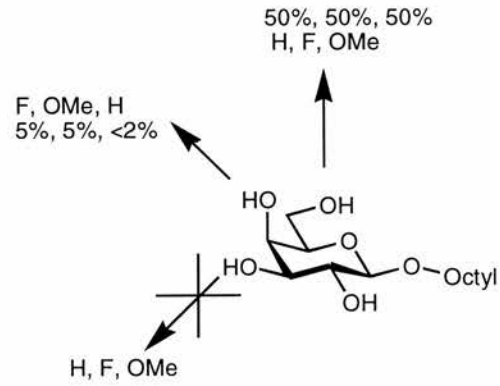


Figure 17: *trans*-Sialidase catalysed sialyation of octyl galactoside derivatives with respect to octyl galactoside (50%).[9]

A library of compounds generated from 1-thio-galactose (**Figure 18**) was tested for recognition by *trans*-sialidase. The results indicate that a very wide variety of substituted groups may be accomodated at the anomeric position.[9]

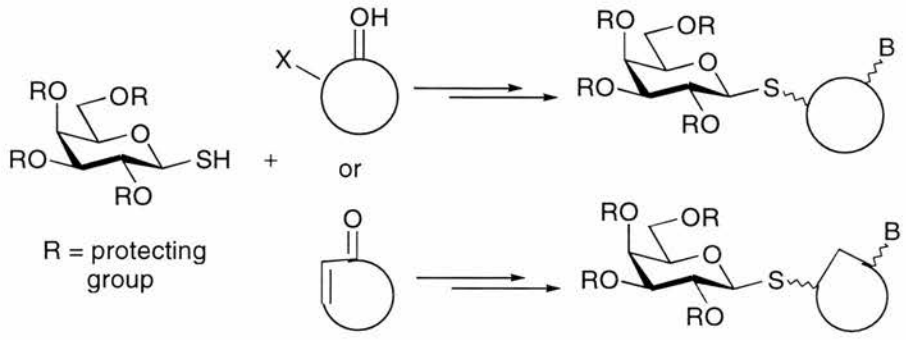


Figure 18: General scheme for the formation of a library of Gal-β-S-X oligosaccharides.[9]

The current working model for the interaction of an acceptor substrate with *trans*-sialidase is outlined in **Figure 19 a.** It is clear from the data which is presented in **Figure 16** and **17** that the 2- and 4-hydroxyl groups of galactose form key polar contacts with the enzyme active site; the 3-hydroxyl group is the site of sialylation. In contrast the 6-hydroxyl group can be replaced by a variety of structures.

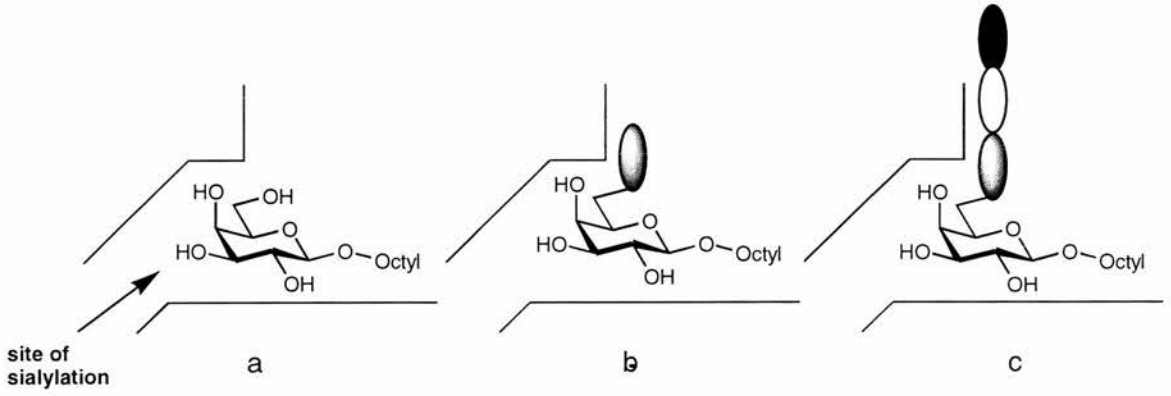


Figure 19: The initial targets and possible interactions with cell surface.

The aim of the study reported in this thesis was to synthesise compounds with enhanced affinity for *trans*-sialidase by incorporating functionality at the 6-position of galactose that might form additional favourable interactions with the enzyme (**Figure 19 b**). If derivatisation of the primary alcohol did not result in enhanced

affinity then a spacer could be introduced (**Figure 19 c**) and a combinatorial approach could be used to introduce diversity. Further details of the chemistry to be investigated are outlined in subsequent sections.

2.3 Aims and Objectives

The aim of this study was to develop methods for the generation of C-6 functionalised libraries based on octyl galactoside. Two approaches were considered. Method A would incorporate traditional carbohydrate chemistry in the formation of octyl galactoside (**12**). Enzymatic chemistry would then be used in the synthesis of the aldehyde (**13**), which could be used for formation of oxime derivatives (**14**). Method B would incorporate traditional carbohydrate chemistry throughout followed by peptide bond formation to gain the derivatised compounds.

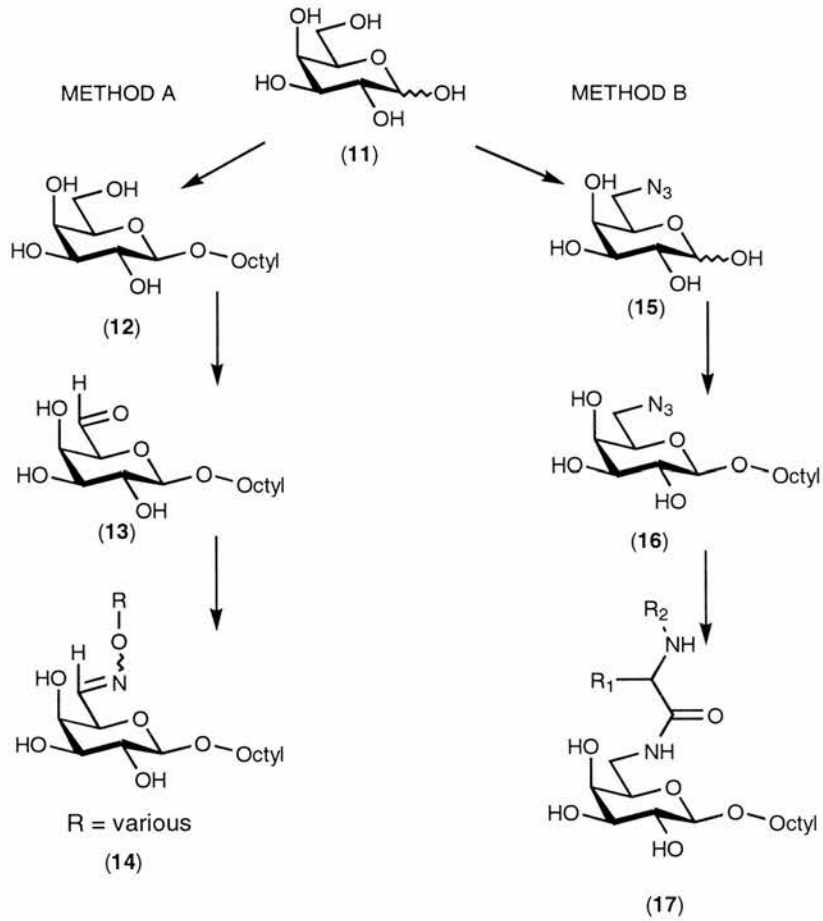


Figure 20: A general outline to the formation of the initial targets.

2.4 Attempted Enzymatic Synthesis of Glycomimetics (14).

Our enzymatic strategy for preparation of glycomimetics starts with the synthesis of octyl galactoside (**12**) (**Figure 21**). The aim behind this approach was to oxidise the primary alcohol to the aldehyde (**13**) and then to perform a chemoselective derivatisation of this functionality without the use of protecting groups (**Figure 22**).

2.4.1 Synthesis of octyl galactoside (12)

The octyl galactopyranoside (**12**) synthesis was non-problematic, utilising standard methods for acetylation, bromination and coupling (**Figure 21**).[10-12]

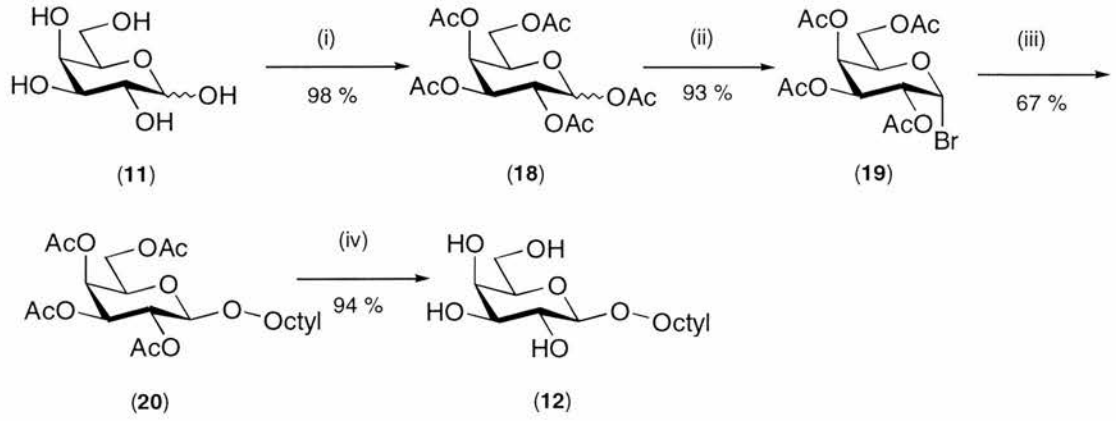
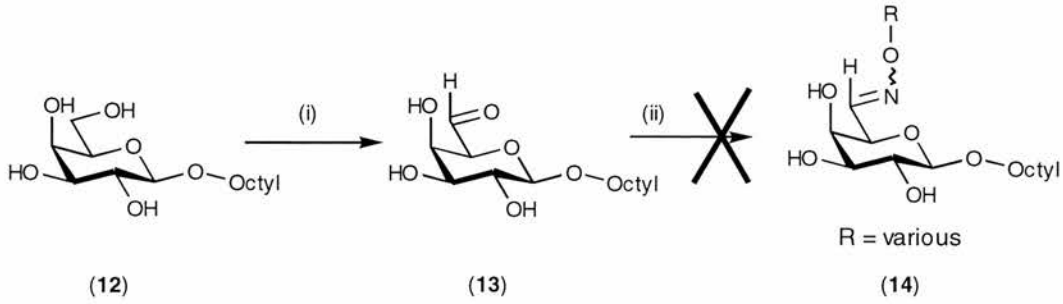


Figure 21: Synthesis of octyl galactoside (**12**).
Reagents: i) I_2, Ac_2O; ii) DCM, 45% w/v HBr/acetic acid; iii) CH_3CN, OctOH, $Hg(CN)_2$, $HgBr_2$, $CaSO_4$; iv) MeOH, Na(s).

Acetylation of galactose using iodine and acetic anhydride gave an excellent yield of (**18**)[10] which was subsequently brominated using 45% HBr/acetic acid in high yield. The resulting glycosyl bromide[11] (**19**) was coupled to octanol, which was achieved using mercuric cyanide and mercuric bromide.[12] This reaction went cleanly to form octyl tetra-*O*-acetyl-β-D-galactoside (**20**) [^{1}H NMR: δ_H 4.41, $J_{1,2}$ 7.9 Hz] in 67 % yield. The next stage of the synthesis involved deprotection of peracetate (**20**) using sodium methoxide in methanol,[13] to yield the unprotected octyl galactopyranoside (**12**).[14]

2.4.2 Attempted enzymatic modification of primary alcohol in octyl galactoside.

The aldehyde (**13**) can be formed using galactose oxidase in aqueous buffer; hydrogen peroxide formed during the reaction can be removed with catalase.[15]



(**12**) (**13**) (**14**)

Figure 22: Reagents: i) Buffer A, Galactose oxidase, Catalase; ii) Et_3N, $RONH_2$.

Our aim was initially to explore oxime formation from aldehyde (**13**). NMR data shows a signal in the aldehydic region corresponding to the H-6 proton (**13**) [1H NMR, δ_H 7.72] on oxidation of octyl galactoside (**12**) with galactose oxidase. However, either by direct reaction with *O*-methyl hydroxylamine or following isolation of aldehyde (**13**) and subsequent reaction with the same reagent, *O*-methyl oxime (**14**) could not be obtained. We assume that either aldehyde hydration or cross-condensation with another sugar molecule may be complicating factors in these experiments.

Due to these initial complications associated with an enzymatic approach, a more classical chemical synthesis approach was adopted.

2.5 Chemical Synthesis of Octyl 6-Azido-6-deoxy-β-D-Galactopyranoside (16)

Schemes for the synthesis of azido-substituted octyl galactopyranoside (**16**) are outlined below in **Figure 23**. The first approach to (**16**) was to follow Route A, *via* octyl galactoside (**12**),[14] and then introduce the azido moiety in place of the primary alcohol. The second route (Route B) proceeds *via* the 6-azido-6-deoxy sugar (**15**), followed by subsequent coupling with octanol.

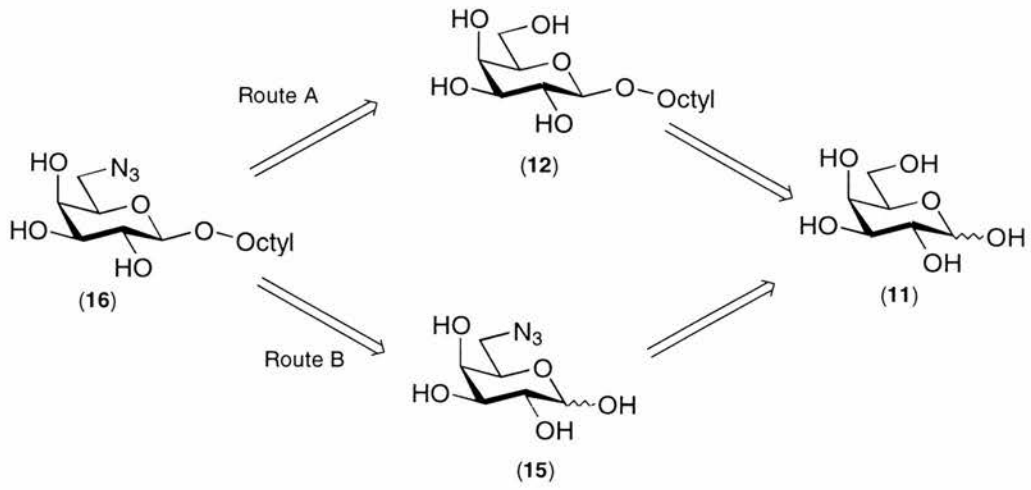


Figure 23 Retrosynthetic Analysis of Target (**16**).

2.5.1 Synthesis of azido sugar (16) *via* Route A.

The synthesis of octyl galactoside (**12**)[14] was as outlined in section **2.4.1**. Synthesis of 6-azido-6-deoxy-sugar (**16**) from octyl galactoside (**12**) without use of protecting groups was attempted using a method described by Wong.[16] This involves selective mono-*O*-tosylation of the primary alcohol of (**12**),[18] to give (**21**), followed by azide displacement, to give (**16**).

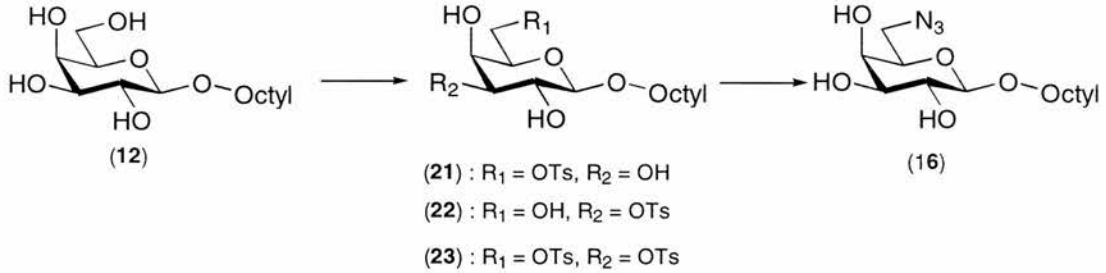


Figure 24: Tosylation reaction using unprotected octyl galactoside (**12**).
Reagents: i) TsCl, pyridine, DCM; ii) NaN_3, $EtOH/H_2O$, NH_3Cl.

When this method was attempted the reaction was not clean and gave a mixture of tosylated compounds, presumed to be the di-tosylate (**23**) and two mono tosylated sugars, presumably (**21**) and (**22**). The primary alcohol was the initial site of tosylation, but position 3 was also tosylated at a slower rate, giving (**22**) (**Figure 24**). Purification (silica gel) of the tosylates was difficult since the two mono- and the di-tosylated compounds had surprisingly similar Rf values. Azide displacement on the

crude mixture of the tosylates gave some of the desired azido sugar, (**16**) [ν_{max}/cm^{-1} 2098 (N_3)], but it again proved difficult to purify. A change in synthetic strategy was therefore required.

Protection of the octyl galactoside (**12**) was therefore necessary. The silylation used a method described by Danishefsky.[18] The primary alcohol of (**12**) was protected using a bulky silyl group (TBDMS) to form compound (**24**) [CI-MS: *m/z* 407 $(M + H)^+$]. Benzoylation of (**24**), using a method described by Brimacombe,[19] was carried out using pyridine, DMAP and benzoyl chloride to form compound (**25**); The TBDMS group was removed with aqueous acetic acid to form compound (**26**) (**Figure 25**)

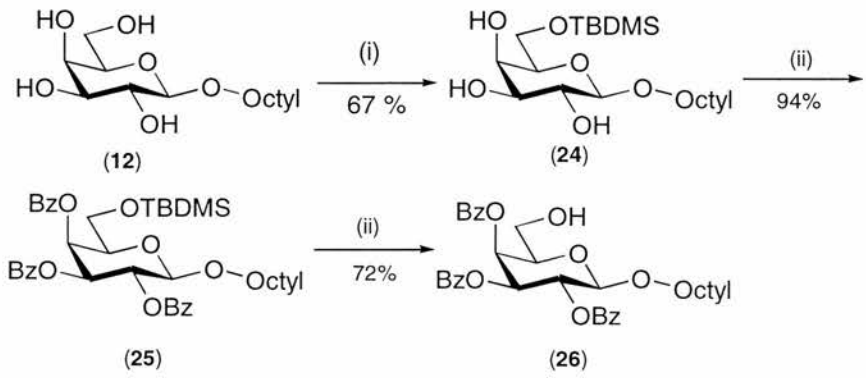


Figure 25: Synthesis of octyl 6-azido-6-deoxy-β-D-galactoside – part 1.
Reagents: i) TBDMSCl, pyridine; ii) BzCl, DMAP, pyridine; iii) AcOH/H_2O (80:20);

The next stage of the synthesis was to make the tosylate (**27**), which was carried out using pyridine, acetone and *p*-tosyl chloride.[17] Azide displacement[16] of the tosyl group gave (**28**) in 77 % yield. Sodium methoxide deprotection[13] of the tri-*O*-benzoyl sugar (**28**) gave the octyl 6-azido-6-deoxy-β-D-galactoside (**16**), (**Figure 26**), in overall yield of 8 % (10 steps) from galactose.

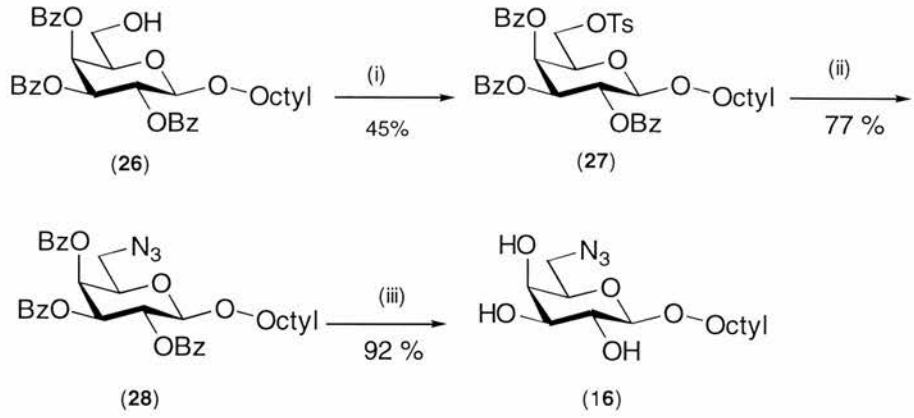


Figure 26: Synthesis of octyl 6-azido-6-deoxy-β-D-galactosidse – part 2. Reagents: i) *p*TsCl, Pyr, Ac_2O; ii) NaN_3, DMF; iii) MeOH, Na(s).

2.5.2 Synthesis of octyl 6-azido-6-deoxy-galactopyranoside (16) *via* Route B.

Partial protection of galactose with isopropylidene groups was chosen because the regiocontrol of protection can be directed, either to give the 1,2:3,4- or 1,2:4,6-di-*O*-isopropylidene galactose. The 1,2:4,6- protected sugar is the kinetically favored product, whilst the 1,2:3,4 protected sugar (**29**) is favoured thermodynamically. 1,2:3,4-Di-isopropylidenation of galactose (**29**) was carried out using acetone and iodine.[20] TLC showed initial formation of the kinetic product, but after leaving the reaction overnight the thermodynamic product was formed exclusively. Isopropylidenation of galactose using the conventional copper (II) sulfate, acetone and concentrated sulfuric acid procedure was unsuccessful in our hands.[21]

The next stages of the synthesis were associated with the primary alcohol of the protected sugar (**29**). The intention was to introduce the azide group using sodium azide in DMF, *via* the tosylated sugar (**30**).[22] Tosylation of the primary alcohol of protected galactose (**29**) was successfully carried out with *p*-toluenesulfonyl chloride in pyridine and acetone in good yield. Nucleophilic displacement of the tosyl group with azide proceeded non-problematically to give known 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**31**) in 97% yield [ν_{max}/cm^{-1} 2100 (N_3)].[16] Removal of the isopropylidene protection in groups of (**31**) was carried out with aqueous TFA (80%) to give the desired 6-azido-6-deoxy-sugar (**15**).[23] Isopropylidene acetal cleavage occurred at different rates, therefore it was possible to follow this stepwise reaction by TLC.

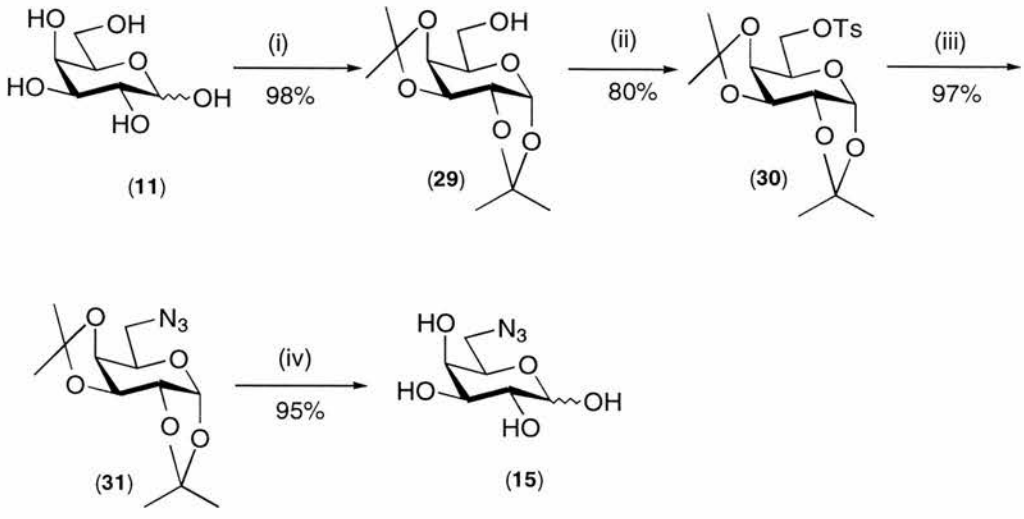


Figure 27: Synthesis of 6-azido-6-deoxy galactose (**15**).
Reagents: i) I_2, $(CH_3)_2CO$; ii) $(CH_3)_2CO$, pyridine, p-toluensulfonyl chloride; iii) DMF, NaN_3; iv) TFA (aq).

The next step in the synthesis involved the acetylation of unprotected azide (**15**) to give per-*O* -acetate (**31**) (**Figure 28**).

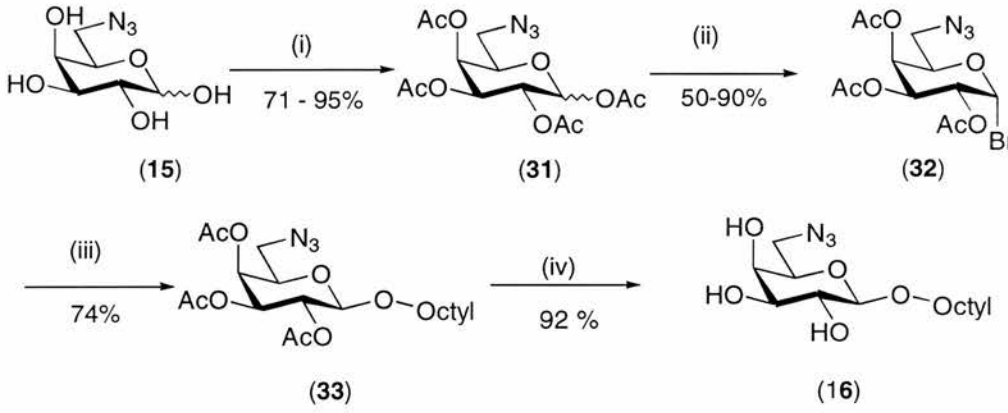


Figure 28: Synthesis of octyl 6-azido-6-deoxy-galactose (**16**).
Reagents: i) a) I_2, Ac_2O; b) pyridine, Ac_2O; ii) a) DCM, HBr/AcOH; b) (i+ii) Ac_2O, HBr/AcOH; c) $TiBr_4$, DCM; iii) I_2, DDQ, CH_3CN, or CH_3CN, $Hg(CN)_2$, $HgBr_2$, $CaSO_4$; iv) MeOH, Na(s).

Acetylation of the free 6-azido-6-deoxy-galactose (**15**) was carried out by two different methods. The first method used iodine and acetic anhydride and the

experiment was rapid and efficient.[10] Use of pyridine and acetic anhydride[24] was slower than the iodine procedure,[23] but the yields were superior.

Formation of the anomeric bromide (**32**) and the subsequent glycosylation reaction with octanol was more difficult than for the formation of octyl galactoside (**12**). Three attempts were made at the formation of the azido-galactosyl bromide (**32**). Method **1** utilising the standard HBr/acetic acid method.[11] Method **2** used a one-pot method of acetylation and bromination.[26] Method **3** used titanium tetrabromide.[28]

2.5.2.1 Method 1: HBr/acetic acid method for bromination of acetylated 6-azido-6-deoxy-galactose (31)[11]

Anomeric bromination of the acetylated sugar (**31**) was not as efficient as expected. Using HBr/acetic acid in DCM gave the bromo compound (**32**) in only modest yield.[11] TLC showed that the majority of the compound was the glycosyl bromide, but after column chromatography only 50% of a mixture of glycosyl bromide (**32**), tetra-acetate (**31**) and the corresponding hemi-acetal was obtained. It was noted that bromide (**32**) is not as easy to prepare as it is less stable than the acetobromogalactose prepared previously (Route A).

A mixture of compounds including octyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranoside (**33**) and per acetate (**31**), were recovered from the impure glycosyl bromide (**32**) in a Helferich glycosylation reaction.[12,25] Due to the inadequate yields of the two step synthesis of glycosyl bromide from compound (**15**), an alternative approach was considered.

2.5.2.2 Method 2: 'One-pot method' for bromination of acetylated 6-azido-6-deoxy-galactose (31)[26]

A 'one-pot' method was used to make the glycosyl bromide from 6-azido-6-deoxy-galactopyranose (**15**).[26] This method involved using acetic anhydride as the acetylating agent and HBr/acetic acid as the catalyst, utilising TLC to monitor the reaction. Once the first reaction was complete, more HBr/acetic acid was added to

facilitate bromination of compound (**31**). Glycosyl bromide (**32**) was taken directly, without purification, onto glycosylation with octanol in an attempt to make octyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranoside (**33**). The glycosylation strategy used was a combination of that of Helferich [12] and Brimacombe.[25] The reaction again gave a mixture of compounds, which were inseparable. NMR data shows the disappearance of the bromide H-1 signal [δ_H: 6.60 $J_{1,2}$ 5.0] and the appearance of a downfield H-1 signal, along with the appearance of the octyl chain; [δ_H($CDCl_3$) 0.91 ($C_7H_{14}CH_3$), 1.2 ((CH_2)$_5CH_3$), 1.55 (OCH_2CH_2), 1.8-2.1 (3 x AcO), 5.55 ($J_{1,2}$ 7.8)]. Coupling the glycosyl bromide (**32**) to octanol proved difficult using both an iodine/DDQ method[27] and the mercuric salts method.[12,25] Another change in bromination procedure was attempted to synthesise bromide (**32**) in better yield and purity.

2.5.2.3 Method 3: Titanium tetrabromide method for bromination of acetylated 6-azido-6-deoxy-galactose (31)[28]

This method for the formation of the anomeric bromide utilises titanium tetrabromide as the halide activator.[28] This reaction proceeds slowly (days rather than hours) but cleanly, with few degradation products. Once the reaction had gone to completion a simple work-up and purification yielded the pure azido-bromo sugar (**32**) in 90 % yield. Glycosylation with octanol was performed under Helfrich conditions.[12.25] With cleaner starting material the glycosylation went quickly and cleanly to yield the octyl sugar (**33**) in 74 % [δ_H; 5.55, $J_{1,2}$ 7.8]. Deprotection of octyl glycoside (**33**) was performed using sodium methoxide to yield the free octyl 6-azido-6-deoxy-galactopyranoside (**16**) in an overall yield of 42 % (8 steps) from galactose.[13]

2.6 Coupling of Azido-bromo galactose (32) with Isopropylidenated galactose (29)

Utilising building blocks that had already been synthesised, preparation of disaccharide (**34**) was attempted to gain experience in oligosaccharide coupling. The azido-bromo sugar (**32**), synthesised via the titanium tetrabromide method,[28] was

coupled to the isopropylidenated sugar (**29**) using a Helferich glycosylation procedure.[12,25]

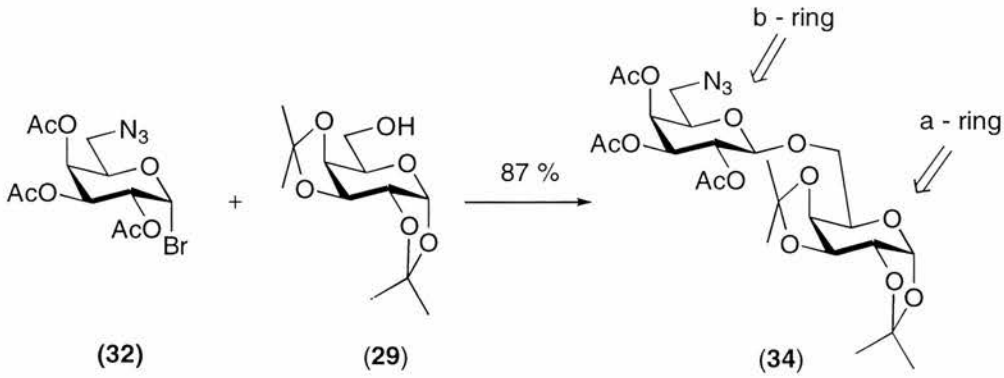


Figure 29: Glycosylation reaction. Reagents: $Hg(CN)_2$, $HgBr_2$, DCM, 4 Å MS, $CaSO_4$.

The reaction proceeded, with only a few degradation products, in 87 % yield. IR and NMR spectroscopy [ν_{max}/cm^{-1} 2100, (N_3); NMR; $\delta_H(CDCl_3)$: 4.62 ($J_{1b,2b}$ 7.8, H-1b), 5.50 ($J_{1a,2a}$, 5.1, H-1a); δ_C: 96.3, 102.1] and mass spectrometry [MALDI-TOF: *m/z* 596 $(M + Na)^+$] confirmed the formation of the derived disaccharide (**34**).

2.7 Derivatisation of 1:2,3:4-di-*O*-isopropylidene galactose (29)

Oxidation of the isopropylidene galactose (**29**) was carried out using two different oxidation procedures. Firstly the primary alcohol was oxidised using nicotinium dichromate, which gave two compounds, the starting material in 38 % and the oxidised sugar (**35**) in 47 % yield.[29] With increased amount of oxidising agent this reaction would prehaps be more successful. Swern oxidation was also attempted on the primary alcohol. Again a mixture of compounds was produced.[30]

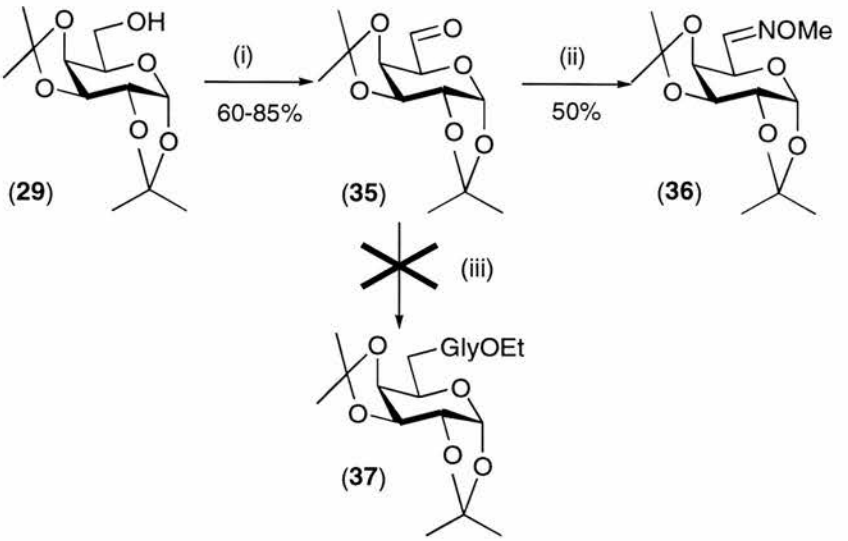


Figure 30: Derivatisation of isopropylidene galactose (**29**).
Reagents: i) a) nictotinium dichromate, pyridine, toluene; b) DMSO, DCM, TFAA, TEA; ii) *N*-methoxyimine hydrochloride, pyridine; iii) MeCN, AcOH, glycine ethyl ester, $NaBH_3CN$.

Derivatisation of the oxidised compound (**35**) was attempted. Firstly oxime chemistry was attempted using *O*-methyl hydroxylamine which gave the resulting oxime (**36**) in 50 % yield [^{1} NMR; 6.71 and 7.38 (H-6), *E/Z* isomers]. The reductive coupling of glycine ethyl ester to aldehyde (**35**) was also attempted, but this proved unsuccessful.[31]

2.8 References

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Chapter 3

Experimental

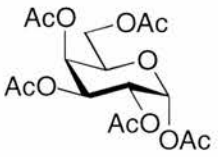
3.1 General Method

All reagents and solvents were dried prior to use according to standard methods[1]. Commercial reagents were otherwise used without further purification. Analytical TLC was performed on silica gel 60-F_{254} (Merck) with detection by fluorescence and/or by charring following immersion in a dilute ethanolic solution of sulphuric acid. Orcinol dipping reagent, prepared by the careful addition of conc. Sulfuric acid (20 cm^3) to an ice cold solution of 3,5-dihydroxytoluene (360 mg) in EtOH (150 cm^3) and water (10 cm^3), was used for deprotected compounds. Column chromatography was performed with silica gel 60 (Fluka).

Optical rotations were measured at the sodium D-line and at ambient temperature, with an Optical Activity AA-1000 polarimeter. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}. Melting points were measured using a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded as thin films on NaCl plates using a Perkin-Elmer 1710 FT-IR spectrometer. Electospray mass spectra (ES-MS) were recorded on a Fisons VG Biotech electrospray mass spectrometer or a FINNIGAN MAT900. Chemical ionisation mass spectra (CI-MS) were recorded on a Fisons VG Autospec. Unless stated otherwise, ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 300 MHz or 500 MHz, and 75 MHz, respectively. ^{1}H NMR spectra were referenced to the following internal standards: $CHCl_3$, δ_H 7.26 in $CDCl_3$; CD_2HOD, δ_H 3.35 in CD_3OD, 4.75 in D_2O. ^{13}C NMR spectra were referenced to the following internal standards: $CDCl_3$ δ_C 76.9 in $CDCl_3$; CD_3OD δ_C 49.0 in CD_3OD. *J* values are given in Hz. For disaccharides, the monosaccharide residues are labelled *a* and *b* from the reducing terminus. Only partial (diagnostic) NMR data are given for some compounds; other spectral features were in accord with the proposed structures.

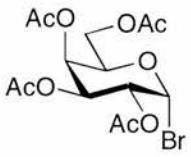
3.2 Synthetic Methods

Penta-*O*-acetyl-α-D-galactopyranose (18)[2]



A suspension of finely ground D-galactose (**11**) (10 g, 55.5 mmol) and molecular iodine (450mg, 1.78 mmol) in acetic anhydride (450 ml) was stirred at room temperature for 2 h, when TLC [EtOAc - pet. ether (1:1)] showed the reaction to be complete. The resulting solution was diluted with sodium thiosulphate solution and extracted with DCM. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Crystallization gave penta-*O*-acetyl-α-D-galactopyranose (**18**) as white prisms (21.2 g, 98%), mp 94-96°C (diethyl ether) (lit.,[2] 95.5°C); $[\alpha]_D$ +106 (*c* 1.0, $CHCl_3$) (lit.,[2] +107); $\delta_H(CDCl_3)$: 2.00 (3 H, s, CH_3), 2.05 (3 H, s, CH_3), 2.10 (3 H, s, CH_3), 2.14 (3 H, s, CH_3), 2.20 (3 H, s, CH_3), 4.09 (2 H, m, H-6,6'), 4.51 (1 H, m, H-5), 5.12 (1 H, dd, $J_{1,2}$ 4.9, $J_{2,3}$ 7.0, H-2), 5.40 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 3.2, H-3), 5.55 (1 H, d, $J_{3,4}$, H-4), 5.8 (1 H, d, $J_{1,2}$, H-1).

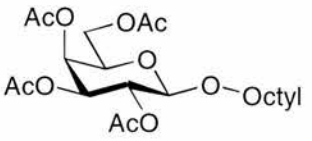
Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (19)[3]



A solution of protected sugar (**18**) (10 g, 25.6 mmol) was dissolved in anhydrous DCM (100 ml) and cooled (0°C) before a solution of 45% HBr in acetic acid (50 ml) was slowly added. The reaction mixture was stirred under nitrogen until TLC [pet. ether-ethyl acetate (3:2)] showed the reaction to be complete (approx. 3 h). The solution was then diluted with toluene and concentrated under reduced pressure. Recrystallisation gave tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**19**) (9.75 g, 93%); mp 82-84°C (diethyl ether) (lit.,[3] 84-85°C); $[\alpha]_D$ +218 (*c* 1.0, $CHCl_3$) (lit.,[3]

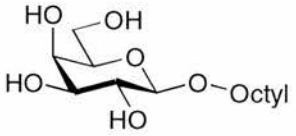
+217); $\delta_H(CDCl_3)$: 2.05 (3 H, s, CH_3), 2.10 (3 H, s, CH_3), 2.14 (3 H, s, CH_3), 2.20 (3 H, s, CH_3), 4.18 (2 H, m, H-6,6'), 4.49 (1 H, br t, *J* 8.0, H-5), 5.10 (1 H, dd, $J_{1,2}$ 4.4, $J_{2,3}$ 7.9, H-2), 5.40 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 4.3, H-3), 5.55 (1 H, m, H-4), 6.68 (1 H, d, $J_{1,2}$, H-1).

Octyl tetra-*O*-acetyl-β-D-galactopyranoside (20)



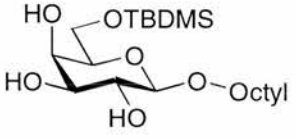
Compound (**20**) was prepared using an standard Helferich glycosylation procedure.[4,5] The glycosyl bromide (**19**) (5.84 g, 14.19 mmol) was stirred at room temperature under nitrogen in anhydrous acetonitrile (25 ml), containing octanol (13 ml) and Drierite (5.84 g). After 2 h mercuric cyanide (4.3 g, 1.2 mol equiv., 17.02 mmol) and mercuric bromide (0.5 g, 0.1 mol equiv., 1.42 mmol) were added, and the solution was stirred under nitrogen at room temperature overnight. Once TLC [pet. ether-EtOAc (4:1)] showed the reaction had gone to completion, the inorganic salts were removed by filtration and the resulting solution was concentrated to dryness. Column chromatography (silica gel; pet. ether-EtOAc, 20:1→ 4:1) gave the *octyl tetra-O-acetyl-β-D-galactopyranoside* (**19**) as a syrup (5 g, 76%); $[\alpha]_D$ +101 (*c* 1.0, $CHCl_3$) $\delta_H(CDCl_3)$: 0.82 (3 H, t, CH_3), 1.22 (10 H, m, 5 x CH_2), 1.51 (2 H, m, OCH_2CH_2), 1.92 (3 H, s, CH_3), 1.98 (6 H, s, 2 x CH_3), 2.08 (3 H, s, CH_3), 3.41 (1 H, m, OCH_2), 3.76-4.18 (4 H, m, OCH_2, H-5, 6, 6'), 4.41 (1 H, d, $J_{1,2}$ 7.9, H-1), 4.96 (1 H, dd, $J_{2,3}$ 7.1, $J_{3,4}$ 3.3, H-3), 5.14 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.32 (1 H, m, H-4).

Octyl β-D-galactopyranoside (12)[6]



Compound (**12**) was prepared using a standard methoxide deprotection.[7] A solution of the peracetylated octyl galactoside (**20**) (1 g, 2.18 mmol) in methanol (10 ml) containing sodium metal (trace) was stirred at room temperature for 2 h. Once TLC [DCM-MeOH (6:1)] showed the reaction to be complete, the resulting solution was neutralised with a Amberlite IR120 (H^+) ion exchange resin. The resulting solution was filtered and concentrated to give *octyl galactoside* (**12**) as a white solid (0.49 g, 80%); mp 115-118 ^{0}C (methanol) (lit.[6], 116-119); $[\alpha]_D$ –15.7 (*c* 0.85, MeOH), (lit.[6], -16) ; $\delta_H(CD_3OD)$: 0.82 (3 H, t, CH_3), 1.2 (10 H, m, 5 x CH_2), 1.51 (2 H, m, OCH_2CH_2), 3.41 (1 H, m, OCH_2), 4.18 (1 H, d, $J_{1,2}$ 7.4, H-1); ^{1}H NMR in accord with literature data.[6]

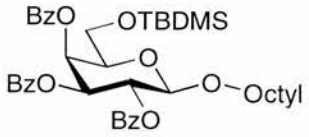
Octyl 6-*O*-(*t*-butyldimethylsilyl)-β-D-galactopyranoside (24)



Compound (**24**) was prepared using a standard silylation protocol.[8] A solution of compound (**12**) (0.512 g, 1.76 mmol) in anhydrous pyridine (11 ml) containing dissolved TBDMSCl (0.4 g, 2.65 mmol) was stirred at 0 °C and allowed to warm slowly to room temperature (approx. 5 h). The solution was cooled and methanol was added. The resulting solution was coevaporated with toluene to give a syrup. Column chromatography (silica gel; pet. ether-EtOAc, 12:1→ 2:1) gave *octyl 6-O-(t-butyldimethylsilyl)-β-D-galactopyranoside* (**24**) as a syrup (0.479 g, 67%); $[\alpha]_D$ – 21.0 (*c* 0.77, $CHCl_3$); $\delta_H(CDCl_3)$: 0.05 (6 H, s, 2 x CH_3Si), 0.86 (12 H, m, $C_7H_{14}CH_3$, t*Bu*), 1.24 (10 H, s, 5 x CH_2), 1.55 (2 H, m, OCH_2CH_2), 4.18 (1 H, d, $J_{1,2}$ 7.4, H-1),

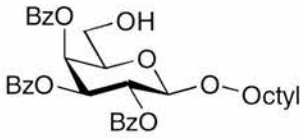
4.64 (1 H, m, H-4); $\delta_C(CDCl_3)$: -5.4, 14.0, 18.2, 22.6, 25.8, 25.9, 29.3, 29.4, 29.6, 31.8, 62.4, 68.9, 69.9, 71.6, 73.8, 74.8, 103.0; CI-MS: *m/z* 407 $(M + H)^+$. (Found: $[M + H]^+$ 407.282289. $C_{20}H_{43}O_6Si$ requires *m/z* 407.28297).

Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-(t.butyldimethylsilyl)-β-D-galactopyranoside (25)



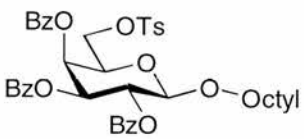
Compound (**25**) was prepared using a standard benzolylation protocol.[9] The partially protected sugar (**24**) (0.236 g, 0.58 mmol) was dissolved in anhydrous pyridine (2.4 ml) and cooled (0°C) before benzoyl chloride (0.45 ml, 246 mmol) and DMAP (71 mg, 0.58 mmol) were added slowly. The reaction mixture was allowed to warm up to room temperature and was stirred overnight. The resulting solution was coevaporated with methanol and toluene to give the protected sugar as a syrup. The syrup was dissolved in DCM and washed with dilute HCl. The organic layer was separated and washed with sodium hydrogen carbonate solution and water, dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; pet. ether-EtOAc, 12:1→2:1) gave *octyl 2,3,4-tri-O-benzoyl-6-O-(t.butyldimethylsilyl)-β-D-galactopyranoside* (**25**) as a syrup (392mg, 94%); $[\alpha]_D$ + 89.6 (*c* 0.99, $CHCl_3$); ν_{max}/cm^{-1} 1750 (CO); $\delta_H(CDCl_3)$: -0.05 (3 H, s, $SiCH_3$), 0.00 (3 H, s, $SiCH_3$), 0.86 (12 H, t, $C_7H_{14}CH_3$, t*Bu*), 1.24 (10 H, s, 5 x CH_2), 1.55 (2 H, m, OCH_2CH_2), 3.58 (1 H, m, OCH_2) 3.78-4.08 (3 H, m, H-5,6,6'), 4.78 (1 H, d, $J_{1,2}$ 8.0, H-1), 5.62 (1 H, dd, $J_{2,3}$ 7.2, $J_{3,4}$ 3.3 , H-3), 5.78 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.96 (1 H, m, H-4), 7.2-7.6 (9 H, m, Ar), 7.8, 7.95, 8.1 (6 H, 3 x d, *J* 7.1, Ar); $\delta_C(CDCl_3)$: -1.4, 13.8, 18.0, 22.6, 25.7, 29.1, 29.2, 29.4, 31.7, 60.4, 69.2, 70.0, 70.6, 71.8, 73.9, 101.5, 127.9, 128.5, 129.7, 130.1, 133.3, 133.8, 140.1, 165.5, 165.6, 166.8 CI-MS: *m/z* 719 $(M + H)^+$. (Found: $[M + H]^+$ 719.3610. $C_{41}H_{35}O_9Si$ requires *m/z* 719.36161).

Octyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (26)



Compound (**26**) was prepared using a modified de-silylation protocol.[8] The protected sugar (**25**) (380 mg, 0.53 mmol) was dissolved in 80% aqueous acetic acid (5 ml) and was stirred at room temperature overnight. The resulting sugar was coevaporated with toluene to give a syrup. Column chromatography (silica gel; pet. ether-EtOAc, 12:1→ 2:1) gave *octyl 2,3,4-tri-O-benzoyl-β-D-galactopyranoside* (**26**) as a colourless crystals (230 mg, 72%); mp 67-69 ^{O}C (ethyl acetate); $[\alpha]_D$ +188.2 (*c* 0.77, $CHCl_3$); $\delta_H(CDCl_3)$: 0.86 (3 H, t, $C_7H_{14}CH_3$), 1.24 (10 H, s, 5 x CH_2), 1.55 (2 H, m, OCH_2CH_2), 2.61 (1 H, br m, OH), 3.58 (1 H, m, OCH_2) 3.68 (1 H, d, $J_{5,6/6'}$ 6.9, H-6/6'), 3.84 (1 H, d, $J_{5,6/6'}$ 6.9, H-6/6'), 3.96 (1 H, m, OCH_2), 4.04 (1 H, dd, $J_{4,5}$ 3.2, $J_{5,6/6'}$ H-5), 4.80 (1 H, d, $J_{1,2}$ 8.0, H-1), 5.61 (1 H, dd, $J_{2,3}$ 7.1, $J_{3,4}$ 3.3 , H-3), 5.86 (2 H, m, H-2, H-4), 7.2-7.6 (9 H, m, Ar), 7.8 (2 H, d, *J* 7.1, Ar), 7.95 (2 H, d, *J* 7.1, Ar), 8.1 (2 H, d, *J* 7.1, Ar); $\delta_C(CDCl_3)$: 14.0, 22.6, 25.8, 29.0, 29.2, 29.4, 31.7, 60.6, 69.1, 70.0, 70.6, 71.9, 73.9, 101.8, 128.3, 128.6, 129.7, 130.1, 133.3, 133.8, 140.0, 165.5, 165.6, 166.9; CI-MS: *m/z* 605 $(M + H)^+$. (Found: $[M + H]^+$ 605.275049. $C_{35}H_{41}O_9$ requires *m/z* 605.27514).

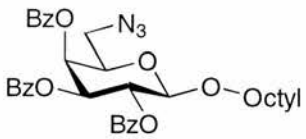
Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-toluenesulfonyl-β-D-galactopyranoside (27)



Compound (**27**) was prepared using a standard tosylation protocol.[10] The protected sugar (**26**) (150 mg, 0.255 mmol) was stirred in acetone (0.5 ml) and anhydrous pyridine (0.3 ml) until all the sugar had dissolved. The solution was cooled in cold water, and *p*-toluenesulfonyl chloride (59 mg, 0.31 mmol) was added in portions over 0.1 h. The reaction mixture was stirred overnight at room temperature, cooled in

ice-water and water (5ml) was added. On stirring, the resulting syrup soon crystallised. The crystals were removed by filtration, washed with water and dried *in vacuo*. Recrystallization gave the *octyl 2,3,4-tri-O-benzoyl-6-O-toluenesulfonyl-β-D-galactopyranoside* (**27**) as white crystals (85 mg, 45%); mp 189-190 ^{O}C (isopropanol); $[\alpha]_D$ +123.3 (*c* 0.3, $CHCl_3$); $\delta_H(CDCl_3)$: 0.82 (3 H, t, $C_7H_{14}CH_3$), 1.18 (10 H, s, 5 x CH_2), 1.55 (2 H, m, OCH_2CH_2), 2.28 (3 H, s, Me of Ts), 3.52 (1 H, m, OCH_2), 3.92 (1 H, m, OCH_2), 4.06-4.21 (3 H, m, H-5, 6, 6'), 4.73 (1 H, d, $J_{1,2}$ 7.7, H-1), 5.51 (1 H, dd, $J_{2,3}$ 7.1, $J_{3,4}$ 3.3 , H-3), 5.66 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.82 (1 H, d, $J_{3,4}$, H-4), 7.21 (5 H, m, Ar), 7.42 (6 H, m, Ar), 7.74 (4 H, m, Ar), 7.92 (4 H, m, Ar); $\delta_H(CDCl_3)$: 18.5, 21.5, 22.6, 25.7, 29.1, 29.2, 29.4, 31.7, 66.7, 67.8, 69.5, 70.6, 71.1, 71.5, 101.7, 127.9, 128.2, 128.3, 128.5, 129.6, 129.7, 129.8, 129.9, 133.2, 133.5, 145.0, 165.1, 165.4, 165.5; CI-MS: *m/z* 759 $(M + H)^+$. (Found: $[M + H]^+$ 759.2832. $C_{42}H_{47}O_{11}S$ requires *m/z* 759.28399).

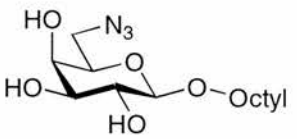
Octyl 6-azido-2,3,4-tri-*O*-benzoyl-6-deoxy-β-D-galactopyranoside (28)



Compound (**27**) was prepared using a modified azide displacement protocol.[11] A solution of compound (**27**) (100 mg, 0.13 mmol) in DMF (2 ml) containing suspended sodium azide (51 mg, 0.78 mmol) was heated under reflux (approx. 4 h). The solution was allowed to cool, diluted with water (2 ml) and extracted with DCM. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give *octyl 6-azido-2,3,4-tri-O-benzoyl-6-deoxy-β-D-galactopyranoside* (**28**) as a syrup (64 mg, 77 %); $[\alpha]_D$ + 142.0 (*c* 0.80 in $CHCl_3$); ν_{max}/cm^{-1} 2100 (N_3); $\delta_H(CDCl_3)$: 0.82 (3 H, t, $C_7H_{14}CH_3$), 1.19 (10 H, m, 5 x CH_2), 1.56 (2 H, m, OCH_2CH_2), 3.28 (1 H, dd, $J_{5,6/6'}$ 4.0, $J_{6/6'}$ 9.0, H-6/6'), 3.58 (1 H, m, OCH_2), 3.66 (1 H, dd, $J_{5,6/6'}$, $J_{6/6'}$, H-6/6'), 4.02 (1 H, m, OCH_2), 4.10 (1 H, m, H-5), 4.78 (1 H, d, $J_{1,2}$ 8.0, H-1), 5.55 (1 H, dd, $J_{2,3}$ 7.5, $J_{3,4}$ 3.0 , H-3), 5.78 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.81 (1 H, d, $J_{3,4}$, H-4), 7.25 (2 H, d, *J* 9.0, Ar), 7.42 (6 H, m, Ar), 7.62 (3

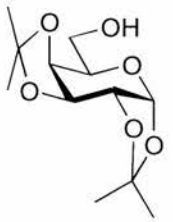
H, t, *J* 7.5, Ar), 7.79 (2 H, d, *J* 7.5, Ar), 7.97 (2 H, d, *J* 7.5, Ar), 8.09 (2 H, d, *J* 7.5, Ar); $\delta_H(CDCl_3)$: 14.0, 22.6, 25.8, 29.1, 29.2, 29.3, 31.7, 50.9, 68.9, 69.7, 70.5, 71.7, 73.5, 101.6, 128.3, 128.6, 129.7, 129.8, 130.0, 133.1, 133.3, 133.7, 165.2, 165.6, 165.7; CI-MS: *m/z* 630 $(M + H)^+$. (Found: $[M + H]^+$ 630.2814. $C_{35}H_{40}N_3O_8$ requires *m/z* 630.28162).

Octyl 6-azido-6-deoxy-β-D-galactopyranoside (16)



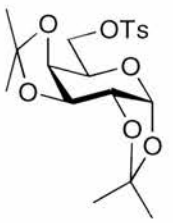
Compound (**16**) was prepared using a standard methoxide deprotection.[7] A solution of the benzoylated octyl galactoside (**28**) (50 mg, 0.08 mmol) in methanol (1 ml) containing sodium metal (trace) was stirred at room temperature for 2 h. Once TLC [DCM-MeOH (6:1)] showed the reaction to be complete, the resulting solution was neutralised with a Amberlite IR120 (H^+) ion exchange resin. The resulting solution was filtered and concentrated to give *azido octyl galactoside* (**16**) as a syrup (23 mg, 92 %); $[\alpha]_D$ – 44.4 (*c* 0.61, $CHCl_3$); ν_{max}/cm^{-1} 2097 (N_3), 3285 (OH); $\delta_H(CDCl_3)$: 0.85 (3 H, t, CH_3), 1.25 (10 H, m, 5 x CH_2), 1.62 (2 H, m, OCH_2CH_2), 3.29 (1 H, dd, $J_{5,6/6'}$ 4.0, $J_{6,6'}$ 9.0, H-6/6'), 3.53 (1 H, m, OCH_2), 3.65 (3 H, m, H-2,3,5), 3.74 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.91 (1 H, m, H-4), 3.94 (1 H, m, OCH_2), 4.26 (1 H, d, $J_{1,2}$ 7.5, H-1); $\delta_C(CDCl_3)$: 13.9, 22.5, 25.8, 29.1, 29.2, 29.4, 31.6, 50.9 (C-6), 68.9, 70.1, 71.5, 72.9, 74.1, 102.6 (C-1); ES-MS: *m/z* 340 $(M + Na)^+$. (Found: $[M + Na]^+$ 340.1844. $C_{14}H_{27}N_3O_5Na$ requires *m/z* 340.18497).

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (29)[12]



A suspension of finely powdered galactose (10 g, 55.5 mmol) and iodine (3 g, 11.8 mmol) in acetone (500ml) was stirred at room temperature until TLC [toluene - EtOAc (2:1)] showed that the reaction was complete (approx. 20 h). The solution was diluted with sodium thiosulphate and extracted with DCM. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give the 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**29**) as a syrup (14.21 g, 98%); $[\alpha]_D$ -54.5 (*c* 1.5 in $CHCl_3$) (lit.,[12] -59); $\delta_H(CHCl_3)$: 1.30 (6 H, s, 2 x CH_3), 1.36 (3H, s, CH_3), 1.40 (3H, s, CH_3), 2.42 (1 H, br s, OH), 3.62-3.90 (3 H, overlapping m, H-5,6,6'), 4.23 (1 H, d, $J_{3,4}$ 3.3, H-4), 4.30 (1 H, dd, $J_{1,2}$ 5.0, $J_{2,3}$ 8.0, H-2), 4.58 (1 H, dd, $J_{2,3}$, $J_{3,4}$, H-3), 5.56 (1 H, d, $J_{1,2}$, H-1); in accord with literature data.[13]

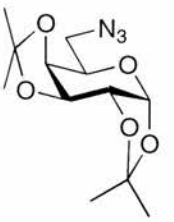
1,2:3,4-Di-*O*-isopropylidene-6-*O*-toluenesulfonyl-α-D-galactopyranose (30)[14]



The protected sugar (**29**) (13.16 g, 0.05 mmol) was stirred in acetone (14 ml) and anhydrous pyridine (16 ml) until all the acetal had dissolved. The solution was cooled in cold water, and with stirring, *p*-toluenesulfonyl chloride (11.44 g, 0.06 mmol) was added in portions over 0.5 h. The reaction mixture was stirred overnight at room temperature then cooled in ice-water, water (5ml) was added and on stirring

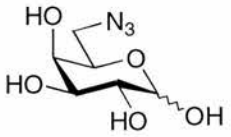
the resulting syrup soon crystallised. The crystals were removed by filtration, washed with water and dried *in vacuo*. Recrystallization gave 1,2:3,4-di-*O*-isopropylidene-6-*O*-toluenesulfonyl-α-D-galactopyranose (**30**) as white needles (16.82 g, 80%), mp 88-91°C (isopropanol), (lit.,[14] 90-92°C); $[\alpha]_D$ -54 (*c* 1.02 in acetone) (lit.,[14] -54.5); $\delta_H(CDCl_3)$: 1.30 (6 H, s, 2 x CH_3), 1.36 (3H, s, CH_3), 1.40 (3H, s, CH_3), 2.40 (3 H, s, Ar*Me*), 3.90-4.10 (3 H, overlapping m, H-5,6,6'), 4.24 (1 H, dd, $J_{1,2}$ 5.0, $J_{2,3}$ 7.6, H-2), 4.30 (1 H, m, H-4), 4.60 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 2.3, H-3), 5.52 (1 H, d, $J_{1,2}$, H-1), 7.31 (2 H, d, *J* 6.0, Ar), 7.82 (2 H, d, *J* 6.0, Ar).

6-Azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (31)[11]



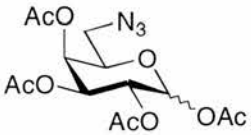
A solution of compound (**30**) (8.8 g, 0.021 mol) in DMF (200 ml) containing suspended sodium azide (20.47 g, 0.32 mol) was heated under reflux (approx. 4 h). The solution was allowed to cool, diluted with water (300 ml) and extracted with DCM. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**31**) as a syrup (5.85 g, 97%); $[\alpha]_D$ -110 (*c* 1.0 in $CHCl_3$) (lit.,[11] – 112); ν_{max}/cm^{-1} 2100 (N_3); $\delta_H(CDCl_3)$: 1.28 (6 H, s, 2 x CH_3), 1.30 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 3.32 (1 H, dd, $J_{5,6/6'}$ 5.4, $J_{6,6'}$ 10.5, H-6'), 3.46 (1 H, dd, $J_{5,6/6'}$ 5.4, $J_{6,6'}$ 10.5, H-6), 3.91 (1 H, m, H-5), 4.18 (1 H, dd, $J_{1,2}$ 4.9, $J_{2,3}$ 7.6, H-2), 4.34 (1 H, m, H-4), 4.61 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 5.4, H-3), 5.56 (1 H, d, $J_{1,2}$, H-1).

6-Azido-6-deoxy-D-galactopyranose (15)[15]



Compound (**15**) was prepared using a standard acetal removal protocol.[15] A solution of compound (**31**) (5.89 g, 0.02 mol) in 80% aqueous TFA (58 ml) was stirred until TLC [DCM - methanol, (7:3)] showed the reaction to be complete (approx. 2 h). The resulting solution was coevaporated with isopropanol to give a syrup. Crystallization gave 6-azido-6-deoxy-D-galactopyranose (**15**) as white needles (4.0 g, 95%); mp 143-148°C (ethyl acetate), (lit.,[15] 145-147°C); $[\alpha]_D$ + 60 (*c* 0.81 in H_2O), (lit.,[23] +110 → +55); $\delta_H(CD_3OD)$: 5.52 (1 H, d, *J* 5.0, H-1); CI-MS: *m/z* 206 $(M + H)^+$. (Found: $[M + H]^+$ 206.077696. $C_6H_{12}N_3O_5$ requires *m/z* 206.07777).

Acetylation of 6-azido-6-deoxy-D-galactopyranose (31)[15]



1. Iodine / acetic anhydride procedure[2]

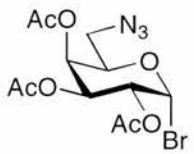
A suspension of azidogalactose (**15**) (300 mg, 1.46 mmol) and molecular iodine (15 mg, 0.05 mmol) in acetic anhydride (15 ml) was stirred at room temperature for 15 min, at which time TLC [EtOAc - pet. ether (1:1)] showed the reaction to be complete. The resulting solution was diluted with sodium thiosulphate solution and extracted with DCM. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranose (**31**) as a syrup (385 mg, 71%), $[\alpha]_D$ + 102 (*c* 1.04 in CH_2Cl_2), (lit.,[23] + 110); ν_{max}/cm^{-1} 2098 (N_3); $\delta_H(CDCl_3)$: 1.28 (6 H, s, 2 x AcO), 1.40 (3 H, s, AcO), 1.52 (3 H, s, AcO), 3.32 (1 H, dd, $J_{5,6/6'}$ 6.5, $J_{6,6'}$ 10.5, H-6'), 3.47 (1 H, dd, $J_{5,6/6'}$ 6.5, $J_{6,6'}$ 10.5, H-6), 3.88 (1 H, m, H-5), 4.16 (1 H, dd, $J_{1,2}$ 5.0, $J_{2,3}$

7.6, H-2), 4.31 (1 H, dd, $J_{3,4}$ 3.4, H-4), 4.60 (1 H, dd, $J_{2,3}$, $J_{3,4}$, H-3), 5.52 (1 H, d, $J_{1,2}$, H-1); MALDI-TOF: *m/z* 396 $(M + Na)^+$, ($C_{14}H_{19}N_3O_9$ requires *m/z* 373).

2. Pyridine / acetic anhydride procedure[3]

A solution of the unprotected sugar (**15**) (4.0 g, 19.5 mmol), anhydrous pyridine (40 ml) and acetic anhydride (20 ml) was stirred at room temperature overnight. The reaction mixture was coevaporated with toluene and taken up in DCM. The resulting solution was washed with dilute HCl and $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated under reduced pressure to give 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranose (**31**) as a syrup (6.95 g, 95%). Spectroscopic data were identical to those reported above.[15]

2,3,4-Tri-*O*-Acetyl-6-azido-6-deoxy-α-D-galactopyranosyl bromide (32)[16]



1. Using the protected sugar (31)[3]

A solution of protected sugar (**31**) (4.96 g, 13.3 mmol) was dissolved in anhydrous DCM (100 ml) and cooled (0°C) before a solution of 45% HBr in acetic acid (30 ml) was slowly added. The reaction mixture was stirred under nitrogen until TLC [pet. ether-ethyl acetate (3:2)] showed that the reaction to be complete (approx. 3 h). The solution was then diluted with toluene and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; pet. ether - EtOAc, 3:1-1:1) gave a mixture, including *2,3,4-tri-O-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl bromide* (**32**) as a syrup (2.7 g, 50%) $\delta_H(CDCl_3)$: 1.98 (3 H, s, AcO), 2.01 (3 H, s, AcO), 2.13 (3 H, s, AcO), 3.25 (1 H, dd, $J_{5,6,6'}$ 5.5, $J_{6,6'}$ 7.3, H-6/6'), 3.47 (1 H, dd, $J_{5,6,6'}$ 5.5, $J_{6,6'}$ 7.3, H-6/6'), 4.37 (1 H, dt, $J_{4,5}$ 1.2, $J_{5,6,6'}$, H-5), 5.03 (1 H, dd, $J_{1,2}$ 3.9, $J_{2,3}$ 6.6, H-2), 5.38 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 3.3, H-3), 5.47 (1 H, dd, $J_{3,4}$, $J_{4,5}$, H-4), 6.68 (1 H, d, $J_{1,2}$, H-1); $\delta_C(CDCl_3)$: 20.5, 20.6, 49.9, 67.65, 67.7, 67.9, 72.3, 87.9, 169.8,

170.0, 170.2.

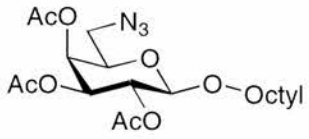
2. Using the free sugar (15) - 'one pot synthesis'[17]

The free sugar (**15**) (2.0 g, 9.75 mmol) was stirred in anhydrous acetic anhydride (10 ml) and 45% HBr in acetic acid (2 ml) at room temperature until TLC [EtOAc-pet.ether (1:1)] showed that acetylation was complete (approx. 4 h). Once complete, more 45% HBr in acetic acid (10 ml) was added and the mixture was stirred until reaction was shown to be complete by TLC [EtOAc - pet. ether (1:1)] (approx. 6-8 h). The resulting solution was then diluted with toluene and concentrated under reduced pressure (this was repeated several times) to give a mixture of compounds, including *2,3,4-tri-O-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl bromide* (**32**) as a syrup (2.3 g, 54%). Spectroscopic data were as reported above.

3. Titanium Bromide[18]

1,2,3,4-Tetra-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranose (**31**) (502 mg, 1.35 mmol) was dissolved in DCM:EtOAc (9:1) (5 ml) and cooled (0^0C) before titanium tetrabromide (593 mg, 1.61 mmol) was slowly added. The solution was stirred at room temperature until TLC [Hexane:EtOAc, (2:1)] showed the reaction to be complete (approx. 48 h). Once complete sodium acetate (1.2 g) was added and the solution was stirred for 1 h. The solution was then diluted with DCM and washed with $NaHCO_3$, water, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; hexane-EtOAc, 10-1—1:1) gave the *2,3,4-tri-O-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl bromide* (**32**) (480 mg, 90%) as a syrup. Spectroscopic data were as reported above

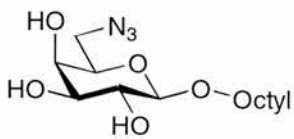
Octyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranoside (33)



Compound (**33**) was prepared using an standard Helferich glycosylation procedure.[4,5] The impure glycosyl bromide (**32**) (1.03 g, 2.62 mmol) was stirred at room

temperature under nitrogen in anhydrous acetonitrile (4.5 ml), octanol (2.7 ml) and Drierite (1.06 g). After 2 h mercuric cyanide (0.78 g, 1.2 mol equiv., 3.14 mmol) and mercuric bromide (0.09 g, 0.1 mol equiv., 0.262 mmol) were added, and the solution was stirred under nitrogen at room temperature overnight. Once TLC [pet. ether-EtOAc (4:1)] showed that the reaction had gone to completion, the inorganic salts were removed by filtration and the resulting solution was concentrated. Column chromatography (silica gel; pet. ether-EtOAc, 20:1→ 4:1) gave *octyl 2,3,4-tri-O-acetyl-6-azido-6-deoxy-β-D-galactopyranoside* (**33**) as a syrup (858 mg, 74 %); $[\alpha]_D$ – 8.58 (*c* 0.98, $CHCl_3$); $\delta_H(CDCl_3)$: 0.85 (3 H, t, $C_7H_{14}CH_3$), 1.25 (10 H, m, $(CH_2)_5CH_3$), 1.55 (2 H, m, OCH_2CH_2), 1.96 (3 H, s, AcO), 2.01 (3 H, s, AcO), 2.14 (3 H, s, AcO), 3.10 (1 H, dd, $J_{5,6/6'}$ 3.9, $J_{6,6'}$ 9.0, H-6/6'), 3.50 (2 H, m, OCH_2, H-6/6'), 3.80 (1 H, m, H-5), 3.91 (1 H, m, OCH_2), 4.48 (1 H, d, $J_{1,2}$ 7.8, H-1), 5.02 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 3.3, H-3), 5.22 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.31 (1 H, dd, $J_{3,4}$, $J_{4,5}$ 0.9, H-4); $\delta_C(CDCl_3)$: 13.9, 20.5, 20.6, 20.7, 22.6, 25.8, 29.2, 29.3, 31.7, 50.6, 68.1, 68.9, 70.3, 70.9, 72.9, 101.4, 169.5, 170.3, 170.5; CI-MS: *m/z* 466 $(M + H)^+$. (Found: $[M + H]^+$ 444.2352. $C_{20}H_{34}N_3O_8$ requires *m/z* 444.23467).

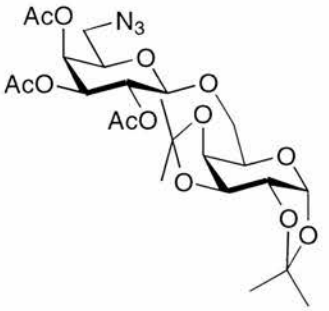
Octyl 6-azido-6-deoxy-β-D-galactopyranoside (16)[6]



Compound (**16**) was prepared using a standard methoxide deprotection.[7] A solution of octyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranoside (**33**) (100 mg, 0.26 mmol) in methanol (2 ml) containing sodium metal (trace) was stirred at room temperature for 2 h. Once TLC [DCM-MeOH (6:1)] showed the reaction to be complete, the resulting solution was neutralised with a Amberlite IR120 (H^+) ion exchange resin. The resulting solution was filtered and concentrated to give *octyl 6-azido-6-deoxy-β-D-galactopyranoside* (**16**) as a syrup (66 mg, 92 %); $\delta_H(CDCl_3)$: 0.86 (3 H, t, CH_3), 1.24 (10 H, m, 5 x CH_2), 1.63 (2 H, m, OCH_2CH_2), 3.29 (1 H, dd, $J_{5,6/6'}$ 4.0, $J_{6,6'}$ 9.0, H-6/6'), 3.53 (1 H, m, OCH_2), 3.65 (3 H, m, H-2,3,5), 3.74 (1 H,

dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.91 (1 H, m, H-4), 3.94 (1 H, m, OCH_2), 4.26 (1 H, d, $J_{1,2}$ 7.4, H-1); $\delta_C(CDCl_3)$: 13.9, 22.5, 25.9, 29.1, 29.2, 29.4, 31.6, 50.8 (C-6), 68.9, 70.2, 71.5, 72.9, 74.1, 102.7 (C-1); in accord with previous results (**page 42**).

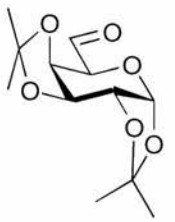
2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl-(1-6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (34)



2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl-(1-6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**34**) was prepared using an standard Helferich glycosylation procedure.[4,5] 2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl bromide (**32**) (324 mg, 0.80 mmol) was stirred at room temperature under nitrogen in anhydrous DCM (10 ml) containing 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**29**) (270 mg, 1.04 mmol) and Drierite (500 mg). After 2 h mercuric cyanide (242 mg, 0.96 mmol) and mercuric bromide (29 mg, 0.08 mmol) were added, and the solution was stirred under nitrogen at room temperature overnight. Once TLC [pet. ether-EtOAc (4:1)] showed that the reaction had gone to completion, the inorganic salts were removed by filtration and the resulting solution was concentrated. Column chromatography (silica gel; pet. ether-EtOAc, 20:1→ 4:1) gave the *2,3,4-tri-O-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl-(1-6)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose* (**34**) as a white solid (398 mg, 87 %); mp 67-69 ^{O}C (DCM); $[\alpha]_D$ –45.2 (*c* 2.14, $CHCl_3$); (Found: C, 50.26; H, 6.15; N, 6.96 %. $C_{24}H_{35}N_3O_{13}$ requires C, 50.26; H, 6.15; N, 7.33 %); ν_{max}/cm^{-1} 2098 (N_3); $\delta_H(CDCl_3)$: 1.31 (3 H, s, CH_3), 1.4 (3 H, s, CH_3), 1.55 (3 H, s, CH_3), 2.00 (3 H, s, OAc), 2.01(3 H, s, OAc), 2.15 (3 H, s, OAc), 3.23 (1 H, dd, $J_{5b,6b,6b'}$ 7.5, $J_{6b,6b'}$ 12.6,

H-6b/6b'), 3.53 1 H, dd, $J_{5b,6b,6b'}$ 7.5, $J_{6b,6b'}$ 12.6, H-6b/6b'), 3.72 (1 H, dd, $J_{5a,6a,6a'}$ 7.5, $J_{6a,6a'}$ 11.4, H-6a/6a'), 3.82 (1 H, dd, $J_{4b,5b}$ 0.9, $J_{5b,6b,6b'}$, H-5b), 3.94 (1 H, dd, $J_{4a,5a}$ 1.8, $J_{5a,6a,6a'}$, H-5a), 4.05 (1 H, dd, $J_{5a,6a,6a'}$, $J_{6a,6a'}$, H-6a/6a'), 4.16 (1 H, dd, $J_{3a,4a}$ 2.1, $J_{4a,5a}$, H-4a), 4.29 (1 H, dd, $J_{1a,2a}$ 5.1, $J_{2a,3a}$ 5.0, H-2a), 4.58 (1 H, dd, $J_{2a,3a}$, $J_{3a,4a}$, H-3a), 4.62 (1 H, d, $J_{1b,2b}$ 7.8, H-1b), 5.01 (1 H, dd, $J_{2b,3b}$ 8.1, $J_{3b,4b}$ 2.4, H-3b), 5.22 (1 H, dd, $J_{1b,2b}$, $J_{2b,3b}$, H-2b), 5.34 (1 H, dd, $J_{3b,4b}$, $J_{4b,5b}$, H-4b), 5.50 (1 H, d, $J_{1a,2a}$, H-1a); $\delta_C(CDCl_3)$: 20.5, 20.6, 20.7, 24.3, 25.0, 25.9, 26.0, 50.5, 68.0, 68.6, 69.7, 70.3, 70.5, 70.7, 70.8, 71.35, 72.7, 96.3, 102.1, 108.7, 109.5, 169.9, 170.3, 170.4; MALDI-TOF: *m/z* 596 $(M + Na)^+$, ($C_{24}H_{35}N_3O_{13}Na$ requires *m/z* 596).

1,2:3,4-Di-*O*-isopropylidene-α-D-galacto-6-ulose (35)[19]



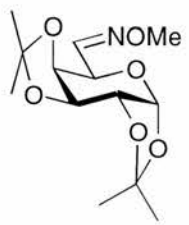
The title compound (**35**) was prepared using a Swern oxidation procedure.[20] A solution of DMSO (1.08 ml, 15.28 mmol) was stirred in anhydrous DCM (15 ml) and cooled (-65°C) before a solution of TFAA (1.62 ml, 11.46 mmol) in anhydrous DCM (10 ml) was slowly added with continuous stirring under nitrogen (approx. 10 min). A solution of alcohol (**29**) (2 g, 7.68 mmol) in anhydrous DCM (20 ml) was slowly added (approx. 1 h). The reaction mixture was allowed to warm up to room temperature (approx. 1 h). TEA (15 ml) was slowly added (approx. 0.5 h). The solution was washed with water, extracted with DCM, dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; pet. ether - EtOAc, 10:1→ 5:2) gave two compounds; starting material (**29**) (400 mg, 20 %) and the *title compound* (**35**) (800 mg, 40%) as a syrup; $[\alpha]_D$ –90.0 (*c* 1.1, $CHCl_3$); ν_{max}/cm^{-1} 1678 (CO); $\delta_H(CDCl_3)$: 1.30 (3 H, s, C*H*$_3$), 1.33 (3 H, s, C*H*$_3$), 1.42 (3 H, s, C*H*$_3$), 1.49 (3 H, s, C*H*$_3$), 4.17 (1 H, d, $J_{4,5}$ 2.5, H-5), 4.37 (1 H, dd, $J_{3,4}$ 2.5, $J_{4,5}$, H-4), 4.57 (1 H, dd, $J_{1,2}$ 5.0, $J_{2,3}$ 2.5, H-2), 4.63 (1 H, dd, $J_{2,3}$, $J_{3,4}$, H-3), 5.28 (1 H, d, $J_{5,6}$ 5.0, H-6), 5.65 (1 H, $J_{1,2}$ 5.0, H-1); $\delta_C(CDCl_3)$: 24.2, 24.8, 25.8, 25.9, 70.4, 70.5,

71.7, 73.2, 97.8, 108.9, 109.9, 200.2; MALDI-TOF: *m/z* 281 $(M + Na)^+$, ($C_{12}H_{18}O_6Na$ requires *m/z* 281)

1,2:3,4-Di-*O*-isopropylidene-α-D-galacto-6-ulose (35)[19]

A solution of sugar (**29**) (1.4 g, 5.37 mmol), nicotinium dichromate (3.64 g, 16.14 mmol) and pyridine (2.5 ml, 32.27 mmol) was dissolved in toluene (36 ml) and stirred at room temperature. The solution was then heated (approx. 80°C) until TLC [toluene – EtOAc, (2:1)] showed the reaction to be complete (approx. 1 h). The reaction mixture was filtered through Celite and the resulting solution was concentrated under reduced pressure. Column chromatography (silica gel; toluene - EtOAc, 10:1→ 2:1) gave two compounds; starting material (**29**) (532 mg, 38%) and the *title compound* (35) (646 mg, 47%) as a syrup. Spectroscopic data were identical to those reported above.

1,2:3,4-Di-*O*-isopropylidene-α-D-galacto-6-ulose-*O*-methyl oxime (36)



A solution of the aldehyde (**35**) (159 mg, 0.62 mmol) and *N*-methoxyimine hydrochloride (257 mg, 3.08 mmol) was dissolved in pyridine (5 ml) and stirred at room temperature overnight. The resulting solution was coevaporated with toluene under reduced pressure to give the *oxime* as a syrup. Column chromatography (silica gel; pet. ether - EtOAc, 10:1→ 5:2) gave *oxime* (**36**) as a syrup (88 mg, 50 %); $[\alpha]_D$ – 113.3 (*c* 1.11, $CHCl_3$); ν_{max}/cm^{-1} 1750 (CN); $\delta_H(CDCl_3)$: 1.30 (6 H, s, 2 x CH_3), 1.41 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 3.85 (3 H, s, OMe (*E* or *Z* isomer)), 3.86 (3 H, s, OMe (*E* or *Z* isomer)), 4.20-4.90 (4 H, H-2, 3, 4, 5), 5.52 (1 H, d, $J_{1,2}$ 4.5, H-1), 6.71 (1 H, sd, H-6 *E/Z* isomer), 6.72 (1 H, d, $J_{5,6}$ 4.5, H-6, *E/Z* isomer), 7.4 (1 H, d, $J_{5,6}$ 4.5, H-6, *E/Z* isomer); $\delta_C(CDCl_3)$: 24.3, 24.9, 25.9, 26.0, 61.7, 63.7, 66.6, 70.3, 70.7,

73.3, 96.3, 108.9, 109.8, 152.0; CI-MS: *m/z* 288 $(M + H)^+$. (Found: $[M + H]^+$ 288.14471. $C_{13}H_{22}NO_6$ requires *m/z* 288.14479).

3.3 References

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Chapter 4

Verotoxin

4.1 The Rediscovery of Shiga Toxin and its Role in Clinical Disease

Kiyoshi Shiga identified the etiological agent of epidemic dysentery (Japan, 1898) and named it *Bacillus dysenteriae*.[1-3] The genus name was changed to *Shigella*, in honour of its discoverer, and *S. dysenteriae* type 1 is often referred to as Shiga's bacillus.[1] A major dysentery epidemic began in Guatemala and Mexico in 1968, with a sharp increase of dysentery related deaths.[1] Dr. Leonardo Mata identified that *S. dysenteriae* type 1 was the cause of the outbreak and treatment was changed to antibiotics. Mortality rates soon plummeted from 20-25 %, similar figures to that of the epidemics of 1890's, to under 1 %.[1] In 1977, Keusch reported evidence for a cell surface "Shigella toxin" receptor.[1-2] Certain strains of *E. coli* produce a Vero cell cytotoxin, which subsequently became known as Verotoxin (VT).[1] *Shigella dysenteriae* produce Shiga toxin, whereas certain enterohemorrrhagic *Escherichia coli* (EHEC) produce Shiga-like toxins, SLTs. The SLTs can be divided into SLTs-I and SLTs-II, and the SLT-II edema variant (SLT-IIe or pig edema toxin).[1-2]

In 1982, a fast food restaurant in the USA was the cause of two outbreaks of hemorrhagic colitis diarrhoea, so-called hamburger disease, which was caused by a previously rare serotype of *E. coli* 0157:H7.[1-2] It was shown that this strain produced a VT-like cytotoxin and was neutralised by antibody to Shiga-like toxin.[1-2] There are two related serologically distinct Shiga-like toxins, each encoded by a separate transforming phage designated SLT-1 (VT-1) and SLT-II (VT-2), (**Table 1**).[4]

The verotoxin family includes VT1, VT2 and VT2c which all bind to globotriaosylceramide [Gb_3; Galα(1-4)Galβ(1-4)Glcβceramide] (**38**) (**Figure 31**).[5] All of these toxins are implicated in the microvascular pathologies hemorrhagic colitis and hemolytic uremic syndrome (HUS). The remaining verotoxin is VT2e (pig edema disease toxin), which binds to both globotriaosyl ceramide (**38**) and also globotetraosyl ceramide [Gb_4; GalNAcβ1-3-Galα1-4Galβ1-4Glc-ceramide] (**39**) (**Figure 31**).[5-6]

Microbial Source	Gene Designation	Toxin Name	Prior Name
S. dysenteriae type 1	Stx	Shiga toxin (Stx)	Shiga toxin
E.coli	stx 1	Shiga toxin 1 (Stx 1)	Shiga-like toxin I Verotoxin 1
	stx 2	Shiga toxin 2 (Stx 2)	Shiga-like toxin II Verotoxin 2
	stx 2c*	Shiga toxin 2c (Stx 2c)	Shiga-like toxin IIc Verotoxin 2c
	stx 2d*	Shiga toxin 2d (Stx 2d)	Shiga-like toxin IId Verotoxin 2d
	stx 2e**	Shiga toxin 2e (Stx 2e)	Shiga-like toxin IIe Verotoxin 2e

* human STx2 variant toxins

** porcine edema disease Stx2 variant toxin

Table 1: Nomenclature for the Shiga Family of Toxins.[1]

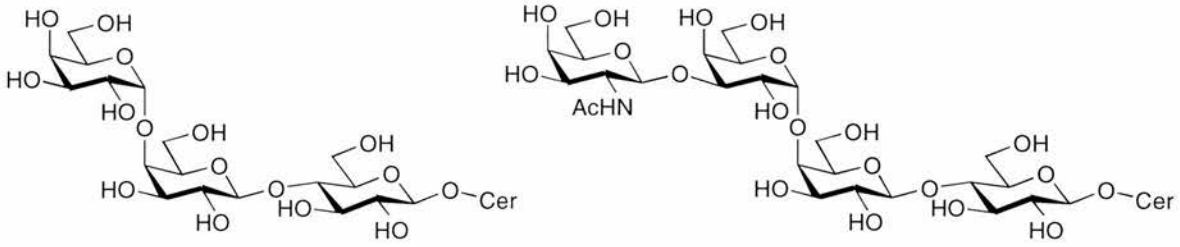


Figure 31: Chemical structures of Gb_3 (**38**) and Gb_4 (**39**).

4.1.1 Symptoms of Toxin Infection

The symptoms of any *S. dysenteriae* or EHEC outbreak are initially diarrhoea, but frequently due to EHEC progressing to hemorrhagic colitis (about 10 % of all cases) and further to HUS.[5-6] HUS is the major cause of renal failure in infected children (mortality rate is 2.7-5.7%) and is a direct result of SLT-induced kidney damage.[6] There are no conventional anti-microbial therapies available for HUS; therefore, there is a need for development of therapies for the treatment of *S. dysenteriae* and EHEC infection.[5-6]

4.1.2 *E.coli* 0157:H7 Toxin Outbreak in Wishaw

An *E. coli* 0157:H7 outbreak in Wishaw, central Scotland, started on Friday November 22 1996.[7] The first indications reported were for 9 people, of whom 8 had

consumed food from J. Barr and Son Butchers of Wishaw.[7] As shown by the epidemic curve for this outbreak, the number of cases increased dramatically (**Figure 32**).

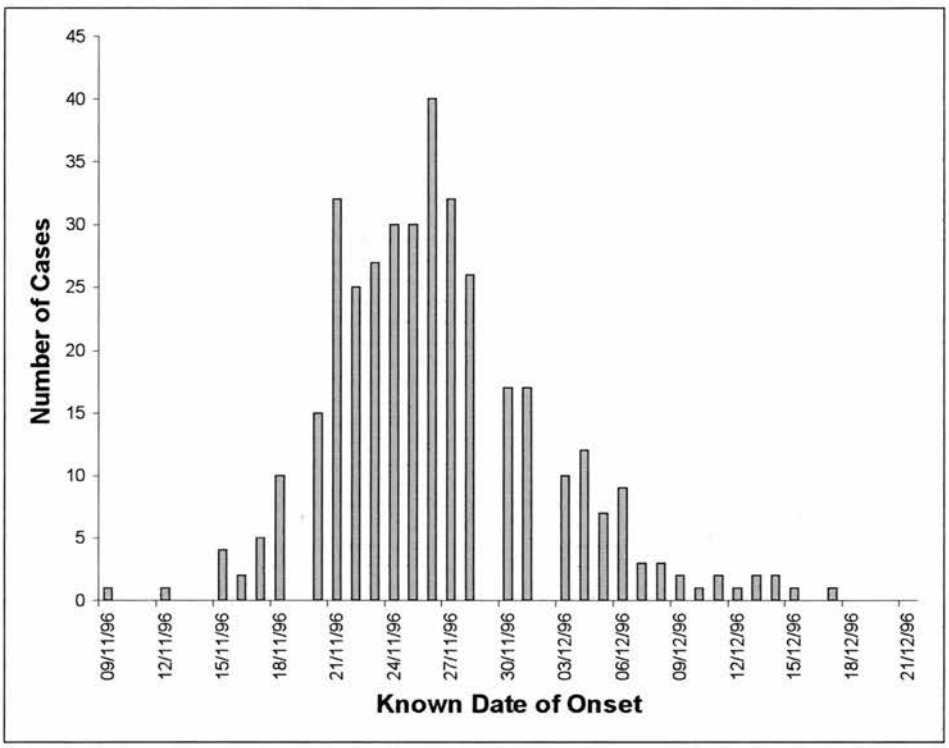


Figure 32: Epidermic curve for the *E.coli* outbreak in Wishaw (1996).[7]

Epidemiological and microbiological evidence for the outbreak indicates that there were several separate but related incidents arising from a lunch (attended by approx. 100 people) held in the Wishaw Church hall. Individuals from the outbreak had isolates of *E. coli* 0157 belonging to phage type 2 that possessed the verocytotoxin-encoding gene.[7] There were a total of 969 people reported to a clinic in Wishaw with diarrhoea, 127 people were hospitalized and 13 required dialysis.[7] Out of all the cases reported, 27 people were diagnosed as having either hemolytic uraemic syndrome or thrombotic thrombocytopaenia purpura.[7] There were 20 adult deaths associated with this particular outbreak (the highest number of deaths associated with an outbreak of *E. coli* 0157 infection in the world), of which 8 had attended the luncheon at the town hall. The age range of the adults who died was 69 to 93 years.[7]

Outbreaks across the UK have been associated with the consumption of a variety of foods including minced beef, milk, yogurt, cheese and water, with most of these

cases affecting fewer than 10 people.[7] Prior to the outbreak in Wishaw, the largest reported case was in West Lothian in 1994.[7] This was associated with the consumption of contaminated pasteurized milk, where more than 100 people were affected and one child died.[7]

4.1.3 Where Verotoxin can be Found

A wide range of animals, both wild and domestic, can carry the *E. coli* 0157 bacterium. The rumen and intestines of cattle and sheep are generally accepted as the main reservoir of the organism.[7] The organism can exist in animal manure and slurry, and is a possible source of contamination in the slaughter house environment.[7] It is also possible for animal to animal cross contamination to occur. The organism can survive frozen storage, but is killed by heating thoroughly, a classic situation for bacteria associated with food poisoning.[7]

4.1.4 General Shiga Toxin Family Structure and Mechanism of Action

The Shiga family belong to the A-B class of bacterial toxins, which all have a bipartite structure, although their amino-acid sequence and pathogenic mechanisms differ.[5,6] These structures consist of an enzymatic A subunit associated with a B oligomer which binds to specific cell-surface receptors.[5,6] Verotoxin was demonstrated to be a heterodimeric protein consisting of an A subunit that inhibited protein synthesis in a cell free system, and a pentamer of 5 B subunits that mediate binding of the toxin to sensitive tissues culture cells.[5,6]

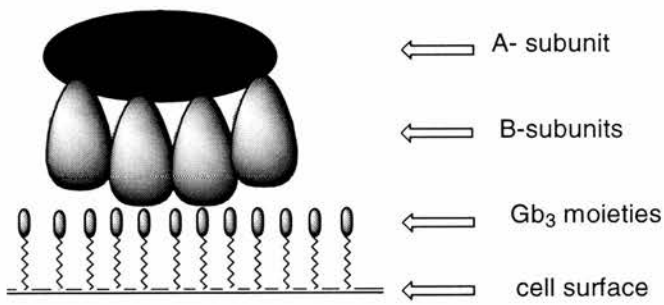


Figure 33: AB_5 structure

Toxin uptake is known to proceed via receptor mediated endocytosis at coated pits.[8] Vesicles containing the toxin are then transported to the Golgi region, and in

retrograde fashion to the endoplasmic reticulum (ER).[8] In the ER the enzymatically active A subunit is released, gaining access to the cytoplasm, where it catalytically and irreversibly inactivates ribosomal protein synthesis.[8] The specific mechanism is still unknown.[8]

4.1.5 Composition of Subunit's of Verotoxin

The SLTs consist of AB_5 protein structures.[6,9-15] The A unit which is the enzymatically active component, is approximately 32 kDa in size, binds non-covalently to the B-5 component on the opposite face to the cell surface binding.[6,9,12-15] In SLTs, the A-subunit is an *N*-glycosidase that inhibits protein synthesis by specifically removing the adenine base at position 4324 of the 28S rRNA.[6,8,9] The B-subunit is composed of five identical units, each approximately 7.5 kDa in size, and is responsible for the binding of the protein to its globo-series glycosphingolipid receptor, globotriaosyl ceramide (Gb_3).[6,12-15] The B-subunit monomer contains 69 amino acid residues and has a typical oligomer-binding (OB) fold that consists of a six-stranded antiparallel β-barrel capped by an α-helix.[6,9,12-15]

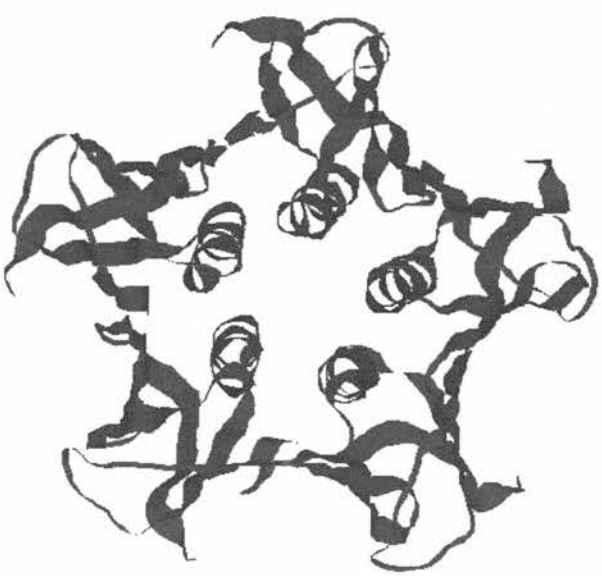


Figure 34: B-subunit indicating α-helixs and β-sheets.

Interaction of the three-stranded β-sheets of neighbouring monomers across the subunit interface forms six-stranded sheets, whereas along the 5-fold axis the five helixes line a central pore (**Figure 34**).[6,9,12] The central pore of the B-pentamer consists of five Trp 34 residues.[6,9] The absence of the A-subunit causes no

structurally deformities to the B-subunits, but the protein is catalytically inactive.[6] Gb_3 is the functional receptor of the SLT family. Cells which express Gb_3 on their cell surface are susceptible to SLTs, whereas cells, which do not express this glycolipid on their surface are resistant to these toxins.[6,12] The crystal structure of SLT-I B pentamer with a complexed Gb_3 analogue shows 3 distinct carbohydrate binding sites per B-subunit monomer (**Figure 35**).[6]

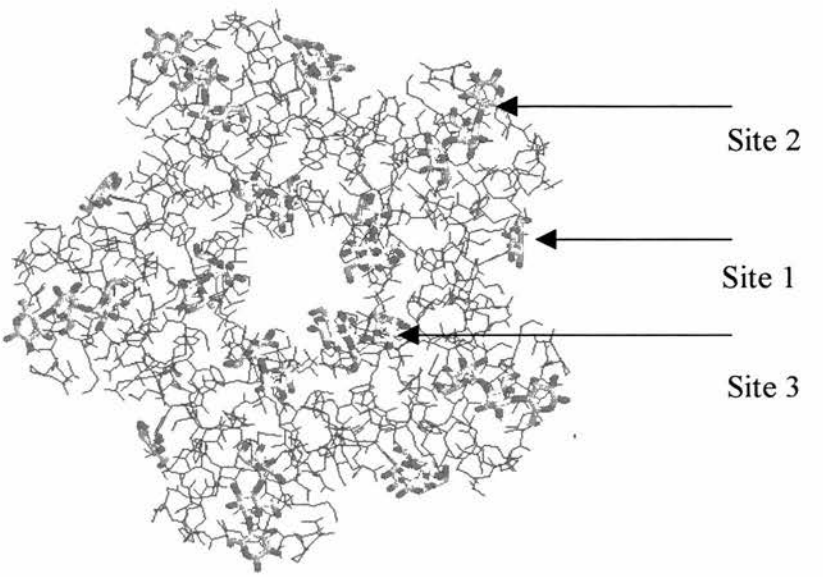


Figure 35: Indicating 3-binding sites per B-subunit.

All of the Gb_3 binding sites are located on the same flat face of the B-component, opposite to the binding site for the A-subunit (**Figure 36**).[6]

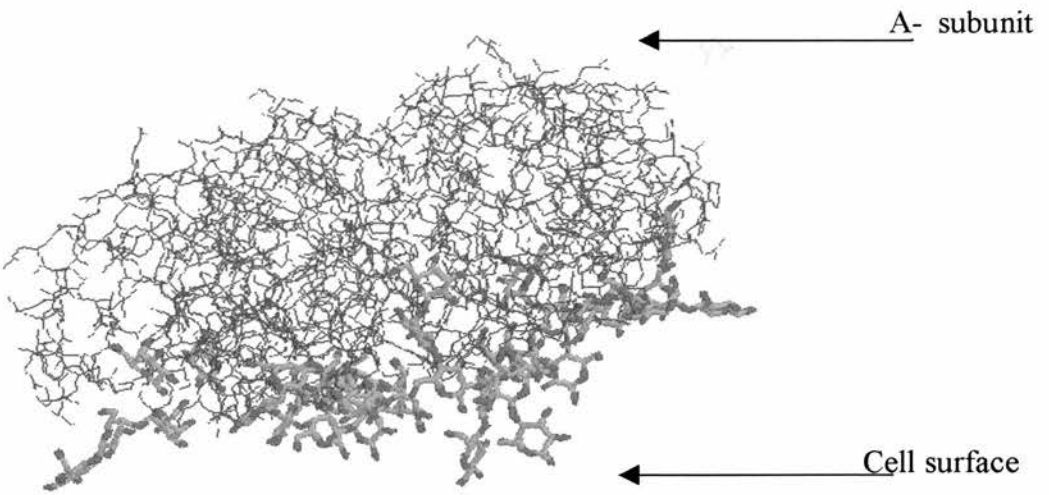


Figure 36: Indicating Gb_3 binding on same side, opposite from A-subunit.

The Gb_3 trisaccharide binds to the three binding sites separately, with no interactions between sites indicating that there is no co-operativity between these sites.[6]

4.2 Binding Affinity of Verotoxin for Gb_3.

Free Gb_3 trisaccharide binds very weakly (K_d 10^{-3} M) to verotoxin compared to the lipid bound Gb_3 (K_d 10^{-9} M).[6] The minimum structural unit for recognition is the galabiose disaccharide [Galα(1-4)Gal].[5-6,14]

It was found that amino acid substitution (Asp-17-Glu, Gly-62-Thr, Trp-34-Ala) in the B-pentamer disrupted and significantly reduced the binding capacity of Gb_3 with verotoxin.[12] The cytotoxicity was also reduced with these changes.[12]

Ligand protein contacts are summarised in the following sub-sections, and in **Tables 2** and **3**.

4.2.1 Binding Site 1 of Verotoxin.

Sugar – protein interactions in site 1 are located within the β3 and β4 strands, and loops β2-β3 and β5-β6.[6,9,12] Mutagenesis of Asp 17 to Asn has a great effect on the binding of the trisaccharide.[6] The main interactions in site 1 are hydrophobic interactions of Phe 30, which stacks with Gal 2.[6,12] The Phe 30 residue is extremely important in binding of Gb_3, which is supported by data for the Phe-30-Ala mutant.[6,9] This shows reduced affinity towards the trisaccharide (4 fold), together with 10^5-fold reduction in cytotoxicity.[6,9] Site 1 is situated in the cleft between the side of the phenyl ring of Phe-30, opposite site 2, and the side chain of Asp-17.[6,12] The Galα1-4Gal moiety fits snugly into the crevice lined by Phe30, Thr21 and Asn15 in site 1. Gb_3 and VT1 has numerous hydrogen bonds located within site 1: Thr21 OH to GalαO6, Galβ6OH to Asp17, Galα6OH H-bond to the side chain of Glu28, Galα2OH H-bond to Asp17, Galα4OH to Lys13 and Galβ2OH to Gly60.[6]

4.2.2 Binding Site 2 of Verotoxin.

This binding site is located parallel to the protein surface, which allows all three sugar moieties to interact with the protein.[6,12] Site 2 is situated in a crevice behind the phenyl ring of the Phe-30 residue and shows only weak van der Waal interactions.[6,12]

There is no aromatic stacking associated within site 2, therefore binding is dominated by numerous hydrogen bonds made by Gal1.[6,12] The protein components which constitute this binding site are loops β2-β3, β4-α helix and β5-β6 from a neighbouring subunit.[6,9,12]

4.2.3 Binding Site 3 of Verotoxin.

This site contains the fewest number of contacts located, due to its orientation (i.e. perpendicular) to the cell surface, therefore has the lowest affinity for Gb_3 binding.[6] The amino acids responsible for ligand binding are situated at the *N*-terminus of the α-helix and around the central pore in loop β2-β3.[6] The main contacts within this site are numerous hydrophobic interactions.[6,12] Site 3 is formed by the stacking of the hydrophobic face of Gal 1 against the indole ring of Trp-34, which points away from the B-unit central pore.[6,12]

Site	Sugar residue	Protein residue
Site 1	Gal 1	Leu29
	Gal 2	Phe30[a]
	Glc	Phe30
Site 2	Gal 1	Phe30, Thr31, Gly62, Ser64
	Gal 2	Thr1, Thr54, Ala56, Gly62
	Glc	Asn55
Site 3	Gal1	Trp34, Trp34[b]
	Gal2	Trp34[a]
[a] = Aromatic stacking interaction. [b] = Residue comes from the adjacent B- subunit		

Table 2: Hydrophobic interactions between Sugar and Protein.[6]

				B1[b]	B2[b]	B3[b]	B4[b]	B5[b]
Sugar atom		Protein atom		()	()	()	()	()
Site 1								
Gal 1	O4	Thr 21	OG1	3.39	3.12	2.66	2.90	2.54
Gal 1	O5	Thr 21	OG1	3.29	3.11	(3.94)	3.50	(4.01)
Gal 1	O6	Glu 28	OE2	(3.83)	3.07	2.93	2.90	3.43
Gal 1	O6	Gly 60	N	3.41	(3.88)	3.30	3.17	(3.86)
Gal 2	O3	Gly 60	O	2.73	C	C	2.79	C
Gal 2	O6	Asp 17	OD2	2.76	C	C	2.57	C
Site 2								
Gal 1	O2	Asp 16[d]	OD2	2.74	2.78	2.87	2.75	2.81
Gal 1	O3	Arg 33	NH2	2.99	3.00	3.13	2.95	2.91
Gal 1	O4	Asn 32	OD1	2.60	2.99	2.92	2.73	2.84
Gal 1	O4	Arg 33	NE	3.02	2.86	2.95	2.90	2.87
Gal 1	O4	Arg 33	NH2	3.30	3.09	3.01	3.12	3.02
Gal 1	O5	Phe 63	N	3.19	3.26	3.46	3.41	3.34
Gal 1	O6	Asn 32	N	3.19	3.18	3.31	3.30	3.36
Gal 1	O6	Asn 32	OD1	3.12	2.99	2.82	3.01	3.04
Gal 1	O6	Phe 63	O	3.04	2.73	3.06	2.69	2.74
Gal 2	O6	Asn 55	N	2.92	3.18	3.11	2.93	3.21
Gal 2	O6	Asn 55	OD1	3.30	3.23	3.28	3.41	3.38
Site 3								
Gal 1	O4	Asp 18[d]	OD1	2.98	3.12	2.97	3.07	3.15
Gal 1	O4	Asp 18[d]	OD2	2.99	2.85	2.80	2.94	2.95
Gal 1	O5	Trp 34	N	3.12	3.14	3.07	3.22	3.31
Gal 1	O6	Trp 34	N	3.09	3.07	3.11	3.16	3.21
Gal 1	O6	Asn 35	N	2.77	2.95	2.76	3.13	2.90

[a]Donor-acceptor distances less than 3.5 A are considered potential hydrogen bonds and the distances of >3.5 A are listed in parentheses. [b]Distance between potential hydrogen bonding partners in B-subunits B1-B5 of the first of the four B-pentamers. [c]Subunits B2, B3 and B5 have incomplete sugar models that lead to some missing hydrogen bonds. [d]These residues are part of a neighboring subunit.

Table 3: Potential Hydrogen Bonds in Binding Sites of Pentamer 1.[6]

4.3 Recent Synthesis of Gb_3 and Analogues thereof.

There has been numerous reported syntheses of Gb_3 trisaccharide and analogues there of.[16-21] Shimizu and co-workers have reported the total synthesis of ^{13}C-labelled Gb_3 trisaccharide.[22] A selection of Gb_3 and analogue syntheses are shown below.

4.3.1 Synthesis of Gb_3 Analogues by Crout and co-workers

Crout and co-workers synthesised the trisaccharide thioglycoside building block (**43**).[23] The trisaccharide is chemically stable and easy to derivatise. The thioglycoside (**43**) was converted to a benzyloxycarbonylamino-hexyl derivative (**44**). Once the amino ester had been prepared, straight forward hydrogenation produced the free amino-hexyl derivative (**45**), which was coupled to a solid support.[23]

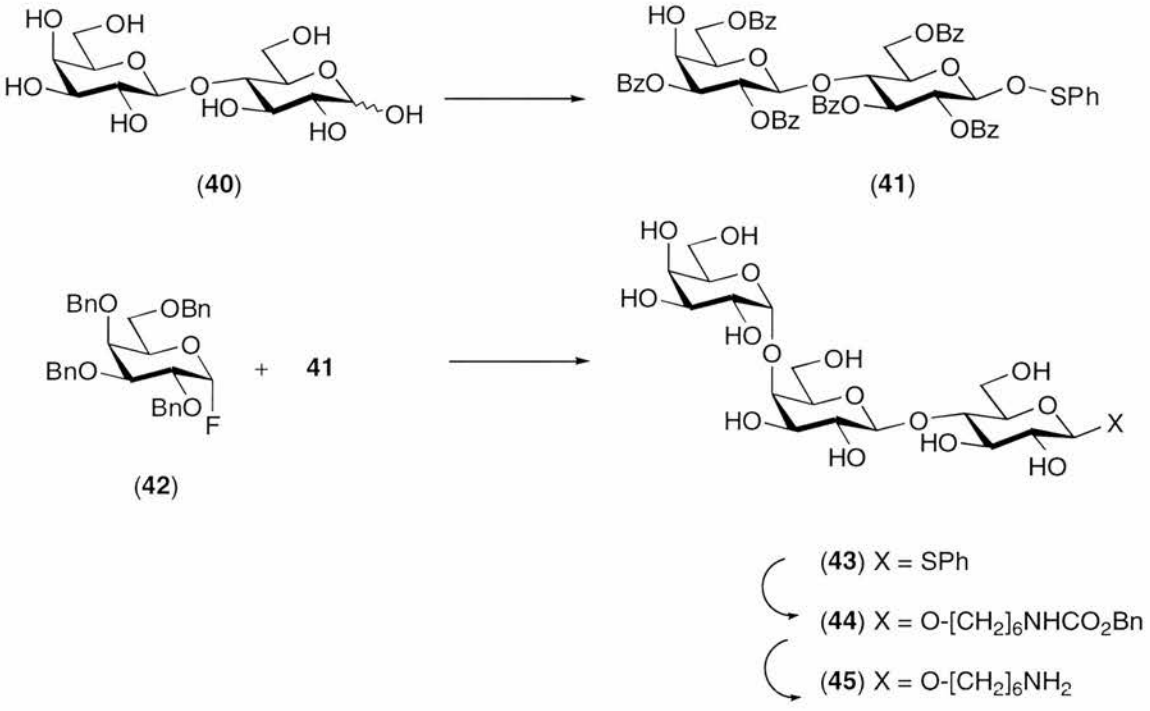


Figure 37: Amino-hexyl Gb_3 synthesis (**45**).[23]

4.3.2 Synthesis of Gb_3 Analogues by Magnusson and co-workers

Magnusson and co-workers have developed syntheses of numerous galabiose derivatives.[24] In the bound form HO-2' and HO-6 of galabiose are situated in close proximity to charged amino acid residues in the *Pseudomonas pillus* $PapG_{J96}$ protein and Verotoxin receptor binding sites.[24] The hydroxyl groups were replaced with amino and carboxyl groups in these specific sites. Figures **38, 39** and **40** indicate the building blocks for the synthesis of specific galabiose derivatives.[24]

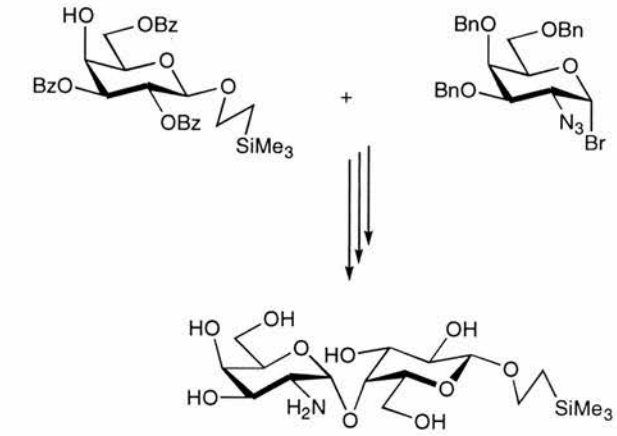


Figure 38: Glycosylation with 2'-amino sugar.[24]

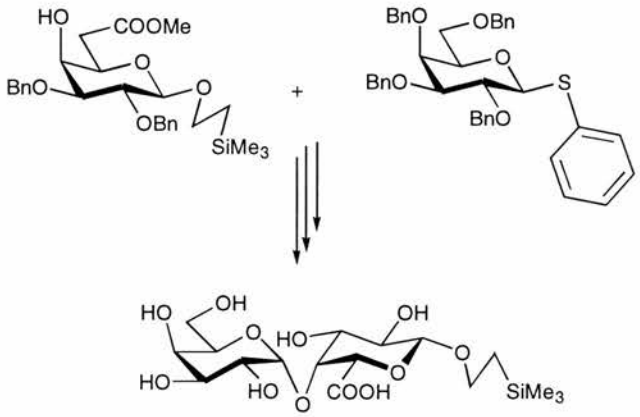


Figure 39: Glycosylation with 6-carboxy sugar.[24]

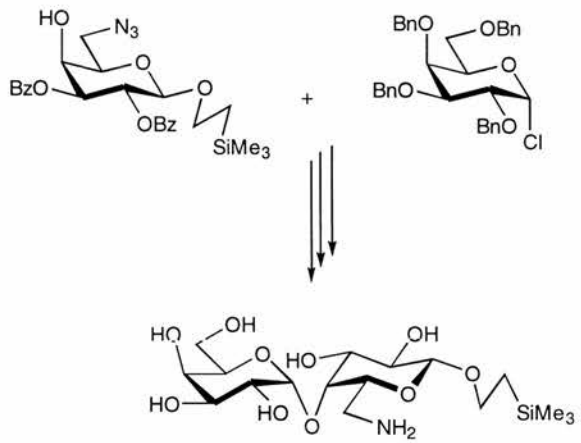


Figure 40: Glycosylation with 6-amino sugar.[24]

Inhibitory efficiency of the derivatised compounds were determined using an ELISA assay. A galabioside possessing a short linker was covalently bound to a microtiter plate, giving a so-called 'glycoplate' (**Figure 41**).[24] Derivatised compounds were

were serially diluted into the wells of the plates and purified $PapG_{J96}$ adhesin was added. ELISA using an anti-$PapG_{J96}$ antibody determined the amount of bound protein.[24]

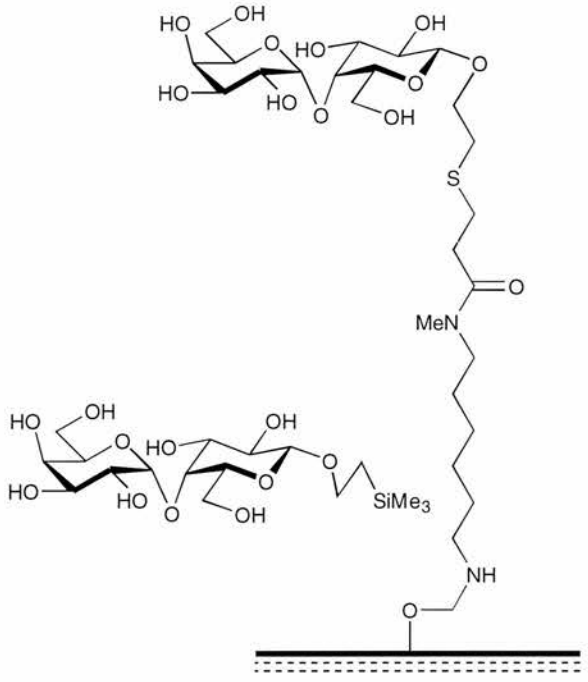


Figure 41: Gycoplate involved in $PapG_{J96}$ assay.[24]

The 2'-amino derivatised galabiose was approximately 50 % more effective than the parent galabiose compound in this assay.[24] The 6-amino compound only retained 13 % effectiveness and the 6-carboxy compound was ineffective as an inhibitor.[24] **Figure 42** (a) indicates the hydrogen bonding associated with galabiose and the protein. The postulated hydrogen-bonding pattern of 2'-amino galabiose is shown in **Figure 42** (b).[24] The salt bridge, which is formed in the 2'-amino galabiose, is the postulated reason for the increase in inhibition.[24]

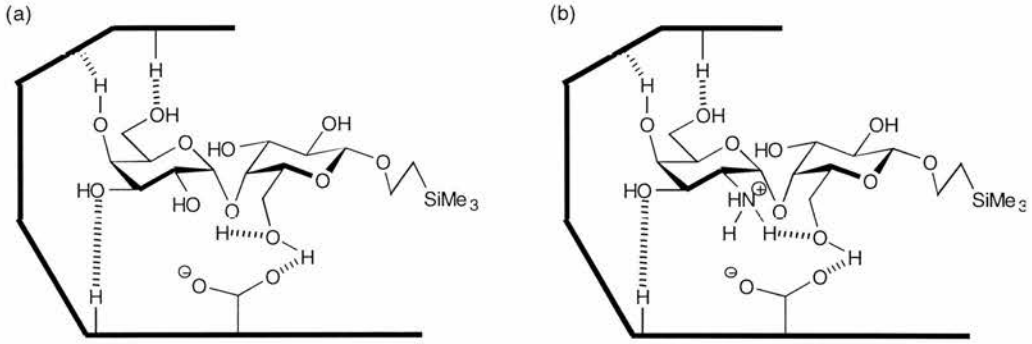


Figure 42: (a) H-bonding pattern of galabiose with $PapG_{J96}$,
(b) Postulated H-bonding pattern with $PapG_{J96}$.[24]

4.3.3 Synthesis of Gb_3 Analogues by Toone and co-workers

Toone and co-workers have synthesised *C*-glycosides with amino acid functionality in an attempt to increase binding affinity of a Gb_3 analogue. **Figure 43** shows the key intermediate in the synthesis of the Gb_3 analogues.[25] No binding data has been reported to date.

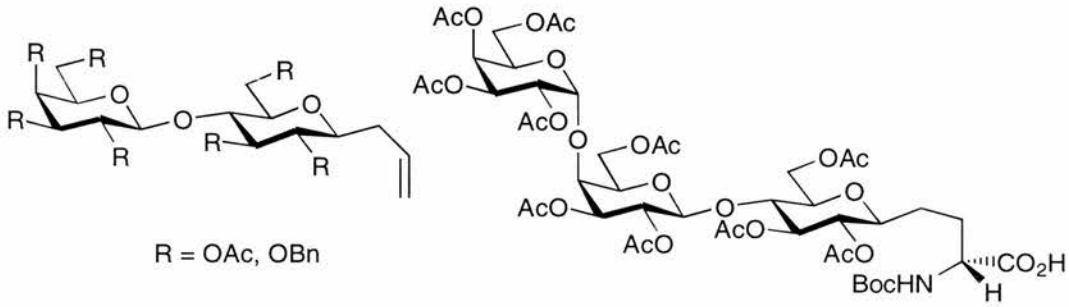


Figure 43: Shows the main building block (a) and the final Gb_3 analogue (b).[25]

4.4 Synthesis of Multi-valent Gb_3 Constructs.

Various research groups have developed multi-valent Gb_3 ligands for toxin inhibition because of the moderate binding affinity of Gb_3.

4.4.1 Synthesis of Gb_3 Analogues by Arya and co-workers

Many research groups have modified their strategy to synthesise multivalent Gb_3 fragments to gain higher binding affinity towards VT. Arya and co-workers decided to use peptides and α-linked galactoside moieties to try and enhance binding affinity to VT-1.[26] The concept of using only one carbohydrate building block (acetylated galactose) reduces the number of chemical manipulations required in synthesis. Peptide chemistry in solution or on solid-phase has been long established and is relatively straight forward. **Figure 44** shows a selection of glycopeptides synthesised during this research.[26] The binding data for the amino sugars was inconclusive.

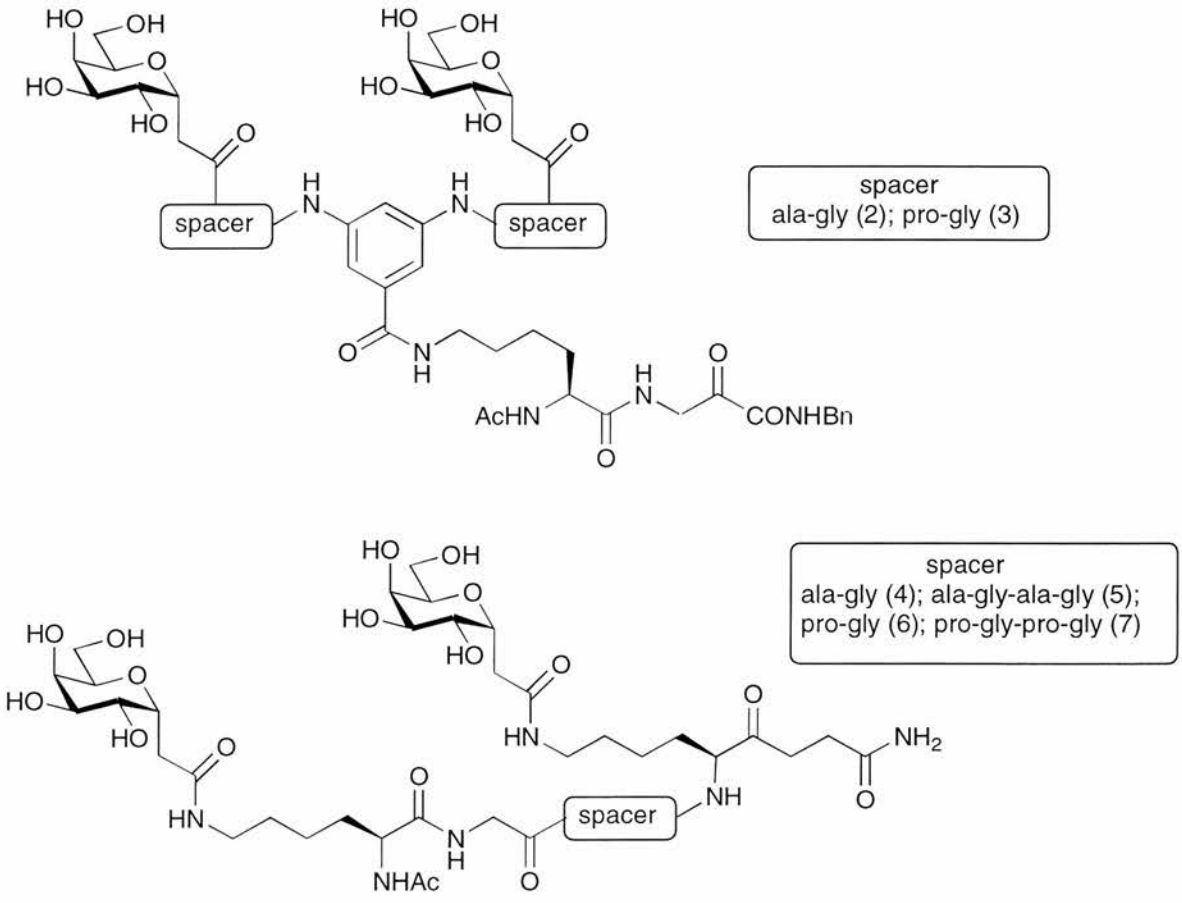


Figure 44: A selection synthesis of neo-glycopeptide.[26]

4.4.2 Synthesis of Multi-valent Gb_3 Analogues by Matsuoka and co-workers

Matsuoka and co-workers have synthesised an artificial ligand display system for VT.[27] This consists of clustering Gb_3 moieties together on a dendrimer core.[27] The support which Matsuoka used was a carbosilane dendrimer, which has been recently developed and found to have numerous unique properties: (1) simplicity of the synthetic process to extend the generation; (2) access to the polymer with defined molecular weight and a definite number of terminal functions which depend on the polymer generation; (3) neutral nature in contrast to the usual polyamine-type dendrimers; and (4) biological inertness.[27] **Figure 45** shows the retrosynthetic analysis of one of the polymer based compounds. Compound **A** showed some potency against VT1 and VT2 but no biological data has been reported.[27]

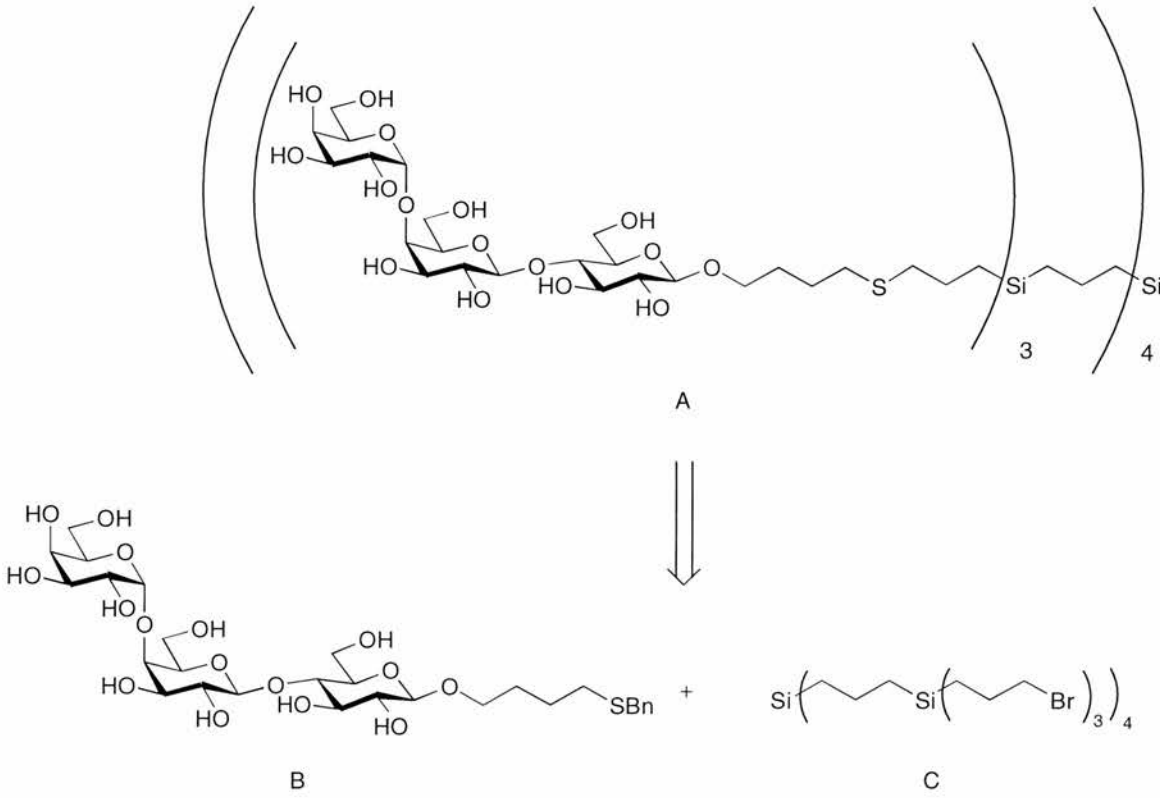


Figure 45: Retrosynthetic analysis of a Gb_3 carbosilane dendrimer.[32]

4.4.3 Synthesis of Multi-valent Gb_3 Analogues by Bundle and co-workers.[28]

The free energy of binding for Gb_3 methyl glycoside is moderate at 3.6 kcal mol^{-1} corresponding to a millimolar dissociation constant (K_d).[15] Bundle has developed a multivalent approach for inhibition of VT.[28] NMR studies have indicated that sites 1 and 2 are the most important binding sites for soluble Gb_3.[29,30] Bundle decided to tether divalent Gb_3 ligands to simultaneously occupy sites 1 and 2 (**Figure 46**).[28] The tethered compound (**Figure 46**), (K_d 10^{-5} M) had a 40 fold increase in binding over the univalent Gb_3 against VT-1.[28] This is still very poor compared to the affinity (K_d 10^{-9} M) of the B-pentamer for cells which express Gb_3.[28] Magnusson and co-workers have also used the tethered approach for increased binding, but with only modest success against Pap_{GJ96}.[31,32]

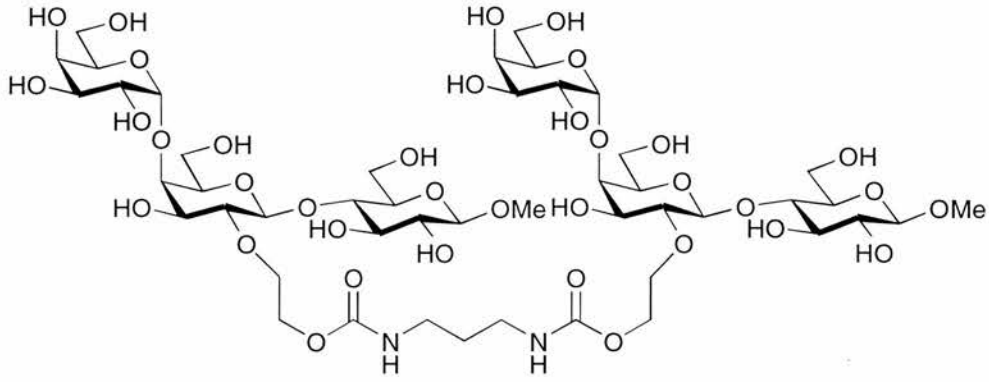


Figure 46: Structure of the divalent Gb_3 ligand.[28]

Bundle's next approach was to tether the bridged saccharides together to make a poly valent saccharide structure (STARFISH) containing 10 trisaccharides (**Figure 47**).[28] The central core of the starfish is a glucose molecule with 'arms' spanning the radius of the B-pentamer.[28] A million-fold increase in inhibition over the free trisaccharide was obtained with the starfish construct.[28] Comparison was also made between VT-I and VT-II. IC_{50} values were recorded against both toxins and the starfish moiety had IC_{50} of 4 x 10^{-10} M with VT-I and also had a 6 x 10^{-9} M with VT-II.[28] This also confirmed that the ligand for VT-II was the Gb_3 trisaccharide.[28]

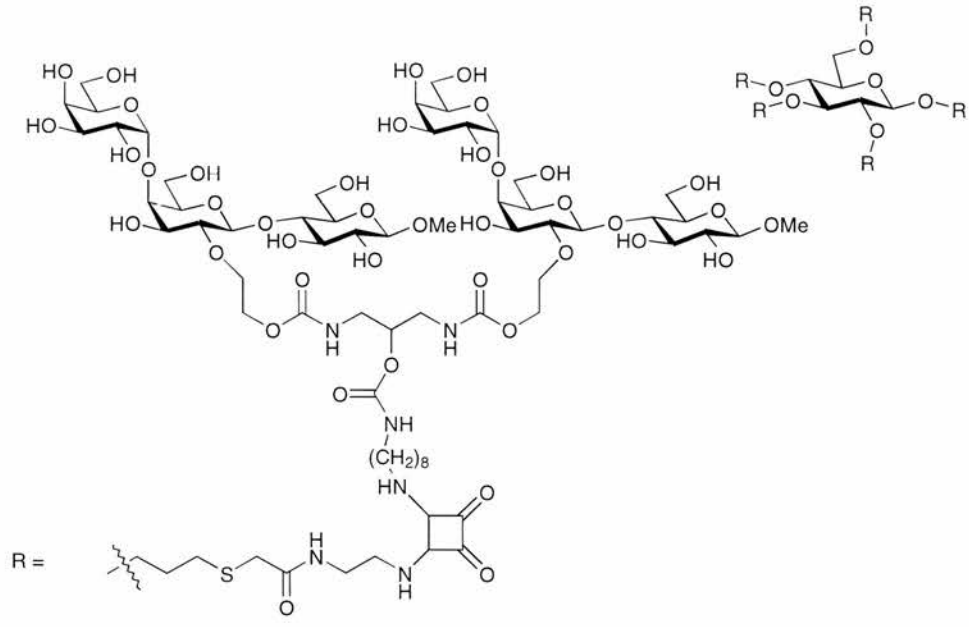
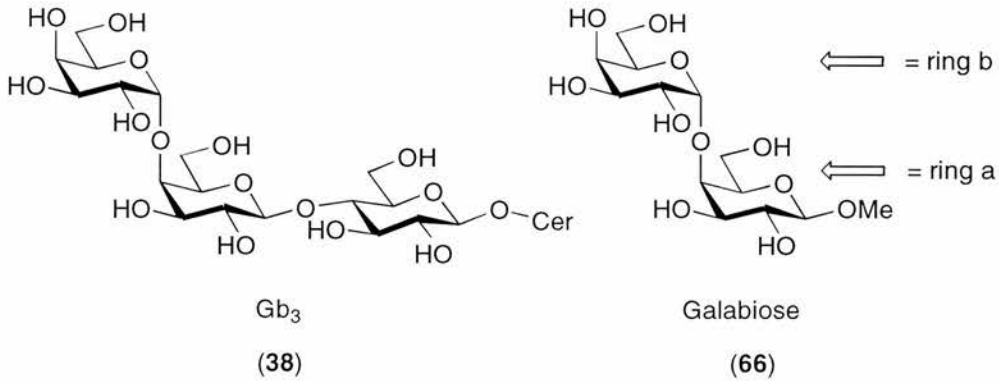


Figure 47: Structure of the Starfish compound.[28]

The crystal structure for the binding of starfish to Vt-1 revealed that it was not 1 starfish to 1 VT-I, but 1 starfish bound to 2 VT-I toxins.[28] The starfish moiety only binds to site 2 in both toxins, with alternate formation of a B5-starfish-B5 sandwich.[28]

4.5 Aims and Objectives of Galabiose Mimics

Gb_3 (**38**) (**Figure 48**) is a known ligand for verotoxin.[6] With its complexity and the number of chemical manipulations required to synthesise Gb_3,[16-22] a more effective study based on the derivatisation of a smaller saccharide [e.g. galabiose (**66**), **Figure 48**] was attractive.



Gb_3 (**38**) Galabiose (**66**)

Figure 48: Chemical structures of Gb_3 (**38**) and Galabiose (**66**).

The crystal structure of the VT-Gb_3 trisaccharide complex indicates potential areas where binding could be increased with simple chemical modifications to the saccharide structure.[6] As shown in **Figure 49,** the majority of hydrogen bonds are located around the non-reducing terminal galactose residue. There is only one very weak (i.e. long) hydrogen bond to the primary alcohol of Gal 6a. No hydrogen bonds were located at the 2a or 3a positions of this galactose residue, offering potential for derivatisation at these sites. Derivatisation at the 2a position is also connected with binding data for Bundle's starfish (**Figure 47**).[28]

The aim of this study was to establish a general route for the synthesis of amino-substituted galabiose (**67**) (**Figure 50**). With this methodology established, a small library of compounds would be synthesised and tested for binding to VT-1.

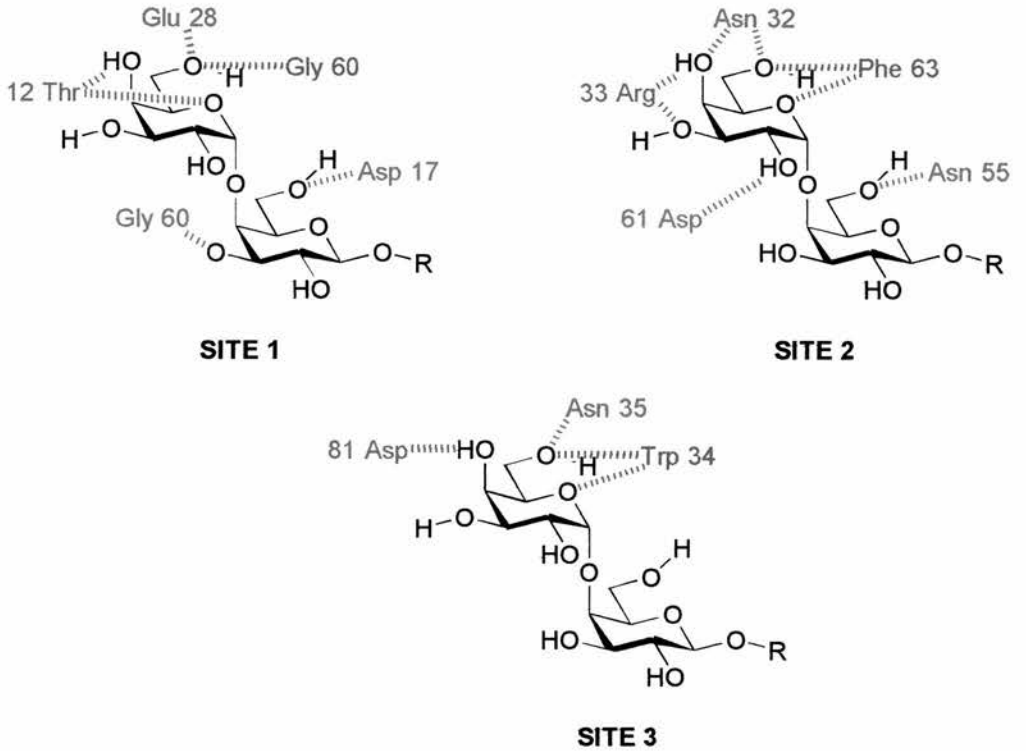


Figure 49: Hydrogen Bonds to galabiose in the VT-1 crystal structure.

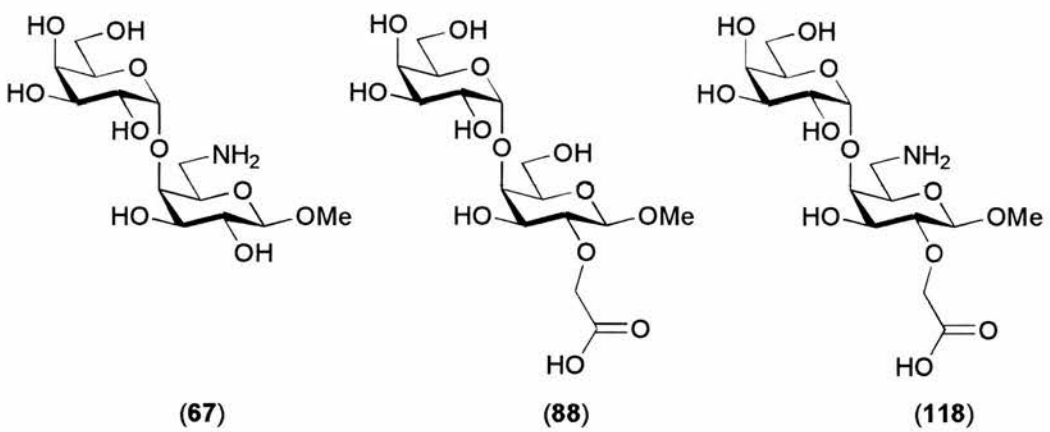


Figure 50: Chemical structures of amino-functionalised galabiose (**67**), carboxy methylene-functionalised galabiose (**88**) and di-functionalised galabiose (**118**).

In addition general methods for the synthesis of other modified galabiose compounds would be established. The 2a position of galabiose (**88**) could be modified to introduce more diversity to the saccharide backbone (**Figure 50**). The di-functionalised galabiose (**118**) would contain amino functionality at C-6a, as well as a carboxylic acid function at C-2a of galabiose (**Figure 50**). This structure could be suitable for use in established peptide chemistry and in the formation of a

combinatorial library of galabiose derivatives, whilst also being suitable for attachment to a solid support.

4.6 CHEMICAL SYNTHESIS

The syntheses of building blocks for galabiose and galabiose mimics are outlined below (**Figure 51**). Section **4.7** will discuss the synthesis of the galabiose building blocks, section **4.8** the synthesis of azido galabiose building blocks, section **4.9** the glycosylation strategy involved in making disaccharides and section **4.10** the derivatisation of azido-substituted galabiose (**62**). Synthesis of the carboxy methyl-functionalised galabiose (**88**) will be discussed in section **4.11**. Di-functionalised galabiose (**118**) will be discussed in section **4.12.**

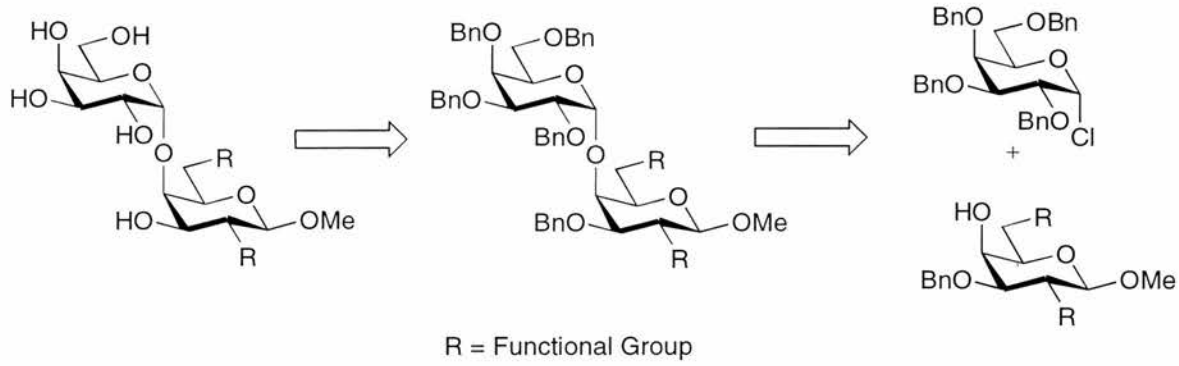


Figure 51: General Retrosynthetic Approach to the Galabiose and Galabiose Mimics.

4.7 Analysis of galabiose building block (50).

The method for the preparation of known methyl 2,3,6-tri-*O*-benzyl-β-D-galactoside (**50**)[33] used commercially available methyl galactopyranoside with selective protection of the sugar exposing the 4-OH for glycosylation. Methyl galactopyranoside (**46**) was protected via benzylidene acetal formation on the 4,6 positions of the sugar. This allows the possibility of selective opening of the acetal to leave the 4-OH free, once the other positions are protected. Benzyl protection of the sugar was chosen for the ease of a final one step deprotection method.

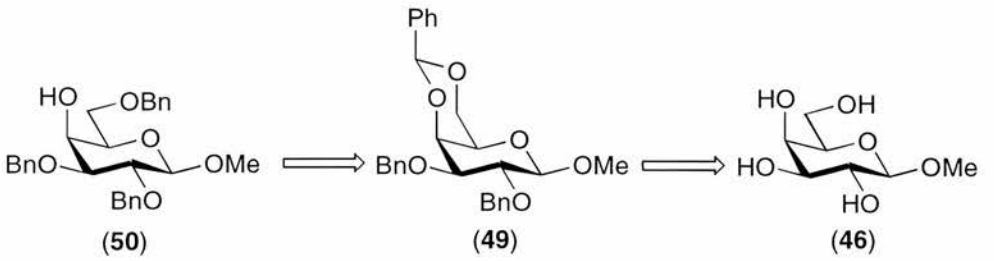


Figure 51: Reterosynthetic analysis of methyl 2,3,6-tri-*O*-benzyl-β-D-galactoside (**50**).

4.7.1 Synthesis of methyl 2,3,6-tri-*O*-benzyl-β-D-galactoside (50)

Methyl galactopyranoside (**46**) being relatively inexpensive, was chosen as the starting material for the synthesis of the tri-*O*-benzylated sugar (**50**).[33] Methyl galactopyranoside (**46**) was converted into the benzylidene acetal-protected sugar (**47**) in 95 % yield with benzaldehyde dimethyl acetal and a catalytic amount of camphor-sulfonic acid.[34] A small amount of (**47**) was acetylated with acetic anhydride/pyridine to prove the acetal was formed on the 4,6-diol [^{1}H NMR, 4.98 ($J_{2,3}$ 8.1, $J_{3,4}$ 3.6, H-3), 5.37 ($J_{1,2}$ 7.8, $J_{2,3}$, H-2)].[35] The 2,3 diol (**47**) was then protected using benzyl bromide and sodium hydride in 94 % yield to give methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**49**).[36]

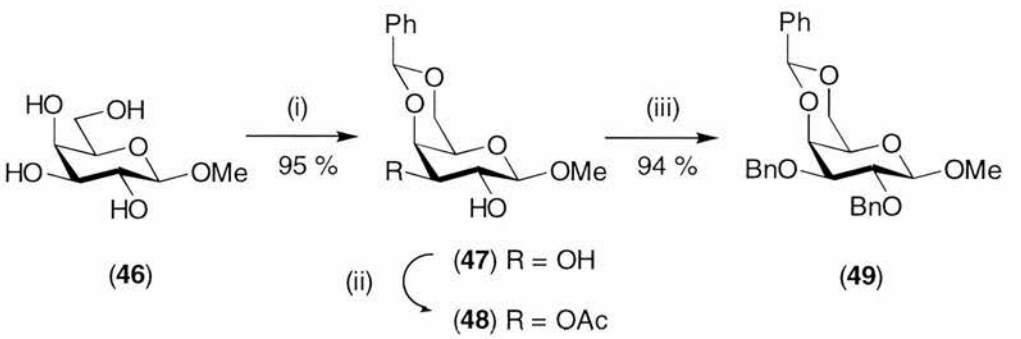


Figure 53: Synthesis of benzylated galactoside (**50**).
Reagents: i) $C_6H_5CH(OCH_3)_2$, CSA, CH_3CN; ii) Ac_2O, pyridine; iii) BnBr, NaH, DMF.

4.7.2 Selective benzylidene acetal opening

Benzylidene acetal protecting groups can be selectively opened to form either 4-OH or 6-OH compounds, depending on the reaction conditions. Outlined in this section are the different conditions used to gain the selectivity in acetal opening.

Method (a) uses a lithium aluminium hydride-aluminium chloride acetal opening method, which leads to the 6-OH compound.[37] This is due to the larger steric demand of the Lewis acid compared with the proton in method (b). Method (b) uses sodium cyanoborohydride-hydrogen chloride reduction, where the proton has a smaller steric demand than the previous method.[37] The relative basicity of O-4 and O-6 of the sugar governs the orientation of acetal opening.

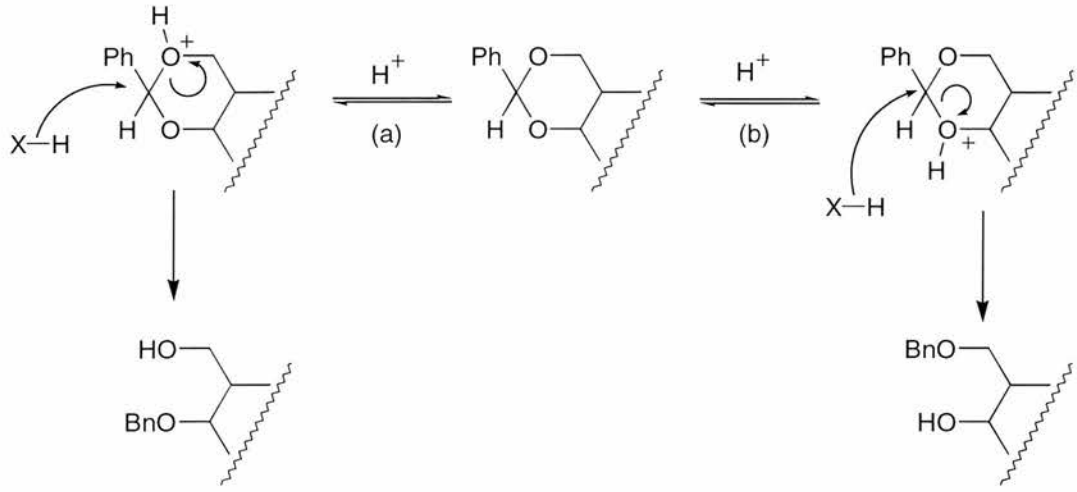


Figure 54: Benzylidene acetal opening mechanism using; (a) lithium aluminium hydride-aluminium chloride and (b) sodium cyanoborohyride-hydrogen chloride.

Oscarson reported that the opening a benzylidene acetal with borane:trimethylamine-aluminium chloride was solvent dependent.[38] The acetal opens to form the 4-OH in THF, but forms the 6-OH when toluene is used as a solvent.

A combination of sodium cyanoborohride and THF was chosen for use in a selective benzylidene acetal opening procedure. Acetal (**49**) was reduced in a 70 % yield, to give the tri-*O*-benzyl-protected sugar (**50**).[33] To confirm the acetal had opened to give the 4-OH and not the 6-OH, a small amount of the product was acetylated with acetic anhydride/pyridine[5] to give the 4-*O*-acetate (**51**) [^{1}H NMR, $CDCl_3$; δ_H 4.25 (4-OH) to δ_H 5.55 (4-OAc)].

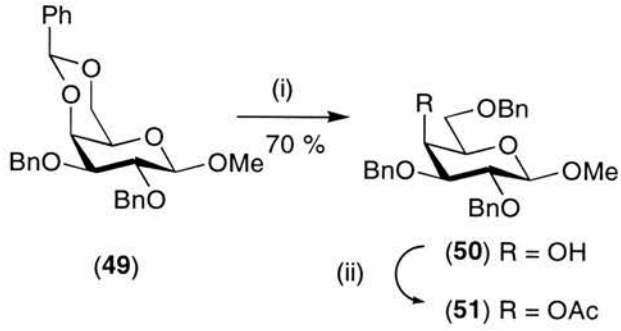


Figure 55: Selective acetal opening of (**49**).
Reagents: i) $NaCNBH_3$, $HCl:Et_2O$, THF; ii) Ac_2O, pyridine.

4.8 Analysis of Azido-substituted Galabiose building block (55)

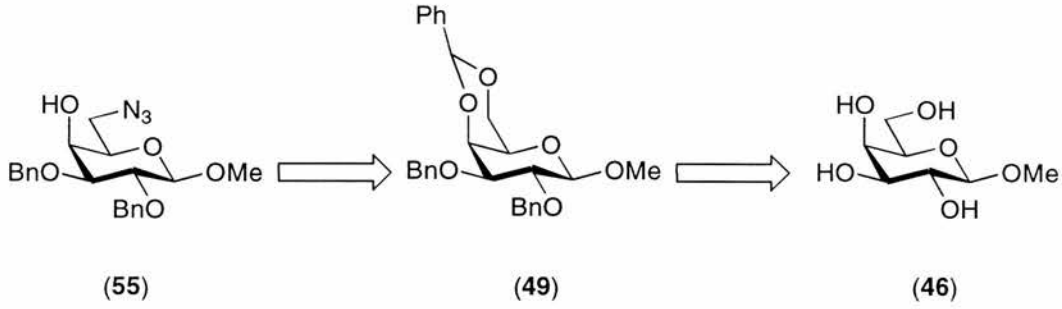


Figure 56: Retrosynthetic analysis of azido galactoside (**55**).

4.8.1 Synthetic analysis: The method for the preparation of methyl 6-azido-2,3-di-*O*-benzyl-6-deoxy-β-D-galactoside (**55**) was to use methyl galactopyranoside (**46**) as the starting material. This compound was protected with a benzylidene acetal on the 4,6-positions of the sugar. The 2,3-diol was benzyl protected before cleavage of the acetal. Selective tosylation of the primary alcohol and displacement of the tosyl group with azide gave the azido galactoside (**55**).

4.8.2 Synthesis of methyl 6-azido-2,3,-di-*O*-benzyl-6-deoxy-β-D-galactoside (55)

Synthesis: Methyl galactopyranoside (**46**) was chosen as the starting material for the synthesis of azido galactose (**55**). This was converted into the benzylidene acetal protected sugar (**47**) in 95 % yield with benzaldehyde dimethyl acetal and a catalytic amount of camphor-sulfonic acid.[34] The 2,3-diol was protected with benzyl bromide and sodium hydride in 94 % yield to give methyl 2,3-di-*O*-benzyl-4,6-*O*-

benzylidene-β-D-galactopyranoside (**49**).[36] Aqueous acetic acid (80 %) was used in the deprotection of the acetal to yield the 4,6-diol (**52**) in 83 %.[36]

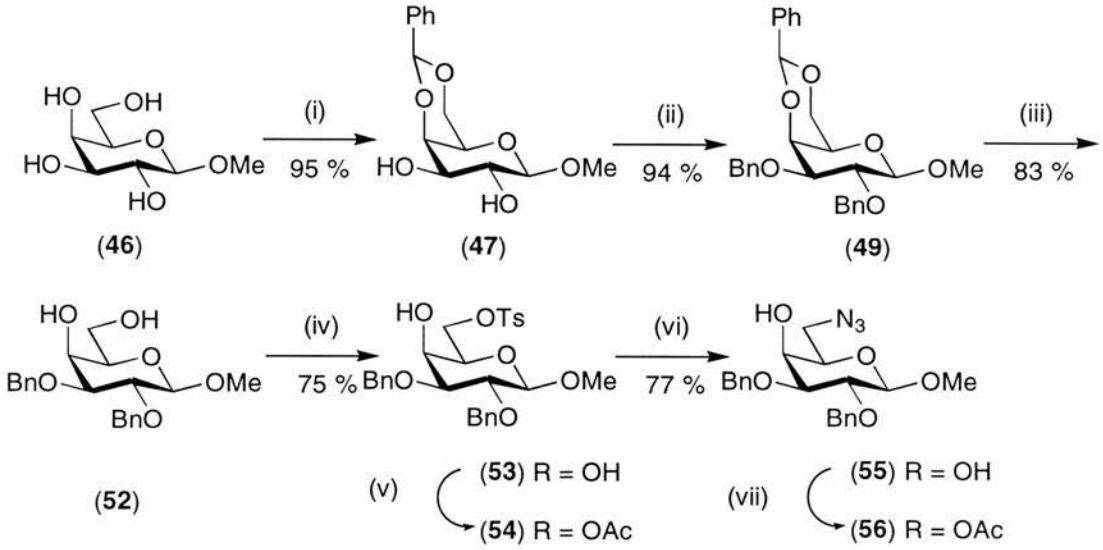


Figure 57: Synthesis of 6-azido-6-deoxy galactoside (**55**).
Reagents: i) $C_6H_5CH(OCH_3)_2$, CSA, CH_3CN; ii) BnBr, NaH, DMF; iii) 80 % AcOH (aq); iv) *p*TsCl, $(CH_3)_2CO$, pyridine; v) Ac_2O, pyridine; vi) NaN_3, DMF; vii) Ac_2O, pyridine.

Two different methods were attempted to introduce the azide functionality on the 6-OH.

4.8.2.1 Method 1: Formation of 6-azide function *via* Nucleophilic displacement.

Method one was the addition of a good leaving group followed by nucleophilic displacment. A tosyl group was chosen as the leaving group due to the selective nature of this group. Tosylation was carried out with *p*-toluenesulfonyl chloride in pyridine and acetone in good yield.[36] A small amount of the tosylated sugar (**53**) was acetylated with acetic anhydride/pyridine to yield the 4-*O*-acetate (**54**) [^{1}H NMR, $CDCl_3$; δ_H 3.91 (4-OH) to δ_H 5.30 (4-OAc)]. Nucleophilic displacement of the tosyl group (**53**) with sodium azide gave the 6-azido sugar (**55**) in 76 % yield. Infrared spectroscopy showed absorption at 2100cm^{-1} indicating azide functionality was present in the product.[33]

4.8.2.2 Method 2: Formation of 6-azide functionality *via* Mitsonobu reaction.

Method two was the introduction of azide function via a Mitsonobu reaction (see **Figure 58**).[39] However, this was unsuccessful, giving a mixture of compounds.

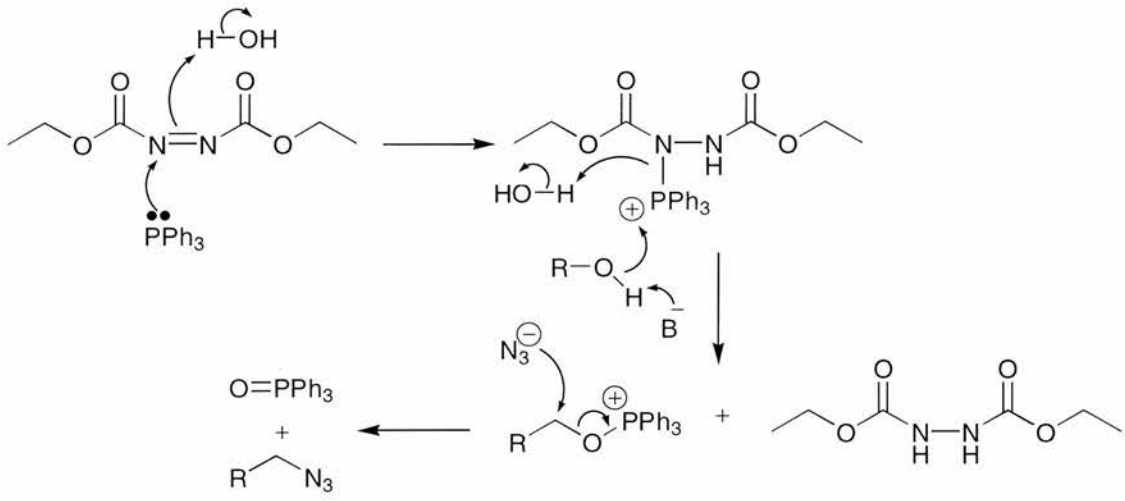
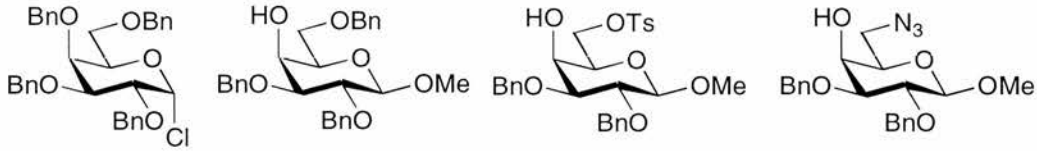


Figure 58: Mitsunobu mechanism.

No credible results were obtained using this reaction, so method 1 was the choice route of synthesis.

4.9 Glycosylation reaction: using previously synthesised building blocks

The glycosylation between tetra-*O*-benzyl-galactosyl chloride (**57**) and partially protected acceptor building blocks (**Figure 59**) are discussed in more detail.



(57) **(50)** **(53)** **(55)**

Figure 59: Building blocks for glycosylation.

4.9.1 Retrosynthetic Analysis of Galabiose.

The glycosylation strategy, as indicated in **Figure 60**, was as follows: Commercially available tetra-*O*-benzyl galactopyranose was converted into the anomeric chloride to give the glycosyl donor (**57**).[40] Glycosylation with previously synthesised glycosyl acceptors (**Figure 59**) employed a modified silver triflate glycosylation procedure. Deprotection was via a one step procedure using a palladium on charcoal catalyst, yielding the free disaccharides.

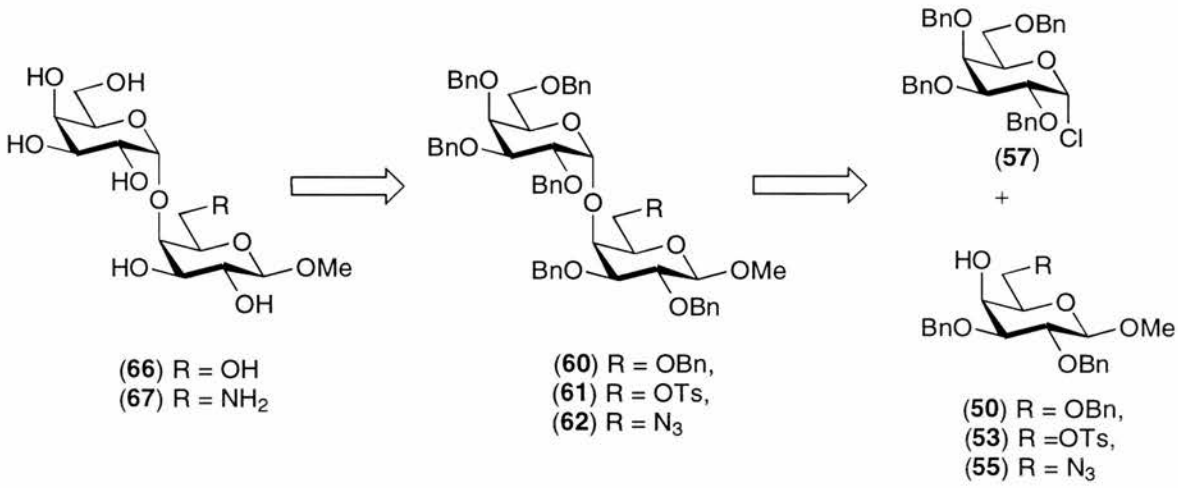


Figure 60: Retrosynthetic analysis of glycosylation.

4.9.2 Koenigs-Knorr glycosylation

A Koenigs-Knorr method for glycosylation was chosen.[41-43] Silver triflate promoted glycosylation was preferred because of previously reported Gb_3 syntheses utilising this methodology.[16-22] The α-selectivity varied within each reported synthesis, therefore attempts were made to try and gain a higher yielding and a more stereoselective glycosylation reaction.

The concept behind the Knoenigs-Knorr method for glycosylation is to activate an anomeric halide with a heavy metal, then glycosylate with an alcohol.[41-43] The group situated at position O-2 of a sugar determines the stereospecificity and the rate of reaction. If acetate or any electron-withdrawing group is present at O-2, then there is a 'disarming' effect towards the anomeric center, which decreases the reaction rate of glycosylation.[44] Neighbouring group participation occurs when acetate is present at

O-2 (**Figure 61**). This effect blocks the α-face of C-1, so the β-face is the only one available for reaction with alcohol/acceptor (**Figure 61**).

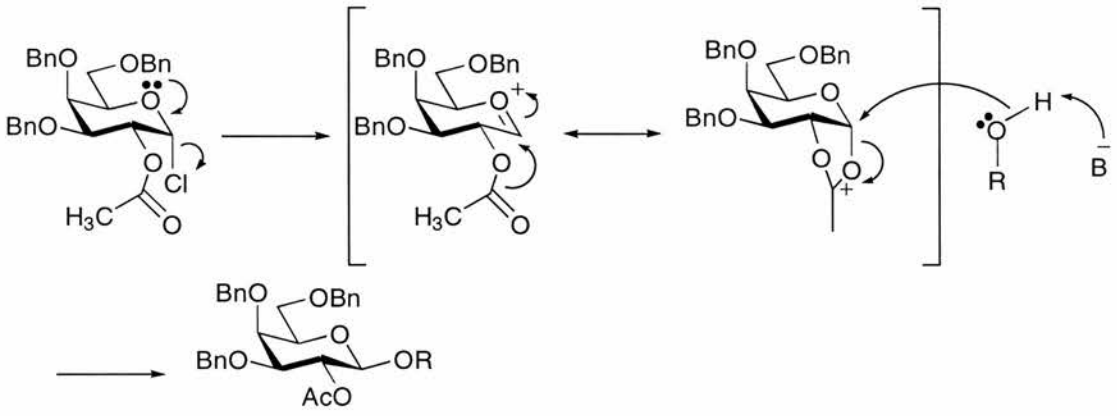


Figure 61: An electron-withdrawing group at C-2 participating in the formation of the glycoside bond.

If an electron-donating group (e.g. benzyl ether) is present at O-2 on the sugar then there is an 'arming' effect towards glycosylation, which enhances the reaction rate.[44] Benzyl ethers do not participate in neighboring group effects; consequently both faces are available for glycosylation. The α-face is generally the side most favoured for glycosylation since it forms the thermodynamically more stable anomer (**Figure 62**).

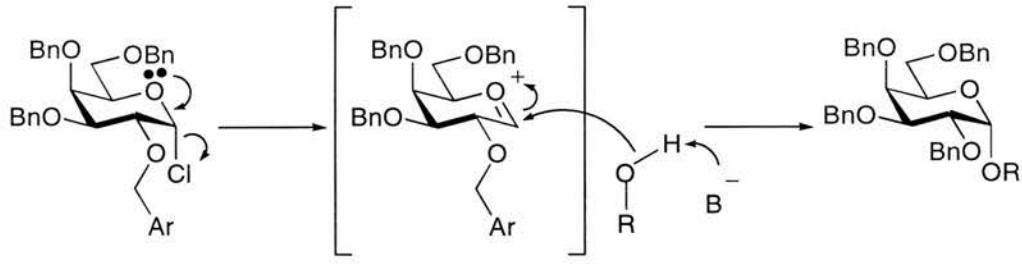


Figure 62: An electron-donating group present at C-2 during glycosylation.

Benzyl protection was chosen for the synthesis of galabiose and galabiose mimics because of the α-selectivity which is given during glycosylation. The presence of benzyl groups on both reducing and non-reducing terminal sugars was advantageous for ease of deprotection.

4.9.3 Solvent Effects on Glycosylation Selectivity

The solvent effect on glycosylation was investigated closely due to recently published results by Boons and co-workers,[45] who reported that a variable degree of stereoselectivity was observed when different solvents was used in glycosylation reactions. The reactions (outlined in **Figure 63**) investigated various solvent mixtures to find out which gave the greatest α-stereoselectivity.

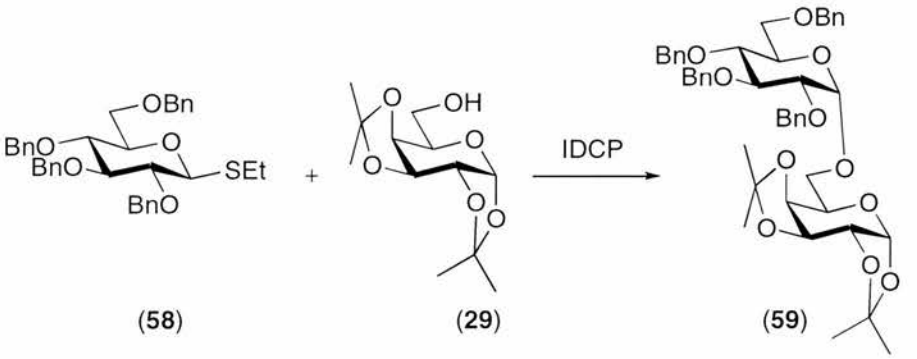


Figure 63: Solvent effect on glycosylation.[45]

Solvent System (v/v)	α : β Ratio
DCM	0.7 : 1
DCM-Ether (1:1)	2.0 : 1
DCM-Ether (1:4)	3.5 : 1
DCM-Dioxane (1:3.2)	3.4 : 1
DCM-Cyclohexane (1:4.1)	1.2 : 1
Toluene-Ether (1:2.4)	12.0 : 1
Toluene-Dioxane (1:2.0)	15.3 : 1

Table 4: Solvent Effect on Glycosylation.[45]

All glycosylations were performed in the presence of IDCP and 4 Å molecular sieves at room temperature. There is a greater α-selectivity when diethyl ether or dioxane was used as a solvent, which may be explained as outlined in **Figure 64**. When toluene is present in place of DCM, the α-selectivity is approximately 4 fold greater (**Table 4**).[45]

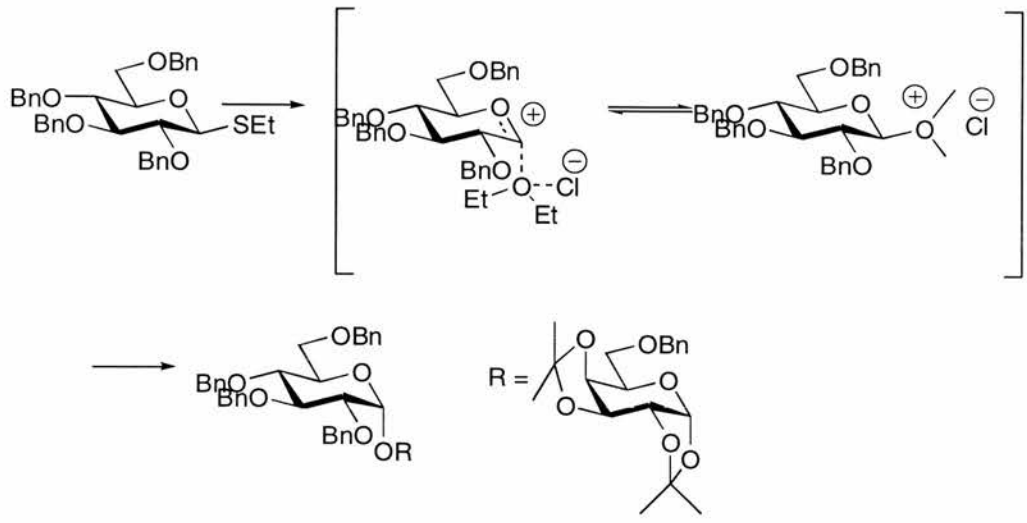


Figure 64: Indicating solvent effect on glycosylation.

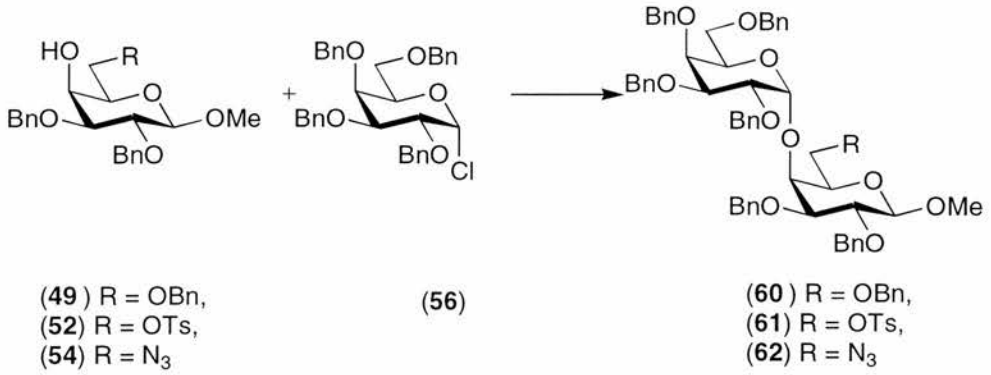
A selection of solvents and solvent mixtures were chosen for use in glycosylation reactions in the current study.

4.9.4 Synthesis of Galabiose and Mono-derivatised Galabiose

Commercially available tetra-*O*-benzyl galactose was converted to the anomeric chloride (**57**), using oxalyl chloride, in 97 % yield.[40] All glycosylations were promoted using silver triflate activation (**Figure 65**).[41-43] Alcohol [**50, 53, 55**] (1 mol eq), chloride (**57**) (1.2 mmol. eq.), collidine (1.83 mol eq) and molecular sieves were stirred in anhydrous solvent. Then 1.8 equivalents of silver triflate were added to promote the reaction. Silver triflate is light sensitive therefore it was essential that the reaction vessel was covered so that no light could be absorbed by the solution. The reactions were carried out at low temperatures to try and enhance α-selectivity by slowing the reaction rate down. Further details can be found in **Table 5**.

The initial attempt at glycosylation looked promising, by TLC, but there was no dissacharide formed. This was due to the pH of the reaction mixture being too acidic, so cleaving the newly formed glycoside bond before work-up/purification. Modification of the glycosylation procedure included addition of an aliquot of collidine (0.5 mmol eq.) once the reaction mixture was complete to ensure that the

pH was only moderately acidic, so preventing cleavage of the newly formed glycoside linkage.



(**49**) R = OBn,
(**52**) R = OTs,
(**54**) R = N_3

(**56**)

(**60**) R = OBn,
(**61**) R = OTs,
(**62**) R = N_3

Figure 65: Glycosylation reaction; Reagents: Collidine, AgOTf, 4 Å MS, -78 ^{O}C→ room temp..

	COMPOUND (α : β ratio) and YIELD		
	H-6a-OBn (**60**)	H-6a-OTs (**61**)	H-6a-N_3 (**62**)
DCM	2:1 (77 %)	N.D	N.D.
TOLUENE	4.5:1 (76 %)	N.D.	N.D.
TOLUENE / ETHER (1:2)	10:1 (82 %)	7:1 (90 %)	10:1 (89 %)
TOLUENE / DIOXANE (1:3)	1:1 (41 %)	N.D.	N.D.
ETHER	3:1 (70 %)	7:1 (89 %)	4:1 (81 %)
ETHER / DCM (2:1)	2:1 (67 %)	4:1 (84 %)	10:1 (89 %)

Table 5: Results of solvent effect on glycosylation.

All glycosylation reaction products were purified by silica gel chromatogaphy (toluene:EtOAc, 12:1). TLC analysis (toluene:EtOAc, 10:1, run 3 times) of the fractions showed separation of the α:β mixture, but there were another two spots present in small amounts (less than 1 %). (**Figure 66**). These compounds were later found to be 1,1'-linked disaccharides, details of which can be found in **section 4.12.8**.[46] The toluene/dioxane was poor compared to Boon's results due to the low temperature required for selectivity.

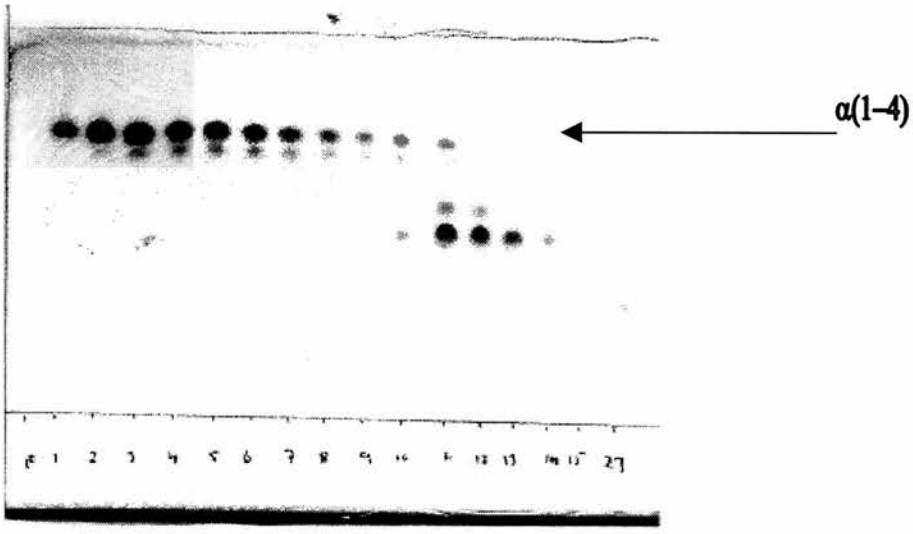


Figure 66: TLC indicating the difficulty in separation of compounds.

4.9.5 Displacement of 6-tosyl galabiose (58).

Azido galabiose (**62**) was synthesised by two different methods. Method one formed the azido galactose sugar (**62**) before glycosylation (as outlined in the previous section). Method two introduced the azide function once the galabiose inter-sugar glycoside linkage had been synthesised. There were few difficulties making the tosylated galabioside (**61**).

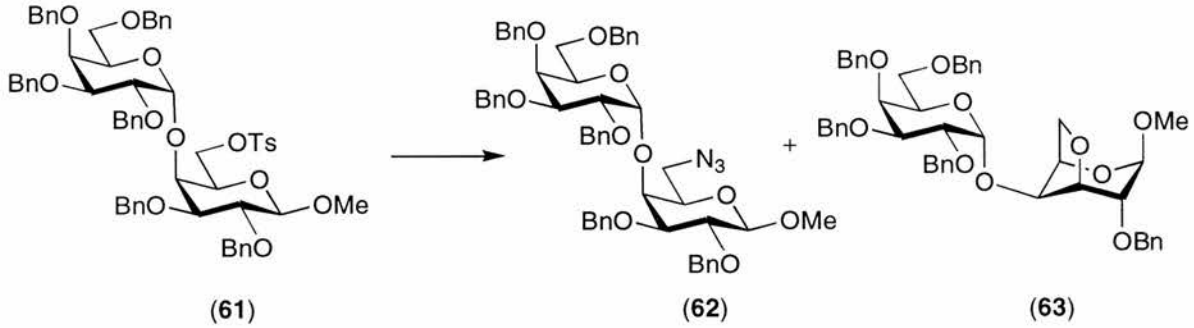


Figure 67: Nucleophilic displacement of 6-tosyl on galabiose; Reagents: NaN_3, DMF.

However nucleophilic displacement of the tosyl group on galabiose (**61**) with sodium azide gave the azido-substituted galabiose (**62**) in only 28 % yield [ν_{max}/cm^{-1} 2099 (N_3)].[33] The poor yield was due to the formation of 3,6-anhydro sugar (**63**) in 30 %

yield [no azide or tosyl group present as judged by IR spectroscopy].[36] The ability of benzyl protected oxygen atoms in carbohydrates to act as nucleophiles in cyclisation reactions is well established.[36]

Pyranoses typically sit in one of two chair conformations, $^4C_1$ and $^1C_4$. Normally, groups would sit equatiorial, as in the 6-tosyl monosaccharide (**53**) (**Figure 68**). Once glycosylation has been carried out the large bulky group, tetra-benzyl galactose, is sitting axial and therefore in an unfavoured conformation. In order for the sugar to sit in a lower energy conformation, disaccharide (**61**) conformation might flip from $^4C_1$ to $^1C_4$. This is not evident from a standard 1 H NMR spectrum run at room temperature, but the azide displacement reaction is conducted in refluxing DMF. Under reflux conditions, the $^4C_1$ conformation in the reducing terminal sugar residue in (**61**) might well be more relevant. The formation of the anhydro sugar is therefore simple because the oxygen at C-3 is in prime position for attack on the carbon at C-6. As there is a good leaving group situated on C-6, the ring closure occurs.

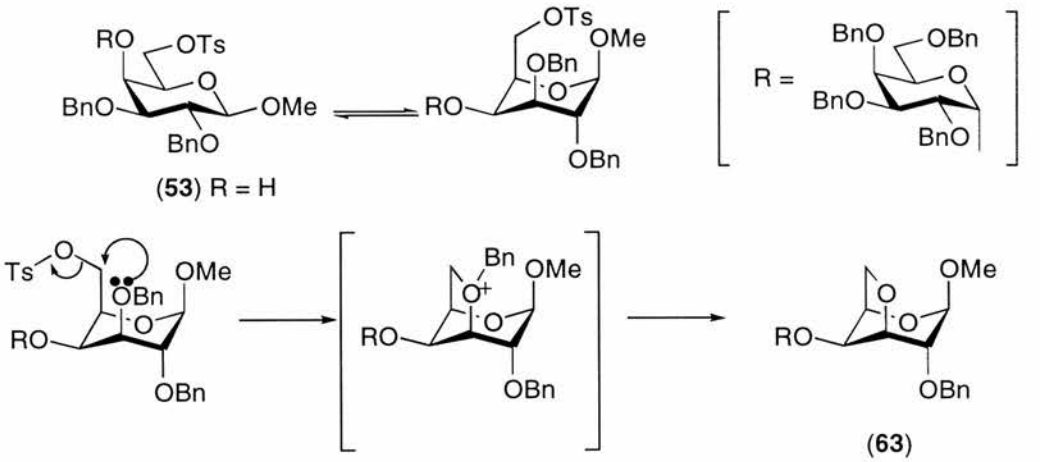


Figure 68: Mechanistic explanation of the formation of the 3,6-anhydro sugar (**63**).

4.9.6 Deprotection and Acetylation of 3,6-Anhydro sugar (63)

The benzylated anhydro sugar (**63**) was deprotected using catalytic hydrogenolysis (Pd-OH on charcoal/H_2/ethanol) to give free anhydro (**64**) in 93 % yield.[47] A small amount of the sugar was acetylated, to give (**65**), using acetic anhydride/pyridine to

help in the assignment [^{1}H NMR: δ_H 4.28 (s, H-4a), 4.45 (s, H-1a), 4.52 ($J_{2,3}$ 5.0, H-3a), 4.99 (H-2a); δ_C 56.0 (OMe), 96.5 (C-1b), 100.7 (C-1a)].

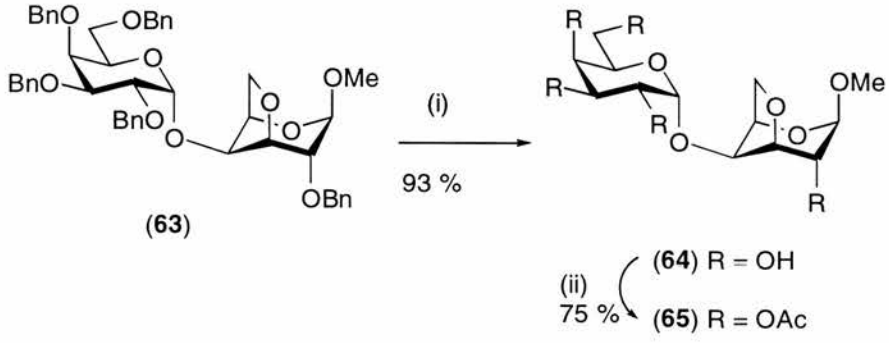


Figure 69: Reagents: i) EtOH, H_2, Pd-OH; ii) Ac_2O, pyridine.

4.9.7 Deprotection of Galabiose (60) and Azido Galabiose (62)

The benzyl-protected galabiose (**60**) was deprotected using catalytic hydrogenolysis (Pd on charcoal/H_2/acetic acid/ethanol) to give the known free methyl galabioside (**66**) in 80 % yield (**Figure 70**).[48] ^{1}H NMR showed the disappearance of the aromatic protons and mass spectrometry gave an appropriate molecular ion; methyl galabioside (**66**) [δ_H 3.53 (OMe), 4.20 ($J_{1a,2a}$ 7.5, H-1a), 4.96 ($J_{1b,2b}$ 3.0, H-1b); δ_C 56.1 (OMe), 101.1 (C-1), 104.7 (C-1); MALDI-TOF: *m/z* 379 $(M + Na)^+$].

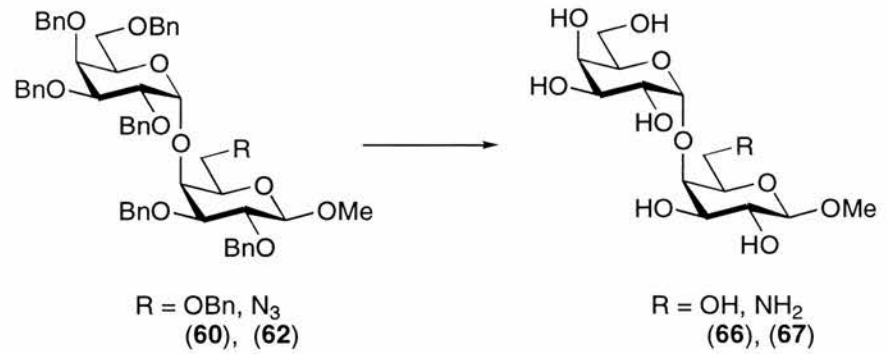


Figure 70: Deprotection of Galabiose (**60**) and Azido Galabiose (**62**); Reagents: EtOH:AcOH, H_2, Pd-C.

Azido galabiose (**62**) was deprotected under the same reaction conditions (**Figure 70**).[47] NMR and mass spectrometry data indicates the presence of methyl 6-amino-6-deoxy- galabioside (**67**) [4.20 ($J_{1a,2a}$ 7.2, H-1a), 4.96 ($J_{1b,2b}$ 3.6, H-1b); δ_C 55.9

(OMe), 101.5 (C-1b), 104.5 (C-1a); MALDI-TOF: *m/z* 378 $(M + Na)^+$].

4.10 Derivatisation of Azido Galabiose

Derivatisation of amino galabioside (**67**) was performed because the crystal structure of verotoxin showed an area that could be exploited for increased binding (**Figure 71**).[6] As shown in the crystal structure data for galabiose, there are few interactions around the O-6a area and the hydrogen bonding which is present is very weak.

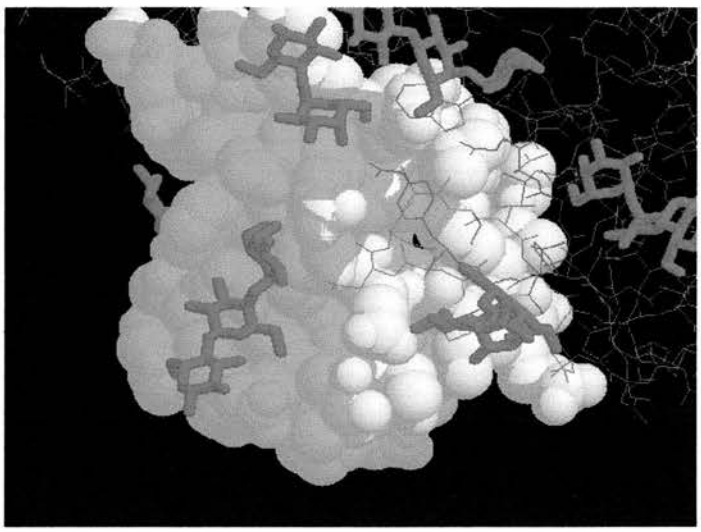


Figure 71: Indicating space available in Site 2 of VT.

The initial groups of derivatised compounds (**72**)-(**74**) were used to investigate changes in the energy of binding. A small but varied panel of acylating agents were investigated, including acetic anhydride, methanesulfonyl chloride and benzoyl chloride (**Figure 72**). These initial compounds would give a broad outline of any favorable or unfavorable interactions that may be present in the VT-inhibitor complex.

During the azide reduction reactions, progress was monitored using the Kaiser test to show the presence of an amine.[49] This is a colour test, which shows whether amine is present in the reaction mixture (blue) or not (yellow). This test is critical in any solid phase reaction because it is a quick and easy method for the determination of the presence of amines.

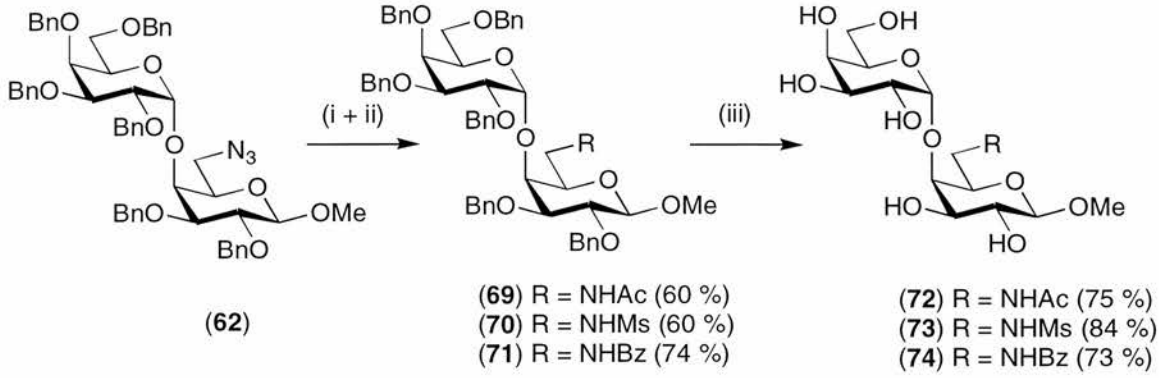


Figure 72: Derivatisation of Azido Galabiose (**62**).
Reagents: i) a) PPh_3{on solid support}, H_2O, THF; b) DTT, DBU, DMF; ii) a) DBU, DMF, Ac_2O; b) DBU, DMF, MsCl; c) DBU, DMF, BzCl; iii) EtOH, H_2, Pd-OH.

Two different methods were applied to reduce the azide functionality of (**62**). The first method involved solid-supported triphenylphosphine (**Figure 73**).[50] The solid support is polystyrene, cross-linked with 2 % divinylbenzene and has a loading of 3 mmol Phosphorous/g resin. This was a successful but time consuming reaction [ν_{max}/cm^{-1} showed the disapperance of the $2100cm^{-1}$ bond from azide and the apperance of 3400 bond from amine].

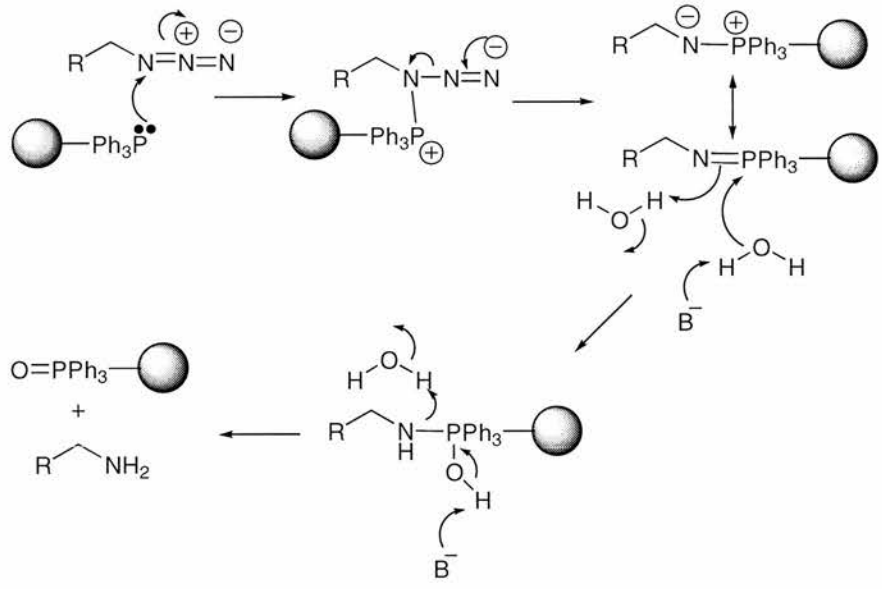


Figure 73: Triphenylphosphine reduction of Azide.

The second method used DTT reduction (**Figure 74**).[51] The reduction was very quick but not as clean as the phosphine procedure due to the presence of a by-product [oxidised form of DTT]. The subsequent acylation reaction was carried out *in situ*. The by-product from DTT reduction was unimportant, as long as there was a large excess of the acylating agent present (10 mole equivalents).

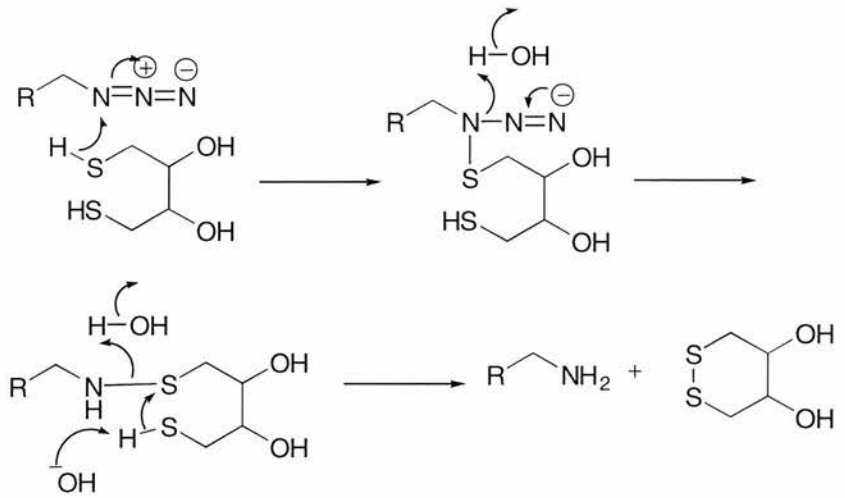


Figure 74: DTT reduction of Azide.

The DTT reduction was selected as the method of choice as it was quick and the acylation reactions could be conducted *in situ*. Table **6** indicates the main characterisation details from the derivatives performed, (**69**) – (**71**).

The derived galabiose analogues were deprotected using palladium-hydroxide/H_2 in ethanol.[47] The free sugars were gel purified (Sephadex LH-20 in methanol) to yield pure acylated galabiosides (**72**) – (**74**).

	NHAc		NHMs		NHBz	
	OBn (**69**)	OH (**72**)	OBn (**70**)	OH (**73**)	OBn (**71**)	OH (**74**)
Mass Spec $(M + Na)^+$	960	379	996	420	1022	482
Yield	60 %	75 %	60 %	84 %	74 %	73 %
NMR						
NH	6.06		5.68		6.70	
H-1a	4.23, *J* 8.0	4.16, *J* 7.5	4.23, *J* 7.5	4.19, *J* 7.5	4.26, *J* 8.0	4.20, *J* 6.9
H-1b	5.05, *J* 3.5	4.96, *J* 3.5	4.93, *J* 3.5	4.96, *J* 3.5	4.99, *J* 3.0	5.02, *J* 3.6
NHR	1.65	1.95	2.55	3.16	7.55, 8.05	7.48, 7.84
IR	3055 NH, 1672 CO		3056 NH, 1266 SO		3057 NH, 1657 CO	

Table 6: Selected data for compounds derivatised from 6-azido-6-deoxy-galabioside (**62**).

4.11 Synthesis of 2-*O*-carboxymethyl-Galabiose (88)

4.11.1 Retrosynthetic Analysis of 2-*O*-carboxymethyl galabiose (88)

Route A

The synthetic strategy for the synthesis of the galabiose containing a carboxylic acid functionality at the 2a position was to initially synthesise the 4,6-*O*-benzylidene derivative (**80**) (**Figure 75**). Selective opening of the acetal, followed by glycosylation would yield the fully protected galabiose with the methyl ester functionality on the 2a position. Deprotection of the disaccharide would yield the target sugar (**Figure 75**).

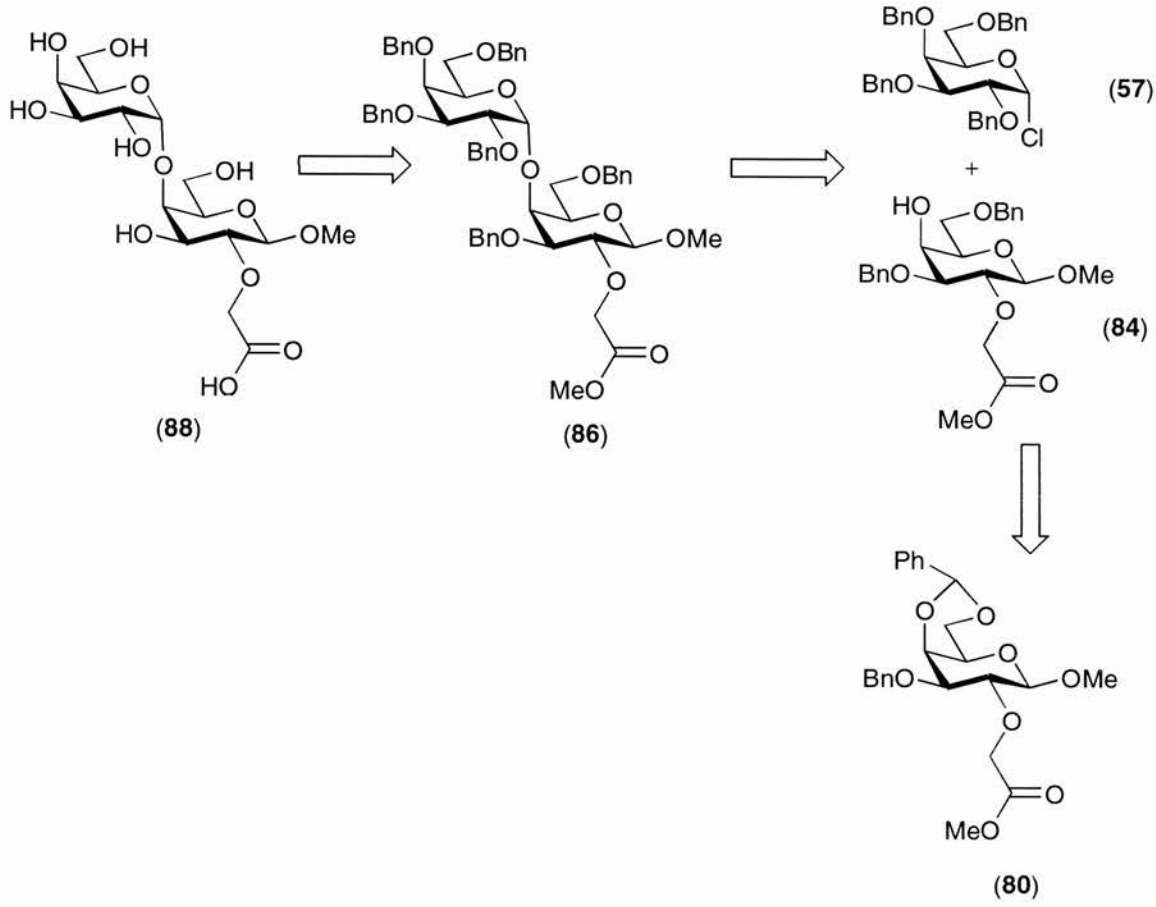


Figure 75: Retrosynthetic analysis of 2-*O*-carboxymethyl-galabiose

Route B

The fully protected sugar (**80**) was synthesised via two routes. Route A used methyl galactoside (**46**), which was acetal protected. Once the 2-OH was alkylated, the acetals were removed allowing 4,6-*O*-benzylidene acetal protection to give (**78**). The 3-OH position was free to be protected with a benzyl group.

Route B also used methyl galactoside (**46**) as starting material, but in this method it was selectively benzyl protected at the 3-OH position with the aid of stannylene acetal chemistry, benzylidene acetal protection on the 4,6-diol and finally alkylation of the 2-OH.

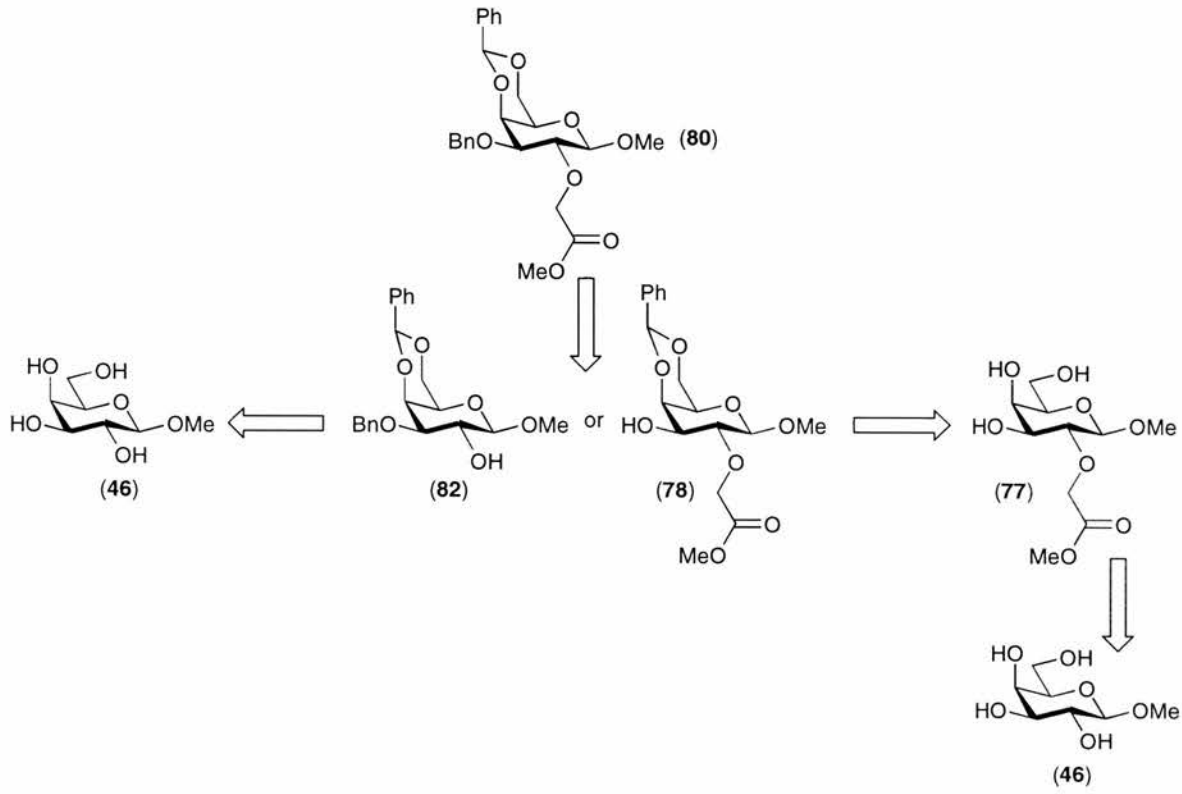


Figure 76: Retrosynthetic approach to the synthesis of Benzylidene acetal (**80**).

4.11.2 Method A: Synthesis of 2-*O*-methoxycarbonylmethyl galactoside (80)

Methyl galactopyranoside (**46**) was chosen as the starting material for the preparation of methyl ester (**77**). The initial protection used 2,2-dimethoxypropane and a catalytic amount of camphor sulfonic acid to form the 3,4-acetal with the 6-position protected with a 2-methoxyisopropyl group (**75**).[52] This latter protective group is very labile, therefore care must be taken when using this method. This compound was carried through, without purification, to be alkylated with methyl bromoacetate in 74 % yield to give (**76**) [MALDI-TOF $(M + Na)^+$ 401]. The labile 2-methoxypropyl group and acetal protection were then removed with 50 % aqueous HBF_4 in 86 % yield to give the triol (**77**).[53] Benzylidene acetal protection of the triol was carried out using a standard protection protocol to give (**78**).[34] To confirm that the protection was successful, a small amount of acetal (**78**) was acetlyated with acetic anhydride/pyridine to give the 3-*O*-acetate (**79**) [^{1}H NMR; H-3 3.75 ppm (**78**) to 5.05 ppm, ($J_{2,3}$ 8.0 $J_{3,4}$ 3.5 Hz), (**79**)]. Benzylation of the 3-OH of (**78**) was carried out using benzyl bromide/sodium hydride in 55 % yield to give (**80**).[36] Fully

protected sugar (**80**) was formed in 5 steps in 22 % yield from methyl galactopyranoside (**46**). Due to the low yielding alkylation step and the lability of the methoxypropane protection, a different synthetic strategy was investigated.

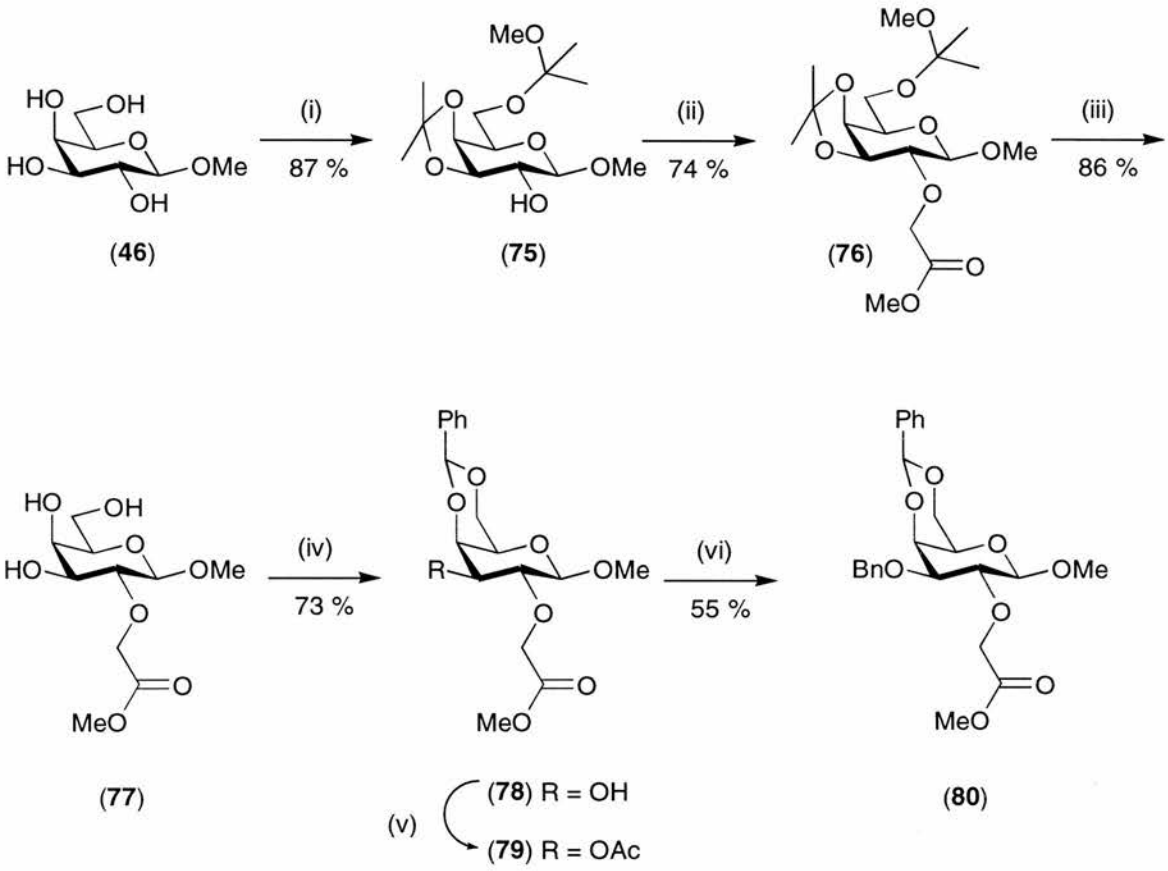


Figure 77: Reagents: i) $Pr^i(OMe)_2$, *p*-TsOH; ii) NaH, methyl bromoacetate, DMF; iii) 50 % HBF_4 (aq), MeOH; iv) CH_3CN, $C_6H_5CH(OMe)_2$, camphor sulfonic acid; v) Ac_2O, pyridine; vi) BnBr, DMF, NaH.

4.11.3 Method B: Synthesis of 2-*O*-methoxycarbonylmethyl galactoside (80)

Methyl galactopyranoside (**46**) was again used as the starting point for this synthesis. The initial step was selective benzylation using tin acetal chemistry. Methyl galactoside (**46**) was dissolved in methanol and refluxed with dibutyltin oxide.[54] Once the dibutyltin oxide had fully dissolved, the tin acetal was presumed to have formed across the 3,4-diol. The reaction mixture was concentrated and resuspended in toluene. The resulting solution was then refluxed with benzyl bromide and tetrabutylammonium bromide. This yielded the mono-3-*O*-benzylated sugar (**81**) in 86 % yield. Benzylidene acetal protection of the 4,6-diol was performed using

standard protection conditions in 87 % yield to give (**82**).[55,56] To confirm that the structure has the 2-OH free, a small amount of (**82**) was acetylated with acetic anhydride/pyridine to give the 2-*O*-acetate (**83**) [^{1}H NMR, 2-OH 3.99 ppm to 2-OAc 5.37 ppm ($J_{1,2}$ 8.0, $J_{2,3}$ 7.1 Hz)].

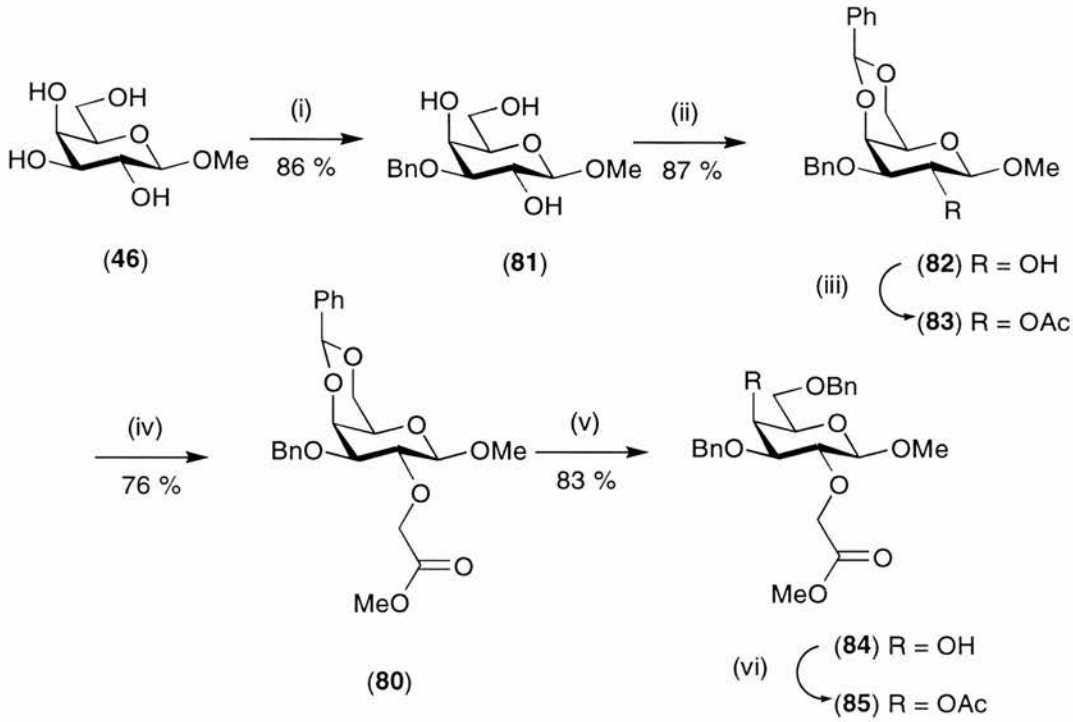


Figure 78: Reagents: i) MeOH, Bu_2SnO, then BnBr, Bu_4NBr, toluene; ii) $C_6H_5CH(OMe)_2$, camphor sulfonic acid, CH_3CN; iii) Ac_2O, pyridine; iv) DMF, NaH, methyl bromoacetate; v) THF, $NaCHBH_3$, 4 Å MS, HCl:diethyl ether; vi) Ac_2O, pyridine.

Alkylation of the 2 hydroxyl was achieved using methyl bromoacetate and sodium hydride in 76 % yield to give (**77**). The benzylidene acetal was selectively opened using sodium cyanoborohyride in 83 % yield to give (**84**).[37,38] To confirm that the acetal had opened to give the 4-OH and not the 6-OH, a small amount of (**84**) was acetylated with acetic anhydride/pyridine to give the 4-*O*-acetate (**85**) [^{1}H NMR, H-4: 3.98 ppm to 5.55 ppm ($J_{3,4}$ 3.3 Hz)]. The 4-OH sugar (**84**) was formed in 4 steps in 47 % yield from methyl galactopyranoside (**46**), a significant improvement over route A.

4.11.4 Glycosylation of the 2-*O*-methoxycarbonylmethyl galactose (84) and galactosyl chloride (57)

Commercially available 2,3,4,6-tetra-*O*-benzyl galactose was converted to the

anomeric chloride (**57**) using oxalyl chloride.[40] The glycosylation between the 2-*O*-methoxycarbonylmethyl galactose (**84**) and the anomeric chloride (**57**) used a silver triflate promotor.[41-43] The 2-*O*-methoxycarbonylmethyl galactose (**84**) (1 mol eq), chloride (**57**) (1.2 mol eq), collidine (1.83 mol eq) and 4 Å molecular sieves were stirred in anhydrous toluene/diethyl ether. This solvent mixture was chosen to give the best α-selectivity during glycosylation. The reaction mixture was cooled to –78 ^{0}C, silver triflate (1.8 mol eq) was added and the reaction was stirred slowly allowing the temperature to increase. After approximatly 2 h the reaction had gone to completion to give the disaccharide (**86**) in 75 % yield; [^{1}H NMR δ_H: 3.59 (OMe), 4.28 (H-1a), 4.94 ($J_{1b,2b}$ 3.0, H-1b); δ_C 56.9 (OMe), 100.3, 104.2; MALDI-TOF: *m/z* 991 $(M + Na)^+$].

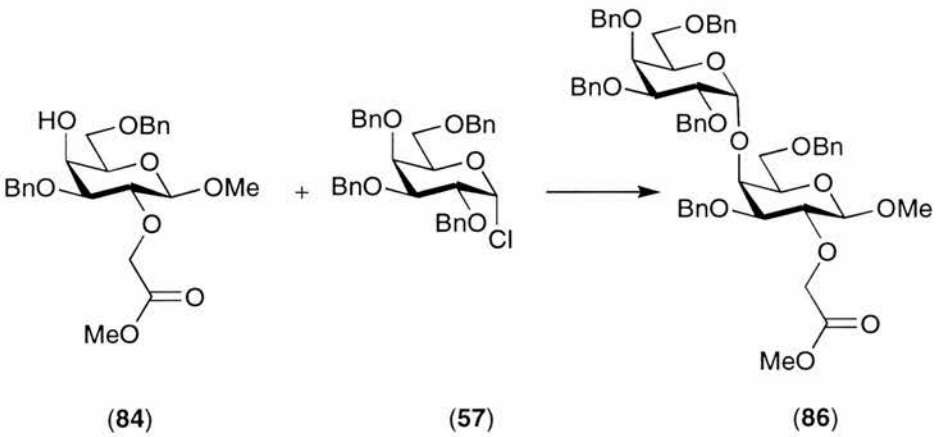


Figure 79: Glycosylation of 2-*O*-methoxycarbonylmethyl galactose (**84**) and galactosyl chloride (**57**).
Reagents: Toluene, diethyl ether, collidine, AgOTf, 4 Å MS.

4.11.4 Reduction of the methyl 2-*O*-methoxycarbonylmethyl galabioside (86)

The benzyl protected galabiose (**86**) was deprotected using catalytic hydrogenolysis (Pd on charcoal/H_2/ethanol)[47] to give the free methyl ester (**87**) in 68 % yield [^{1}H NMR: δ_H: 3.49 (OMe), 3.74 (OMe), 4.28 ($J_{1a,2a}$ 7.5, H-1a), 4.94 ($J_{1b,2b}$ 3.5, H-1b); δ_C: 50.95, 55.85 (OMe), 100.9 (C-1b), 104.2 (C-1a), 172.45 (CO); MALDI-TOF: *m/z* 451 $(M + Na)^+$]. Cleavage of the methyl ester using lithium hydroxide[25] gave the free carboxylic acid (**88**) in 95 % yield; [^{1}H NMR δ_H: 3.49 (3 H, s, OMe), 4.31 ($J_{1a,2a}$ 7.8,

H-1a), 4.99 ($J_{1b,2b}$ 3.0, H-1b); δ_C: 55.9 (OMe), 100.9 (C-1b), 104.3 (C-1a), 173.9 (CO); MALDI-TOF: *m/z* 437 $(M + Na)^+$].

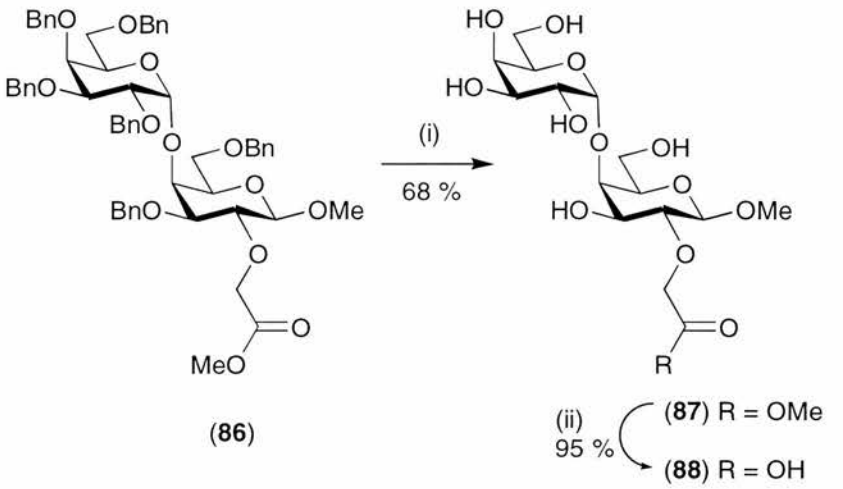


Figure 80: Reagents: i) EtOH, H_2, Pd-C; ii) LiOH, MeOH, H_2O.

2-Carboxymethyl galabioside (**88**) was formed in 7 steps in 23 % overall yield from methyl galactoside (**46**).

4.12 Analysis of the Di-functionalised Galabiose

4.12.1 Retrosynthetic analysis of di-functionalised galabiose (118): Figure 81

The first aim in the synthesis of the di-functionalised disaccharide (**118**) was to make the protected azido sugar (**95**) (**Figure 82**). The 2-OH would be free for the introduction of the methoxycarbonylmethyl group, the masked carboxylic acid function. Deprotection of the acetal followed by lactonisation would give alcohol (**105**), ready for glycosylation with tetra-benzyl galactosyl chloride (**57**). Deprotection of the benzyl groups and ester bond cleavage would yield the free disaccharide (**118**).

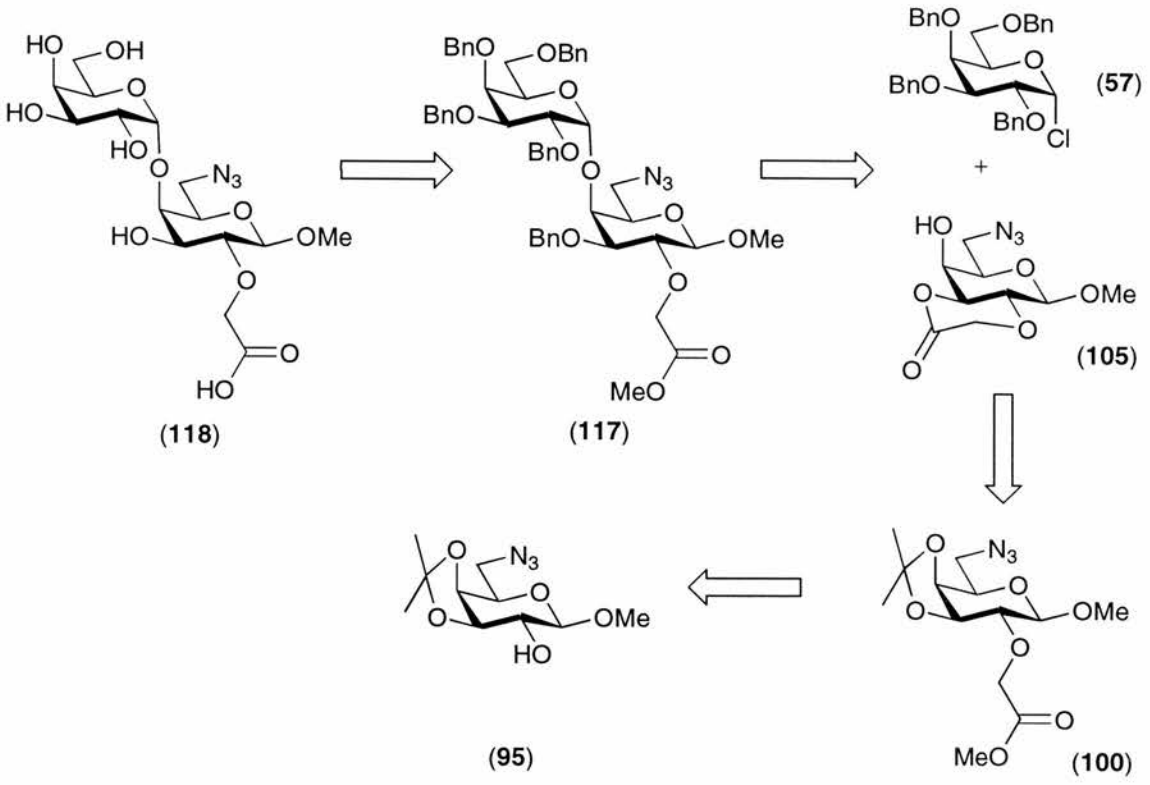


Figure 81: Retrosynthetic analysis of di-functionalised galabiose.

4.12.2 Retrosynthetic analysis of azido galactoside (95)

Two different synthetic strategies were considered to make the protected azido sugar (**95**). The first stage to form the silylated sugar (**89**). Benzoyl protection followed by deprotection of the silyl group would yield the 6-OH (**91**). Protection of the primary alcohol with a tosyl group followed by azide displacement and benzoyl deprotection would yield the azido (**94**). A simple acetal formation across the 3,4-diol would yield the azido galactoside (**95**). The second method made the acetal (**96**), followed by tosylation to give (**97**). Displacement with azide on the tosylate (**97**) would yield the desired azido galactoside (**95**).

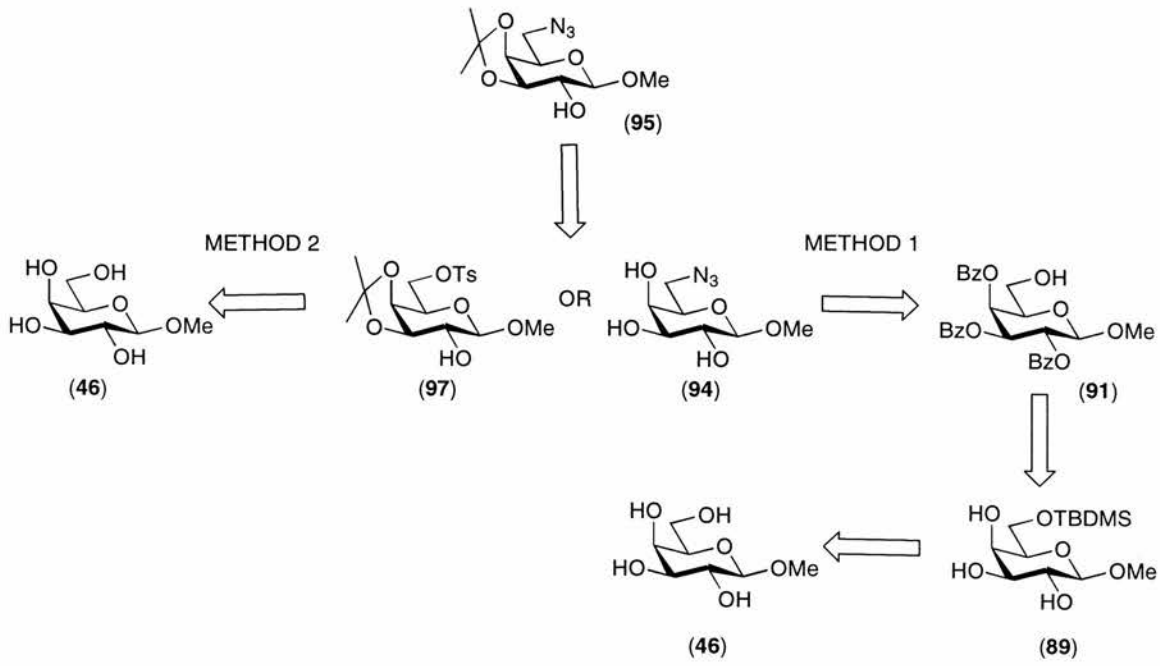


Figure 82: Retrosynthetic analysis of 3,4-acetal-6-azido-6-deoxy galactoside (**95**).

4.12.3 Method 1: Synthesis of azido galactoside (95)

This synthetic strategy incorporated simple protection and de-protection steps but was time consuming. Commercially available methyl galactoside (**46**) was selectively silylated using TBDMSCl/pyridine to give the 6-*O*-TBDMS sugar (**89**) in a 69 % yield.[59,60] A standard benzoylation of the silylated sugar gave fully protected sugar (**90**),[60,61] which was de-silylated with 80 % aqueous acetic acid to give the 2,3,4-tri-*O*-benzoyl sugar (**91**) in 69 % yield over 2 steps.[62] Tosylation of primary alcohol (**91**) was conducted according to a literature procedure.[33] Nucleophilic displacement with sodium azide gave the azide derivative (**93**) in good yield [IR: ν_{max}/cm^{-1} 2100 (N_3)].[33] A standard benzoyl deprotection was carried out to give the triol (**94**). Acetal formation on this azido sugar with iodine and acetone gave (**95**), formed in 7 steps and 29 % overall yield from methyl galactopyranoside (**46**).

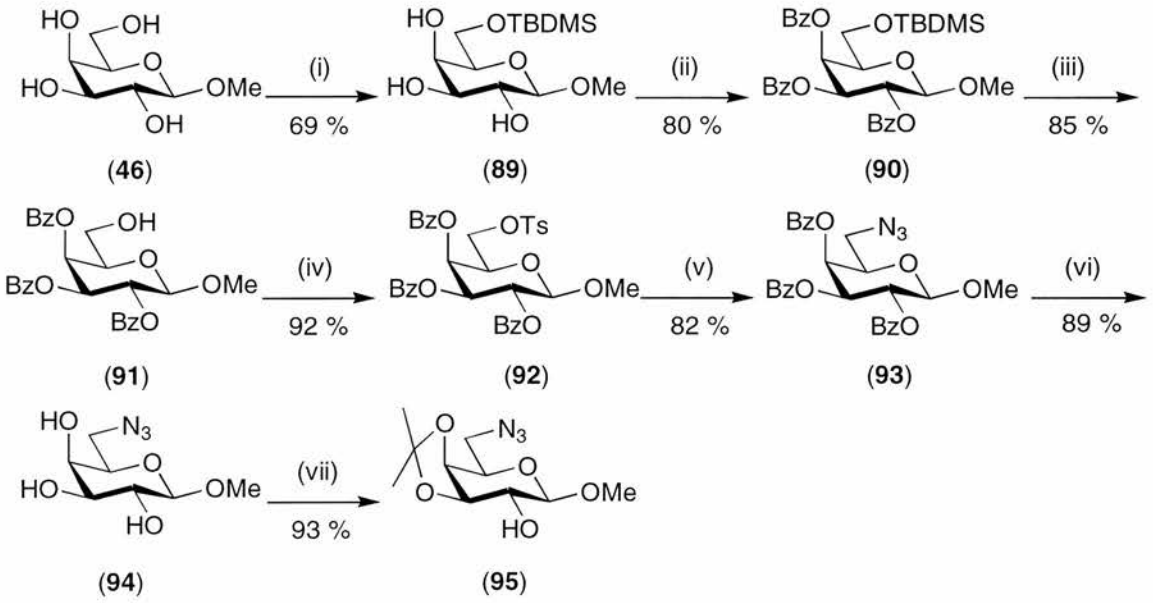


Figure 83: Synthesis of 3,4-acetal-6-azido-6-deoxy-galactose (**95**).
Reagents: i) TBDMSCl, pyridine; ii) BzCl, pyridine; iii) 80 % AcOH (aq); iv) *p*-TsCl, $(CH_3)_2CO$, pyridine; v) NaN_3, DMF; vi) MeOH, Na (trace); vii) I_2, $(CH_3)_2CO$.

4.12.4 Method 2: Synthesis of azido galactoside (95)

The methodology in this synthetic scheme was quicker than in method 1 but the initial protection may be problematic. Commercially available methyl galactoside was protected with 2,2-dimethoxypropane and *p*-toluenesulfonic acid to give acetal on protected sugar.[52] The syrup was dissolved in DCM and 50 % aqueous TFA was added to selectively deprotect the methoxyisopropyl group situated on the 6-OH, to give the 3,4-acetal (**96**) in 93 % yield.[63] Care is needed in the selective deprotection to avoid the 3,4-*O*-acetal also being removed. Tosylation followed by azide displacement gave the protected azido sugar (**95**),[33] which was reacted with methyl bromoacetate and sodium hydride to yield the target compound (**100**) in 5 steps in 59 % overall yield from methyl galactopyranoside (**46**).

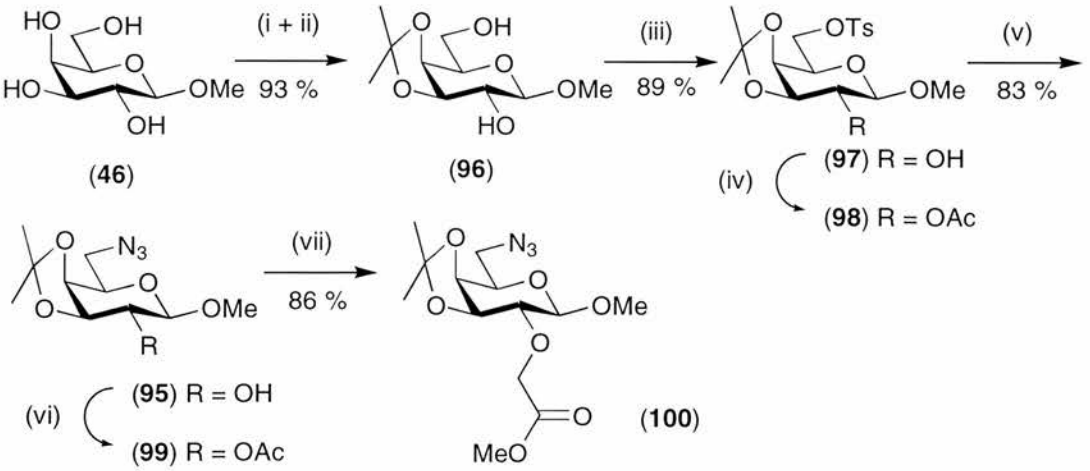


Figure 84: Reagents: i) *p*-TsOH, $Pr^{i}(OMe)_2$; ii) 50 % TFA (aq), DCM; iii) *p*-TsCl, $(CH_3)_2CO$, pyridine; iv) Ac_2O, pyridine; v) NaN_3, DMF; vi) Ac_2O, pyridine; vii) methyl bromoacetate, NaH, DMF.

Deprotection of the acetal was initially attempted with 80 % aqueous TFA but this gave a mixture of products (**Figure 85**).[35] The main product was the free carboxylic acid (**101**), in 69 % and the methyl ester (**103**), in 19 %. Small samples of the two compounds were acetylated with acetic anhydride/pyridine to help in assignment of the structures (**Table 7** for data). The main component did not show the methyl ester peak and once acetylated showed only one acetate, but H-3 and 4 were both moved downfield (**Table 7** for NMR data). This indicates that the lactone (**105**) had been formed under acetylation conditions.

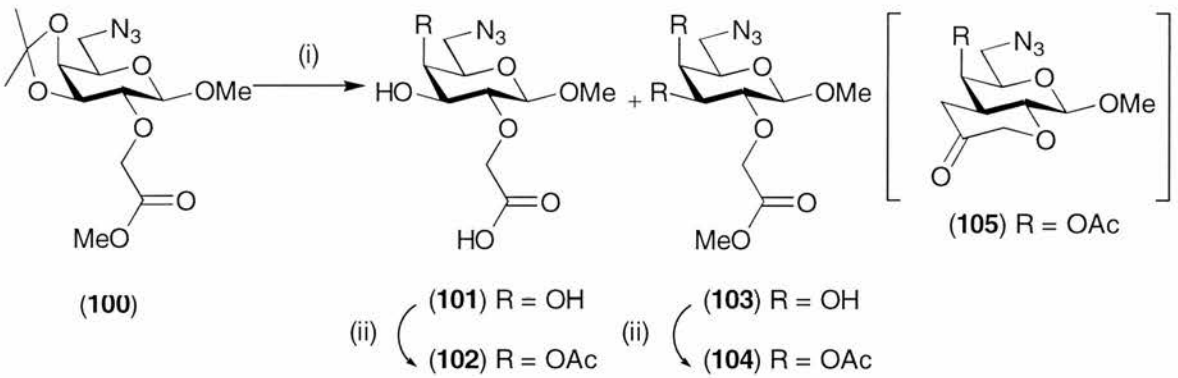


Figure 85: Isopropylidene acetal removal.
Reagents: i) 80 % TFA:DCM; ii) Ac_2O, pyridine.

	Me ester (**104**)	Carboxylic acid (**102**)
OAc	2.05, 2.15	2.05
OMe	3.73	-
H-3	5.01 $J_{2,3}$ 8.1, $J_{3,4}$ 3.5	4.50 $J_{2,3}$ 7.5, $J_{3,4}$ 3.3
H-4	5.30 $J_{3,4}$, $J_{4,5}$ 1.0	5.39 $J_{3,4}$

Table 7: Selective ^{1}H NMR data from the acetylation of the compounds after acetal removal.

The second attempt of acetal removal with 50 % aqueous HBF_4 and methanol gave the methyl ester (**103**) in 90 % yield (**Figure 86**).[53]

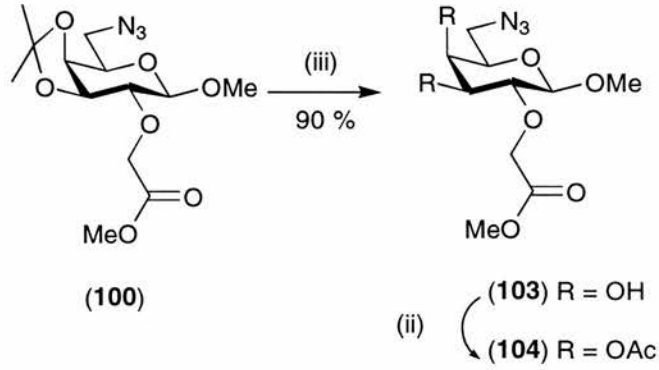
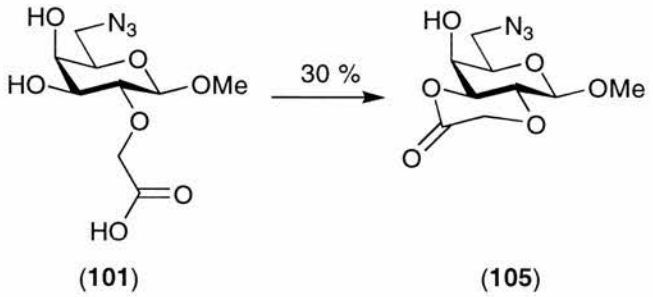


Figure 86: i) 50 % HBF_4 (aq), MeOH; ii) Ac_20, pyridine.

This compound was also acetylated to give (**104**) with acetic anhydride/pyridine to prove that the product was the methyl 6-azido-6-deoxy-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**103**); ^{1}H NMR 5.01 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 3.5, H-3), 5.30 (1 H, d, $J_{3,4}$, $J_{4,5}$ 1.0, H-4). Ester bond cleavage with LiOH gave the free carboxylic acid (**101**) in good yield.[57]

Numerous attempts to form the lactone (**105**) were made but most were unsuccessful. The lactone was formed once during a trial reaction using a DCC-type coupling (**Figure 87**).[64] NMR data shows changes in H-3 shift from 3.56 to 4.38 ppm and a carbonyl shift from 170.8 to 165.8 ppm. The reaction was low yielding and proved not to be a reliable method for blocking the hydroxyl function at C-3 of galactose.

Therefore a different protecting group strategy was required. Selective protection of the 3-OH was attempted next.

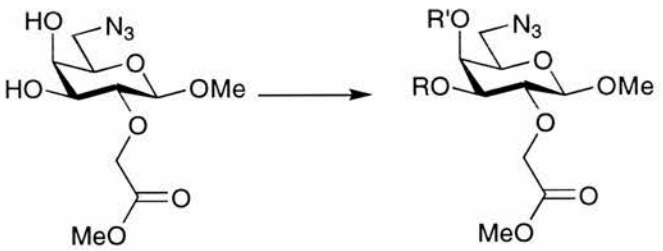


(**101**) (**105**)

Figure 87: 2,3-Lactone formation. Reagents: CMCT, DMF.

4.12.5 Selective protection of the 3-hydroxyl group of galactose (103).

The reactivity of the 3-OH (equatorial) and 4-OH (axial) of galactoside (**103**) are different because equatorial hydroxyls are more reactive, therefore selective protection of the H-3 should be possible.[65] Acetylation of the H-3 with acetic anhydride/pyridine at –20 ^{0}C gave a mixture of compounds, the 3-OAc (**106**), in 43 % and the 3,4-di-*O*-acetyl (**107**) in 31 % (**Figure 88**). The selectivity of the acetylation was poor, therefore it was decided to try the benzoylated analogues. Again in these trial reactions a mixture of products; mainly the mono (**108**) and the di benzoyl (**109**) compounds (**Figure 88**), were formed.



(**103**)

(**106**) R = Ac, R' = H
(**107**) R = Ac, R' = Ac
(**108**) R = Bz, R' = H
(**109**) R = Bz, R' = Bz

Figure 88: Selective protection of azido galactoside (**103**).
Reagents: Ac_2O, pyridine or BzCl, pyridine.

4.12.6 Selective benzyl protection of 3-OH of galactose (103).

The problems associated with selective protection with acetate and benzoate caused a change in protection strategy and tin acetal chemistry was considered (**Figure 88**).[54]

A tin acetal should form across the 3,4-diol on refluxing the sugar (**103**) with dibutyltin oxide in methanol. A benzyl bromide / tetrabutylammonium bromide promoted alkylation of the stannylene acetal was attempted. However, on purification the main product proved to be benzyl ester (**110**). The expected methyl ester functionality was lost (no signal in ^{1}H NMR). ^{1}H NMR showed that there were 10 protons present in the aromatic region and an extra CH_2 signal present [ν_{max}/cm^{-1} 1752 (*CO*OBn), 2100 (N_3) 3474 (OH); δ_H($CDCl_3$): 3.45 (OMe), 4.28 ($J_{1,2}$ 7.5, H-1), 4.40 (J 16.5 + 35.5, CH_2), 4.75 (J 11.5 + 34, CH_2), 5.18 (J 12.5 + 15, CH_2); δ_C($CDCl_3$): 56.7 (OMe), 103.8 (C-1), 170.1 (CO); MALDI-TOF: *m/z* 480 (M + Na)$^+$]. A small sample of benzyl ester (**110**) was acetylated with acetic anhydride/pyridine (**111**) to prove the tin acetal was opened to form the 3-OBn (**110**) and not the isomeric 4-OBn compound [NMR data 4-OH 3.79 ppm (**110**) to 4-OAc 5.36 ppm, ($J_{3,4}$ 3.3), (**111**)].

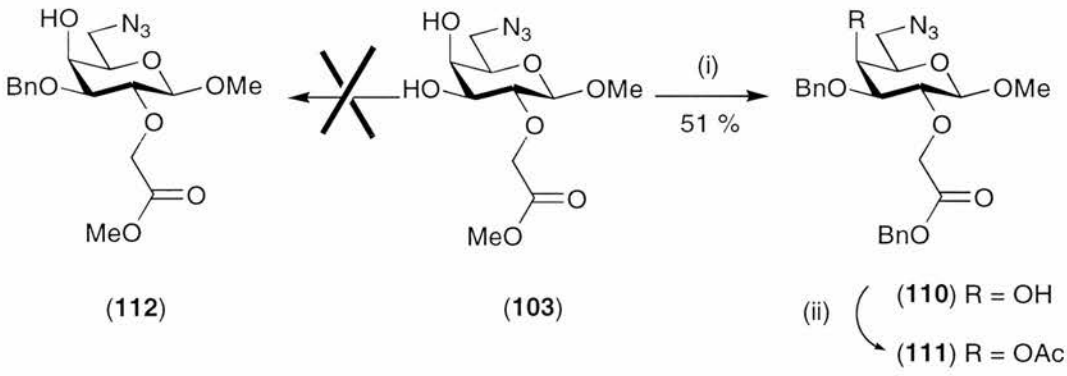


Figure 89: Selective benzylation of galactose (**103**).
Reagents: i)a) MeOH, Bu_2SnO; b) toluene, BnBr, Bu_4NBr; ii) Ac_2O, pyridine.

Clearly the methyl ester group had been cleaved and replaced by a benzyl group. There are at least two mechanistic explanations that could be postulated, which are shown in **Figure 90** and **Figure 91**. In both postulated mechanisms the benzyl

functionality at C-3 is formed in standard tin acetal chemistry, with the tin acetal forming between the 3,4-*trans* diol.

4.12.6.1 MECHANISM 1

Figure 90 suggests attack on the carbonyl function from the tin acetal therefore cleaving the methyl ester functionality. Once the ester is cleaved the carboxylic acid can then attack benzyl bromide to form a new ester bond.

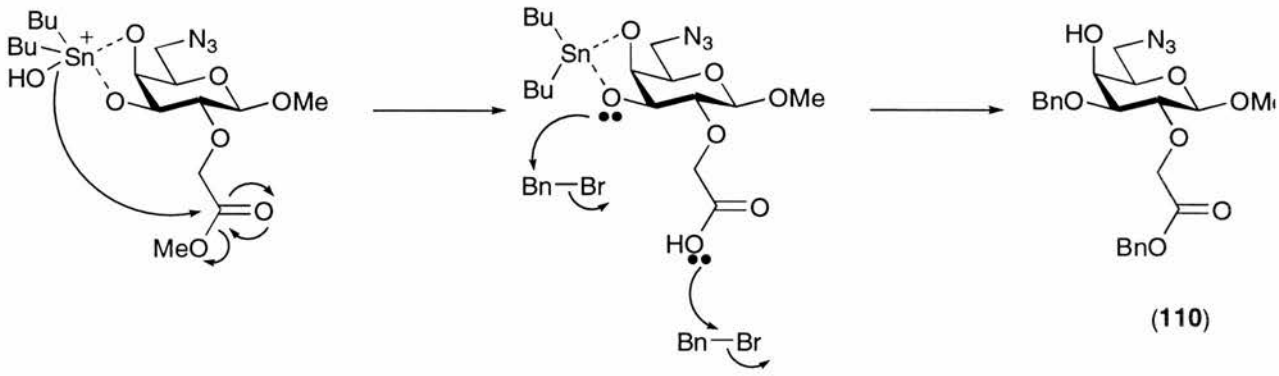


Figure 90: Postulated explanation of benzyl ester function.

4.12.6.2 MECHANISM 2

Figure 91 suggests that the nucleophilicity of O-3 would attack the carbonyl to form a lactone. The lactone is then opened with water to form the free carboxylic acid. Again the acid function could again attack benzyl bromide to form the new ester function.

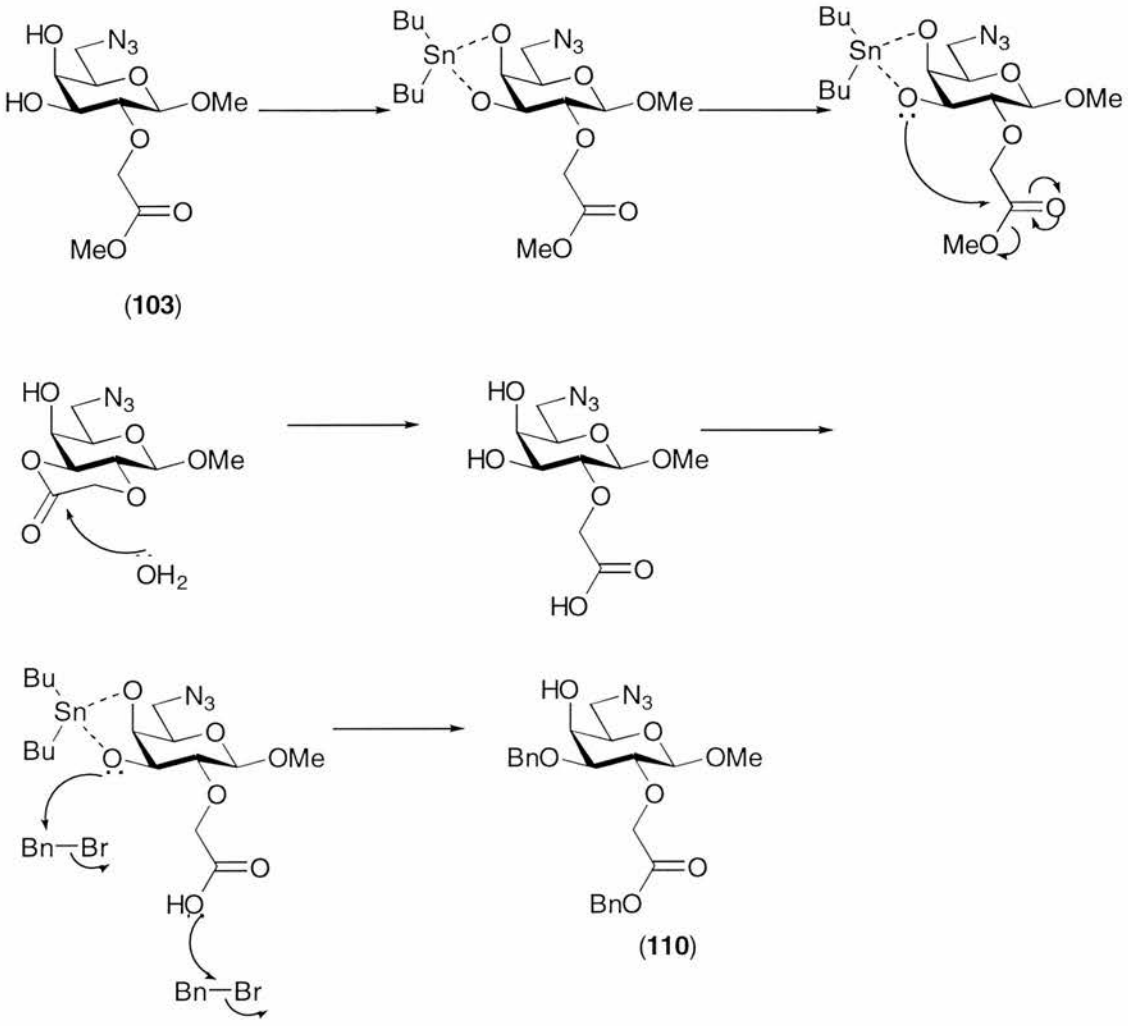


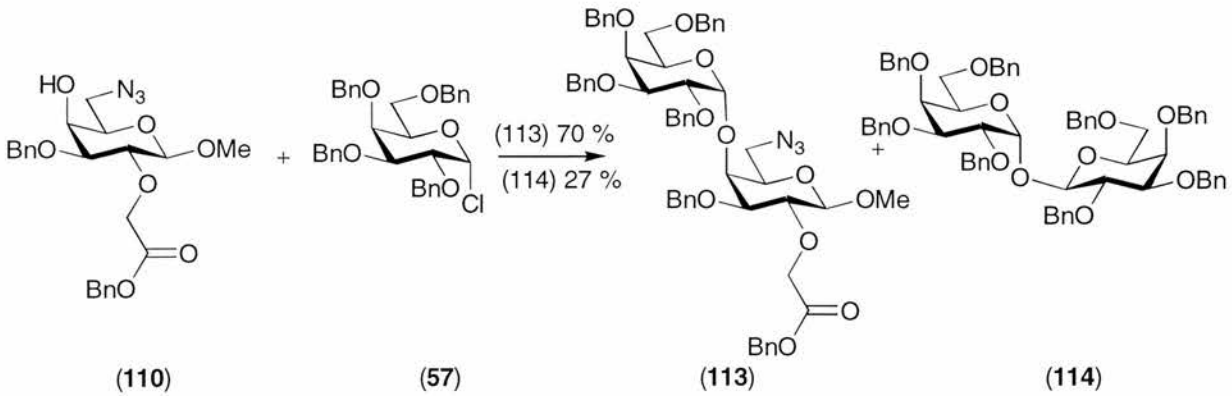
Figure 91: Postulated mechanism of benzyl ester function.

Figure 90 indicates the more likely explanation of how the methyl ester (**112**) was cleaved and the two-benzyl groups were incorporated (**110**).

4.12.7 Glycosylation of the benzyl ester (110) with tetra-benzyl galactosyl chloride (57)

Commercially available tetra-benzyl galactosyl hemi-acetal was converted to the anomeric chloride (**57**) *via* oxalyl chloride.[40] The glycosylation between the 6-azido compound (**110**) and the anomeric chloride (**57**) used a silver triflate promoted Koeings-Knorr method of glycosylation (**Figure 92**).[41-43] The 6-azide (**110**) (1 mol eq), chloride (**57**) (1.2 mol eq), collidine (1.83 mol eq) and 4 Å molecular sieves were stirred in anhydrous toluene:diethyl ether (1:2 ratio). This solvent mixture was chosen to give the best α-selectivity during glycosylation. Silver triflate (1.8 mol eq)

was then added and the reaction was stirred at –78 ^{0}C, slowly allowing the temperature to increase.



(**110**) (**57**) (**113**) (**114**)

Figure 92: Glycosylation reaction of benzyl ester (**110**) and the galactosyl chloride (**57**); Reagents: Toluene:Diethyl ether, AgOTf, Collidine, 4 Å MS, -78 ^{O}C.

Once the reaction was complete and purified, it was found that there were two compounds present. The first compound being the di-functionalised galabiose (**113**) [ν_{max}/cm^{-1} 1757 (*CO*OBn), 2100 (N_3); δ_H($CDCl_3$): 3.48 (OMe), 4.25 ($J_{1a,2a}$, 8.0, H-1a), 4.94 ($J_{1b,2b}$ 3.3, H-1b); δ_C($CDCl_3$): 56.9 (OMe), 100.8 (C-1b), 104.3 (C-1a), MALDI-TOF: *m/z* 1002 $(M + Na)^+$]. The second product from the glycosylation was the known 1,1'-linked disaccharide (**114**)[15]; [^{1}H NMR 5.23 ($J_{1b,2b}$ 3.0, H-1b); δ_C($CDCl_3$): 99.8 (C-1a), 103.7 (C-1b); MALDI-TOF: *m/z* 1085 $(M + Na)^+$].

4.12.8 Deprotection and Acetylation of 1,1'-linked disaccharide (114)

The benzyl protected 1,1'-linked disaccharide (**114**) was deprotected using catalytic hydrogenolysis (Pd on charcoal/H_2/acetic acid/ethanol)[47] to give the free 1,1'-linked disaccharide (**115**) in 89 % yield [$[\alpha]_D$ +58 (*c* 0.22, H_2O), (lit.[46], + 56); ^{1}H NMR: δ_H 4.20 ($J_{1a,2a}$ 7.8, H-1a), 5.15 ($J_{1b,2b}$ 3.0, H-1b)]. To help in assignment of the dissacharide, a small amount was acetylated with acetic anhydride/pyridine to give per-*O*-acetylated 1,1'-linked disaccharide (**116**).

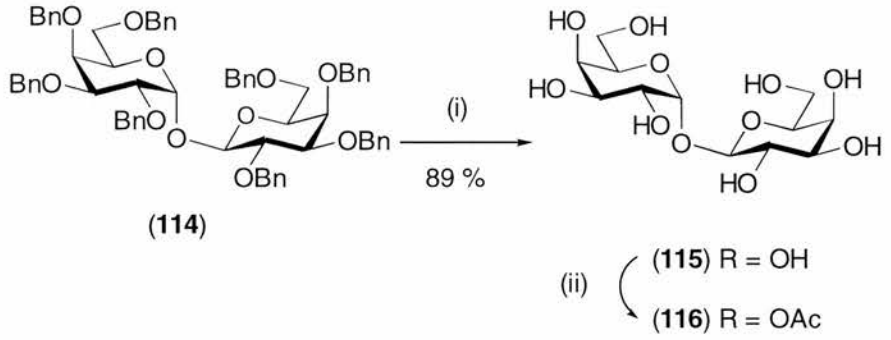


Figure 93: Reagents: i) EtOH, AcOH, H_2, Pd-C, ii) Ac_2O, pyridine.

4.12.9 Displacement of benzyl ester with methoxide on the di-functionalised galabiose (113)

A small amount of the di-functionalised sugar (**113**) was dissolved in methanol and a small amount of sodium metal was added to inspect if the ester bond was still present. The carbonyl functionality would be attacked by methoxide and displace the benzyl ester to form the methyl ester (**117**). The infra-red spectrum of the methyl ester (**117**) shows the presence of a carbonyl stretch at 1760 cm^{-1} and MALDI-TOF mass spectrometry gives a peak 926 $(M + Na)^+$.

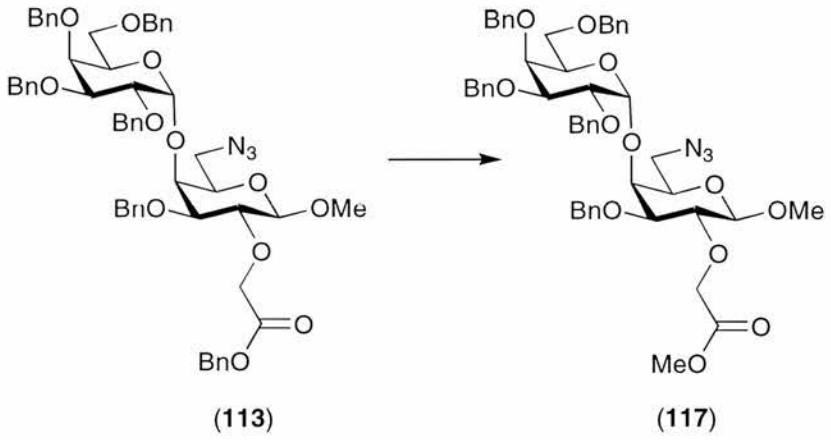
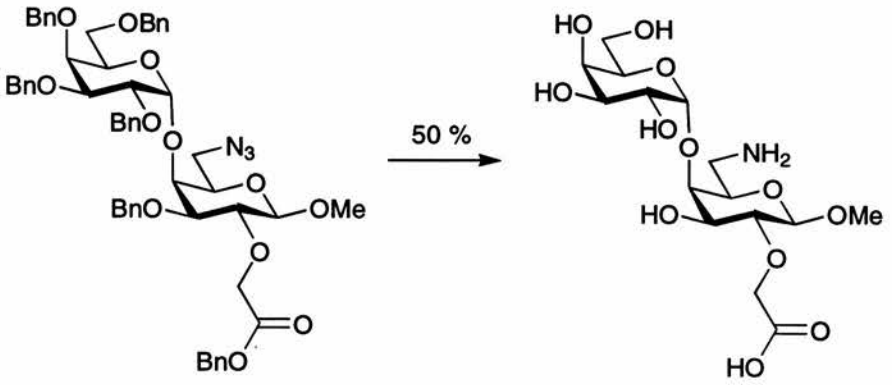


Figure 94: Reagents: MeOH, Na (metal).

4.12.10 Reduction of di-functionalised galabiose (113)

The benzyl protected galabiose (**113**) was deprotected using catalytic hydrogenolysis (Pd on charcoal/H_2/acetic acid/ethanol)[47] to give the free di-functionalised methyl galabiose (**118**), [^{1}H NMR; 4.35 ($J_{1a,2a}$7.6, H-1a), 5.01 ($J_{1b,2b}$ 3.4, H-1b); $\delta_C(CD_3OD)$: 101.7 (C-1b), 104.7 (C-1a); MALDI-TOF; $(M + Na)^+$ 436].



(113) (118)

Figure 95: Reagents: EtOH, AcOH, H_2, Pd-C.

Overall di-functionalised galabioside (**118**) was formed in 9 steps in 9.5 % overall yield from methyl galactoside (**46**).

4.13 References

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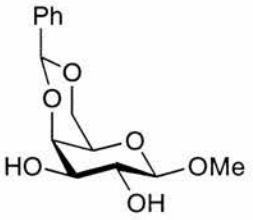
Chapter 5

Experimental - Verotoxin

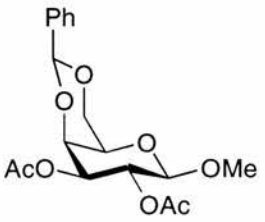
5.1 Synthetic Methods

General experimental procedures were described in Chapter 3 (**page 35**).

Methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (47)[1]



The title compound was prepared using a standard benzylidene acetal preparation. Methyl β-D-galactopyranoside (**46**) (3.0 g, 15.5 mmol) was dissolved in acetonitrile (40 ml) and stirred before benzaldehyde dimethyl acetal (2.32 ml, 18.5 mmol) and camphor sulfonic acid (0.72 g, 3.1 mmol) were slowly added. The reaction mixture was stirred at room temperature until TLC [DCM - MeOH, (6:1)] showed the reaction to be complete. The solution was neutralized with saturated sodium hydrogen carbonate solution and the organic extract was washed with water, dried and concentrated under reduced pressure to give a white solid. Crystallization gave methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**47**) as white prisms (4.15 g, 94.5 %); mp 186-188^{0}C (EtOH) (lit.[1], 187 ^{0}C); $[\alpha]_D$ –39.8 (*c* 0.93, $CHCl_3$) (lit.[1], -35.1); δ_H (CD_3OD): 3.26 (1 H, m, H-5), 3.62 (3 H, s, OMe), 4.0 (1 H, dd, $J_{5,6/6'}$ 1.5, $J_{6,6'}$ 12.6, H-6/6'), 4.12 (1 H, d, $J_{1,2}$ 6.9, H-1), 4.22 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 5.48 (1 H, s, PHC*H*), 7.23 (3 H, m, *Ph*CH), 7.41 (2 H, m, *Ph*CH); δ_C (CD_3OD) : 55.9, 66.6, 68.8, 70.8, 72.3, 76.1, 101.0, 104.4, 126.2, 127.5, 128.4, 138.3.

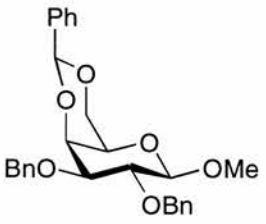


48

A sample of the benzylated acetal (**47**) was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^{1}H NMR spectroscopy to be methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**48**); δ_H ($CDCl_3$): 2.07 (6 H, s, 2

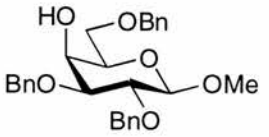
x OAc), 3.62 (4 H, m, OMe + H-5), 4.09 (1 H, dd, $J_{5,6/6'}$ 1.5, $J_{6,6'}$ 12.6, H-6/6'), 4.33 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.37 (1 H, m, H-4), 4.45 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.98 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 3.6, H-3), 5.37 (1 H, d, $J_{1,2}$, $J_{2,3,}$ H-2), 5.48 (1 H, s, PHC*H*), 7.23 (3 H, m, *Ph*CH), 7.41 (2 H, m, *Ph*CH).

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (49)[2]

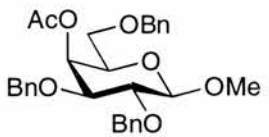


The title compound was prepared using a standard benzylation procedure.[2] A solution of methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**47**) (2.0 g, 7.09 mmol) was dissolved in DMF (10 ml) and cooled (0°C) before sodium hydride (60 % w/w suspension in mineral oil) (0.68 g, 17.02 mol) was slowly added. After 0.5 h BnBr 2.02 ml, 17.02 mmol) was slowly added and the solution was stirred until TLC [pet.ether - EtOAc, (3:1)] showed the reaction had gone to completion. The reaction mixture was cooled (0°C) before methanol (30 ml) was added. The solution was stirred for 0.5 h. and concentrated under reduced pressure to a syrup. The syrup was diluted with water and extracted with diethyl ether. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Crystallization gave methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**49**) (3.08 g, 94.0 %); mp 131-133°C (EtOH) (lit.[2], 130-132 °C); $[\alpha]_D$ +45 (*c* 1.1, $CHCl_3$) (lit.[2], +49); δ_H ($CDCl_3$): 3.28 (1 H, d, $J_{5,6/6'}$ 1.8, H-5), 3.60 (4 H, m,H-3, OMe), 3.85 (1 H, dd, $J_{1,2}$ 9.0, $J_{2,3}$ 7.8, H-2), 4.03 (1 H, dd, $J_{5,6/6'}$, $J_{6/6'}$ 12.0, H-6/6'), 4.13 (1 H, d, $J_{3,4}$ 3.6, H-4), 4.32 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6,6'), 4.34 (1 H, d, $J_{1,2}$, H-1), 4.78 (2 H, s, O*CH*$_2$) 4.81 (1 H, d, *J* 11.1, O*CH*$_2$), 4.93 (1 H, d, *J* 11.1, O*CH*$_2$), 5.51 (1 H, s, PhC*H*), 7.22-7.42 (13 H, m, *Ph*CH and 0CH_2*Ar*), 7.52 (2 H, m, *Ph*CH); δ_C($CDCl_3$): 57.1 (OMe), 66.2, 69.3, 72.1, 74.0, 76.1, 78.8, 79.1, 101.9 (C-1), 105.8, 127.3, 127.6, 127.8, 128.0, 128.1, 128.2, 128.4, 128.6, 138.1, 138.7, 138.9 (2 x OBn).

Methyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (50)[3]



Compound (**49**) was prepared using a selective acetal opening procedure.[3] A solution of methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**49**) (1.0 g, 2.16 mmol) was dissolved in anhydrous THF (20 ml) and stirred before sodium cyanoborohydride (475 mg, 7.57 mmol) and molecular sieves (4 A) (1.0 g) were added. Diethyl ether saturated with anhydrous HCl was added dropwise to the reaction mixture, until the methyl orange indicator turned pink. The resulting solution was stirred at room temperature until TLC [pet. ether – EtOAc, (3:1)] showed the reaction had gone to completion. The reaction mixture was diluted with DCM, filtered through Celite, washed with water and then with saturated aqueous $NaHCO_3$ solution. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; pet. ether – EtOAc, 12:1--2:1) gave two compounds, unreacted starting material (73 mg, 8 %) and methyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**50**) (697 mg, 70 %); $[\alpha]_D$ + 2.7 (*c* 1.0, $CHCl_3$) (lit.[3], + 3.0); $\delta_H(CDCl_3)$: 3.50 (1 H, dd, $J_{2,3}$ 9.3, $J_{3,4}$ 3.3, H-3), 3.57 (4 H, m, OMe, H-2), 3.64 (1 H, t, $J_{5,6,6'}$ 6.0, H-5), 3.74 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 12.0, H-6'/6), 3.82 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.26 (1 H, d, $J_{3,4}$, H-4), 4.28 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.58 (2 H, s, OCH_2Ar), 4.72 (2 H, s, OCH_2Ar), 4.72 (1 H, d, *J* 10.8, OCH_2Ar), 4.89 (1 H, d, *J* 10.8, OCH_2Ar), 7.25-7.4 (15 H, m, 3 x OCH$_2$*Ar*); $\delta_C(CDCl_3)$: 56.9, 66.9, 69.2, 72.4, 73.2, 73.7, 75.1, 79.0, 80.6, 104.7, 127.6, 127.8, 128.1, 128.3, 128.5, 137.9, 138.0, 138.7.

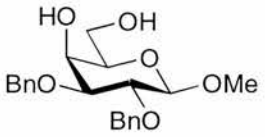


51

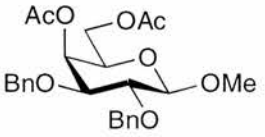
A sample of alcohol (**50**) was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^{1}H NMR specroscopy to be methyl 4-*O*-acetyl-2,3,6-tri-*O*-

benzyl-β-D-galactopyranoside (**51**) $\delta_H(CDCl_3)$: 1.23 (3 H, s, OAc), 3.50 (1 H, dd, $J_{2,3}$ 9.0, $J_{3,4}$ 3.3, H-3), 3.57 (4 H, m, OMe, H-2), 3.64 (1 H, t, $J_{5,6,6'}$ 6.0, H-5), 3.74 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 12.0, H-6'/6), 3.82 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.28 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.58 (2 H, s, OCH_2Ar), 4.72 (2 H, s, OCH_2Ar), 4.72 (1 H, d, *J* 11.0, OCH_2Ar), 4.89 (1 H, d, *J* 11.0, OCH_2Ar), 5.55 (1 H, d, $J_{3,4}$, H-4), 7.25-7.4 (15 H, m, 3 x OCH_2Ar),

Methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside (52)[2]



The title compound was prepared using a standard benzylidene acetal cleavage reaction.[2] A solution of compound (**49**) (200 mg, 0.43 mmol) in 80% aqueous acetic acid (5 ml) was heated (90°C) and stirred until TLC [pet.ether - EtOAc, (3:1)] showed the reaction to be complete (approx. 1 h). The resulting solution was coevaporated with toluene to give a syrup. Crystallisation gave the methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside (**52**) (135 mg, 83 %) mp 66-68°C (haxane) (lit.[2], 65-67°C); $[\alpha]_D$ +14 (*c* 0.82, $CHCl_3$) (lit.[2], +12); δ_H ($CDCl_3$): 2.87 (2 H, br s, 2 x OH), 3.58 (3 H, s, OMe), 3.44-3.85 (5 H, m, H-2, 3, 5, 6, 6'), 4.00 (1 H, d, $J_{3,4}$ 3.5, H-4), 4.30 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.75 (2 H, s, OCH_2), 4.76 (1 H, d, *J* 11.4, OCH_2), 4.90 (1 H, d, *J* 11.4, OCH_2), 7.22-7.42 (10 H, m, $0CH_2Ar$); $\delta_C(CDCl_3)$: 57.2 (OMe), 62.3, 67.4, 72.5, 73.9, 75.2, 78.9, 80.5, 104.9 (C-1), 127.8, 128.0, 128.1, 128.2, 128.3, 128.5, 137.9, 138.8.

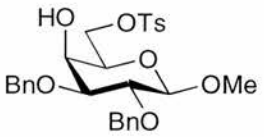


52a

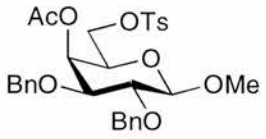
A sample of **52** was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^1H NMR specroscopy to be methyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (**52a**).δ_H ($CDCl_3$): 2.06 (3 H, s, CH_3), 2.12 (3 H, s,

CH_3), 3.58 (45H, m, OMe, H-2, 3), 3.77 (1 H, t, $J_{5,6/6'}$ 6.6, H-5), 4.19 (2 H, d, $J_{5,6/6'}$, H-6/6'), 4.17 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.53 (1 H, d, *J* 11.4, OCH_2), 4.73 (1 H, d, *J* 8.0, OCH_2), 4.77 (1 H, d, *J* 8.7, OCH_2), 4.85 (1 H, d, *J* 11.1, OCH_2), 5.50 (1 H, m, H-4), 7.22-7.42 (10 H, m, $0CH_2Ar$).

Methyl 2,3-di-*O*-benzyl-6-*O*-toluenesulfonyl-β-D-galactopyranoside (53)[2]



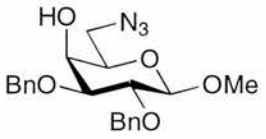
A standard tosylation procedure was preformed to give the title compound.[2] Methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside (**52**) (655 mg, 1.75 mmol) was stirred in anhydrous acetone (3.0 ml) and anhydrous pyridine (2.0 ml) until all the diol had dissolved. The solution was cooled in cold water, and with stirring, *p*-toluenesulfonyl chloride (402 mg, 2.11 mmol) was added in portions over 0.5 h. The reaction mixture was stirred overnight at room temperature then cooled in ice-water, water (3.0 ml) was added and on stirring the resulting syrup soon crystallised. The crystals were removed by filtration, washed with water and dried *in vacuo*. Recrystallization gave methyl 2,3-di-*O*-benzyl-6-*O*-toluenesulfonyl-β-D-galactopyranoside (**53**) as white needles (694 mg, 74.8 %); mp 86-88°C (aq MeOH), (lit.[2], 88-89 °C); $[\alpha]_D$ +5 (*c* 1.16, $CHCl_3$) (lit.[2], +4 +/-1°); $\delta_H(CDCl_3)$: 2.44 (3 H, s, Me of OTs), 3.50 (1 H, dd, $J_{2,3}$ 7.8, $J_{3,4}$ 3.3 , H-3), 3.52 (3 H, s, OMe), 3.55 (1 H, dd, $J_{1,2}$ 7.5, $J_{2,3}$, H-2), 3.65 (1 H, t, $J_{5,6/6'}$ 6.1, H-5), 3.91 (1 H, d, $J_{3,4}$, H-4), 4.25 (1 H, d, $J_{1,2}$, H-1), 4.25 (2 H, m, H-6, 6'), 4.69 (3 H, m, OCH_2Ar +OCH_2Ar), 4.89 (1 H, d, *J* 11.1, OCH_2Ar), 7.33 (12 H, m, Ar), 7.82 (2 H, d, *J* 8.1, OTs); $\delta_{II}(CDCl_3)$: 21.9, 57.8 (OMe), 66.2, 68.5, 71.9, 72.9, 75.2, 78.5, 80.1, 104.6, 127.8, 128.2, 128.4, 128.6, 128.8, 129.2, 129.8, 133.0, 137.8, 138.5, 145.8.



54

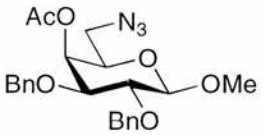
A sample of tosylate (**53**) was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^{1}H NMR specroscopy to be methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-tosyl-β-D-galactopyranoside (**54**); $\delta_H(CDCl_3)$: 2.05 (3H, s, OAc), 2.44 (3 H, s, Me of OTs), 3.50 (1 H, dd, $J_{2,3}$ 7.8, $J_{3,4}$ 3.3 , H-3), 3.52 (3 H, s, OMe), 3.55 (1 H, dd, $J_{1,2}$ 7.5, $J_{2,3}$, H-2), 3.65 (1 H, t, $J_{5,6/6'}$ 6.1, H-5), 4.25 (1 H, d, $J_{1,2}$, H-1), 4.25 (2 H, m, H-6, 6'), 4.69 (3 H, m, OCH_2Ar +OCH_2Ar), 4.89 (1 H, d, *J* 11.1, OCH_2Ar), 5.30 (1 H, d, $J_{3,4}$, H-4), 7.33 (12 H, m, Ar), 7.82 (2 H, d, *J* 8.1, OTs).

Methyl 6-azido-2,3-di-*O*-benzyl-6-deoxy-β-D-galactopyranoside (55)



A standard azide displacement was utilised in the formation of the title compound.[4] A solution of compound (**54**) (610 mg, 1.15 mmol) in DMF (5 ml) containing suspended sodium azide (1.12g, 17.3 mmol) was heated under reflux (approx. 4 h). The solution was allowed to cool, diluted with water (50 ml) and extracted with diethyl ether. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; hexane-EtOAc, 12-1→2-1) gave two compounds, the unreacted starting material (42 mg, 9%) and *methyl 6-azido-2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside* (**55**) as crystals (355 mg, 77%); mp 79-81°C (EtOAc); $[\alpha]_D$ –36.9 (*c* 0.91, $CHCl_3$); ν_{max}/cm^{-1} 2100 (N_3); (Found: C, 63.21; H, 6.30; N, 10.18 %. $C_{21}H_{25}N_3O_5$ requires C, 63.14; H, 6.31; N, 10.52 %); $\delta_H(CDCl_3)$: 3.29 (1 H, dd, $J_{5,6/6'}$ 8.4, $J_{6,6'}$ 12.9, H-6/6'), 3.50 (1 H, dd, $J_{2,3}$ 7.8, $J_{3,4}$ 3.3, H-3), 3.55 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$, H-2), 3.60 (3 H, s, OMe), 3.65 (1 H, t, $J_{5,6/6'}$, H-5), 3.77 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.86 (1 H, d, $J_{3,4}$, H-4), 4.32 (1 H, d, $J_{1,2}$, H-1), 4.72 (3 H, m, OCH_2Ar, OCH_2Ar), 4.94 (1 H, d, *J* 11.1,

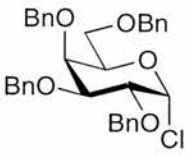
OCH_2Ar), 7.33 (10 H, m, Ar); δ_C($CDCl_3$): 51.2 (C-6), 57.0 (OMe), 67.3, 72.8, 73.9, 75.2, 78.8, 80.2, 104.8 (C-1), 127.8, 128.0, 128.2, 128.4, 128.45, 128.5, 128.6, 128.7 (2 x OBn), 137.8 (quat. C), 138.7 (quat. C); MALDI-TOF: *m/z* 422 $(M + Na)^+$,($C_{62}H_{66}O_{13}SNa$ requires *m/z* 422).



56

A sample of azide (**55**) was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^{1}H NMR specroscopy to be *methyl 4-O-acetyl-6-azido-2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside* (**56**); δ_H($CDCl_3$): 2.1 (3 H, s, OAc), 3.29 (1 H, dd, $J_{5,6/6'}$ 8.4, $J_{6,6'}$ 12.9, H-6/6'), 3.50 (1 H, dd, $J_{2,3}$ 7.8, $J_{3,4}$ 3.3, H-3), 3.55 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$, H-2), 3.60 (3 H, s, OMe), 3.65 (1 H, m, H-5), 3.77 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.32 (1 H, d, $J_{1,2}$, H-1), 4.72 (3 H, m, OCH_2Ar, OCH_2Ar), 4.94 (1 H, d, *J* 11.1, OCH_2Ar), 5.28 (1 H, d, $J_{3,4}$, H-4), 7.33 (10 H, m, Ar)

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl chloride (57)[5]



A solution of commercially available 2,3,4,6-tetra-*O*-benzyl-galactopyranose (500 mg, 0.93 mmol) was dissolved in anhydrous DCM (20 ml) and cooled (O^0C) before oxalyl chloride (242 µl, 2.8 mmol) and DMF (338 µl, 4.6 mmol) were slowly added. The reaction mixture was stirred under nitrogen until TLC [pet. ether – EtOAc, (3:1)] showed the reaction to be complete (approx. 4 h). The solution was then diluted with water and extracted with DCM. The organic extract was dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; pet. ether – EtOAc, 12:1→2:1) gave the labile 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl chloride (**57**) as a syrup (500 mg, 97 %); δ_H($CDCl_3$): 3.58, 4.00,

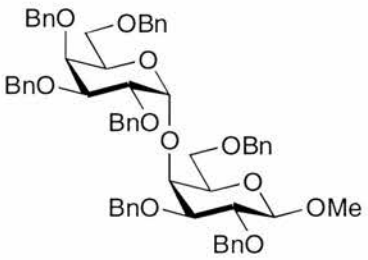
4.30 (3 x 2 H, 3 x m, H-2, 3, 4, 5, 6, 6'), 4.51 (1 H, d, *J* 12.0, OCH_2Ar), 4.53 (1 H, d, *J* 12.0, OCH_2Ar), 4.60 (1 H, d, *J* 11.4, OCH_2Ar), 4.76 (3 H, m, OCH_2Ar, OCH_2Ar), 4.83 (1 H, d, *J* 11.4,OCH_2Ar), 4.97 (1 H, d, *J* 8.1,OCH_2Ar), 6.18 (1 H, d, $J_{1,2}$ 3.6, H-1), 7.52 - 7.82 (20 H, m, 4 X OCH$_2$*Ar*). δ_C($CDCl_3$) 68.1, 72.5, 73.1, 73.5, 78.6, 74.6, 75.1, 76.3, 78.4, 95.1 (H-1), 127.8-128.8 (Ar), 137.8, 138, 138.4, 138.6 (4 x OBn).

Glycosylation Reactions

Glycosylation reactions were performed utilizing a standard silver triflate promoted procedure.[6-9] Reactions were typically carried out on 1 mmol acceptor. A typical procedure is as follows:

To a mixture of acceptor [(**50**), (**53**), (**55**)] (1.0 mol eq), collidine (1.83 mol eq), molecular sieves (4 Å), and anhydrous solvent (3 ml) was added a solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**57**) (1.2 mol eq) in anhydrous solvent (1 ml). The mixture was cooled (–78^{O}C) and stirred for 1 h under dry nitrogen, then silver triflate (1.8 mol eq) was quickly added. The mixture was stirred under nitrogen for approximately 2 h, allowing the temperature to slowly rise to room temperature. Once TLC [pet. ether – EtOAc, (3:1)] indicated the reaction to be complete, collidine (5 µl, 0.04 mmol, 0.5 mol eq) was added and stirring was continued for approx. 0.5 h. The mixture was diluted with DCM, filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 1 M HCl and extracted with DCM. The combined organic extracts were washed with saturated $NaHCO_3$ solution and water, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; toluene – EtOAc, 20:1→8:1) gave the glycosylated product.

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-2,3,6-tri-*O*-benzyl–β-D-galactopyranoside (60) – from glycosylation in toluene:ether ratio. Column chromatography (silica gel; toluene – EtOAc, 20:1→8:1) gave the glycosylated product (**60**) in 75 % yield.



$[\alpha]_D$ +31.5 (*c* 1.65, $CHCl_3$); (Found: C, 75.8; H, 6.74 %. $C_{62}H_{66}O_{11}$ requires C, 75.43; H, 6.74 %); $\delta_H(CDCl_3)$: 3.18 (1 H, dd, $J_{5b,6b/6b'}$ 4.9, $J_{6b,6b'}$ 8.4, H-6b/6b'), 3.31 (1 H, dd, $J_{2a,3a}$ 7.2, $J_{3a,4a}$ 2.8, H-3a), 3.41 (1 H, t, $J_{5a,6a/6a'}$ 6.65, $J_{6a,6a'}$ 6.65, H-5a), 3.48 (3 H, s, OMe), 3.44-3.51 (2 H, m, H-6a/6a', H-6b/6b'), 3.59 (1 H, $J_{1a,2a}$ 8.3, $J_{2a,3a}$, H-2a), 3.90 (1 H, dd, $J_{5a,6a/6a'}$, H-6a/6a'), 3.95 (1 H, d, $J_{3a,4a}$, H-4a), 3.99-4.05 (3 H, m, H-2b, 3b, 4b), 4.07 (1 H, d, *J* 11.6, OCH_2Ar), 4.10 (1 H, d, *J* 11.6, OCH_2Ar), 4.16 (1 H, d, *J* 11.6, OCH_2Ar), 4.18 (1 H, d, $J_{1a,2a}$, H-1a), 4.20 (1 H, d, *J* 12.2, OCH_2Ar), 4.35 (1 H, m, H-5b), 4.47 (1 H, d, *J* 11.1, OCH_2Ar), 4.61 (1 H, d, *J* 12.2, OCH_2Ar), 4.69 (5 H, m, 2.5 x OCH_2Ar), 4.80 (3 H, m, 1.5 x OCH_2Ar), 4.96 (1 H, d, $J_{1b,2b}$ 3.3, H-1b), 7.04 - 7.36 (35 H, m, 7 x OCH_2Ar); $\delta_C(CDCl_3)$: 57.1 (OMe), 67.9, 68.0, 69.3, 72.3, 72.4, 73.0, 73.2, 73.6, 73.8, 74.7, 74.8, 74.9, 75.1, 77.0, 78.8, 79.1, 80.8, 100.5 (C-1b), 104.9 (C-1a), 127.3, 127.4, 127.4, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.4, 138.0, 138.1, 138.6, 138.7, 138.9 (7 x OBn); MALDI-TOF: *m/z* 1009 $(M + Na)^+$,($C_{62}H_{66}O_{11}Na$ requires *m/z* 1009)

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-2,3,-di-*O*-benzyl-6-*O*-tosyl–β-D-galactopyranoside (61) - from glycosylation in toluene:ether ratio. Column chromatography (silica gel; toluene – EtOAc, 20:1→8:1) gave the glycosylated product (**61**) in 79 % yield.

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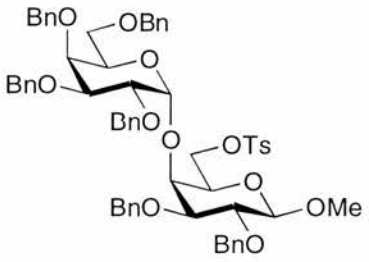

$[\alpha]_D$ +13.9 (*c* 0.52, $CHCl_3$); (Found: C, 70.57; H, 5.94 %. $C_{62}H_{66}O_{13}S$ requires C, 70.84; H, 6.33 %); $\delta_H(CDCl_3)$: 2.39 (3 H, s, OArC*H*$_3$), 3.19 (1 H, dd, $J_{5b,6b/6b'}$ 4.9, $J_{6b,6b'}$ 8.4, H-6b/6b'), 3.35 (1 H, dd, $J_{2a,3a}$ 7.2, $J_{3a,4a}$ 2.8, H-3a), 3.48 (3 H, s, OMe), 3.47-3.62 (2 H, m, H-5a, H-6b/6b'), 3.59 (1 H, $J_{1a,2a}$ 8.3, $J_{2a,\ 3a}$, H-2a), 3.95 (1 H, d, $J_{3a,4a}$, H-4a), 3.99-4.05 (3 H, m, H-2b, 3b, 4b), 4.12 (1 H, d, *J* 11.5, O*CH*$_2$Ar), 4.18 (1 H, d, *J* 11.5, O*CH*$_2$Ar), 4.22 (1 H, d, $J_{1a,2a}$, H-1a), 4.30 (1 H, m, H-6a/6a'), 4.35 (1 H, m, H-5b), 4.59 (1 H, m, H-6a/6a'), 4.54 (3 H, m, 1.5 x O*CH*$_2$Ar), 4.60 (1 H, d, *J* 12.5, O*CH*$_2$Ar), 4.70 (4 H, m, 2 x *OCH*$_2$Ar), 4.83 (1 H, d, *J* 11.0, O*CH*$_2$Ar), 4.87 (1 H, d, *J* 13.0, O*CH*$_2$Ar), 4.92 (1 H, d, $J_{1b,2b}$ 3.3, H-1b), 7.04-7.36 (32 H, m, 7 x OCH$_2$*Ar* and OTs), 7.63 (2 H, d, OTs); $\delta_C(CDCl_3)$: 28.4 ($ArCH_3$), 57.1 (OMe), 66.2, 68.0, 68.1, 69.6, 72.4, 72.5, 73.15, 73.5, 74.7, 74.8, 74.9, 76.2, 76.8, 78.5, 79.1, 80.3, 100.0 (C-1b), 105.0 (C-1a), 127.3, 127.5, 127.7, 127.8, 127.9, 128.1, 128.3, 129.8, 133.0, 138.0, 138.4, 138.6, 138.7, 138.9 (6 x OBn); MALDI-TOF: *m/z* 1073 $(M + Na)^+$,($C_{62}H_{66}O_{13}SNa$ requires *m/z* 1073)

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-azido-2,3,-di-*O*-benzyl-6-doexy–β-D-galactopyranoside (62) - from glycosylation in toluene:ether ratio. Column chromatography (silica gel; toluene – EtOAc, 20:1→8:1) gave the glycosylated product (**62**) in 81 % yield.

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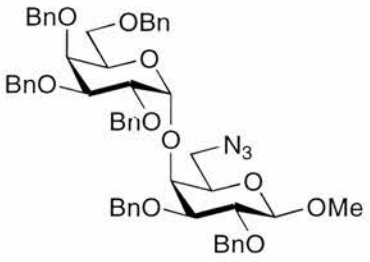
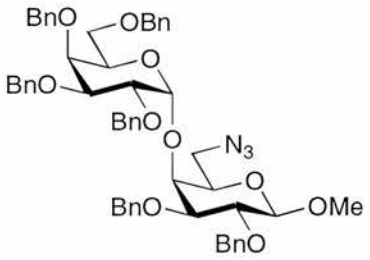


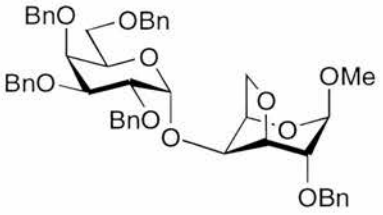
$[\alpha]_D$ +36.6 (*c* 0.95, $CHCl_3$); (Found: C, 70.52; H, 6.05; N, 4.29 %. $C_{55}H_{59}N_3O_{10}.H_2O$ requires C, 70.27; H, 6.34; N, 4.47 %); ν_{max}/cm^{-1} 2099 (N_3); $\delta_H(CDCl_3)$: 3.23 (1 H, dd, $J_{5a,6a/6a'}$ 4.9, $J_{6a,6a'}$ 8.4, H-6a/6a'), 3.30 (1 H, dd, $J_{5b,6b/6b'}$ 4.9, $J_{6b,6b'}$ 8.4, H-6b/6b'), 3.39 (1 H, dd, $J_{2a,3a}$ 7.2, $J_{3a,4a}$ 2.8, H-3a), 3.42 (1 H, m, H-5a), 3.57 (1 H, t, $J_{5b,6b/6b'}$, $J_{6b,6b'}$, H-6b/6b'), 3.58 (3 H, s, OMe), 3.62 (1 H, $J_{1a,2a}$ 8.3, $J_{2a,3a}$, H-2a), 3.72 (1 H, dd, $J_{5a,6a/6a'}$, $J_{6a,6a'}$, H-6a/6a'), 3.80 (1 H, d, $J_{3a,4a}$, H-4a), 4.05-4.12 (3 H, m, H-2b, 3b, 4b), 4.20 (1 H, d, *J* 11.7, OCH_2Ar), 4.24 (1 H, d, *J* 11.7, OCH_2Ar), 4.26 (1 H, d, $J_{1a,2a}$, H-1a), 4.36 (1 H, m, H-5b), 4.55 (1 H, d, *J* 12.1, OCH_2Ar), 4.66 (1 H, d, *J* 11.4, OCH_2Ar), 4.77 (4 H, m, OCH_2Ar), 4.86 (1 H, d, *J* 11.1, OCH_2Ar), 4.91 (3 H, m, 1.5 x OCH_2Ar), 4.92 (1 H, d, $J_{1,2}$ 3.3, H-1b), 7.04-7.36 (30 H, m, 6 x OCH_2Ar); $\delta_C(CDCl_3)$: 50.9 (C-6a), 57.2 (OMe), 68.1, 69.7, 72.3, 72.7, 73.3, 74.5, 74.7, 75.0, 75.1, 76.4, 76.7, 77.1, 78.8, 79.1, 80.5, 100.0 (C-1b), 105.0 (C-1a), 127.6, 127.7, 127.75, 127.8, 127.95, 128.0, 128.15, 128.2, 128.3, 128.35, 128.45, 128.5, 138.2, 138.7, 138.8, 139.1 (6 x OBn). MALDI-TOF: *m/z* 944 $(M + Na)^+$,($C_{55}H_{59}N_3O_{10}Na$ requires *m/z* 944).

	COMPOUND (α : β ratio) and YIELD		
	H-6a-OBn (**60**)	H-6a-OTs (**61**)	H-6a-N_3 (**62**)
DCM	2:1 (77 %)	N.D	N.D.
TOLUENE	4.5:1 (76 %)	N.D.	N.D.
TOLUENE / ETHER (1:2)	10:1 (82 %)	7:1 (90 %)	10:1 (89 %)
TOLUENE / DIOXANE (1:3)	1:1 (41 %)	N.D.	N.D.
ETHER	3:1 (70 %)	7:1 (89 %)	4:1 (81 %)
ETHER / DCM (2:1)	2:1 (67 %)	4:1 (84 %)	10:1 (89 %)

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-azido-2,3,-di-*O*-benzyl-6-deoxy–β-D-galactopyranoside (**62**) – from azide displacement of the tosylate (**61**)



62

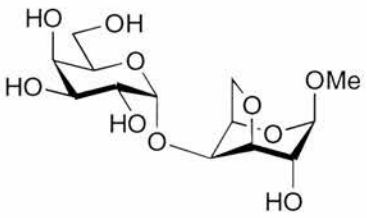


63

The azide sugar (**62**) was formed using a standard azide displacement procedure.[5] A solution of compound (**61**) (114 mg, 0.11 mmol) in DMF (2 ml) containing suspended sodium azide (71 mg, 1.1 mmol) was heated under reflux for 4 h. The solution was allowed to cool, diluted with water (2 ml) and extracted with diethyl ether. The organic extract was washed with salt solution, water, dried (Na_2SO_4) and concentrated under reduced pressure to a give *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-3,6-anhydro-2-O-benzyl–β-D-galactopyranoside* (**63**) (27.7 mg, 30 %); $[\alpha]_D$ +23.9 (*c* 0.75, $CHCl_3$); $\delta_H(CDCl_3)$: 3.37 (1 H, s, OMe), 3.51 (2 H, dd, $J_{5b,6b/6b'}$ 6.6, $J_{6b,6b'}$ 6.6, H-6b/6b'), 3.78 (1 H, d, $J_{2a,3a}$ 4.9, H-3a), 3.89 (1 H, dd, $J_{2b,3b}$ 9.3, $J_{3b,4b}$ 3.3, H-3b), 3.94 (1 H, dd, $J_{5a,6a/6a'}$ 10.6, $J_{6a,6a'}$ 10.6, H-6a/6a'), 3.96 (1 H, d, $J_{3b,4b}$, 1.6, H-4b), 4.01 (1 H, dd, $J_{1b,2b}$ 3.6, $J_{2b,3b}$, H-2b), 4.05 (1 H, m, H-5b), 4.12 (1 H, dd, $J_{5a,6a/6a'}$, $J_{6a,6a'}$, H-6a/6a'), 4.31 (1 H, m, H-5a), 4.34 (1 H, s, H-4a),

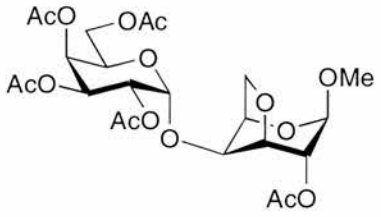
4.35 (1 H, d, *J* 11.5, O*CH*$_2$Ar), 4.38 (1 H, d, *J* 11.7, O*CH*$_2$Ar), 4.44 (1 H, d, *J* 11.6, O*CH*$_2$Ar), 4.54 (1 H, s, H-1a), 4.58 (1 H, d, $J_{2a,3a}$, H-2a), 4.58 (2 H, m, O*CH*$_2$Ar), 4.63 (1 H, d, *J* 12.0, O*CH*$_2$Ar), 4.71 (1 H, d, *J* 11.7, O*CH*$_2$Ar), 4.80 (2 H, m, O*CH*$_2$Ar), 4.94 (1 H, d, *J* 11.2, O*CH*$_2$Ar), 4.98 (1 H, d, $J_{1b,2b}$, H-1b), 7.04-7.36 (30 H, m, 6 x OCH$_2$*Ar*); δ_C($CDCl_3$) 55.6 (OMe), 69.1, 69.5, 70.7, 72.1, 72.9, 73.3, 74.7, 74.9, 75.9, 76.4, 77.5, 77.9, 78.7, 79.8, 82.3, 98.3 (C-1b), 101.3 (C-1a), 127.3, 127.4, 127.5, 127.6, 127.7, 128.1, 128.3, 129.6, 137.5, 137.9, 138.5, 138.6 (5 x OBn); MALDI-TOF: *m/z* 811 (M + Na)$^+$,($C_{48}H_{52}O_{10}Na$ requires *m/z* 811) and *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-6-azido-2,3,-di-O-benzyl-6-deoxy–β-D-galactopyranoside* (**62**) as a syrup (24 g, 28 %); $[\alpha]_D$ +36.9 (*c* 0.97, $CHCl_3$); ν_{max}/cm^{-1} 2099 (N_3). As data reported on **page 125**.

Methyl α-D-galactopyranosyl-(1-4)-3,6-anhydro-β-D-galactopyranoside (64)



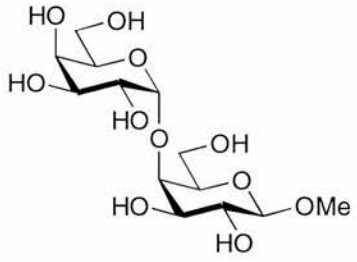
The anhydro sugar (**63**) was deprotected using a standard hydrogenation procedure.[10] The anhydro sugar (**63**) (25 mg, 0.03 mmol) and palladium on charcoal (25 mg; 10 % w/w) in EtOH (1 ml) and stirred under an atmosphere of hydrogen. After 12 h the mixture was filtered through Celite and concentrated under reduced pressure to give *methyl α-D-galactopyranosyl-(1-4)-3,6-anhydro-β-D-galactopyranoside* (**64**) (12.0 mg, 93 %) $[\alpha]_D$ +30.8 (*c* 0.65, MeOH); δ_H(CD_3OD): 3.28 (2 H, m, H-6b/6b'), 3.31 (3 H, s, OMe), 3.67 (1 H, dd, $J_{2b,3b}$ 7.5, $J_{3b,4b}$ 3.0, H-3b), 3.71 (1 H, d, $J_{3b,4b}$, H-4b), 3.77 (1 H, $J_{1b,2b}$ 4.0, $J_{2b,3b}$, H-2b), 3.87(1 H, s, H-5b), 3.91 (1 H, d, $J_{2a,3a}$ 6.5, H-3a), 3.92 (1 H, d, $J_{5a,6a/6a'}$, $J_{6a,6a'}$, H-6a/6a'), 4.11 (1 H, d, $J_{5a,6a/6a'}$, $J_{6a,6a'}$, H-6a/6a'), 4.37 (1 H, d, $J_{2a,3a,}$, H-2a), 4.38 (1 H, m, H-5a), 4.43 (1 H, s, H-1a), 5.01 (1 H, d, $J_{1b,2b}$, H-1b); δ_C(CD_3OD): 54.5 (OMe), 61.3, 68.5, 69.5, 70.2, 71.3, 72.6, 75.5, 76.5, 79.6, 99.1 (C-1), 103.4 (C-1); MALDI-TOF: *m/z* 361 (M + Na)$^+$, ($C_{13}H_{22}O_{10}Na$ requires *m/z* 361).

Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1-4)-2-*O*-acetyl-3,6-anhydro–β-D-galactopyranoside (65)



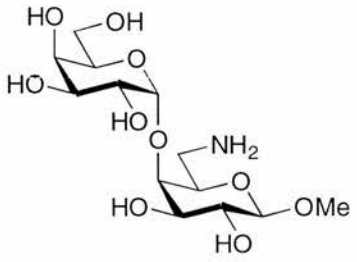
The anhydro sugar (**64**) was protected using a standard acetylation procedure.[11] The reduced compound (**64**) (10 mg, 0.03 mmol) was dissolved in pyridine (1 ml) and acetic anhydride (0.5 ml) and stirred at room temperature overnight. The reaction mixture was coevaporated with toluene and taken up in DCM. The resulting solution was washed with dilute HCl and $HaHCO_3$ solution, dried (Na_2SO_4) and concentrated under reduced pressure to give *methyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl-(1-4)-2-O-acetyl-3,6-anhydro–β-D-galactopyranosid*e (**65**) as a syrup (12.1 mg, 75 %); $[\alpha]_D$ +68.6 (*c* 0.40, $CHCl_3$); $\delta_H(CDCl_3)$: 1.97 (3 H, s, OAc), 2.055 (3 H, s, OAc), 2.065 (3 H, s, OAc), 2.10 (3 H, s, OAc), 2.13 (3 H, s, OAc), 3.40 (3 H, s, OMe), 3.89 (1 H, dd, $J_{5a,6a,6a'}$ 3.0, $J_{6a,6a'}$ 9.5, H-6a/6a'), 4.04 (1 H, dd, $J_{5b,6b/6b'}$ 6.0, $J_{6b,6b'}$ 11.0, H-6b/6b'), 4.12 (1 H, dd, $J_{5b,6b/6b'}$, $J_{6b,6b'}$, H-6b/6b'), 4.26 (3 H, m, H-5a, 5b, 6a/6a'), 4.28 (1 H, s, H-4a), 4.45 (1 H, s, H-1a), 4.525 (1 H, $J_{2a,3a}$ 5.0, H-3a), 4.99 (1 H, $J_{2a,3a}$, H-2a), 5.07 (1 H, $J_{1b,\ 2b}$ 3.5, $J_{2b,3b}$ 10.5, H-3b), 5.26 (1 H, $J_{2b,\ 3b}$, $J_{3b,4b}$, H-3b), 5.32 (1 H, $J_{1b,2b}$ 3.5, H-1b), 5.44 (1 H, $J_{3b,4b}$, H-4b); $\delta_C(CDCl_3)$: 20.6, 20.7, 20.8, 29.6, 56.0 (OMe), 61.8, 67.0, 67.3, 67.5, 67.9, 70.7, 73.5, 75.5, 76.5, 78.5, 96.5 (C-1b), 100.7 (C-1a), 169.2, 169.9, 170.0, 170.3, 170.5 (5 x Ac); ES-MS: *m/z* 571 $(M + Na)^+$. (Found: $[M + Na]^+$ 571.1643. $C_{23}H_{32}O_{15}Na$ requires *m/z* 571.17413).

Methyl α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside (66)[12]



Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**60**) (73.9 mg, 0.075 mmol) and palladium on charcoal (74 mg) were stirred in EtOH (5 ml) under a hydrogen atmosphere. After 12 h the mixture was filtered through Celite and concentrated under reduced pressure to give methyl α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside (**66**) (21.4 mg, 80 %); $\delta_H(CD_3OD)$: 3.30 (2 H, m, H-6b/6b'), 3.53 (3 H, s OMe), 3.44-3.85 (4 H, m, H-2a, 2b, 3a, 3a), 3.62 (1 H, t, , $J_{5a,6a/6a'}$ 6.5, H-5a), 3.76 (2 H, m, H-6a/6a'), 3.91 (1 H, d, $J_{3b,4b}$, 1.5, H-4b), 3.99 (1 H, d, $J_{3a,4a}$ 2.5, H-4a), 4.2 (1 H, d, $J_{1a,2a}$ 7.5, H-1a), 4.29 (1 H, t, $J_{5a,6a/6a'}$ 6.5, H-5b), 4.96 (1 H, d, $J_{1b,2b}$ 3.0, H-1b); $\delta_C(CD_3OD)$: 56.1 (OMe), 59.5, 61.2, 69.3, 69.65, 69.9, 71.2, 71.4, 73.25, 74.7, 77.7, 101.1 (C-1), 104.7 (C-1); MALDI-TOF: *m/z* 379 $(M + Na)^+$, ($C_{13}H_{24}O_{11}Na$ requires *m/z* 379); NMR data in accord with the literature.[12]

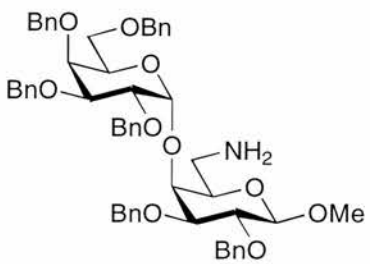
Methyl (α-D-galactopyranosyl)-(1-4)-6-amino-6-deoxy-β-D-galactopyranoside (67)



Sugar (**62**) was deprotected using a standard hydrogenation procedure.[10] Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-azido-6-deoxy-2,3,4-tri-*O*-

benzyl-β-D-galactopyranoside (**62**) (30 mg, 0.084 mmol) was dissolved in a mixture of EtOH (2 ml) and acetic acid (2 ml), and hydrogenated (H_2, Pd-C, 30 mg, 1 atm). After 12 h the mixture was filtered through Celite and concentrated under reduced pressure to give *methyl α-D-galactopyranosyl-(1-4)-6-amino-6-deoxy-β-D-galactopyranoside* (**67**) (9.4 mg, 81 %); $\delta_H(CD_3OD)$: 3.30 (2 H, m, H-6b/6b'), 3.53 (3 H, s OMe), 3.44-3.85 (4 H, m, H-2a, 2b, 3a, 3a), 3.62 (1 H, m, H-5a), 3.76 (2 H, m, H-6a/6a'), 3.91 (1 H, m, H-4b), 3.91 (1 H, m, H-4a), 4.2 (1 H, d, $J_{1a,2a}$ 7.2, H-1a), 4.29 (1 H, m, H-5b), 4.96 (1 H, d, $J_{1b,2b}$ 3.6, H-1b); $\delta_C(CD_3OD)$ 39.2, 55.9 (OMe), 61.2, 64.5, 69.3, 69.6, 71.3, 71.7, 72.4, 73.3, 79.3, 101.5 (C-1), 104.5 (C-1); MALDI-TOF: *m/z* 378 $(M + Na)^+$, ($C_{13}H_{25}NO_{10}Na$ requires *m/z* 378). High-Res ES-MS not found.

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-galactopyranoside (68)



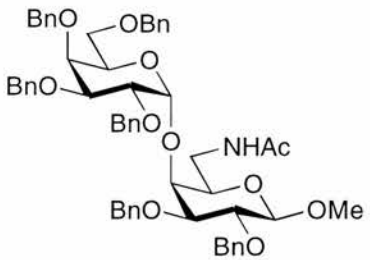
Method 1 : Azide Reduction with Triphenylphosphine[13]

A solution of the azido disaccharide (**62**) (30 mg, 0.03 mmol) in THF (5 ml, 0.1 % H_20) containing triphenylphosphine {on solid support} (25.6 mg) was stirred for 30 h at 50 ^OC. Once TLC [hexane:EtOAc (3:1)] showed the reaction to be complete, the solid support was filtered and the resulting solution was concentrated to dryness to give *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-6-amino-2,3,4-tri-O-benzyl-6-deoxy-β-D-galactopyranoside* (**68**) (29 mg, 99%). Kaiser Test showed the production of amine; ν_{max}/cm^{-1} 3400 (NH_2), no absorption at 2100 (N_3).[14] This material was used without further purification.

Method 2 : Azide Reduction with DTT[15]

A solution of methyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1-4)-6-azido-6-deoxy-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**62**) (49.4 mg, 0.054 mmol) in anhydrous DMF (1 ml) containing DTT (38.9 mg, 0.27 mmol) and DBU (40 µl, 0.27mmol) was stirred at room temperature for 1 h (approx.). Once TLC [hexane:EtOAc (3:1)] indicated the absence of starting material and the Kaiser test showed amine was present, the crude product was reacted with a panel of reagents.[14]

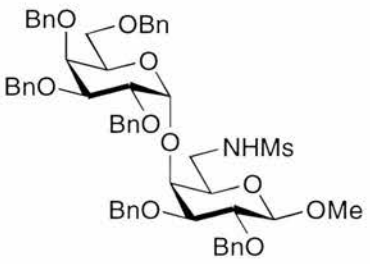
Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-acetamido-2,3-di-*O*-benzyl-6-deoxy-β-D-galactopyranoside (69)



The crude reaction mixture from reduction of azide containing DBU (80 µl, 0.54 mmol) and acetic anhydride (50.5 µl, 0.54 mmol) was stirred at 0^{O}C and allowed to warm slowly to room temperature. Once TLC [hexane-EtOAc, (3:1)] indicated the reaction to be complete, the reaction mixture was cooled and methanol was added. The resulting solution was concentrated and then dissolved in diethyl ether. The solution was washed with salt solution and water, dried (Na_2SO_4) and concentrated to give *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-6-acetamido-2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside* (**69**) (30 mg, 60%); $[\alpha]_D$ +31.2 (*c* 1.15, $CHCl_3$); ν_{max}/cm^{-1} (3055 NH, 1672 CO); (Found: C, 70.99; H, 7.03; N, 1.37 %. $C_{57}H_{63}NO_{11}.H_2O$ requires C, 71.5; H, 6.85; N, 1.46 %); $\delta_H(CDCl_3)$: 1.65 (3 H, s, NAc), 3.25 (1 H, m, H-6a/6a'), 3.33 (1 H, dd, $J_{5b,6b/6b'}$ 5.0, $J_{6b,6b'}$ 9.0, H-6b/6b'), 3.38 (1 H, dd, $J_{2a,3a}$ 7.0, $J_{3a,4a}$ 2.5, H-3a), 3.55 (3 H, s, OMe), 3.57 (4 H, m, H-2a, 5a, 6a/6a', 6b/6b'), 3.78 (1 H, d, $J_{3a,4a}$, H-4a), 4.05 (1 H, dd, $J_{2b,3b}$ 7.5, $J_{3b,4b}$ 3.0, H-3b), 4.11 (1 H, d, $J_{3b,4b}$, H-4b), 4.12 (1 H, dd, $J_{1b,2b}$ 3.5, $J_{2b,3b}$, H-2b), 4.23 (1 H, d, $J_{1a,2a}$

8.0, H-1a), 4.30 (3 H, m, H-5b, OCH_2), 4.56-4.95 (10 H, m, 5 x OCH_2), 5.05 (1 H, d, $J_{1b,2b}$, H-1b), 6.06 (1 H, m, NH), 7.18-7.42 (30 H, m, 6 x OCH_2Ar); $\delta_C(CDCl_3)$: 22.6 (NHAc), 39.2 (C-6a), 57.3 (OMe), 68.2, 69.9, 71.8, 71.95, 73.0, 73.25, 74.5, 74.9, 75.0, 76.3, 77.0, 77.8, 79.0, 79.1, 81.0, 100.4 (C-1b), 104.9 (C-1a), 127.5, 127.6, 127.7, 127.95, 128.05, 128.1, 128.3, 128.4, 128.6, 138.1, 138.4, 138.5, 138.7, 138.8 (6 x OBn), 170.7 (CO); MALDI-TOF: *m/z* 960 $(M + Na)^+$, ($C_{57}H_{63}NO_{11}Na$ requires *m/z* 960).

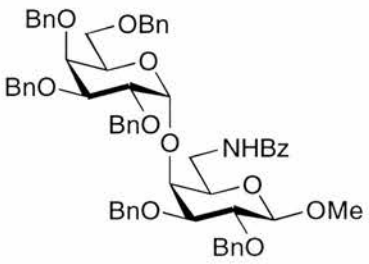
Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-2,3-di-*O*-benzyl-6-deoxy-6-methylsulfonamido-β-D-galactopyranoside (70)



The crude reaction mixture from reduction of azide containing DBU (80 µl, 0.54 mmol) and methanesulfonyl chloride (41.5 µl, 0.54 mmol) was stirred at 0^OC and allowed to warm slowly to room temperature. Once TLC [hexane-EtOAc, (3:1)] indicated the reaction to be complete, the reaction mixture was cooled and methanol was added. The resulting solution was concentrated and then dissolved in diethyl ether. The solution was washed with salt solution and water, dried (Na_2SO_4) and concentrated to give *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-2,3-di-O-benzyl-6-deoxy-6-N-methylsulfonyl-β-D-galactopyranoside* (**70**) (31 mg, 60 %); $[\alpha]_D$ +23.1 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} (3056 NH, 1639 SO); (Found: C, 67.49; H, 7.00; N, 1.44 %. $C_{56}H_{63}NO_{12}S.H_2O$ requires C, 67.79; H, 6.60; N, 1.41 %); $\delta_H(CDCl_3)$: 2.55 (3 H, s, SO_2CH_3), 3.27 (3 H, m, H-6a/6a', H-6b/b'), 3.41 (1 H, dd, $J_{2a,3a}$ 7.5, $J_{3a,4a}$ 2.5, H-3a), 3.55 (3 H, s, OMe), 3.57 (3 H, m, H-2a, 5a, 6b/6b'), 3.99 (1 H, d, $J_{3a,4a}$, H-4a), 4.05 (1 H, dd, $J_{2b,3b}$ 7.5, $J_{3b,4b}$ 3.0, H-3b), 4.11 (2 H, m, H-2b, 4b), 4.23 (1 H, d, $J_{1a,2a}$ 7.5, H-1a), 4.24 (3 H, m, H-5b + OCH_2), 4.56-4.95 (10 H, m, 5 x OCH_2), 4.93 (1 H, d, $J_{1b,2b}$ 3.5, H-1b), 5.03 (1 H, d, *J* 11.5, OCH_2), 5.68 (1 H, m,

NH), 7.18-7.42 (30 H, m, 6 x OCH_2Ar); $\delta_C(CDCl_3)$: 29.7 (SO_2CH_3), 39.5 (C-6a), 42.4, 57.4 (OMe), 68.05, 69.9, 72.1, 72.6, 73.1, 73.3, 74.4, 74.7, 74.8, 75.1, 76.1, 78.85, 79.4, 80.79, 100.8 (C-1b), 104.9 (C-1a), 127.4, 127.4, 127.6, 127.7, 127.9, 128.05, 128.2, 128.3, 128.4, 128.6, 128.6, 138.0, 138.3, 138.6, 138.7 (6 x OBn); MALDI-TOF: *m/z* 996 $(M + Na)^+$, ($C_{56}H_{63}NO_{12}SNa$ requires *m/z* 996).

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-benzamido-2,3-di-*O*-benzyl-6-deoxy-β-D-galactopyranoside (71)



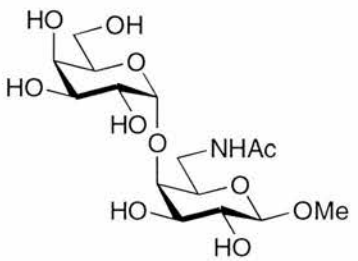
The crude reaction mixture from reduction of azide containing DBU (80 µl, 0.54 mmol) and benzoyl chloride (62.2 µl, 0.54 mmol) was stirred at 0^OC and allowed to warm slowly to room temperature. Once TLC [hexane:EtOAc (3:1)] indicated the reaction to be complete, the reaction mixture was cooled and methanol was added. The resulting solution was concentrated and then dissolved in diethyl ether. The solution was washed with salt solution and water, dried (Na_2SO_4) and concentrated to give *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-6-benzamido-2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside*the (**71**) (39.4 mg, 74%); $[\alpha]_D$ +27.6 (*c* 1.2, $CHCl_3$); ν_{max}/cm^{-1} (3057 NH, 1657 CO); (Found: C, 72.82; H, 6.78; N, 1.75 %. $C_{21}H_{25}N_3O_5.H_2O$ requires C, 73.09; H, 6.63; N, 1.37 %); $\delta_H(CDCl_3)$: 3.29 (1 H, dd, $J_{5b,6b/6b'}$ 5.0, $J_{6b,6b'}$ 8.5, H-6b/6b'), 3.39 (1 H, dd, $J_{2a,3a}$ 7.0, $J_{3a,4a}$ 2.5, H-3a), 3.55 (3 H, s, OMe), 3.57 (3 H, m, H-2a, 6a/6a', 6b/6b'), 3.74 (1 H, m, H-5), 3.83 (1 H, d, $J_{3a,4a}$, H-4a), 4.05 (3 H, m , H-2b, 3b, 4b), 4.26 (1 H, d, $J_{1a,2a}$ 8.0, H-1a), 4.28 (2 H, s, OCH_2), 4.33 (1 H, m, H-5b), 4.56-4.95 (10 H, m, 5 x OCH_2), 4.99 (1 H, d, $J_{1b,2b}$ 3.0, H-1b), 6.70 (1 H, m, NH), 7.18-7.42 (30 H, m, 6 x OCH_2Ar) 7.55 (3 H, m , COBz), 8.05 (2 H, d, *J* 11.5, COBz); $\delta_C(CDCl_3)$ 39.8 (C-6a), 57.4 (OMe), 67.9, 69.7, 72.0,

72.2, 72.7, 73.2, 74.4, 74.6, 74.95, 75.1, 76.1, 76.5, 78.9, 79.3, 80.8. 100.1 (C-1b), 104.9 (C-1a), 126.9, 127.0, 127.4, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 129.5, 130.1, 131.5, 138.0, 138.5, 138.7, 138.8, 167.9 (CO); MALDI-TOF: *m/z* 1022 $(M + Na)^+$, ($C_{62}H_{65}NO_{11}Na$ requires *m/z* 1022).

Deprotection of disaccharide (69), (70) and (71)

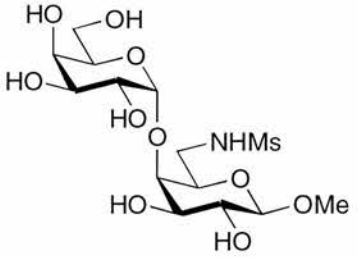
The panel of compounds [(**69**) (**70**) (**71**)] were reduced using a standard hydrogenation procedure.[10] The compounds were individually dissolved in EtOH (1 ml) and hydrogenated (H_2, Pd-OH, 1 atm). The reaction mixture was stirred at room temperature until TLC [$CHCl_3$-MeOH-H_2O, (10:10:3)] showed the reaction to be complete (approx. 12 h). The reaction mixture was then filtered through Celite and concentrated under reduced pressure to yield the reduced compound.

Methyl α-D-galactopyranosyl-(1-4)-6-acetamido-6-deoxy-β-D-galactopyranoside (72)



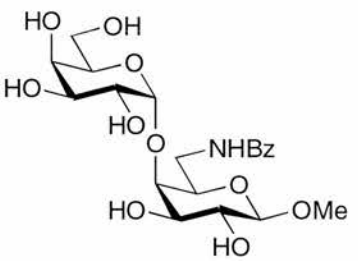
$\delta_H(CD_3OD)$: 1.95 (3 H, s, NHAc), 3.45 (4 H, m, H-2a, 5a, 6a/6a'), 3.52 (4 H, m, H-3a, OMe), 3.66–3.85 (4 H, m, H-2b, 3b, 6b/6b'), 3.85 (1 H, d, $J_{3b,4b}$ 3.5, H-4b), 3.92 (1 H, d, $J_{3a,4a}$ 3.0, H-4a), 4.16 (1 H, d, $J_{1a,2a}$ 7.5, H-1a), 4.19 (1 H, t, $J_{5a,6a/6a'}$ 6.0, H-5b), 4.96 (1 H, d, $J_{1b,2b}$ 3.5, H-1b); $\delta_C(CD_3OD)$: 21.1 (NHAc), 38.5, 55.9 (OMe), 61.2, 69.2, 69.6, 69.7, 71.3, 71.7, 72.4, 73.4, 79.3, 101.6 (C-1), 104.6 (C-1); ES-MS: *m/z* 415 $(M + NH_4)^+$. (Found: $[M + NH_4]^+$ 415.1920. $C_{15}H_{31}N_2O_{11}$ requires *m/z* 415.19001).

Methyl α-D-galactopyranosyl-(1-4)-6-deoxy-6-methylsulfonamido-β-D-galactopyranoside (73)



δ_H(CD_3OD): 3.16 (3 H, s, $NHSO_2CH_3$), 3.45 (4 H, m, H-2a, 5a, 6a/6a'), 3.52 (4 H, m, H-3a, OMe), 3.71 (2 H, m, H-6b/6b'), 3.78 (1 H, dd, $J_{2b,3b}$ 9.0, $J_{3b,4b}$, 3.0, H-3b), 3.83 (1 H, dd, $J_{1b,2b}$ 3.5, $J_{2b,3b}$, H-2b), 3.92 (1 H, d, $J_{3b,4b}$ 2.5, H-4b), 3.95 (1 H, d, $J_{3a,4a}$ 2.5, H-4a), 4.19 (1 H, d, $J_{1a,2a}$ 7.5, H-1a), 4.195 (1 H, t, $J_{5a,6a/6a'}$ 6.5, H-5b), 4.96 (1 H, d, $J_{1b,2b}$, H-1b); δ_C(CD_3OD): 38.5, 42.4, 55.9 (OMe), 61.2, 69.2, 69.6, 69.7, 71.3, 71.7, 72.4, 73.4, 78.9, 101.4 (C-1), 104.7 (C-1); ES-MS: *m/z* 451 $(M + NH_4)^+$. (Found: $[M + NH_4]^+$ 451.1595. $C_{14}H_{31}N_2O_{12}S$ requires *m/z* 451.16374).

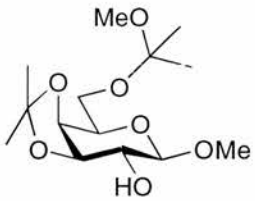
Methyl α-D-galactopyranosyl-(1-4)-6-deoxy-6-benzamido-β-D-galactopyranoside (74)



δ_H(CD_3OD): 3.45 (4 H, m, H-2a, 5a + 6a/6a'), 3.52 (4 H, m, H-3a, OMe), 3.71 (2 H, m, H-6b/6b'), 3.86 (3 H, m, H-2b, 3b, 4b), 3.95 (1 H, m, H-4a), 4.20 (1 H, d, $J_{1a,2a}$ 6.9, H-1a), 4.20 (1 H, m, H-5b), 5.02 (1 H, d, $J_{1b,2b}$ 3.6, H-1b) 7.48 (3 H, m, NHBz) 7.84 (2 H, m, NHBz); δ_C(CD_3OD): 39.4, 55.9 (OMe), 61.2, 69.3, 69.8, 69.9, 71.4, 71.9, 72.1, 73.5, 79.4, 101.7 (C-1), 104.6 (C-1), 126.9, 128.2, 131.3, 133.9, 169.1;

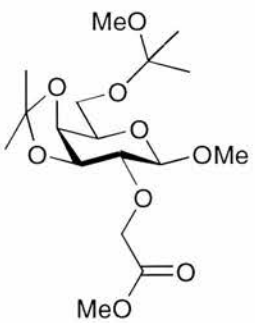
ES-MS: *m/z* 482 $(M + NH_4)^+$. (Found: $[M + NH_4]^+$ 482.1640. $C_{20}H_{33}N_2O_{11}$ requires *m/z* 482.16383).

Methyl 3,4-*O*-isopropylidene-6-*O*-(2-methoxyisopropyl)-β-D-galactopyranoside (75)



Sugar (**46**) was protected using a standard acetal formation procedure.[16] Methyl β-D-galactopyranoside (**46**) (5 g, 25.75 mmol) and *p*-toluenesulfonic acid (250 mg, 1.31 mmol) were dissolved in 2,2-dimethoxypropane (375 ml) and the mixture was stirred for 24 h. The reaction mixture was quenched with TEA (5 ml) and concentrated under reduced pressure to give *methyl 3,4-O-isopropylidene-6-O-(2-methoxyisopropyl)-β-D-galactopyranoside* (**75**) (6.84 g, 87 %) as a syrup which was used in subsequent reactions without purification, δ_H ($CDCl_3$): 1.42 (9 H, s, 3 x CH_3), 1.52 (3 H, s, CH_3), 3.23 (3 H, s, OMe), 3.40 (1 H, m, H-5), 3.55 (3 H, s, OMe), 3.56 (1 H, m, H-6/6'), 3.80 (1 H, m, H-6/6'),4.16 (2 H, m, H-3 + 4), 4.22 (1 H, d, $J_{1,2}$ 7.8, H-1).

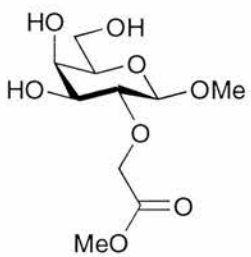
Methyl 2-*O*-methyl-acetate-3,4-*O*-isopropylidene-6-*O*-(2-methoxyisopropyl)-β-D-galactopyranoside (76)



A mixture methyl 3,4-*O*-isopropylidene-6-*O*-(2-methoxyisopropyl)-β-D-

galactopyranoside (**75**) (2.0 g, 6.53 mmol) and sodium hydride in oil (60 % of sodium hydride by weight) (392 mg, 9.80 mmol) in DMF (25 ml) were cooled (0°C) and stirred. After 0.5 h bromo methoxycarbonylmethyl (1.24 ml, 13.06 mmol) was slowly added and the solution was stirred until TLC [pet.ether - EtOAc, (3:1)] showed the reaction to be complete (approx. 3 h). The reaction mixture was cooled (0°C) before methanol (20 ml) was added and the solution was stirred for 0.5 h. and concentrated under reduced pressure to a syrup. The syrup was diluted with water and extracted with diethyl ether, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; pet. ether - EtOAc, 8:1--1:1) gave *methyl 2-O-methyl-acetate-3,4-O-isopropylidene-6-O-(2-methoxyisopropyl)-β-D-galactopyranoside* (**76**) (1.83 g, 74 %); $[\alpha]_D$ – 4.88 (*c* 1.41, $CHCl_3$); δ_H ($CDCl_3$): 1.42 (9 H, s, 3 x CH_3), 1.52 (3 H, s, CH_3), 3.14 (3 H, s, OMe), 3.27 (1 H, m, H-5), 3.40 (3 H, s, OMe), 3.61 (2 H, m, H-6/6'), 3.66 (3 H, s, OMe), 3.66-3.75 (2 H, m, H-3, 4), 4.11 (2 H, m, O*C*H_2), 4.16 (1 H, d, $J_{1,2}$ 8.4, H-1), 4.29 (2 H, dd, *J* 3.9 + 14.0, O*C*H_2); δ_C ($CDCl_3$): 24.3, 24.3, 26.1, 27.9, 48.4, 51.6, 56.3 (OMe), 60.25, 68.9, 72.2, 73.8, 78.3, 81.95, 100.1, 103.1 (C-1), 110.0, 170.8 (CO); MALDI-TOF: *m/z* 401 $(M + Na)^+$, ($C_{17}H_{30}O_9Na$ requires *m/z* 401).

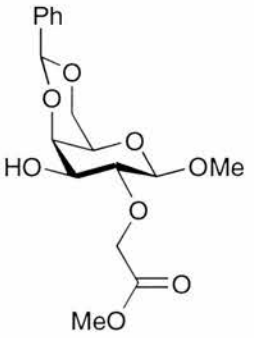
Methyl 2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (77)



Sugar (**76**) was deprotected using a standard acetal deprotection procedure.[17] A solution of methyl 2-*O*-methoxycarbonylmethyl-3,4-*O*-isopropylidene-6-*O*-(2-methoxyisopropyl)-β-D-galactopyranoside (**76**) (1.0 g, 2.64 mmol) in methanol (15 ml) and 50 % aqueous HBF_4 (225 µl) was stirred at room temperature until TLC [DCM-MeOH, (6:1)] indicated the reaction to be complete. $NaHCO_3$ (300 mg) was added and the mixture was stirred for 1 h and then concentrated. The compound was extracted with DCM and the combined extracts were washed with water, dried

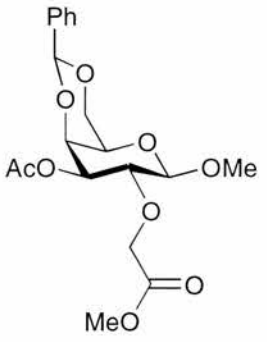
(Na_2SO_4) and concentrated to yield *methyl 2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**77**) (605 mg, 86 %); $[\alpha]_D$ +6.15 (*c* 1.24, MeOH); $\delta_H(CD_3OD)$: 3.30 (1 H, m, H-2), 3.47 (3 H, s, OMe), 3.48 (1 H, m , H-3), 3.60 (1 H, dd, $J_{5,6,6'}$ 3.3, $J_{6,6'}$ 6.3, H-6/6'), 3.75 (5 H, m, H-5, 6/6', OMe), 3.86 (1 H, d, $J_{3,4}$ 2.7, H-4), 4.24 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.34 (2 H, s, OCH_2); $\delta_C(CD_3OD)$: 51.1, 55.7, 61.0, 68.5, 68.7, 72.6, 75.1, 81.1, 104.3, 173.0; CI-MS: *m/z* 267 $(M + H)^+$. (Found: $[M + H]^+$ 267.1079. $C_{10}H_{19}O_8$ requires *m/z* 267.1080).

Methyl 4,6-*O*-benzylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (78)



The title compound was prepared using a standard benzylidene acetal reaction.[1] Methyl 2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**77**) (1.03 g, 3.86 mmol) was dissolved in acetonitrile (15 ml) and stirred before benzaldehyde dimethyl acetal (695 µl, 4.63 mmol) and camphor sulfonic acid (179 mg, 0.77 mmol) were slowly added. The reaction mixture was stirred at room temperature until TLC [DCM - MeOH, (6:1)] showed the reaction to be complete. The solution was neutralized with saturated sodium hydrogen carbonate solution and the organic extract was washed with water, dried and concentrated under reduced pressure to give the *methyl 4,6-O-benzylidene-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**78**) as a solid (1.0 g, 73 %); mp 111-113^{0}C; $[\alpha]_D$ +1.83 (*c* 1.16, $CHCl_3$); δ_H ($CHCl_3$): 3.41 (1 H, d, $J_{5,6/6'}$ 1.5, H-5), 3.49 (1 H, dd, $J_{1,2}$ 7.5, $J_{2,3}$ 8.0, H-2), 3.53 (3 H, s, OMe), 3.75 (4 H, m, H-3, OMe), 4.05 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 11.0, H-6/6'), 4.24 (1 H, d, $J_{3,4}$ 3.5, H-4), 4.29 (1 H, d, $J_{1,2}$, H-1), 4.32 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.38 (2 H, s, OCH_2), 5.51 (1 H, s, PHC*H*), 7.23 (3 H, m, *Ph*CH), 7.41 (2 H,

m, *Ph*CH); δ_C ($CHCl_3$): 52.1, 57.05 (OMe), 66.5, 68.8, 69.1, 71.7, 75.3, 80.65, 101.3, 104.1 (C-1), 126.5, 128.05, 128.9, 137.7, 172.7 (CO); CI-MS: *m/z* 355 $(M + H)^+$. (Found: $[M + H]^+$ 355.1392. $C_{17}H_{23}O_8$ requires *m/z* 355.1393).



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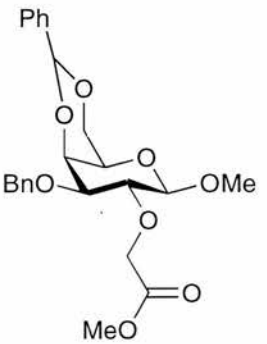
A sample of methyl 4,6-*O*-benzylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**78**) was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^{1}H NMR spectroscopy to be *methyl 3-O-acetyl-4,6-O-benzylidene-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**79**); δ_H ($CDCl_3$) 2.07 (3 H, s, OAc), 3.41 (1 H, d, $J_{5,6/6'}$ 1.5, H-5), 3.53 (3 H, s, OMe), 3.75 (3 H, s, OMe), 3.77 (1 H, dd, $J_{1,2}$ 7.5, $J_{2,3}$ 8.0, H-2), 4.05 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 11.0, H-6/6'), 4.24 (1 H, d, $J_{3,4}$ 3.5, H-4), 4.29 (1 H, d, $J_{1,2}$, H-1), 4.32 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.38 (2 H, s, O*CH*$_2$), 5.05 (1 H, dd, $J_{2,3}$, $J_{3,4}$, H-3), 5.51 (1 H, s, PHC*H*), 7.23 (3 H, m, *Ph*CH), 7.41 (2 H, m, *Ph*CH).

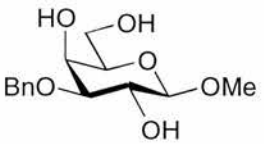
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (80) - [from (**78**)]



The title compound was prepared using a standard benzylation procedure.[2] A solution of methyl 4,6-*O*-benzylidene-2-*O*-methoxycarbonylmethyl-β-D-

galactopyranoside (**78**) (900 mg, 2.54 mmol) was dissolved in DMF (10 ml) and cooled (0°C) before sodium hydride in oil (60 % w/w solution in mineral oil) (122 mg, 3.05 mmol) was slowly added. After 0.5 h BnBr (362 µl, 3.05 mmol) was slowly added and the solution was stirred until TLC [pet.ether - EtOAc, (3:1)] showed the reaction had gone to completion. The reaction mixture was cooled (0°C) before methanol (15 ml) was added. The solution was stirred for 0.5 h. and concentrated under reduced pressure to a syrup. The syrup was diluted with water and extracted with diethyl ether. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Crystallization with ethanol gave *methyl 3-benzyl-4,6-benzylidene-2O--methoxycarbonylmethyl-β-D-galactopyranoside* (**80**) (624 mg, 55 %); mp 89-91°C (EtOH); $[\alpha]_D$ +19.1 (*c* 1.15, $CHCl_3$); δ_H ($CDCl_3$): 3.28 (1 H, dd, $J_{5,6/6'}$ 1.2, H-5), 3.5 (3 H, s, OMe), 3.62 (1 H, dd, $J_{2,3}$ 7.2, $J_{3,4}$ 3.6, H-3), 3.66 (1 H, dd, $J_{1,2}$ 7.2, $J_{2,3}$, H-2), 3.70 (3 H, s, OMe), 4.00 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 12.3, H-6/6'), 4.09 (1 H, d, $J_{3,4}$, H-4), 4.27 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6,6'), 4.31 (1 H, d, $J_{1,2}$, H-1), 4.40 (2 H, dd, *J* 12.0 + 16.2, OCH_2) 4.76 (2 H, s, OCH_2), 5.49 (1 H, s, PhC*H*), 7.22-7.42 (8 H, m, *Ph*CH and $0CH_2Ar$), 7.52 (2 H, m, *Ph*CH); δ_C ($CDCl_3$): 51.6 (OMe), 56.8 (OMe), 66.4, 69.1, 70.2, 72.1, 73.95, 78.7, 79.8, 101.3, 103.9 (C-1), 126.6, 127.8, 128.2, 128.5, 129.0, 137.9, 138.6, 171.1; CI-MS: *m/z* 445 $(M + H)^+$. (Found: $[M + H]^+$ 445.1862. $C_{24}H_{29}O_8$ requires *m/z* 445.1863).

Methyl 3-*O*-benzyl-β-D-galactopyranoside (81)[18]



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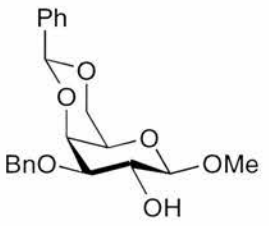
A solution of methyl-β-D-galactopyranoside (**46**) (500 mg, 2.58 mmol) in methanol (25 ml) containing suspended dibutyltin oxide (641 mg, 2.58 mmol) was heated under reflux for 1 h and then concentrated under reduced pressure. Benzyl bromide (1.53 ml, 12.87 mmol) and tetrabutylammonium bromide (415 mg, 1.29 mmol) were added to a solution of the residue in toluene (25 ml) and heated under reflux (approx. 1.5 h). Once TLC [DCM - MeOH, (7:1)] showed the reaction to be complete, the

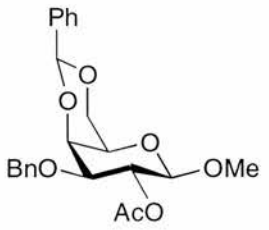
solution was concentrated under reduced pressure. Column chromatography (silica gel; DCM – MeOH, 20:1→6:1) gave the *methyl 3-O-benzyl-β-D-galactopyranoside* (**81**) (611 mg, 86 %) as a syrup; $[\alpha]_D$ +18.9 (*c* 1.24, MeOH); δ_H (CD_3OD): 3.37 (1 H, dd, $J_{2,3}$ 6.3, $J_{3,4}$ 3.3, H-3), 3.28 (1 H, m, H-5), 3.5 (3 H, s, OMe), 3.66 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$, H-2), 3.75 (2 H, dd, $J_{5,6/6'}$ 6.6, $J_{6,6'}$ 8.7, H-6/6'), 4.02 (1 H, d, $J_{3,4}$, H-4), 4.15 (1 H, d, $J_{1,2}$, H-1), 4.70 (2 H, dd, *J* 12.0 + 20.4, OCH_2), 7.29 (3 H, m, OCH_2Ar), 7.45 (2 H, d, *J* 6.9, OCH_2Ar); δ_C (CD_3OD): 55.8 (OMe), 61.0, 65.7, 70.3, 71.1, 75.1, 80.9, 104.5, 127.2, 127.6, 127.8, 138.5; MALDI-TOF: *m/z* 307 $(M + Na)^+$, ($C_{14}H_{20}O_6Na$ requires *m/z* 307).

Methyl 4,6-*O*-benzylidene-3-*O*-benzyl-β-D-galactopyranoside (82)[19]



The title compound was prepared using a standard benzylidene acetal reaction.[1] Methyl 3-*O*-benzyl-β-D-galactopyranoside (**81**) (550 mg, 1.94 mmol) was dissolved in acetonitrile (15 ml) and stirred before benzaldehyde dimethyl acetal (349 mg, 2.32 mmol) and camphor sulfonic acid (90 mg, 0.39 mmol) were slowly added. The reaction mixture was stirred at room temperature until TLC [DCM - MeOH, (6:1)] showed the reaction to be complete. The solution was neutralized with saturated sodium hydrogen carbonate solution and the organic extract was washed with water, dried and concentrated under reduced pressure to give *methyl 4,6-O-benzylidene-3-O-benzyl-β-D-galactopyranoside* (**82**) (627 mg, 87 %); mp 198-199^{0}C (EtOAc:Hexane) (lit.[19], 199-200); $[\alpha]_D$ +58.4 (*c* 1.0, $CHCl_3$) (lit.[19], 56.2); δ_H ($CHCl_3$): 3.37 (1 H, d, $J_{5,6/6'}$ 1.2, H-5), 3.48 (1 H, dd, $J_{2,3}$ 6.0, $J_{3,4}$ 2.7, H-3), 3.56 (3 H, s, OMe), 3.99 (2 H, m, H-2 + 6/6'), 4.14 (1 H, d, $J_{3,4}$, H-4), 4.25 (1 H, d, $J_{1,2}$, H-1), 4.34 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 11.1, H-6/6'), 4.75 (2 H, dd, *J* 3.6 + 12.0, OCH_2), 5.46 (1 H, s, OC*H*Ar), 7.29 (8 H, m, $0CH_2Ar$), 7.45 (2 H, d, *J* 6.9, OCH_2Ar); δ_C ($CHCl_3$): 56.9 (OMe), 66.6. 69.2, 69.9, 71.5, 73.0, 79.3, 101.1, 103.8, 126.35, 127.85, 128.1,

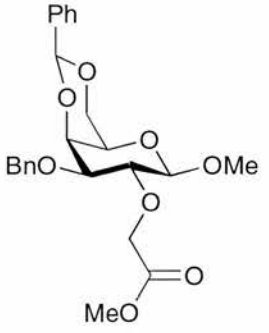
128.5, 128.9, 137.7, 137.9; MALDI-TOF: *m/z* 395 $(M + Na)^+$, ($C_{21}H_{24}O_6Na$ requires *m/z* 395).



83

A sample of methyl 4,6-*O*-benzylidene-3-*O*-benzyl-β-D-galactopyranoside (**82**) was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^1H NMR spectroscopy to be *methyl 2-O-acetyl-4,6-O-benzylidene-3-O-benzyl-β-D-galactopyranoside* (**83**); δ_H ($CDCl_3$): 2.07 (3 H, s, OAc), 3.37 (1 H, d, $J_{5,6,6'}$ 1.5, H-5), 3.49 (3 H, s, OMe), 3.60 (1 H, dd, $J_{2,3}$ 7.05, $J_{3,4}$ 3.5, H-3), 4.03 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 10.5, H-6/6'), 4.17 (1 H, d, $J_{3,4}$, H-4), 4.34 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$ 11.1, H-6/6'), 4.37 (1 H, d, $J_{1,2}$ 8.0, H-1), 4.67 (2 H, dd, *J* 12.5 + 23.5, OCH_2), 5.37 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.40 (1 H, s, OC*H*Ar), 7.29 (8 H, m, $0CH_2Ar$), 7.45 (2 H, d, *J* 6.9, OCH_2Ar).

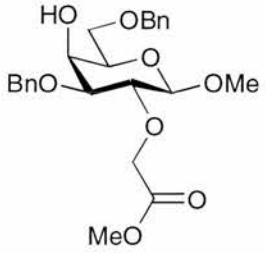
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (80) - [from (**82**)]



A solution of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**82**) (1.0 g, 2.68 mmol) was dissolved in DMF (15 ml) and cooled (0°C) before sodium hydride in oil (60 % w/w solution in mineral oil) (215 mg, 5.37 mmol) was slowly added. After 0.5 h bromo methoxycarbonylmethyl (1.27 ml, 13.43 mmol) was slowly added and the solution was stirred until TLC [pet.ether - EtOAc, (3:1)] showed the

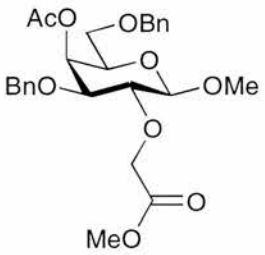
reaction had gone to completion. The reaction mixture was cooled (0°C) before methanol (30 ml) was added. The solution was stirred for 0.5 h. and concentrated under reduced pressure to a syrup. The syrup was diluted with water and extracted with diethyl ether. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Crystallization with ethanol gave *methyl 3-O-benzyl-4,6-O-benzylidene-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**80**) (910 mg, 76 %); δ_H ($CDCl_3$): 3.28 (1 H, dd, $J_{5,6,6'}$ 1.2, H-5), 3.5 (3 H, s, OMe), 3.62 (1 H, dd, $J_{2,3}$ 7.2, $J_{3,4}$ 3.6, H-3), 3.66 (1 H, dd, $J_{1,2}$ 7.2, $J_{2,3}$, H-2), 3.70 (3 H, s, OMe), 4.00 (1 H, dd, $J_{5,6,6'}$, $J_{6,6'}$ 12.3, H-6/6'), 4.09 (1 H, d, $J_{3,4}$, H-4), 4.27 (1 H, dd, $J_{5,6,6'}$, $J_{6,6'}$, H-6,6'), 4.31 (1 H, d, $J_{1,2}$, H-1), 4.40 (2 H, dd, *J* 12.0 + 16.2, OCH_2) 4.76 (2 H, s, OCH_2), 5.49 (1 H, s, PhC*H*), 7.22-7.42 (8 H, m, *Ph*CH and $0CH_2Ar$), 7.52 (2 H, m, *Ph*CH); δ_C ($CDCl_3$): 51.6 (OMe), 56.8 (OMe), 66.4, 69.1, 70.2, 72.1, 73.95, 78.7, 79.8, 101.3, 103.9 (C-1), 126.6, 127.8, 128.2, 128.5, 129.0, 137.9, 138.6, 171.1; MALDI-TOF: *m/z* 468 $(M + Na)^+$,($C_{24}H_{29}O_8Na$ requires *m/z* 468); in accord with data (see **page 139**).

Methyl 3,6-di-*O*-benzyl-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (84)



Compound (**84**) was prepared using a selective acetal opening procedure[3]. A solution of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**80**) (1.14 g, 2.56 mmol) was dissolved in anhydrous THF (20 ml) and stirred before sodium cyanoborohydride (483 mg, 7.69 mmol) and molecular sieves (4 Å) (1.14 g) were added. Diethyl ether saturated with anhydrous HCl, which was then added to the reaction mixture, until the methyl orange indicator turned pink. The resulting solution was stirred at room temperature until TLC [pet. ether – EtOAc, (3:1)] showed the reaction had gone to completion. The reaction mixture was

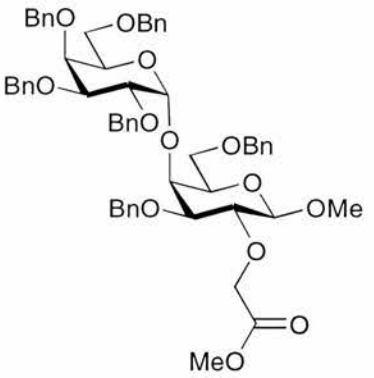
diluted with DCM and water, filtered through Celite, washed with water and then with saturated aqueous $NaHCO_3$ solution. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; pet. ether – EtOAc, 12:1→2:1) gave *methyl 3,6-di-O-benzyl-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**84**) (1.15 g, 83 %); $[\alpha]_D$ + 25.5 (*c* 1.26, $CHCl_3$); δ_H($CDCl_3$): 3.49 (3 H, s, OMe), 3.53 (3 H, m, H-2, 3, 5), 3.70 (4 H, m, H-6/6', OMe), 3.79 (1 H, dd, $J_{5,6/6'}$ 4.5, $J_{6,6'}$ 6.0, H-6/6'), 3.98 (1 H, d, $J_{3,4}$ 2.9, H-4), 4.27 (1 H, d, $J_{1,2}$ 7.0, H-1), 4.34 (2 H, dd, *J* 13.5 + 16.2, OCH_2), 4.57 (2 H, s, OCH_2) 4.75 (2 H, dd, *J* 5.4 + 12.0, OCH_2), 7.22-7.42 (10 H, m, *Ph*CH and 0CH_2Ar); δ_C ($CDCl_3$): 51.6 (OMe), 56.7 (OMe), 67.1, 69.1, 70.1, 72.7, 73.1, 73.7, 79.9, 80.7, 103.9 (C-1), 127.9, 128.0, 128.5, 128.5, 128.6, 128.6, 138.1, 138.2, 170.9 (CO); MALDI-TOF: *m/z* 469 $(M + Na)^+$, ($C_{24}H_{30}O_8Na$ requires *m/z* 469).



85

A sample of methyl 3,6-di-*O*-benzyl-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**84**) was acetylated with acetic anhydride/pyridine and the crude product was confirmed by ^1H NMR specroscopy to be *methyl 4-O-acetyl-3,6-di-O-benzyl-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**85**); δ_H($CDCl_3$): 2.05 (3, s, OAc), 3.49 (3 H, s, OMe), 3.53 (3 H, m , H-2, 3, 5), 3.70 (4 H, m, H-6/6', OMe), 3.79 (1 H, dd, $J_{5,6/6'}$ 4.5, $J_{6,6'}$ 6.0, H-6/6'), 4.27 (1 H, d, $J_{1,2}$ 7.0, H-1), 4.34 (2 H, dd, *J* 13.5 + 16.2, OCH_2), 4.57 (2 H, s, OCH_2) 4.75 (2 H, dd, *J* 5.4 + 12.0, OCH_2), 5.55 (1 H, d, $J_{3,4}$ 3.3, H-4), 7.22-7.42 (10 H, m, *Ph*CH and 0CH_2Ar).

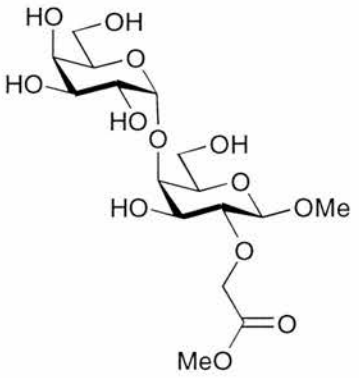
Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-3,6-di-*O*-benzyl-2-methoxycarbonylmethyl-β-D-galactopyranoside (86)



The glycosylation reactions utilized a standard silver triflate promoted glycoylation.[6-9] To a mixture of methyl 3,6-di-*O*-benzyl-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**84**) (318 mg, 0.71 mmol), collidine (173 μl, 1.30 mmol), molecular sieves (4 Å) and diethyl ether:toluene (10 ml, 2:1) was added a solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**57**) (559 mg, 0.99 mmol) in toluene (1 ml). The mixture was cooled (–78^{O}C) and stirred for 1 h under dry nitrogen, then silver triflate (329 mg, 1.28 mmol) was quickly added. The mixture was stirred under nitrogen for approximately 2 h, allowing the temperature to slowly rise to room temperature. Once TLC [pet. ether – EtOAc, (3:1)] indicated the reaction to be complete, collidine (47 μl, 0.35 mmol) was added and stirring continued for approx. 0.5 h. The mixture was diluted with DCM, filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 1 M HCl and extracted with DCM. The combined organic extracts were washed with saturated $NaHCO_3$ solution and water, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; toluene – EtOAc, 20:1→8:1) gave the glycosylated compound *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-3,6-di-O-benzyl-2-methoxycarbonylmethyl-β-D-galactopyranoside* (**86**) (520 mg, 75 %); $[\alpha]_D$ +25.5 (*c* 1.26, $CHCl_3$); ν_{max}/cm^{-1} 1757 (*CO*OMe); δ_H($CDCl_3$): 3.18 (1 H, m, H-6b/6b'), 3.59 (3 H, s, OMe), 3.51 (5 H, m, H-2a, 3a, 5, 6a/6a', 6b/6b'), 3.73 (3 H, s, OMe), 3.98 (1 H, m, H-6a/6a'), 4.03 (1 H, $J_{3,4}$ 3.0, H-4a), 4.03 (3 H, m, H-2b, 3b, 4b), 4.18 (2 H, s, OCH_2), 4.28 (3 H, m, H-1a,

OCH_2), 4.44 (3 H, m, H-5b, OCH_2), 4.60 (2 H, t, *J* 12.0, OCH_2), 4.70 (1 H, d, *J* 13.0, OC*H*), 4.77 (2 H, s, OCH_2), 4.85 (1 H, d, *J* 13.0, OC*H*), 4.92 (2 H, dd, *J* 3.5 + 11.5, OCH_2), 4.94 (1 H, d, $J_{1b,2b}$ 3.0, H-1b), 7.04-7.36 (30 H, m, 6 x OCH$_2$*Ar*); δ_C($CDCl_3$): 51.6, 56.9 (OMe), 68.0, 68.1, 69.4, 70.1, 70.3, 72.3, 72.6, 73.1, 73.2, 73.6, 73.7, 74.8, 74.9, 76.6, 79.0, 80.1, 80.7, 100.3, 104.2, 127.4, 127.6, 127.8, 127.85, 127.9, 128.05, 128.1, 128.3, 128.4, 138.1, 138.7, 138.75, 138.85, 138.9, 170.75; MALDI-TOF: *m/z* 991 $(M + Na)^+$, ($C_{58}H_{64}O_{13}Na$ requires *m/z* 991)

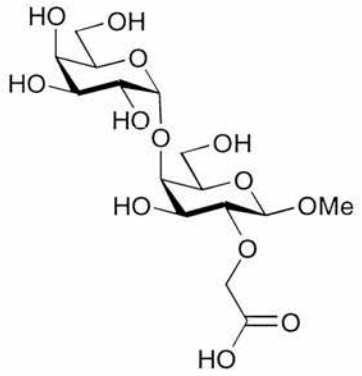
Methyl α-D-galactopyranosyl-(1-4)-2-methoxycarbonylmethyl-β-D-galactopyranoside (87)



Sugar (**86**) was deprotected using a standard hydrogenation procedure.[10] Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-3,6-di-*O*-benzyl-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**86**) (50 mg, 0.052 mmol) and palladium on charcoal (50 mg) were stirred in EtOH (5 ml) under a hydrogen atmosphere. After 12 h the mixture was filtered through Celite and concentrated under reduced pressure to give *methyl α-D-galactopyranosyl-(1-4)-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**87**) (15 mg, 68 %); $[\alpha]_D$ +56.1 (*c* 0.66, MeOH); δ_H(CD_3OD): 3.34 (2 H, m, H-6b/6b'), 3.49 (3 H, s, OMe), 3.51-3.80 (7 H, m, H-2a, 3a, 5, 6a/6a', 2b + 3b,), 3.74 (3 H, s, OMe), 4.03 (1 H, $J_{3a,4a}$ 3.0, H-4a), 4.03 (3 H, d, $J_{3b,4b}$ 2.5, 4b), 4.28 (1H, d, $J_{1a,2a}$ 7.5, H-1a), 4.36 (1 H, m, H5-b), 4.94 (1 H, d, $J_{1b,2b}$ 3.5, H-1b); δ_C(CD_3OD): 50.95, 55.85 (OMe), 59.4, 61.3, 68.7, 69.4, 69.7, 69.95, 71.2, 72.3, 74.5, 76.95, 80.95, 100.9 (C-1b), 104.2 (C-1a), 172.45 (CO); ES-MS: *m/z* 451 $(M + Na)^+$. (Found: $[M + Na]^+$ 451.1425. $C_{16}H_{28}O_{13}Na$

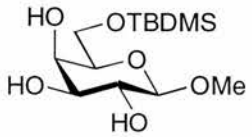
requires *m/z* 451.14277).

Methyl α-D-galactopyranosyl-(1-4)-2-*O*-carbonylmethyl-β-D-galactopyranoside (88)



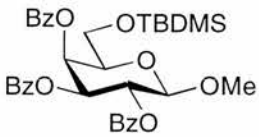
Sugar (**87**) was deprotected using a standard ester clevage procedure.[20] Methyl α-D-galactopyranosyl-(1-4)-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**87**) (12 mg, 0.028 mmol) was dissolved in 50 % aqueous methanol (2 ml) and LiOH was added until pH 11. The solution was stirred at room temperature for 1 h. Once TLC [DCM-MeOH, (6:1)] showed the rection to be complete, the resulting solution was neutralised with Amberlite IR120 (H^+) ion exchange resin. The resulting solution was filtered and concentrated under reduced pressure to give *methyl α-D-galactopyranosyl-(1-4)-2-O-carbonylmethyl-β-D-galactopyranoside* (**88**) (11 mg, 95 %); $[\alpha]_D$ +42.5 (*c* 0.56, MeOH); $\delta_H(CD_3OD)$: 3.34 (2 H, m, H-6b/6b'), 3.49 (3 H, s, OMe), 3.51-3.80 (7 H, m, H-2a, 3a, 5, 6a/6a', 2b + 3b), 3.99 (1 H, $J_{3a,4a}$ 3.0, H-4a), 4.04 (3 H, d, $J_{3b,4b}$ 3.3, 4b), 4.31 (1H, d, $J_{1a,2a}$ 7.8, H-1a), 4.33 (1 H, s, H5-b), 4.99 (1 H, d, $J_{1b,2b}$ 3.0, H-1b); $\delta_C(CD_3OD)$: 55.9 (OMe), 59.4, 61.3, 68.5. 69.4, 69.75, 69.9, 71.0, 72.2, 74.6, 76.7, 80.8, 100.9 (C-1b), 104.3 (C-1a), 173.9 (CO); ES-MS: *m/z* 437 $(M + Na)^+$. (Found: $[M + Na]^+$ 437.1282. $C_{15}H_{26}O_{13}Na$ requires *m/z* 437.12711).

Methyl 6-*O*-(*t*-butyldimethylsilyl)-β-D-galactopyranoside (89)[21]



Compound (**89**) was prepared using a standard silylation protocol.[21,22] A solution of compound (**46**) (2.0 g, 10.30 mmol) in anhydrous pyridine (20 ml) containing TBDMSCl (1.86 g, 12.4 mmol) was stirred at 0°C and allowed to warm slowly to room temperature (approx. 5 h). The solution was cooled and methanol was added. The resulting solution was concentrated and coevaporated with toluene to give a syrup. Column chromatography (silica gel; pet. ether-EtOAc, 12:1→2:1) gave the *silylated sugar* (**89**) as a syrup (2.19 g, 69 %); $[\alpha]_D$ - 28.6 (*c* 1.05 $CHCl_3$) (lit.[21], -30.5); $\delta_H(CDCl_3)$: -0.05 (6 H, s, 2 x CH_3Si), 0.70 (9 H, s, t*Bu*Si), 3.52 (5 H, m, H-2, 3, OCH_3), 3.56 (1 H, m , H-5), 3.75 (2H, m, H-6, 6'), 3.90 (1 H, d, $J_{3,4}$ 3.3, H-4), 4.16 (1 H, d, $J_{1,2}$ 7.5, H-1).

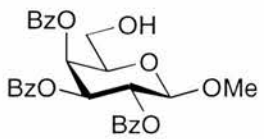
Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(t.butyldimethylsilyl)-β-D-galactopyranoside (90)



Sugar (**89**) was protected using a standard benzoylation procedure.[23] Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(t.butyldimethylsilyl)-β-D-galactopyranoside (**90**) was prepared using a standard benzolylation protocol.[7] The silylated sugar (**89**) (2.1 g, 6.81 mmol) was dissolved in anhydrous pyridine (21 ml) and cooled (0°C) before benzoyl chloride (3.55 ml, 31 mmol) was added slowly. The reaction mixture was allowed to warm up to room temperature and was stirred overnight. The resulting solution was coevaporated with methanol and toluene to give a syrup which was dissolved in DCM and washed with dilute HCl. The organic layer was separated and washed with sodium hydrogen carbonate solution and water, dried (Na_2SO_4) and concentrated

under reduced pressure to give a syrup. Column chromatography (silica gel; pet. ether-EtOAc, 12:1→2:1) gave the *protected sugar* (**90**) as colourless crystals (3.36 g, 80 %); mp 98-100 ^{0}C (hexane-EtOAc); $[\alpha]_D$ +138.9 (*c* 0.93, $CHCl_3$); $\delta_H(CDCl_3)$: 0.00 (6H, s, 2 x $SiCH_3$), 0.70 (9 H, t, t*Bu*Si), 1.24 (10 H, s, 5 x CH_2), 3.60 (3 H, s, OCH_3) 3.76-3.92 (2 H, m, H-6/6'), 4.03 (1 H, m, H-5), 4.72 (1 H, d, $J_{1,2}$ 7.8, H-1), 5.63 (1 H, dd, $J_{2,3}$ 7.8, $J_{3,4}$ 3.3, H-3), 5.76 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.98 (1 H, d, $J_{3,4}$, H-4), 7.21 (2 H, m, Ar), 7.44 (6 H, m, Ar), 7.50 (1 H, m, Ar), 7.80 (2 H, d, *J* 8.1, Ar), 8.00 (2 H, d, *J* 8.1, Ar), 8.10 (2 H, d, *J* 8.1, Ar); $\delta_H(CDCl_3)$: 18.6, 26.0, 57.9 (OMe), 62.0, 67.8, 70.0, 72.0, 74.4, 103.5, 128.6, 128.7, 128.8, 128.9, 129.4, 130.1, 130.2, 130.3, 130.4, 133.6, 133.7, 133.8, 165.6, 165.7, 165.8; CI-MS: *m/z* 621 $(M + H)^+$. (Found: $[M + H]^+$ 621.24987. $C_{34}H_{41}OSi$ requires *m/z* 621.25206).

Methyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (91)

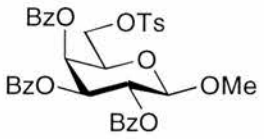


91

Methyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (**91**) was prepared using a modified de-silylation protocol.[21] The protected sugar (**90**) (3.36 g, 5.41 mmol) was dissolved in 80% aqueous acetic acid (35 ml) and was stirred at room temperature overnight. The resulting sugar was coevaporated with toluene to give a syrup. Column chromatography (silica gel; pet. ether-EtOAc, 12:1--2:1) gave two compounds, unreacted starting material (393 mg, 14.5 %) and *methyl 2,3,4-tri-O-benzoyl-β-D-galactopyranoside* (**91**) as a syrup (2.33 g, 85%); $[\alpha]_D$ +202.4 (*c* 1.02, $CHCl_3$); $\delta_H(CDCl_3)$: 2.48 (1 H, brs, 6-OH), 3.59 (3 H,s, OMe), 3.68 (1H, dd, $J_{5,6/6'}$ 4.8, $J_{6,6'}$ 11.7, H-6/6'), 3.86 (1H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.06 (1 H, m, H-5), 4.75 (1 H, d $J_{1,2}$ 7.8, H-1), 5.61 (1H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 2.4, H-4), 5.84 (1H, d, $J_{3,4}$, H-4), 5.84 (1H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 7.26 (2H, m, Ar), 7.36-7.64 (7 H, m, Ar), 7.80 (2 H, d, *J* 8.4, Ar), 7.98 (2 H, d, *J* 8.4, Ar), 8.10 (2 H, d, *J* 8.4, Ar); $\delta_C(CDCl_3)$: 57.3, 60.6, 69.0, 69.9, 71.8, 73.9, 102.5, 128.4, 128.6, 129.7, 130.0, 133.2, 133.3, 133.8, 165.4, 165.5; Ci-MS: *m/z* 220 $(M + H)^+$. (Found: $[M + H]^+$ 507.16432. $C_{28}H_{27}O_9$ requires *m/z*

507.16559).

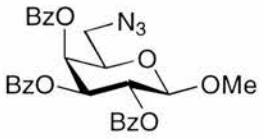
Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-toluenesulfonyl-β-D-galactopyranoside (92)[24]



92

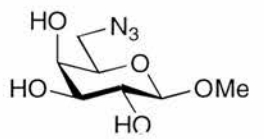
Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-toluenesulfonyl-β-D-galactopyranoside (**92**) was prepared using a standard tosylation protocol.[24] The protected sugar (**91**) (2.18 g, 4.31 mmol) was stirred in acetone (2.31 ml) and anhydrous pyridine (1.47 ml) until all the sugar had dissolved. The solution was cooled in cold water, and *p*-toluenesulfonyl chloride (1.23 g, 6.47 mmol) was added in portions over 10 min. The reaction mixture was stirred overnight at room temperature, cooled in ice water and water (2 ml) was added. The reaction mixture was poured into ice water and the resulting syrup soon crystallised. The crystals were removed by filtration, washed with water and dried *in vacuo*. Recrystallization gave *methyl 2,3,4-tri-O-benzoyl-6-O-toluenesulfonyl-β-D-galactopyranoside* (**92**) as white crystals (2.61 g, 92 %); mp. 194-5^{O}C (isopropanol), (lit.[24], 194); $[\alpha]_D$ + 133.1 (*c* 0.96, $CHCl_3$), (lit.[24], 148.4); $\delta_H(CDCl_3)$: 2.28 (3 H, s, Me of Ts), 3.55 (1 H, m, OCH_3), 4.10 (1 H, dd, $J_{5,6/6'}$ 4.8, H-5), 4.20- 4.35 (2 H, m, H-6/6'), 4.70 (1 H, d, $J_{1,2}$ 8.1, H-1), 5.52 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 3.3 , H-3), 5.69 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.83 (1 H, d, $J_{3,4}$, H-4), 7.21 (4 H, m, Ar), 7.44 (6 H, m, Ar), 7.50 (1 H, m, Ar), 7.72 (4 H, m, Ar), 7.96 (4 H, m, Ar); $\delta_C(CDCl_3)$: 22.0, 57.9 (OMe), 67.8, 68.1, 69.4, 71.0, 71.4, 103.5, 128.6, 128.7, 128.8, 128.9, 129.2, 129.3, 129.4, 130.1, 130.2, 130.3, 130.4, 132.2, 133.6, 133.7, 133.8, 145.0, 165.6, 165.7, 165.8; MALDI-TOF: *m/z* 643 $(M + Na)^+$, ($C_{34}H_{40}O_9SiNa$ requires *m/z* 643).

Methyl 6-Azido-2,3,4-tri-*O*-benzoyl-6-deoxy-β-D-galactopyranoside (93)[25]



A solution of compound (**92**) (2.24 g, 3.4 mol) in DMF (30 ml) containing suspended sodium azide (2.21 g, 34 mol) was heated under reflux for 4 h. The solution was allowed to cool, diluted with water (20 ml) and extracted with DCM. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give methyl 6-azido-2,3,4-tri-*O*-benzoyl-6-deoxy-β-D-galactopyranoside (**93**) as a syrup (1.46 g, 82 %); $[\alpha]_D$ + 197 (*c* 0.91, $CHCl_3$) (lit.[25], + 209); ν_{max}/cm^{-1} 2100 (N_3); $\delta_H(CDCl_3)$: 3.29 (1 H, dd, $J_{5,6/6'}$ 4.5, $J_{6,6'}$ 13.2, H-6/6'), 3.61 (3 H, m, OCH_3), 3.65 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.10 (1 H, dd, $J_{5,6/6'}$, H-5), 4.76 (1 H, d, $J_{1,2}$ 8.1, H-1), 5.58 (1 H, dd, $J_{2,3}$ 6.9, $J_{3,4}$ 3.3, H-3), 5.79 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 5.83 (1 H, d, $J_{3,4}$, H-4), 7.22 (2 H, m, Ar), 7.32-7.52 (6 H, m, Ar), 7.61 (1 H, m, Ar), 7.78 (2 H, d, *J* 7.8, Ar), 7.96 (2 H, d, *J* 7.8, Ar), 8.08 (2 H, d, *J* 7.8, Ar); $\delta_C(CDCl_3)$: 50.9, 57.3, 68.8, 69.6, 71.6, 73.6, 102.4, 128.3, 128.4, 128.6, 128.8, 129.3, 129.7, 130.0, 133.2, 133.3, 133.7, 165.5, 165.6, 165.7; CI-MS: *m/z* 532 $(M + H)^+$. (Found: $[M + H]^+$ 532.17199. $C_{28}H_{26}N_3O_8$ requires *m/z* 532.1720).

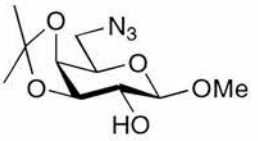
Methyl 6-azido-6-deoxy-β-D-galactopyranoside (94)



The title compound (**94**) was prepared using a standard sodium methoxide deprotection.[26] A solution of the benzoylated methyl galactoside (**93**) (1.1 g, 2.07 mmol) in methanol (12 ml) containing sodium metal (trace) was stirred at room temperature for 2 h. Once TLC [DCM-MeOH, (6:1)] showed the reaction to be complete, the resulting solution was neutralised with Amberlite IR120 (H^+) ion

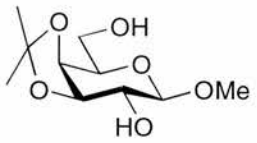
exchange resin. The resulting solution was filtered and concentrated to give *methyl 6-azido-6-deoxy-β-D-galactopyranoside* (**94**) as a white solid (404 mg, 89 %); $[\alpha]_D$ -79.5 (*c* 0.92, MeOH); $\delta_H(CD_3OD)$: 3.26 (1 H, dd, $J_{5,6/6'}$ 3.0, $J_{6,6'}$ 9.5, H-6/6'), 3.32 (1 H, m, H-5), 3.56 (3 H, m, OCH_3), 3.80 (3 H, m, H-2, 3, 6/6'), 4.01 (1 H, m, H-4), 4.41 (1 H, d, $J_{1,2}$ 7.0, H-1); $\delta_C(CD_3OD)$: 49.6, 54.3, 67.9, 69.4, 71.8, 72.9, 102.9; MALDI-TOF: *m/z* 242 $(M + Na)^+$, ($C_7H_{13}N_3O_5Na$ requires *m/z* 242).

Methyl 6-azido-6-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranoside (95)



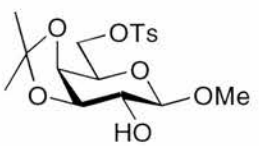
Sugar (**94**) was protected using a standard acetal formation procedure.[27] A solution of methyl 6-azido-6-deoxy-β-D-galactopyranoside (**94**) (300 mg, 1.37 mmol) and iodine (100 mg, 0.39 mmol) in acetone (15 ml) was stirred at room temperature until TLC [toluene - EtOAc (2:1)] showed that the reaction was complete (approx. 20 h). The solution was diluted with sodium thiosulphate and extracted with DCM. The organic extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give *methyl 6-azido-6-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside* (**95**) as a syrup (329 mg, 93 %); mp 80-81 ^OC (DCM); $[\alpha]_D$ –16.0 (*c* 0.95, $CHCl_3$); ν_{max}/cm^{-1} 2097 (N_3); requires C, 46.60; H 6.59; N, 15.75; found C, 46.63; H, 6.61; N, 16.21; $\delta_H(CDCl_3)$: 1.36 (3 H, s, CH_3), 1.48 (3 H,s, CH_3),2.85 (1 H, br s, OH), 3.39 (1 H, dd, $J_{5,6,6'}$ 4.5, $J_{6,6'}$ 12.9,H-6/6') 3.49 (1 H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 8.4, H-2), 3.51 (3 H, s, OMe), 3.69 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.90 (1 H, m H-5), 4.01-4.08 (2 H, m, H-3, 4), 4.08 (1 H, d, $J_{1,2,}$ H-1); $\delta_C(CDCl_3)$: 26.2 (CH_3),27.9 (CH_3), 51.05 (C-6), 57.0 (OMe), 72.9, 73.5, 73.8, 78.9, 103.3 (C-1), 110.5 ($C(CH_3)_2$); CI-MS: *m/z* 260 $(M + H)^+$. (Found: $[M + H]^+$ 260.12465. $C_{10}H_{17}N_3O_5$ requires *m/z* 260.1247).

Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (96)[2]



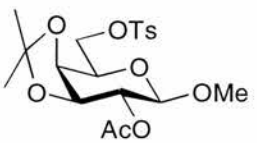
Methyl β-D-galactopyranoside (**46**) (10 g, 51.5 mmol) was suspended in 2,2 dimethoxypropane (225 ml) and a catalytic amount of *p*-tolunenesulfonic acid monohydrate (490 mg, 2.6 mmol) was added. The reaction mixture was stirred at room temperature until TLC [DCM - MeOH, (6:1)] showed the reaction to be complete, TEA was added to neutralise the solution. The solution was then concentrated under reduced pressure to a syrup which was then dissolved in DCM (100 ml) and acidified with 50% aqueous TFA (1.5 ml). Once TLC [DCM – MeOH, (6:1)] showed the reaction to be complete, TEA was added to neutralise the solution. The solution was concentrated under reduced pressure to a syrup and column chromatography (silica gel; EtOAc – pet. Ether, 1:3→5:1) gave the methyl 3,4-O-isopropylidene-β-D-galactopyranoside (**96**) (11.16 g, 93 %) mp ^{o}C 133-135^{o}C (acetone:hexane), (lit.[2], 134-135 ^{o}C); $[\alpha]_D$ + 7.61 (*c* 1.2, Acetone) (lit.[2], + 8.0); δ_H(CD_3OD): 1.31 (3 H, s, CH_3), 1.46 (3H, s, CH_3), 3.37 (1 H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 7.8, H-2), 3.52 (3 H, s, OMe), 3.76 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.78 (1 H, d, $J_{3,4}$ 2.7, H-4), 3.83 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.01 (1 H, dd, $J_{5,6/6'}$, H-5), 4.1 (1 H, d, $J_{1,2}$, H-1), 4.18 (1 H, dd, $J_{2,3}$, $J_{3,4}$, H-3).

Methyl 3,4-*O*-isopropylidene-6-*O*-toluenesulfonyl-β-D-galactopyranoside (97)[2]



The acetal (**96**) (11.0 g, 47.0 mmol) was stirred in acetone (13.0 ml) and anhydrous pyridine (8.3 ml) until all the acetal had dissolved. The solution was cooled in cold water, and *p*-toluenesulfonyl chloride (10.7 g, 56.3 mol) was added in portions over

0.5 h. The reaction mixture was stirred overnight at room temperature then cooled in ice-water. Water (5 ml) was added and on stirring the resulting syrup soon crystallised. The crystals were removed by filtration, washed with water and dried *in vacuo*. Recrystallization gave *methyl 3,4-O-isopropylidene-6-O-toluenesulfonyl-β-D-galactopyranoside* (**97**) as white needles (16.3 g, 89 %), mp 154-155°C (ethyl acetate:hexane), (lit.[2],154-155 °C); $[\alpha]_D$ 0.0 (*c* 1.0, $CHCl_3$) (lit.[2], 0.0, $CHCl_3$); $\delta_H(CD_3OD)$: 1.27 (3 H, s, CH_3), 1.40 (3H, s, CH_3), 2.45 (3H, s, CH_3 of OTs), 3.32 (1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.0, H-2), 3.44 (3 H, s, OCH_3), 4.00 (1 H, t, $J_{5,6/6'}$ 7.2, H-5), 4.07 (1 H, d, $J_{1,2}$, H-1), 4.05-4.20 (3 H, m, H-4, 6/6'), 4.22 (1 H, dd, $J_{2,3}$, $J_{3,4}$, H-3), 7.43 (2 H, d, *J* 8.1, Ar), 7.80 (2 H, d, *J* 8.1, Ar).



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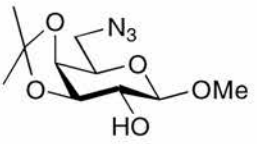
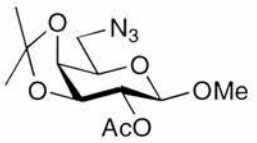
A sample of (**97**) was acetylated with acetic anhydride/pyridine and the crude product, was identified by ^{1}H NMR spectroscopy to be *methyl 3,4-O-isoproylidene-6-O-toluenesulfonyl-β-D-galactopyranoside* (**98**); $\delta_H(CDCl_3)$: 1.28 (3 H, s, CH_3), 1.42 (3H, s, CH_3), 2.06 (3 H, s, OAc), 2.42 (3H, s, CH_3 of OTs), 3.41 (3 H, s, OCH_3), 4.05 (1 H, m, H-5), 4.11-4.3 (4 H, m, H-3, 4, 6/6'), 4.21 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.88 (1 H, dd, $J_{1,2}$, $J_{2,3}$ 8.1, H-2), 7.34 (2 H, d, *J* 8.1, Ar), 7.80 (2 H, d, *J* 8.1, Ar).

Methyl 6-azido-6-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranoside (95)



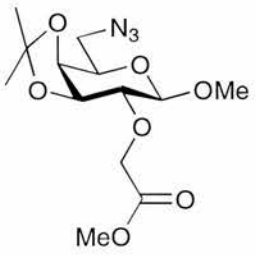
Sugar (**95**) was formed using a standard azide displacment procedure.[4] A solution of compound (**97**) (6.71 g, 17.3 mol) in DMF (155 ml) containing suspended sodium azide (6.74 g, 104 mol) was heated under reflux for 4 h. The solution was allowed to cool, diluted with water (100 ml) and extracted with diethyl ether. The organic

extract was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give *methyl 6-azido-6-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside* (**95**) as a syrup (3.72 g, 83 %); ν_{max}/cm^{-1} 2097 (N_3); $\delta_H(CDCl_3)$: 1.35 (3 H, s, CH_3), 1.48 (3 H,s, CH_3), 2.84 (1 H, br s, OH), 3.39 (1 H, dd, $J_{5,6,6'}$ 4.4, $J_{6,6'}$ 12.9,H-6/6') 3.49 (1 H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 8.3, H-2), 3.51 (3 H, s, OMe), 3.69 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.90 (1 H, m H-5), 4.01-4.08 (2 H, m, H-3, 4), 4.08 (1 H, d, $J_{1,2,}$ H-1); $\delta_C(CDCl_3)$: 26.2 (CH_3),27.9 (CH_3), 51.05 (C-6), 56.9 (OMe), 72.8, 73.5, 73.8, 78.8, 103.3 (C-1), 110.5 ($C(CH_3)_2$); in accord with previous results, **page 152**.



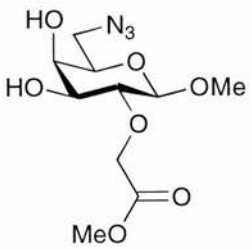
A sample of (**95**) was acetylated with acetic anhydride/pyridine and the crude product, was identified by ^1H NMR spectroscopy to be *methyl 2-O-acetyl-6-azido-6-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside* (**96**); $\delta_H(CDCl_3)$: 1.36 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 2.10 (3 H, s, OAc), 3.39 (1 H, dd, $J_{5,6/6'}$ 4.5, $J_{6,6'}$ 12.9, H-6'/6), 3.50 (3 H, s, OMe), 3.73 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.92 (1 H, m, H-5), 4.11 (1 H, dd, $J_{3,4}$ 3.3, $J_{4,5}$ 2.1, H-4), 4.18 (1 H, dd, $J_{2,3}$ 7.2, $J_{3,4}$, H-3), 4.28 (1 H, d, $J_{1,2}$ 8.0, H-1), 4.96 (1 H, t, $J_{1,2}$, $J_{2,3}$, H-2); $\delta_C(CDCl_3)$: 20.5 (OAc), 26.2 (CH_3), 27.9 (CH_3), 51.0 (C-6), 57.0 (OMe), 72.9, 73.5, 73.8, 78.9, 103.3 (C-1), 110.6 ($C(CH_3)_2$), 164.2 (OAc).

Methyl 6-azido-6-deoxy-3,4-*O*-isopropylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (100)

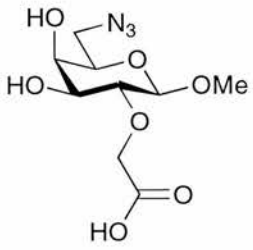


A solution of methyl 6-azido-6-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranoside (**95**) (280 mg, 1.1 mmol) was dissolved in DMF (8 ml) and cooled (0^{O}C) before sodium hydride (60% w/w suspension in mineral oil) (52 mg, 1.3 mmol) was slowly added. After 0.5 h methyl bromoacetate (133 μl, 1.40 mmol) was slowly added and the solution was stirred until TLC [pet. ether – EtOAc, (3:1)] showed the reaction had gone to completion. The reaction mixture was cooled (0^{O}C) before methanol (3 ml) was added to quench the reaction. The solution was stirred for 0.5 h and concentrated under reduced pressure to a syrup. The syrup was diluted with water and extracted with diethyl ether. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; pet. ether – EtOAc, 10:1--2:1) gave *methyl 6-azido-6-deoxy-3,4-di-O-isopropylidene-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**100**) as a solid (307 mg, 86 %), mp 76-79^{O}C; $[\alpha]_D$ – 16.4 (*c* 1.26, $CHCl_3$); ν_{max}/cm^{-1} 1734 (*CO*OMe), 2097 (N_3); $\delta_H(CDCl_3)$: 1.30 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 3.31 (1 H, dd, $J_{5,6'/6}$ 4.5, $J_{6,6'}$ 10.2, H-6'/6), 3.34 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$ 6.6, H-2) 3.50 (3 H, s, OMe), 3.70 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.74 (3 H, s, OCH_2COOCH_3), 3.87 (1 H, m, H-5), 4.08 (1 H, dd, $J_{3,4}$ 3.6, $J_{4,5}$ 2.1, H-4), 4.21 (1 H, dd, $J_{2,3}$, $J_{3,4}$, H-3), 4.26 (1 H, d, $J_{1,2}$, H-1), 4.33 (1 H, d, OCH_2COOCH_3), 4.40 (1 H, d, OCH_2COOCH_3); $\delta_C(CDCl_3)$: 26.1 (CH_3), 27.7 (CH_3), 50.9 (C-6), 56.5 (OMe), 68.9 (COOMe), 72.6, 73.5, 73.7, 78.3, 81.6, 103.3 (C-1), 110.6 ($C(CH_3)_2$), 170.8 (CO); MALDI-TOF: *m/z* 354 $(M + Na)^+$, ($C_{13}H_{21}N_3O_7Na$ requires *m/z* 354).

Method 1 : Acetal removal[28]

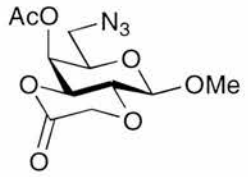


103



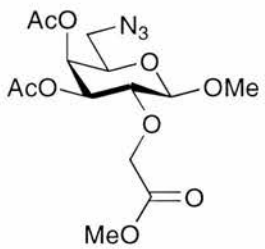
101

A solution of compound (**100**) (1.0 g, 3.0 mmol) in TFA-DCM (8:2) (10 ml) was stirred until TLC [pet. ether – EtOAC, (3:1)] showed the reaction to be complete (approx 15 min). The resulting solution was neutralised with TEA and concentrated under reduced pressure. Column chromatography (silica gel; pet. ether –EtOAc, 1:1—1:10) gave two compounds, *methyl 6-azido-6-deoxy-2-O-carboxymethyl-β-D-galactopyranoside* (**101**) (616 mg, 69 %); $[\alpha]_D$ -3.92 (*c* 0.97, MeOH); ν_{max}/cm^{-1} 2097 (N_3); $\delta_H(CD_3OD)$: 3.22 (1 H, d, $J_{6,6'}$ 9.0, H-6/6'), 3.32 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$ 7.4, H-2), 3.53 (3 H, s, OMe), 3.57 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 3.3, H-3), 3.61 (1 H, d, $J_{6,6'}$ 9.0, H-6/6'), 3.66 (1 H, s, H-5), 3.74 (1 H, dd, $J_{3,4}$, H-4) 4.07 (2 H, d, *J* 17.8, OCH_2COOMe), 4.26 (1 H, d, $J_{1,2}$, H-1); $\delta_C(CDCl_3)$: 51.2 (C-6), 55.8 (OMe), 68.8, 69.0, 72.5, 74.3, 79.9, 104.1 (C-1), 173.0 (CO); MALDI-TOF: *m/z* 300 $(M + Na)^+$, ($C_9H_{15}N_3O_7Na$ requires *m/z* 300) and *methyl 6-azido-6-deoxy-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**103**) (164 mg, 19 %) mp 103-104 ^oC (DCM); $[\alpha]_D$ –7.41 (*c* 0.61, $CHCl_3$); ν_{max}/cm^{-1} 2097 (N_3); requires C, 41.24; H, 5.88; N, 14.43; found C, 41.40; H, 5.87; N, 14.21; $\delta_H(CDCl_3)$: 3.26 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 3.6 H-3), 3.32 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$, H-2), 3.55 (3 H, s, OMe), 3.60-3.69 (3 H, m, H-5 + H-6/6') 3.76 (3 H, s, OCH_2COOMe), 3.85 (1 H, d, $J_{3,4}$, $J_{4,5}$ 1.2, H-4), 4.25 (1 H, d, $J_{1,2,}$ H-1) 4.36 (2 H, dd, *J* 17.5 + 6.3, OCH_2COOMe); $\delta_C(CD_3OD)$: 51.1 (C-6), 55.7 (OMe), 68.6, 69.1, 72.2, 74.4, 80.7, 104.1 (C-1), 173.0 (CO); CI-MS: *m/z* 292 $(M + H)^+$. (Found: $[M + H]^+$ 292.114475. $C_{10}H_{18}N_3O_7$ requires *m/z* 292.11455).



102

A sample of compound (**101**) was acetylated with acetic anhydride/pyridine and the crude product was identified by ^{1}H NMR to be *methyl 4-O-acetyl-6-azido-6-deoxy-2,3-lactone-β-D-galactopyranoside* (**102**); $\delta_H(CDCl_3)$: 3.60 (1 H, dd, $J_{5,6/6'}$ 8.4, $J_{6,6'}$ 13.2, H-6/6'), 3.48 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.46 (3 H, s, OMe), 3.73 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$ 7.5, H-2), 3.84 (1 H, dd, $J_{5,6/6'}$, H-5), 4.34 (1 H, d, $J_{1,2}$, H-1), 4.34 (1 H, d, *J* 17.8, OCH_2COOMe), 4.50 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 3.3, H-3), 4.55 (1 H, d, *J* 17.8, OCH_2COOMe) 5.39 (1 H, d, $J_{3,4}$, H-4).



104

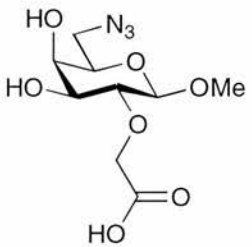
A sample of methyl 6-azido-6-deoxy-2-methoxycarbonylmethyl-β-D-galactopyranoside (**103**) was acetylated with acetic anhydride/pyridine and the crude product was identified by ^{1}H NMR to be *methyl 3,4-di-O-acetyl-6-azido-6-deoxy-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**104**); $\delta_H(CDCl_3)$: 2.05 (3 H, s, OAc), 2.15 (3 H, s, OAc), 3.07 (1 H, dd, $J_{5,6/6'}$ 4.0, $J_{6,6'}$ 9.0, H-6/6'), 3.49–3.55 (2 H, m, H-2, 6/6'), 3.55 (3 H, s, OMe), 3.73 (3 H, s, OCH$_2$COO*Me*), 3.81 (1 H, m, H-5), 4.30 (2 H, dd, *J* 16.0 + 7.5, OCH_2COOMe), 4.43 (1 H, d, $J_{1,2}$, 7.5, H-1), 5.01 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 3.5, H-3), 5.30 (1 H, d, $J_{3,4}$, $J_{4,5}$ 1.0, H-4).

Method 2 : Acetal removal[17]

A solution of methyl 6-azido-6-deoxy-3,4-*O*-isopropylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**100**) (704 mg, 2.13mmol) in methanol (12 ml) and 50 % aqueous HBF_4 (0.2 ml) was stirred at room temperature until TLC [DCM-MeOH, (6:1)] indicated the reaction to be complete. $NaHCO_3$ (150

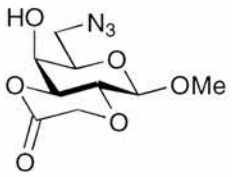
mg) was added and the mixture was stirred for 1 h and then concentrated. The compound was extracted with DCM and the combined extracts were washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to yield *methyl 6-azido-6-deoxy-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**103**) (417 mg, 80 %); data as reported on **page 156**.

Methyl 6-azido-6-deoxy-2-*O*-carboxymethyl-β-D-galactopyranoside (101)



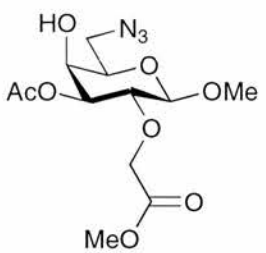
Sugar (**103**) was deprotected using a standard ester cleavage procedure.[20] The resulting syrup (**103**) (100 mg, 0.34 mmol) was dissolved in MeOH : H_2O (1:1) (2 ml) and LiOH was added until pH 11. The solution was stirred at room temperature for 1 h. Once TLC [DCM-MeOH, (6:1)] showed the rection to be complete, the resulting solution was neutralised with Amberlite IR120 (H^+) ion exchange resin. The resulting solution was filtered and concentrated under reduced pressure to give *methyl 6-azido-6-deoxy-2-O-carboxymethyl-β-D-galactopyranoside* (**101**) (83 mg, 88 %); $[\alpha]_D$ -3.92 (*c* 0.97, MeOH); ν_{max}/cm^{-1} 1670 (CO), 2097 (N_3); $\delta_H(CD_3OD)$: 3.22 (1 H, d, $J_{6,6'}$ 9.0, H-6/6'), 3.32 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$ 7.4, H-2), 3.53 (3 H, s, OMe), 3.57 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 3.3, H-3), 3.61 (1 H, d, $J_{6,6'}$ 9.0, H-6/6'), 3.66 (1 H, s, H-5), 3.74 (1 H, dd, $J_{3,4}$, H-4) 4.07 (2 H, d, *J* 17.8, OCH_2COOMe), 4.26 (1 H, d, $J_{1,2}$, H-1); $\delta_C(CDCl_3)$: 51.2 (C-6), 55.8 (OMe), 68.8, 69.0, 72.5, 74.3, 79.9, 104.1 (C-1), 173.0 (CO); MALDI-TOF: *m/z* 300 $(M + Na)^+$, ($C_9H_{15}N_3O_7Na$ requires *m/z* 300).

Formation of lactone (105) from Methyl 6-azido-6-deoxy-2-*O*-carboxymethyl-β-D-galactopyranoside (101)

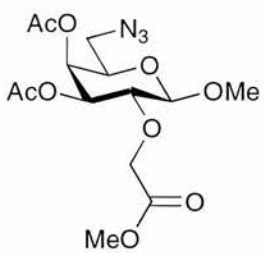


A solution of sugar (**101**) (120 mg, 0.43 mmol) was dissolved in DMF (2 ml) and stirred before 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (220 mg, 0.52 mmol) was added. The resulting solution was stirred at room temperature until TLC [pet. ether – EtOAc, (1:1)] showed the reaction had gone to completion. The reaction mixture was diluted with diethyl ether and was washed with water and salt solution, dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; EtOAc) gave the *lactone* (**105**) (20 mg, 16 %), $[\alpha]_D$ – 15.9 (*c* 0.36, $CHCl_3$); ν_{max}/cm^{-1} 1720 (CO), 2100 (N_3); $\delta_H(CDCl_3)$: 3.35 (1 H, dd, $J_{5,6,6'}$ 2.1, $J_{6,6'}$ 7.8, H-6/6'), 3.60 (3 H, s, OMe), 3.74 (1 H, m, $J_{5,6,6'}$, H-5), 3.75 (2 H, m, H-2, 6/6'), 4.07 (1 H, dd, $J_{3,4}$ 2.7, H-4), 4.38 (1 H, dd, $J_{2,3}$ 6.6, $J_{3,4,}$ H-3), 4.42 (1 H, d, $J_{1,2}$ 7.5, H-1), 4.43 (1 H, d, *J* 18.0, OCH_2COOMe) 4.62 (1 H, d, *J* 18.0, OCH_2COOMe); $\delta_C(CDCl_3)$: 50.7 (C-6), 57.1 (OMe), 66.2, 67.1, 71.5, 74.0, 79.7, 101.3 (C-1), 165.8 (CO); CI-MS: *m/z* 260 $(M + H)^+$. (Found: $[M + H]^+$ 260.07699. $C_9H_{13}N_3O_6$ requires *m/z* 260.08834).

Acetylation of Methyl 6-azido-6-deoxy-2-*O*-carboxymethyl-β-D-galactopyranoside (101)



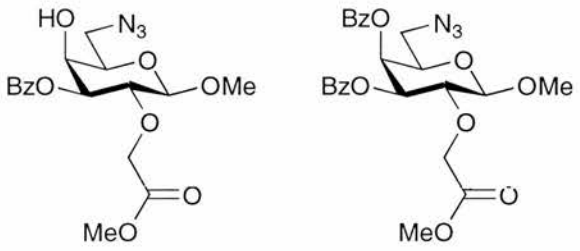
106



107

A solution of methyl 6-azido-6-deoxy-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**103**) (63 mg, 0.22 mmol), anhydrous pyridine (19 µl, 0.24 mmol) and acetic anhydride (22 µl, 0.24 mmol) in DCM (1 ml) were cooled (-45 ^{0}C) and stirred overnight. The reaction mixture was coevaporated with toluene and taken up in DCM. The resulting solution was washed with dilute HCl and $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; pet. ether - EtOAc, 3:1-1:1) gave a mixture of two compounds, *methyl 3,4-di-O-acetyl-6-azido-6-deoxy-2-O-methoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside* (**107**) as a syrup (22 mg, 31 %); $[\alpha]_D$ – 11.0 (*c* 0.73, $CHCl_3$); $\delta_H(CDCl_3)$: 2.05 (3 H, s, OAc), 2.15 (3 H, s, OAc), 3.07 (1 H, dd, $J_{5,6/6'}$ 4.0, $J_{6,6'}$ 9.0, H-6/6'), 3.49–3.55 (2 H, m, H-2, 6/6'), 3.55 (3 H, s, OMe), 3.73 (3 H, s, OCH_2COOMe), 3.81 (1 H, m, H-5), 4.30 (2 H, dd, *J* 16.0 + 7.5, OCH_2COOMe), 4.43 (1 H, d, $J_{1,2}$ 7.5, H-1), 5.01 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 3.5, H-3), 5.30 (1 H, d, $J_{3,4}$, $J_{4,5}$ 1.0, H-4); $\delta_C(CDCl_3)$: 20.6, 50.7, 51.7, 57.1, 68.3, 69.6, 71.4, 72.8, 78.0, 104.1, 169.9, 170.1, 170.4; MALDI-TOF: *m/z* 398 $(M + Na)^+$, ($C_{14}H_{21}N_3O_9Na$ requires *m/z* 398) and *methyl 3-O-acetyl-6-azido-6-deoxy-2-O-methoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside* as a syrup (**106**) (31 mg, 43 %); $[\alpha]_D$ – 22.5 (*c* 1.48, $CHCl_3$); $\delta_H(CDCl_3)$: 2.15 (3 H, s, OAc), 3.07 (1 H, dd, $J_{5,6/6'}$ 4.0, $J_{6,6'}$ 9.0, H-6/6'), 3.49–3.55 (2 H, m, H-2, 6/6'), 3.55 (3 H, s, OMe), 3.73 (3 H, s, OCH_2COOMe), 3.81 (1 H, m, H-5), 3.96 (1 H, m, H-4).4.30 (2 H, s, OCH_2COOMe), 4.43 (1 H, d, $J_{1,2}$ 8.0, H-1), 5.01 (1 H, dd, $J_{2,3}$ 6.6, $J_{3,4}$ 3.5, H-3); $\delta_H(CDCl_3)$: 20.8, 51.1, 52.3, 57.3 (OMe), 68.4, 69.5, 70.9, 73.5, 81.1, 104.1 (C-1), 170.5 (CO), 173.2 (CO); MALDI-TOF: *m/z* 356 $(M + Na)^+$, ($C_{12}H_{19}N_3O_8Na$ requires *m/z* 356).

Benzoylation of Methyl 6-azido-6-deoxy-2-*O*-carboxymethyl-β-D-galactopyranoside (101)

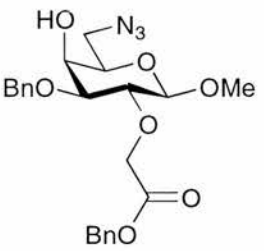


108 **109**

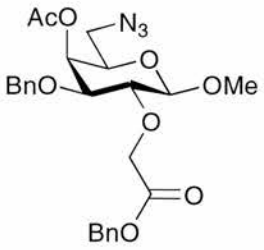
A solution of methyl 6-azido-6-deoxy-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**103**) (63 mg, 0.22 mmol), anhydrous pyridine (19 µl, 0.24 mmol) and benzoyl chloride (28 µl, 0.24 mmol) in DCM (1 ml) were cooled (-45 ^{0}C) and stirred overnight. The reaction mixture was coevaporated with toluene and taken up in DCM. The resulting solution was washed with dilute HCl and $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; pet. ether - EtOAc, 3:1-1:1) gave a mixture of two compounds, *methyl 6-azido-3,4-di-O-benzoyl-6-deoxy-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* (**109**) as a syrup (28 mg, 33%); $[\alpha]_D$ + 100.2 (*c* 0.76, $CHCl_3$); $\delta_H(CDCl_3)$: 3.19 (1 H, dd, $J_{5,6/6'}$ 3.6, $J_{6,6'}$ 9.3, H-6/6'), 3.55 (3 H, s, OMe), 3.56 (1 H, m, H-5 or 6/6'), 3.62 (3 H, s, OCH_2COOMe), 3.82 (1 H, dd, $J_{1,2}$ 7.5, $J_{2,3}$ 7.8, H-2), 3.99 (1 H, m, H-5 or 6/6'), 4.30 (2 H, s, OCH_2COOMe), 4.56 (1 H, d, $J_{1,2,}$, H-1), 5.41 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 3.6, H-3), 5.71 (1 H, d, $J_{3,4}$, H-4), 7.26 (2 H, m, Ar), 7.49 (3 H, m, Ar), 7.87 (2 H, m, Ar), 8.03 (3 H, m, Ar); $\delta_C(CDCl_3)$: 50.9, 51.6, 57.3, 69.1, 69.8, 72.3, 73.4, 78.5, 104.3 (C-1), 128.4, 128.7, 129.9, 130.1, 133.3, 133.8; MALDI-TOF: *m/z* 522 $(M + Na)^+$, ($C_{24}H_{25}N_3O_9Na$ requires *m/z* 522) and *methyl 6-azido-3-O-benzoyl-6-deoxy-2-O-methoxycarbonylmethyl-β-D-galactopyranoside* as a syrup (**108**) (38 mg, 45 %); $[\alpha]_D$ + 12.1 (*c* 0.76, $CHCl_3$); $\delta_H(CDCl_3)$: 3.19 (1 H, dd, $J_{5,6/6'}$ 1.2, $J_{6,6'}$ 8.7, H-6/6'), 3.55 (5 H, m, H-5, 6/6', OMe), 3.62 (4 H, m, H-2, OCH_2COOMe), 3.99 (1 H, m, H-5 or 6/6'), 4.13 (1 H, d, $J_{3,4}$ 3.3, H-4), 4.30 (2 H, s, OCH_2COOMe), 4.47 (1 H, d, $J_{1,2,}$ 7.8, H-1), 5.19 (1 H, dd, $J_{2,3}$ 7.8, $J_{3,4}$, H-3), 7.49 (2

H, m, Ar), 7.56 (1 H, m, Ar), 8.10 (2 H, m, Ar); $\delta_H(CDCl_3)$: 50.9, 51.6, 56.9 (OMe), 67.9, 69.5, 73.7, 74.7, 77.9, 104.2 (C-1), 128.5, 129.9, 133.4, 165.6, 170.4 (CO); MALDI-TOF: *m/z* 418 $(M + Na)^+$, ($C_{17}H_{21}N_3O_8Na$ requires *m/z* 418).

Methyl 6-azido-3-*O*-benzyl-2-*O*-benzoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside (110)



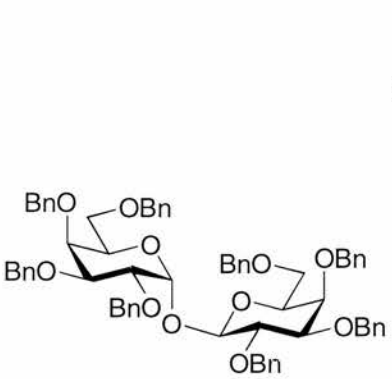
Sugar (**103**) was protected using a standard benzylation procedure.[18] A solution of methyl 6-azido-6-deoxy-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside (**103**) (440 mg, 1.51 mmol) in methanol (25 ml) containing suspended dibutyltin oxide (376 mg, 1.51 mmol) was heated under reflux for 1 h and then concentrated under reduced pressure. Benzyl bromide (898 µl, 7.55 mmol) and tetrabutylammonium bromide (243.5 mg, 0.75 mmol) were added to a solution of the residue in toluene (25 ml) and heated under reflux (approx. 1.5 h). Once TLC [DCM - MeOH, (7:1)] showed the reaction to be complete, the solution was concentrated under reduced pressure. Column chromatography (silica gel; DCM – MeOH, 20:1—6:1) gave the *methyl 6-azido-3-O-benzyl-2-O-benzoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside* (**110**) (351 mg, 51%) as a syrup, $[\alpha]_D$ – 42.92 (*c* 0.89, $CHCl_3$); ν_{max}/cm^{-1} 1752 (*CO*OBn), 2100 (N_3) 3474 (OH); $\delta_H(CDCl_3)$: 3.23 (1 H, dd, $J_{5,6'/6}$ 4.0, $J_{6,6'}$ 9.0, H-6/6'), 3.45 (3 H, s, OMe), 3.50-3.56 (3 H, m, H-2, 3 + 5) 3.72 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 3.79 (1 H, m, H-4), 4.28 (1 H, d, $J_{1,2}$ 7.5, H-1), 4.38 (1 H, d, *J* 16.5, CH_2), 4.42 (1 H, d, *J* 16.5, CH_2) 4.73 (1 H, d, *J* 11.5, CH_2), 4.75 (1 H, d, *J* 11.5, CH_2), 5.17 (1 H, d, *J* 12.5, CH_2) 5.19 (1 H, d, *J* 15, CH_2), 7.25-7.36 (10 H, m, 2 x OBn); $\delta_C(CDCl_3)$: 51.1, 56.7 (OMe), 66.4, 67.5, 70.0, 73.05, 73.8, 79.4, 80.55, 103.8 (C-1), 127.85, 127.9, 128.4, 128.5, 135.5, 137.8, 170.1 (CO); MALDI-TOF: *m/z* 480 $(M + Na)^+$, ($C_{23}H_{27}N_3O_7Na$ requires *m/z* 480).



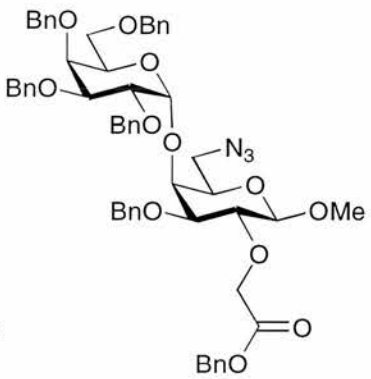
111

A sample of methyl 6-azido-3-*O*-benzyl-2-*O*-benzoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside-β-D-galactopyranoside (**110**) was acetylated with acetic anhydride/pyridine and the crude product was identified by ^{1}H NMR to be *methyl 4-O-acetyl-6-azido-3-O-benzyl-2-O-benzoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside* (**111**) $\delta_H(CDCl_3)$: 2.12 (3 H, s, OAc), 3.10 (1 H, dd, $J_{5,6'/6}$ 3.6, $J_{6,6'}$ 9.3, H-6/6'), 3.45 (3 H, s, OMe), 3.53-3.65 (3 H, m, H-2, 3 + 5) 3.66 (1 H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6'), 4.36 (1 H, d, $J_{1,2}$ 7.5, H-1), 4.39 (1H, d, *J* 9.0, CH_2), 4.41 (1H, d, *J* 16.2, CH_2), 4.62 (1 H, d, *J* 11.4, CH_2), 4.64 (1H, d, *J* 11.4, CH_2), 5.14 (1 H, dd, *J* 9.9, CH_2) 5.16 (1H, d, *J* 12.0, CH_2), 5.36 (1 H, d, $J_{3,4}$ 3.3, H-4), 7.26-7.34 (10 H, m, OBn).

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-azido-3-*O*-benzyl-2-*O*-benzoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside (113)



114



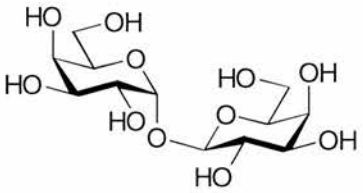
113

This glycosylation reaction was performed utilizing a standard silver triflate promoted glycosylation.[6-9] To a mixture of methyl 6-azido-3-*O*-benzyl-2-*O*-benzoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside (**110**) (250 mg, 0.55

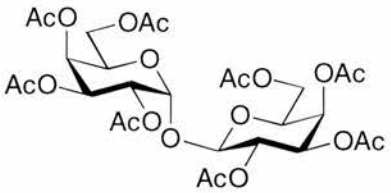
mmol), collidine (132 µl, 1.00 mmol), molecular sieves (4 Å), and diethyl ether/toluene (10 ml, 2:1) was added a solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride () (459 mg, 0.82 mmol) in toluene (1 ml). The mixture was cooled (–78^{O}C) and stirred for 1 h under dry nitrogen, then silver triflate (1.8 mol eq) was quickly added. The mixture was stirred under nitrogen for approximately 2 h, allowing the temperature to slowly rise to room temperature. Once TLC [pet. ether – EtOAc, (3:1)] indicated the reaction to be complete, collidine (36 µl, 0.27 mmol) was added and stirring continued for approx. 0.5 h. The mixture was diluted with DCM, filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 1 M HCl and extracted with DCM. The combined organic extracts were washed with saturated $NaHCO_3$ solution and water, dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; toluene – EtOAc, 20:1→8:1) gave two compounds: *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-3-O-benzyl-6-azido-6-deoxy-2-benzoxycarbonylmethyl-β-D-galactopyranoside* (**113**) (378 mg, 70 %); $[\alpha]_D$ +22.7 (*c* 0.99, $CHCl_3$); ν_{max}/cm^{-1} 1757 (*CO*OBn), 2100 (N_3); δ_H($CDCl_3$): 3.18 (1 H, dd, $J_{5a,6a/6a'}$ 4.5, $J_{6a,6a'}$ 8.1, H-6a/6a'), 3.32 (1 H, t, $J_{5b,6b/6b'}$5.1, $J_{6b,6b'}$5.1, H-6b/6b'), 3.48 (3 H, s, OMe), 3.42-3.47 (2 H, m, H-3a + H-5a), 3.59 (2 H, m, H-2a + H-6b/6b'), 3.68 (1 H, dd, $J_{5a,6a/6a'}$, $J_{6a,6a'}$, H-6a/6a'), 3.75 (1 H, d, $J_{3a,4a}$, 3.5, H-4a), 3.99-4.01 (3 H, m, H-2b, 3b + 4b), 4.20-4.25 (2 H, dd, O*CH*$_2$Ar), 4.25 (1 H, d, $J_{1a,2a}$, 8.0, H-1a), 4.35 (3 H, m, H-5b + O*CH*$_2$), 4.54-4.80 (4 H, m, 2 x O*CH*$_2$Ar), 4.86-4.94 (2 H, m, O*CH*$_2$Ar), 4.94 (1 H, d, $J_{1b,2b}$ 3.3, H-1b), 5.15 (2 H, dd, *J* 9.3 + 12.6, O*CH*$_2$), 7.04-7.36 (30 H, m, 6 x OCH$_2$*Ar*); δ_C($CDCl_3$): 50.9, 56.9 (OMe), 66.4, 68.2, 69.8, 70.25, 72.2, 73.0, 73.3, 74.4, 74.7, 74.8, 74.9, 76.4, 77.8, 78.9, 79.6, 80.7, 100.8 (C-1b), 104.3 (C-1a), 127.6, 127.7, 127.8, 127.9, 128.2, 128.2, 128.3, 128.5, 128.7, 135.8, 138.3, 138.5, 138.7, 138.8, 139.1, 170.3; ES-MS: *m/z* 997 $(M + NH_4)^+$. (Found: $[M + NH_4]^+$ 997.4609. $C_{57}H_{61}N_3O_{12}$ requires *m/z* 997.4583) and *2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-1)-2,3,4,6-tetra-O-benzyl-β-D-galactopyranoside* (**114**) (150 mg, 27 %); $[\alpha]_D$ +40.1 (*c* 1.74, $CHCl_3$); δ_H($CDCl_3$): 3.42 (1 H, m, H-6a/6a'), 3.60 (5 H, m, OMe, H-3a, 6a/6a'), 4.00 (3 H, m, H-2b, 3b, 4b), 4.40 (4 H, m, H-5a, 5b, 6b, 6b'), 4.58 (3 H, m, OCH_2Ar, H-1a), 4.94 (2 H, dd, *J* 11.5, *J* 11.5, O*CH*$_2$Ar), 5.06 (1 H, d, *J* 11.5, O*CH*$_2$Ar), 5.23 (1 H, d, $J_{1b,2b}$ 3.0, H-1b), 7.18-7.41 (40 H, m, 8 x OAr);

$\delta_C(CDCl_3)$: 68.3, 68.6, 69.8, 72.6, 72.7, 73.0, 73.2, 73.3, 73.4, 74.6, 74.7, 74.8, 75.1, 76.0, 76.5, 78.9, 79.0, 82.4, 99.8, 103.7, 127.4, 127.5, 127.7, 128.0, 128.2, 128.3, 128.4, 138.0, 138.3, 138.6, 138.7, 138.8, 138.9, 139.0, 139.1; MALDI-TOF: *m/z* 1085 $(M + Na)^+$, ($C_{68}H_{70}O_{11}Na$ requires *m/z* 1085).

α-D-Galactopyranosyl-(1-1)-β-D-galactopyranoside (115)[30]



2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1-1)-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (**114**) (116 mg, 0.109 mmol) and palladium on charcoal (116 mg) were stirred in EtOH:Acetic acid (10 ml, 1:1) under a hydrogen atmosphere. After 12 h the mixture was filtered through Celite and concentrated under reduced pressure to give α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside (**115**) (33.2 mg, 89 %); $[\alpha]_D$ + 58 (*c* 0.23, H_2O) (lit.[30], + 56); $\delta_H(CD_3OD)$: 4.20 (1 H, d, $J_{1\beta,2\beta}$ 7.8, H-1a), 5.15 (1 H, d, $J_{1\alpha,2\alpha}$ 3.0, H-1b); $\delta_C(CD_3OD)$: 61.6, 61.8, 69.0, 69.1, 69.9, 70.0, 71.1, 71.8, 73.1, 75.9, 101.1 (C-1α), 104.5 (C-1β); ES-MS: *m/z* 365 $(M + Na)^+$. (Found: $[M + Na]^+$ 365.1059. $C_{12}H_{22}O_{11}Na$ requires *m/z* 365.11672).

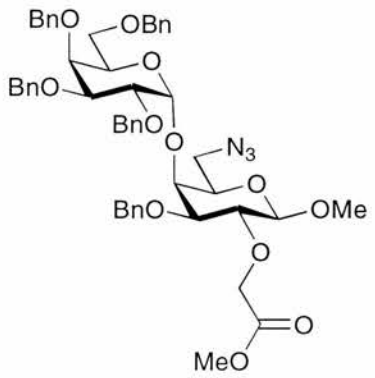


116

A sample of α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside (**115**) was acetylated with acetic anhydride/pyridine and the crude product was identified by ^1H NMR to be *2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl-(1-1)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside* (**116**); $\delta_H(CDCl_3)$: 1.98 (3 H, s, OAc), 1.99 (3 H, s,

OAc), 2.02 (3 H, s, OAc), 2.03 (6 H, s, 2 x OAc), 2.06 (3 H, s, OAc), 2.13 (3 H, s, OAc), 2.19 (3 H, s, OAc), 3.93 (1 H, m, H-5b), 4.13 (4 H, m, H-6β, 6β', 6α, 6α'), 4.53 (1 H, t, $J_{5\beta,6\beta/6\beta'}$ 6.5, H-5β), 4.67 (1 H, $J_{1\beta,2\beta}$ 8.0, H-1β), 5.01 (2 H, m, H-2α, 3β), 5.24 (1 H, d, $J_{1\beta,2\beta}$, $J_{2\beta,3\beta}$ 8.0, H-2β), 5.38 (3 H, m, H-1α, 3α, 4β), 5.47 (1 H, m, H-4α); $\delta_C(CDCl_3)$: 20.6, 20.6, 20.7, 60.9, 61.4, 66.8, 66.9, 67.3, 67.7, 68.2, 70.4, 71.3, 77.9. 97.1, 99.7, 168.9, 169.8, 170.1, 170.2, 170.3, 170.4, 170.5.

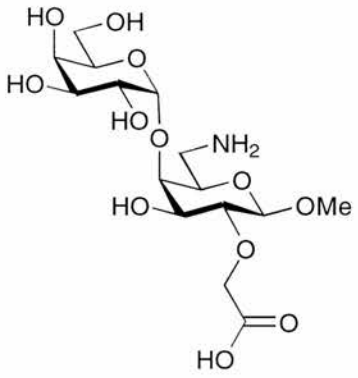
Methyl 2,3,4,6-*O*-tetra-benzyl-α-D-galactopyranosyl-(1-4)-3-*O*-benzyl-6-azido-6-deoxy-2-methoxycarbonylmethyl-β-D-galactopyranoside (117)



Sugar (**117**) was formed using a standard transesterification procedure on the corresponding benzyl ester.[26] A solution of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-3-*O*-benzyl-6-azido-6-deoxy-2-benzoxycarbonylmethyl-β-D-galactopyranoside (**113**) (30 mg, 0.03 mmol) in methanol (2 ml) containing sodium metal (trace) was stirred at room temperature for 1 h. Once the reaction was complete, the solution was neutralised with Amberlite IR 120 (H^+) ion exchange resin. The resulting solution was filtered and concentrated under reduced pressure to give *methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1-4)-3-O-benzyl-6-azido-6-deoxy-2-methoxycarbonylmethyl-β-D-galactopyranoside* (**117**) (18 mg, 65 %) as a syrup; $[\alpha]_D$ +17.1 (*c* 0.73, $CHCl_3$); ν_{max}/cm^{-1} 1760 (*CO*OMe), 2100 (N_3); $\delta_H(CDCl_3)$: 3.18 (1 H, dd, $J_{5a,6a/6a'}$ 4.5, $J_{6a,6a'}$ 8.1, H-6a/6a'), 3.32 (1 H, t, $J_{5b,6b/6b'}$ 5.1, $J_{6b,6b'}$ 5.1, H-6b/6b'), 3.48 (3 H, s, OMe), 3.42-3.47 (2 H, m, H-3a + H-5a), 3.59 (2 H, m, H-2a + H-6b/6b'), 3.68 (1 H, dd, $J_{5a,6a/6a'}$, $J_{6a,6a'}$, H-6a/6a'), 3.70 (3 H, s, OMe), 3.75 (1 H, d, $J_{3a,4a}$, 3.5, H-4a), 3.99-4.01 (3 H, m, H-2b, 3b + 4b), 4.20-4.25 (2 H, dd, O*CH*$_2$Ar),

4.25 (1 H, d, $J_{1a,2a}$, 8.0, H-1a), 4.35 (3 H, m, H-5b + OCH_2), 4.86-4.94 (2 H, m, OCH_2Ar), 4.94 (1 H, d, $J_{1b,2b}$ 3.3, H-1b), 5.15 (2 H, dd, *J* 9.3 + 12.6, OCH_2), 7.04-7.36 (25 H, m, 5 x OCH$_2$*Ar*); δ_C($CDCl_3$): 50.8, 51.6, 56.9 (OMe), 68.1, 69.7, 70.1, 72.1, 72.9, 73.2, 74.4, 74.6, 74.7, 74.9, 76.1, 76.8, 78.8, 79.6, 80.5, 100.7 (C-1b), 104.1 (C-1a), 127.4, 127.6, 127.7, 127.8, 128.0, 128.2, 128.3, 138.0, 138.4, 138.5, 138.6, 138.6, 170.7; ES-MS: *m/z* 921 $(M + NH_4)^+$. (Found: $[M + NH_4]^+$ 921.43246. $C_{51}H_{65}N_4O_{12}$ requires *m/z* 921.43250).

Methyl α-D-galactopyranosyl-(1-4)-6-amino-2-acetate-6-deoxy-β-D-galactopyranoside (118)



Sugar (**113**) was deprotected using a standard hydrogenation procedure.[10] Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1-4)-6-amino-3-*O*-benzyl-2-*O*-benzoxycarbonylmethyl-6-deoxy-β-D-galactopyranoside (**113**) (40 mg, 0.04 mmol) and palladium on charcoal (40 mg) were stirred in EtOH (3ml) and acetic acid (3 ml) under a hydrogen atmosphere. After 12 h the mixture was filtered through Celite and concentrated under reduced pressure to give *methyl α-D-galactopyranosyl-(1-4)-6-amino-2-O-carboxymethyl-6-deoxy-β-D-galactopyranoside* (**118**) (8.5 mg, 50 %); δ_C(CD_3OD): 3.51 (3 H, s, OMe), 4.31 (2 H, m, OCH_2), 4.35 (1 H, d, $J_{1a,2a}$7.6, H-1a), 5.01 (1 H, d, $J_{1b,2b}$ 3.4, H-1b); δ_C(CD_3OD): 51.5, 56.4, 60.3, 69.2, 69.4, 70.1, 70.6, 71.8, 78.3, 101.7 (C-1b), 104.7 (C-1a), 169.5; MALDI-TOF: *m/z* $(M + Na)^+$ 436, ($C_{15}H_{27}NO_{12}Na$ requires *m/z* 436). High res. ES-MS was not found.

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Chapter 6

Conclusions and Future Work

6.0 Conclusions and Future Work

6.1 *trans*-Sialidase: conclusions and future work

Octyl 6-azido-6-deoxy-galactoside (**6**) has been successfully synthesized by two different methods. Each method had difficulties, which were overcome. At this point the synthesis of the derivatised compounds was not followed up because the crystal structure of *trans*-sialidase has not yet been elucidated.

Once the crystal structure has been published then derivatisation of the octyl azido-galactoside (**6**) can take place. This can be achieved using peptide chemistry extending *via* the reduced azide function (**Figure 97**).

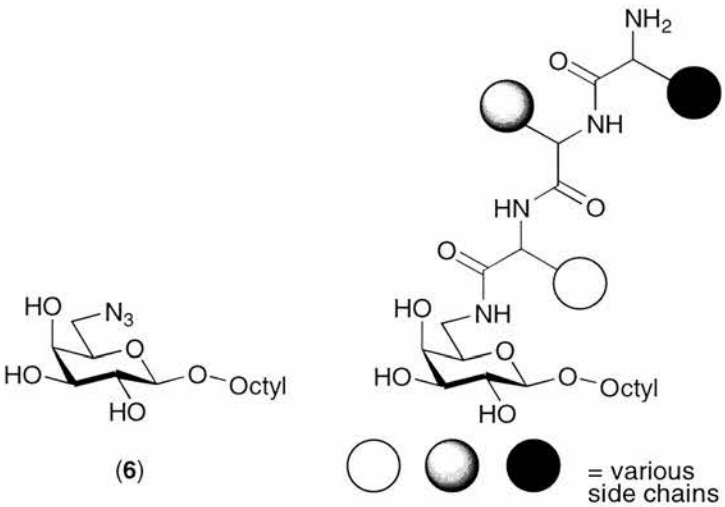

Figure 97: Indicating peptide chain which could be synthesised from the octyl azido-galactoside (**6**) sugar.

Another approach could be to synthesise a polymeric or dendrimeric compound based on the azido-isopropylidene disaccharide (**34**), which has already been synthesised elsewhere in this project.

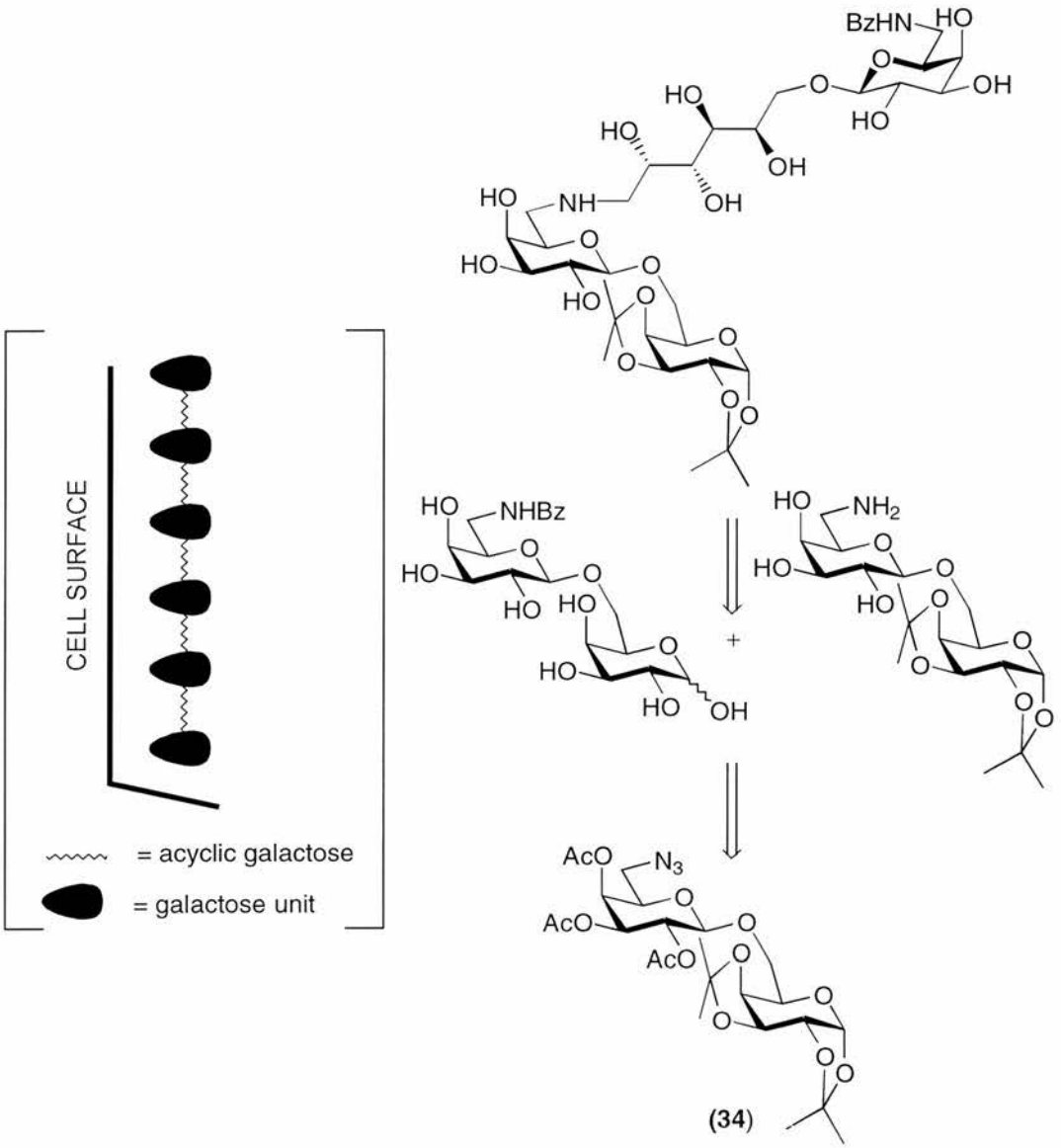

Figure 98: Retrosynthetic analysis of carbohydrate dendrimer, based on disaccharide (**34**) and schematic cell surface representation.

The disaccharide (**34**), which was synthesised (section 2.6), could be used in a similar manner, (**Figure 98**) to increase binding to the cell surface of *trans*-sialidase. The multi-valent saccharide would be advantageous because of the numerous copies of *trans*-sialidase present on the cell surface.[1]

Figure 99 indicates how a saccharide-based polymer dendrimer has been synthesised by Turnbull and Stoddart.[2] This involved simple saccharide units with azido functionality situated on the primary alcohol (**122**).[2] The trisaccharide (**122**) was deprotected to the free amine trisaccharide (**123**) or to the benzoyl protected amine

derivative (**124**). The deprotected galactose unit situated in trisaccharide (**124**) was reduced to the open chain form of galactose. The free amines in trisaccharide (**123**) attacked the reduced sugar to form the polysaccharide (**125**).

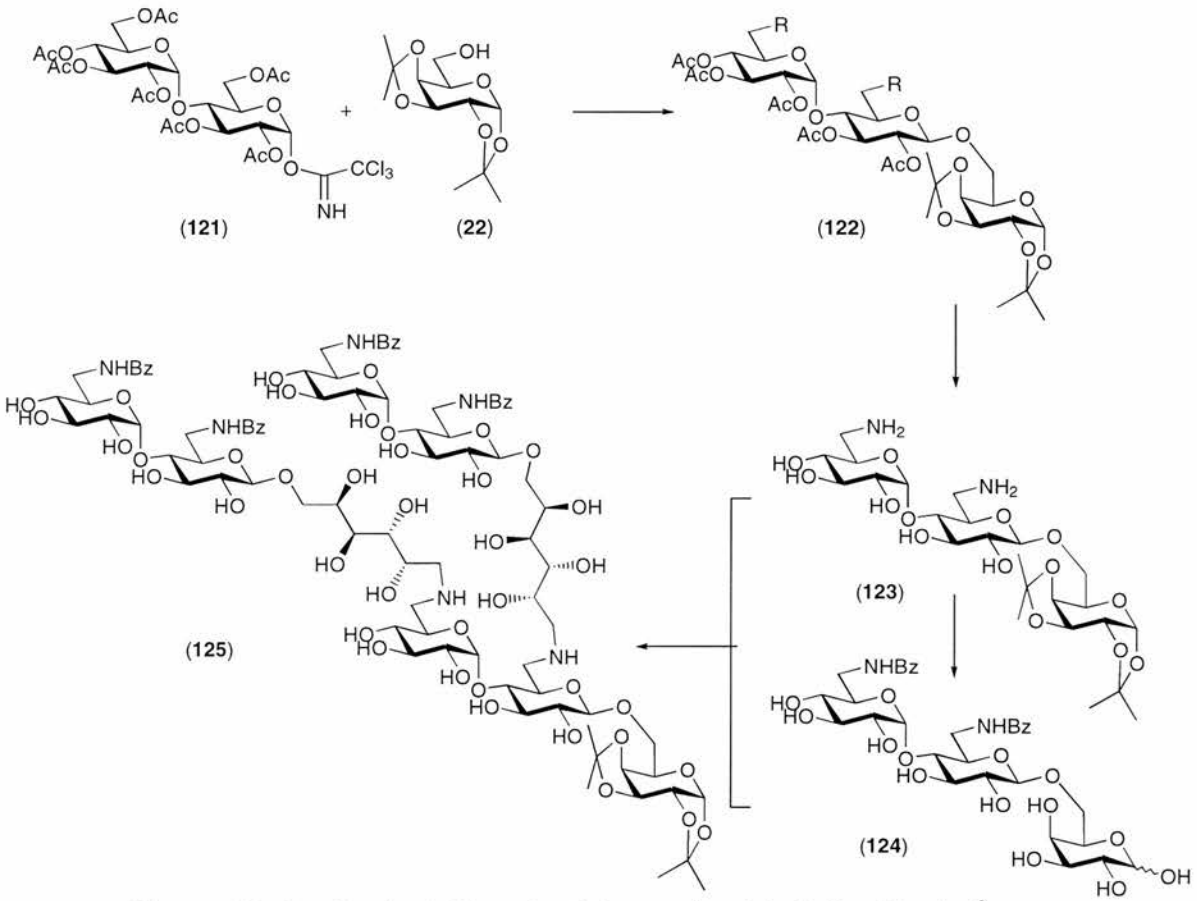

Figure 99: Synthesised Glycodendrimer using 2 building blocks.[2]

6.2 Verotoxin: conclusions and future work

A similar approach to that used in the *trans*-sialidase study was also employed in the successful synthesis of derivatised saccharides for the inhibition of verotoxin (**Figure 100**). The reason for the change in the biological target was due to publication of the crystal and NMR structures of verotoxin,[3] which indicated scope for increased binding through site 2a and 6a of galabiose.

Numerous disaccharide templates and a small array of the amino functionalised compounds were successfully synthesised (**Figure 100**).

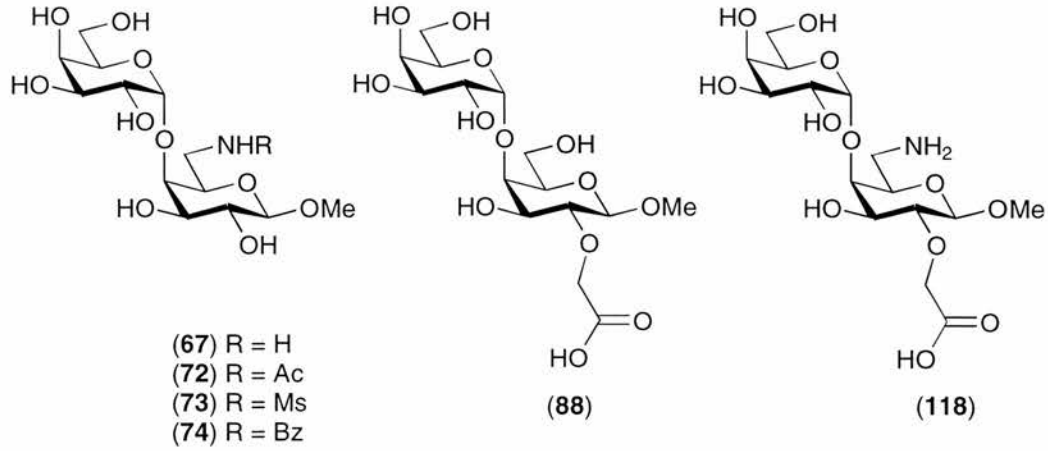

Figure 100: Mono- and di- functionalised galabiose templates, and derivatives thereof.

The potential to synthesise a library of compounds would be substantial due to the multi-functionalised galabiose sugar (**118**) (**Figure 101**). Such a library could be synthesised *via* peptide coupling in solution or on solid-phase resin. The testing of the compounds against VT could then be carried out on or off the resin.[4,5]

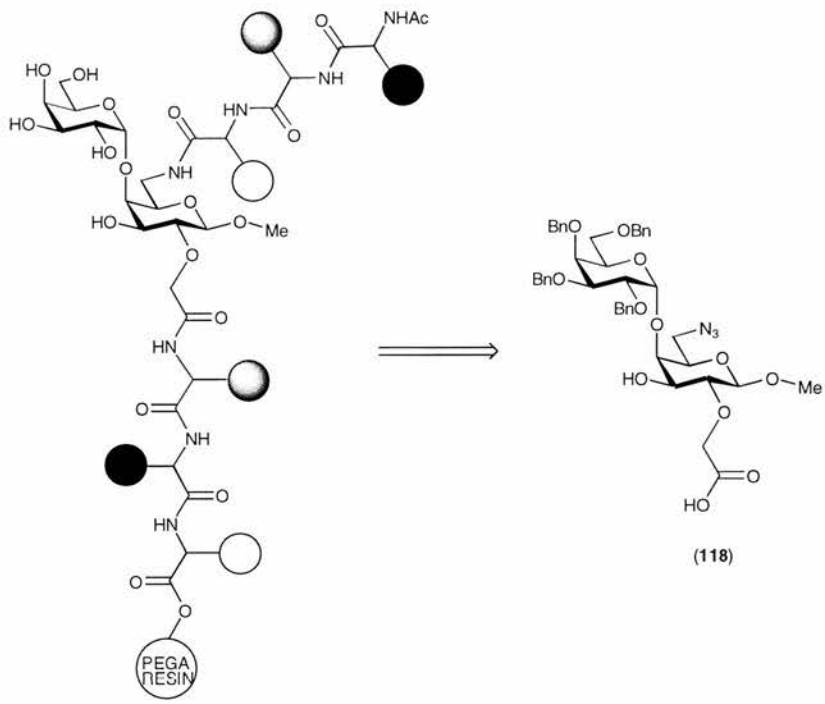

Figure 101: Retrosynthetic analysis of multifunctionalised disaccharide.

Another stage of derivatisation could be to synthesise a dendrimer-type structure to increase inhibition, as illustrated in recent multi-valent sugar results published by Bundle (details of synthesis **section 3.4.2**).[6] A recent survey of multivalency in glycobiology was recently published by Borman.[7]

A schematic representation of a cyclic compound is indicated in **Figure 102**. The cyclic structure could be composed of 5 galabiose units being linked together with either a string of peptides or a polyethylene glycol chain.[8]

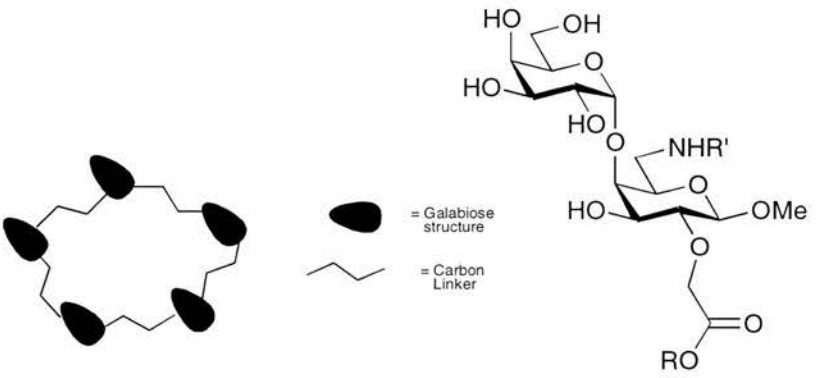

Figure 102: Cyclisation of Galabiose units.

A schematic representation of a polyvalent structure using a PAMAM core is indicated in **Figure 103**.[9]

Peptide coupling with the terminal amine from the G1 PAMAM core and the carboxylic acid from the galabiose unit (**88**) will give a polyvalent galabiose unit with numerous sugar units at the edge of the dendrimer core.

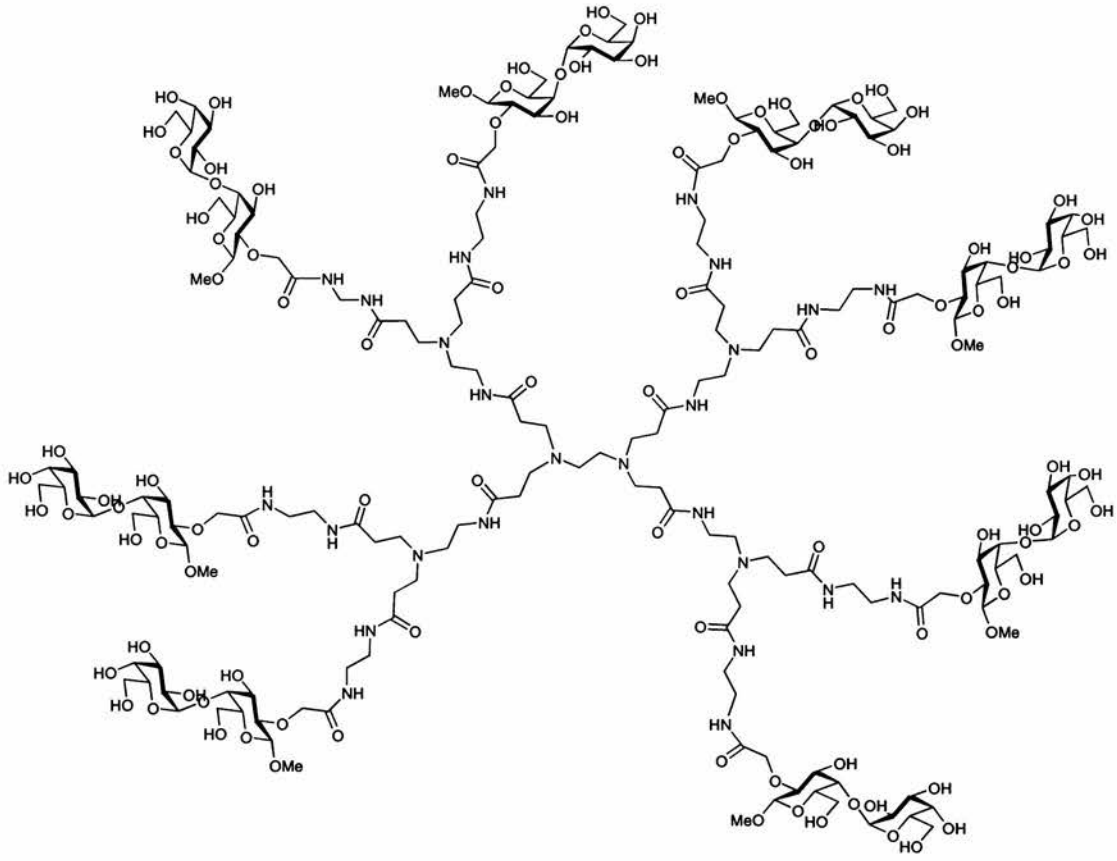

Figure 103: G1 PAMAM core derivatised using Galabiose unit (**88**)

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