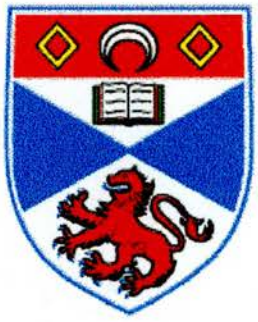

Development of potential inhibitors of mycobacterial cell wall biosynthesis

A Thesis Submitted for the Degree of Doctor of Philosophy

Giles Newbury

University of St. Andrews

April 2002

Abstract

Herein is described the design and synthesis of arabinofuranose and galactofuranose analogues as potential inhibitors of arabinofuranosyl transferases and UDP-galactopyranose mutase, respectively. Towards this aim a number of (i) acyclic azasugar mimics and (ii) disaccharides were sythesised.

(i) 2-Amino-2-deoxy-D,L-erythritols **(2.72)-(2.78)**, 2-amino-2-deoxy-D,L-threitols **(2.41)-(2.48)** and 2-aminomethylene-2-deoxy-D,L-threitol **(2.86)** were synthesized by epoxidation of 1,4-di-*O*-(4-methoxybenzyl)-(*Z*)-but-2-ene-1,4-diol **(2.29)**, 1,4-di-*O*-(4-methoxybenzyl)-(*E*)-but-2-ene-1,4-diol **(2.63)** and epoxide ring opening with amines thereof. Once synthesised these *erythro* and *threo* target compounds were subjected to biological evaluation with UDP-galactopyranose mutase, *M. tuberculosis* and various commercially available glycosidases. Preliminary studies showed that 2-benzylamino-2-deoxy-D,L-threitol **(2.48)** has an ability to inhibit the growth of *M. tuberculosis* H37Rv, for this reason the asymmetric synthesis of 2-benzylamino-2-deoxy-L-threitol **(2.102)** was undertaken.

(ii) Octyl 6-*O*-(5-*O*-phospho-α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.11)** and methyl 4-*O*-(5-phospho-α-D-arabinofuranosyl)-α-D-glucopyranoside **(4.9)** were synthesised from octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)**, 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide **(4.24)**, and methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(4.31)**, 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide **(4.24)** respectively. Glycoside couplings with arabinofuranosyl glycosyl donor sugars were performed in anhydrous DCM at –30°C with silver trifluoromethanesulfonate as promoter. It was also noted that octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** is reported as a plant natural product this is surprising since arabinose in plants is generally L-configured. This suggested that an initial strategy of generating octyl 6-*O*-(5-*O*-phospho-α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.11)** via octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** would allow confirmation of the unusual D-Ara*f* composition of octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)**. There was disagreement between optical rotation values of synthetic

octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** and the literature (synthetic $[\alpha]_D$ +25.7, lit., $[\alpha]_D$ -75.0). These findings, encouraged synthesis of the closely related known compound, octyl 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranoside **(4.69)** and octyl 6-*O*-(α-D-arabinopyranosyl)-β-D-glucopyranoside **(4.68)** and to compare optical rotations. Octyl 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranoside **(4.69)** and octyl 6-*O*-(α-D-arabinopyranosyl)-β-D-glucopyranoside **(4.68)** were synthesized from octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)**, 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl bromide **(4.72)** and octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)**, 2,3,4-tri-*O*-benzoyl-β-D-arabinopyranosyl bromide **(4.76)** respectively. Glycoside couplings with arabinopyranosyl glycosyl donor sugars were performed in anhydrous MeCN at –20°C with iodine monobromide as promoter. Synthesis of four isomeric forms of the natural product octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** has been successful. From the characterisation of these compounds it suggests the structural assignment of the natural product, octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)**, to be incorrect. Indeed, the data generated in this report does not support the claim that this natural product is composed of a 1,6-linked D-arabinofuranose-D-glucopyranose disaccharide.

Declaration

(i) I, Giles Newbury, hereby certify that this thesis, which is approximately 47,000 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.

date ..22:5:02..... signature of candidate

(ii) I was admitted as a research student in October 1998 and as a candidate for the degree of Doctor of Philosophy in October 1999; the higher study for which this is a record. The work was carried out at the University of St. Andrews between 1998 and 2001 and the University of East Anglia between 2001 and 2002.

date ..22:5:02..... signature of candidate

(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.

date ..20 May 02..... signature of supervisor

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Acknowledgements

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I owe an enormous debt of gratitude to my mentor and guiding spirit Rob, for his advice and guidance, support and patience over the last 3 years or so. Thank you Rob. I am similarly indebted to Ravi (a.k.a The Guv'nor), for so much help and encouragement in the lab. Ravi your collaboration so freely given was absolutely crucial to me. A special word of thanks must go to the EPSRC National Mass Spectrometry service centre in Swansea for providing me with mass spectra of my compounds. Similarly I must thank Melanya Smith of the University of St-Andrews for running a large number of NMR spectra for me. I must acknowledge the efforts of Alan Haines of the Carbohydrate Research Centre at the University of East Anglia, all the Field group members, past and present. and also to Russ in St-Andrews for making (our!) lab such a fun environment to work in. Thanks to the BMS crowd for their friendship, especially Alessia, Brett, Darren, Ellis, Jill, Lesley, Mike, Simon, Steve and Tiger.

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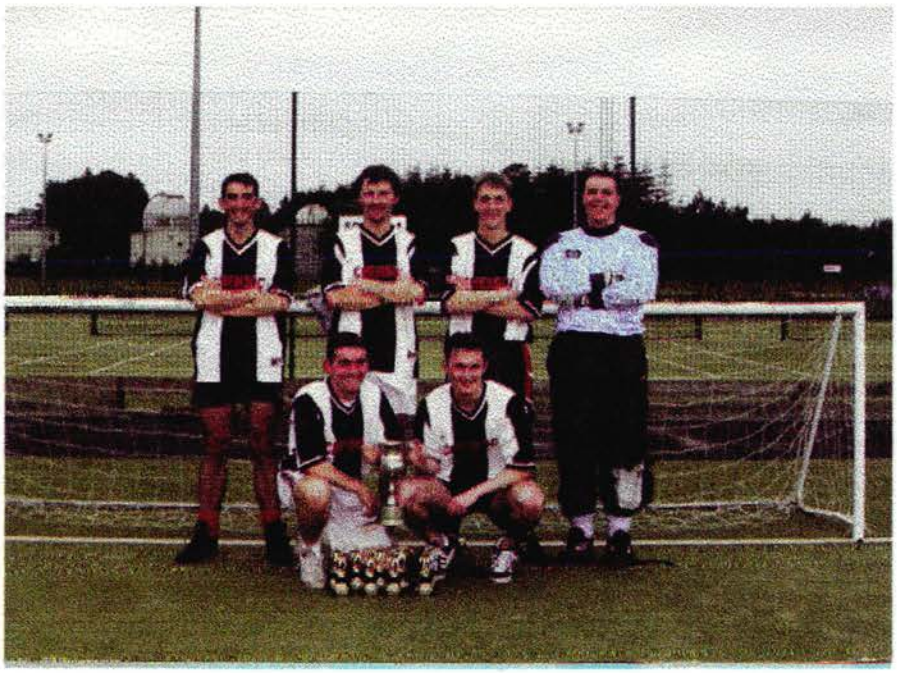


The mighty Steaua Needarest

Last but certainly not least I would like to thank Joana for all her love, kindness and for making my time in St. Andrews so happy.

ThanX

I would like to dedicate this thesis to my Mum and Dad.

Thank you for all your kindness, love and support through the good and the bad times.

Abbreviations Used in Text:

AG	Arabinogalactan
AIDS	Acquired immuno-deficiency syndrome
Ara*f*	Arabinofuranose
BCG	Bacille-Calmette-Guerin
CI-MS	Chemical Ionisation Mass Spectroscopy
DCB	Dichlorobutane
DOTS	Directly Observed Treatment Short Course
DNJ	1-Deoxynojirimycin
DpA	β-D-Arabinofuranosyl-1-monophosphoryldecaprenol
DTBT	Di-*tert*-Butyl Tartrate
Emb	Ethambutol
ES-MS	Electrospray Mass Spectrometry
Eth	Ethionamide
FAB-MS	Fast Atom Bombardment Mass Spectrometry
FAD	Flavin Adenine Dinucleotide
Gal*f*	Galactofuranose
Gal*p*	Galactopyranose
GP	Glycogen Phosphorylase
HIV	Human immunodefiency virus
HR-MS	High Resolution Mass Spectrometry
Inh	Isoniazid
LAM	Lipoarabinomannan
MALDI-TOF	Matrix-Asisted Laser-Desorption-Ionisation Time-Of-Flight
MDR-TB	Multi Drug Resistant Tuberculosis
MIC	Minimum Inhibitory Concentration
MurNAc	*N*-Acetylmuramic acid
pApp	5-phosphoarabinosyl pyrophosphate
PIM	Phosphatidylinositol mannoside
pRpp	5-phosphoribosyl pyrophosphate

Rib*f*	Ribofuranose
TB	Tuberculosis
WHO	World Health Organisation

Table of Contents:

Chapter 1

General Introduction

1.0 General Background

Tuberculosis (TB) is a disease that has plagued mankind throughout the ages; it was known to the ancient Greeks as *phthisis* (or consumption) due to the generally debilitating nature of the disease.[1] Down the centuries the disease has wreaked havoc, and despite earlier optimism about permanent eradication, it continues to do so in the present day. Societies worldwide have suffered from this disease but little was known until comparatively recently about the exact nature of the disease and the mode of transmission. By the eighteenth century it was recognized that prolonged contact with an infected person was instrumental in spreading the infection.[2] Owing to insanitary and overcrowded housing conditions TB was rife, frequently decimating families who lacked the financial resources to escape their surroundings.

1.1 The nature of the disease

Pulmonary TB is the best known form of the disease and it is characterised by a cough, sometimes with blood in the sputum, accompanied by weakness or fatigue, chest pain, weight loss, fever and night sweats. The disease can also spread internally, via the blood, becoming 'milliary' or 'disseminated' TB, and infecting internal organs, skin, glands, bone and brain (TB meningitis). The disease follows a similar pattern in animals, such as cattle.[3,4] It has been suggested that the primary host of mycobacteria in the U.K is the badger.[5] The pathogenesis of bovine tuberculosis in many countries has been well documented.[6]

On entering the lung, the mycobacteria are taken up by macrophages (immune system cells that engulf invading organisms and destroy them with toxic chemicals).[7] In a few people this macrophage defence mechanism is strong enough to defeat the mycobacteria, and the disease does not progress. In others, the disease process can go one of two ways. In the first scenario, the macrophages are unable to destroy the mycobacteria and the host's immune system is unable to contain the infection. In this case the mycobacteria divide and multiply inside the macrophages, eventually rupturing them. The mycobacteria then burst out in large numbers and in the process release toxic enzymes from the dead macrophages, which can damage surrounding tissues. This results in the formation of cavitary lesions (or tubercules) within the lung, in which the mycobacteria are able to multiply extracellularly. This is the active and

contagious form of the disease.[2] The alternative scenario occurs when the macrophages in the lung are unable to destroy the mycobacteria but the hosts immune system is still able to halt the spread of the disease. It does this by hemming in the infected macrophages behind a barrier of phagocytes and lymphocytes (white blood cells), known as granuloma. This is the latent form of the disease.[7] This stalemate can persist for years, until the host's immune system becomes compromised, either through old age, general ill health or the development of an immunodeficiency disorder. In such an event, the mycobacteria may then once again start to divide and spread, and the host will develop the active form of the disease. This is a particular danger for HIV-positive individuals. The spread of the HIV epidemic contributes significantly to the worsening of the situation. Co-infection with TB and HIV results in special diagnostic and therapeutic problems and uses up larger amounts of medical resources in developing countries. Outbreaks of MDR-TB (MDX-TB) were first reported from American centers caring for HIV patients.[8] The synergy between TB and the AIDS epidemic, and the surge of MDR clinical isolates of *M. tuberculosis* have reaffirmed TB as a primary health care threat.[9]

In Western Europe the TB epidemic is under control, but increasing incidence rates in migrants raise new problems in these countries. TB is uncontrolled in large parts of the former Soviet Union and Africa due to the socio-economic breakdown in these countries. Only rigorous infection contol measures on a worldwide scale will prevent further detoriation of this situation.[8] Recently suspicion has fallen upon long haul air travel as a source of disease promotion. Most aircraft only take in a fresh charge of air every twelve minutes providing plenty of opportunity for an infected passenger to pass on the bacillus to other travellers. It has been calculated that up to ten people could be infected during a long haul flight.[10] An obvious, although expensive prophylactic measure could be to increase the amount of fresh air available by a more frequent intake; the failure of airlines to do this is further evidence of lack of insight into the magnitude of the problem.

It is relevant at this juncture to examine how the fight has been carried to TB over the years and why the disease is still so problematic.

1.2 Chronology of treatments.

The first really effective treatment for *Mycobacterium tuberculosis* infection was found to be bed rest, open air and a proper diet.[11-13] This clearly was impractical for much of the worlds population living a precarious existence below the poverty line. Further progress was however forthcoming; by 1865 it was shown that TB could pass the species barrier,[5] thereby demonstrating the disease to be a form of contagion rather than a spontaneously occurring event within man. By 1882 the TB bacillus had been isolated for the first time, raising hopes of an eventual treatment. Such a treatment was partly realised through the advent of the Bacille-Calmette-Guerin vaccine (BCG)[14] but the real breakthrough was not to come until the middle of World War II with the discovery of antibiotics and the first chemotherapy for infectious diseases. Spectacular success followed, so much so that TB was, within a short period, to become an extremely treatable disease and not the universal scourge of earlier times. The first drug shown to be effective was streptomycin (**Figure 1.1**).

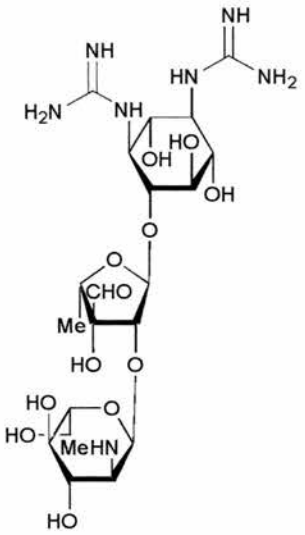
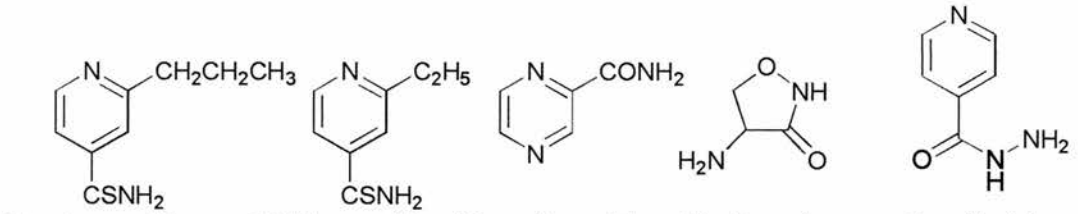
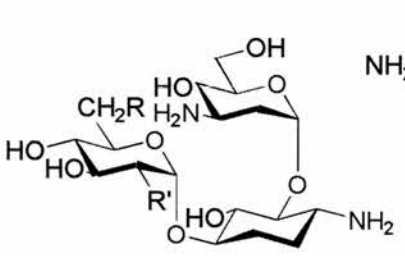


Figure 1.1: Streptomycin

A rapid succession of anti-TB drugs appeared in the following years (**Figure 1.2**). Resistant mutants[15] to streptomycin monotherapy began to appear within a few months of its introduction endangering the success of this particular antibiotic therapy.

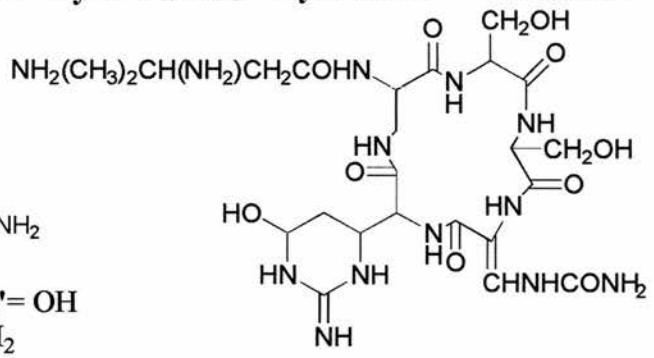


Protionamide **Ethionamide** **Pyrazinamide** **Cycloserine** **Isoniazid**

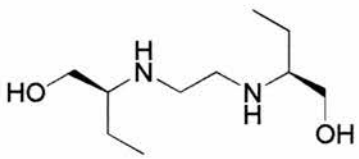


Kanamycin A, **R**= NH_2, **R'**= OH
Kanamycin B, **R**= **R'**= NH_2

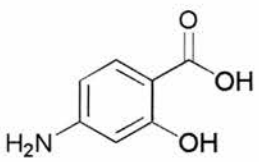
Kanamycin



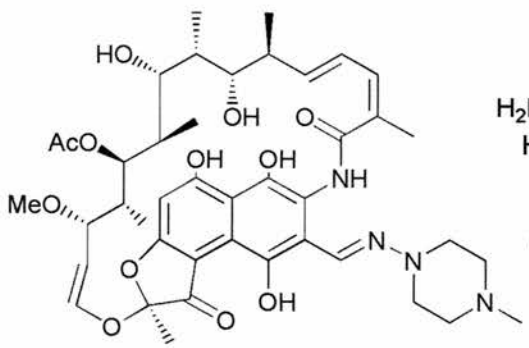
Viomycin



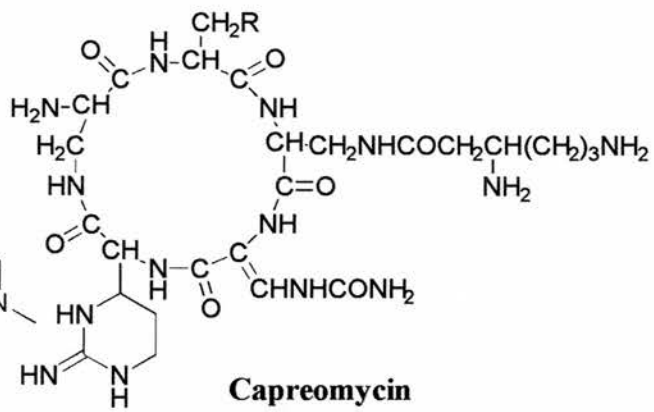
(+)Ethambutol



***p*-aminosalicylic acid**



Rifampicin



Capreomycin

Capreomycin 1A, **R** = OH
Capreomycin 1B, **R** = H
Capreomycin 2A and 2B
lack β lysine residues

Figure 1.2: Toxic antimycobacterial agents (taken from references 39).

However, it was soon demonstrated that this problem could be overcome with combination therapy [16,17] where the patient in question would be administered with two sets of medication, one set to stem the flow of the disease and the second set would prevent resistant mutants occuring. Following streptomycin, *p*-aminosalicylic acid (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955), ethambutol (1962) and rifampin (rifampicin; 1963) were introduced as anti-TB agents (**Figure 1.2**).[18-23] Two properties are important in anti-TB drugs antibacterial activity (highest in isoniazid, rifampin and streptomycin) and capacity to inhibit the development of resistance (highest in isoniazid, rifampin and ethambutol). With the proper four drug combination therapy, a rapid clinical improvement and a significant fall in the bacterial count have been demonstrated.[16] If TB becomes active again in a previously treated patient, there is a high chance that the bacteria will now be drug resistant. Any current therapy should be suspended until multiple drugs are found to which the pathogen is fully sensitive, and treatment can be resumed with the addition of these drugs to the original regimen.[17] A single drug should never be added to a failing regimen; this strategy has been shown to be counter productive. If the microorganism is resistant to the standard drugs, then more toxic medications such as, ethionamide, protionamide, pyrazinamide, cycloserine, capreomycin, viomycin and kanamycin (**Figure 1.2**) should be administered. The aforementioned drugs were extremely effective and whilst resistant mutants of *M. tuberculosis* were known, these yield to combination therapy of two or three drugs and TB was considered a problem solved.

1.2.1 Molecular action of anti-mycobacterial agents:[24,25]

The cell wall of mycobacteria, in its full structural and functional integrity, is essential for growth and survival in the infected host. Consequently, some of the most effective antimycobacterial drugs including isoniazid (Inh) and ethambutol (Emb) are known to inhibit the biogenesis of the cell wall components.[26] Ethambutol, was previously shown to inhibit the synthesis of arabinans of both the cell wall arabinogalactan (AG) and lipoarabinomannan (LAM) of mycobacterium-TB and other mycobacteria. In particular, the primary mode of action of ethambutol was thought to be the inhibition of the polymerisation step of AG

biosynthesis[27,28] although more recent opinion is that ethambutol functions by targeting the EmbB protein, where EmbB is a gene encoding an arabinosyltransferase,[29,30]

Inh and Ethionamide (Eth) are also used in the treatment of TB. The primary biochemical effect of Inh is on mycolic acid synthesis. Inh is activated by *katG* catalase-peroxidase activities.[31]

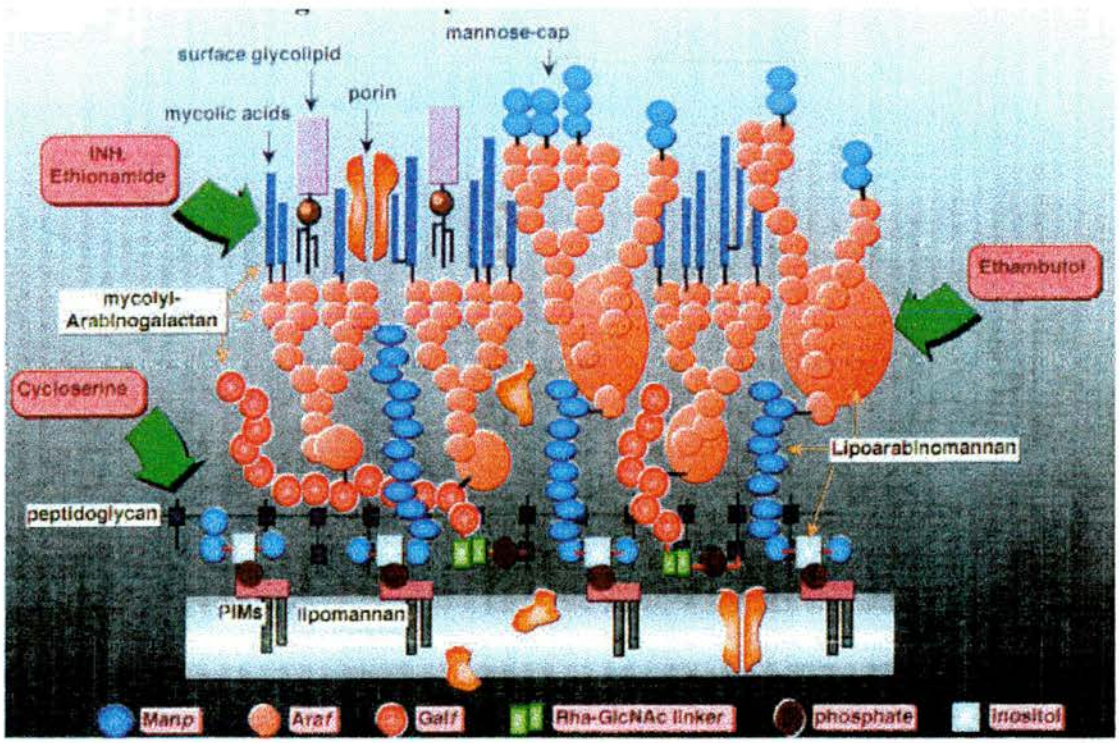


Figure 1.3: Mode of action of front line anti-TB drugs (Taken from reference 32)

D-Cycloserine blocks peptidoglycan synthesis by competitive inhibition of D-alanine racemase (*alr*) and D-alanyl-D-alanine synthetase (D-alanine ligase) (*ddlA*). In *M. tuberculosis*, the biosynthesis of the cell-wall core, the AG-peptidoglycan complex, is inhibited by cycloserine.[33]

1.3 Resurgence of the disease.

Given the efficacy of proven therapies, there was reason to believe that TB should have been either in decline or at least under control on a worldwide basis. However, after a period of rapid progress in the fight it was discovered comparatively recently that TB had returned in a virulent drug resistant form.[34]

There are eight million new infections and three and a half million deaths from TB every year,[34] more adults die each year from TB than from malaria, diarrhoea, HIV and tropical diseases combined.[17] In 1997, more people died of TB than in any other year in history. Data presented recently by the WHO[35] estimates that between 2000 and 2020, 1 billion people will become newly infected, 200 million will get sick from the disease and 35 million people will die. If present trends continue, the WHO estimates that annual deaths will exceed four million by 2004. Once thought to have been consigned to the history books, TB is definitely back with a vengeance.

What is surprising about these alarming statistics is that infection by *M. tuberculosis* is basically treatable. Short course multidrug therapies, such as Directly Observed Treatment (DOTS),[35] are both cheap and up to 95 % effective; the WHO claim cure rates of up to 95%. DOTS involve a regimen of four front-line antimycobacterial drugs taken intensively for two months, followed by a further four months of isoniazid and rifampicin.

The current resurgence in TB levels is mainly a problem of man's own creation. Multi-drug resistant (MDR-TB), for instance, is a result of patients failing to complete a full course of therapy.[36] This provides the selection mechanism needed for the development of resistant strains of mycobacteria not destroyed by the drugs. MDR-TB therapy requires antibiotics that are much more toxic and expensive than are needed for drug-sensitive strains. Dramatic outbreaks of MDR-TB in HIV infected patients in the USA, and in Europe, have focused international attention on the emergence of MDR strains of *M. tuberculosis*, and their threat to clinical management and to control programs.[2]

Drug resistance in TB occurs as a result of tubercle bacillus mutations.[37] These mutations are not dependent on the presence of the drug. Exposed to a single effective anti-TB medication, the predominant bacilli sensitive to that drug, are killed. The few drug resistant mutants likely to be present if the bacterial population is large and will multiply freely. Since it is

improbable that a single bacillus will spontaneously mutate to resistance to more than one drug, giving multiple effective drugs simultaneously will inhibit the multiplication of these resistant mutants. Therefore it is absolutely essential to treat TB patients with the recommended four drug regimen. Furthermore, over-reliance on the existing front and second line anti-mycobacteria drugs, and a belief that TB was a purely Third World disease, meant that from the end of the 1960's to the beginning of the 1990's there was little advance in the research and development of TB drugs. Clearly a more sophisticated approach is required if we are to solve this global emergency.[38]

1.4 A new approach

1.4.1 *M. tuberculosis* Cell Envelope

Recent research has concentrated on the mycobacterial cell wall or envelope as a possible therapeutic target (**Figure 1.4**).[38]

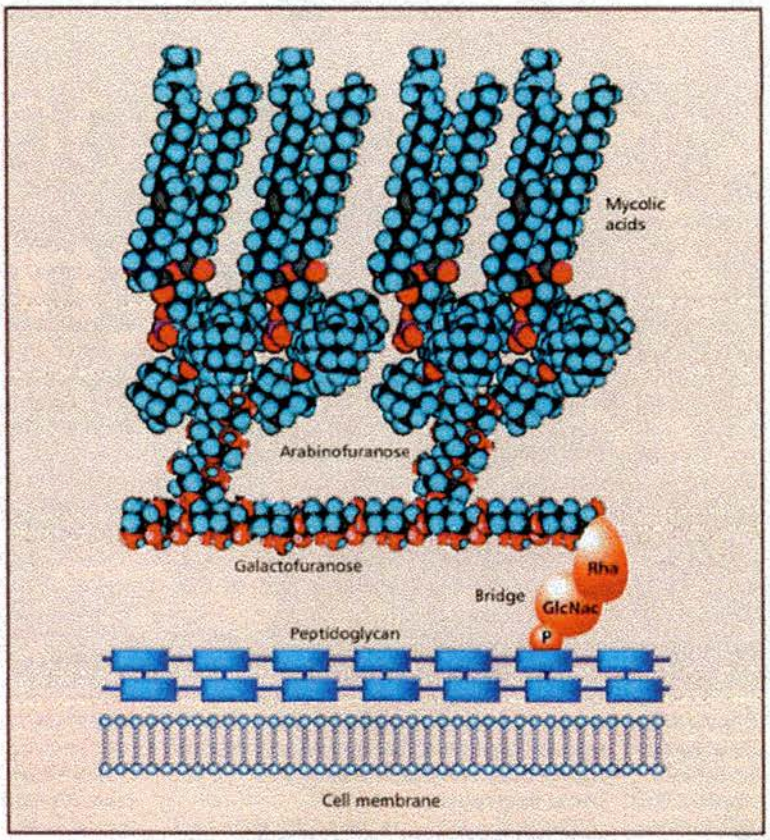


Figure 1.4: Mycobacterial Cell Envelope (taken from reference 39).

The cell wall has a thick waxy structure which provides a dense barrier to anti-TB agents and protection from host defenses.[38,40] Electron microscopy studies have shown the cell envelope to comprise five layers.[24,36] The two inner most regions represent sections of the plasma membrane which are connected to an electron dense and an electron transparent layer respectively. The electron dense layer is thought to be represented in the cell wall by a peptidoglycan moiety. The electron transparent layer is composed of a lipoarabinogalactan

polymer, connected to the electron dense layer via a disaccharide linkage. The composition of the final/outer layer is not fully known but is thought to consist of a glycopeptidolipid.[41,42] Many recent anti-mycobacterial drugs work by interrupting the synthesis of this envelope, and the enzymes involved in that synthesis still represent a rich source of potential drug targets. The cell envelope structure comprises five classes of polymer: lipoarabinomannan, mycolic acids, arabinogalactan and peptidoglycan which are connected to the innermost section of the mycobacterial cell envelope, the cell membrane.[24,36] The basic structure has been known for some time, but the biosynthetic processes involved in the construction process have only recently begun to be determined.

1.4.2.1 Cell Membrane

As alluded to earlier, the cell membrane consists of a bilayer with the inner layer being the thinner of the two. Studies of membrane composition have proved inconclusive but it is believed that the outer layer of the cell membrane is augmented by various carbohydrates, namely phosphatidylinositol mannosides (PIM's).[40,43] Linkage of the phosphodiester to the inositol occurs at the one-position. The two and six positions of the ring are glycosidically mannosylated (PIM_2). Higher homologues of PIM_2 have been found, most notably $PIM_{3\text{-}6}$. Polyterpene based moieties are also located within the plasma membrane.

1.4.2.2 Peptidoglycan

The next layer outwards from the plasma membrane is the peptidoglycan polymer (**Figure 1.5**). This polymer is made up of a series of alternating shorter polymers, i.e GlcNAc-(β1-4)-MurNAc cross linked via peptide bridges, connected at the 3-position of MurNAc (**Figure 1.5**).[36,40]

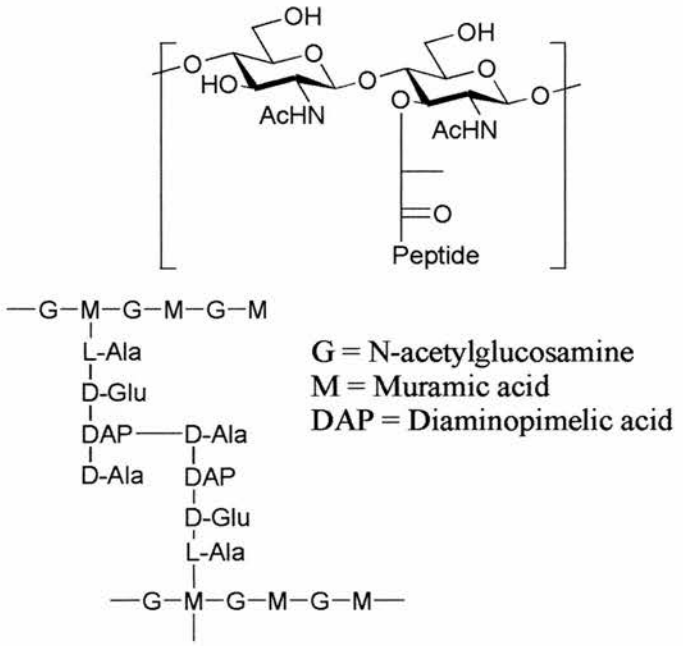


Figure 1.5: Peptidoglycan polymer.

The peptidoglycan found in *M. tuberculosis* is generally similar to that found in other bacteria and consists of linear polysaccharide chains, extensively cross-linked by short peptides.[40] Peptidoglycan is linked to the next polymer in the cell envelope, arabinogalactan, by a unique diglycosylphosphoryl bridge, containing rhamnose and *N*-acetyl glucosamine (see **Figure 1.6**).[44]

1.4.2.3 Bridge region

A small percentage of muramic acid residues in the peptidoglycan are linked to the Rha-(α1-3)-GlcNAc disaccharide via a phosphodiester bridge. This is known as the linker region.[40] The linker region is the bridge between the peptidoglycan and arabinogalactan polymers (**Figure 1.6**).

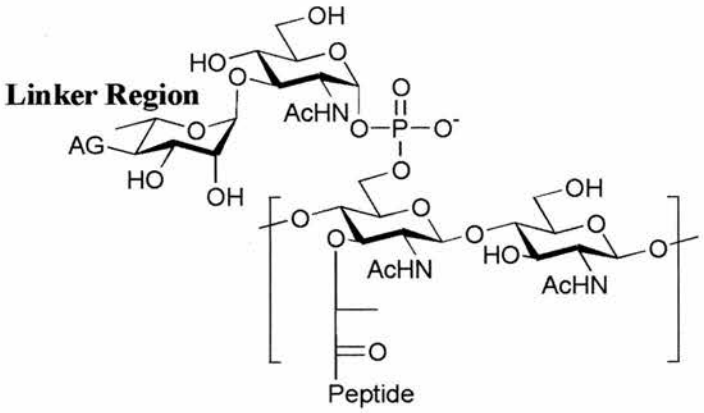


Figure 1.6: The bridge region of the mycobacterial cell wall.

A linear galactan polymer of alternating $\beta_{1\text{-}5}$ and $\beta_{1\text{-}6}$ galactofuranose residues is connected to the rhamnose sugar at the 4-position.[40] The linear galactan contains arabinan branches, in the form of arabinofuranose units linked $\beta_{1\text{-}2}$, $\alpha_{1\text{-}3}$ and $\alpha_{1\text{-}5}$ respectively. This section of the mycobacterial cell wall containing the linear galactan and branched arabinan polymers is referred to as arabinogalactan (**Figure 1.7**).[40]

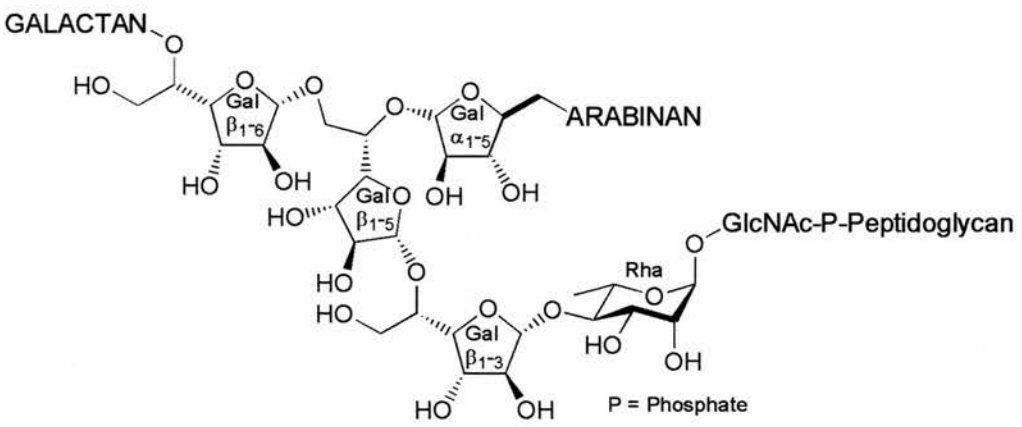


Figure 1.7: Linkage of arabinan to galactan and galactan to rhamnose.

1.4.2.4 Arabinogalactan

Arabinogalactan consists of a galactose backbone (**Figure 1.7**) with arabinose branches (**Figure 1.8**).[40] This structure differs from others due to both the arabinose and galactose sugars being in the furanose form (mycobacteria are the only known pathogens that contain both galactofuranose and arabinofuranose in an essential structural component of the cell wall).[38] Galactofuranose is seldom found in nature and knowledge of the biosynthetic pathways involved in formation of same is limited. One enzyme which has been identified is UDP-Gal*p* mutase[40] which is responsible for the interconversion of UDP-adducts of galactopyranose and galactofuranose.[45]

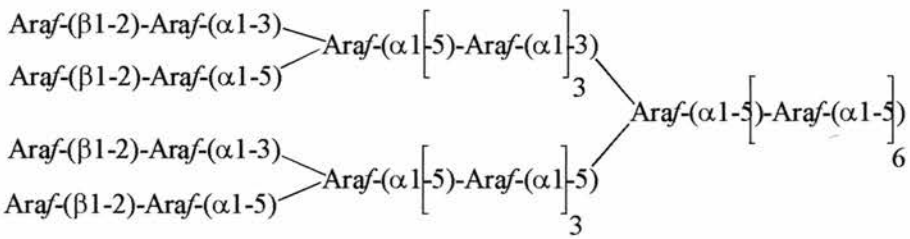


Figure 1.8: Branched arabinan.

The arabinan also contains branches from it's 3- and 5-positions resulting in the formation of two four-unit (α1-5) chains which in turn also branch off at the 3- and 5- positions.[40,46] The branches are then linked to (β1-2) Ara*f* disaccharides. These arabinans attach only towards the reducing end of the galactan (**Figure 1.8**).[40]

1.4.2.5 Mycolic Acids

Mycolic acids are found at the non-reducing end of the arabinan.[40] These compounds, the single largest component of the mycobacterial cell wall, are 3-hydroxyl, 2-alkyl-branched fatty acids that contain sixty to ninety carbon atoms.[40] The branches range from twenty to

twenty five carbon atoms. They do not have a single structure but comprise a number of different forms, and are covalently attached by ester linkages to a hexa-arabinose motif found at the terminus of the branched arabinogalactan. These high molecular weight polymers are responsible for the waxy appearance of the mycobacteria. Other molecules found within the mycolic acids include cyclopropane rings, double bonds, methoxy groups, ketones and branched methyl groups.

1.5 Target enzymes:

Due to an increasing resistance of mycobacteria to existing therapies, a new approach to the treatment of TB is required. A novel strategy would be to target an enzyme/enzymes not currently inhibited by front line anti-TB medications, for example UDP-Gal*p* mutase (enzyme responsible for the interconversion of UDP-Gal*p* and UDP-Gal*f*). Inhibition of this enzyme might involve synthesis of galactofuranose mimics. As galactofuranose is not found in man, selectivity is therefore more probable.

Another possible approach would be to synthesise Ara*f* analogues, i.e. compounds capable of inhibition of arabinofuranosyltransferases. In fact the front line anti-tuberculosis medication ethambutol is known to operate in this manner.[28] Takayama et al reported[47] ethambutol to have an inhibitory effect on mycolic acid biosynthesis. In more recent work[28] it was reported that ethambutols primary antibacterial activity was on arabinogalactan biosynthesis, radiolabelled experiments showed that ethambutol inhibits the inclusion of Ara*f* into the cell wall of *M. smegmatis*. More specifically, they showed that the role of ethambutol was to inhibit the polymerisation step in arabinogalactan biosynthesis. It is now widely accepted that ethambutol achieves this via inhibition of arabinosyltransferases. In 1995 ethambutol was shown to inhibit a different arabinosyltransferase and affect LAM biosynthesis in doing so.[27] Many ethambutol analogues have been synthesised, some of which have been active against TB strains.[48] However due to the increasing resistance of *M. tuberculosis* to these treatments greater efficacy is required. Inhibition of this class of enzymes may have an adverse effect on the arabinogalactan biosynthetic pathway

1.6 UDP-Galactose mutase

1.6.1 Galactofuranose biosynthesis

Galactofuranose (Gal*f*) is rarely found in nature, but does exist in a few macromolecules, such as bacterial *O*-antigens[49,50] and in the cell wall of mycobacteria.[51,52] The first polysaccharide found to contain Gal*f* was galactocarolose, a polysaccharide produced by *Penicillium charlesii*. Experiments showed that *P. charlesii* could not utilise exogenous galactose for galactocarolose biosynthesis and cell extracts of this organism were found to incorporate glucose or glucose-1-phosphate into galactocarolose.[53] In the presence of zinc or fluoride ions, polysaccharide synthesis was inhibited and traces of UDP-Gal*f* were detected, suggesting the sugar nucleotide moiety to be a precursor in Gal*f* biosynthesis. Gal*f* is also present in the T1-antigen of *Salmonella typhimurium*, in which it is known that α-D-Gal*f* is synthesised from a derivative of Gal*p*. It is also known that this contraction does not take place at the free sugar level.[54,55] These observations led to speculation that interconversion takes place at the UDP-sugar level.[56] It has been shown that the UDP-Gal*p* mutase and UDP-Gal*f* transferase systems play a critical role in cell wall biosynthesis in three different species of microbe namely *E. coli, Klebsiella pneumonia* and *P. charlesii*.[57,58]

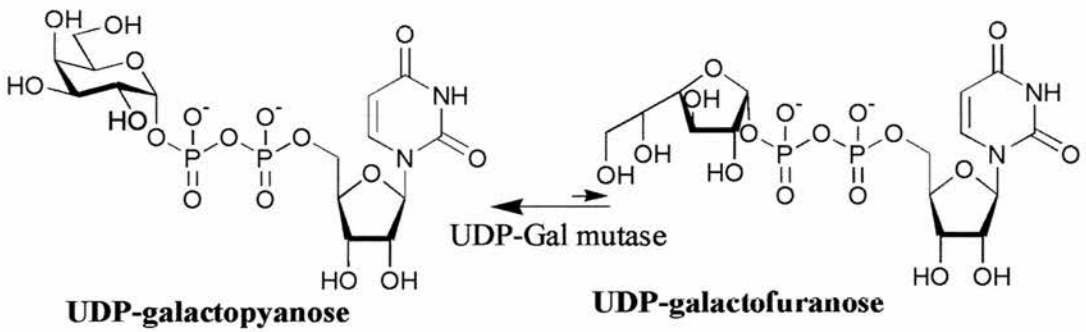


Figure 1.9: Interconversion of UDP-Gal*p* and UDP-Gal*f*.

1.6.2 Mechanism of action of UDP-galactose mutase[59,60,61,62,63]

The role of UDP-Gal*p* mutase is to convert UDP-galactopyranose to UDP-galactofuranose and *vice versa*. Once converted, various galactofuranosyltransferases incorporate the Gal*f* sugar unit in to the cell wall. The mechanism of action of the mutase is not fully understood, however, it is known that the mutase enzyme contains a flavin co-factor (FAD). Flavins are known to participate in a range of redox processes, such as electron transfer and the activation of molecular oxygen for oxygenation reactions. A review of the known and hypothesised mechanisms contains detailed information regarding these processes.[61,62,63]

1.7 Aims and Objectives

1.7.1 Sugar-nucleotide mimetics

The aim of this project is to synthesise galactofuranose and arabinofuranose mimics capable of inhibiting UDP-galactose mutase and arabinosyltransferases, respectively. UDP-Gal*f* has only recently been chemically synthesized by Liu,[64] Kiessling,[65] and Nikolaev.[66]

1.7.1.1 Galactofuranose mimics

Recent reports in the literature showed that various azasugar analogues (**Figure 1.10** and **Table 1.1**) cause substantial inhibition of UDP-Gal*p* mutase at low concentrations.[67]

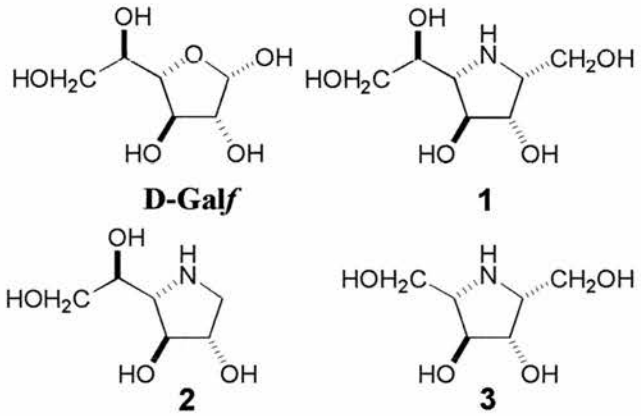


Figure 1.10: Pyrrolidine derivatives that mimic Gal*f*.

Compound 200 μg/ml	% Inhibition of mycobacteria-galactan biosynthesis	% Inhibition of UDP-Gal*p* to UDP-Gal*f*	% Inhibition of UDP-Gal*f* to UDP-Gal*p*
1	63	64	67
2	56	36	81
3	16	-	-

Table 1.1: Pyrrolidine derivatives as inhibitors of mycobacterial galactan biosynthesis.

Given these results it may prove beneficial to synthesise simpler acyclic aminothreitol derivatives (**Figure 1.11**). The synthesis of these aminothreitol compounds could be achieved more efficiently in as few as four steps. Once synthesised the amino compounds could be subjected to biological testing with UDP-Gal*p* mutase, this, in fact, constituted the first objective of this project.

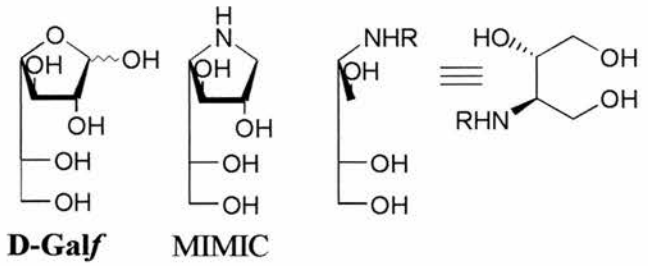


Figure 1.11: acyclic aminothreitol compounds.

1.7.1.2 Arabinofuranose mimics

Recently a key mycobacterial arabinose donor, β-D-arabinofuranosyl-1-monophosphoryldecaprenol **(Figure 1.12)** was identified within the lipid extracts of *M. smegmatis* and implicated in the biogenesis of two major cell wall polysaccharides, arabinogalactan and LAM.[68] This compound is not readily available from natural sources due to lability. Successful efforts have been made in chemically synthesising[69] a [^{14}C]- labelled form of this compound. Using the labelled compound an arabinosyltransferase assay has been developed using a membrane fraction of *M. smegmatis.*

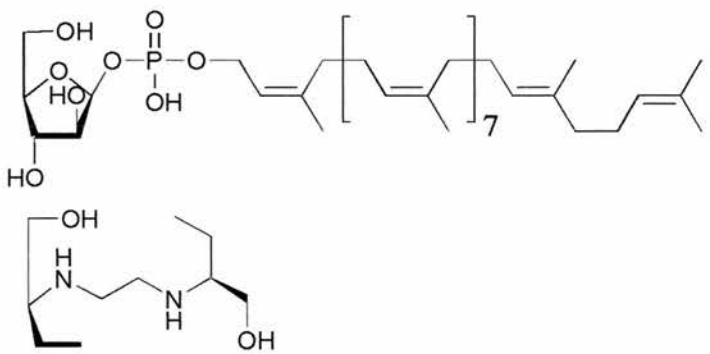


Figure 1.12: β-D-arabinofuranosyl-1-monophosphosphoryldecaprenol and ethambutol.

A time-course experiment showed smooth, time-dependent incorporation of the donor sugar into the cell wall material. Ethambutol, a front line anti-TB treatment, is thought to operate

by mimicking the structure of β-D-arabinofuranosyl-1-monophosphoryldecaprenol, thereby inhibiting arabinosyltransferase activity[38,70,71]

With this in mind, the synthesis of acyclic aminodeoxyerythritol (**Figure 1.13**) compounds similar in structure to ethambutol was undertaken.

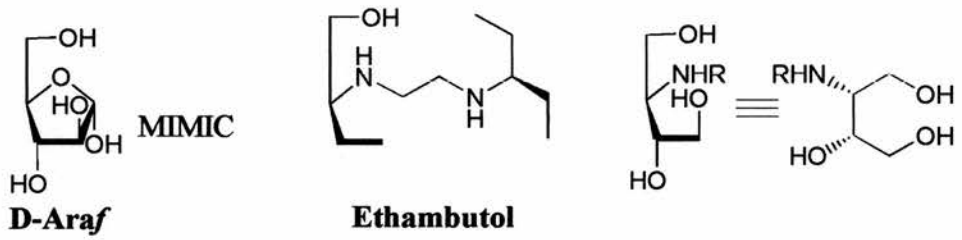


Figure 1.13: acyclic aminoerythritol target compounds.

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Chapter 2

Acyclic azasugars as Gal*f* and Ara*f* mimics

2.1 Justification of the investigation of acyclic azasugar analogues:

The synthesis of acyclic analogues of biologically important compounds has already been shown to be worthwhile, most notably in the case of deoxynojirimycin **(2.1)** (**Figure 2.1**).[1] 1-Deoxynojirimycin (DNJ)[2] (**Figure 2.1**) shows potent competitive inhibition of the endoplasmic reticulum α-glycosidase II which is involved in glycoconjugate synthesis. Acyclic analogues of 1-deoxynojirimycin can readily be achieved, by applying the disconnection between C2-C3 and C3-C4 to give its acyclic equivalent **(2.2)** (**Figure 2.1**). The design of the acyclic compound **(2.2)** can be encouraged by the fact that short chain alditols are already known to act as glycosidase inhibitors (**Table 2.1**).[3]

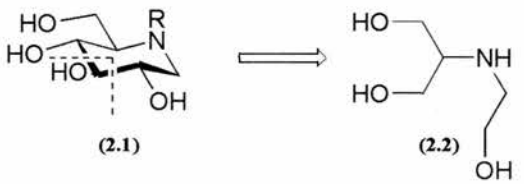


Figure 2.1: 1-Deoxynojirimycin and acyclic equivalent.[1]

Empirical Formula	Alditol	K_i (mM)
$C_6H_{14}O_6$	D-Glucitol	1300
"	D-Galactitol	> 4000
"	D-Mannitol	3100
$C_6H_{14}O_5$	L-Rhamnitol	370
$C_5H_{12}O_5$	D-Xylitol	210
"	Ribitol	200
$C_5H_{12}O_5$	D-Arabinitol	44
"	L-Arabinitol	60
$C_5H_{12}O_4$	Pentaerythritol	225
$C_4H_{10}O_4$	D,L-Threitol	215
"	Erythritol	22

Table 2.1: Inhibition of sweet almond β-glucosidase by alditols (pH 6.2, 27°C).[3]

However, when tested for activity against ß-D-glucosidase, the inhibition constants obtained indicated that 1-deoxynojirimycin **(2.1)** was a more effective inhibitor of sweet almond ß-D-glucosidase than its acyclic counterpart **(2.2)** ($K_i = 1.8 \times 10^{-5}$ M for **(2.1)** and $K_i = 9.1 \times 10^{-4}$

M for **(2.2)**). Compound **(2.2)** and other acyclic compounds however showed competitive inhibition when tested against yeast α-glucosidase, proving that the synthesis of acyclic analogues of 1-deoxynojirimycin to be worthwhile (**Figure 2.2**) and (**Table 2.2**).

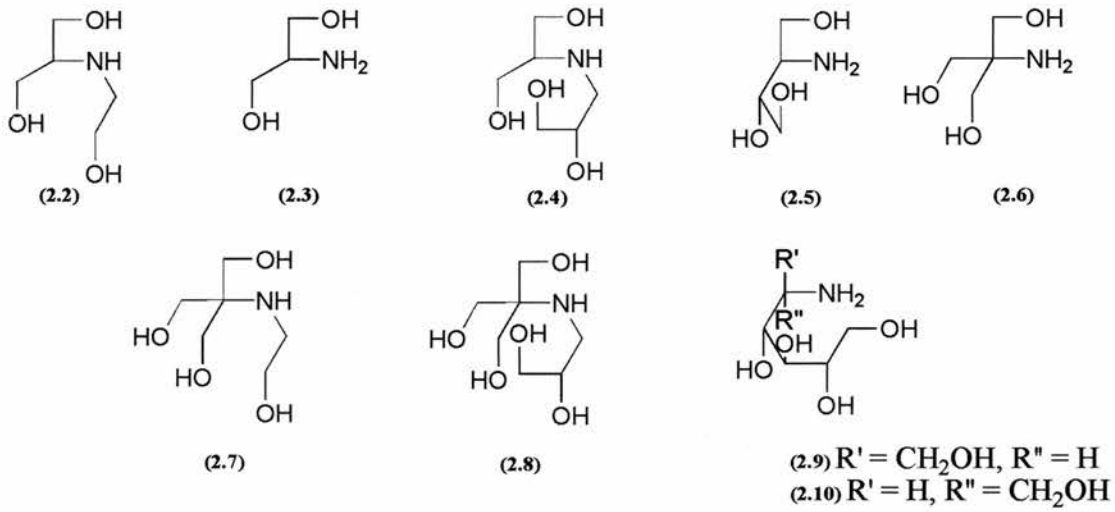


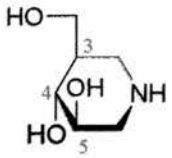
Figure 2.2: Compounds tested for inhibition of yeast α-glucosidase.[4]

Compound	K_i (μM)	Compound	K_i (μM)
(2.2)	4	**(2.7)**	3556
(2.3)	702	**(2.8)**	652
(2.4)	10	**(2.9)**	3.6
(2.5)	82	**(2.10)**	216
(2.6)	379		

Table 2.2: Inhibition constants K_i for the inhibition of yeast α-glucosidase by acyclic analogues of 1-deoxynojirimycin. In μmol dm^{-3}.[4]

2.1.1 Iminosugars as glycogen phosphorylase inhibitors:

Iminosugars have attracted much attention due to their ability to act as powerful glycosidase inhibitors,[5] most notably in the cases where the glycosidases are involved in intestinal break down of sugars.[6,7,8] Glucose is produced by the liver in two different modes, gluconeogenesis and glycogenolysis (breakdown of glycogen). It has been reported[9] that a large portion of the glucose produced by gluconeogenesis is cycled through the glycogen storage before efflux from the liver. This makes inhibition of glycogenolysis a prime target, in the development of new anti-hyperglycemic agents. Isofagomine **(2.11) (Figure 2.3)** has been identified as a strong liver glycogen phosphorylase (GP) inhibitor.



(2.11)

Figure 2.3: Isofagomine, a potent glycogen phosphorylase inhibitor.[5]

Several other iminosugar type compounds have been synthesised and tested for their ability to inhibit this enzyme (GP).[10] The results of the biological assays suggest that to achieve an inhibitory effect in the low μM range, it is essential that compounds contain the *3 R, 4 R, 5 R-* or *3 S, 4 S, 5 S-* (as is the case with isofagomine).

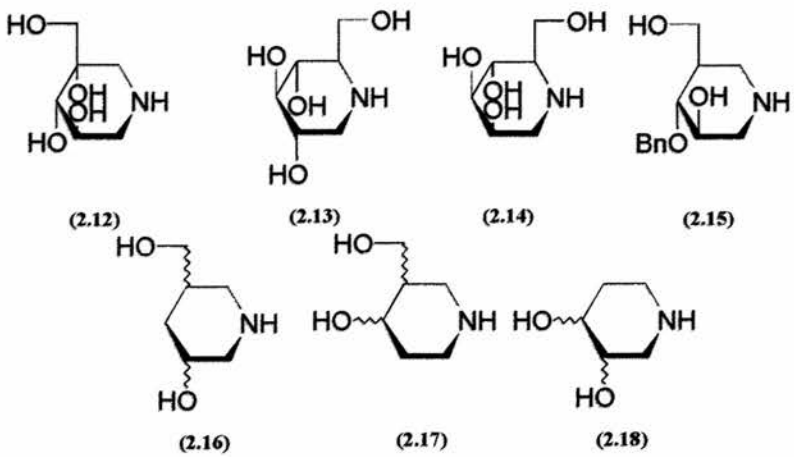


Figure 2.4: Iminosugars incapable of inhibiting GP.

In addition to these requirements, it is evident from the biological studies[10] that no extra OH groups are tolerated, **(2.12)**, **(2.13)** and **(2.14)** (**Figure 2.4**) nor is is *O*-substitution tolerated **(2.15)** (**Figure 2.4**). It is also clear that all 3 hydroxyl groups should be present, since compounds **(2.16)**, **(2.17)** and **(2.18)** were inactive (**Figure 2.4**). Substitution at other places in the piperidine ring is also not permitted, suggesting that the compounds may bind in the catalytic site in the same fashion as the natural ligand, with the 3 OH groups mimicking the hydroxylmethyl and hydroxyl groups in the 3 and 4 positions of the isofagomine moiety. Substitution on nitrogen always resulted in an equal or less inhibitory effect, indicating that *N*-substitution does not destroy the interaction with the binding site.

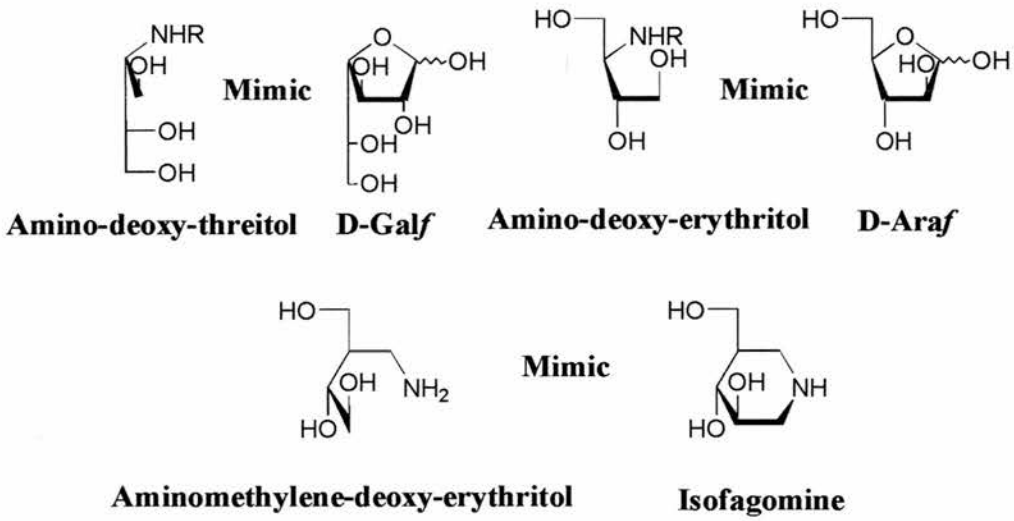


Figure 2.5: D-Galactofuranose, D-arabinofuranose, isofagomine and their acyclic azasugar equivalents.

These preceeding favourable results indicate that it would be logical to adopt a similar approach in the synthesis of other acyclic mimics of biologically important compounds such as galactofuranose, arabinofuranose and isofagomine (**Figure 2.5**).

2.2 Aims and Objectives

The main aim of this project was to design short syntheses for three sets of target materials, 2-amino-2-deoxy-D,L-erythritols, 2-amino-2-deoxy-D,L-threitols and 2-aminomethylene-2-deoxy-D,L-erythritol targets (**Figure 2.6**).

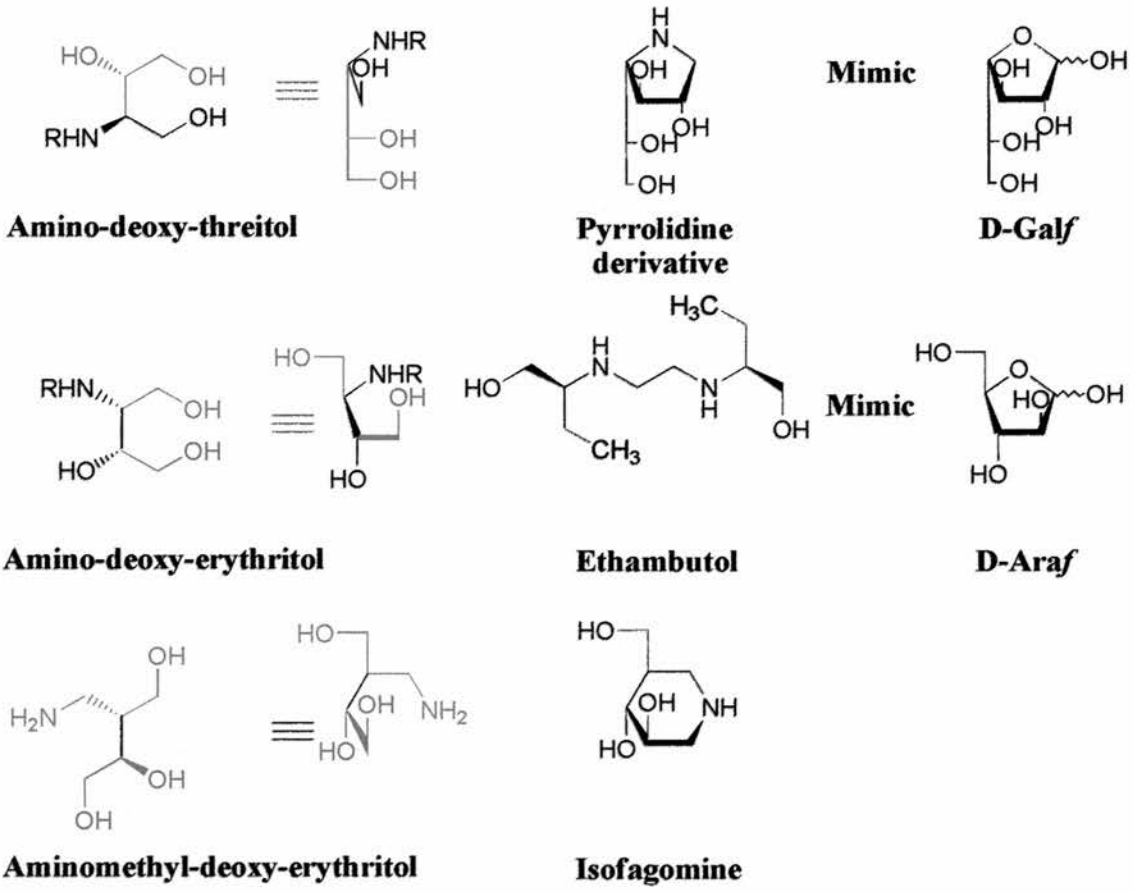


Figure 2.6: 2-Amino-2-deoxy-D,L-erythritol, 2-amino-2-deoxy-D,L-threitol and 2-aminomethylene-2-deoxy-D,L-erythritol targets.

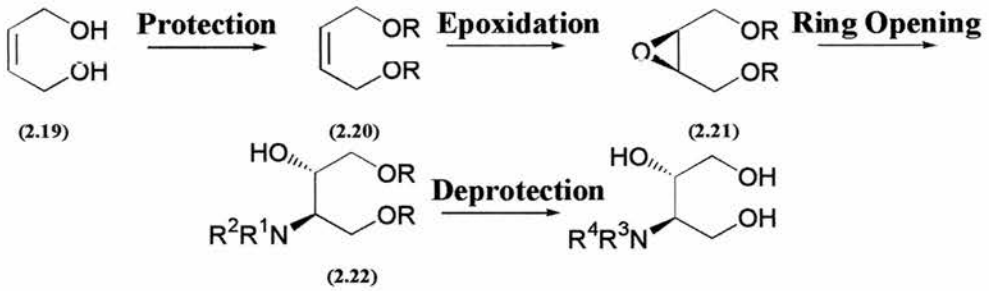
The amino-deoxy-threitol, amino-deoxy-erythritol and aminomethylene-deoxy-erthritol target materials are racemates by design. Should any of the compounds show biological activity then synthesis of the individual enantiomers could be achieved with the aid of asymmetric epoxide ring opening with Jacobsen's catalyst.[11,12]

2.3 2-Amino-2-deoxy-D,L-threitols:

2.3.1 Strategy for the preparation of 2-amino-2-deoxy-D,L-threitols:

The proposed syntheses of the *threo* target compounds comprises four steps from the commercially available starting material, (*Z*)-2-butene-1,4-diol (**Scheme 2.1**). The reaction

pathway involves protection of the diol **(2.19)**, epoxidation of the protected diol **(2.20)**, ring opening of the resulting epoxide **(2.21)** with various primary and secondary amines with deprotection of the ring opened epoxide **(2.22)** to yield the ultimate target compounds. The reaction pathway is outlined in **Scheme 2.1**.

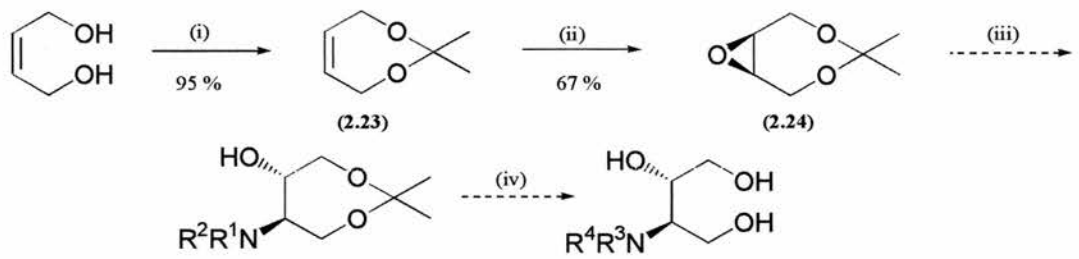


R = $C(CH_3)_2$, Bn or PMB; $\mathbf{R}^{1\text{-}4}$ = Various

Scheme 2.1: General strategy for the preparation of 2-amino-2-deoxy-D,L-threitols.

2.3.2 Discussion of the synthesis of 2-amino-2-deoxy-D,L-threitols:

Two different syntheses of 2-amino-2-deoxy-D,L-threitols were attempted, the first involved use of a known isopropylidene-protected diol **(2.23)**[13]**(Scheme 2.2)**. Isopropylidenation was accomplished by the action of camphorsulfonic acid and 2,2-dimethoxypropane on the commercially available diol **(2.19)**. [13]



Scheme 2.2: (i) 2,2-Dimethoxypropane, Camphorsulfonic acid; (ii) DCM, *m*-CPBA (2.4 mol eq); (iii) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (iv) H^+.

Acetal formation worked well and in excellent yield (95%), however the reaction must be viewed closely giving **(2.23)** by T.L.C as acetal formation is a reversible process. Immediately the starting material was consumed, the reaction mixture was neutralised and purified by distillation. It was noted that the reaction did not proceed when *p*-toluenesulfonic acid was used as catalyst, but did when camphorsulfonic acid was used. This may be due to complications arising from the water present in commercial tosic acid (i.e. it is typically sold as a monohydrate). Two different methods were used in carrying out the purification, conventional distillation and distillation with a Kügelrohr apparatus. Epoxidation of the resulting protected acetal, **(2.23)**, gave the known epoxide **(2.24)** in good yield (67%) **(Scheme 2.2)**.

The next stage in the target synthesis was the epoxide ring opening of epoxide **(2.24)** with primary and secondary amines. This reaction needs to be promoted and will not proceed at room temperature unaided.

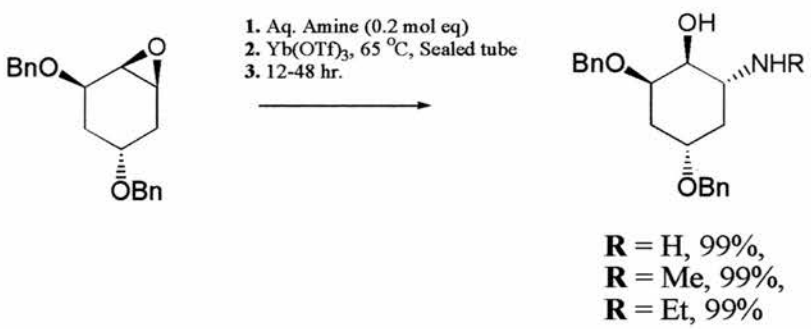


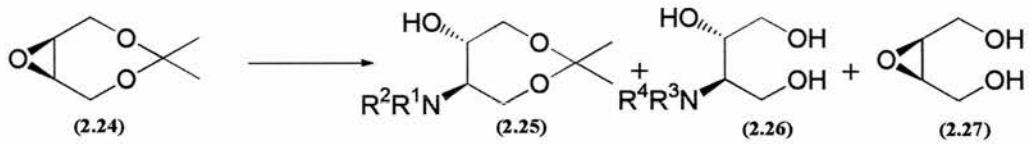
Figure 2.7: Ring opening of cyclohexene oxide derivatives with amines. [15]

Reports in the literature have shown both lithium perchlorate[14] and ytterbium (III) triflate[15] to be excellent promoters for epoxide ring openings with various primary and secondary amines. Literature reports (**Table 2.3** and **Figure 2.7**) cite lanthanide (III) trifluoromethanesulfonates as effective promoters for epoxide ring openings with primary and secondary amines. [14,15,16]

Entry	epoxide	$Ln(OTf)_3$	amine	solvent	Rxn. time	yield
1	Cyclohexene oxide	$Yb(OTf)_3$	$NHEt_2$	CH_2Cl_2	1 hr	98%
2	"	$Yb(OTf)_3$	t-$BuNH_2$	CH_2Cl_2	5 hr	97%
3	"	$Gd(OTf)_3$	t-$BuNH_2$	CH_2Cl_2	6 hr	98%
4	"	$Nd(OTf)_3$	t-$BuNH_2$	CH_2Cl_2	18 hr	85%
5	"	$LiClO_4$	t-$BuNH_2$	CH_3CN	18 hr	95%
6	"	$Yb(OTf)_3$	$(i\text{-}Pr)_2NH$	CH_2Cl_2	18 hr	90%
7	"	$LiClO_4$	$(i\text{-}Pr)_2NH$	CH_3CN	64 hr	86%

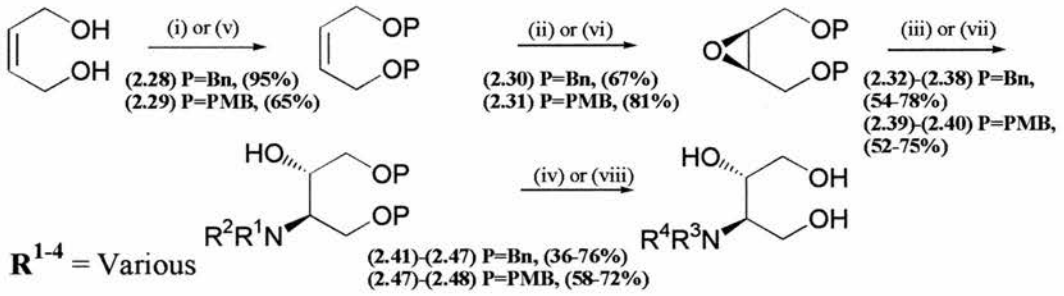
Table 2.3: Ring openings of epoxides using Lewis acids as catalysts. [14]

It has also recently been shown[14] that common metal salts such as $LiClO_4$ promote efficient direct aminolysis of different 1,2-epoxides with various primary and secondary amines, however an equimolar amount of the metal salt had to be used. Utilising this information from the literature, it was decided to attempt the ring opening of epoxide **(2.24)** in the presence of two different catalysts, namely with ytterbium (III) triflate and lithium perchlorate. Ring opening reactions carried out on **(2.24)** were unsatisfactory, due to the high lability of the acetal protecting group under Lewis acidic conditions, and generation of mixtures of compounds as a result. **(Scheme 2.3)**. In the presence of catalytic amounts of Lewis acid (0.2 eq), the isopropylidine protecting group cleaved.



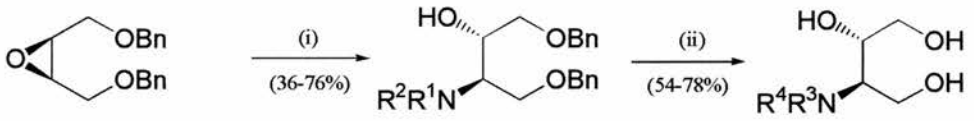
Scheme 2.3: Products obtained from epoxide ring opening of **(2.24)**.

As a result an alternative protecting group strategy was employed as in **(Scheme 2.4)**.



Scheme 2.4: (i) DMF, NaH (2.4 mol eq), BnBr (2.4 mol eq); (ii) DCM, *m*CPBA (2.4 mol eq); (iii) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (iv) 10% Pd-C, H_2, MeOH; (v) DMF, NaH (2.2 mol eq), PMB-Cl (2.2 mol eq); (vi) DCM, *m*CPBA (2.4 mol eq); (vii) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (viii) DCM/TFA (9:1).

The protection of (*Z*)-2-butene-1,4-diol using sodium hydride and BnBr/PMB-Cl, proceeded smoothly and in excellent yield. Compounds **(2.28)** and **(2.29)** were treated with *m*-CPBA generating epoxides **(2.30)** and **(2.31)** in good yields. The epoxide ring-opening step proved to be problematic since the $Yb(OTf)_3$ catalysed reaction did not proceed at room temperature in DCM. A series of trial reactions were carried out to establish the optimum conditions for the ring opening of epoxides **(2.30)** and **(2.31)**. DCM, AcN, DCE, IPA and 1,4-dioxane were the solvents used in the trial reactions, the ratio of epoxide to amine was varied (1.0 - 2.4 mol eq) and an increase in reaction rates was observed with an excess of amine. A control experiment proved that IPA was not sufficiently nucleophilic to ring open either **(2.30)** or **(2.31)**. It was found that the catalyst, or more correctly the promoter, had to be used in excess (2 mol eq) for the reaction to proceed efficiently. Contrary to literature reports[14] lithium perchlorate showed greater activity than ytterbium (III) triflate in our hands. It was decided, therefore, to use lithium perchlorate in all subsequent reactions as it demonstrated greater activity, was cheap and readily available. Both primary and secondary amines were used in the reactions. Reaction rates of secondary amines were lower than primary amines, which can be attributed to steric hindrance. From the results of trial reactions, the optimum conditions were found to be 2.4 mole equivalents of amine, 2 mole equivalents of $LiClO_4$ promoter and Bn/PMB ether protected epoxide in refluxing anhydrous 1,4-dioxane. The first amines to be used under the optimised conditions are detailed in (**Tables 2.4** and **2.5** and **Schemes 2.5** and **2.6**).

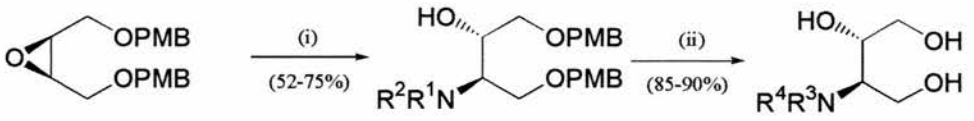


(2.30) **(2.32)-(2.38)** **(2.41)-(2.47)**

Scheme 2.5: (i) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (ii) 10% Pd-C, H_2, MeOH.

Amine	R^1	R^2	Intermediate	Yield	R^3	R^4	Prod.	Yield
Benzylamine	$C_6H_5CH_2$	H	**(2.32)**	61%	H	H	**(2.41)**	57%
Octylamine	C_8H_{17}	H	**(2.33)**	58%	C_8H_{17}	H	**(2.42)**	64%
Ethanolamine	CH_2CH_2OH	H	**(2.34)**	78%	CH_2CH_2OH	H	**(2.43)**	58%
Allylamine	C_3H_5	H	**(2.35)**	54%	C_3H_7-	H	**(2.44)**	76%
Piperidine	-$CH_2(CH_2)_3CH_2$-	R^1	**(2.36)**	72%	-$CH_2(CH_2)_3CH_2$-	H	**(2.45)**	36%
Morpholine	-$CH_2CH_2OCH_2CH_2$-	R^1	**(2.37)**	68%	-$CH_2CH_2OCH_2CH_2$-	H	**(2.46)**	61%
Diphenylethylamine	$(C_6H_5)_2CHCH_2$	H	**(2.38)**	71%	$(C_6H_5)_2CHCH_2$	H	**(2.47)**	72%

Table 2.4: Amines used in epoxide ring opening of **(2.30)**.



(2.31) **(2.39)-(2.40)** **(2.47)-(2.48)**

Scheme 2.6: (i) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (ii) DCM/TFA (9:1).

Amine	R^1	R^2	Intermediate	Yield	R^3	R^4	Prod.	Yield
Benzylamine	$C_6H_5CH_2$	H	**(2.39)**	52%	$C_6H_5CH_2$	H	**(2.48)**	90%
Diphenylethylamine	$(C_6H_5)_2CHCH_2$	H	**(2.40)**	75%	$(C_6H_5)_2CHCH_2$	H	**(2.47)**	85%

Table 2.5: Amines used in epoxide ring opening of **(2.31)**.

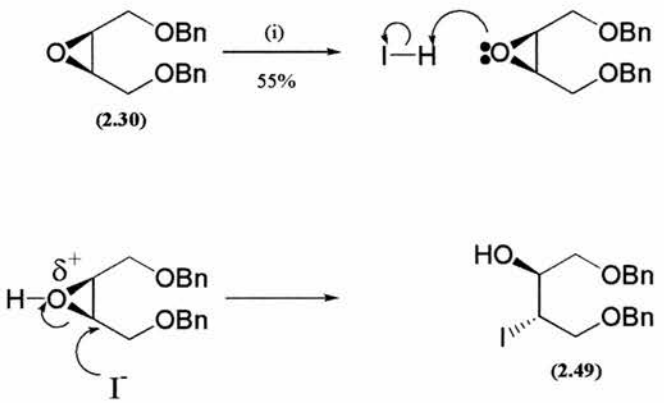
Most epoxide ring opening reactions worked well, the primary amines reacting within three to four hours and the secondaries within twelve hours. These protected benzyl/PMB-protected amino alditols **(2.32) – (2.40)** were not purified by column chromatography since they had a tendency to stick to the silica gel. When a silica chromatography step was included poor yields of material were realized in most cases. Some epoxide ring openings involving amines with two amino groups (phenylhydrazine and piperazine) did not work

well, with a combination of products being obtained, making eventual purification very difficult.

Deprotection of compounds **(2.32) - (2.38)** and **(2.39) - (2.40)** was achieved by catalytic hydrogenation or reaction with TFA, respectively. Hydrogenation was successful in all but one case. De-*O*-benzylation of the benzylamine adduct, **(2.32)** proved troublesome. More forceful conditions were applied and the *O,N*-deprotected free amine **(2.41)** was obtained. Synthesis of the benzylamine adduct **(2.48)** was eventually optimised by the acid-catalysed deprotection of **(2.39)** with TFA.

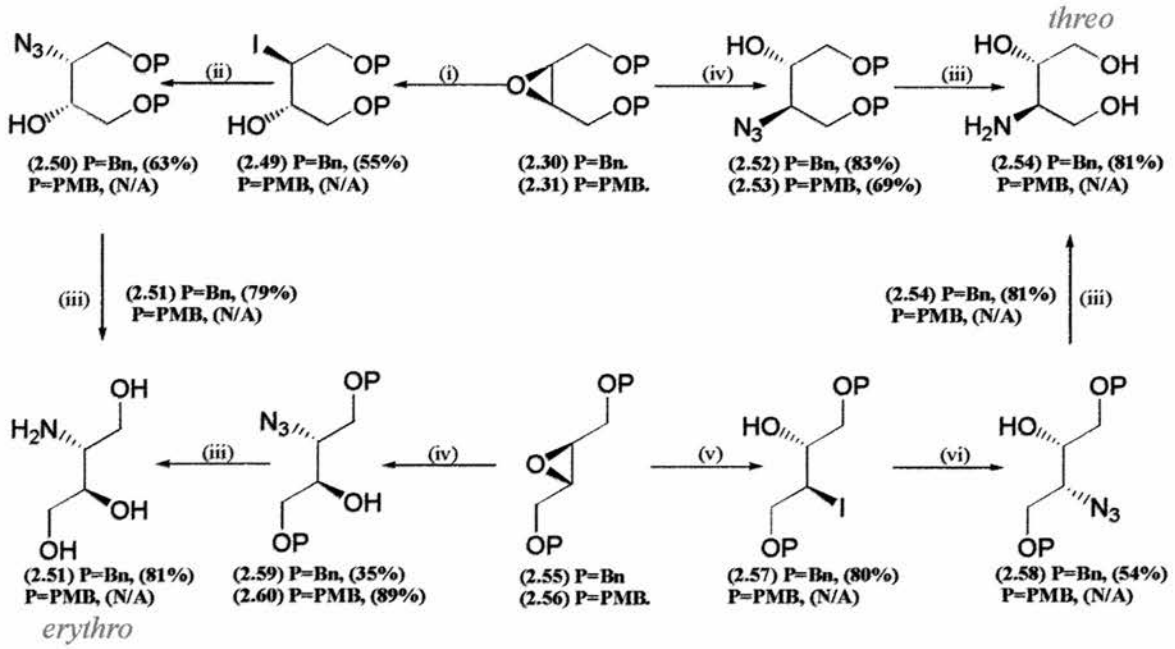
2.3.3 Synthesis of 2-amino-2-deoxy-D,L-erythritols via iodine ring opening of 2,3-anhydro-1,4-di-*O*-benzyl-erythritol, (2.30)

Previously iodine had been used extensively within the Field group as a mild Lewis acid in the activation of both oxygen and sulfur leaving groups for glycosylation chemistry.[17] An attempt to open epoxide **(2.30)** with diisopropylamine in the presence of iodine (as promoter) gave only the iodide, **(2.49)**. This conclusion is supported by HRMS; *m/z* (CI) 413 (M+1, 100%); HRMS: Found: 413.0613 $C_{18}H_{22}IO_3$ ($M+H^+$) Requires 413.0613 and IR ν_{max}/cm^{-1} 3500 (OH) (no NH stretch observed). Formation of the iodide probably resulted from the action of hydrogen iodide (generated from the reaction between diisopropylamine and molecular iodine) on epoxide **(2.30)**. The reaction outlined in **Scheme 2.7**.



Scheme 2.7: (i) I_2 (10 mol eq), Diisopropylamine (2.5 mol eq), 1,4-dioxane.

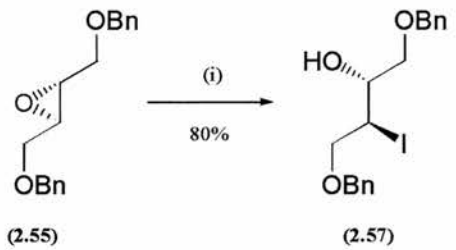
In principle, this finding provides a convenient route to *erythro* compounds. Iodide should easily be displaced from **(2.49)** with azide which can in turn be reduced via hydrogenation to yield the erythritol **(2.51)** (**Scheme 2.8**), thus permitting interconversion between *erythro* and *threo* series.



Scheme 2.8: (i) I_2 (10 mol eq), diisopropylamine (2.5 mol eq), 1,4-dioxane, reflux; (ii) NaN_3 (4 mol eq), Bu_4NOTf (0.3 mol eq), DMF, 80 °C; (iii) 10% Pd-C, H_2, MeOH; (iv) NaN_3 (10 mol eq), NH_4Cl (10 mol eq), DMF, reflux; (v) LiI (1.7 mol eq), AcOH, THF; (vi) NaN_3 (10 mol eq), 18-crown-6, THF.

As mentioned previously initial iodinations were achieved by the action of iodine and diisopropylamine in dioxane on epoxide **(2.30)**. Since this reaction was unexpected, a series of trial reactions were performed in an attempt to enhance yields and reaction times. All iodinations were followed very closely by T.L.C, since excessive reaction times might lead to the re-generation of epoxide starting materials. Iodinations were initially attempted in DCM with molecular iodine (1-10 mol eq) as nucleophile in the presence of a crown ether (dibenzyl-18-crown-6). The reactions were unsuccessful at room temperature consequently the system was heated to reflux. The system was still unreactive so diisopropylamine (2.5 mol eq) was added. This was the key to the success of the reaction, with the iodide **(2.49)** subsequently being generated in moderate yield (55%). A second, more successful iodination

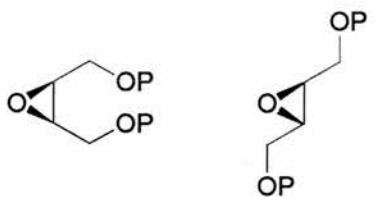
method was identified. Literature reports suggest that the action of lithium iodide/acetic acid in anhydrous THF on epoxides leads to the formation of iodides in good yield.[18] This indeed proved to be the case with iodide **(2.57)** being generated in good yield (80%), **(Scheme 2.9)**.



(2.55) (2.57)

Scheme 2.9: (i) LiI (1.7 mol eq), AcOH, THF.

Following literature protocols,[19] azide formation (Compound **(2.50)**, **Scheme 2.8**) from iodide starting materials (Compound **(2.49)**, **Scheme 2.8**) proved facile [ν_{max}/cm^{-1} 2093 (N_3), 3500 (OH); *m/z* (CI) 328 (M+1, 100%); HRMS: Found: 328.1661 $C_{18}H_{22}N_3O_3$ ($M+H^+$) Requires 328.1661.]. In contrast azide formation from epoxide starting materials proved troublesome. Sodium azide (4-10 mol eq) was the nucleophile of choice for epoxide ring openings with **(2.30)**, **(2.31)**, **(2.55)** and **(2.56)**.



(2.30) P=Bn **(2.55) P=Bn**
(2.31) P=PMB. **(2.56) P=PMB.**

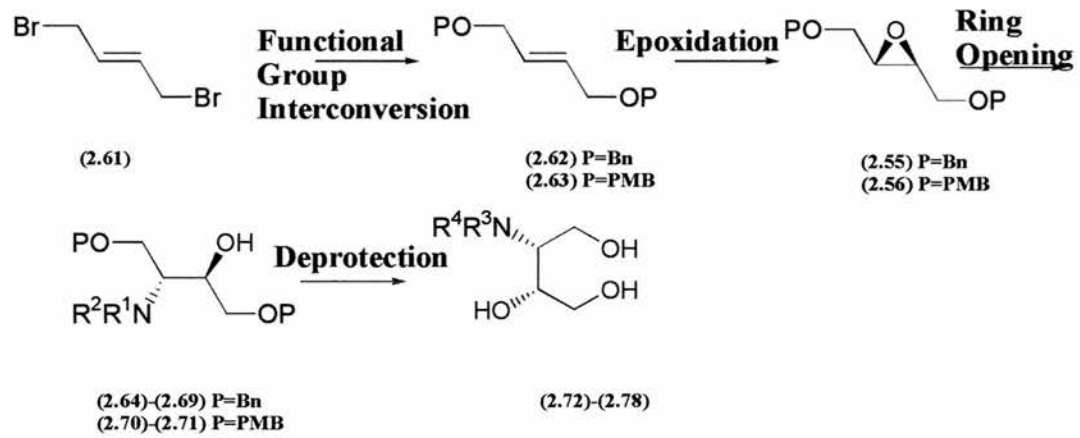
A series of analytical scale reactions were performed in order to identify the optimum reaction conditions. Lithium perchlorate[14] and tetrabutylammonium triflate[19] were unable to promote epoxide ring opening, whilst the reaction was found to be unsuccessful i.e. too lethargic in 1,4-dioxane and THF. When gently heated (50°C) in DMF, with tetrabutylammonium triflate[19] as promotor a quantity of azide was generated. The reaction

was then repeated in DMF with ammonium chloride[20] as promotor (10 mol eq). These conditions led to the formation of azide **(2.52)** in 63% yield.

2.4 2-amino-2-deoxy-D,L-erythritols:

2.4.1 Alternative approach for the preparation of 2-amino-2-deoxy-D,L-erythritols

As was the case in the preparation of 2-amino-2-deoxy-D,L-threitols, syntheses of the proposed *erythro* target compounds comprised four classes of protecting group interconversion (**Scheme 2.10**). The reaction pathway involved protection of the commercially available (*E*)-1,4-di-bromo-butene **(2.61)**, epoxidation of the protected olefins **(2.62)** and **(2.63)** ring opening of the resulting epoxides **(2.55)** and **(2.56)** with various primary and secondary amines and finally deprotection of the ring opened epoxides **(2.64 – 2.71)** to yield the target compounds. The reaction pathway is illustrated in **Scheme 2.10**.



$\mathbf{R^{1-4}}$ = Various
P = PMB or Bn PG's

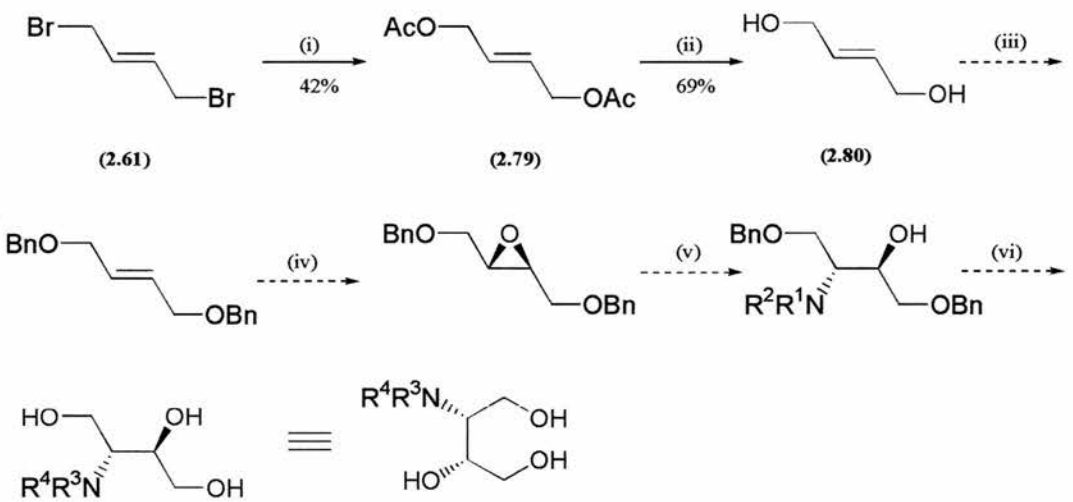
Scheme 2.10: Synthetic route to 2-amino-2-deoxy-D,L-erythritols.

Compound Number	Corresponding Amine
(2.64)	Benzylamine
(2.65)	Octylamine
(2.66)	Allylamine
(2.67)	Piperidine
(2.68)	Morpholine
(2.69)	Diphenylethylamine
(2.70)	Benzylamine
(2.71)	Diphenylethylamine
(2.72)	Amine
(2.73)	Octylamine
(2.74)	Propylamine
(2.75)	Piperidine
(2.76)	Morpholine
(2.77)	Diphenylethylamine
(2.78)	Benzylamine

Table 2.6: Amines used in epoxide ring openings of compounds **(2.55)** and **(2.56)**.

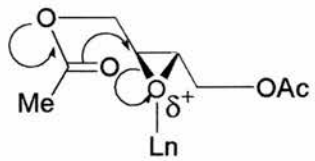
2.4.2 Synthesis of 2-amino-2-deoxy-D,L-erythritols:

An initial aim was to prepare the target *erythro* compounds from 1,4-dibromo-but-2-ene, as detailed in **Scheme 2.11**.



Scheme 2.11: (i) NaOAc, 18-Crown-6, Dry DCM; (ii) NaOMe/MeOH; (iii) NaH (2.4 mol eq), BnBr (2.4 mol eq), DMF; (iv) *m*CPBA (2.4 mol eq), DCM; (v) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (vi) 10% Pd-C, H_2, MeOH.

Attempts to convert the dibromide, **(2.61)** to the diacetate, **(2.79)** using anhydrous sodium acetate in dichloromethane proved unsuccessful. However on addition of 18-crown-6 the reaction proceeded in average (42%) yield. It was also considered vitally important to protect the diol, **(2.80)** with benzyl ether protecting groups, since ring opening of the diacetate epoxide, **(2.81)** with amines in the presence of a Lewis acid is likely to be unsuccessful due to the possibility of ring opening of the epoxide by the acetate group, as per (**Figure 2.8**).



(2.81)

Figure 2.8: Ring opening of *threo* epoxide, **(2.81)** by acetate.

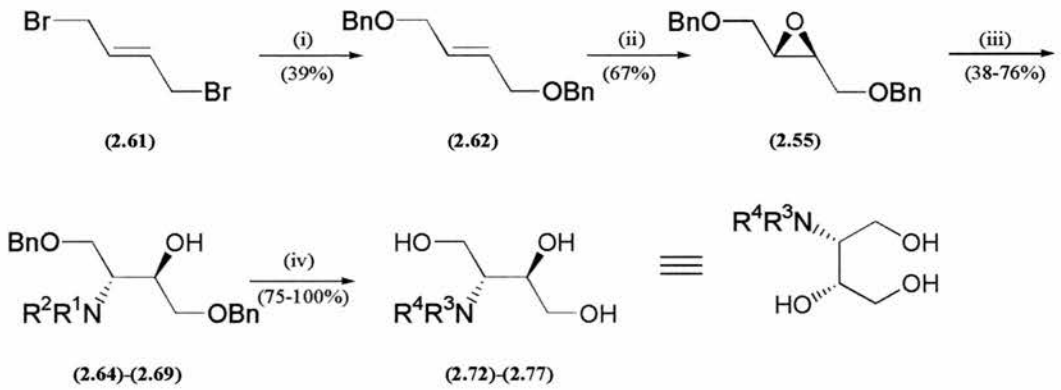
In the event of the ring opening of diacetate epoxide **(2.81)** proving successful there remains the prospect of acetyl migration from alcohol to amine, which would limit opportunities for further derivitisation on nitrogen (**Figure 2.9**).

(2.82)

Figure 2.9: Acetyl migration of **(2.82)**.

Deprotection of the diacetate **(2.79)** with sodium methoxide proceeded in moderate yield (69%). Benzylation of **(2.80)** in the presence of both NaH (2.4 mol eq) and BnBr (2.4 mol eq) failed. Direct benzylation of the dibromo starting material **(2.61)** using the sodium salt of benzyl alcohol, was similarly unsuccessful, with the starting material proving unreactive. By modification of a previously reported procedure,[21] the dibromo starting material **(2.61)**, was protected using crushed KOH/18-C-6 and BnOH/PMBOH. In most cases protection with PMB protecting groups was the preferred option, since PMB deprotection can be achieved almost immediately when treated with TFA. An added advantage with this step is that

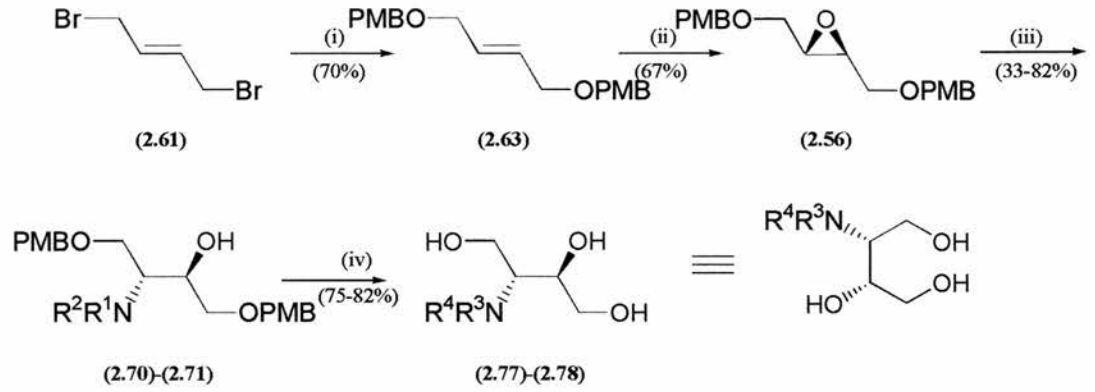
synthesis of the diacetate **(2.79)** and diol **(2.80)** is avoided. The next step in the synthesis was the epoxide ring opening with lithium perchlorate and various amines. As indicated previously in section **2.3.2** this proved to be the most difficult step in the generation of the acyclic target materials. The final step is a PMB/benzyl ether deprotection. The final deprotection steps proceeded well and in high yield. The advantage with PMB protection over benzyl ether protection is that deprotection of the PMB ether protecting group is achieved almost immediately.



Scheme 2.12: (i) Crushed KOH (4 mol eq), BnOH (4 mol eq), 18-crown-6, anhydrous THF; (ii) DCM, *m*CPBA (2.4 mol eq); (iii) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (iv) 10% Pd-C, H_2, MeOH.

Amine	R^1	R^2	Intermediate	Yield	R^3	R^4	Prod.	Yield
Benzylamine	$C_6H_5CH_2$	H	**(2.64)**	73%	H	H	**(2.72)**	81%
Octylamine	C_8H_{17}	H	**(2.65)**	73%	C_8H_{17}	H	**(2.73)**	94%
Allylamine	C_3H_5	H	**(2.66)**	38%	C_3H_7-	H	**(2.74)**	100%
Piperidine	$-CH_2(CH_2)_3CH_2-$	-	**(2.67)**	70%	$-CH_2(CH_2)_3CH_2-$	-	**(2.75)**	100%
Morpholine	$-CH_2CH_2OCH_2CH_2-$	-	**(2.68)**	76%	$-CH_2CH_2OCH_2CH_2-$	-	**(2.76)**	76%
Diphenylethylamine	$(C_6H_5)_2CHCH_2$	H	**(2.69)**	75%	$(C_6H_5)_2CHCH_2$	-	**(2.77)**	82%

Table 2.7: Amines used in epoxide ring opening of **(2.55)**.



Scheme 2.13: (i) Crushed KOH (4 mol eq), PMBOH (4 mol eq), 18-crown-6, anhydrous THF; (ii) DCM, *m*CPBA (2.4 mol eq); (iii) $LiClO_4$ (2 mol eq), R^2R^1NH (2.4 mol eq), 1,4-dioxane, 105 °C; (iv) DCM/TFA (9:1).

Amine	R^1	R^2	Intermediate	Yield	R^3	R^4	Prod.	Yield
Benzylamine	$C_6H_5CH_2$	H	**(2.70)**	33%	$C_6H_5CH_2$	H	**(2.78)**	95%
Diphenylethylamine	$(C_6H_5)_2CHCH_2$	H	**(2.71)**	82%	$(C_6H_5)_2CHCH_2$	H	**(2.77)**	88%

Table 2.8: Amines used in epoxide ring opening of **(2.56)**.

2.5 Strategy for the preparation of open chain isofagomine analogues:

As previously mentioned in section **2.1.1**, the third and final short racemic synthesis involved attempting to synthesise the acyclic isofagomine derivative **(2.83)** **(Figure 2.10)**.

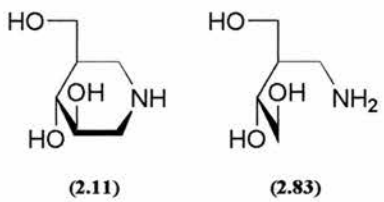
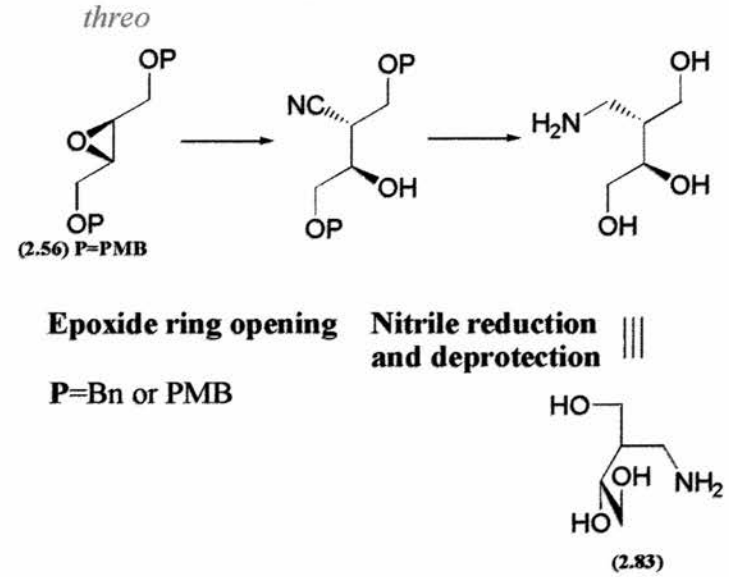


Figure 2.10: Isofagomine **(2.11)** and its acyclic equivalent **(2.83)**.

The proposed syntheses of the acyclic isofagomine derivative comprises two steps from the previously synthesised epoxide, 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol, **(2.56)** **(Scheme 2.14)**. The reaction pathway involves epoxide ring opening of **(2.56)** with

nitriles, nitrile reduction and PMB deprotection by hydrogenolysis to yield the target compound **(2.83)**. The reaction pathway is outlined in **Scheme 2.14**.

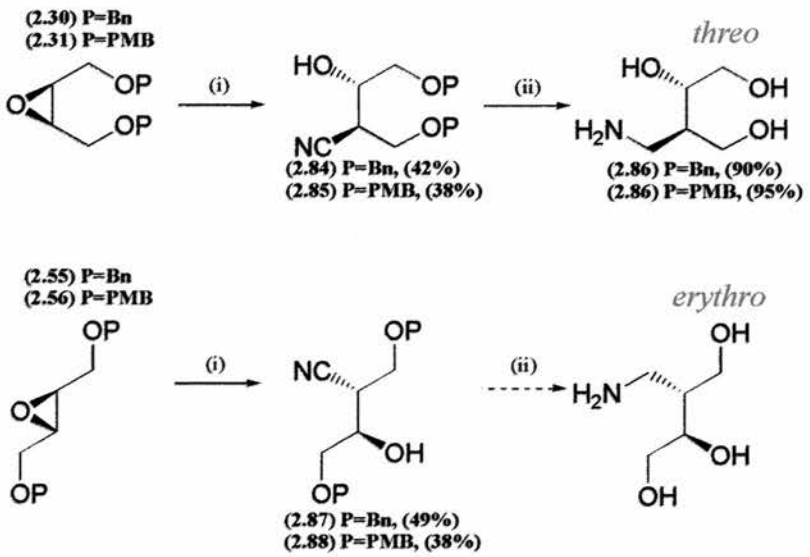


Scheme 2.14: General strategy for the preparation of acyclic isofagomine mimic **(2.83)**.

2.5.1 Discussion of the synthesis of 2-aminomethylene-2-deoxy-D,L-erythritol (2.83)

Initial epoxide ring openings with nitriles were performed on *erythro*-epoxides, **(2.30)** and **(2.31)** since they were more readily available. Should reaction be successful then it would be repeated on the *trans*-epoxide, 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol **(2.56)** to generate the desired target compound, **(2.83)**. The reaction was first performed in THF, using potassium cyanide[22] (3 mol eq) as the nucleophile and lithium perchlorate[14] (2 mol eq) as the promotor of choice. The reaction was unsuccessful. Repeating the reaction with the addition of 18-crown-6 proved similarly unsuccessful. The solvent was changed to DMF and the catalyst to tetrabutylammonium trifluoromethanesulfonate[19] but once again the reaction conditions were ineffective. The nucleophile was then changed to TMS-cyanide[23] and the promotor to BF_3:Et_2O. Once again the reaction did not yield the desired nitrile product. The epoxide ring opening was repeated with Et_2AlCN[24] (3 mol eq) in anhydrous toluene, this reaction was only partially successful as it would not proceed to completion, presumably due

to complexation between the desired nitrile product and the promoter (42% conversion with epoxide **(2.30)**). Confirmation of the nitrile linkage was obtained from IR spectroscopy, HRMS and ^{13}C NMR: ν_{max}/cm^{-1}: 2250 (CN), 3500 (OH); δ_c ($CDCl_3$), 118.0 (*C*N); *m/z* (CI) 312 (M+1, 100%); HRMS: Found: 312.1599. $C_{19}H_{22}NO_3$ ($M+H^+$) Requires 312.1599. Hydrogenolysis of *threo* intermediate, **(2.84)** yielded the *threo* target material, **(2.86)**. Hydrogenolysis of *erythro* intermediates, **(2.87)** and **(2.88)** has not yet been undertaken.



Scheme 2.15: (i) Et_2AlCN (3 mol eq), anhydrous toluene, N_2; (ii) 10% Pd-C, H_2, MeOH.

Thus far, several 2-amino-2-deoxy-D,L-threitol and-erythritol's and 2-aminomethylene-2-deoxy-D,L-erythritol/threitol's have been synthesized. These open chain compounds were then subjected to biological evaluation in various enzyme and whole cell assays. The results of these biological assays are briefly discussed in section **2.6**.

2.6 Biological Assays:

Work carried out so far has focused on the synthesis of 2-amino-2-deoxy-D,L-threitols/erythritols and 2-aminomethylene-2-deoxy-D,L-erythritol/threitols with various biological assays having been performed on these *threo* and *erythro* acyclic target materials. Although the target compounds are primarily arabinofuranose, galactofuranose and isofagomine mimics they were also subjected to assays against other freely, commercially available enzymes. All mycobacterial assays were performed in collaboration with Prof. Mike McNeil at Colorado State University and Dr. Rob Young at GSK. All glycosidase assays were performed in house by James Errey. Thus far all acyclic *threo* and *erythro* compounds have been subjected to mycobacterial enzyme assays with UDP-Gal*p* mutase and whole cell assays with *M. tuberculosis*. In addition the compounds have also been subjected to enzyme assays with various commercially available enzymes such as α-glucosidase, β-glucosidase and α-arabinofuranosidase.

2.6.1 Mycobacterial assays:

Two different mycobacterial assays were performed on the amino-deoxy-threitol/erythritol drug candidates. Mycobacterial whole cell assays were performed with *M. tuberculosis* H37Rv. Preliminary studies show that compound **(2.48)** has an ability to inhibit the growth of *M. tuberculosis* H37Rv. Inhibition by this compound was thirty fold weaker in comparison with the front line anti TB drug ethambutol. Work is ongoing in an attempt to find a more effective inhibitor and to ascertain the mode of action of same. Mycobacterial enzyme assays were performed with *Klebsiella pneumonia* UDP-Gal*p* mutase. In order to perform said assay, the enzyme was incubated with isotopically (^{3}H) labeled substrate (UDP-Gal*f*) and an azasugar drug candidate. This enzymatic reaction was performed and after a given time period was quenched with $NaIO_4$. From the level of isotopically labeled formaldehyde produced the turnover of the enzyme was established thereby enabling calculation of inhibition levels of the compounds. Four of the compounds assayed showed >90% inhibition of UDP-Gal*p* mutase at a concentration of 1 mM.

2.6.2 Glycosidase enzyme assays:

In addition to mycobacterial assays, several assays were performed against various commercially available enzymes. All glycosidase enzymes involved were purchased from Sigma. Assays were performed at pH 6.2 unless otherwise stated. Substrate concentration was 10% of K_M. The K_M values obtained for baker's yeast α-glucosidase, sweet almond β-glucosidase and *Asperigillius niger* α-arabinofuranosidase were 140 μM, 1.12 mM and 200μM respectively. Assays were performed using a fluorometric microplate assay procedure employing the same substrate as indicated above. The results of all of the biological assays (glycosidase and mycobacterial) are illustrated in **Tables 2.9** and **2.10**, respectively. Exact experimental details for the glycosidase assays performed will be reported in due course.[25]

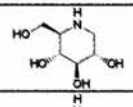
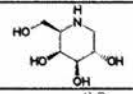
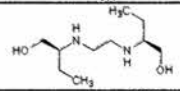
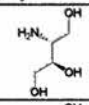
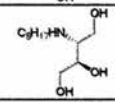
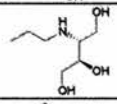
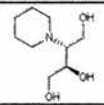
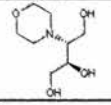
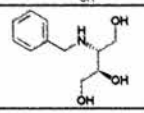
Compound No.	Structure	Bakers Yeast α-Glucosidase (IC_{50})	Sweet Almond β-Glucosidase (IC_{50})	*Aspergillius niger* α-Arabinofuranosidase (IC_{50})	*Klebsiella Pneumonia* UDP-Gal mutase (% turnover)*	*M. tuberculosis* H37Rv (M.I.C)**
DNJ		9 μM	11 μM	N	8%	N/A
GalDNJ		N	68 μM	N	N/A	N/A
Ethambutol		N	N	N	N/A	N/A
(2.72)		N	283 μM	271 μM	N/A	1000
(2.73)		N	577 μM	135 μM	6%	250
(2.74)		N	N	87 μM	79%	500
(2.75)		N	N	N	81%	>1000
(2.76)		N	N	N	N/A	1000
(2.78)		N	N	N	4%	1000

Table 2.9: Ability of 2-amino-2-deoxy-D,L-erythritols, * = % turnover of UDP-Gal*p* mutase at 1 mM and ** = MIC for *M. tuberculosis* H37Rv (μg/ml). **N** = compound does not inhibit, **N/A** = data not available.

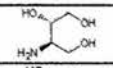
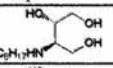
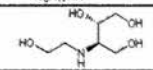
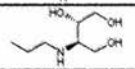
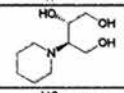
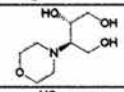
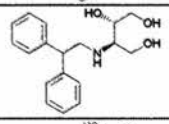
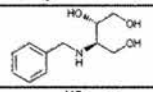
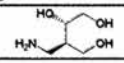
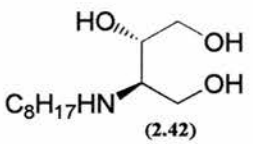
Compound No.	Structure	Bakers Yeast α-Glucosidase (IC_{50})	Sweet Almond β-Glucosidase (IC_{50})	*Aspergillius niger* α-Arabinofuranosidase (IC_{50})	*Klebsiella Pneumonia* UDP-Gal mutase (% turnover)*	*M. tuberculosis* H37Rv (M.I.C)**
(2.41)		N	N	N	N/A	1000
(2.42)		50 μM	160 μM (7 μM at pH 7)	315 μM	N/A	>1000
(2.43)		N	N	N	N/A	>1000
(2.44)		N	N	N	N/A	>1000
(2.45)		N	N	N	N/A	>1000
(2.46)		N	N	N	N/A	>1000
(2.47)		29 μM	N	N	5%	N/A
(2.48)		N	N	N	6%	63
(2.86)		N	N	N	N/A	N/A

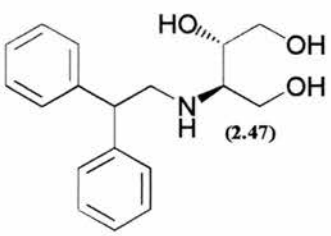
Table 2.10: Ability of 2-amino-2-deoxy-D,L-threitols, * = % turnover of UDP-Gal*p* mutase at 1 mM and ** = MIC for *M. tuberculosis* H37Rv (μg/ml). **N** = compound does not inhibit, **N/A** = data not available.

2.6.3 Conclusions:

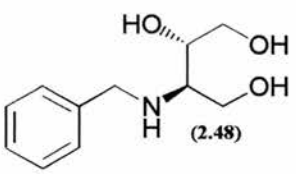
A number of potent inhibitors of have been identified. The best inhibitors of the glycosidases demonstrated IC_{50} values of 9 μM (DNJ), 7 μM (compound **(2.42)**) and 90 μM (compound **(2.74)**) for α-glucosidase, β-glucosidase and L-arabinofuranosidase, respectively. None of the acyclic materials assayed showed significant inhibition of α-mannosidase at a concentration of 1 mM. Compound **(2.48)** was identified as having an ability to inhibit the growth of *M. tuberculosis* H37 Rv. Compounds **(2.47)**, **(2.48)**, **(2.73)** and **(2.78)** showed greater than 90% inhibition of UDP-Gal*p* mutase at a concentration of 1 mM. Experimental methods for all the biological assays performed will be reported in due course.[25]



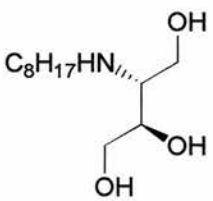
(2.42)



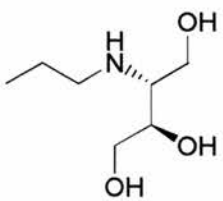
(2.47)



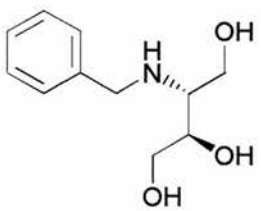
(2.48)



(2.73)



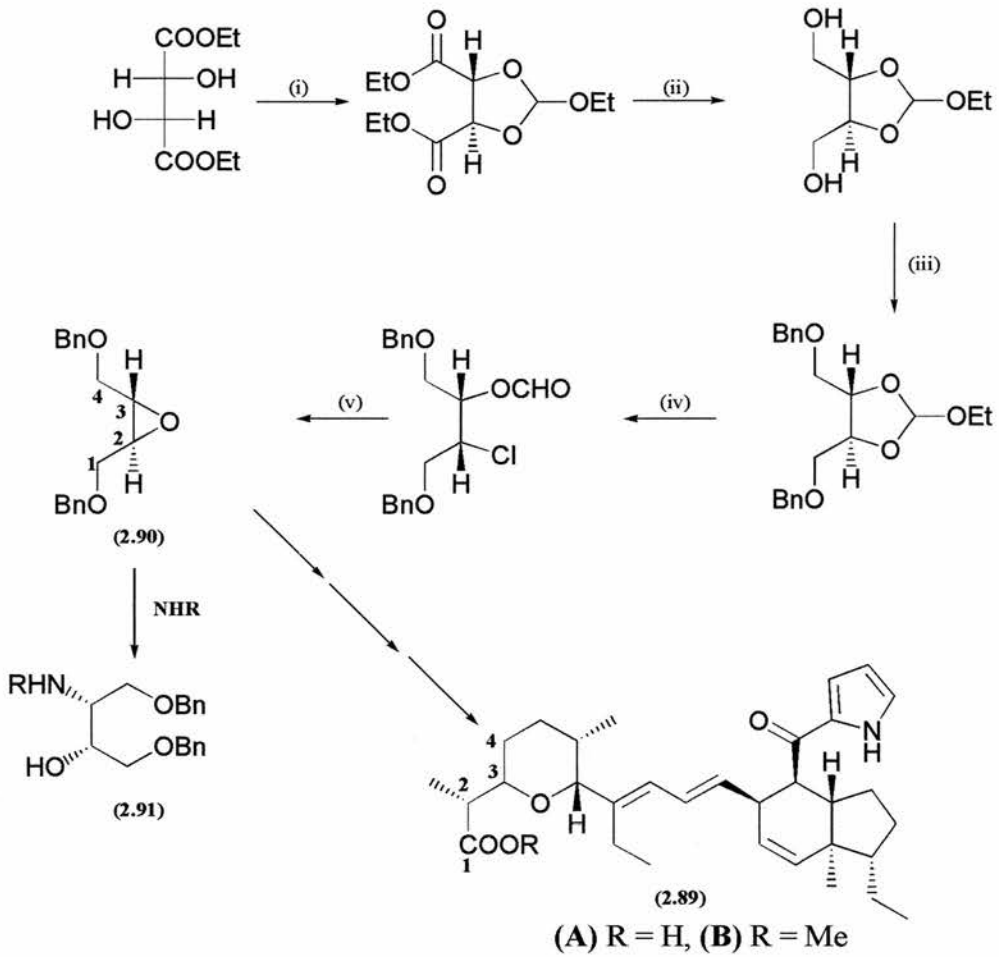
(2.74)



(2.78)

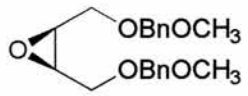
2.7 Asymmetric synthesis of 2-amino-2-deoxy-D,L-threitols:

Since some of the *erythro* or *threo* target compounds demonstrated biological activity then the individual stereoisomers needed to be synthesised in homochiral form. Nicolaou outlined a total synthesis of the ionophore antibiotic X-14547A[1], **(2.89)**[26] **(Scheme 2.16)** via ring opening of the enantiomerically pure epoxide **(2.90)**, which was synthesized as outlined in **Scheme 2.16**.[4,26] Ring opening of **(2.90)** with amines could be used to generate the *erythro* target compounds, **(2.91)** in homochiral form. The *threo* series of target compounds could be developed in a similar manner by repeating the synthesis with D-(-)-diethyl tartrate.



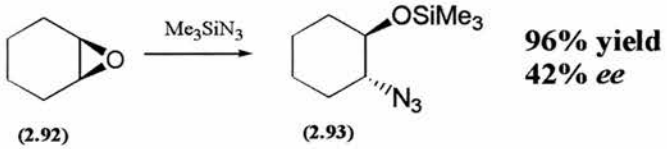
Scheme 2.16: (i) $(EtO)_3CH$, AcOH, $PhCH_3$, reflux; (ii) $LiAlH_4$, THF, 0°C; (iii) BnBr, NaH, THF; (iv) PCl_5 (1.2 mol eq), DCM, 0°C; (v) K_2CO_3 (2.5 mol eq), MeOH, 25°C.

Alternatively, instead of preparing an enantiopure epoxide, synthesis of the desired homochiral amino alcohols could be achieved by asymmetric ring opening (ARO) of the meso epoxide **(2.31)**.



(2.31)

The first examples of enantioselective ring opening of achiral epoxides by nitrogen based nucleophiles was reported by Yamashita and co-workers in 1987.[27,28] Yamashita details the ARO of various epoxides with nucleophiles such as aniline and TMS-N_3 in the presence of Zn(II) and Cu(II) tartrate (**Scheme 2.17**)

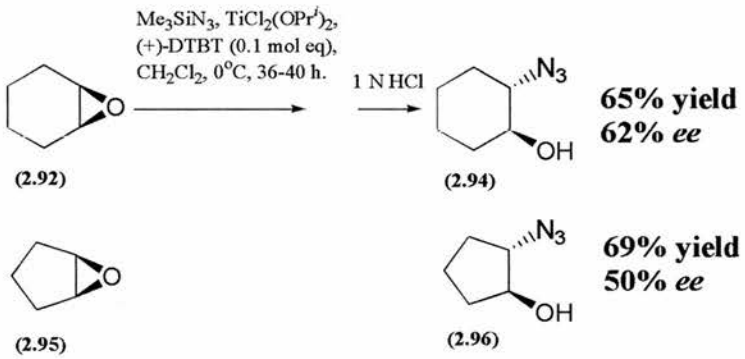


(2.92) **(2.93)**

Zn(II) (2 *R*, 3 *R*)-tartrate (0.1 mol eq),
Benzene, RT, 14 d.

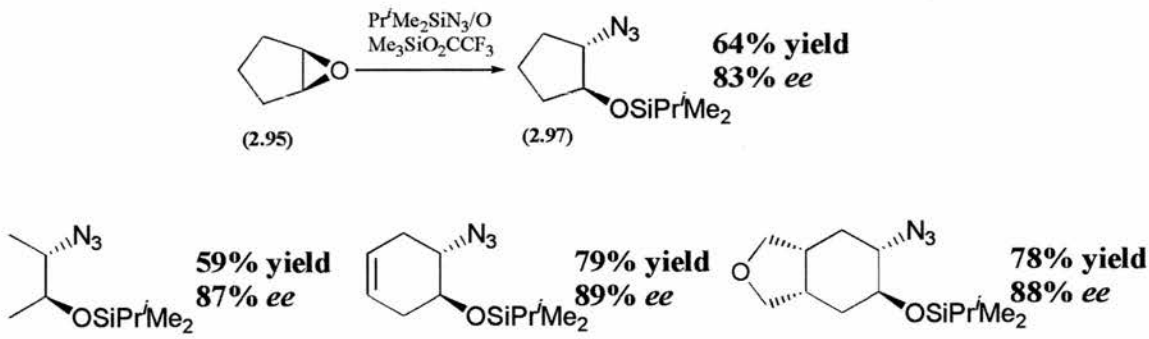
Scheme 2.17: Asymmetric ring opening of meso epoxides with Zn(II) tartrate catalysts.

Following on from this, Sinou decided to repeat the reaction on cyclohexene oxide, using titanium tetraisopropoxide in the presence of chiral diols or aminoalcohols, to generate *trans*-azidocyclohexanol in up to 24% *ee*.[29] Oguni and co-workers subsequently reported that titanium complexes of dialkyl tartrate, such as the complex formed from $TiCl_2(OPr^i)_2$ and di-tert-butyl tartrate (DTBT), could be used for the ARO of meso epoxides with TMS-N_3 **(Scheme 2.18)**[30]



Scheme 2.18: Asymmetric ring opening of meso epoxides with titanium complex catalysts.

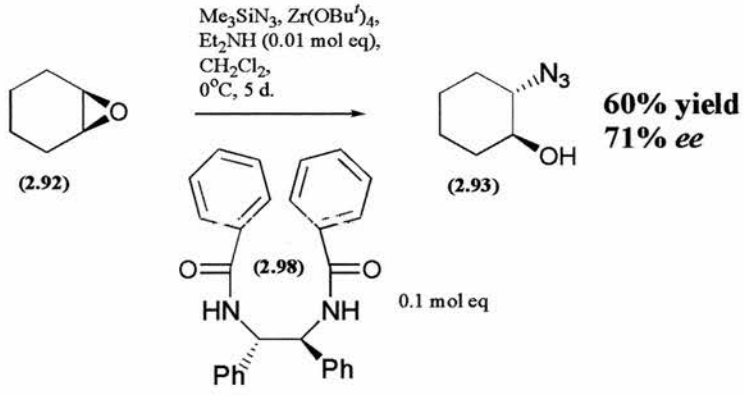
Details of ARO of achiral epoxides with the use of zirconium cataysts was reported by Nugent in 1992.[31] Nugent used zirconium cataysts, prepared from $Zr(OBu^t)_4$ and the tetradentate C_3 symmetric ligand (*S,S,S*)-triisopropanolamine. Reaction of cyclohexene oxide **(2.92)**, TMS-N_3, the zirconium catalyst (0.1 mol eq), and a trace of trimethylsilyl trifluoroacetate in 1,2-DCB (at 25 °C for 18 h) resulted in the generation of the desired azido alcohol **(2.94)**, in 86% *ee*. Enantioselectivity of the ARO was bettered by lowering the reaction temperature and by the use of bulkier azido nucleophiles, such as $Pr^iMe_2SiN_3$. Higher *ee's* were observed with various epoxides. **(Scheme 2.19)**



Scheme 2.19: Asymmetric ring opening of meso epoxides with zirconium catalysts.

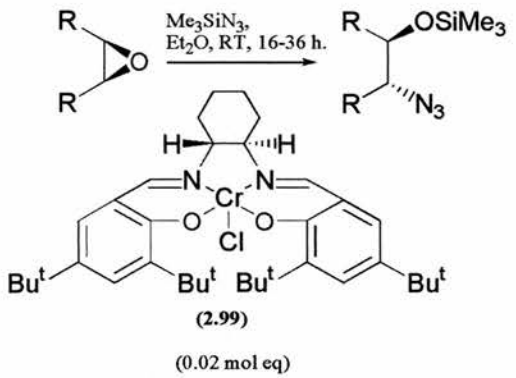
Adolffson and Moberg used a similar theme in their ARO of cyclohexene oxide, they employed the use of titanium and zirconium complexes of bis-picolinic amides as catalysts in the ring opening of **(2.92)** with TMS-N_3.[32] Optimum conditions for the ARO were reported

after using a catalyst prepared from ligand **(2.98)**, $Zr(OBu^t)_4$ and a trace of a secondary amine.



Scheme 2.20: Asymmetric ring opening of meso epoxides with titanium and zirconium complex as catalysts.

The most effective procedure for the ARO of meso epoxides reported to date involves the use of (salen)Cr(III) complexes, such as **(2.99)** developed by Jacobsen and co-workers.[33] Apart from good percentage yields (65-90%) and excellent *ee's* (81-98%), the procedure is highly efficient in the use of the catalyst.



Scheme 2.21: Asymmetric ring opening of meso epoxides with Jacobsen's Cr(III) catalyst.

This procedure developed by Jacobsen was used on a variety achiral epoxides (**Table 2.11**).

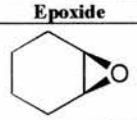
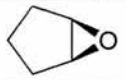
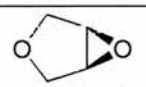
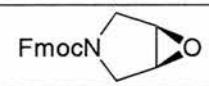
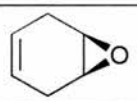
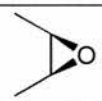
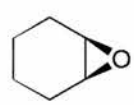
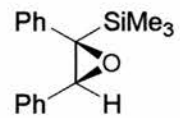
Epoxide	Time/ h	Yield/ %	*ee*/ %
	18	80	88
	28	80	94
	18	80	98
	36	80	95
	16	90	95
	46	72	81
	30	65	82

Table 2.11: Achiral epoxides used in the Jacobsen ARO with Cr(III) catalysts.

Jacobsen and co workers also showed that the Cr(III) catalyst **(2.99)** could be recovered and reused in a solvent free process (**Table 2.12**) Upon purification of the product by distillation, the remaining catalyst can be treated with more TMS-N_3 and epoxide, and the process repeated several times.



(2.92)

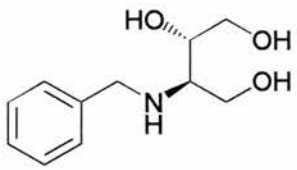


(2.100)

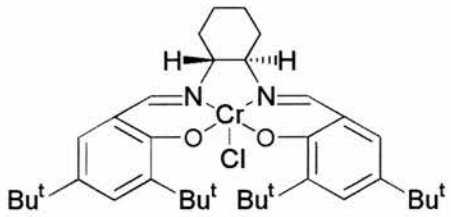
(2.101)

Cycle	Epoxide	Time/ h	Yield/ %	*ee*/ %
1	**(2.92)**	18	86	84
2	**(2.92)**	21	88	87
3	**(2.92)**	20	91	88
4	**(2.100)**	4	81	94
5	**(2.101)**	18	75	83

Table 2.12: Quantitative recovery of catalyst **(2.99)** in a solvent free atmosphere.



(2.48)



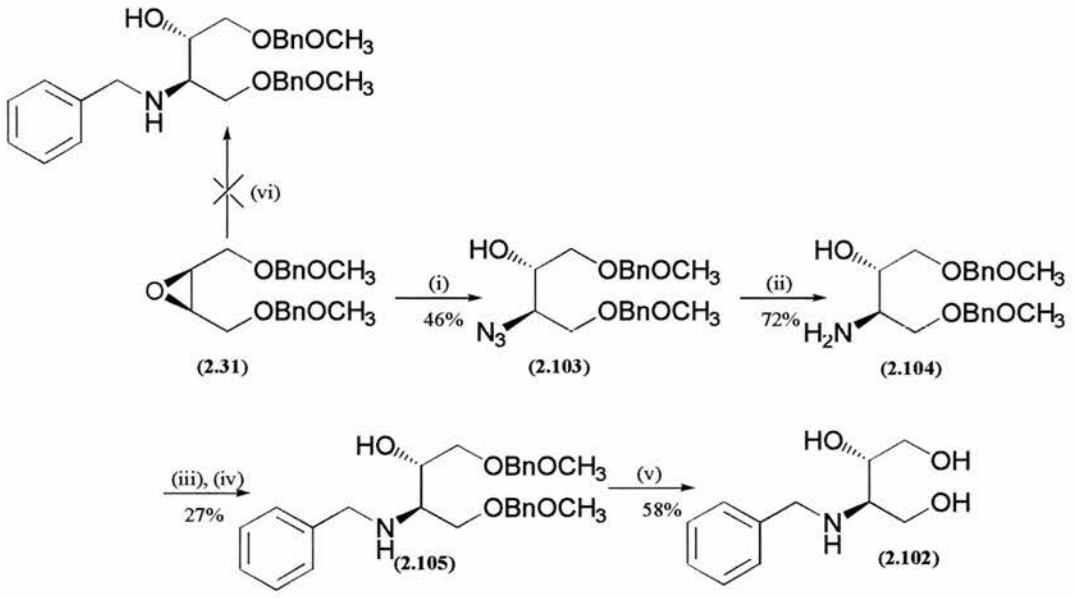
(2.99)

Jacobsen and Leighton have also used other Cr(III) complexes to prepare an important precursor for prostaglandin synthesis.[34]

Given that Cr(III) complexes have successfully been used in the synthesis of near homochiral azido alcohols, it was deemed appropriate to employ (salen)Cr(III) catalysts in an attempt to synthesise 2-benzylmino-2-deoxy-L-threitol **(2.102)**. The racemic moiety **(2.48)** was the most biologically active of the open chain amino alditols so synthesis of the individual enantiomers is required. The proposed synthesis of 2-benzylmino-2-deoxy-L-threitol **(2.102)** is shown in **Scheme 2.22**.

2.8 Preparation of 2-benzyalmino-2-deoxy-L-threitol (2.102):

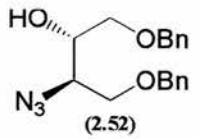
Certain reactions utilised in the 2-benzyalmino-2-deoxy-L-threitol synthetic pathway have been accounted for in the preparation of the racemic *threo* and *erythro* amino alcohols, previously discussed in section **2.3.2** and **2.4.1**. The first step in the pathway involved ARO of the meso epoxide, 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-erythritol **(2.31)** in the presence of Jacobsen's Cr(III) catalyst {(*R,R*)-*N,N'*-bis(3,5-di-*tert*-butyl-salicylidene)-1,2-cyclohexane-diaminochromium(III) chloride)}.[11,12]



Scheme 2.22: (i) TMS-N_3 (1.05 mol eq), (*R,R*)-*N,N'*-bis(3,5-di-*tert*-butyl-salicylidene)-1,2-cyclohexane-diaminochromium(III) chloride (0.05 mol eq), anhydrous toluene, N_2; (ii) PPh_3 (3 mol eq), THF/H_2O (10:1), 80 °C; (iii) DCE, benzaldehyde (1 mol eq); (iv) Sodium triacetoxyborohydride (1.4 mol eq); (v) DCM/TFA (9:1).

Initially the reaction was performed on a scale of 100 mg epoxide/1 mls of solvent (since the reagents would not dissolve in the absence of a solvent). This proved to be ineffective, however following a tenfold increase in system concentration reactivity was noted to increase although remaining lethargic. The reaction was then repeated a third time with a minimum amount of solvent being used (50 µl of solvent for 300 mg of epoxide), this proved to be the key to the success of the reaction. The ARO was left overnight. When checked by T.L.C the reaction appeared to be 50% complete. The reaction was concentrated following which purification by column chromatography yielded the desired azido alcohol (ν_{max}/cm^{-1} 2099 (N_3), 3436 (OH); *m/z* (ES) 405 (M, 100%); HRMS: Found: 405.2133. $C_{20}H_{29}N_4O_5$ ($M+NH_4$) Requires 405.2138), 2-azido-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-L-threitol **(2.103)** in average yield (46%). This ARO of 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-erythritol **(2.31)** in the presence of Jacobsen's Cr(III) catalyst was repeated with benzylamine. As in the previous reaction, concentration of the reaction mixture was varied although the reaction, as judged by T.L.C., was unsuccessful.

Azide reduction of **(2.103)** was attempted by using of a well established literature procedure.[35] Disappearance of the azido starting material was observed on T.L.C., however, after work up and purification only side products and reagents were seen to remain. As a result, a second, less harsh azide reduction procedure[36] was attempted on a previously synthesised azide {2-azido-1,4-di-*O*-benzyl-2-deoxy-D,L-threitol **(2.52)**}.



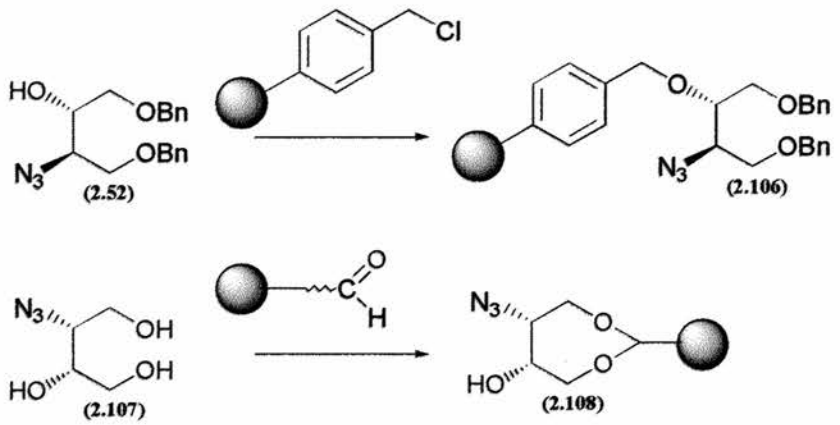
(2.52)

This PPh_3 azide reduction worked well, and was therefore repeated on the enantioenriched azide **(2.103)**. This procedure generated the protected amino alcohol **(2.104)** in good yield (72%). Azide reduction was confirmed by HRMS and IR-spectroscopy (ν_{max}/cm^{-1} 3369 (NH and OH); *m/z* (ES) 362 (M+1, 100%); HRMS: Found: 362.1968 $C_{20}H_{28}NO_5$ ($M+H^+$) Requires 362.1967). A literature protocol was used for the reductive amination reaction of amino alcohol **(2.104)**,[37] reaction conditions involved the use of sodium triacetoxyborohydride[38] in DCE, which resulted in the formation of the desired benzylamino adduct **(2.105)**. Deprotection of the two PMB protecting groups with a TFA/DCM solution yielded the enantioenriched target material, 2-benzyalmino-2-deoxy-L-threitol **(2.102)**. Confirmation of the formation of the *N*-benzyl linkage was obtained from NMR and HRMS (δ_c (CD_3OD): 50.4, 58.7, 61.8, 64.9, 68.4 (3 x CH_2 and 2 x *C*H), 130.0 (2 C), 130.2, 130.8 (2 C) (5 C, Ar), 132.4 (2 C, 2 x Quat. Ar. C); *m/z* (ES) 212 (M+1, 100%); HRMS: Found: 212.1284 $C_{11}H_{18}NO_3$ ($M+H^+$) Requires 212.1286). Proof of the desired configuration was given by a close agreement in literature values for the optical rotations of the hydrochloride salts {$[\alpha]_D$ +9.6° (*c* 0.3, CH_3OH, lit.[39], $[\alpha]_D$ +12.0° (*c* 0.5, CH_3OH)}. This close agreement in optical rotations suggests that we were successful in generating the desired L-configuration. However the two and a half degree difference in optical rotations values indicates that we obtained an *ee* and not a homochiral product. Compound **(2.102)** will be subjected to various mycobacterial and glycosidase biological assays. The results of which will be reported in due course.

2.9 Conclusions and recommendations for further work:

2.9.1 Combinatorial approach:

Since the biological assays yielded positive results it is of interest to investigate the structure/activity relationship for these acyclic aza sugars. The best way to achieve this would be to apply combinatorial methods on both acyclic materials, in order to generate large libraries of, potential inhibitors. Tripeptides could be used as an initial pilot study, as described in Bols work on 1-azafagomine and isofagomine.[40,41,42] Attachment of the acyclic skeletons to a standard Merrifield resin could be achieved by use of a linker. Ether, **(2.106)** or acetal linkages, **(2.108)** could be used to attach the acyclic backbone to the resin, **(Scheme 2.23)**.



Scheme 2.23: Attachment of acyclic skeletons to a solid support by use of ether and acetal linkages.

Ether linkages are very stable to both acidic and basic conditions, therefore cheap Boc-protected amino acids can be used in the coupling steps instead of the more expensive Fmoc-protected amino acids. Alternatively, an acetal linker could be used, **(2.108)**. Such a linker has been reported in the literature for attachment of carbohydrates to a solid phase. **(Figure 2.11)**.[43]

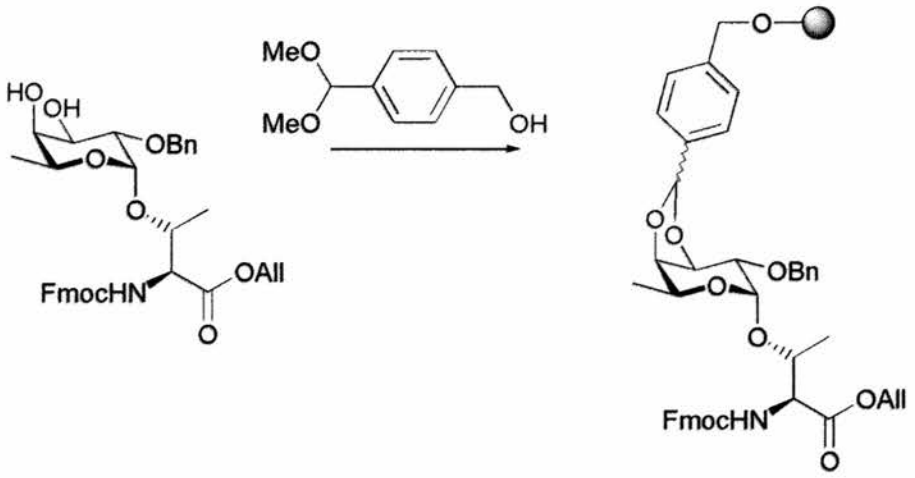


Figure 2.11: Attachment of a glycopeptide on to a solid phase using an acetal linker.

Once attached, reduction of the azide would generate an amine which could subsequently be used in amino acid couplings to generate a tripeptide. Given the importance of the amino functionality for enzyme inhibition, the first amide linkage would have to be reduced to an amine before subsequent amino acid coupling steps could take place. Only relatively hydrophobic (facilitates permeation through the mycobacterial cell wall) amino acids would be used. The amino acids of choice would be glycine, alanine, phenylalanine, serine, leucine and valine. These amino acids would allow the quick generation of a large number of new compounds. Once synthesized, the ether and acetal protected tripeptides could be freed from the solid support by treatment with $SnCl_4$[44] and mild acid,[43] respectively. Using such forcing conditions as these ($SnCl_4$) may give rise to problems, such as racemisation or peptide cleavage. As a result less forceful conditions such as hydrogenolysis could be used so as to ensure the stereochemistry remains unaffected. Cleavage with mild acid would rule out the possibility of racemisation and peptide cleavage. However, under such acid labile conditions the more expensive Fmoc amino acids would have to be used in the coupling steps.

2.10 References

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Chapter 3

Experimental – Acyclic azasugar mimics

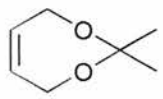
3.1 General Methods

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 Whatman precoated K6F silica gel 60A (layer thickness 0.25 mm) TLC plates, with detection by fluorescence and/or by charring following immersion with a dilute ethanolic solution of sulphuric acid or a dilute ethanolic solution of phosphomolybdic acid (12 g/250 cm^3). 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 Sorbsil C60 40/60A silica gel. Purification of deprotected compounds was achieved by use of a Sephadex LH-20 gel filtration column (2.8 x 73 cm), or by use of Dowex 120 (H^+) ion exchange resin.

Optical rotations were measured at the sodium D-line and at ambient temperature, on a Jasco DIP-370 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. Electrospray mass spectra (ES-MS) and Chemical ionisation mass spectra (CI-MS) were recorded on either a Finnigan MAT900 XLT high resolution double focusing (EB) mass spectrometer or an Autospec double focusing mass spectrometer at either the Mass Spectrometry service at the University of St. Andrews or at the EPSRC National Mass Spectrometry service centre, at the University of Wales, Swansea. Combustion analysis were run in house at the Microanalysis service at the University of St. Andrews and the University of East Anglia. Unless stated otherwise, ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 300 MHz, and 75 MHz respectively. ^1H 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 references 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.

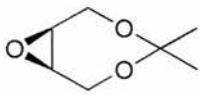
3.2 Synthetic Methods

1,4-*O*-Isopropylidene-(*Z*)-but-2-ene-1,4-diol (2.23)[2]



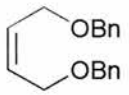
(*Z*)-2-Butene-1,4-diol (21.5 g, 244 mmol) and camphorsulfonic acid (2 mg) were added to 2,2-dimethoxypropane (65 ml). The reaction mixture was immediately separated by fractional distillation to give the known title compound as a clear liquid (25.5 g, 82%), b.p. 142 - 145°C (15 mm Hg); {lit., [2] b.p. 144 - 146°C (15 mm Hg); ν_{max}/cm^{-1} no absorption near 3600; δ_H (300 MHz, $CDCl_3$): 1.41 (6 H, s, 2 x C*H*$_3$), 4.22 (4 H, d, *J* 1.7, 2 x C*H*$_2$), 5.63 (2 H, t, *J* 1.7, 2 x C*H*).

2,3-Anhydro-1,4-isopropylidene-erythritol (2.24)[2]



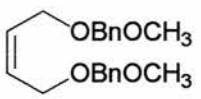
A solution of 1,4-*O*-isopropylidene-(*Z*)-but-2-ene-1,4-diol **(2.23)** (5.04 g, 39.3 mmol) in DCM (25 ml) was added over half an hour to a solution of 3-chloroperoxybenzoic acid (5.4 g, 54.5 mmol) in DCM (25 ml). The mixture was refluxed for 6 hrs. When the reaction had gone to completion, as judged by TLC [hexane/ethyl acetate (2:1)]. The mixture was allowed to cool to room temperature and excess 3-chloroperoxybenzoic acid and 3-chlorobenzoic acid precipitated out of solution. The resulting solid was removed by filtration and the filtrate was washed with 10 % (w/v) aqueous K_2SO_3 solution (2 x 125 ml), saturated $NaHCO_3$ solution (3 x 125 ml), 5% (w/v) NaOH solution (100 ml) and saturated NaCl solution (100 ml). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by fractional distillation to give the known title compound **(2.24)** as a clear liquid (3.1 g, 55 %), b.p. 77 - 79°C (15 mm Hg); {lit., [2] b.p. 78 - 80°C (15 mm Hg)}; ν_{max}/cm^{-1} no absorption near 3600; δ_H (300 MHz, $CDCl_3$): 1.35 (3 H, s, C*H*$_3$), 1.40 (3 H, s, C*H*$_3$), 3.23 (2 H, m, C*H*OC*H*), 4.03 (2 H, dd, *J* = 1.2 and 14.4, C*H*$_2$), 4.07 (2 H, dd, *J* = 1.8 and 14.4 Hz, CH_2).

1,4-Di-*O*-benzyl-(*Z*)-but-2-ene-1,4-diol (2.28)[3-8]



Sodium hydride (5.63 g, 140 mmol of a 45 % w/w dispersion in mineral oil), was slowly added to a solution of (Z)-2-butene-1,4-diol (5.14 g, 58.3 mmol) in DMF (35 ml). Benzyl bromide (16.65 ml, 139.9 mmol) was then added dropwise to the reaction mixture, which was stirred overnight at room temperature. Methanol (20 ml) was then carefully added and after stirring for a few minutes the resulting solution was washed with water (3 x 20 ml) and the product was extracted into diethyl ether (3 x 20 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by column chromatography [toluene/ethyl acetate (25:1)] to yield the known title compound as a clear colourless oil (14.85 g, 95%); ν_{max}/cm^{-1} no absorption near 3600; δ_H (300 MHz, $CDCl_3$): 4.00 (4 H, dd, *J* 1.2 and 5.1, 2 x CH_2OBn), 4.42 (4 H, s, 2 x OCH_2Ph), 5.73 (4 H, dd, *J* 5.1 and 1.2, 2 x *CHCH*), 7.19 - 7.27 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$) 65.9 (2 C), 72.3 (2 C) (4 C, 2 x CH_2Ph and 2 x $CHCH_2$), 127.8 (3 C), 128.0 (3 C), 128.6 (3 C), 129.7 (3 C) (12 C, 2 x *C*H of olefin and 10 Ar C) 138.5 (2 C, 2 x Quat Ar C); MALDI-TOF (+ve): *m/z* 291.1 $(M+Na)^+$, ($C_{18}H_{20}O_2Na$ requires *m/z* 291.1).

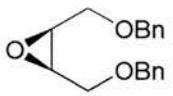
1,4-Di-*O*-(4-methoxybenzyl)-(*Z*)-but-2-ene-1,4-diol (2.29)[9]



Sodium hydride (5.6 g, 140 mmol of a 45 % w/w dispersion in mineral oil), was slowly added to a solution of (*Z*)-2-butene-1,4-diol (5.1 g, 58.3 mmol) in DMF (35 ml). Methoxybenzyl chloride (16.7 ml, 140 mmol) was then added dropwise to the reaction mixture, which was stirred overnight at room temperature. Methanol (20 ml) was then added and after stirring for a few minutes the resulting solution was washed with water (3 x 20 ml) and the product was extracted into diethyl ether (3 x 20 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by column chromatography [Hexane/ethyl acetate (10:1)] to yield the known title compound as a clear colourless oil (3.6 g, 65%). δ_H ($CDCl_3$): 3.81 (6 H, s, 2 x OCH_3), 4.05 (4 H, d, *J* 5.0, 2 x $CHCH_2$), 4.44 (4 H, s, 2 x CH_2Ph), 5.79 – 5.81 (2 H, m, 2 x *CH*), 6.88 – 6.91, 7.26 – 7.29 (8

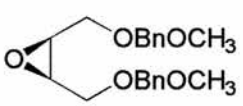
H, 2 x m, Ar); δ_C ($CDCl_3$): 55.3 (2 C, 2 x OCH_3), 65.5 (2 C), 72.0 (2 C) (4 C, 2 x $CHCH_2$ and 2 x $CH_2C_6H_5$), 113.9 (2 C, Ar), 129.5 (5 C) , 129.7 (3 C) (8 C, 2 x *C*H and 6 x Ar C), 130.4 (2 C), 159.5 (2 C) (4 C, 4 x quat. Ar C); *m/z* (ES) 346 ($M+NH_4^+$, 100%); HRMS: Found: 346.2021 $C_{20}H_{28}NO_4$ ($M+NH_4^+$) Requires 346.2018.

2,3-Anhydro-1,4-di-*O*-benzyl-erythritol (2.30) [8,10]



3-Chloroperoxybenzoic acid (4.12 g, 41.9 mmol) was added to a solution of 1,4-di-*O*-benzyl-(*Z*)-but-2-ene-1,4-diol **(2.28)** (6.1 g, 22.7 mmol) in dry DCM (40 ml) and the mixture was stirred overnight at room temperature. During this time complete disappearance of **(2.28)** occurred, as judged by TLC [hexane/ethyl acetate (8:1)] along with precipitation of 3-chlorobenzoic acid. Solids were removed by filtration and the filtrate was successively washed with 10% w/v K_2SO_3 solution (2 x 25 ml), saturated $NaHCO_3$ solution (3 x 25 ml), 5% NaOH solution (20 ml) and saturated aq. NaCl solution (20 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by column chromatography [hexane/ethyl acetate (8:1)] to yield the known title compound **(2.30)** as a clear oil (4.3 g, 67%); ν_{max}/cm^{-1} 740, 840, 1080 (COC); δ_H (300 MHz, $CDCl_3$): 3.19 (2 H, m, C*H*OC*H*), 3.46 (2 H, dd, *J* 11.4 and 6.3, *H*-1a and *H*-4a), 3.61 (2 H, dd, *J* 11.4 and 3.6, *H*-1b and *H*-4b), 4.44 (2 H, d, *J* 12.1, OCH_2Ph), 4.54 (2 H, d, *J* 12.1, OCH_2Ph), 7.23 - 7.29 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 54.5 (2 C), 68.1 (2 C), 73.3 (2 C), 127.9 (6 C, Ar), 128.6 (4 C, Ar), 137.9 (2 C, 2 x Quat. Ar C); MALDI-TOF (+ve): *m/z* 307.1 $(M+Na)^+$, ($C_{18}H_{20}O_3Na$ requires *m/z* 307.1).

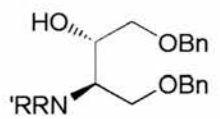
2,3-Anhydro-1,4-di-*O*-(4-methoxybenzyl)-erythritol (2.31)



3-Chloroperoxybenzoic acid (155 mg, 0.9 mmol) was added to a solution of 1,4-di-*O*-(4-methoxybenzyl)-(*Z*)-but-2-ene-1,4-diol **(2.29)** (200 mg, 0.6 mmol) in dry DCM (3 ml) and the mixture was stirred overnight at room temperature. During this time complete disappearance of the starting material occurred, as judged by TLC [hexane/ethyl acetate

(3:1)] along with precipitation of 3-chlorobenzoic acid. Solids were removed by filtration and the filtrate was successively washed with 10% w/v aq. K_2SO_3 solution (2 x 25 ml), saturated aq. $NaHCO_3$ solution (3 x 25 ml), 5% aq. NaOH solution (20 ml) and saturated aq. NaCl solution (20 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by column chromatography [hexane/ethyl acetate (3:1)] to yield the *title compound* as a white solid (170 mg, 81%); mp 54 - 56° C (Pet. ether); (Found: C, 69.61; H, 6.94. $C_{20}H_{24}O_5$. Requires: C, 69.75; H, 7.02%); δ_H ($CDCl_3$): 3.22 – 3.24 (2 H, m, 2 x C*H*), 3.47 – 3.52 (2 H, m, CH_2), 3.66 (2 H, dd, *J* 11.2 and 3.9, CH_2), 4.44, 4.54 (4 H, 2 x d, *J* 11.5, 2 x OCH_2Ph), 6.56 – 6.89 (4 H, m, Ar), 7.24 - 7.27 (4 H, m, Ar); δ_c ($CDCl_3$), 54.5 (2 C, 2 x O*C*H$_3$), 55.3 (2 C, 2 x *C*H), 67.8 (2 C), 72.9 (2 C) (4 C, 2 x *C*H_2Ph and 2 x CH*C*H_2), 114.0 (4 C, Ar), 129.6 (4 C, Ar), 130.0 (2 C, 2 x quat. Ar. C), 159.6 (2 C, 2 x quat. Ar. C).

General procedure for the generation of *N*-substituted 2-amino-1,4-di-*O*-benzyl-2-deoxy-D,L-threitol derivatives (2.32-2.38)



R = $C_6H_5CH_2$-, R' = H **(2.32)**, R = C_8H_{17}-, R' = H **(2.33)**, R = HOC_2H_4-, R' = H **(2.34)**, R = C_3H_5-, R' = H **(2.35)**, R, R' = -$CH_2(CH_2)_3CH_2$- **(2.36)**, R, R' = -$CH_2CH_2OCH_2CH_2$- **(2.37)**, R = $(C_6H_5)_2CHCH_2$-, R' = H **(2.38)**.

2,3-Anhydro-1,4-di-*O*-benzyl-erythritol **(2.30)** (51.6 mg, 45 mmol), amine (96 mmol) and lithium perchlorate (195 mmol, 39 µl of a 5 M solution in diethyl ether) were added to 1,4-dioxane (2 ml) under a nitrogen atmosphere. The reaction mixtures were heated to 105°C, and the progress of the reactions was followed by TLC. On completion the resulting solutions were diluted with DCM (100 ml) and washed successively with water (2 x 100 ml) and saturated aq. $NaHCO_3$ solution (1 x 100 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting materials were purified by column chromatography [hexane/ethyl acetate] to yield the *title compounds*.

R = $C_6H_5CH_2$-, R' = H (2.32)

Clear oil (98 mg, 61%); ν_{max}/cm^{-1} 2725 (NH), 3400 (OH); δ_H (300 MHz, $CDCl_3$): 2.95 (1 H, q, *J* 5.0, C*H*NH-), 3.45 - 3.91 (7 H, m, 3 x CH_2 and C*H*OH), 4.45 - 4.60 (4 H, m, 2 x OCH_2Ph), 7.22 - 7.42 (15 H, m, Ar); δ_c (75 MHz, $CDCl_3$): 51.9, 58.4, 68.3, 69.5, 71.7, 73.4, 73.5, 127.4 (2 C), 127.8 (2 C), 127.9 (3 C), 127.9 (3 C), 128.5 (3 C), 128.6 (2 C) (15 C, Ar), 138.1, 138.3, 139.5 (3 x Quat Ar C); MALDI-TOF (+ve): *m/z* 392.2 $(M+H)^+$, ($C_{25}H_{31}NO_3$ requires *m/z* 392.2); *m/z* (CI) 392 ($M+H^+$, 100%); HRMS: Found: 392.2225 $C_{25}H_{30}NO_3$ ($M+H^+$) Requires 392.2225.

R = C_8H_{17}-, R' = H (2.33)

Clear oil (231 mg, 58 %); ν_{max}/cm^{-1} 3050 (NH), 3350 (OH); δ_H (300 MHz, $CDCl_3$): 0.85 - 0.91 (3 H, m, C$H_3$$CH_2$-), 1.26 - 1.43 (12 H), 2.44 - 2.82 (3 H), 3.41 - 3.73 (5 H), 4.48 - 4.58 (4 H, m, 2 x CH_2Ph), 7.25 - 7.36 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$); 14.1, 22.6, 27.2, 29.3, 29.5, 30.5, 31.8, 48.0, 59.0, 68.8, 69.6, 71.9, 73.3, 73.5, 127.7 (2 C), 127.8 (2 C), 127.9 (4 C), 128.5 (2 C) (10 C, Ar), 138.2, 138.4 (2 x Quat Ar C); *m/z* (CI) 414 ($M+H^+$, 100%); HRMS: Found: 414.3005 $C_{26}H_{40}NO_3$ ($M+H^+$) Requires 414.3008.

R = CH_2CH_2OH, R' = H (2.34)

Oil (200 mg, 77.5%); ν_{max}/cm^{-1} 3000 (NH), 3400 (OH); δ_H (300 MHz, $CDCl_3$): 2.67 - 2.95 (6 H), 3.44 - 3.81 (7 H), 4.49 (2 H, s, OCH_2Ph), 4.52, 4.53 (2 H, d, *J* 12.0, OCH_2Ph), 7.26 - 7.37 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 49.6, 58.3, 61.3, 69.7, 70.3, 71.7, 73.5 (2 C), 127.9 (2 C), 127.9 (2 C), 128.0 (2 C), 128.6 (2 C), 128.6 (2 C) (10 C, Ar), 137.9, 138.2 (2 x Quat Ar C); MALDI-TOF (+ve): *m/z* 368.2 $(M+Na)^+$, ($C_{20}H_{27}NO_4Na$ requires *m/z* 368.2); *m/z* (CI) 346 ($M+H^+$, 100%); HRMS: Found: 346.2018 $C_{20}H_{28}NO_4$ ($M+H^+$) Requires 346.2018.

R = CH_2CHCH_2-, R' = H (2.35)

Oil (205 mg, 54%); ν_{max}/cm^{-1} 3050 (NH), 3400 (OH); δ_H (300 MHz, $CDCl_3$ + D_2O): 2.84 - 2.90 (1 H), 3.17 - 3.61 (7 H), 3.73 - 3.80 (1 H), 4.44 - 4.58 (4 H, m, 2 x OCH_2Ph), 5.06 - 5.19 (2 H, m, CH_2CH), 5.80 - 5.91 (1 H, m, CH_2C*H*), 7.25 - 7.36 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 50.4, 58.2, 68.6, 69.8, 71.7, 73.4, 73.5, 76.7, 116.5, 127.8 (2 C), 127.9 (4 C), 128.5

(2 C), 128.6 (2 C), 138.1, 138.3 (2 x Quat. Ar C); *m/z* (ES) 342 ($M+H^+$, 100%); HRMS: Found: 342.2066 $C_{21}H_{28}NO_3$ ($M+H^+$) Requires 342.2069.

R, R' = -$CH_2(CH_2)_3CH_2$- (2.36)

Oil (190 mg, 72%); ν_{max}/cm^{-1} 3400 (OH); δ_H (300 MHz, $CDCl_3$): 1.44 -1.62 (6 H), 2.50 - 2.86 (5 H), 3.40 - 3.80 (5 H, 2 x CH_2 and 1 x C*H*), 4.40, 4.44 (2 H, 2 x d, *J* 11.7, CH_2Ph), 4.53, 4.60 (2 H, 2 x d, *J* 12.3, CH_2Ph), 7.26 - 7.37 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 24.5, 26.7, 29.8, 50.2, 65.7, 66.7, 66.8, 71.8, 73.2, 73.5, 76.7, 127.6 (2 C), 127.7 (2 C), 127.9 (2 C), 128.5 (4 C) (10 C, Ar), 138.3, 138.6 (2 x Quat. Ar C); MALDI-TOF (+ve): *m/z* 392.2 $(M+Na)^+$, ($C_{23}H_{31}NO_3Na$ requires *m/z* 392.2); *m/z* (CI) 370 ($M+H^+$, 100%); HRMS: Found: 370.2382 $C_{23}H_{32}NO_3$ ($M+H^+$) Requires 370.2382.

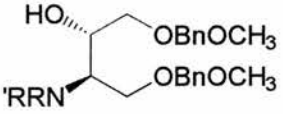
R, R' = -$CH_2CH_2OCH_2CH_2$- (2.37)

Oil (193 mg, 68%); ν_{max}/cm^{-1} 3400 (OH); δ_H (300 MHz, $CDCl_3$ + D_2O): 2.52 - 2.63 (2 H), 2.80 - 2.94 (3 H), 3.43 - 3.72 (9 H), 4.35 - 4.63 (4 H, m, 2 x CH_2Ph), 7.25 - 7.37 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 50.0, 65.4, 66.4, 66.7 (2 C), 67.7 (2 C), 71.2, 73.4, 73.5, 127.7 (2 C), 127.8 (2 C), 127.9 (2 C), 128.0 (2 C), 128.5, 128.6 (10 C, Ar), 138.4 (2 C, 2 x Quat. Ar C). MALDI-TOF (+ve): *m/z* 394.2 $(M+Na)^+$, ($C_{22}H_{29}NO_4Na$ requires *m/z* 394.2); *m/z* (CI) 372 ($M+H^+$, 100%); HRMS: Found: 372.2174 $C_{22}H_{30}NO_4$ ($M+H^+$) Requires 372.2174.

R = $(C_6H_5)_2CHCH_2$-, R' = H (2.38).

Syrup (362 mg, 71 %); δ_H (300 MHz, $CDCl_3$): 2.93 (1 H, d, *J* 4.9, C*H*NH), 3.20 (1 H, m, C*H*OH), 3.40 – 3.70, 4.14 – 4.20 (7 H, 2 x m, 2 x CH_2, CH_2NH and C*H*$(Ph)_2$), 4.44 – 4.55 (4 H, m, 2 x CH_2Ph), 7.20 – 7.40 (20 H, m, Ar); δ_c (75 MHz, $CDCl_3$) 51.9, 52.8, 59.3, 69.0, 69.7, 71.7, 73.4, 73.5 (5 x *C*H_2 and 3 x *C*H),126.6, 126.8, 127.8 (2 C), 127.9 (2 C), 128.0 (2 C), 128.2 (2 C), 128.3 (2 C), 128.5 (2 C), 128.6 (2 C), 128.7 (2 C), 128.8 (2 C) (20 C, Ar), 138.2, 138.4, 142.8, 143.2 (4 x quat. Ar C); *m/z* (CI) 482 ($M+H^+$, 100%); HRMS: Found: 482.2695. $C_{32}H_{36}NO_3$ ($M+H^+$) Requires 482.2695.

General procedure for the generation of *N*-substituted 2-amino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol derivatives (2.39, 2.40)



To a solution of 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-erythritol **(2.31)** (100 mg, 0.3 mmol) in anhydrous 1,4-dioxane (1 ml), was added lithium perchlorate solution (136 µl of a 5 M solution in diethyl ether, 0.6 mmol) and amine (82 mg, 0.76 mmol). The reaction mixtures were heated to 105°C with continuous stirring until TLC [Hexane – EtOAc, (1:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with equal volumes H_2O, 5 % aq. acetic acid and 10 % aq. Na_2CO_3. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc) gave *the title compound*s **(2.39)** and **(2.40)**.

R = $C_6H_5CH_2$-, R' = H (2.39)

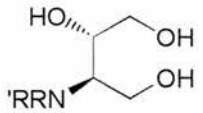
Syrup (70 mg, 52 %); δ_H (300 MHz, $CDCl_3$): 2.75 (1 H, br s, O*H*), 2.87 (1 H, q, *J* 4.5, C*H*NH), 3.41 – 3.62 (4 H, m, 2 x CH_2), 3.70, 3.85 (2 H, d, *J* 13.2, CH_2), 3.74 – 3.83 (7 H, m, 2 x OCH_3, C*H*OH), 4.40 – 4.46 (4 H, m, 2 x CH_2Ph), 6.85 – 6.88, 7.20 – 7.31 (13 H, 2 x m, Ar); δ_c (75 MHz, $CDCl_3$), 51.9, 55.3, 58.3 (4 C, *C*HNH, 2 x O*C*H$_3$ and *C*H$_2$NH), 68.5, 70.1, 71.4, 73.0, 73.1 (4 x *C*H$_2$ and *C*HOH), 113.9 (2 C), 114.0 (2 C) (4 C, Ar), 127.2 (2 C), 128.0, 128.3 (3 C), 128.5 (3 C), 129.5 (3 C), 129.6, 130.2, 130.5, (Ar), 140.4 (2 C, 2 x quat. Ar C), 159.4 (2 C, 2 x quat. Ar C); *m/z* (CI) 452 ($M+H^+$, 100%); HRMS: Found: 452.2436. $C_{27}H_{34}NO_5$ ($M+H^+$) Requires 452.2424.

R = $(C_6H_5)_2CHCH_2$-, R' = H (2.40).

Syrup (363 mg, 75 %); δ_H (300 MHz, $CDCl_3$): 2.81 – 2.90, 3.16, 3.35 – 3.63, 4.14 (9 H, 2 x m, dd, *J* 7.2 and *J* 11.7, t, *J* 7.8, 3 x CH_2 and 3 x C*H*), 3.80, 3.81 (6 H, 2 x s, 2 x OCH_3), 4.13 – 4.45 (4 H, m, 2 x CH_2Ph), 6.85 – 6.89, 7.13 – 7.32 (18 H, m, Ar); δ_c (75 MHz, $CDCl_3$) 51.8, 52.7, 55.3 (2C), 59.3, 68.6, 69.6, 71.3, 73.0, 73.1 (10 C, 5 x *C*H$_2$, 2 x O*C*H$_3$ and 3 x *C*H), 113.9 (9 C), 126.6 (2 C), 126.7 (2 C), 128.1 (2 C), 128.3 (2 C), 128.7 (2 C), 128.8 (5 C), 129.4 (2 C), 129.5 (2 C) (18 C, Ar), 130.2, 130.5 (2 x quat. Ar C), 142.7, 143.1 (2 x quat.

Ar C), 159.5 (2 C, 2 x quat. Ar C); *m/z* (CI) 542 ($M+H^+$, 100%); HRMS: Found: 542.2906. $C_{34}H_{40}NO_5$ ($M+H^+$) Requires 542.2906.

General procedure for the generation of *N*-substituted derivatives of 2-amino-2-deoxy-D,L-threitol (2.41-2.48)



R = C_8H_{17}-, R' = H **(2.42)**, R = HOC_2H_4-, R' = H **(2.43)**, R = C_3H_7-, R' = H **(2.44)**, R, R' = -$CH_2(CH_2)_3CH_2$- **(2.45)**, R, R' = -$CH_2CH_2OCH_2CH_2$- **(2.46)**. R = R' = H **(2.41)**, R = $(C_6H_5)_2CHCH_2$-, R' = H **(2.47)** and R = $(C_6H_5)CH_2$-, R' = H **(2.48).**

To a solution of the respective benzyl ether **(2.33)-(2.39)** (0.33 mmol) methanol (4.5 ml), 10% $Pd(OH)_2$ catalyst (Pearlman's catalyst) (25 mg, 20% mol) and TFA (2 drops) were added. The system was saturated with H_2 gas and stirred at room temperature until the reaction was complete, as judged by TLC [ethyl acetate/ethanol/water (9:2:1)]. The crude material was then filtered through Celite and purified by Sephadex LH-20 gel filtration using methanol as eluent to yield the *title compounds* **(2.41)-(2.48)**.

R = R' = H (2.41)

Oil (23 mg, 57%). * δ_H (300 MHz, D_2O): 3.27 - 3.36 and 3.52 - 3.80 (all *H*, 2 x m); δ_c (75 MHz, D_2O), 54.6, 58.9, 62.8, 68.0; *m/z* (CI) 122 ($M+H^+$, 100%); HRMS: Found: 122.0823 $C_4H_{12}NO_3$ ($M+H^+$) Requires 122.0817.

*Although there are many references[11-15] to the individual isomers of this compound in the literature, no data is supplied for the racemic mixture.

R = C_8H_{17}-, R' = H (2.42)

Oil (49 mg, 64%). δ_H (300 MHz, D_2O): 0.70 (3 H, br s, CH_3), 1.10 (10 H, br s, 5 x CH_2), 1.59 (2 H, br s, CH_2), 3.02 - 3.80 (remaining protons, m); δ_c (75 MHz, D_2O), 13.3, 21.9, 25.4, 25.6, 28.1, 28.2, 31.0, 45.3, 56.4, 60.2, 62.8, 67.7; *m/z* (FAB) 234 ($M+H^+$, 100%); HRMS: Found: 234.2054 $C_{12}H_{28}NO_3$ ($M+H^+$) Requires 234.2069.

R = HOC_2H_4-, R' = H (2.43)

Oil (31 mg, 58%). δ_H (300 MHz, D_2O): 3.24 - 3.84 (all *H*, 2 x m); δ_c (75 MHz, D_2O), 46.9, 56.6, 56.6, 60.6, 62.9, 67.6; *m/z* (ES) 166 ($M+H^+$, 100%); HRMS: Found: 166.1078 $C_6H_{16}NO_4$ ($M+H^+$) Requires 166.1079.

R = C_3H_7-, R' = H (2.44)

Oil (41 mg, 76%). δ_H (300 MHz, D_2O): 0.87 (3 H, t, *J* 6.6, $CH_2CH_2CH_3$), 1.62 (2 H, br s, $CH_2CH_2CH_3$), 2.88 - 3.08 (2 H, m), 3.18 - 3.37 (1 H, m), 3.56 - 3.84 (5 H, m); δ_c (75 MHz, D_2O): 10.1, 19.1, 46.8, 56.4, 60.2, 62.7, 67.7; *m/z* (FAB) 164 ($M+H^+$, 100%); HRMS: Found: 164.1300 $C_7H_{18}NO_3$ ($M+H^+$) Requires 164.1287.

R, R' = -$CH_2(CH_2)_3CH_2$- (2.45)

Oil (22 mg, 36%). δ_H (300 MHz, D_2O): 1.31 - 1.90 (6 H, m, 3 x $CH_2(CH_2)_3CH_2$), 3.08 - 3.98 (10 H, m); δ_c (75 MHz, D_2O), 21.3, 23.4, 23.5, 50.1, 53.5, 55.7, 62.9, 66.2, 68.0; *m/z* (ES) 190 ($M+H^+$, 100%); HRMS: Found: 190.1446 $C_9H_{20}NO_3$ ($M+H^+$) Requires 190.1443.

R, R' = -$CH_2CH_2OCH_2CH_2$- (2.46)

Oil (39 mg, 61%). δ_H (300 MHz, D_2O): 3.23 - 4.01 (all *H*, m); δ_c (75 MHz, D_2O), 49.0, 51.5, 55.6, 62.8, 64.1, 64.2, 66.1, 68.2; *m/z* (FAB) 192 ($M+H^+$, 100%); HRMS: Found: 192.1237 $C_8H_{18}NO_4$ ($M+H^+$) Requires 192.1236.

R = $(C_6H_5)_2CHCH_2$-, R' = H (2.47).

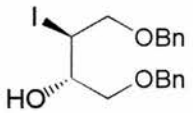
Oil (195 mg, 72%). δ_H (300 MHz, D_2O): 3.32 - 3.98 and 4.31 – 4.84 (9 H, 2 x m), 7.34 – 7.43 (10 H, Ar); δ_c (75 MHz, D_2O): 48.0, 50.0 (*C*H), 58.2, 62.8 (*C*H), 64.4, 67.2, 127.4, 127.6, 127.8 (2 C), 128.1 (2 C), 129.0 (2 C), 129.3 (2 C) (10 C, Ar), 139.7, 140.2 (2 x Quat. Ar C); *m/z* (CI) 302 ($M+H^+$, 100%); HRMS: Found: 302.1753 $C_{18}H_{24}NO_3$ ($M+H^+$) Requires 302.1756.

R = $C_6H_5CH_2$-, R' = H (2.48)

To a solution of 2-benzylamino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol **(2.39)** (591 mg, 1.3 mmol) in DCM (7.2 ml), was added TFA (800 μl). The reaction mixture was

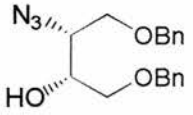
allowed to stir until TLC [EtOAc – EtOH, (2:1)] showed the reaction to be complete. The mixture was concentrated and coevaporated with IPA (to remove any remaining acid) to yield 2-benzylamino-2-deoxy-D,L-threitol **(2.48)** as a syrup (249 mg, 90%); δ_H (CD_3OD): 3.78 – 4.78 (8 H, 3 x m, 3 x CH_2 and 2 x C*H*), 7.84 - 7.94 (5 H, Ar); δ_c (CD_3OD): 50.4, 58.7, 61.8, 64.9, 68.4 (3 x CH_2 and 2 x C*H*), 130.0 (2 C), 130.2, 130.8 (2 C) (5 C, Ar), 132.4 (Quat. Ar. C); *m/z* (ES) 212 ($M+H^+$, 100%); HRMS: Found: 212.1286 $C_{11}H_{18}NO_3$ ($M+H^+$) Requires 212.1286.

1,4-Di-*O*-benzyl-2-deoxy-2-iodo-D,L-threitol (2.49)[16]



To a solution of 2,3-anhydro-1,4-di-*O*-benzyl-erythritol **(2.30)** (206 mg, 0.72 mmol) in dry 1,4-dioxane (20 ml), was added iodine (1.9 g, 7.5 mmol, 10 eq) and diisopropylamine (0.24 ml, 1.8 mmol, 2.5 eq). The reaction mixture was heated under reflux overnight. The resulting solution was diluted in DCM (100 ml) and washed with 10% $Na_2S_2O_3$ solution (2 x 100 ml) and saturated aq. $NaHCO_3$ solution (2 x 100 ml). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*, the resulting oil was purified by column chromatography [hexane/ethyl acetate (7:1)] to yield the known title compound **(2.49)** as a clear oil (200 mg, 55%); ν_{max}/cm^{-1} 3500 (OH); δ_H (300 MHz, $CDCl_3$): 2.62 (1 H, d, *J* 4.8 Hz, O*H*), 3.38 - 3.44 (1 H, m, C*H*OH), 3.45 - 3.57 (2 H, 2 x dd, *J* 6.0 and 8.7, OCH_2CHOH), 3.83 - 3.95 (2 H, m, OCH_2CHI), 4.42 - 4.48 (1 H, m, C*H*), 4.52 - 4.63 (4 H, m, 2 x PhCH_2), 7.26 - 7.44 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 36.7, 69.9, 73.4 73.6, 73.9, 74.3, 127.9 (2 C), 128.0 (2 C), 128.1 (2 C), 128.6 (2 C), 128.7 (2 C), 137.7, 137.9 (2 x Quat Ar C); *m/z* (CI) 413 ($M+H^+$, 100%); HRMS: Found: 413.0613 $C_{18}H_{22}IO_3$ ($M+H^+$) Requires 413.0613.

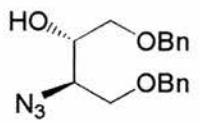
2-Azido-1,4-di-*O*-benzyl-2-deoxy-D,L-erythritol (2.50)



To a solution of 1,4-di-*O*-benzyl-2-deoxy-2-iodo-D,L-threitol **(2.49)** (110 mg, 0.264 mmol) in DMF (1 ml) was added NaN_3 (68 mg, 1.1 mmol, 4 eq) and Bu_4NOTf[17] (34 mg, 0.09

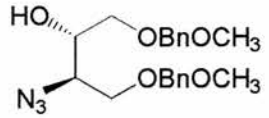
mmol, 0.3 mol equiv.). The reaction mixture was heated to 80°C. The progress of the reaction was followed by TLC [hexane/ethyl acetate (3:1)]. On completion the solution was diluted with an equal volume of DCM and washed with water (2 x 5 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*, the resulting oil was purified by column chromatography [hexane/ethyl acetate (8:1)] to yield the *title compound* **(2.50)** as a clear oil (54 mg, 63%); ν_{max}/cm^{-1} 2093 (N_3), 3500 (OH); δ_H (300 MHz, $CDCl_3$): 2.69 (1 H, br s, CHO*H*), 3.46 - 3.86 (6 H, m, 2 x CH_2 and 2 x C*H*), 4.56, 4.58 (4 H, 2 x s, CH_2Ph), 7.32 - 7.39 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 62.3, 70.2 (2 C), 70.9, 73.6 (2 C) {(4 x CH_2 and 2 x *C*H)}, 127.8, 128.0 (3 C), 128.1, 128.6 (3 C), 128.7 (2 C) {(10 C, Ar)}, 137.8, 137.8 (2 x quat. Ar C); MALDI-TOF (+ve): *m/z* 350.2 $(M+Na)^+$, ($C_{18}H_{21}N_3O_3Na$ requires *m/z* 350.2); *m/z* (CI) 328 ($M+H^+$, 100%); HRMS: Found: 328.1661 $C_{18}H_{22}N_3O_3$ ($M+H^+$) Requires 328.1661.

2-Azido-1,4-di-*O*-benzyl-2-deoxy-D,L-threitol (2.52)



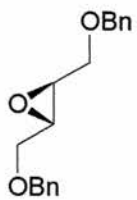
To a solution of 2,3-anhydro-1,4-di-*O*-benzyl-erythritol **(2.30)** (750 mg, 2.6 mmol) in anhydrous DMF (8 ml), was added sodium azide (1.71 g, 26 mmol) and ammonium chloride[18] (1.39 g, 26 mmol). The reaction mixture was heated to 120°C and was then allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave the *title compound* **(2.52)** as a syrup (705 mg, 83 %); ν_{max}/cm^{-1}: 2093 (N_3), 3500 (OH); δ_H ($CDCl_3$): 3.55 (2 H, d, *J* 8.0, CH_2), 3.65 – 3.78 (3 H, m, CH_2 and CHN_3), 3.95 (1 H, m, C*H*OH), 4.57 (4 H, d, *J* 6.0, 2 x CH_2Ph), 7.32 - 7.38 (10 H, m, Ar); δ_c ($CDCl_3$), 62.3 (*C*N_3), 70.5 (2 C, 2 x *C*H_2), 71.1 (*C*HOH), 73.6 (2 C, 2 x *C*H_2Ph), 127.9 (2 C), 128.0 (2 C), 128.1 (2 C), 128.7 (4 C) (10 C, Ar), 137.7, 137.8 (2 x quat. Ar. C); MALDI-TOF (+ve): *m/z* 350.1 $(M+Na)^+$, ($C_{18}H_{21}N_3O_3Na$ requires *m/z* 350.1); *m/z* (ES) 345 ($M+NH_4$, 100%); HRMS: Found: 345.1930. $C_{18}H_{25}N_4O_3$ ($M+NH_4^+$) Requires 345.1927.

2-Azido-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol (2.53)



To a solution of 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-erythritol **(2.31)** (163 mg, 0.47 mmol) in anhydrous DMF (2 ml), was added sodium azide (308 mg, 4.7 mmol) and ammonium chloride[18] (251 mg, 4.7 mmol). The reaction mixture was heated to 120°C and was then allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave the *title compound* **(2.53)** as a syrup (126 mg, 69 %); ν_{max}/cm^{-1}: 2100 (N_3), 3500 (OH); δ_H ($CDCl_3$): 2.45 (1 H, br s, CHO*H*), 3.49 (2 H, d, *J* 5.8, CH_2), 3.60 – 3.77 (3 H, m, CH_2 and C*H*), 3.80 (6 H, s, 2 x OCH_3), 4.49, 4.53 (4 H, 2 x s, 2 x CH_2Ph), 6.87 – 7.10, 7.22 - 7.27 (8 H, 2 x m, Ar); δ_c ($CDCl_3$), 55.3 (2 C, 2 x CH_3), 62.2 (CHN_3), 70.2, 70.6, 70.7 (2 x CH_2 and 1 x C*H*), 73.3 (2 C, 2 x CH_2Ph), 114.0 (2 C), 129.5 (3 C), 129.6 (2 C), 129.9 (8 C, Ar), 130.5 (2 C, 2 x quat. Ar. C), 159.6 (2 C, 2 x quat. Ar. C); *m/z* (ES) 405 (M+NH_4, 100%); HRMS: Found: 405.2133. $C_{20}H_{29}N_4O_5$ (M+NH_4) Requires 405.2138.

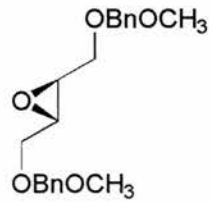
2,3-Anhydro-1,4-di-*O*-benzyl-D,L-threitol (2.55)[15,19,20,21]



3-Chloroperoxybenzoic acid (7.23 g, 25.2 mmol) was added to a solution of 1,4-di-*O*-benzyloxy-2-(*E*)-butene **(2.62)** (2.7 g, 10.1 mmol) in dry DCM (30 ml) and the mixture was stirred overnight at room temperature. During this time complete disappearance of the starting material occurred, as judged by TLC [hexane/ethyl acetate (8:1)] along with precipitation of 3-chlorobenzoic acid. Solids were removed by filtration and the filtrate was successively washed with 10% w/v K_2SO_3 solution (2 x 25 ml), saturated aq. $NaHCO_3$ solution (3 x 25 ml), 5% aq. NaOH solution (20 ml) and saturated aq. NaCl solution (20 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was

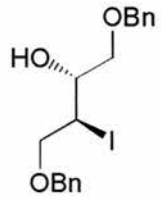
purified by column chromatography [hexane/ethyl acetate (8:1)] to yield the known title compound **(2.55)** as a clear oil (1.9 g, 67%). δ_H ($CDCl_3$): 3.13 (2 H, m, 2 x C*H*), 3.51 (2 H, dd, *J* 11.5 and 5.2, *H*-1a and *H*-4a), 3.76 (2 H, dd, *J* 11.6 and 2.8, *H*-1b and *H*-4b), 4.57 (2 H, d, *J* 12.1, $OCH_2C_6H_5$), 4.61 (2 H, d, *J* 12.1, $OCH_2C_6H_5$), 7.35 - 7.36 (10 H, m, Ar); δ_c ($CDCl_3$), 54.5 (2 C, 2 x *C*O), 69.9 (2 C), 73.4 (2 C) (4 C, 2 x CH_2Ph and 2 x CH*C*H_2), 127.9 (6 C), 128.6 (4 C) (10 C, Ar), 138.1 (2 C, 2 x quat. Ar. C); MALDI-TOF (+ve): *m/z* 307.1 $(M+Na)^+$, ($C_{18}H_{20}O_3Na$ requires *m/z* 307.1).

2,3-Anhydro-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol (2.56)[9,22]



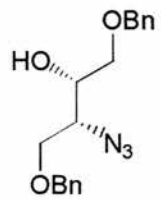
3-Chloroperoxybenzoic acid (2.6 g, 15.2 mmol) was added to a solution of 1,4-di-*O*-(4-methoxybenzyl)-(*E*)-but-2-ene-1,4-diol **(2.63)** (2.7 g, 10.1 mmol) in dry DCM (30 ml) and the mixture was stirred overnight at room temperature. During this time complete disappearance of the starting material occurred, as judged by TLC [hexane/ethyl acetate (8:1)] along with precipitation of 3-chlorobenzoic acid. Solids were removed by filtration and the filtrate was successively washed with 10% w/v aq. K_2SO_3 solution (2 x 25 ml), saturated aq. $NaHCO_3$ solution (3 x 25 ml), 5% aq. NaOH solution (20 ml) and saturated aq. NaCl solution (20 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by column chromatography [hexane/ethyl acetate (8:1)] to yield the title compound **(2.56)** as a clear oil (1.9 g, 67%). (Found: C, 69.88; H, 6.92. $C_{20}H_{24}O_5$. Requires: C, 69.75; H, 7.02%); δ_H ($CDCl_3$): 3.13 (2 H, m, 2 x C*H*), 3.51 (2 H, dd, *J* 11.5 and 5.2 , *H*-1a and *H*-4a), 3.76 (2 H, dd, *J* 11.6 and 2.8, *H*-1b and *H*-4b), 4.57 (2 H, d, *J* 12.1, OCH_2Ph), 4.61 (2 H, d, *J* 12.1, OCH_2Ph), 7.35 - 7.36 (10 H, m, Ar); δ_c ($CDCl_3$): 54.5 (2 C, 2 x CH_3), 55.2 (2 C, 2 x *C*H), 69.7 (2 C), 72.9 (2 C) (4 C, 2 x CH_2Ph and 2 x CH*C*H_2), 113.9 (4 C, Ar), 129.6 (4 C) (8 C, Ar), 130.2 (2 C, 2 x quat. Ar. C); 159.5 (2 C, 2 x quat. Ar. C).

1,4-Di-*O*-benzyl-2-deoxy-2-iodo-D,L-erythritol (2.57)[23]



To a solution of 2,3-anhydro-1,4-di-*O*-benzyl-D,L-threitol **(2.55)** (300 mg, 1.06 mmol) in THF (3 mls), was added lithium iodide (226 mg, 1.7 mmol) and acetic acid (0.18 ml, 3.17 mmol). The reaction mixture was allowed to stir until TLC [Hexane – EtOAc, (8:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with equal volumes of 10 % aq. $Na_2S_2O_3$ and 10 % aq. Na_2CO_3. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 8:1) gave the known title compound **(2.57)** as a syrup (351 mg, 80 %); δ_H ($CDCl_3$): 3.08 (1 H, br s, CHO*H*), 3.67 – 3.78, 3.79 – 3.94 (4 H, 2 x dd, 2 x m, 2 x CH_2), 3.94 – 4.02 (1 H, m, C*H*OH), 4.34 – 4.42 (1 H, m, C*H*I), 4.56, 4.58 (4 H, 2 x s, 2 x CH_2Ph), 7.30 - 7.36 (10 H, m, Ar); δ_c ($CDCl_3$): 32.2 (*C*I), 72.8, 73.0, 73.1, 73.3, 73.6 (4 x CH_2 and 1 x *C*H), 127.6 (2 C), 127.7 (2 C), 127.9, 128.1, 128.3 (4 C) (10 C, Ar), 137.3, 137.5 (2 x quat. Ar. C); MALDI-TOF (+ve): *m/z* 435.0 $(M+Na)^+$, ($C_{18}H_{21}IO_3Na$ requires *m/z* 435.0); *m/z* (ES) 430 ($M+NH_4^+$, 100%); HRMS: Found: 430.0875. $C_{18}H_{25}NIO_3$ ($M+NH_4$) Requires 430.0879.

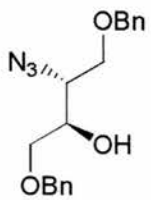
2-Azido-1,4-di-*O*-benzyl-2-deoxy-D,L-threitol (2.58)



To a solution of 1,4-di-O-benzyl-2-iodo-2-deoxy-D,L-erythritol **(2.57)** (341 mg, 0.83 mmol) in anhydrous THF (3.5 ml), was added 18-crown-6 (50 mg) and sodium azide (537 mg, 8.3 mmol). The reaction mixture was allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc,

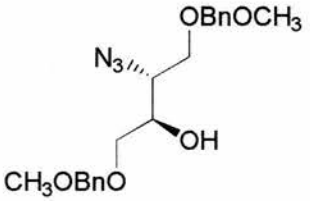
4:1) gave the *title compound* **(2.58)** as a syrup (146 mg, 54 %); ν_{max}/cm^{-1}: 2093 (N_3), 3490 (OH); δ_H ($CDCl_3$): 3.55 (2 H, d, *J* 5.8, CH_2), 3.64 – 3.80 (3 H, m, CH_2 and CH), 3.96 (1 H, br s, CH), 4.55, 4.57 (4 H, 2 x s, 2 x CH_2Ph), 7.31 - 7.37 (10 H, m, Ar); δ_c ($CDCl_3$), 62.2 (CN_3), 70.6 (2 C, CH_2 and CHOH), 71.0 (CH_2), 73.6 (2 C, 2 x CH_2), 127.8 (2 C), 128.0 (2 C), 128.0 (2 C), 128.6 (4 C) (10 C, Ar), 137.4, 137.5 (2 x quat. Ar. C); MALDI-TOF (+ve): *m/z* 350.1 $(M+Na)^+$, ($C_{18}H_{21}N_3O_3Na$ requires *m/z* 350.1); *m/z* (ES) 345 ($M+NH_4^+$, 100%); HRMS: Found: 345.1925. $C_{18}H_{25}N_4O_3$ ($M+NH_4$) Requires 345.1927.

2-Azido-1,4-di-*O*-benzyl-2-deoxy-D,L-erythritol (2.59)



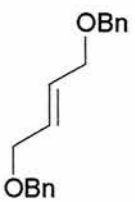
To a solution of 2,3-anhydro-1,4-di-*O*-benzyl-D,L-threitol **(2.55)** (300 mg, 1.06 mmol) in anhydrous DMF (4 ml), was added sodium azide (689 mg, 10.6 mmol) and ammonium chloride[18] (567 mg, 10.6 mmol). The reaction mixture was heated to 120°C and allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave the *title compound* **(2.59)** as a syrup (121 mg, 35 %); ν_{max}/cm^{-1}: 2093 (N_3), 3490 (OH); δ_H ($CDCl_3$): 3.54 (2 H, d, *J* 5.8, CH_2), 3.64 – 3.79 (3 H, m, CH_2 and CHN_3), 3.94 (1 H, m, CHOH), 4.54, 4.56 (4 H, 2 x s, 2 x CH_2Ph), 7.30 - 7.36 (10 H, m, Ar); δ_c ($CDCl_3$), 62.1 (CN_3), 70.0 (2 C, CH_2 and CHOH), 70.7 (CH_2), 73.4 (2 C, 2 x CH_2), 127.5 (2 C), 127.7 (2 C), 127.8 (2 C), 128.3 (2 C), 128.4 (2 C) (10 C, Ar), 137.4, 137.5 (2 x quat. Ar. C); MALDI-TOF (+ve): *m/z* 350.1 $(M+Na)^+$, ($C_{18}H_{21}N_3O_3Na$ requires *m/z* 350.1); *m/z* (CI) 328 ($M+H^+$, 100%); HRMS: Found: 328.1661. $C_{18}H_{22}N_3O_3$ ($M+H^+$) Requires 328.1661.

2-Azido-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-erythritol (2.60)



To a solution of 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol **(2.56)** (484 mg, 1.4 mmol) in anhydrous DMF (5 ml), was added sodium azide (914 mg, 14 mmol) and ammonium chloride[18] (749 mg, 14 mmol). The reaction mixture was heated to 120°C and was then allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave the *title compound* **(2.60)** as a syrup (481 mg, 89 %); ν_{max}/cm^{-1}: 2080 (N_3), 3500 (OH); δ_H ($CDCl_3$): 2.75 (1 H, br s, CHO*H*), 3.54 – 3.56, 3.63 – 3.65, 3.75 – 3.77 (6 H, 3 x m, 2 x CH_2 and 2 x C*H*), 3.79 – 3.80 (6 H, m, 2 x OCH_3), 4.47, 4.50 (4 H, 2 x s, 2 x CH_2Ph), 6.87 – 6.90, 7.22 - 7.28 (8 H, 2 x m, Ar); δ_c ($CDCl_3$), 55.3 (2 C, 2 x *C*H_3), 62.4 (*C*HN_3), 69.8, 70.2, 70.6 (2 x *C*H_2 and 1 x *C*HOH), 73.2 (2 C, 2 x *C*H_2Ph), 114.0 (2 C), 129.4 (2 C), 129.6 (2 C), 129.9 (2 C) (8 C, Ar), 129.9 (2 C, 2 x quat. Ar. C), 159.5, 159.6 (2 x quat. Ar. C); *m/z* (ES) 405 (M+ NH_4^+, 100%); HRMS: Found: 405.2143. $C_{20}H_{29}N_4O_5$ (M+NH_4) Requires 405.2138.

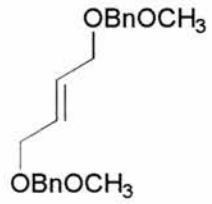
1,4-Di-*O*-benzyl-(*E*)-but-2-ene-1,4-diol (2.62)[19,20,24]



To a stirred solution of benzyl alcohol (238 mg, 2.2 mmol) in anhydrous THF (3 ml) was added crushed potassium hydroxide powder (222 mg, 3.9 mmol), 18-crown-6 (21 mg, 0.08 mmol) and 1,4-dibromo-(*E*)-but-2-ene (214 mg, 1 mmol). The reaction mixture was stirred at room temperature until TLC [Hexane – EtOAc, (9:1)] showed the reaction to be complete. The reaction mixture was then diluted in DCM (200 ml) and washed with an equal volume of water. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced

pressure. Column chromatography (silica gel; Hexane – EtOAc, 9:1) gave the known 1,4-di-*O*-benzyl-(*E*)-but-2-ene-1,4-diol **(2.62)** as a syrup (106 mg, 39 %); δ_H ($CDCl_3$): 4.09 – 4.10 (4 H, m, 2 x CHC*H*$_2$), 4.56 (4 H, s, 2 x C*H*$_2$Ph), 5.92 – 5.97 (2 H, m, 2 x C*H*), 7.32 – 7.38 (10 H, m, Ar); δ_C ($CDCl_3$) 70.0 (2 C), 72.1 (2 C) (4 C, 2 x CH*C*H$_2$ and 2 x *C*H$_2$Ph), 127.8 (2 C), 127.9 (4 C), 128.6 (4 C), 129.7 (2 C) (12 C, 2 x *C*H and Ar), 138.5 (2 C, 2 x quat. C); MALDI-TOF (+ve): *m/z* 291.1 $(M+Na)^+$, ($C_{18}H_{20}O_2Na$ requires *m/z* 291.1).

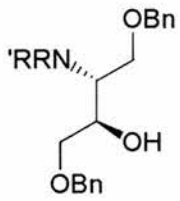
1,4-Di-*O*-(4-methoxybenzyl)-(*E*)-but-2-ene-1,4-diol (2.63)



To a stirred solution of 4-methoxybenzyl alcohol (23 ml, 187 mmol) in anhydrous THF (100 ml) was added crushed potassium hydroxide powder (10.5 g, 187 mmol), 18-crown-6 (100 mg, 0.08 mmol) and 1,4-dibromo-(*E*)-but-2-ene (10 g, 47 mmol). The reaction mixture was stirred at room temperature until TLC [Hexane – EtOAc, (10:1)] showed the reaction to be complete. The reaction mixture was then diluted in DCM (200 ml) and washed with an equal volume of water. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 10:1) gave *1,4-di-O-(4-methoxybenzyl)-(E)-but-2-ene-1,4-diol** **(2.63)** as a syrup (10.7g, 70 %); δ_H ($CDCl_3$): 3.82 (6 H, s, 2 x OC*H*$_3$), 4.01 – 4.02 (4 H, m, 2 x CHC*H*$_2$), 4.45 (4 H, s, 2 x C*H*$_2$Ph), 5.82 – 5.88 (2 H, m, 2 x C*H*), 6.86 – 6.89, 7.25 – 7.26 (8 H, 2 x m, Ar). δ_C ($CDCl_3$) 55.3 (2 C, 2 x O*C*H$_3$), 69.9 (2 C), 71.9 (2 C) (4 C, 2 x CH*C*H$_2$ and 2 x *C*H$_2$C$_6$H$_5$), 113.9 (2 C, Ar), 129.5, 129.7 (10 C, 2 x *C*H and 8 x Ar C); EI-MS (+ve): *m/z* 328 $(M)^+$, ($C_{20}H_{24}O_4$ requires *m/z* 328). EI-MS (+ve): *m/z* 328.1679 $(M)^+$, ($C_{20}H_{24}O_4$ requires *m/z* 328.1675).

*Although this is a new compound, details of the cis/trans mixture have been reported.[22]

General procedure for the generation of *N*-substituted 2-amino-1,4-dibenzyl-2-deoxy-D,L-erythritol derivatives (2.64-2.69)



R = $C_6H_5CH_2$-, R' = H **(2.64)**, R = C_8H_{17}-, R' = H **(2.65)**, R = C_3H_5-, R' = H **(2.66)**, R, R' = -$CH_2(CH_2)_3CH_2$- **(2.67)**, R, R' = -$CH_2CH_2OCH_2CH_2$- **(2.68)**, R = $(C_6H_5)_2CHCH_2$-, R' = H **(2.69)**.

2,3-Anhydro-1,4-di-*O*-benzyl-D,L-threitol **(2.55)** (51.6 mg, 45 mmol), amine (96 mmol) and lithium perchlorate (195 mmol, 39 µl of a 5 M solution in diethyl ether) were added to 1,4-dioxane (2 ml) under a nitrogen atmosphere. The reaction mixtures were heated to 105°C, and the progress of the reactions was followed by TLC. On completion the resulting, solutions were diluted with DCM (200 ml) and washed successively with with water (2 x 10 ml) and saturated aq. $NaHCO_3$ solution (1 x 10 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting materials were purified by column chromatography [hexane/ethyl acetate] to yield the *title compounds*.

R = $C_6H_5CH_2$-, R' = H (2.64)

Colourless oil (100 mg, 73 %). δ_H (300 MHz, $CDCl_3$): 2.97 (1 H, q, *J* 4.7, C*H*NH), 3.00 - 3.05 (1 H, br s, O*H*), 3.59 - 3.64 (4 H, 2 x d, *J* 9.6, *J* 9.9, 2 x CH_2), 3.83 (2 H, s, NCH_2), 3.99 (1 H, q, *J* 5.7, C*H*OH), 4.49, 4.53 (4 H, 2 x s, 2 x CH_2Ph), 7.26 -7.38 (15 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 51.4, 58.3, 68.7, 69.6, 71.9, 73.4, 73.5 (5 x CH_2 and 2 x *C*H), 127.2, 127.9 (6 C), 128.4 (3 C), 128.6 (5 C) (15 C, Ar), 138.2, 138.3, 140.2 (3 x quat. Ar C); MALDI-TOF (+ve): *m/z* 414.2 $(M+Na)^+$, ($C_{25}H_{29}NO_3Na$ requires *m/z* 414.2); *m/z* (CI) 392 ($M+H^+$, 100%); HRMS: Found: 392.2225. $C_{25}H_{30}NO_3$ ($M+H^+$) Requires 392.2225.

R = C_8H_{17}-, R' = H (2.65)

Colourless oil (320 mg, 73 %). δ_H (300 MHz, $CDCl_3$): 0.90 (3 H, t, *J* 6.9, CH_3), 1.21 -1.39 (10 H, m, 5 x CH_2), 1.40 – 1.51 (2 H, m, NCH$_2$CH_2), 2.52 - 2.68 (3 H, m, NCH_2 and O*H*),

2.87 (1 H, q, *J* 6.0, NHC*H*), 3.52 – 3.61 (4 H, m, 2 x CH_2), 3.95 (1 H, q, *J* 6.0, C*H*OH), 4.48 – 4.64 (4 H, m, 2 x CH_2Ph), 7.29 – 7.35 (10 H, Ar); δ_c (75 MHz, $CDCl_3$) 14.1 (*C*H_3), 22.6, 27.3, 29.3, 29.5, 30.3, 31.8, 47.6 (7 x *C*H_2), 59.1 (*C*H), 68.9 (*C*H_2), 69.4 (*C*H), 71.9 (*C*H_2), 73.5 (2 C, 2 x *C*H_2), 127.8 (3 C), 127.9 (3 C), 128.5 (3 C), 128.6 (10 C, Ar), 138.2, 138.3 (2 x quat. Ar C); MALDI-TOF (+ve): *m/z* 436.2 $(M+Na)^+$, ($C_{26}H_{39}NO_3Na$ requires *m/z* 436.2); *m/z* (CI) 414 ($M+H^+$, 100%); HRMS: Found: 414.3008. $C_{26}H_{40}NO_3$ ($M+H^+$) Requires 414.3008.

R = CH_2CHCH_2-, R' = H (2.66)

Oil (46 mg, 38 %). δ_H (300 MHz, $CDCl_3$): 2.72 (1 H, br s, CHO*H*), 2.91 – 3.03 (2 H, 2 x q, *J* 6.0, C*H*OH and C*H*NH), 3.26 – 3.28 (2 H, m, NCH_2), 3.58 (4 H, d, 5.2, 2 x CH_2), 4.49 – 4.54 (4 H, m, 2 x CH_2Ph), 5.07 – 5.23 (2 H, m, CH=CH_2), 5.86 (1 H, m, C*H*=CH$_2$), 7.29 – 7.38 (10 H, Ar); δ_c (75 MHz, $CDCl_3$): 50.0, 58.2, 69.0, 69.6, 71.9, 73.4, 73.5 (5 x *C*H_2 and 2 x *C*H), 116.2, 137.0 (*C*H=CH$_2$ and CH=*C*H$_2$), 127.9 (4 C), 128.6 (6 C) (10 C, Ar), 138.3 (2 C, 2 x quat. Ar C); MALDI-TOF (+ve): *m/z* 364.1 $(M+Na)^+$, ($C_{21}H_{27}NO_3Na$ requires *m/z* 364.1); *m/z* (CI) 342 ($M+H^+$, 100%); HRMS: Found: 342.2069. $C_{21}H_{28}NO_3$ ($M+H^+$) Requires 342.2069.

R, R' = -$CH_2(CH_2)_3CH_2$- (2.67)

Oil (85 mg, 70 %). δ_H (300 MHz, $CDCl_3$): 1.41 -1.50 (2 H, m, $NCH_2CH_2CH_2$), 1.64 – 2.03 (4 H, m, 2 x $NCH_2CH_2CH_2$), 2.69 – 3.06 (5 H, 2 m, 2 x $NCH_2CH_2CH_2$ and NHC*H*), 3.54 – 3.89 (4 H, 2 x m, 2 x $CHCH_2$), 4.25 (1 H, q, *J* 6.0, C*H*OH), 4.49 – 4.52 (4 H, m, 2 x CH_2Ph), 7.29 – 7.35 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 23.9, 25.5 (2 C), 29.9, 52.2, 66.4, 66.6, 68.7, 72.0, 73.4 (2 C), 127.8 (3 C), 127.9 (3 C), 128.5 (2 C), 128.6 (2 C) (10 C, Ar), 138.1, 138.3 (2 x quat. Ar C); MALDI-TOF (+ve): *m/z* 392.2 $(M+Na)^+$, ($C_{23}H_{31}NO_3Na$ requires *m/z* 392.2); *m/z* (CI) 370 ($M+H^+$, 100%); HRMS: Found: 370.2382. $C_{23}H_{32}NO_3$ ($M+H^+$) Requires 370.2382.

R, R' = -$CH_2CH_2OCH_2CH_2$- (2.68)

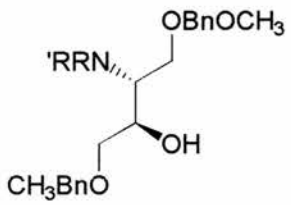
Oil (51 mg, 76 %). δ_H (300 MHz, $CDCl_3$): 2.55 – 2.80 (5 H, 2 x m, 2 x NCH_2 and NC*H*), 3.56 – 3.78 (8 H, 2 x m, 2 x CH_2 and 2 x CH_2OCH_2), 3.95 – 4.00 (1 H, m, C*H*), 4.50 – 4.60

(4 H, m, 2 x CH_2Ph), 7.26 – 7.40 (10 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 50.9 (2 C), 65.1, 67.3, 67.6 (2 C), 69.5, 72.4, 73.4 (2 C), 127.8 (2 C), 127.9 (4 C), 128.5 (4 C) (10 C, Ar), 138.2, 138.3 (2 x quat. Ar C); MALDI-TOF (+ve): *m/z* 394.2 $(M+Na)^+$, ($C_{22}H_{29}NO_4Na$ requires *m/z* 394.2); *m/z* (CI) 372 ($M+H^+$, 100%); HRMS: Found: 372.2174. $C_{22}H_{30}NO_4$ ($M+H^+$) Requires 372.2174.

R = $(C_6H_5)_2CHCH_2$-, R' = H (2.69).

Oil (384 mg, 75 %). δ_H (300 MHz, $CDCl_3$): 2.98, 3.94 (2 H, 2 x q, *J* 5.5, CH_2), 3.27 - 3.55 (2 H, m, CH_2), 3.54 – 3.58 (4 H, m, 2 x C*H* and 1 x CH_2), 4.18 (1 H, t, *J* 7.1, C*H*), 4.41, 4.51 (4 H, 2 x s, CH_2Ph), 7.21 – 7.39 (20 H, m, Ar); δ_c (75 MHz, $CDCl_3$), 51.8, 52.5, 59.4, 69.0, 69.6, 71.8, 73.3, 73.5 (5 x *C*H_2 and 3 x *C*H), 126.6 (2 C), 126.7 (2 C), 127.8 (3 C), 127.9 (2 C), 128.2 (2 C), 128.3 (2 C), 128.6 (3 C), 128.7 (2 C), 128.8 (2 C) (20 C, Ar), 138.2, 138.3, 142.9, 143.3 (4 x quat. Ar C); *m/z* (ES) 482 ($M+H^+$, 100%); HRMS: Found: 482.2690. $C_{32}H_{36}NO_3$ (M+H) Requires 482.2695.

General procedure for the generation of *N*-substituted-2-amino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-erythritol derivatives (2.70, 2.71)



To a solution of 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol **(2.56)** (271 mg, 0.79 mmol) in anhydrous 1,4-dioxane (3 ml), was added lithium perchlorate solution (420 µl of a 5 M solution in diethyl ether, 1.8 mmol) and amine (222 mg, 2.3 mmol). The reaction mixtures were heated to 105°C with continuous stirring until TLC [Hexane – EtOAc, (1:1)] showed the reaction to be complete. The mixture was diluted with DCM (200 ml) and washed with equal volumes of H_2O, 5 % aq. acetic acid and 10 % aq. Na_2CO_3. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc) gave the *title compounds* **(2.70, 2.71)**.

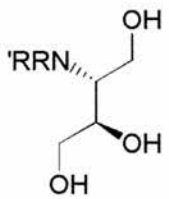
R = $C_6H_5CH_2$-, R' = H (2.70)

Syrup (118 mg, 33 %); δ_H (300 MHz, $CDCl_3$): 2.84 (1 H, m, C*H*NH), 3.50 – 3.58 (4 H, m, 2 x CH_2), 3.71 - 3.78 (8 H, m, 2 x OCH_3 and NHCH_2Ph), 3.86 (1 H, m, C*H*OH), 4.35 – 4.41 (4 H, m, 2 x CH_2Ph), 6.83 – 6.88, 7.19 – 7.25 (13 H, 2 x m, Ar); δ_c (75 MHz, $CDCl_3$), 51.1, 54.4 (2 x O*C*H_3), 54.4, 58.4, 68.3, 69.9, 71.6, 72.5, 72.6 (5 x *C*H_2 and 2 x *C*H), 113.5 (2 C), 126.8 (3 C), 128.2 (2 C), 129.3 (2 C), 129.4 (4 C) (13 C, Ar), 130.3 (NHCH_2*C*), 139.9 (2 C, 2 x quat. Ar C), 159.6 (2 C, 2 x quat. Ar C); *m/z* (ES) 452 ($M+H^+$, 100%); HRMS: Found: 452.2439. $C_{27}H_{34}NO_5$ ($M+H^+$) Requires 452.2437.

R = $(C_6H_5)_2CHCH_2$-, R' = H (2.71)

Syrup (388 mg, 82 %); (Found: C, 75.33; H, 7.30; N, 2.51 $C_{34}H_{39}NO_5$. Requires: C, 75.39; H, 7.26; N, 2.59%); δ_H (300 MHz, $CDCl_3$): 2.80, 3.11 – 3.19, 3.35 – 3.39, 3.78, 4.03 (9 H, 4 x m, t, *J* 7.4, 3 x CH_2 and 3 x C*H*), 3.70 (6 H, s, 2 x OCH_3), 4.23, 4.31 (4 H, 2 x s, 2 x CH_2Ph), 6.73 – 7.21 (18 H, Ar); δ_C (75 MHz, $CDCl_3$): 51.6, 52.2, 55.2 (2 C), 59.2, 68.4, 69.4, 71.3, 72.8, 72.9 (10 C, 2 x *C*H_3, 5 x *C*H_2 and 3 x *C*H), 113.8 (2 C), 126.4 (2 C), 126.5 (2 C), 128.0 (2 C), 128.1 (2 C), 128.5 (2 C), 128.6 (2 C), 129.3 (2 C), 129.4 (2 C) (18 C, Ar), 130.1, 130.2 (2 x Quat. Ar C), 142.8, 143.1 (2 x Quat. Ar C), 159.3 (2 C, 2 x Quat. Ar C).

General procedure for the generation of *N*-substituted derivatives of 2-amino-2-deoxy-D,L-erythritol (2.72-2.78)



R = R' = H **(2.72)**, R = C_8H_{17}-, R' = H **(2.73)**, R = C_3H_7-, R' = H **(2.74)**, R, R' = -$CH_2(CH_2)_3CH_2$- **(2.75)**, R, R' = -$CH_2CH_2OCH_2CH_2$- **(2.76)**, R = $(C_6H_5)_2CHCH_2$-, R' = H **(2.77)**, R = $C_6H_5CH_2$-, R' = H **(2.78)**.

To a solution of the respective benzyl ether **(2.65)-(2.70)** (0.33 mmol) methanol (4.5 ml), 10% $Pd(OH)_2$ catalyst (Pearlman's catalyst) (25 mg, 20% mol) and TFA (2 drops) were added. The system was saturated with H_2 gas and stirred at room temperature until the

reaction was complete, as judged by TLC [ethyl acetate/ethanol/water (9:2:1)]. The crude material was then filtered through Celite and purified by gel filtration using methanol as eluent to yield the *title compounds* **(2.72)-(2.78)**.

R = R' = H (2.72)

Oil (14 mg, 81 %).* δ_H (300 MHz, D_2O): 3.26 - 3.90 (6 H, m, 2 x CH_2 and 2 x CH); δ_c (75 MHz, D_2O), 55.4, 57.9, 62.7, 68.8 (2 x CH_2 and 2 x CH); *m/z* (CI) 122 ($M+H^+$, 100%); HRMS: Found: 122.0822. $C_4H_{12}NO_3$ ($M+H^+$) Requires 122.0817.

*Although there are many references[19,25,26] to the individual isomers of this compound in the literature, no data is supplied for the D,L-racemic mixture.

R = C_8H_{17}-, R' = H (2.73)

Oil (101 mg, 94%). δ_H (300 MHz, CD_3OD): 0.89 (3 H, t, *J* 6.0, CH_3), 1.31 – 1.36 (10 H, m, 5 x CH_2), 1.72 (2 H, br s, NCH_2CH_2), 3.10 – 3.89 (8 H, 2 x CH_2, 2 x CH and 1 x NHCH_2); δ_c (75 MHz, CD_3OD), 13.0 (CH_3), 22.2, 25.6, 26.2, 28.7 (2 C), 31.4 (5 x CH_2), 45.9, 56.7, 61.3, 62.7, 68.0; *m/z* (ES) 234 ($M+H^+$, 100%); HRMS: Found: 234.2069. $C_{12}H_{28}NO_3$ ($M+H^+$) Requires 234.2069.

R = C_3H_7-, R' = H (2.74)

Oil (24 mg, 100 %). δ_H (300 MHz, CD_3OD): 1.01 (3 H, br s, CH_3), 1.28 – 1.74, 3.09 – 3.98 (10 H, 2 x m, 4 x CH_2 and 2 x CH); δ_c (75 MHz, CD_3OD), 13.7 (CH_3), 23.0 (NCH_2 CH_2), 51.3 (NCH_2CH_2), 60.5 (OCH_2), 65.2 (CH), 66.6 (OCH_2), 71.8 (CH); *m/z* (CI) 164 ($M+H^+$, 100%); HRMS: Found: 164.1295. $C_7H_{18}NO_3$ ($M+H^+$) Requires 164.1289.

R, R' = -$CH_2(CH_2)_3CH_2$- (2.75)

Oil (37 mg, 100 %). δ_H (300 MHz, CD_3OD): 0.80 – 1.93 (6 H, m, 2 x NCH_2CH_2 and 1 x $NCH_2CH_2CH_2$), 3.31 - 4.20 (10 H, m, 2 x OCH_2, 2 x NCH_2, 1 x CHOH and 1 x CHNH); δ_c (75 MHz, CD_3OD), 21.5, 23.4 (2C), 51.2, 52.1, 55.0, 63.3, 67.9, 68.9 (9 C, 7 x CH_2 and 2 x CH); *m/z* (ES) 190 ($M+H^+$, 100%); HRMS: Found: 190.1442. $C_9H_{20}NO_3$ ($M+H^+$) Requires 190.1443.

R, R' = -$CH_2CH_2OCH_2CH_2$- (2.76)

Oil (20 mg, 76 %). δ_H (300 MHz, D_2O): 3.36 - 3.66, 3.90 – 4.04, 4.22 – 4.28 (14 H, 3 x m, 6 x C*H*$_2$ and 2 x C*H*); δ_c (75 MHz, D_2O), 50.1, 54.9, 62.8, 63.9 (2C), 67.6 (2C), 68.0 (8 C, 6 x *C*H$_2$ and 2 x *C*H); *m/z* (ES) 192 ($M+H^+$, 100%); HRMS: Found: 192.1234. $C_8H_{18}NO_4$ ($M+H^+$) Requires 192.1236.

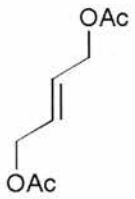
R = $(C_6H_5)_2CHCH_2$-, R' = H (2.77).

Oil (257 mg, 82 %). δ_H (300 MHz, CD_3OD): 2.40 – 3.15, 3.20 – 4.11, 4.35 – 5.02 (9 H, 3 x m, 3 x C*H*$_2$ and 3 x C*H*), 7.26 – 7.32 (10 H, Ar); δ_c (75 MHz, CD_3OD), 36.0, 50.6, 57.1, 63.5, 63.7, 67.3 (3 x *C*H$_2$ and 3 x *C*H), 127.7 (2 C), 128.1 (2 C), 129.2 (2 C), 129.3 (4 C) (10 C, Ar), 139.9, 140.2 (2 x quat. Ar C); MALDI-TOF (+ve): *m/z* 302.1 $(M+H)^+$, ($C_{18}H_{24}NO_3$ requires *m/z* 302.1); *m/z* (ES) 302 ($M+H^+$, 100%); HRMS: Found: 302.1752. $C_{18}H_{24}NO_3$ (M+H) Requires 302.1756.

R = $C_6H_5CH_2$-, R' = H (2.78)

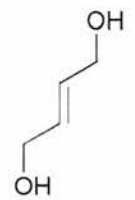
To a solution of 2-benzylamino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-erythritol **(2.70)** (424 mg, 0.94 mmol) in DCM (7.2 ml), was added TFA (800 µl). The reaction mixture was allowed to stir until TLC [EtOAc – EtOH, (2:1)] showed the reaction to be complete. The mixture was concentrated and coevaporated with IPA to yield 2-benzylamino-2-deoxy-D,L-erythritol **(2.78)** as a syrup (188 mg, 95 %). δ_H (300 MHz, CD_3OD): 3.40 - 4.03 (6 H, m, 2 x C*H*$_2$ and 2 x C*H*), 4.35 (2 H, br s, C*H*$_2$Ph), 7.41 – 7.53 (5 H, Ar); δ_c (75 MHz, CD_3OD), 49.7 (*C*HNH), 57.0 (*C*H$_2$NH), 61.0, 63.1, 67.9 (2 x *C*H$_2$ and 1 x *C*H), 129.2 (2 C), 129.5, 130.1 (2 C) (5 C, Ar), 131.3 (1 x quat. Ar C); MALDI-TOF (+ve): *m/z* 234.1 $(M+Na)^+$, ($C_{11}H_{17}NO_3Na$ requires *m/z* 234.1); *m/z* (ES) 212 ($M+H^+$, 100%); HRMS: Found: 212.1287. $C_{11}H_{18}NO_3$ ($M+H^+$) Requires 212.1286.

1,4-Di-acetyl-(*E*)-but-2-ene-1,4-diol (2.79)[27,28]



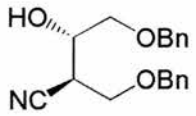
To a solution of 1,4-dibromo-2-(*E*)-butene (1.02 g, 4.8 mmol) in dry DMF (25 ml) anhydrous sodium acetate (4.1 g, 49.4 mmol) and 18-Crown-6 ether (500 mg, 1.9 mmol) were added. The reaction mixture was heated under reflux overnight. The resulting solution was washed with water (3 x 20 ml) and the product was extracted with diethyl ether (60 ml). The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by column chromatography [hexane/ethyl acetate (5:1)] to yield the known title compound as a clear oil (0.35 g, 42%). ν_{max}/cm^{-1} 1795 (C=O); δ_H (300 MHz, $CDCl_3$): 2.07 (6 H, s, 2 x CH_3), 4.57 (4 H, s, 2 x OCH_2CH), 5.82 (2 H, s, 2 x CH_2CH); δ_c (75 MHz, $CDCl_3$): 20.8 (2 C), 63.9 (2 C), 128.2 (2 C), 170.8 (2 C).

(*E*)-2-Butene-1,4-diol (2.80)[28,29,30,31]



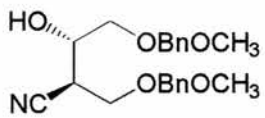
Sodium metal (0.16 g, 4.02 mmol) was added to a solution of **(2.79)** (0.35 g, 2.0 mmol) in MeOH (10 ml). The reaction mixture was allowed to stir over overnight. The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo* to yield the known title compound as a clear oil (0.123 g, 69%). ν_{max}/cm^{-1} 3500 (OH); δ_H (300 MHz, $CDCl_3$): 4.06 (4 H, m, 2 x OCH_2CH), 5.81 (2 H, m, 2 x CH_2CH). δ_c (75 MHz, $CDCl_3$), 61.7 (2 C), 130.1 (2 C).

1,4-Di-*O*-benzyl-2-cyano-2-deoxy-D,L-threitol (2.84)



2,3-Anhydro-1,4-di-*O*-benzyl-erythritol **(2.30)** (100 mg, 0.35 mmol) was dissolved in anhydrous toluene (1 ml). The mixture was cooled (0°C) and stirred for 1 h under nitrogen, then diethylaluminium cyanide (0.14 ml, 1.06 mmol) was added. The reaction mixture was allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete (Note: reaction went to only 50% completion). The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave the *title compound* **(2.84)** as a syrup (46 mg, 42 %); ν_{max}/cm^{-1}: 2250 (CN), 3500 (OH); δ_H ($CDCl_3$): 3.08 (1 H, m, C*H*CN), 3.58 – 3.61 (2 H, m, CH_2), 3.75 – 3.82 (2 H, m, CH_2), 4.10 (1 H, m, C*H*OH), 4.56 - 4.58 (4 H, m, 2 x CH_2Ph) 7.32 - 7.40 (10 H, m, Ar); δ_c ($CDCl_3$), 36.1 (*C*HCN), 67.8 (*C*H_2), 68.4 (*C*HOH), 71.4 (*C*H_2), 73.4 (2 C, 2 x *C*H_2), 118.0 (*C*N), 128.0 (3 C), 128.2 (2 C), 128.3 (2 C), 128.7 (3 C) (10 C, Ar), 137.1, 137.5 (2 x quat. Ar. C); ***m/z*** (CI) 312 ($M+H^+$, 100%); HRMS: Found: 312.1599. $C_{19}H_{22}NO_3$ ($M+H^+$) Requires 312.1599.

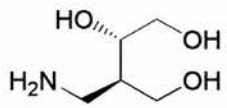
2-Cyano-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol (2.85)



2,3-Anhydro-1,4-di-*O*-(4-methoxybenzyl)-erythritol **(2.31)** (300 mg, 0.87 mmol) was dissolved in anhydrous toluene (3 ml). The mixture was cooled (0°C) and stirred for 1 h under nitrogen, then diethylaluminium cyanide (336 µl, 2.6 mmol) was added. The reaction mixture was allowed to stir until TLC [Hexane – EtOAc, (3:1)] showed the reaction to be complete (Note: reaction only went 50% to completion). The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 3:1) gave the *title compound* **(2.85)** as a syrup (124 mg, 38 %); ν_{max}/cm^{-1}: 2250 (CN), 3500 (OH); δ_H ($CDCl_3$): 3.03 - 3.04, 4.01 – 4.07 (2 H, 2 x m, 2 x C*H*),

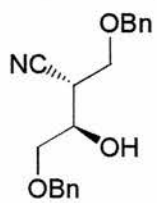
3.48 – 3.56, 3.65 – 3.76 (4 H, 2 x m, 2 x CH_2), 3.86 (6 H, s, 2 x OCH_3), 4.47 - 4.49 (4 H, m, 2 x CH_2Ph), 6.86 – 6.89, 7.22 - 7.26 (8 H, m, Ar); δ_c ($CDCl_3$), 36.3 (2 C, 2 x OCH_3), 55.3, 67.3, 68.0, 71.3, 73.2, 73.3 (2 x CH_2, 2 x CH_2Ph and 2 x CH), 114.1 (2 C), 114.1 (2 C) (4 C, Ar), 118.5 (CN), 129.4 (2 C), 129.6 (2 C), 129.7 (2 C) (6 C, 4 x Ar C and 2 x quat. Ar. C), 159.6, 159.7 (2 x quat. Ar. C); *m/z* (CI) 372 ($M+H^+$, 100%); HRMS: Found: 372.1810. $C_{21}H_{26}NO_5$ ($M+H^+$) Requires 372.1810.

2-Aminomethylene-2-deoxy-D,L-threitol (2.86)



To a solution of 1,4-di-*O*-benzyl-2-cyano-2-deoxy-D,L-threitol **(2.84)** (110 mg, 0.35 mmol) in anhydrous methanol (3 ml), was added 10% $Pd(OH)_2$ catalyst (Pearlman's catalyst) (100 mg, 20% mol) and TFA (2 drops) were added. The system was saturated with H_2 gas and stirred at room temperature until the reaction was complete, as judged by TLC [DCM/methanol/water (10:4:1)]. The crude material was then filtered through Celite and purified by gel filtration using methanol as eluent to yield the *title compound* **(2.86)** (43 mg, 90 %). δ_H (300 MHz, CD_3OD): 2.01 (1 H, m, $CHCH_2NH_2$), 3.15 – 3.30 (2 H, m, CH_2), 3.53 – 3.79 (5 H, m, 2 x CH_2 and 1 x CH); δ_c (75 MHz, CD_3OD), 39.5, 40.9, 61.4, 64.0, 71.1 (3 x CH_2 and 2 x CH); *m/z* (CI) 136 ($M+H^+$, 100%); HRMS: Found: 136.0973. $C_5H_{14}NO_3$ ($M+H^+$) Requires 136.0973.

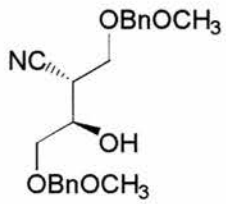
1,4-Di-*O*-benzyl-2-cyano-2-deoxy-D,L-erythritol (2.87)



2,3-Anhydro-1,4-di-*O*-benzyl-D,L-threitol **(2.55)** (300 mg, 1.06 mmol) in anhydrous toluene (3 ml) was cooled (0°C) and stirred for 1 h under nitrogen, then diethylaluminium cyanide (0.41 ml, 3.2 mmol) was added. The reaction mixture was allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete (Note: reaction went 50% to completion). The mixture was diluted with DCM (200 ml) and washed with an equal volume

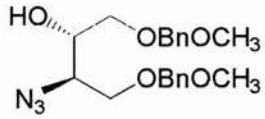
of 10 % aq. $NaHCO_3$. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave the *title compound* **(2.87)** as a syrup (162 mg, 49 %); ν_{max}/cm^{-1}: 2235 (CN), 3500 (OH); δ_H ($CDCl_3$): 3.06 (1 H, m, C*H*CN), 3.68 – 3.73 (2 H, m, CH_2), 3.74 – 3.88 (2 H, m, CH_2), 4.11 (1 H, m, C*H*OH), 4.58 (4 H, d, *J* 4.7, 2 x CH_2Ph) 7.32 - 7.40 (10 H, m, Ar); δ_c ($CDCl_3$), 35.8 (*C*HCN), 66.6 (*C*H_2), 68.4 (*C*HOH), 71.4 (*C*H_2), 73.6, 73.8 (2 x *C*H_2), 118.0 (*C*N), 127.9 (2 C), 128.0 (2 C), 128.1 (2 C), 128.2 (2 C), 128.7 (2 C) (10 C, Ar), 137.7, 137.9 (2 x quat. Ar. C); *m/z* (CI) 312 ($M+H^+$, 100%); HRMS: Found: 312.1599. $C_{19}H_{22}NO_3$ ($M+H^+$) Requires 312.1599.

2-Cyano-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-D,L-erythritol (2.88)



2,3-Anhydro-1,4-di-*O*-(4-methoxybenzyl)-D,L-threitol **(2.56)** (2.0 g, 5.8 mmol) was dissolved in anhydrous toluene (20 ml). The mixture was cooled (0°C) and stirred for 1 h under nitrogen, then diethylaluminium cyanide (2.2 ml, 17.4 mmol) was added. The reaction mixture was allowed to stir until TLC [Hexane – EtOAc, (3:1)] showed the reaction to be complete (Note: reaction did not go to completion). The mixture was diluted with DCM (200 ml) and washed with an equal volume of H_2O. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 3:1) gave the *title compound* **(2.88)** as a syrup (124 mg, 38 %); ν_{max}/cm^{-1}: 2247 (CN), 3468 (OH); δ_H ($CDCl_3$): 2.92 (1 H, m, C*H*), 3.52 – 3.67 (4 H, 2 x m, 2 x CH_2), 3.70 (6 H, s, 2 x OCH_3), 3.94 (1 H, m, C*H*), 4.38 – 4.40 (4 H, m, 2 x CH_2Ph), 6.78 – 6.81, 7.14 - 7.19 (8 H, m, Ar); δ_c ($CDCl_3$), 35.7 (2 C, 2 x O*C*H_3), 55.2, 66.1, 68.3, 71.1, 73.1, 73.3 (2 x *C*H_2, 2 x *C*H_2Ph and 2 x *C*H), 113.9 (2 C), 114.0 (2 C) (4 C, Ar), 118.9 (*C*N), 129.4 (2 C), 129.5 (2 C), 129.6 (2 C) (6 C, 4 x Ar C and 2 x quat. Ar. C), 159.6 (2 C, 2 x quat. Ar. C); *m/z* (CI) 372 ($M+H^+$, 100%); HRMS: Found: 372.1810. $C_{21}H_{26}NO_5$ ($M+H^+$) Requires 372.1810.

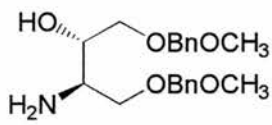
2-Azido-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-L-threitol (2.103)*



To a solution of 2,3-anhydro-1,4-di-*O*-(4-methoxybenzyl)-erythritol **(2.31)** (344 mg, 1 mmol) in anhydrous toluene (2 ml), was added trimethylsilyl azide (121 mg, 1.05 mmol) and(*R,R*)-*N,N'*-bis(3,5-di-*tert*-butyl-salicylidene)-1,2-cyclohexane-diaminochromium(III) chloride[32,33] (13 mg, 0.05 mmol). The reaction mixture was allowed to stir under nitrogen until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete. The mixture was concentrated. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave the *title compound* **(2.103)** as a syrup (180 mg, 46 %); ν_{max}/cm^{-1} 2099 (N_3), 3436 (OH); δ_H ($CDCl_3$): 3.40 (2 H, d, *J* 5.2, C*H*$_2$), 3.55 – 3.60 (4 H, m, C*H*$_2$ and 2 x C*H*), 3.70 (6 H, s, 2 x OC*H*$_3$), 3.80 (1 H, br s, O*H*) 4.37, 4.39 (4 H, 2 x s, 2 x C*H*$_2$Ph), 6.78 – 6.81, 7.13 - 7.18 (8 H, 2 x m, Ar); δ_c ($CDCl_3$), 55.2 (2 C, 2 x *C*H$_3$), 62.1, 70.2, 70.5, 70.7, 73.1, 73.2 (2 x *C*H$_2$, 2 x *C*H, 2 x *C*H$_2$Ph), 113.9 (2 C), 129.5 (2 C), 129.6 (2 C), 129.7 (2 C) (8 C, Ar), 129.8 (2 C, 2 x quat. Ar. C), 159.5 (2 C, 2 x quat. Ar. C); *m/z* (ES) 405 ($M+NH_4^+$, 100%); HRMS: Found: 405.2133. $C_{20}H_{29}N_4O_5$ ($M+NH_4$) Requires 405.2138.

*This compound can also be named as 2-(*R*)-azido-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-butan-3-(*S*)-ol.

2-Amino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-L-threitol (2.104)*

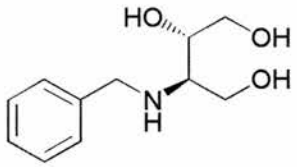


To a solution of 2-azido-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-L-threitol **(2.103)** (400 mg, 1 mmol) in THF (8 ml), was added water (800 µl) and triphenylphosphine[34] (788 mg, 3 mmol). The reaction mixture was heated to reflux and allowed to stir until TLC [Hexane – EtOAc, (2:1)] showed the reaction to be complete. The mixture was concentrated. Column chromatography (silica gel; Hexane – EtOAc, 1:1 – EtOAc – EtOH, 9:1) gave the *title compound* **(2.104)** as a syrup (261 mg, 72 %); ν_{max}/cm^{-1} 3369 (NH and OH); δ_H ($CDCl_3$): 3.09 (1 H, br s, CHO*H*), 3.30 – 3.56 (6 H, m, 2 x C*H* and 2 x C*H*$_2$), 3.71 (6 H, s, 2 x OC*H*$_3$), 4.27 – 4.38 (4 H, m, 2 x C*H*$_2$Ph), 6.75 – 6.78, 7.11 - 7.19 (8 H, 2 x m, Ar); δ_c ($CDCl_3$): 55.2

(2 C, 2 x CH_3), 52.7, 69.5, 71.0, 71.7, 73.0 (2 C) (6 C, 2 x CH_2Ph, 2 x CH_2 and 1 x CH), 113.9 (4 C), 129.3 (2 C), 129.5 (2 C) (8 C, Ar), 130.0, 130.1 (2 x Quat. Ar. C), 159.4 (2 C, 2 x quat. Ar. C); *m/z* (ES) 362 ($M+H^+$, 100%); HRMS: Found: 362.1968 $C_{20}H_{28}NO_5$ ($M+H^+$) Requires 362.1967.

*This compound can also be named as 2-(*R*)-amino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-butan-3-(*S*)-ol.

2-Benzylmino-2-deoxy-L-threitol (2.102)[35]



To a solution of 2-amino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-L-threitol **(2.104)** (95 mg, 0.26 mmol) in DCE (1 ml), was added benzaldehyde (27 µl, 0.26 mmol) and sodium triacetoxyborohydride[36](76 mg, 0.36 mmol, 1.4 mol eq). The reaction mixture was allowed to stir until TLC [EtOAc – EtOH, (2:1)] showed the reaction to be complete. The mixture was concentrated to yield 2-benzylamino-2-deoxy-1,4-di-*O*-(4-methoxybenzyl)-L-threitol **(2.105)** as a syrup, which was used directly, without further characterisation in the next step The syrup was dissolved in a mixture of DCM (7.2 ml) and TFA (800 µl). The reaction mixture was allowed to stir until TLC [EtOAc – EtOH, (2:1)] showed the reaction to be complete. The reaction mixture was concentrated, coevaporated with isopropanol and purified on a Dowex 50XW H^+ ion exchange column to yield the title compound as a syrup (32 mg, 58 %); $[\alpha]_D$ +9.6° (*c* 0.3, CH_3OH), (lit., [35] $[\alpha]_D$ +12.0° (*c* 0.5, CH_3OH)); δ_H (CD_3OD): 3.78 – 4.78 (8 H, 3 x m, 3 x CH_2 and 2 x CH), 7.84 - 7.94 (5 H, Ar); δ_c (CD_3OD): 50.4, 58.7, 61.8, 64.9, 68.4 (3 x CH_2 and 2 x CH), 130.0 (2 C), 130.2, 130.8 (2 C) (5 C, Ar), 132.4 (2 C, 2 x Quat. Ar. C); *m/z* (ES) 212 ($M+H^+$, 100%); HRMS: Found: 212.1284 $C_{11}H_{18}NO_3$ ($M+H^+$) Requires 212.1286.

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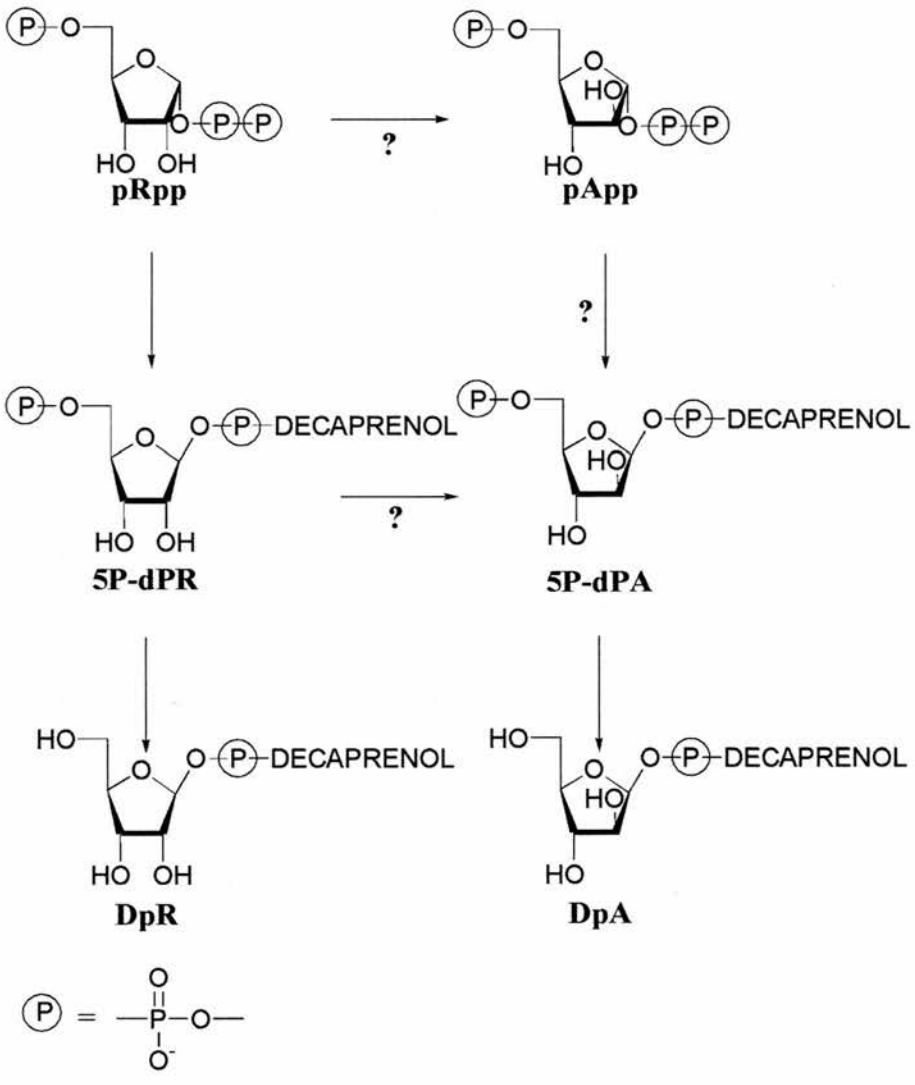
Chapter 4

Disaccharide analogues as pApp and pRpp mimics

4.1 DpA Biosynthesis:

In Chapter 1 it was noted that the biosynthetic origin of the tetramycolylated-hexa-arabinose unit of the mycobacterial cell wall involves the β-D-arabinofuranosyl-1-monophosphoryldecaprenol moiety (DpA) acting as an arabinose donor to the cell wall, DpA is the arabinofuranose donor for the mycobacterial cell wall.[1,2,3] Whilst the natural biosynthetic route to this donor is not known it is thought to proceed *via* one of the pathways shown below.[4]



Scheme 4.1: Hypothetical DpA biosynthetic pathways. [4]

5-Phospho-α-D-arabinosyl pyrophosphate (pApp) is thought to be a key intermediate in the DpA biosynthetic pathway.[4,5] The pApp moiety contains a pyrophosphate group; such pyrophosphates are highly charged and unstable. Therefore it was decided to replace the unstable pyrophosphate linkage with a more stable structural unit, thereby probing the role of pApp, or otherwise in DpA biosynthesis.

4.2 Glycosyltransferase complex mimetics:

Complex oligosaccharides are synthesised by glycosyltransferases in the endoplasmic reticulum and Golgi complex by the sequential transfer of sugar residues from nucleotide or membrane-bound donors to growing polysaccharide chains.[6] It is generally postulated that these enzymatic reactions proceed through a half-chair transition state with substantial sp^2 character at the anomeric carbon (**Figure 4.1**).

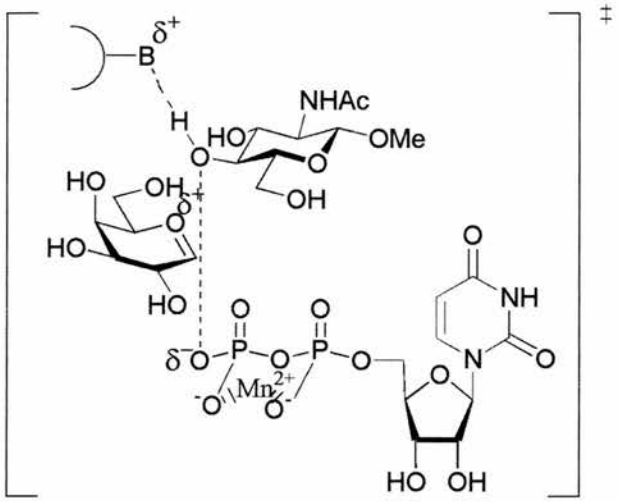


Figure 4.1: Transition state of a galactosyltransferase reaction.

Recent reports in the literature on glycosyltransferases,[7] using β-1,4-galactosyltransferase as a model system, indicated that the transfer of galactose sugar unit from UDP-galactose to an acceptor sugar proceeds through the transition state shown in **Figure 4.1**, with the pyrophosphate group chelating to an essential divalent manganese ion, thereby adopting a six

membered chair type conformation. Wong and co-workers[7] focused their attention on developing three series of compounds capable of imitating this pyrophosphate-metal ion interaction. The three different sets of compounds they chose were compounds with malonic acid, tartaric acid and monosaccharide linkages, schematics of which are represented in (**Figure 4.2**). The suggestion was that both malonic and tartaric esters would form a complex with the divalent manganese ion and the complex would serve as a pyrophosphate mimic.
It is known that the naturally occuring GlcNAc phosphotransferase inhibitor tunicamycin possess a monosaccharide unit (**Figure 4.2**) that is thought to mimic the pyrophosphate-Mn^{2+} complex (**Figure 4.2**).[8]

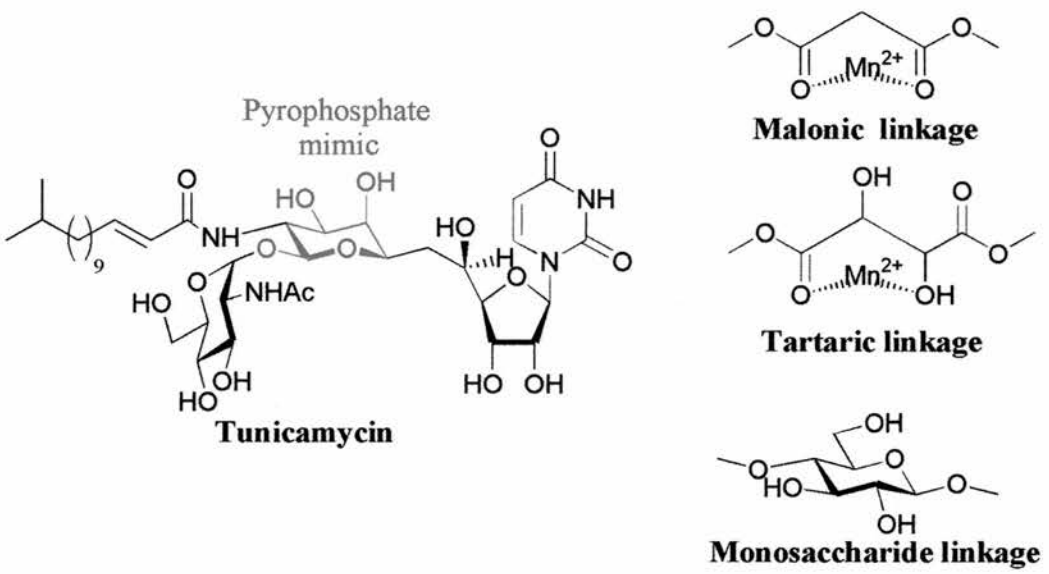


Figure 4.2: (a) Tunicamycin and (b) malonic, tartaric and monosaccharide linkages in Wong's inhibitors.[7]

In his work Wong chose glucose and galactose monosaccharide units as potential pyrophosphate-manganese mimics. Seven inhibitors synthesised by Wong containing these three different types of linkage are depicted in **Figure 4.3**.

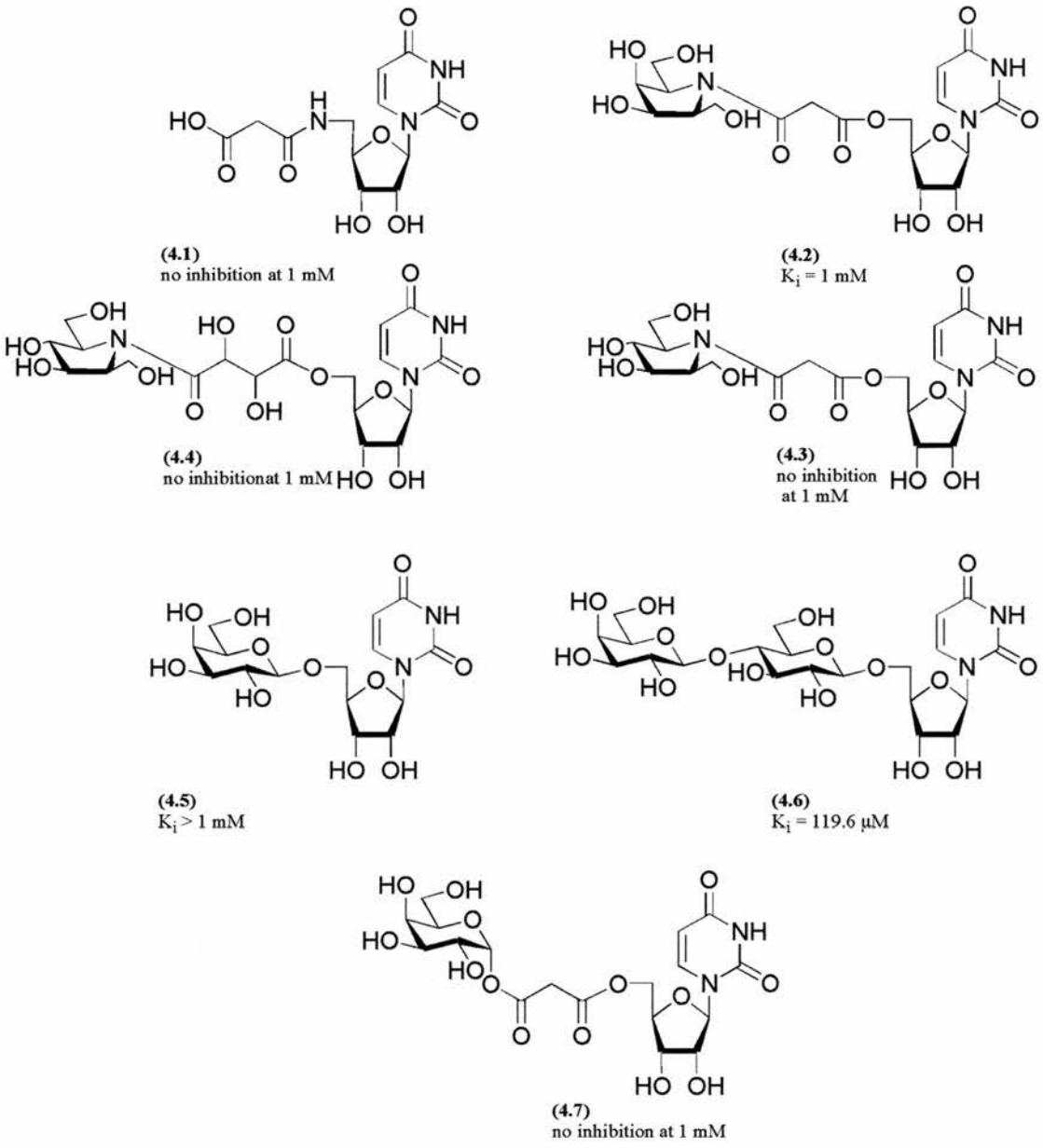


Figure 4.3: β-1,4-galactosyltransferase inhibitors.

Referring to the compounds shown above (**Figure 4.3**), those numbered **(4.1)**, **(4.3)** and **(4.7)** were unable to inhibit β-1,4-galactosyltransferase, whilst compound **(4.2)** did show slight inhibition of the enzyme registering a K_i in the region of 1mM. These results suggest the malonic ester linkage shown in **Figure 4.2** to be incapable of replacing the pyrophosphate-linkage productively. Compound **(4.4)** also demonstrated no inhibition of the

enzyme; this latter result can be attributed to conformational differences between the tartaric ester-Mn^{2+} complex and the pyrophosphate-Mn^{2+} complex. Compound **(4.6)** was an inhibitor of the enzyme, with a K_i of 119.6 μM. Consequently glucose may be thought of as a more than adequate pyrophosphate-Mn^{2+} complex replacement. The other monosaccharide (galactose) based inhibitor, compound **(4.5)** showed poor inhibition of the enzyme, registering a K_i of > 1mM. The foregoing results suggest that a glucose sugar unit is hence capable of acting as a surrogate for the pyrophosphate (**Figure 4.4**).

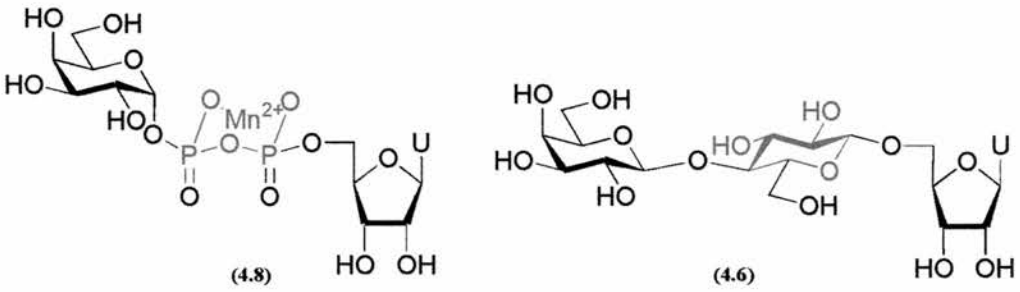


Figure 4.4: Natural complex for galactosyl transferase **(4.8)** and glucose as a pyrophosphate-Mn^{2+} mimic **(4.6)**.

The preceding material formed the starting point for the work in the current study, whereby a series of pApp mimics were prepared in order to determine whether or not glucose could act as an adequate pyrophosphate replacement. If successful, the advantage with this approach would be two fold for not only is glucose a good pyrophosphate replacement but the pApp mimic **(4.9)** contains an acetal linkage which is relatively robust. In contrast pApp has a pyrophosphate linkage which is highly charged and unstable. Previous work within the group has focused on this idea in developing a more stable *C*-phosphonate mimic of pApp, namely pA*C*pp (**Figure 4.5**).[5] pA*C*pp was synthesized and shown to be incapable of interrupting key steps in mycobacterial arabinan formation.[5]

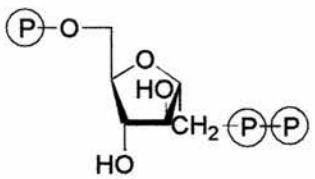


Figure 4.5: pA*C*pp.

Synthesis of arabinofuranose and glucopyranose containing disaccharides was therefore undertaken with the objective of developing a mimic of the pyrophosphate moiety, see **Figure 4.6**. The disaccharides would then be screened as potential inhibitors of *M. tuberculosis* cell wall biosynthesis.

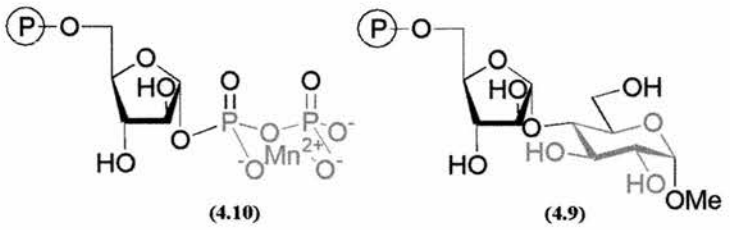


Figure 4.6: pApp/ Mn^{2+} complex **(4.10)** and glucose as a pyrophosphate replacement **(4.9)**.

In the event that glucose unit mimicked the pyrophosphate moiety of the pApp/Mn^{2+} complex, then inhibition of addition of arabinofuranose to the arabinogalactan component would be achieved. To faciltate such investigations it was decided therefore to synthesise pApp mimics **(4.9)** and **(4.11)** shown below.

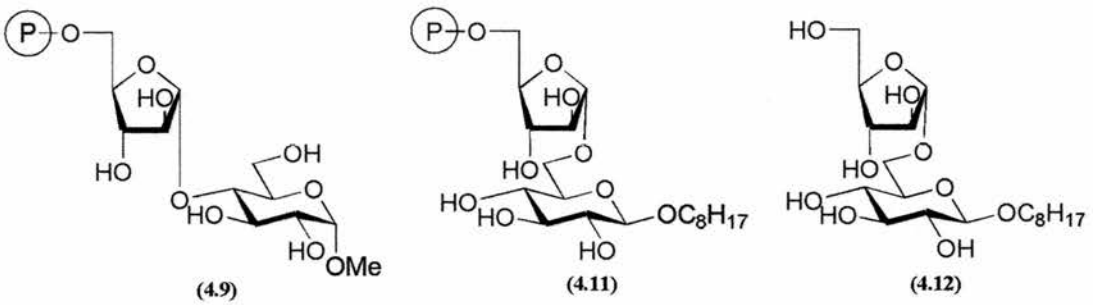
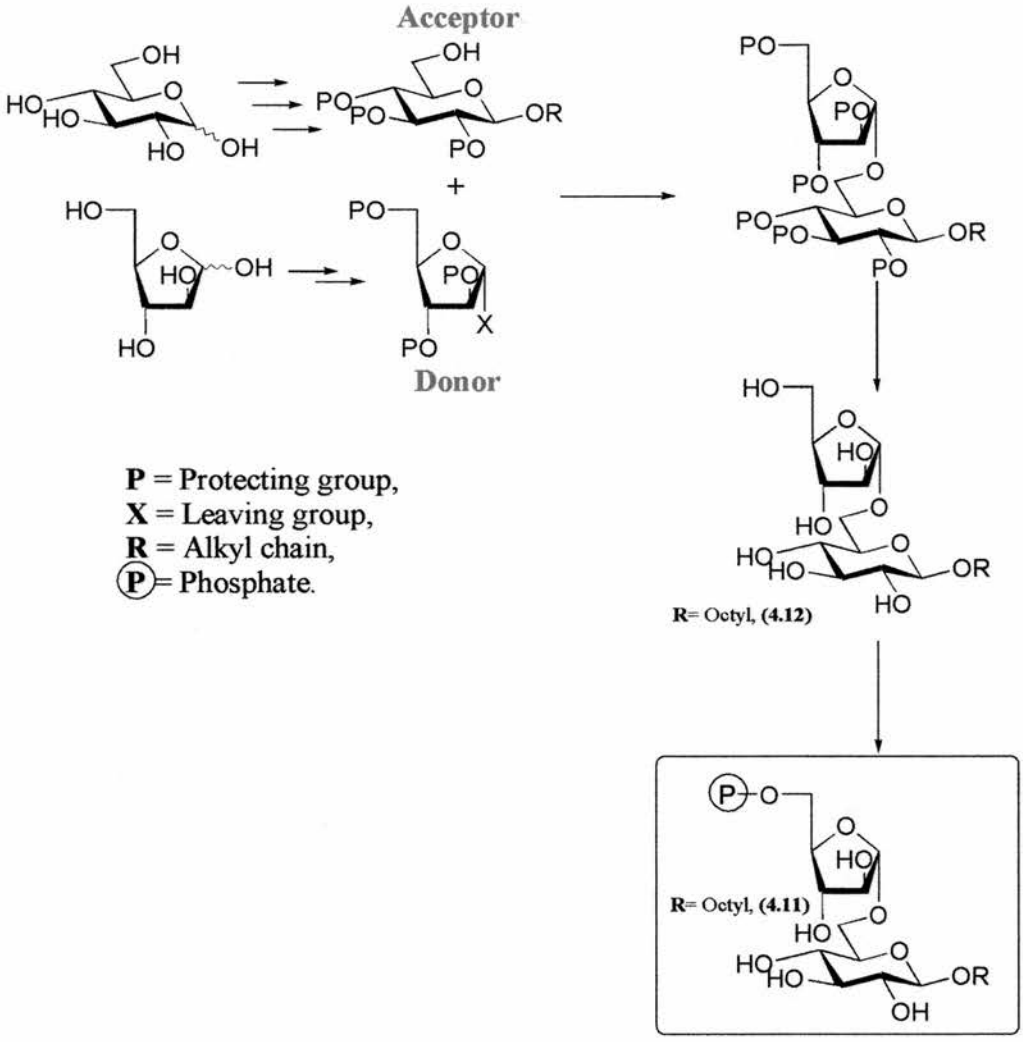


Figure 4.7: Target disaccharides.

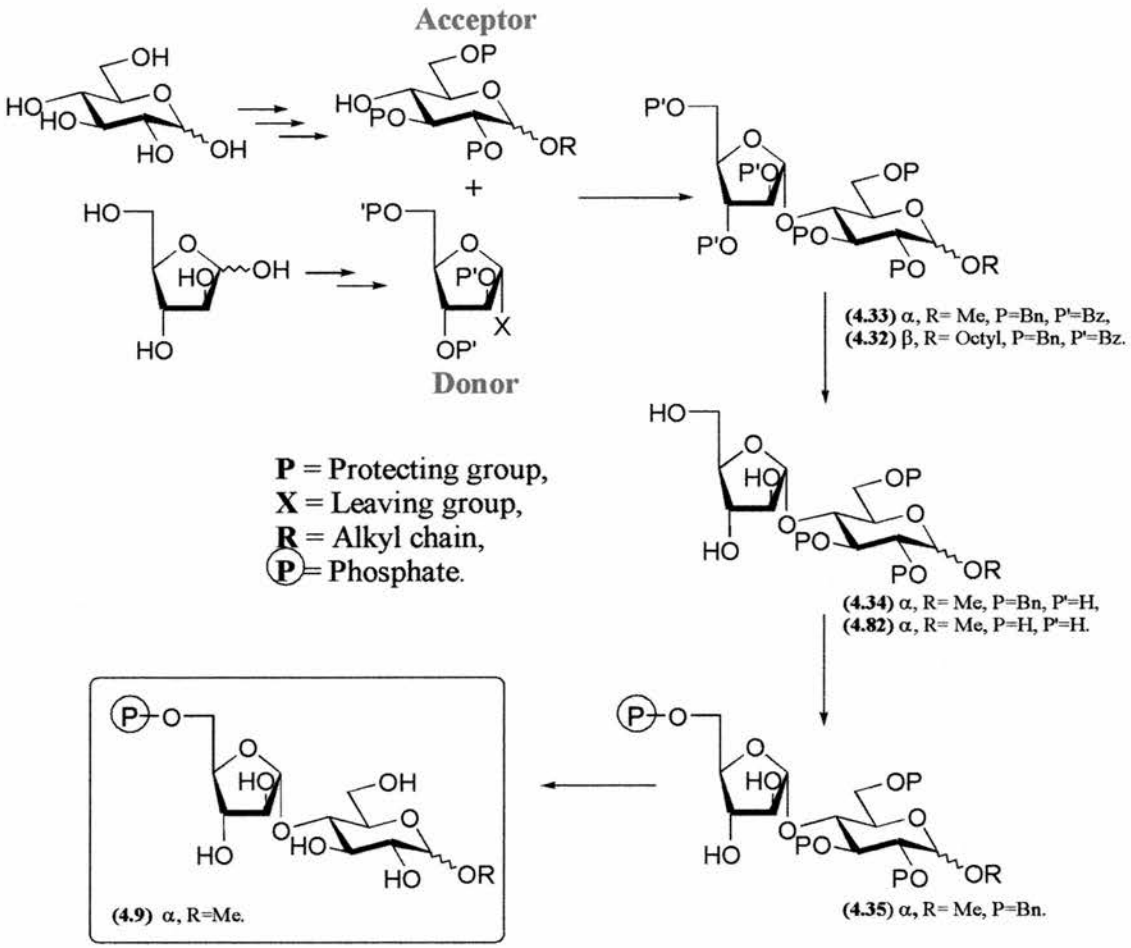
It was also noted that octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** is reported as naturally occurring in the leaves of the perennial plant, *Circaea lutetiana* L. ssp. *Canadensis*.[9] The structure of the natural product **(4.12)** was surprising, since arabinose in plants is generally L-configured and not D-configured as claimed for **(4.12)**. This suggested an initial strategy of generating **(4.11)** via **(4.12)** since the 1,6-linkage is easier to realise in

practice than the 1,4-linkage in **(4.9)**. This would allow confirmation, or otherwise of the unusual D-Ara*f* composition of **(4.12)**. The first target disaccharide **(4.11)** was 1,6-linked and as mentioned previously, the 1,6-linkage is easier to generate and so ideal for use in a pilot study **(Scheme 4.2)**.



Scheme 4.2: General strategy for the preparation of the 1,6-linked arabinofuranose-glucopyranose disaccharide.

An outline synthesis of the second target methyl 4-*O*-(5-phospho-α-D-arabinofuranosyl)-α-D-glucopyranoside **(4.9)** may be seen in **Scheme 4.3**.



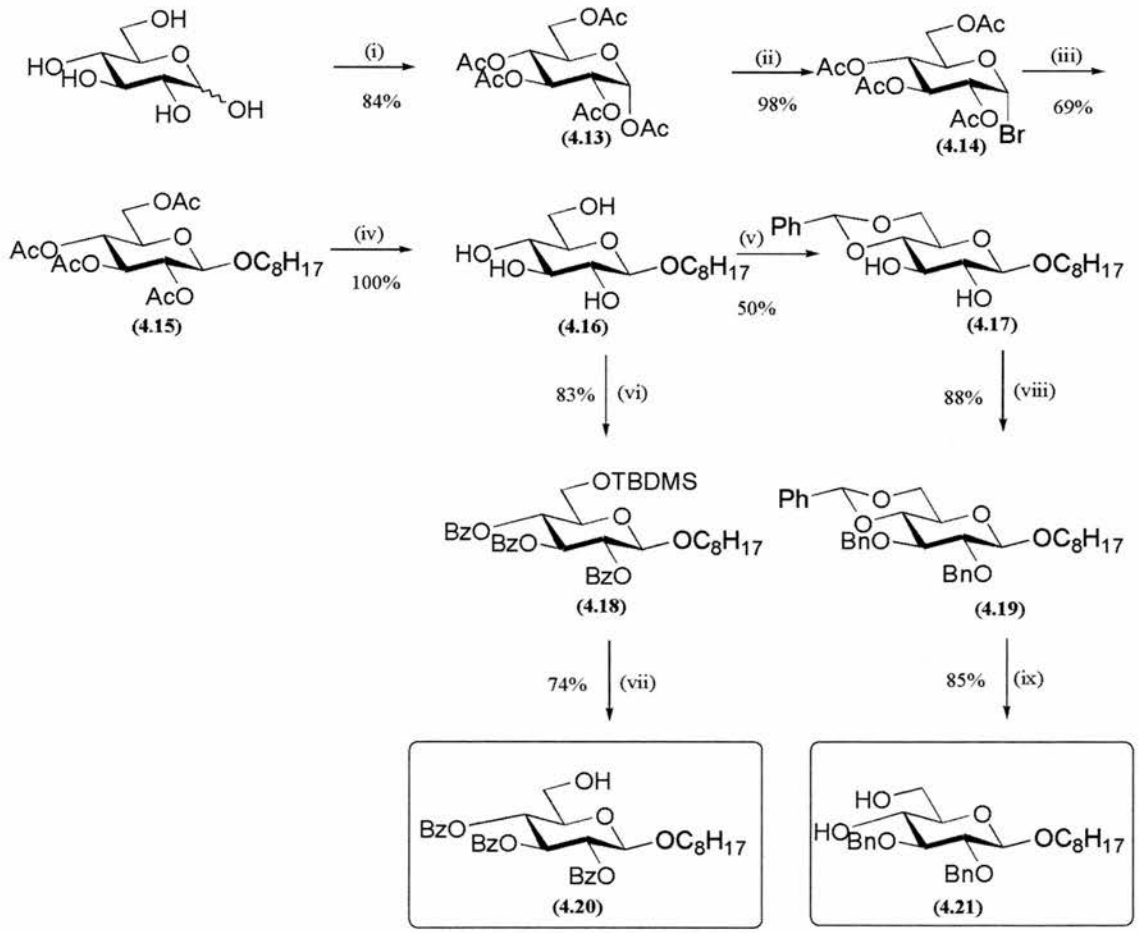
Scheme 4.3: General strategy for the preparation of the 1,4-linked arabinofuranose-glucopyranose disaccharide.

4.3 Preparation of 1,6-linked pApp mimic:

4.3.1 Preparation of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.20) and octyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (4.21):

Two different acceptor sugars were synthesised and subsequently used in coupling reactions; both were derived from D-glucose. The first 1,6-acceptor, **(4.21)** was prepared *via* protection of the 4,6-positions with a benzylidene acetal protecting group and benzyl ether protection at the remaining positions. The second 1,6-acceptor, **(4.20)**, was prepared from a silyl/benzoyl-protected precursor **(4.18)**.

The first acceptor to be synthesised was octyl 2,3-di-*O*-benzyl-β-D-glucopyranoside **(4.21)**. D-Glucose was converted to pentaacetate **(4.13)** in near quantitative yield by using an iodine/acetic anhydride combination.[10] Confirmation of the alpha linkage in **(4.13)** was given by a chemical shift for C-1 of 89.0 ppm and a $J_{1,2}$ coupling constant of 3.8 Hz. The formation of **(4.13)** was further evidenced by the fact that of optical rotation and melting points were in good agreement with the literature (mp 104 - 106° C, (lit.,[11] 109 - 111°C; $[\alpha]_D$ +98.2, lit.,[12] $[\alpha]_D$ +101.6). The pentaacetate was then treated with HBr/AcOH to generate the bromide, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(4.14)**.



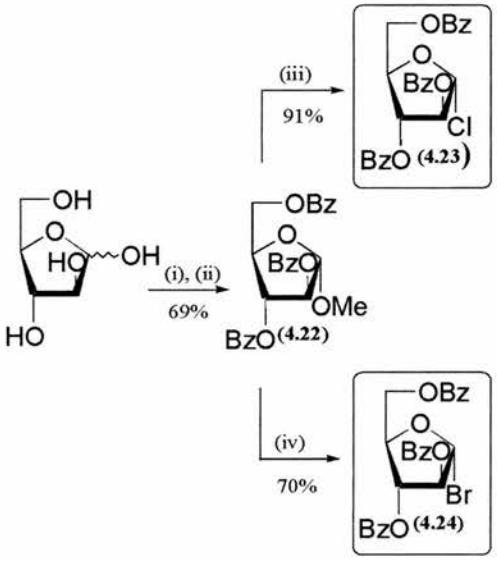
Scheme 4.4: (i) Ac_2O/I_2; (ii) AcOH, HBr.AcOH; (iii) $C_8H_{17}OH$ (1.5 mol eq), $Hg(CN)_2$ (1.2 mol eq) and $Hg(Br)_2$ (0.1 mol eq), MeCN; (iv) NaOMe/MeOH; (v) Benzaldehyde dimethyl acetal (5.9 mol eq), H^+(cat.), MeCN; (vi) Pyridine, TBDMSCl (1.1 mol eq), BzCl (3.3 mol eq); (vii) AcOH; (viii) NaH (2.2 mol eq), BnBr (2.2 mol eq), DMF; (ix) 80% aq. AcOH.

Coupling constant and chemical shift data for compound **(4.14)** suggested an alpha-linked glycosyl halide had been formed (i.e. $J_{1,2} = 4.1$ Hz and $\delta_C = 86.4$ ppm). Optical rotation and melting point data were also consistent with literature values (mp 82 - 84°C, lit.,[13] 83 - 85°C; $[\alpha]_D$ +191.9, lit.,[14] $[\alpha]_D$ +198.0) suggesting retention of the anomeric stereochemistry. The halide **(4.14)** was then reacted with octanol in the presence of mercury salts to give octyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **(4.15)**. Once more confirmation of the formation of **(4.15)** was given by diagnostic shifts in NMR values and agreement with literature data (mp 48-50°C, lit.,[15] 53 - 54°C; $[\alpha]_D$ -22.1, lit.,[16] $[\alpha]_D$ -20.5). Evidence for the beta-linkage in

(4.15) was given by a chemical shift for C-1 of 100.9 ppm and a $J_{1,2}$ coupling constant of 9.0 Hz. Standard deacetylation, using NaOMe/MeOH gave the known octyl β-D-glucopyranoside **(4.16)**.[14,16,17,18,19] Treatment of **(4.16)** with benzaldehyde dimethyl acetal (6 mol eq), catalytic amounts of *p*-toluenesulfonic acid, in AcN resulted in the formation of **(4.17)** which was then treated with sodium hydride and benzyl bromide in DMF resulting in the formation of the fully protected sugar **(4.19)**. The selective benzylidene acetal ring opening of **(4.19)**, required to liberate the primary alcohol at C-6, proved to be extremely difficult. Attempts using TMSCl and $NaCNBH_3$,[20] aluminium chloride and borane trimethylamine[21] proved unsuccessful. Treatment of octyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside **(4.19)** with aqueous acetic acid gave the diol, **(4.21)**, as one of the 1,6-acceptors. Due to the presence of two unprotected hydroxyls, coupling reactions proceeded uncleanly (due to coupling with glycosyl donors at C-4) and in low yield. This problem was overcome by the synthesis of a new 1,6-acceptor, derived from octyl β-D-glucopyranoside **(4.16)**, by silylation and benzoylation which generated **(4.18)** in high yield. Treatment of **(4.18)** with acetic acid formed the new acceptor **(4.20)**. The advantage with this molecule over compound **(4.21)** was two fold. Firstly it coupled cleanly, quickly and in high yield with glycosyl donors at C-6. Secondly only one subsequent benzoyl deprotection step was required (see **Scheme 4.7**).

4.3.2 Preparation of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl chloride (4.23) and 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide (4.24):

As with the acceptor sugars, two donors sugars were synthesized; both were benzoylated arabinofuranosyl halides. The sugars synthesised were the glycosyl chloride **(4.23)** and glycosyl bromide **(4.24)**. In both cases D-arabinose was fully protected to give the known benzoylated methyl arabinofuranoside **(4.22)**.[22,23] When this molecule was reacted with DCMME/stannic chloride, or HBr/AcOH it was found to yield the chloride and bromide derivatives **(4.23)** and **(4.24)**, respectively. Both donor sugars were obtained in high yield.

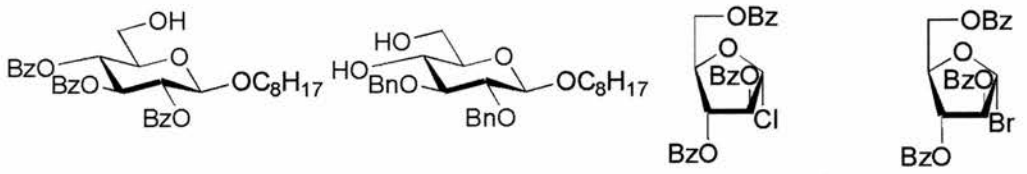


Scheme 4.5: (i) MeOH/ HCl (1 mol eq); (ii) Py., BzCl (6 mol eq); (iii) DCMME (20 mol eq), $SnCl_4$ (1.1 mol eq); (iv) AcOH, HBr.AcOH.

Generation of alpha-stereochemistry in **(4.23)** and **(4.24)** was apparent from NMR and optical rotation data. Substantial changes in chemical shifts for C-1 from 107.0 to 95.6 and 88.9 ppm, in the ^{13}C NMR and change in chemical shifts for H-1 from 5.19 to 6.39 and 6.66 ppm, in the ^{1}H NMR for the chloro and bromo derivatives, **(4.23)** and **(4.24)**, respectively, were recorded. These dramatic shifts in ^{1}H and ^{13}C NMR, together with optical rotations which were in good agreement with the literature values {**(4.24)**: $[\alpha]_D$ +82.9, lit., [22] $[\alpha]_D$ +84.8°) and **(4.23)**: $[\alpha]_D$ +34.1, lit., [24] $[\alpha]_D$ -28.0 for the L-isomer), suggest that we were successful in generating the glycosyl halides **(4.23)** and **(4.24)**, respectively.

4.3.3 Preparation of octyl 6-*O*-(5-*O*-phospho-α-D-arabinofuranosyl)-β-D-glucopyranoside (4.11):

Initial coupling reactions were performed on a 1 mmol scale. Mercury salts [$(Hg(CN)_2$ and $Hg(Br)_2)$][25,26,27] were used as the promoters, anhydrous DCM was the solvent of choice. Two different acceptor sugars, **(4.20)** and **(4.21)**, were used in the synthesis of the 1,6-linked disaccharide target material. The first acceptor to be used in the coupling reactions was the diol **(4.21)**.



(4.20) (4.21) (4.23) (4.24)

Figure 4.8: Donor and acceptor sugars used in the generation of octyl 6-*O*-(5-*O*-phospho-α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.11)**.

Initial couplings with the donor sugar **(4.23)** proved unsuccessful. This was accounted for by the low reactivity of the mercury salts and the instability/purity of the glycosyl halide donor **(4.23)**. Due to their instability, the glycosyl halides **(4.23)** or **(4.24)** were freshly prepared prior to each coupling, however couplings still proved relatively unsuccessful, with low yields of disaccharide products being generated. In an attempt to overcome these problems reaction conditions were varied. Different promoters, such as iodine[28] and silver salts ($AgClO_4$, Ag_2CO_3)[29,30] were employed. DCM and acetonitrile were the solvents used, whilst a range of temperatures were also used in the process. The purity of the donor sugar was found to a significant factor affecting reaction yields. After numerous trial reactions using 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl chloride **(4.23)** as glycosyl donor and anhydrous octanol as glycosyl acceptor, the optimum conditions for the coupling reaction were established, (**Table 4.1**).

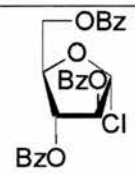
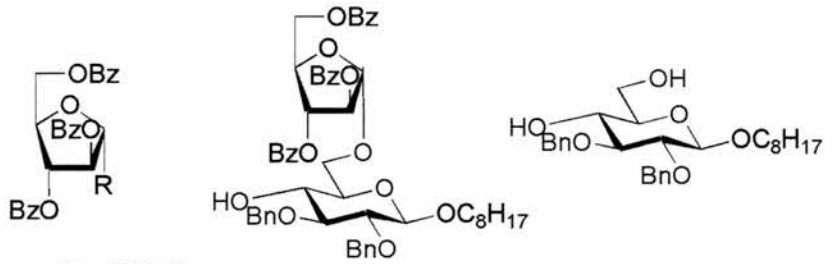
Donor	Acceptor	Promotor	Solvent
 (4.23)	$C_8H_{17}OH$	I_2	DCM or MeCN
"	"	$Ag(ClO_4)$	"
"	"	Ag_2CO_3	"
"	"	$Hg(CN)_2/Hg(Br)_2$	"
"	"	AgOTf	"

Table 4.1: Trial reactions performed on 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl chloride **(4.23)**.

The coupling was found to work best when three equivalents of glycosyl bromide **(4.24)** [due to the irreproducibility of the preparation of the chloride **(4.23)** the bromide, **(4.24)** was preferentially used in all subsequent coupling procedures] one equivalent of acceptor sugar and Drierite in DCM were allowed to stir at room temperature in a dark atmosphere. The reaction mixture was then cooled to –30 °C and five equivalents of AgOTf[31,32] were quickly added. Close monitoring was employed using TLC.

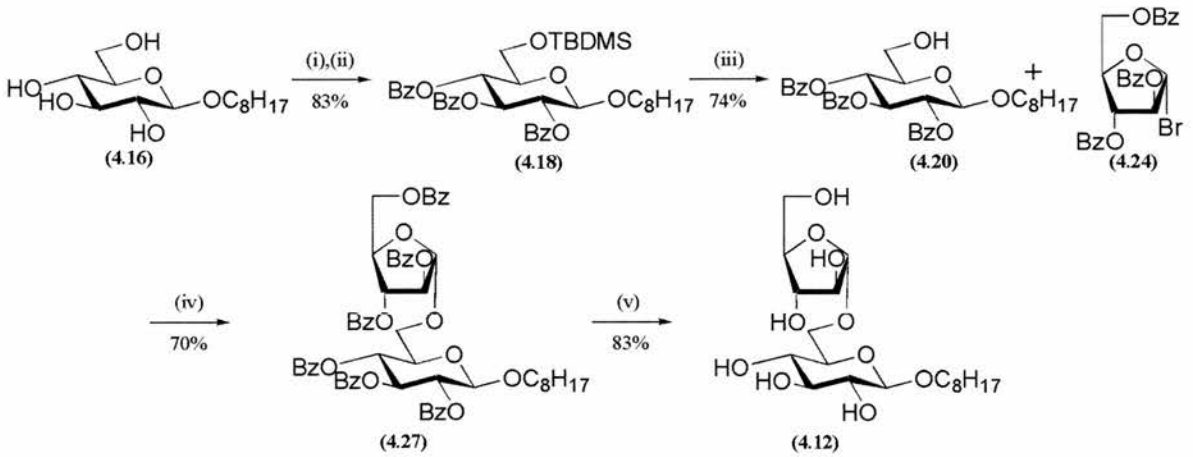


(4.25) R= OC_8H_{17}
(4.24) R= Br

(4.26)

(4.21)

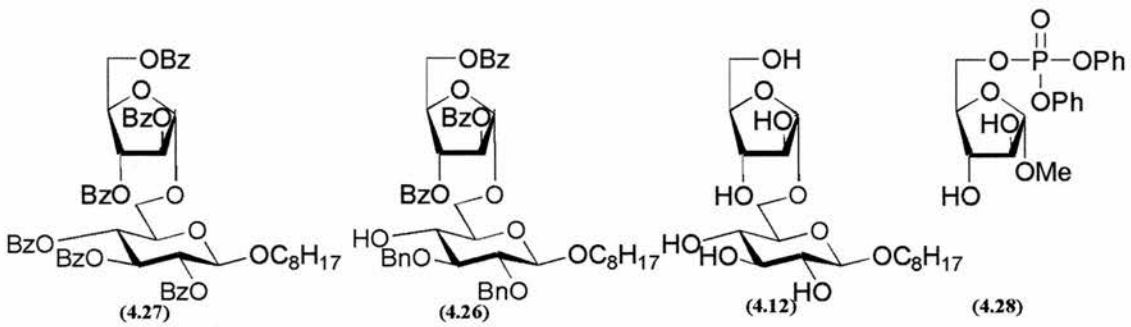
Then, as soon as the acceptor disappeared, the reaction was quenched with collidine; neutralisation and purification yielded the alpha-configured octyl glycoside **(4.25)**, {5.31 (H-1', $J_{1',2'}$ = 0.0 Hz) and δ_C = 105.8 ppm (C-1'), (Found: C, 70.78; H, 6.85. $C_{34}H_{38}O_8$ Requires C, 71.06; H, 6.67%)}. The glycosyl coupling reaction was repeated with octyl 2,3-di-*O*-benzyl-β-D-glucopyranoside **(4.21)** and 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide **(4.24)**. This proved successful, yielding the alpha-linked disaccharide **(4.26)** {5.56 (H-1', $J_{1',2'}$ = 0.0 Hz) and δ_C = 105.7 ppm (C-1')}. Carbon NMR data verified the formation of the 1,6-linkage (C-6 shifted from 61.4 in **(4.21)** to 66.5 ppm upon coupling). However more than one product was generated due to the presence of two free hydroxyls. To obviate this problem a new acceptor molecule with only the primary alcohol free was prepared, octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)**. The methodology employed in this was the temporary protection of C-6 with a silyl protecting group, protection of all other ring hydroxyls, followed by removal of the silyl protecting group (**Scheme 4.6**).



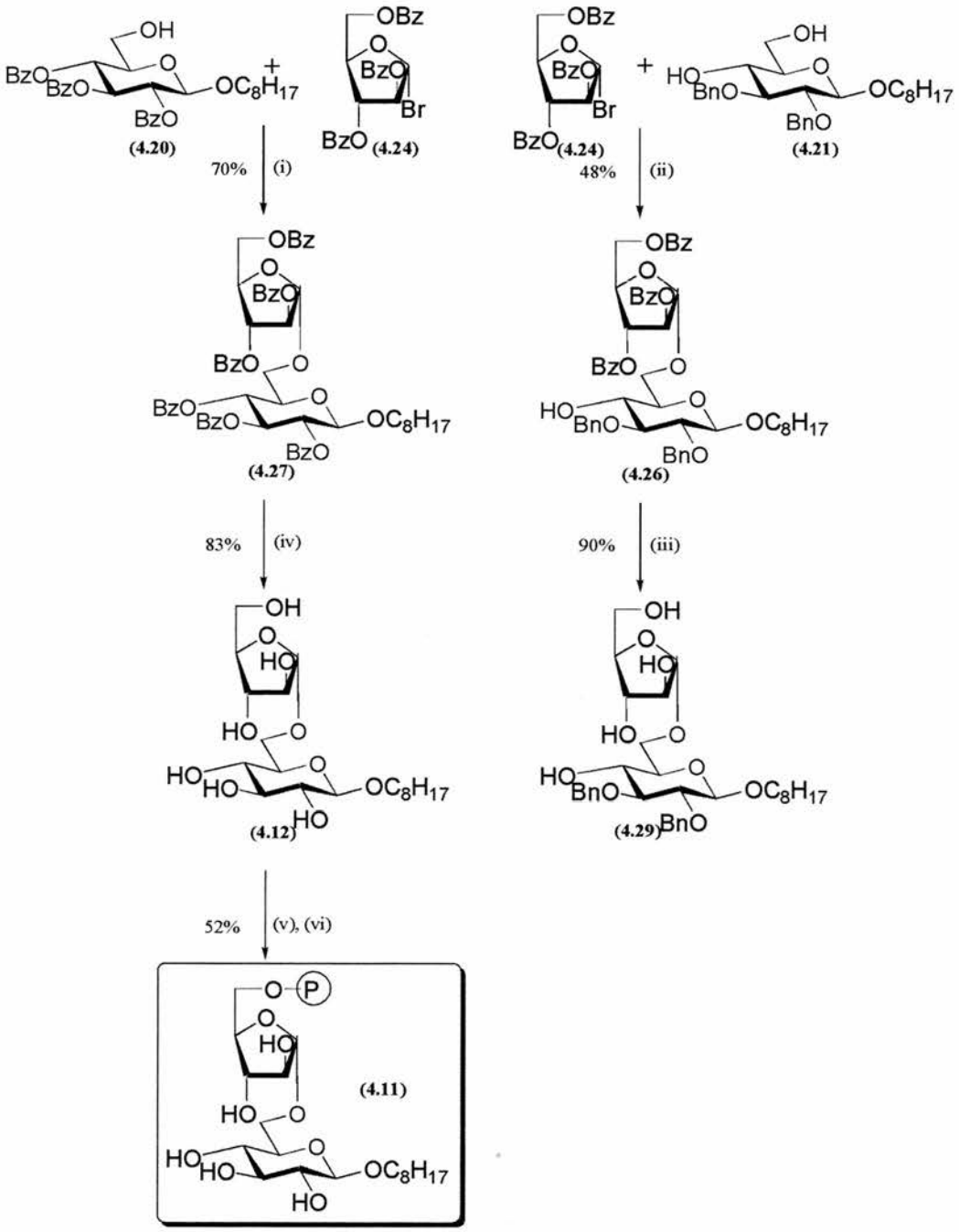
Scheme 4.6: (i) Pyridine, TBDMSCl (1.1 mol eq); (ii) BzCl (3.3 mol eq); (iii) AcOH; **:** (iv) 4 Å mol.sieves, DCM,-30 °C, AgOTf (5 mol eq), collidine (0.5 mol eq); (v) NaOMe/ MeOH;

The advantages conferred by this molecule were three-fold; firstly higher reaction temperatures were used enabling the reaction to proceed quickly. Secondly the disaccharide coupling reaction was clean giving only one product, and thirdly once synthesised and purified the alpha-configured {5.32 (H-1', $J_{1',2'}$ = 0.0 Hz) and δ_C = 105.5 ppm (C-1') protected disaccharide **(4.27)** only required one facile deprotection to yield the completely deprotected disacchaide **(4.12)** (**Scheme 4.7**). Formation of the disaccharide was evidenced by accurate elemental analysis [(Found: C, 70.06; H, 5.76. $C_{61}H_{60}O_{16}$ Requires C, 69.84; H, 5.66%)] and confirmation of the 1,6-linked stereochemistry was obtained from the ^{13}C NMR spectrum. C-6 shifted from 61.5 to 65.6 ppm in the ^{13}C NMR, suggesting that the desired 1,6-linkage was obtained. The last step in the reaction sequence was a phosphorylation step, which proved problematic. Initial reactions involved treating the completely deprotected disaccharide **(4.12)** with 1.1mol eq. of diphenyl chlorophosphate in dry pyridine. The reaction was allowed to stir for 24h. TLC indicated the reaction mixture to contain 100% unreacted starting material. The concentration of phosphorylating agent was next increased from 1.1to 10 mol eq., but the reaction still did not proceed. Repeating the process on a large scale on methyl α-D-arabinofuranoside was observed to proceed more successfully leading to the provision of a 60% overall yield of methyl 5-diphenylphosphoro-α-D-arabinofuranoside **(4.28)**. Formation of **(4.28)** was confirmed by HRMS [*m/z* (ES) 397 ($M+H^+$, 100%); HRMS: Found: 397.1038. $C_{18}H_{21}O_8P$ ($M+H^+$) Requires 397.1052]. Proof of

the linkage was obtained from carbon NMR data. C-5 shifted from 61.9 to 67.9 (1 C, d, $J_{C,P}$ 6.3, C-5) upon coupling.



The reaction was successfully repeated with octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside, **(4.12)**. The keys to this success were found to be the dryness of the solvent and scale of operation, the larger the latter the more successful the outcome. When anhydrous pyridine was used as the solvent (purchased from Aldrich) an increase in activity was observed. It was imperative to prevent atmospheric moisture from contaminating the sample, since the reaction is extremely moisture sensitive. Formation of the phosphate was confirmed by HRMS [*m/z* (ES) 674 ($M+NH_4^+$, 100%); HRMS: Found: 674.2935. $C_{31}H_{49}NO_{13}P$ ($M+NH_4^+$) Requires: 674.2942]. Proof of the linkage was once again obtained from carbon NMR data. C-5' shifted from 63.3 to 69.5 (1 C, d, $J_{C,P}$ 6.3, C-5) upon coupling. Once the primary position of the Ara*f* sugar is phosphorylated, deprotection of the phenyl groups on the phosphate using 10% Pt on C and PtO_2 in acetic acid yielded the 1,6-linked target disaccharide **(4.11)**, **(Scheme 4.7)**.

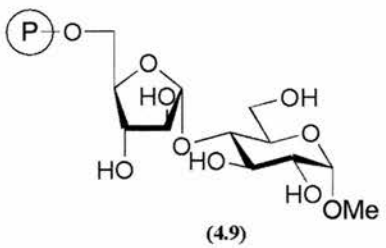


Scheme 4.7: (i) 4 Å mol.sieves, DCM,-30 °C, AgOTf (5 mol eq), collidine (0.5 mol eq); (ii) 4 Å mol.sieves, DCM, ,-70 °C, AgOTf (5 mol eq), collidine (0.5 mol eq); (iii) NaOMe/ MeOH, (iv) NaOMe/ MeOH; (v) Py., $(PhO)_2P(O)Cl$ (1.1 mol eq); (vi) H_2/Pt-C (cat), PtO_2 (cat), AcOH.

4.4 Preparation of 1,4-linked pApp mimic:

4.4.1 Preparation of octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4.30) and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (4.31):

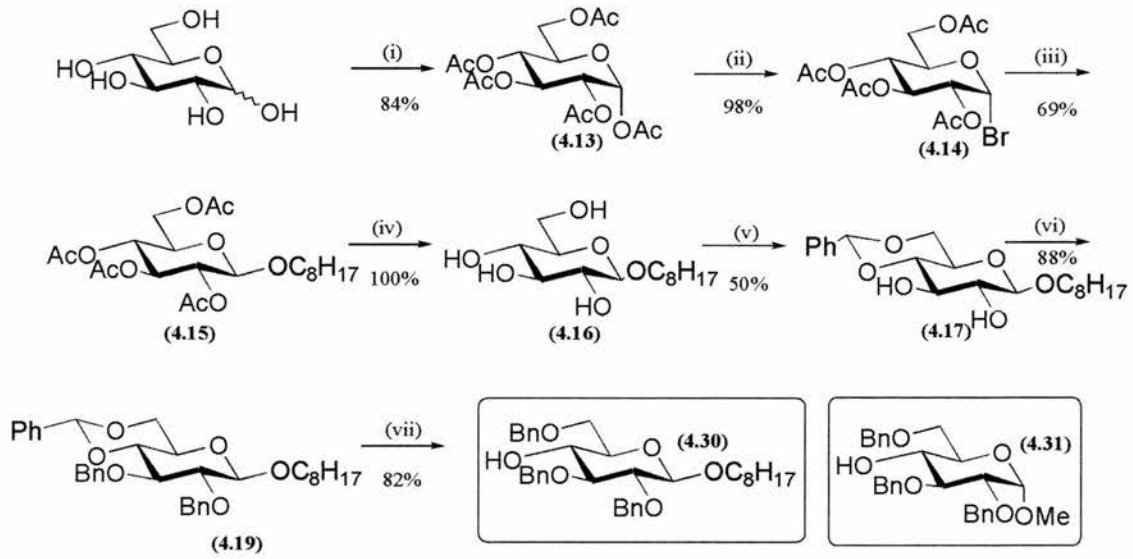
Once the first disaccharide had successfully been synthesised, attention was focused on the generation of a 1,4-linked disaccharide. The 1,4-linkage has the greater biological relevance, since the 1,4-stereochemistry mimics that of the natural substrate pApp.



(4.9)

As with the 1,6-linked disaccharide, 1,4-linked disaccharides required the individual synthesis of two monosaccharide derivatives (donor and acceptor sugars) prior to a coupling step. Two different acceptor sugars were utilised in the formation of the 1,4-arabinofuranosyl glucopyranoside **(4.9)**.

The first acceptor, octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(4.30)** was made from D-glucose. Compound **(4.30)** was consumed for characterisation purposes therefore a second 1,4-linked acceptor sugar, methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[33] **(4.31)** was obtained (Dr. Phil McGurk, University of St. Andrews) and used in all subsequent coupling reactions. Details of the synthesis of **(4.30)** are shown in **Scheme 4.8**.



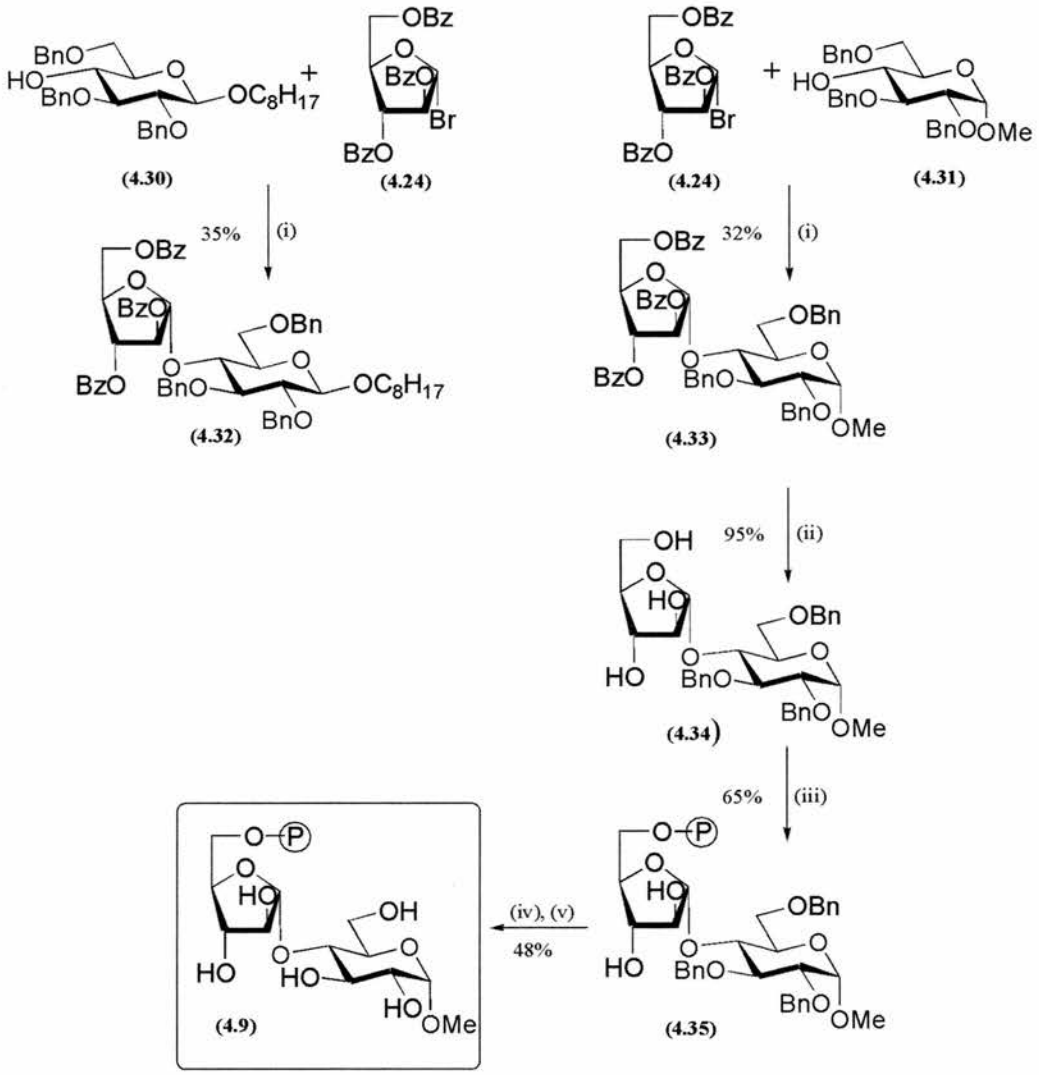
Scheme 4.8: (i) Ac_2O/I_2; (ii) AcOH, HBr.AcOH; (iii) $C_8H_{17}OH$ (1.5 mol eq), $Hg(CN)_2$ (1.2 mol eq) and $Hg(Br)_2$ (0.1 mol eq), MeCN; (iv) NaOMe/MeOH; (v) Benzylidene dimethyl acetal (5.9 mol eq), H^+(cat.), MeCN; (vi) NaH (2.2 mol eq), BnBr (2.2 mol eq), DMF; (vii) TFA (5 mol eq), Et_3SiH, DCM (5 mol eq).

The majority of reactions in the synthesis of the 1,4-linked acceptors have already been detailed in the synthesis of the 1,6-acceptors in section **4.3.1**. Some difficulty was experienced in the selective ring opening of the benzylidene acetal **(4.19)**. Initial attempts using sodium cyanoborohydride (5 mol eq) and TFA (10 mol eq) in DMF proved unsuccessful. TLC indicated the starting material to be left intact in the presence of this combination of reagents. The reaction was repeated with triethylsilane as the electrophile. The acetal then selectively ring opened to generate the desired 4-OH, **(4.30)** product in high yield.

4.4.2 Preparation of 4-*O*-(5-*O*-phospho-α-D-arabinofuranosyl)-α-D-glucopyranoside (4.9):

Two different acceptor sugars, **(4.30)** and **(4.31)** were used in the course of the synthesis of the 1,4-linked disaccharide target material **(4.9)**. The first acceptor to be used was the β-linked octyl glucopyranoside **(4.30)**. As before (see **Table 4.1**, section **4.3.3**) initial couplings with glycosyl halide donor **(4.24)** in the presence of mercury salts were unsuccessful. In

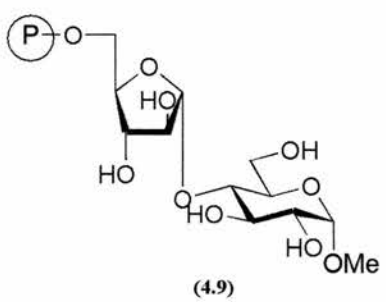
order to overcome these problems the reaction conditions were once again varied. An assortment of promoters, solvents and temperature were used and after numerous trial reactions optimum conditions were established. Coupling was optimized when three equivalents of donor sugar, one equivalent of acceptor sugar and Drierite in DCM were allowed to stir at room temperature in a light free atmosphere. The reaction mixture was then cooled to –20 °C and five equivalents of AgOTf were quickly added.



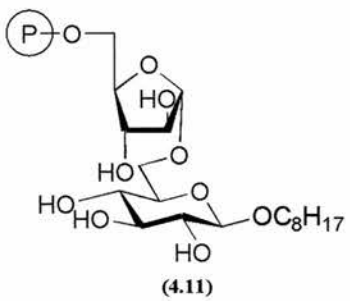
Scheme 4.9: (i) 4 Å mol.sieves, DCM, -20 °C, AgOTf (5 mol eq), collidine (0.5 mol eq); (ii) NaOMe/ MeOH, (iii) Py., $(PhO)_2P(O)Cl$ (1.1 mol eq); (iv) H_2, 10% Pd-C, AcOH; (v) H_2, 10% Pt-C (cat), PtO_2 (cat), AcOH.

Close monitoring by TLC permitted the reaction to be quenched with collidine immediately the acceptor disappeared. Neutralisation and purification yielded the alpha-methyl and beta-octyl glycosides **(4.33)** and **(4.32)**, respectively. Accurate elemental analysis gave proof of their formation {**(4.33)**, Found: C, 71.10; H, 5.80. $C_{54}H_{52}O_{13}$ Requires C, 71.35; H, 5.77%, **(4.32)**, Found: C, 72.54; H, 7.15. $C_{61}H_{66}O_{13}.H_2O$ Requires: C, 72.60; H, 6.79%. As before, the stereochemistry of **(4.32)** and **(4.33)** was assigned on close examination of proton and carbon NMR spectra {**(4.32)**, 5.52 (H-1', $J_{1',2'}$ = 0.0 Hz) and δ_C = 106.7 ppm (C-1'), and **(4.33)**, 5.55 (H-1', $J_{1',2'}$ = 0.0 Hz) and δ_C = 106.9 ppm (C-1'). All of the octyl 1,4-linked acceptor **(4.30)** sugar was consumed in optimising these coupling procedures and in characterisation. The fully protected 1,4-linked disaccharide, octyl 4-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(4.32)** was not deprotected due to the generation of insufficient quantity of material. Only one deprotected 1,4-linked disaccharide was generated, namely methyl 4-*O*-(α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(4.34)**.

The last step in the pathway involved phosphorylation of arabinofuranose primary alcohol. Due to the abundance of free hydroxyls and the moisture sensitive nature of the phosphorylating agent this proved to be a troublesome reaction. Due to the greater reactivity of primary alcohols over secondary alcohols the protected precursor **(4.34)** was phosphorlyated. Optimum phosphorylation conditions were established (see section **4.3.3**) and then employed to generate the phosphate **(4.35)**. Formation of the phosphate was confirmed by HRMS [*m/z* (ES) 846 ($M+NH_4^+$, 100%); HRMS: Found: 846.3222. $C_{45}H_{53}NO_{13}P$ ($M+NH_4^+$) Requires 846.3255]. Proof of the linkage was once again obtained from carbon NMR data. C-5' shifted from 61.4 to 69.4 (1 C, d, $J_{C,P}$ 10.3, C-5) upon coupling. Final deprotection steps using H_2, 10% Pd-C and then H_2, 10% Pt-C and Adams catalyst yielded the phosphorylated target compound **(4.9)**.



(4.9)



(4.11)

pApp mimics **(4.9)** and **(4.11)** are currently under biological evaluation. It is hoped that the results from the biological assays will provide an insight as to whether or not pApp is an intermediate in DpA biosynthesis.

4.5 Preparation of 1,6-linked pRpp mimic:

One molecule which is definitely an intermediate in DpA biosynthesis is pRpp. In consequence of the success obtained in synthesising two pApp mimics using a glucose sugar residue in place of the pyrophosphate the next logical step was to repeat the approach using ribose in place of arabinose, i.e. using the same approach to generate pRpp mimics. The advantage with this set of compounds is that pRpp is a known intermediate, where as pApp is a putative intermediate. The results of the biological assays of these series of compounds (pApp and pRpp mimics) should provide a great insight in to the biosynthetic route to DpA.

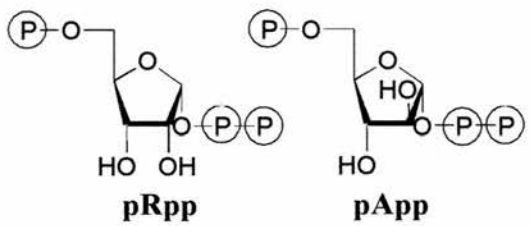


Figure 4.9: pRpp, known intermediate in DpA biosynthesis and pApp, putative intermediate in DpA biosynthsis.

As was the case previously with octyl 6-*O*-(5-*O*-phospho-α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.11)** and methyl 4-*O*-(5-phospho-α-D-arabinofuranosyl)-α-D-glucopyranoside **(4.9)** a ribofuranose-glucopyranose disaccharide would require the synthesis of two individual monosaccharide derivatives (donor and acceptor sugars), which could then be brought together in a coupling step to yield a protected disaccharide. Deprotection and phosphorylation steps would lead to our final target compounds **(4.37)** and **(4.38)**. There was one major difference in this case, the target compound **(4.37)** is a 1,2-*cis* glycoside, whereas the pApp analogues **(4.9)** and **(4.11)** are 1,2-*trans* glycosides. Such 1,2-*cis* glycosides are

much more difficult to generate, consequently it was decided to attempt the synthesis of the more straight forward 1,6-linked glycoside **(4.38)** as an initial step.

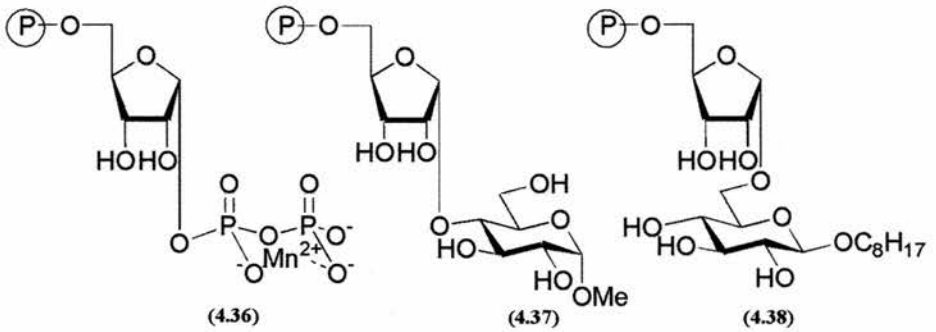


Figure 4.10: pRpp/ Mn^{2+} complex **(4.36)** and glucose complexes as pyrophosphate mimics **(4.37)** and **(4.38)**.

4.5.1 Strategy for the preparation of octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside (4.38):

The target molecule (1,6-linked disaccharide) **(4.38)** is composed of ribofuranose and glucopyranose motifs. These monosaccharides (donor and acceptor sugars) were prepared independently via a series of protecting group interconversions. A vital coupling step could yield a protected disaccharide product. The main obstacle to overcome in the synthesis of this desired 1,6-linked riboside **(4.38)** is the generation of the 1,2-*cis* stereochemistry. These 1,2-*cis* glycosides are reputed to be notoriously difficult to generate and for this reason an attempt was made to synthesise the target compound **(4.38)** by a couple of different methods.

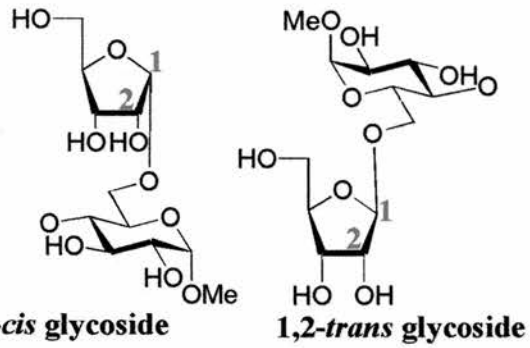
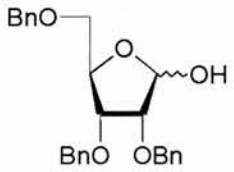


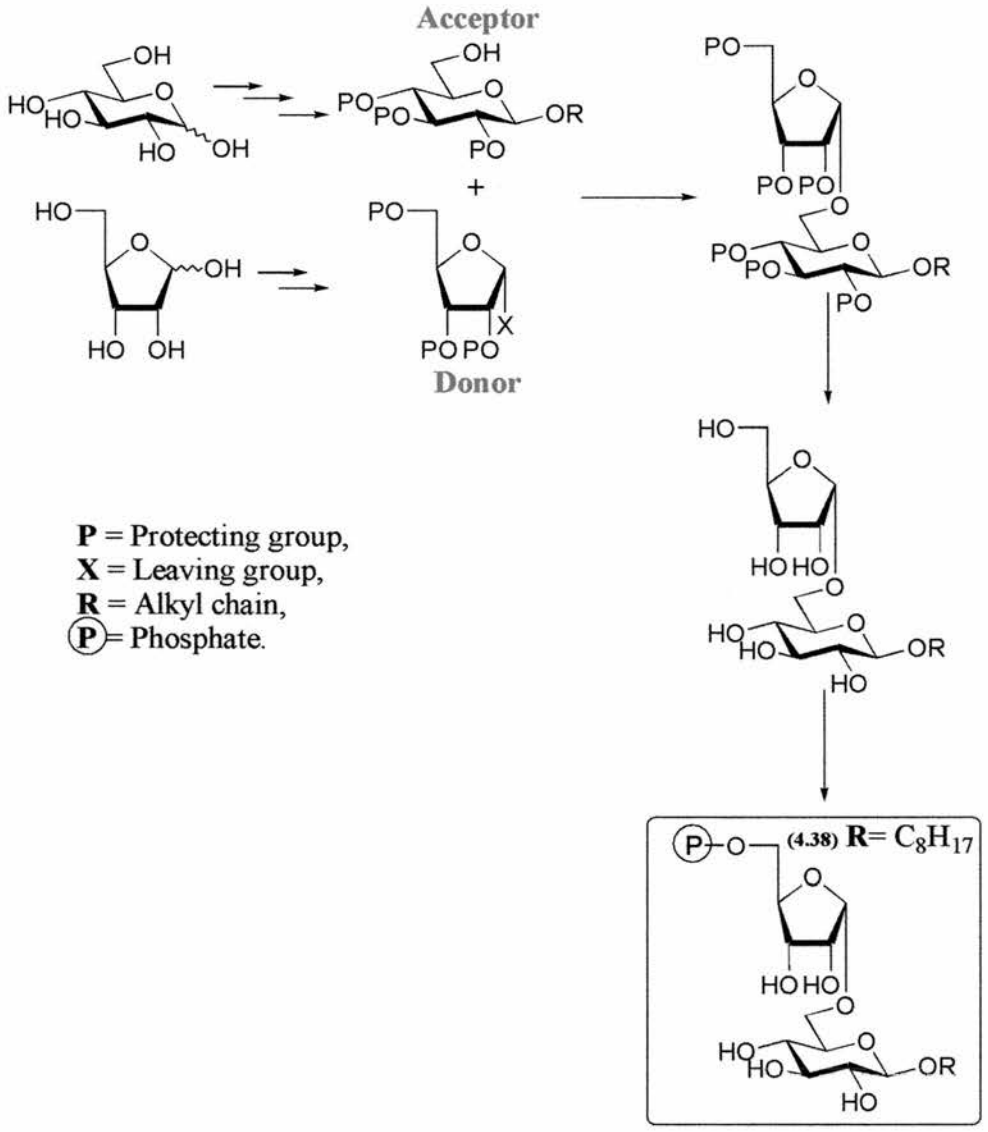
Figure 4.11: 1,2-*cis* and 1,2-*trans* glycosides.

Reports in the literature suggest that benzylated 1,2-*cis* ribofuranosides are readily obtainable by the use of promotors such as diphenyltin sulfide,[34] silver hexafluoroantimonate,[35] [catecholato(2-)-*O,O'*]oxotitanium,[36] lithium bis[(trifluoromethyl)sulfonyl]imide,[37] and tin trifluoromethanesulfonate and trimethylsilyl chloride.[38] Given that all these promotors are rare, and in most cases would need to be synthesised, none of these protocols was followed. An alternative synthesis of the target compound **(4.38)** was attempted.



(4.41)

The first coupling method was a repeat of the previously successful silver triflate coupling (see section **4.3.3**). This procedure was repeated in the expectation that the harsh conditions generated would lead to the formation of at least some of the desired 1,2-*cis* glycoside (**Scheme 4.10**).

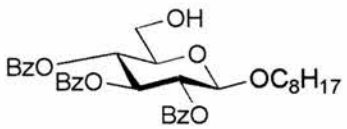


Scheme 4.10: Method 1 for the preparation of octyl 6-*O*-(2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside.

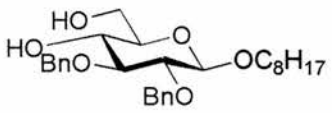
Should this prove unsuccessful a second coupling method that could be used, would be the direct 1-*O*-alkylation of the ribofuanose **(4.41)** (see **Scheme 4.11**). Deprotection of the Rib*f* protecting groups would then lead to the formation of a semi-protected disaccharide. Further deprotection and phosphorylation steps would generate the 1,6-disaccharide product, octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside **(4.38)**, **(Scheme 4.10)**.

4.5.2 Preparation of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.20) and octyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (4.21):

Two different acceptor sugars **(4.20)** and **(4.21)** were used in the formation of the 1,6-ribofuranosyl glucopyranoside containing disaccharide. Both were derived from D-glucose (see **Scheme 4.4**). Details of the synthesis of these acceptors have already been outlined in section **4.3.1**.



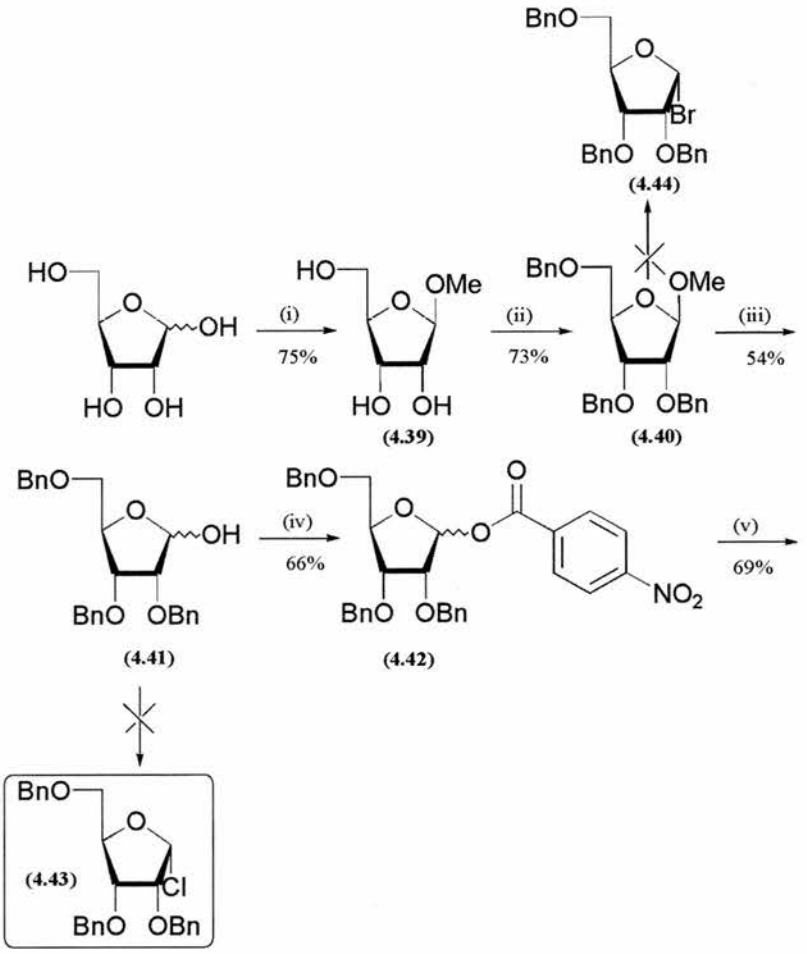
(4.20)



(4.21)

4.5.3 Preparation of 2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl chloride (4.43):

The donor sugar synthesised was the glycosyl chloride 2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl chloride, **(4.43)**. Benzoyl protecting groups could not be used due to the neighbouring group effect at C-2, this prompted use of benzyl ether protecting groups. The disadvantage with such protecting groups is that they are armed, and thus make the sugar more reactive and therefore more difficult to handle. For this reason we chose chloride as our leaving group. Chloride, although less reactive than bromide, is still reactive enough to act as an effective leaving group. D-Ribose was treated with sulphuric acid and anhydrous methanol to generate the known[39,40] beta-linked ($J_{1,2}$ = 0.0 Hz.) methyl glycoside **(4.39)** [mp 78 - 80°C, lit., [39,40] 79 - 80°C; $[\alpha]_D$ -49.3, lit., [39,40] $[\alpha]_D$ -50.0]. For reasons outlined already the methyl glycoside was then protected with benzyl ether protecting groups. Attempts to make the bromide, 2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl bromide **(4.44)** from the protected methyl glycoside **(4.40)** using HBr.acetic acid proved unsuccessful. Similarly attempts to make the chloride, 2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl chloride **(4.43)**, from the protected methyl glycoside using DCMME and zinc chloride also proved unsuccessful. The protected methyl glycoside **(4.40)** was then treated with 80 % aq. TFA in DCM to generate the hemiacetal **(4.41)**{C-1 (α and β) of 95.6 and 99.7 ppm and MALDI-TOF (+ve): *m/z*443.2 $(M+Na)^+$, ($C_{26}H_{28}O_5Na$ requires *m/z* 443.2)}.

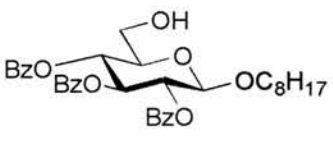


Scheme 4.11: (i) MeOH, H_2SO_4; (ii) NaH (3.3 mol eq), BnBr (3.3 mol eq), DMF; (iii) 80% aq. TFA, DCM; (iv) Py., DCM, *p*-NO_2-BzCl (1.1 mol eq); (v) DCM, HCl.

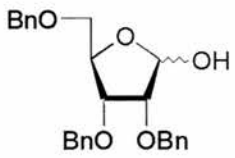
Several attempts to make the desired chloride, 2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl chloride **(4.43)** directly from the hemiacetal using oxalyl chloride in DMF/DCM,[41] and DCMME[42] were also unsuccessful. Next the hemiacetal **(4.41)** was dissolved in anhydrous DCM and treated with pyridine and *p*-nitrobenzoyl chloride. This resulted in the formation of the known anomeric nitrobenzoate **(4.42)**[43,44] {C-1 (α and β) of 96.1 and 100.3 ppm and *m/z* (ES) 587 ($M+NH_4^+$, 100%); HRMS: Found: 587.2393. $C_{33}H_{35}N_2O_8$ ($M+NH_4$) Requires 587.2393.}. Compound **(4.42)** was then dissolved in DCM and a treated with dry HCl gas, yielding **(4.43)**[43] ($J_{1,2}$ = 0.0 Hz, C-1, 95.6 ppm).

4.5.4 Strategy for the preparation of octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside (4.38):

As already mentioned, it was intended to make the desired 1,2-*cis* glycoside, octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside **(4.38)** by two different methods. The first method involved repeating the silver triflate coupling chemistry on the ribofuranosyl donor 2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl chloride, **(4.43)**, and standard glycosyl acceptor octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside, **(4.20)**.



(4.20)

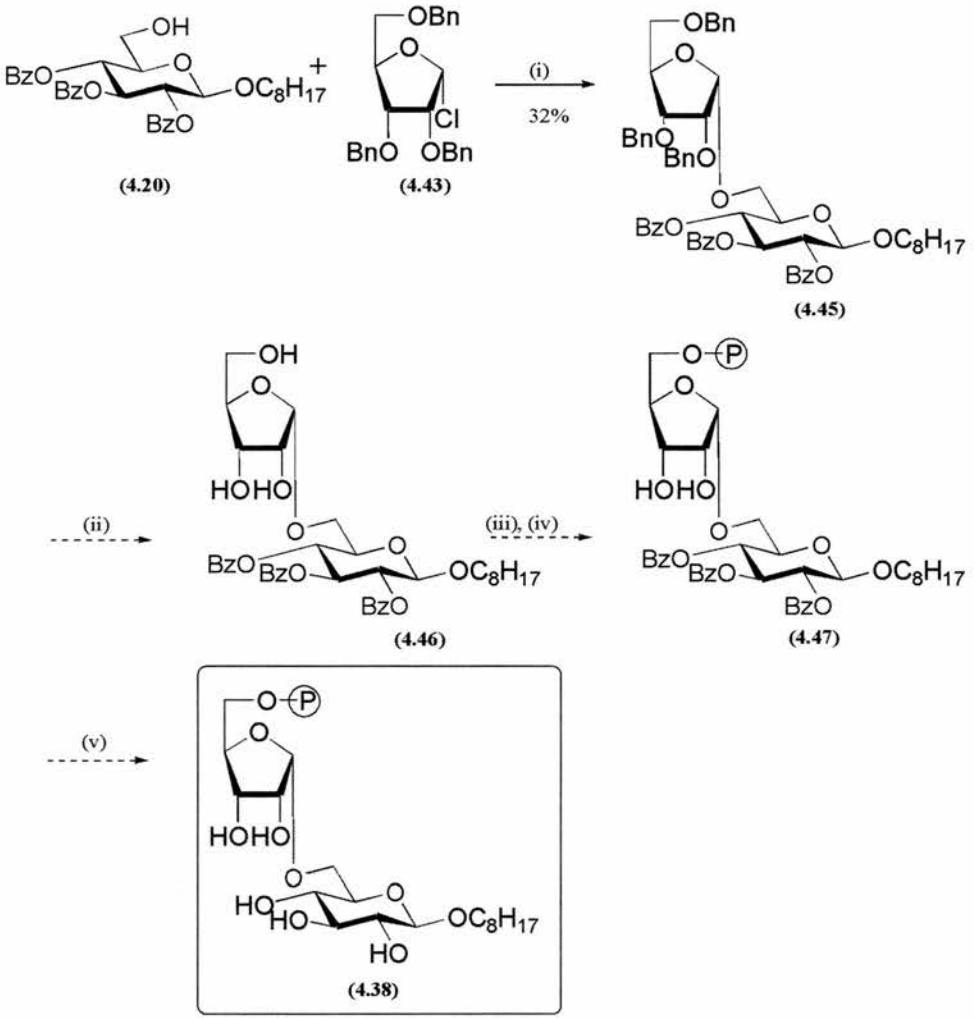


(4.41)

See paragraph **4.5.4.1**. The second method, direct 1-*O*-alkylation of the ribo hemiacetal **(4.41)**, is outlined in **4.6.4**.

4.5.4.1 Method 1 for the preparation of octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside (4.38):

Initial attempts to couple glycosyl donor **(4.43)** and glycosyl acceptor **(4.20)** together proved very successful. Not only did the coupling work (*m/z* (ES) 1024 ($M+NH_4^+$, 100%); HRMS: Found: 1024.4849. $C_{61}H_{70}NO_{13}$ ($M+NH_4$) Requires 1024.4847) but the stereochemistry of the reaction was also in favour of the desired alpha anomer, i.e. unexpectedly the coupling showed a preference for the generation of the 1,2-*cis* glycoside. The alpha:beta ratio for the silver triflate coupling was 3:1. The anomeric configuration of the glycoside products was determined by comparison of the coupling constants of their anomeric protons,[45,46,47] {4.99 (1 H, d, $J_{1',2'}$ 4.0, H-1'(α); 4.98 (1 H, s, H-1' (β)}, chemical shift of the anomeric carbon in the ^{13}C NMR[48] {101.4 (C-1' (α),105.9 (C-1' (β)} and of their optical rotations {$[\alpha]_D$ +33.2° (*c* 0.25, $CHCl_3$) (α), $[\alpha]_D$ +25.0° (*c* 0.1, $CHCl_3$) (β)}.[46,49]



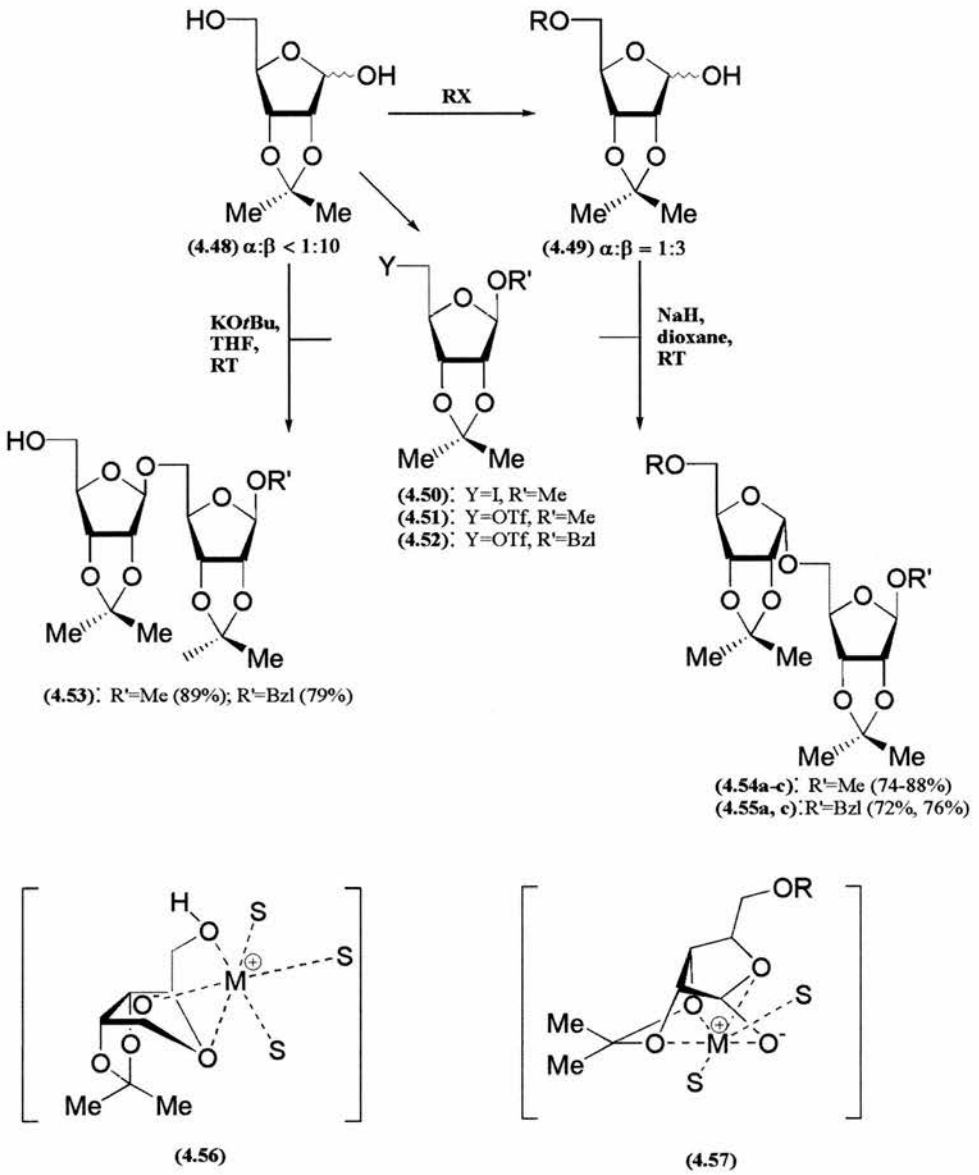
Scheme 4.12: (i) 4 Å mol.sieves, DCM,-30 °C, AgOTf (5 mol eq), collidine (0.5 mol eq); (ii) H_2, 10% Pd-C, AcOH; (iii) Py., $(PhO)_2P(O)Cl$ (1.1 mol eq); (iv) H_2/Pt-C, PtO_2, AcOH; (v) NaOMe/ MeOH.

Time constraints prevented the completion of the synthesis of the desired disaccharide, octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside **(4.38)**.

4.6 Direct 1-*O*-alkylation of D-ribofuranose:

The direct 1-*O*-alkylation with alkylating agents such as, methyl iodide has long been known.[50-57] The stereochemical outcome of the alkylation, i.e generation of α or β glycosides is dependent on reaction conditions. The percentage yield, regio and stereo-control are

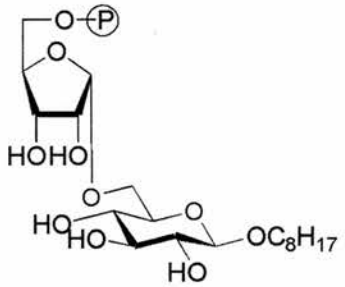
influenced by three factors: 1. the stability of the anion generated, 2. the ring-chain tautomeric equilibrium and related dynamics and 3. the relative reactivites of the three anionic species. Recent work by Schmidt[31,58-61] has shown that the stereochemical outcome of these direct 1-*O*-alkylations can be controlled by the use of bulky protecting groups and the variation of reaction conditions. Schmidt used **(4.50)** as the glycosyl donor, **(4.49a)** as the acceptor and various bases for deprotonation of **(4.49a)**. However the system was not sufficiently reactive, so the iodide leaving group at the primary position of **(4.50)** was replaced with a triflate. Two new glycosyl donors, **(4.51)** and **(4.52)** with triflaes at C-5 were generated. Treatment of glycosyl acceptor **(4.48)** with one mole equivalent of base and glycosyl donors **(4.51)** and **(4.52)** resulted in the excluive generation of β-glycosides **(4.53)** in excellent yield.[61] Schmidt initially reasoned that this outcome was due to the preference of **(4.48)** for adopting the β-furanose form and to the higher acidity of the anomeric hydroxyl group in comparison with the primary hydroxyl group at position C-5. Schmidt then dismissed that explanation, as when bulky protecting groups were introduced at the primary position of the protected ribofuranose **(4.49a-c)**, alkylation with glycosyl triflates afforded the α-glycosides **(4.54a-c)** and **(4.55a-c)** exclusively, even though the β-anomer predominates in the 1-*O*-protonated species. It was suggested that in addition to steric effects at C-5 another influencing factor on the stereochemical outcome of the alkylation is the ability of the glycosyl oxide to form differing intramolecular complexes **(4.56)** and **(4.57)** with metal ions.[61]



Scheme 4.13: a: R= trityl, **b:** R= methoxytrityl, **c:** R= tert-butyldimethylsilyl. S (in **(4.56)** and **(4.57)**) = solvent molecule.

The effect of the intramolecular metal ion complexation was further substantiated by investigations on protected D-mannofuranoses.[58,60]

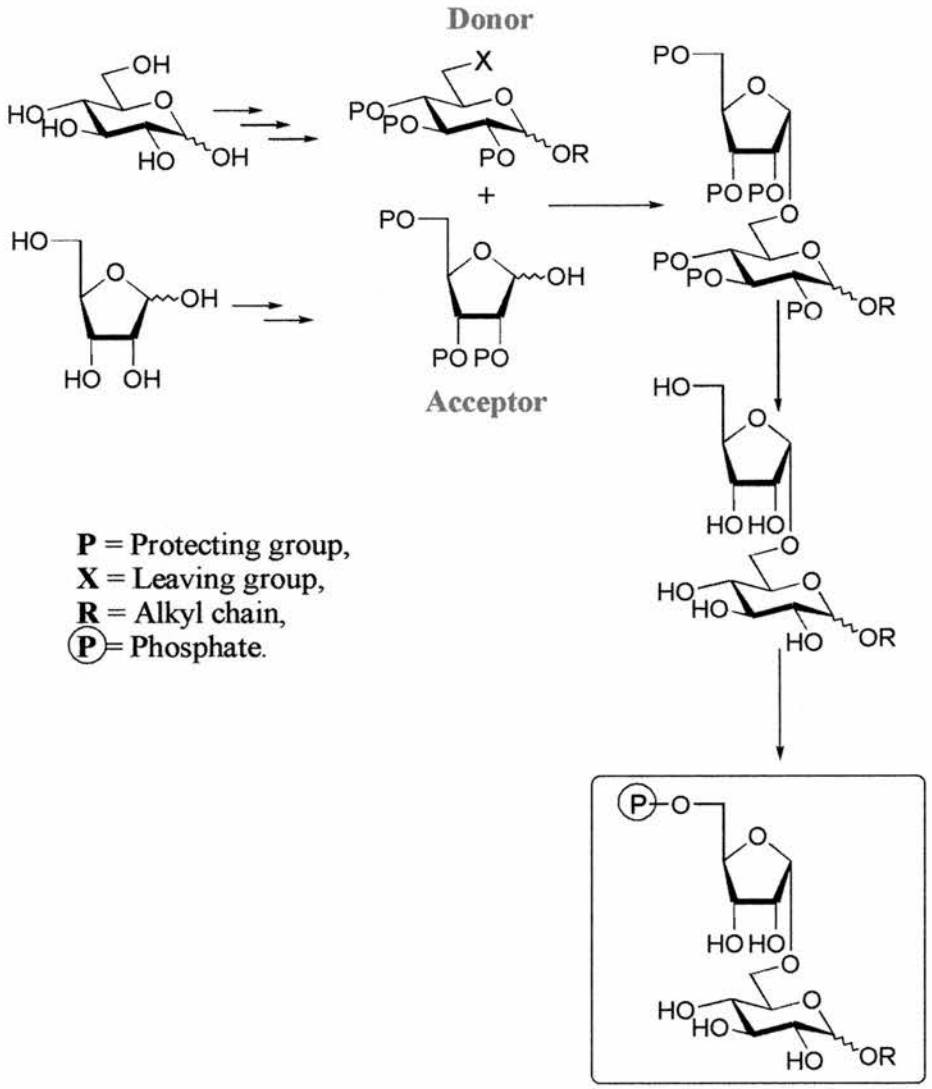
The results of the D-ribofuranose and D-mannofuranose experiments suggest that it may be possible to generate our desired 1,2-*cis* riboside, octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside **(4.38)** without the use of halogenoses and heavy metal salt catalysts.



(4.38)

4.6.1 General strategy for the preparation of octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside (4.38) via direct 1-*O*-alkylation:

Stereo, electronic and anomeric effects favour the formation of 1,2-*trans* glycosides.[62] In order to form the "unfavourable" 1,2-*cis* arrangement these problems need to be solved. One possible way in which this could be achieved would be to use a donor equipped with a good leaving group, such as a triflate, together with an acceptor and a base that induces a "crown ether effect".

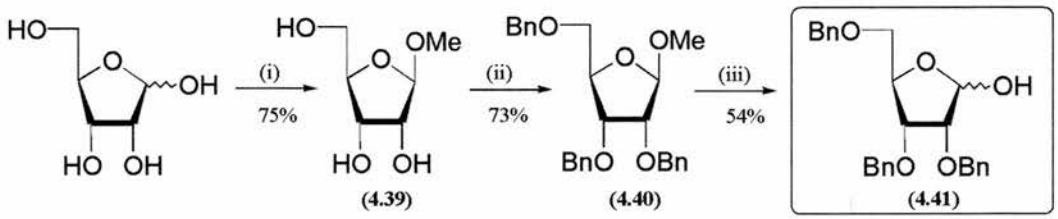


Scheme 4.14: Method 2 for the preparation of octyl 6-*O*-(2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside.

The work done by Schmidt[31] has shown that certain bases such as sodium iodide/sodium hydride can induce this "crown ether effect" resulting in the formation of a distorted anion intermediate. This intermediate then has the correct stereochemistry to react with the donor to generate the desired 1,2-*cis* glycoside product.

4.6.2 Preparation of 2,3,5-tri-*O*-benzyl-D-ribofuranose (4.41):

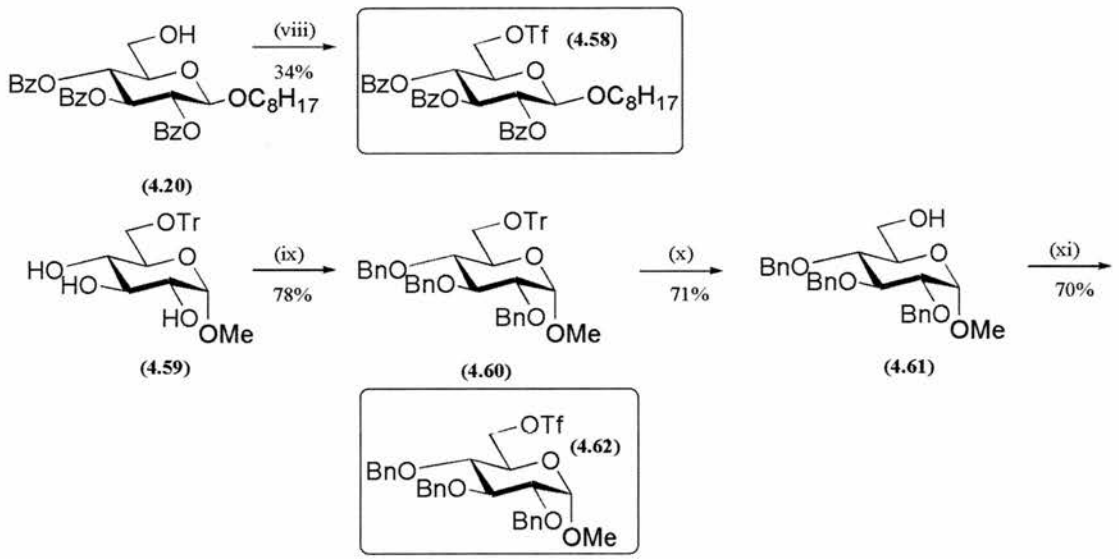
D-Ribose was treated with sulphuric acid and anhydrous methanol to generate the beta configured ($J_{1,2}$ = 0.0 Hz) methyl glycoside, methyl β-D-ribofuranoside **(4.39)**. The methyl glycoside **(4.39)** was then armed with benzyl ether protecting groups and the protected methyl glycoside **(4.40)** was then treated with 80 % aq. TFA in DCM to generate the hemiacetal ({C-1 (α and β) of 95.6 and 99.7 ppm}) acceptor sugar 2,3,5-tri-*O*-benzyl-D-ribofuranose **(4.41)**.



Scheme 4.15: (i) MeOH, H_2SO_4; (ii) NaH (3.3 mol eq), BnBr (3.3 mol eq), DMF; (iii) 80% aq. TFA, DCM.

4.6.3 Preparation of methyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside (4.62) and octyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside (4.58):

Two donor sugars **(4.58)** and **(4.62)** were synthesized, both were glucose derivatives that contained a triflate group at C-6. The first donor to be synthesised was octyl 2,3,4-tri-*O*-benzoyl-6-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside **(4.58)**. Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside **(4.18)** was treated with AcOH. This resulted in deprotection of the silyl protecting group and the generation of the octyl glycoside **(4.20)**.

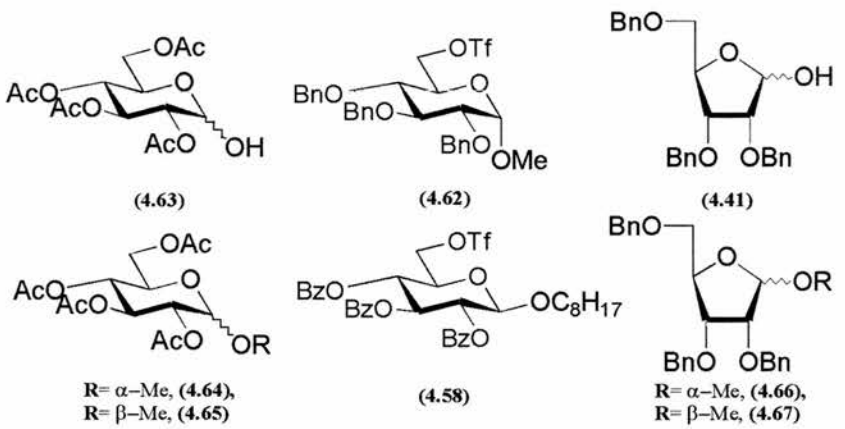


Scheme 4.16: (i) 2,6-di-t-butyl-4-Me-pyridine (1.2 mol eq), DCM, triflic anhydride (1.1 mol eq); (ii) NaH (3.3 mol eq), BnBr (3.3 mol eq), DMF; (iii) 80% TFA, DCM; (iv) 2,6-di-t-butyl-4-Me-pyridine (1.2 mol eq), DCM, triflic anhydride (1.1 mol eq).

The final step in the synthesis of octyl 2,3,4-tri-*O*-benzoyl-6-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside was trifluoromethanesulfonylation of the primary alcohol. This proved to be the most challenging step in the reaction sequence. Initial attempts to generate the triflate involved the use of triflic anhydride and pyridine as the base. Complete degradation was observed, the subsequent black resultant being conjectured as attributable to a pyridinium salt arising from displacement of triflate from **(4.58)**. Only when the reaction temperature was lowered to -20°C and a more bulky non nucleophilic base used (2,6-di-t-butyl-4-methylpyridine)[63] was the reaction successful (C-6 shifted from 62.9 to 69.3 ppm in the ^{13}C NMR spectrum). The second donor to be synthesised was methyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside **(4.62)**. Tritylated gluco derivative, methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside (which was kindly donated by Dr. J. D. Hall, University of East Anglia) **(4.59)** was benzylated using a standard benzylation procedure.[64] The primary alcohol of the benzylated moiety **(4.60)** was then deprotected using an 80% aq. TFA solution. The second donor, methyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside **(4.62)** was obtained by trifluoromethanesulfonylation of the primary alcohol in an identical manner as before.

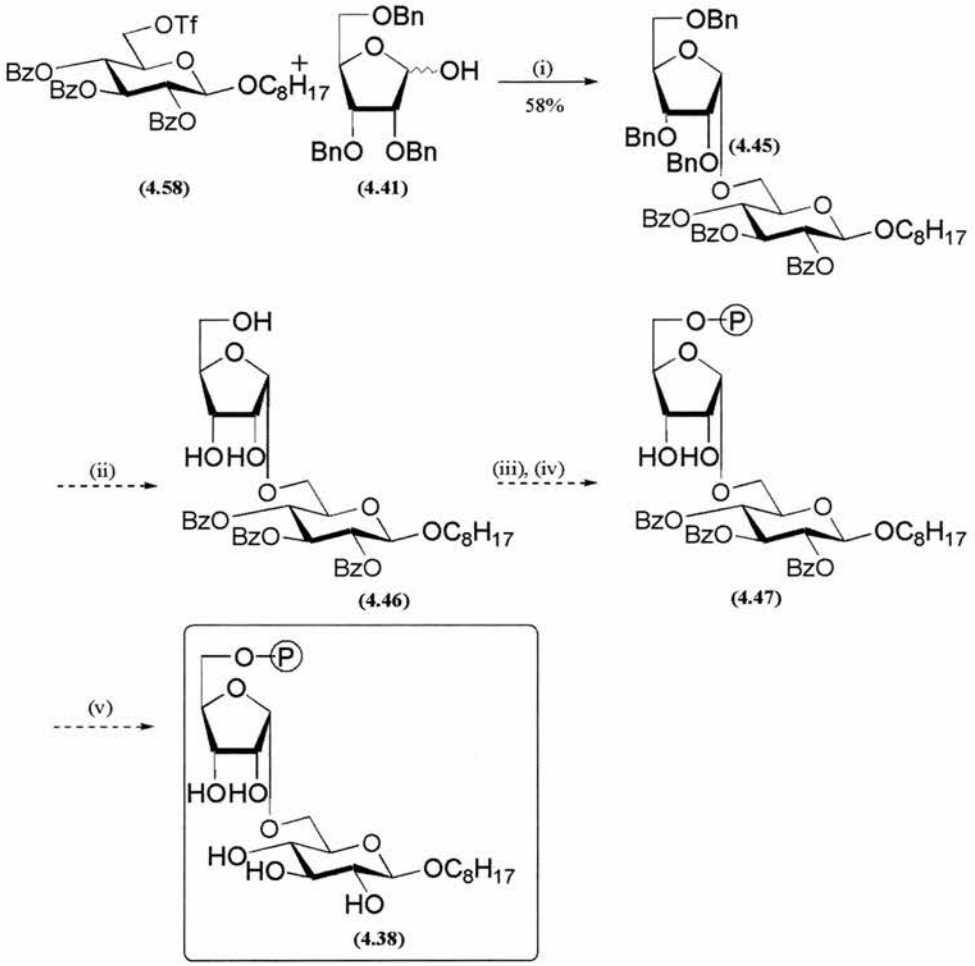
4.6.4 Method 2 for the preparation of octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside (4.38):

A series of trial reactions were set up to establish the optimum coupling conditions. Initially methyl triflate and methyl iodide were the model glycosyl donors of choice. The glycosyl acceptor employed was 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose **(4.63)**. Anhydrous THF and 1,4-dioxane were the solvents used. The acceptor sugar was dissolved in the solvent of choice, cooled (0 °C) and treated with five equivalents of base (sodium hydride). The reaction mixture was allowed to stir for 15 min. Once more the mixture was cooled to (0 °C) and an equimolar amount of donor sugar (methyl triflate) was added. TLC monitored the progress of the reactions, which proved successful in THF but not in dioxane. The reaction was adjudged a success by virtue of a TLC comparison with authentic standards of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside **(4.64)** and methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **(4.65)**. The procedure was repeated in THF using the hemiacetal 2,3,5-tri-*O*-benzyl-D-ribofuranose as glycosyl acceptor **(4.41)** and methyl triflate as glycosyl donor. TLC comparison with authentic standards of methyl 2,3,5-tri-*O*-benzyl-α-D-ribofuranoside **(4.66)** and methyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranoside **(4.67)** verified that the reaction was similarly successful.



Next, analytical scale couplings were performed using methyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside **(4.62)** as the donor sugar (due to the greater quantity of material), and 2,3,5-tri-*O*-benzyl-D-ribofuranose **(4.41)** as glycosyl acceptor. THF was the solvent and sodium hydride the base. The reaction was again followed by TLC

and observed successful, due to the formation of an anomeric mixture of methyl 6-*O*-(2,3,5-tri-*O*-benzyl-D-ribofuranosyl)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranosides. The procedure was repeated preparitively using the other triflate donor, octyl 2,3,4-tri-*O*-benzoyl-6-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside **(4.58)** in the place of methyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside **(4.62)**. The hemiacetal acceptor **(4.41)** was dissolved in a minimum amount of anhydrous THF, reaction mixture was cooled to 0 °C and 5 mol eq. of sodium hydride base was added.



Scheme 4.17: (i) NaH (5 mol eq), THF, 0 °C; (ii) H_2, 10% Pd-C, AcOH; (iii) Py., $(PhO)_2P(O)Cl$ (1.1 mol eq); (iv) H_2, 10% Pt-C, PtO_2, AcOH; (v) NaOMe/ MeOH.

The octyl triflate donor sugar **(4.58)** was added and the progress of the reaction followed by TLC. The crude material was neutralised and then purified by column chromatography. Yields of the desired disaccharides were poor, however both alpha and beta anomers were generated, with the beta disaccharide being the preferred anomer by a ratio of 3:1, β:α. The anomeric configuration of the glycoside products was determined by comparison of the coupling constants of their anomeric protons,[45,46,47] {4.99 (1 H, d, $J_{1',2'}$ 4.0, H-1'(α); 4.98 (1 H, s, H-1' (β)}, chemical shift of the anomeric carbon in the ^{13}C NMR[48] {101.4 (C-1' (α),105.9 (C-1')} and of their optical rotations {$[\alpha]_D$ +33.2° (*c* 0.25, $CHCl_3$) (α), $[\alpha]_D$ +25.0° (*c* 0.1, $CHCl_3$) (β)}.[46,49] Due to time constraints the remaining synthetic steps were not completed.

4.7 Synthesis of arabinose and glucose containing disaccharides:

There was disagreement between optical rotation values of synthetic **(4.12)** and the literature (synthetic $[\alpha]_D$ +25.7, lit.,[9] $[\alpha]_D$ -75.0). Kim and co-workers suggested the closely related arabinopyranoside **(4.68)** to be a known compound.[9] Closer investigation indicated an error in the literature. Octyl 6-*O*-(α-D-arabinopyranosyl)-β-D-glucopyranoside **(4.68)** is in fact not known, whereas octyl 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranoside **(4.69)** is a naturally occuring compound.[65] These findings, together with the disagreement in optical rotations, encouraged us to synthesise the closely related known compound, arabinopyranoside **(4.69)** and compare optical rotations.

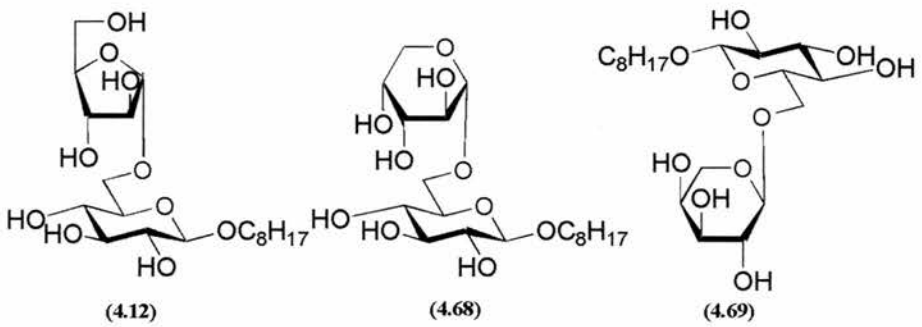
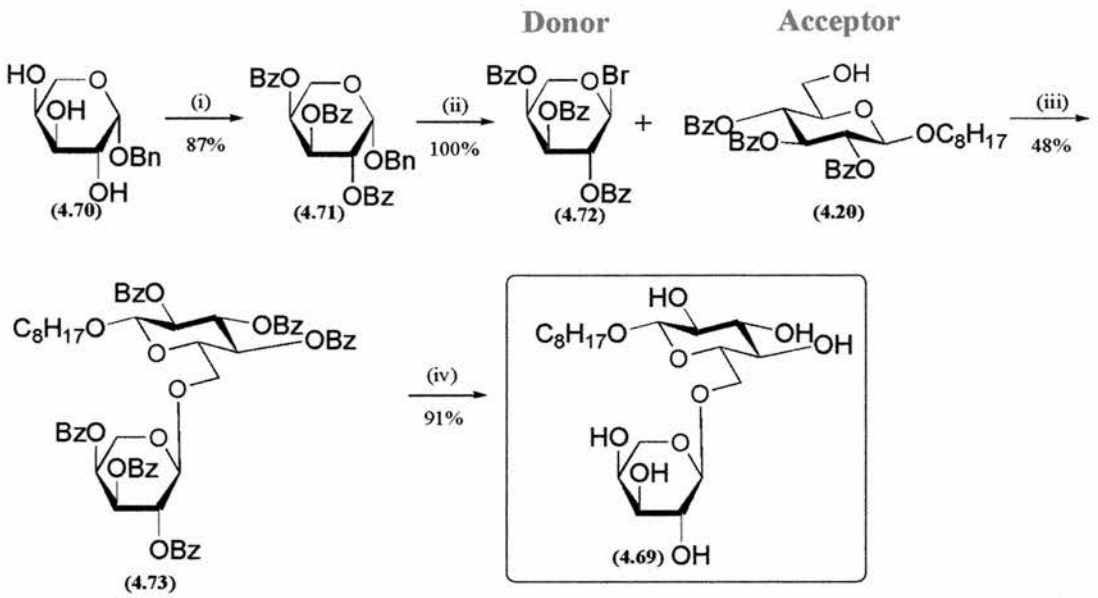


Figure 4.12: Octyl 6-O-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)**, octyl 6-O-(α-D-arabinopyranosyl)-β-D-glucopyranoside **(4.68)** and octyl 6-O-(α-L-arabinopyranosyl)-β-D-glucopyranoside **(4.69)**.

4.7.1 Preparation of octyl 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranoside (4.69):

As usual, the synthesis of this disaccharide, octyl 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranoside **(4.69)** required the synthesis of two individual monosaccharides (donor and acceptor) which were then brought together in a coupling step to yield a protected disaccharide **(4.73)**. Further deprotection steps lead to the generation of the desired disaccharide product **(4.69)**. Dr. A. H. Haines (University of East Anglia) kindly donated compound **(4.70)**[66,67] which was protected in high yield using pyridine and benzoyl chloride. The protected compound **(4.71)** was then treated with HBr.acetic acid leading to the

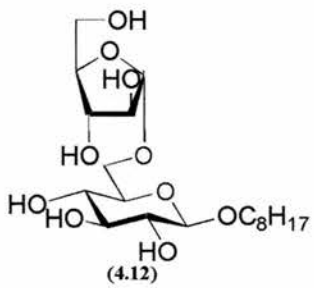
formation of the alpha-configured donor sugar **(4.72)**.[68,69] Formation of the glycosyl halide was evidenced by diagnostic changes in both proton and carbon spectra. A chemical shift for C-1 of 89.8 ppm (C-1 of **(4.72)** appeared at 96.2 ppm) and a $J_{1,2}$ coupling constant of 3.8 Hz for H-1 were observed. These shifts in NMR values, together with an optical rotation which was in close agreement with the literature ($[\alpha]_D$ +207.1, lit., [68] $[\alpha]_D$ +203) and accurate combustion analysis ((Found: C, 59.44; H, 4.09; Br, 15.13. $C_{26}H_{21}BrO_7$ Requires: C, 59.44; H, 4.03; Br, 15.21%)) led us to believe that we were successful in generating the glycosyl bromide **(4.72)**.



Scheme 4.18: (i) Py., BzCl (4 mol eq); (ii) AcOH, HBr.AcOH; (iii) MeCN, IBr (2.5 mol eq); (iv) NaOMe/MeOH.

Initial attempts to couple donor and acceptor sugars together using the standard silver triflate procedure proved fruitless, indicating a need to change the promotor. Prior work carried out within the Field research group has shown iodine monobromide to be a capable promotor.[70] Employing this knowledge led to a second attempted coupling using IBr. Donor (3 mol eq) and acceptor sugars, **(4.72)** and **(4.20)** were dissolved in anhydrous MeCN and the reaction mixture cooled to –20 °C. Iodine monobromide (2.5 mol eq) was added and the reaction followed by TLC. The fully protected disaccharide **(4.73)** was generated in average, 48% yield (*m/z* (ES) 1066 ($M+NH_4^+$, 100%); HRMS: Found: 1066.4194. $C_{61}H_{60}O_{16}$ ($M+NH_4^+$)

Requires 1066.4225). The stereochemistry of the protected disaccharide **(4.73)** was determined by close examination of the coupling constants in the ^{1}H NMR spectrum. A $J_{1',2'}$ coupling constant of 5.9 Hz for H-1' was proof that the desired alpha-linkage had indeed been generated. Deprotection of the six benzoyl protecting groups provided the desired deprotected disaccharide **(4.69)**. The optical rotation value recorded for **(4.69)** was of the same sign and of a similar magnitude to cited literature values {$[\alpha]_D$ -22.9° (*c* 0.1, CH_3OH), (lit., [65] $[\alpha]_D$ -29.2°}. Optical rotation of the plant natural product **(4.12)** quoted in the literature (lit., [9] $[\alpha]_D$ -75.0°) was also levorotatory suggesting a misreporting of either the optical rotation recorded in the literature for **(4.12)** or structural assignment of same.



(4.12)

Given that there is still contradiction in optical rotation values between the value recorded for **(4.12)** and the literature, it was decided to help clarify the situation the synthesis of the arabinose-glucose disaccharide combinations shown in **Figure 4.13** was undertaken.

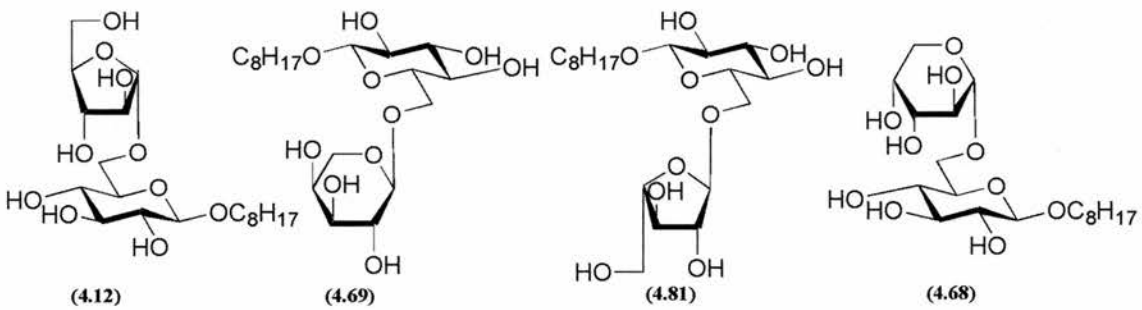
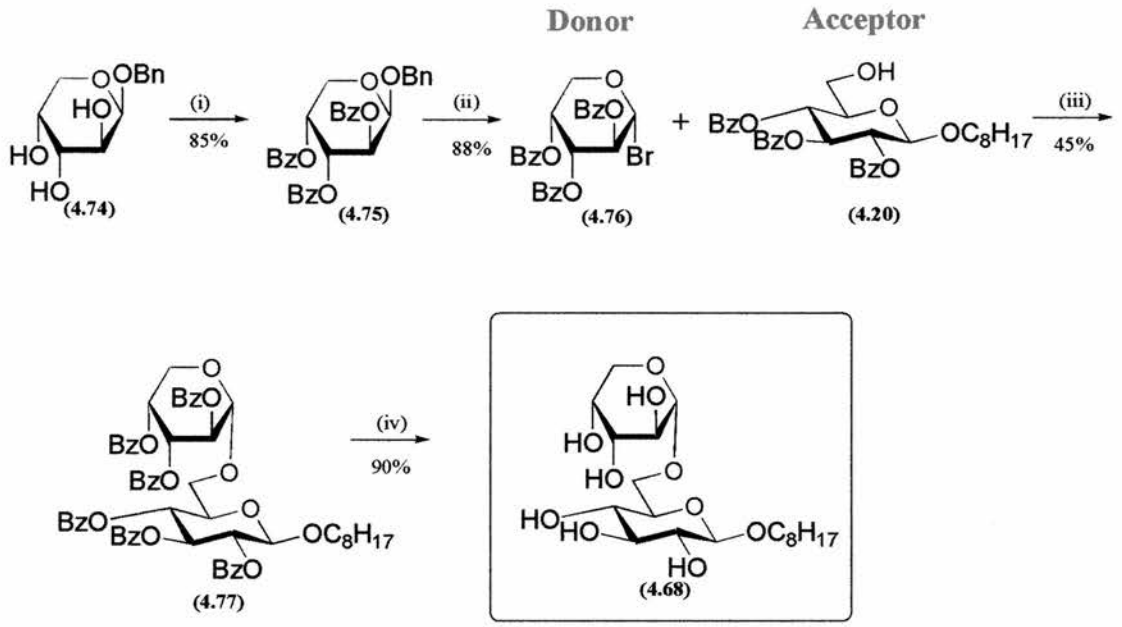


Figure 4.13: arabinose-glucose disaccharide combinations.

4.7.2 Preparation octyl 6-*O*-(α-D-arabinopyranosyl)-β-D-glucopyranoside (4.68):

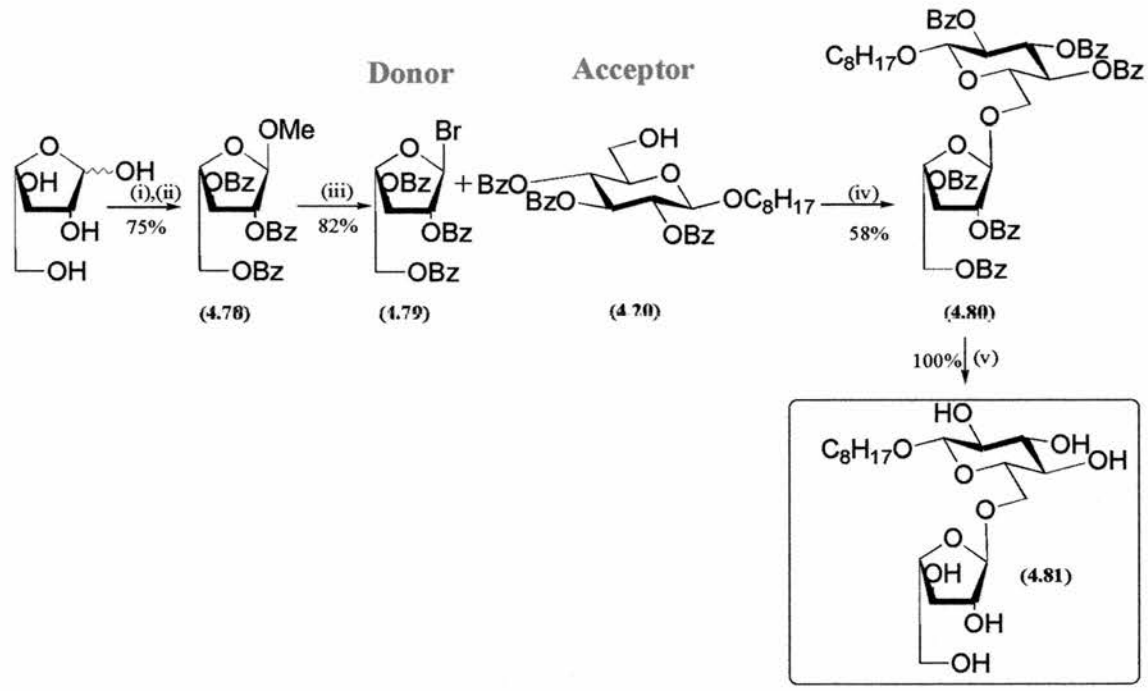
Synthesis of octyl 6-*O*-(α-D-arabinopyranosyl)-β-D-glucopyranoside **(4.68)** was performed in an identical manner to octyl 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranoside **(4.69)**. Compound **(4.74)**[71] was again kindly donated by Dr. A. H. Haines (University of East Anglia). **(4.74)** was treated with pyridine and BzCl. This resulted in the formation of the protected ether **(4.75)**. Treatment of **(4.75)** with HBr.acetic acid gave the known bromide **(4.76)**.[72] Formation of the glycosyl halide was evidenced by diagnostic changes in both proton and carbon spectra. Confirmation of the linkage in **(4.76)** was given by a dramatic change in chemical shift, δ_C C-1 = 89.8 ppm (C-1 of **(4.76)** appeared at 96.1 ppm) and H-1 appeared at 6.85 ppm, with a $J_{1,2}$ coupling constant of 3.9 Hz). This data, together with accurate combustion analysis (Found: C, 59.58; H, 4.10; Br, 14.99. $C_{26}H_{21}BrO_7$ Requires: C, 59.44; H, 4.03; Br, 15.21%); led us to believe we were successful in the synthesis of **(4.76)**. As in the case of the L-isomer the only difficult step proved to be the IBr coupling step, with the alpha configured {4.83 (H-1', $J_{1',2'}$ = 5.1 Hz) and δ_C = 101.2 ppm (C-1')}disaccharide **(4.77)** being generated (*m/z* (ES) 1066 ($M+NH_4^+$, 100%); HRMS: Found: 1066.4221. $C_{61}H_{64}NO_{16}$ ($M+NH_4$) Requires 1066.4225) in a 45% yield. Deprotection of the benzoyl protecting groups gave **(4.68)** in 90% yield.



Scheme 4.19: (i) Py., BzCl (4 mol eq); (ii) AcOH, HBr.AcOH; (iii) MeCN, IBr (2.5 mol eq); (iv) NaOMe.

4.7.3 Preparation of octyl 6-*O*-(α-L-arabinofuranosyl)-β-D-glucopyranoside (4.81):

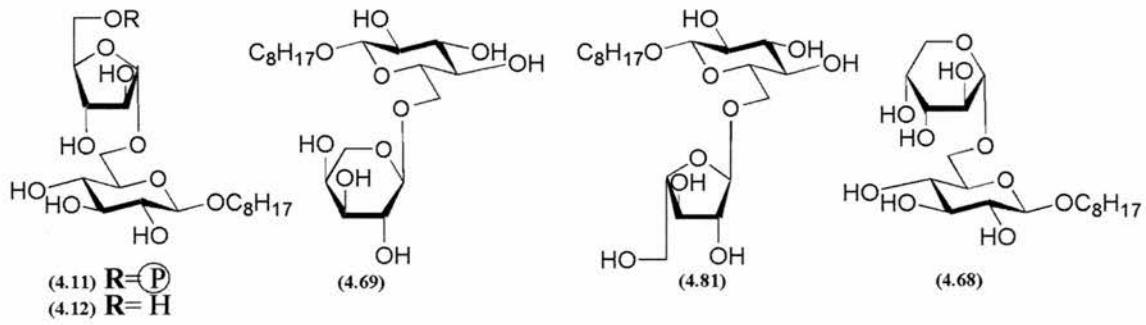
The last disaccharide in the series to be prepared was octyl 6-*O*-(α-L-arabinofuranosyl)-β-D-glucopyranoside **(4.81)**. This compound was synthesised in an identical manner to octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)**. Known donor **(4.79)**[73,74] and acceptor **(4.20)** were prepared independently, in an identical manner as before (see sections **4.3.2** and **4.3.1** respectively). They were then brought together in a coupling step to generate **(4.80)**. Confirmation of same was given by accurate combustion analysis (Found: C, 69.22; H, 5.97. $C_{61}H_{60}O_{16}.0.5\ H_2O$ Requires: C, 69.23; H, 5.81%) and HRMS (*m/z* (ES) 1071 ($M+Na^+$, 100%); HRMS: Found: 1071.3796. $C_{61}H_{60}O_{16}Na$ ($M+Na^+$) Requires 1071.3779); the stereochemical outcome of the silver triflate coupling step was assessed by H-1' coupling constants in the proton NMR spectrum {5.50 (1 H, s, $J_{1',2'}$ 0.0, H-1')} and the chemical shift of C-1' in the carbon spectrum {106.0 (C-1')} and was judged to be alpha-configured.



Scheme 4.20: (i) MeOH/ HCl (1 mol eq); (ii) BzCl (6 mol eq); (iii) AcOH, HBr.AcOH; (iv) 4 Å mol.sieves, DCM,-30 °C, AgOTf (5 mol eq), collidine (0.5 mol eq); (v) NaOMe/ MeOH.

4.7.4 Further evidence of the possible misreporting of the "D-configured" disaccharide, octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside (4.12)

Synthesis of pApp mimics **(4.9)** and **(4.11)** has successfully been completed. These pApp mimics will be evaluated as inhibitors of arabinogalactan biosynthesis in due course. Disagreement in optical rotation values between the natural product **(4.12)** and the literature encouraged us to synthesise four different arabinose-glucose disaccharide combinations (shown below). Synthesis of the four isomeric forms of the natural product **(4.12)** {**(4.12)**, **(4.68)**, **(4.69)** and **(4.81)**}has been successful.



(4.11) R=P
(4.12) R= H

(4.69)

(4.81)

(4.68)

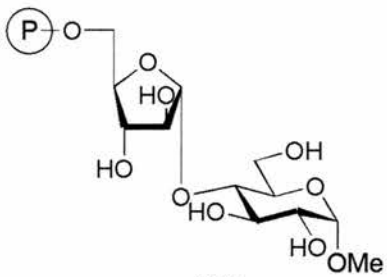
Disaccharide	Optical rotation $[\alpha]_D$ [Lit]	$\delta_H(J_{1',2'})$ [Lit]	δ_C(C-1 and C-1') [Lit]
D-Ara*f*-D-Glc*p* **(4.12)**	+25.7°, [-75.0°][9]	4.94 (0.8 Hz), [4.95 (1.3 Hz)][9]	104.7, 109.8, [104.4, 109.9][9]
L-Ara*f*-D-Glc*p* **(4.81)**	-98.6°, [N/A]	4.86 (0.8 Hz), [N/A]	103.3, 108.8, [N/A]
D-Ara*p*-D-Glc*p* **(4.68)**	-25.6°, [N/A]	4.16 (6.4 Hz), [N/A]	104.2 (2 C), [N/A]
L-Ara*p*-D-Glc*p* **(4.69)**	-22.9°, [-29.2°][75]	4.30 (6.8 Hz) [4.32 (6.6 Hz)][65,75]	104.0, 104.8, [104.3, 105.1][65,75]

Table 4.2: Optical rotation and NMR data for arabinofuranose and glucopyranose containing disaccharides.

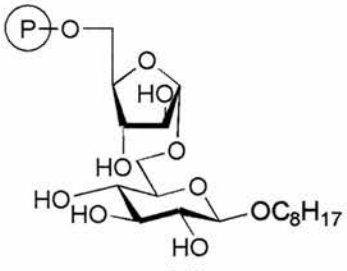
From the characterisation of these compounds (**Table 4.2**) we believe the data reported for the natural product **(4.12)** to be incorrect. The literature claims the plant natural product **(4.12)** contains a D-Ara*f* sugar residue, although D-Arabinose is not commonly found in plants. Our data do not support this structural assignment

4.8 Conclusions and recommendations for further work:

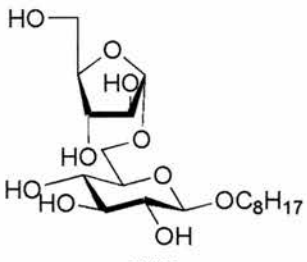
The synthesis of pApp mimics **(4.9)** and **(4.11)** has successfully been completed. Disagreement in optical rotation of **(4.12)** (an intermediate in the synthesis of **(4.11)**) urged synthesis and characterization of the closely related known compound **(4.69)**.



(4.9)

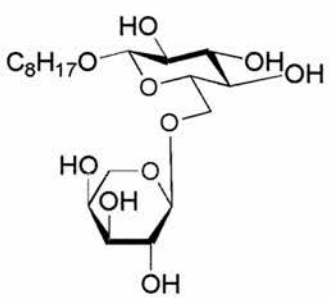


(4.11)



(4.12)

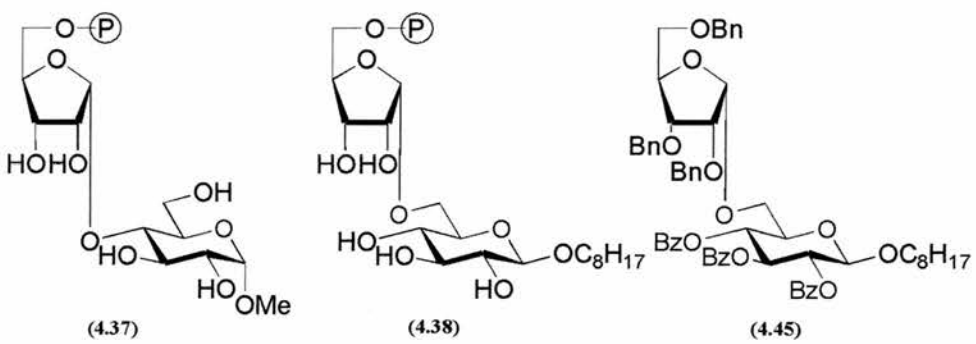
Close agreement in both optical rotations and NMR spectra (proton and carbon) between **(4.69)** and the literature suggested an error to have been made in the literature concerning the characterisation of **(4.12)**.



(4.69)

To help clarify the situation a further four isomeric forms of the disaccharide naturual product **(4.12)** were also synthesised and characterised. From the table of data assembled, it would now appear incontrovertible that an error was made in the literature relating to the characterisation of octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)**. This is not surprising as Kingston claims that octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)**, which is a natural product isolated from the leaves of the perennial plant, *Circaea lutetiana* L. ssp. *Canadensis* contains a D-arabinofuranose sugar residue. D-

Arabinose is not commonly found in plants whilst L-arabinose would demonstrate a rotation of opposite sign, consequently the disparity between optical rotations is explained. Given the disagreement in optical rotations and close agreement in NMR spectra (proton and carbon) between **(4.12)** and the literature, we believe Kingston has incorrectly reported the configuration of the disaccharide, i.e that they have isolated octyl 6-*O*-(α-L-arabinofuranosyl)-β-D-glucopyranoside and not octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** as claimed. The pApp mimics synthesised are currently under biological evaluation and we are hopeful that the results of the biological assays will provide an insight in to the DpA biosynthetic pathway. pRpp is a known intermediate in the DpA biosynthetic pathway and for this reason we decided to repeat the chemistry already used in developing pApp analogues on ribofuranose. This would generate a parallel set of pRpp mimics which could also be the subject of biologial evaluation and thereby allowing comparisons to be drawn between the two sets of epimers. This would also provide a good indication as to the biosynthetic route taken in DpA biosynthesis. The synthesis of the pRpp analogues was not so straight forward, due to nature of the 1,2-*cis* stereochemical linkage in **(4.37)**.



Consequently for this reason it was decided to focus attention initially on the synthesis of the unnatural 1,6-linked disaccharide **(4.38)** (stereochemistry of natural substrate is 1,4-linked). Two different approaches were made in an attempt to synthesise the desired disaccharide, octyl 6-*O*-(2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.45)**. The first method used, was a repeat of the silver triflate chemistry used in the synthesis of pApp mimics. The second method involved attempting a new synthetic strategy.

This strategy incorporated the direct 1-*O*-alkylation of a protected ribofuranose with various carbohydrate based triflates. Both methods proved successful with the silver triflate method yielding greater quantities of the desired 1,2-*cis* glycoside. Time constraints inhibited completion of the synthesis of the pRpp analogue, octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside **(4.38)**. Future work should focus on the completion of the synthesis of the 1,6-linked disaccharide, octyl 6-*O*-(5-*O*-phospho-α-D-ribofuranosyl)-β-D-glucopyranoside **(4.38)**. Once completed, synthesis of the 1,4-linked pRpp mimic, (natural substrate being 1,4-linked) should be undertaken. This should prove achievable as all the chemistry has already been performed in the previous synthesis of 1,4-linked pApp analogue, methyl 4-*O*-(5-phospho-α-D-arabinofuranosyl)-α-D-glucopyranoside **(4.9)**.

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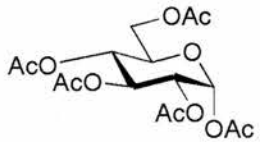
Chapter 5

Experimental - Carbohydrates

5.1 Synthetic Methods

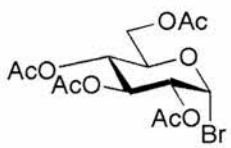
General experimental procedures were described in Chapter 3 (page 62).

Penta-*O*-acetyl-α-D-glucopyranose (4.13)[1]



D-Glucose (10.0 g, 56 mmol) was added portion-wise to an ice cold solution of acetic anhydride (50 ml) and iodine (500 mg).[1] The reaction mixture was stirred at room temperature until TLC [Toluene – EtOAc, (4:1)] showed the reaction to be complete. The reaction mixture was then poured into ice cold 10 % aqueous $Na_2S_2O_3$ solution. The crude product was removed by filtration and washed thoroughly with water to give penta-*O*-acetyl-α-D-glucopyranose **(4.13)** as a white solid (18.25 g, 84 %); mp 104 - 106° C (95% EtOH), (lit., [2] 109 - 111°C); $[\alpha]_D$ +98.2° (*c* 0.2, $CHCl_3$), (lit., [3] $[\alpha]_D$ +101.6° (*c* 5.3, $CHCl_3$)); δ_H ($CDCl_3$): 2.01 (3 H, s, $COCH_3$), 2.02 (3 H, s, $COCH_3$), 2.03 (3 H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 2.09 (3 H, s, $COCH_3$), 4.07 – 4.14 (2 H, m, H-5 and H-6), 4.26 (1 H, dd, $J_{5,6}$ 4.1, $J_{6,6'}$ 12.6, H-6'), 5.09 (1 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.0, H-2), 5.16 (1 H, t, $J_{3,4}$ 10.0, $J_{2,3}$, H-3), 5.47 (1 H, t, $J_{4,5}$ 10.0, $J_{3,4}$, H-4), 6.33 (1 H, d, $J_{1,2}$, H-1). δ_C ($CDCl_3$) 20.5, 20.6, 20.7 (2 C), 20.9 (5 x CH_3), 61.4 (C-6), 67.7 (C-4), 67.8 (C-2), 69.2 (2C, C-3 and C-5), 89.0 (C-1), 168.7, 169.4, 169.6, 170.2, 170.6 (5 x *C*O).

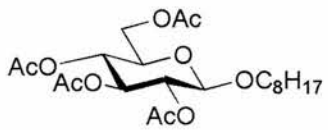
2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.14)[4-8]



To a solution of acetic acid (50 ml) and HBr (50% w/v solution in acetic acid, 50 ml) was added penta-*O*-acetyl-α-D-glucopyranose **(4.13)** (17.75 g, 45 mmol). The reaction mixture was kept at 4°C until TLC [Toluene – EtOAc, (4:1)] showed the reaction to be complete. The resulting solution was concentrated to dryness and coevaporated with toluene to give an off-white solid. The crude product was dissolved in DCM (200 ml) and washed with ice-water

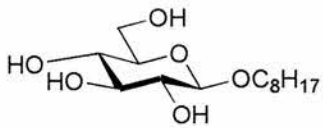
(200 ml) and 5 % $NaHCO_3$ solution (200 ml x 2). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Crystallisation gave 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(4.14)** (18.05 g, 98 %); mp 82 - 84°C (ether/60-80°C pet.ether), (lit., [5] 83 - 85°C); $[\alpha]_D$ +191.9° (*c* 0.51, $CHCl_3$), (lit., [6] $[\alpha]_D$ +198.0° (*c* 2.0, $CHCl_3$)); δ_H ($CDCl_3$): 2.02 (3H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 2.09 (3 H, s, $COCH_3$), 4.13 (1 H, m, H-6'), 4.28 - 4.35 (2 H, m, H-5 and H-6), 4.83 (1H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 10.2, H-2), 5.15 (1 H, t, $J_{3,4}$ 10.2, $J_{4,5}$ 10.2, H-4), 5.55 (1 H, t, $J_{2,3}$, $J_{3,4}$, H-3), 6.60 (1 H, d, $J_{1,2}$, H-1); δ_C ($CDCl_3$) 20.5 (2C), 20.6 (2 C) (4 x CH_3), 60.8 (C-6), 67.0, 70.0, 70.5, 72.0 (C-2 - C-5), 86.4 (C-1), 169.3, 169.7, 169.9, 170.4 (4 x CO);

Octyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (4.15)[9-12]



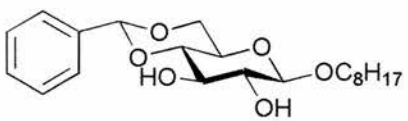
A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(4.14)** (820 mg, 2 mmol), powdered Drierite (870 mg), anhydrous octanol (0.48 ml, 3 mmol, 1.5 eq) and anhydrous acetonitrile (8 ml) was allowed to stir for 2 h under dry nitrogen, then mercury bromide (72 mg, 0.2 mmol, 0.1 mol eq) and mercury cyanide (606 mg, 2.4 mmol, 1.2 mol eq) were added. The reaction mixture was allowed to stir under nitrogen until TLC [Hexane – EtOAc, (4:1)] showed the reaction to be complete (4 h). The mixture was diluted with DCM, filtered through Celite and concentrated under reduced pressure to a syrup. Column chromatography (silica gel; Hexane – EtOAc, 4:1) gave octyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **(4.15)** as a solid (632 mg, 69 %); mp 48 - 50°C (MeOH), (lit., [11] 53 - 54°C); $[\alpha]_D$ -22.1° (*c* 0.1, $CHCl_3$), (lit., [12] $[\alpha]_D$ -20.5° (*c* 0.04, $CHCl_3$)); δ_H ($CDCl_3$): 0.87 (3 H, t, *J* 6.8, CH_2CH_3), 1.25 - 1.30 (10 H, m, 5 x CH_2), 1.53-1.57 (2 H, m, OCH_2CH_2), 1.99, 2.01, 2.03, 2.07 (12 H, 4 x s, 4 x $COCH_3$), 3.46, 3.85 (2 H, 2 x m, OCH_2CH_2), 3.68 (1 H, m, H-5), 4.13 (1 H, dd, $J_{5,6}$ 1.5, $J_{6,6'}$ 7.5, H-6), 4.25 (1 H, dd, $J_{5,6'}$ 2.7, $J_{6,6'}$, H-6'), 4.48 (1 H, d, $J_{1,2}$ 9.0, H-1), 4.97 (1 H, t, $J_{1,2}$, $J_{2,3}$ 9.5, H-2), 5.08 (1H, t, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5, H-4), 5.19 (1 H, t, $J_{2,3}$, $J_{3,4}$, H-3). δ_C ($CDCl_3$) 14.0 (CH_2CH_3), 20.6 (4C, 4 x $COCH_3$), 22.6, 25.7, 29.2, 29.3 (2 C), 31.7 (6C, 6 x CH_2), 62.0 (C-6), 68.5, 70.3, 71.4, 71.8, 72.9 (C-2 - C-5 and OCH_2), 100.9 (C-1), 169.6 (2C), 170.5, 170.8 (4 x CO).

Octyl β-D-glucopyranoside (4.16)[12-16]



A standard deacetylation procedure was performed to give the title compound.[13] To a solution of compound **(4.15)** octyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (3.62 g, 7.9 mmol) in anhydrous methanol (40 ml) was added a catalytic amount of sodium metal. The reaction mixture was allowed to stir until TLC [DCM– MeOH, (9.5:0.5)] showed the reaction to be complete. The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated under reduced pressure to a foam (2.35 g, 100 %); $[\alpha]_D$ -32.3° (*c* 0.15, H_2O), (lit., [12] $[\alpha]_D$ -34.0° (*c* 0.04, H_2O)); δ_H (CD_3OD): 0.89 (3 H, t, *J* 7.1,CH_3), 1.29-1.37 (10 H, m, 5 x CH_2), 1.60 (2 H, m, OCH_2CH_2), 3.16 (1 H, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0, H-2), 3.24 – 3.38 (3 H, m, H-3, H-4, H-5), 3.48 – 3.57, 3.84 – 3.90 (2 H, 2 x m, OCH_2), 3.66 (1 H, m, H-6'), 3.84 (1 H, m, H-6), 4.24 (1 H, d, $J_{1,2}$, H-1). δ_C (CD_3OD) 13.0 (CH_3), 22.3, 25.7, 29.0, 29.2, 29.4, 31.6 (6 x CH_2), 61.4 (C-6), 69.5, 70.3, 73.8, 76.5, 76.8 (C-2, C-3, C-4, C-5 and OCH_2), 103.0 (C-1).

Octyl 4,6-*O*-benzylidene-β-D-glucopyranoside (4.17)[17,18]

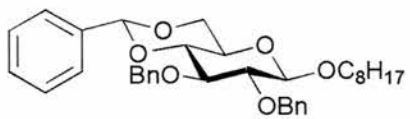


To a solution of octyl β-D-glucopyranoside **(4.16)** (2.35 g, 10.9 mmol) in anhydrous acetonitile (120 ml) was added *p*-toluenesulfonic acid (204 mg, 1.07 mmol) and benzaldehyde dimethyl acetal (7 ml, 64 mmol). The solution was allowed to stir until TLC [DCM– MeOH, (9.5:0.5)] showed the reaction to be complete (aprox. 4 h). The solution was neutralised with triethylamine and added with constant stirring to an ice-water mixture (200 ml). The crude material was allowed to stand overnight. The reaction mixture was then filtered and washed with water several times. Crystallisation gave the *octyl 4,6-O-benzylidene-β-D-glucopyranoside** **(4.17)** (1.99 g, 50 %); mp 146 - 147°C; (Found: C, 64.36; H, 8.29. $C_{21}H_{32}O_6$.(0.5 H_2O) Requires C, 64.74; H, 8.54%); $[\alpha]_D$ -43.9° (*c* 0.1, $CHCl_3$); δ_H (CD_3OD): 0.88 - 0.92 (3 H, t, *J* 6.9,CH_3), 1.31 (10 H, br s, 5 x CH_2), 1.59 - 1.64

(2 H, m, OCH_2CH_2), 3.27 (1 H, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 9.1, H-2), 3.39 – 3.50 (1 H, m, H-5), 3.46, 3.62 (2 H, 2 x t, $J_{2,3}$, $J_{3,4}$ 9.1, $J_{4,5}$ 9.1 H-3, H-4), 3.76 (1H, t, $J_{6,6'}$ 10.4, H-6'), 3.52 – 3.60, 3.79 – 3.89, (2 H, 2 x m, OCH_2), 4.27 (1 H, dd, $J_{5,6}$ 4.4, $J_{6,6'}$, H-6), 4.37 (1 H, d, $J_{1,2}$, H-1), 5.57 (1 H, s, PhC*H*), 7.32 – 7.51 (5 H, Ar). δ_C (CD_3OD) 12.9 (CH_3), 22.3, 25.6, 29.0, 29.1, 29.4, 31.6 (6 x CH_2), 66.2 (C-6), 68.4, 69.8, 73.4, 74.6, 81.0, (C-2, C-3, C-4, C-5 and OCH_2), 101.6 (C-1), 103.8 (Ph*C*), 126.3 (2 C), 127.7 (2 C), 128.6 (5 C, Ar), 137.9 (quat. Ar C); EI-MS (+ve): *m/z* (ES) 403 ($M+Na^+$, 100 %); HRMS: Found: 403.2100. $C_{21}H_{32}O_6Na$ ($M+Na^+$) Requires 403.2097.

*Although there are several references[17,18] to this compound in the literature, neither melting point nor optical rotation are reported.

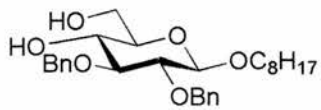
Octyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (4.19)[17,18]



The title compound was prepared using a standard benzylation procedure.[19] A solution of octyl 4,6-*O*-benzylidene-β-D-glucopyranoside **(4.17)** (1.2 g, 3.3 mmol) was dissolved in anhydrous DMF (12 ml) and cooled (0°C) before sodium hydride (60 % w/w suspension in mineral oil) (292 mg, 7.2 mmol, 2.2 mol eq.) was slowly added. After 0.5 h BnBr (0.86 ml, 7.2 mmol) was slowly added and the solution stirred until TLC [toluene– EtOAc, (4:1)] showed the reaction had gone to completion. The reaction mixture was cooled (0°C) before methanol (15 ml) was added. The solution was stirred 0.5 h. and concentrated under reduced pressure to a syrup. The syrup was dissolved in an equal volume of DCM and washed with water (2 x 100 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 20:1 – 5:1) gave *octyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside* **(4.19)** as a white solid (1.62 g, 88 %); mp 66 - 68° C (EtOH); $[\alpha]_D$ -36.5° (*c* 0.243, $CHCl_3$); (lit., [18] $[\alpha]_D$ -39.1° (*c* 1.0, $CHCl_3$)); δ_H ($CDCl_3$): 0.86 - 0.91 (3 H, t, *J* 6.9, CH_3), 1.24 - 1.33 (10 H, m, 5 x CH_2), 1.63 - 1.66 (2 H, m, OCH_2CH_2), 3.34 – 3.50 (2 H, m, H-2, H-5), 3.52 – 3.62, 3.88 – 3.98 (2 H, 2 x m, OCH_2), 3.69 (1 H, t, *J* 8.2. H-3/H-4), 3.75 (1 H, t, *J* 8.2. H-3/H-4), 3.80 (1H, t, $J_{6,6'}$ 10.4, H-6'), 4.36 (1 H, dd, $J_{5,6}$ 4.9, $J_{6,6'}$, H-6), 4.51 (1 H, d, $J_{1,2}$ 7.7, H-1),

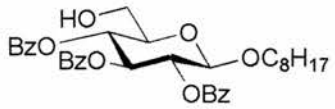
4.77, 4.81 (2 H, 2 x d, *J* 10.7, 2 x (1 H of CH_2Ph)), 4.92 (2 H, d, *J*, 11.0, 2 x (1 H of CH_2Ph)), 5.57 (1 H, s, OC*H*O), 7.26 – 7.39, 7.48 – 7.51 (15 H, Ar); δ_C ($CDCl_3$) 14.0 (CH_3), 22.6, 26.1, 29.2, 29.4, 29.8, 31.8 (6 x CH_2), 66.1, 68.9, 70.7, 75.1, 75.4, 81.0, 81.6, 82.2 (2 x CH_2Ph, 1 x OCH_2, C-2, C-3, C-4, C-5 and C-6), 101.2 (C-1), 104.3 (O*C*O), 126.2 (2 C), 127.7, 127.8, 128.1 (2 C), 128.2 (2 C), 128.4 (4 C), 128.5 (2 C), 129.1 (15 C, Ar), 137.5, 138.6, 138.7 (3 x quat. Ar C).

Octyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (4.21)[17]



The title compound was prepared using a benzylidene acetal hydrolysis reaction on compound **(4.19)**. A solution of octyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside **(4.19)** (1.62 g, 2.9 mmol) in 80 % aqueous acetic acid (30 ml) was heated (80°C) and stirred until TLC [hexane– EtOAc, (3:1)] showed the reaction had gone to completion (approx. 2 h). The resulting solution was concentrated and coevaporated with toluene to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 3:1) gave octyl 2,3-di-*O*-benzyl-β-D-glucopyranoside **(4.21)** as a white solid (1.17 g, 85 %); mp 46 – 48°C (ether/60-80°C pet. ether); $[\alpha]_D$ -34.9° (*c* 0.18, $CHCl_3$); (lit., [17] $[\alpha]_D$ -35.4° (*c* 0.18, $CHCl_3$)); δ_H (CD_3OD): 0.86 (3 H, t, *J* 6.6, CH_3), 1.20 - 1.39 (10 H, m, 5 x CH_2), 1.56 - 1.65 (2 H, m, OCH_2CH_2), 3.25 – 3.30, 3.44 – 3.50 (4 H, 2 x m, H-2, H-3, H-4 and H-5), 3.52 – 3.57, 3.92 –3.98 (2 H, 2 x m, 1 x OCH_2), 3.67, (1 H, dd, $J_{6,6'}$ 11.8 $J_{5,6}$ 5.7, H-6), 3.86 (1H, dd, $J_{5,6'}$ 2.2, $J_{6,6'}$, H-6'), 4.42 (1 H, d, $J_{1,2}$ 8.0, H-1), 4.65 (1 H, d, *J* 11.3, 1 H of CH_2Ph), 4.80 (1 H, d, *J* 11.3, 1 H of CH_2Ph), 4.82 (1 H, d, *J* 11.3, 1 H of CH_2Ph), 4.90 (1 H, d, *J* 11.3, 1 H of CH_2Ph), 7.22 – 7.35 (10 H, m, 2 x C_6H_5). δ_C (CD_3OD) 13.1 (CH_3), 22.3, 26.0, 29.0, 29.1, 29.5, 31.6 (6 x CH_2), 61.4 (C-6), 69.5, 70.4, 74.3, 75.1, 76.4, 81.9, 84.6 (2 x CH_2Ph, 1 x OCH_2, C-2, C-3, C-4 and C-5), 103.5 (C-1), 127.2, 127.3, 127.7 (4 C), 127.9 (2 C), 128.0 (2 C), (10 C, Ar), 138.9, 139.1 (2 x quat. C).

Octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.20)



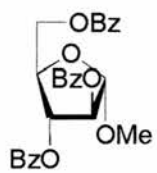
To a cooled (0°C) solution of octyl β-D-glucopyranoside **(4.16)** (650 mg, 2.22 mmol) in pyridine (20 ml), was added *tert.*butyldimethylsilyl chloride (502 mg, mg, 3.33 mmol). The mixture was stirred with the temperature being allowed to rise to ambient over a 4 h period. The progress of the reaction was followed by TLC [DCM – MeOH, (95:5)]. Once the starting material had been consumed the mixture was cooled again (0°C). A solution of benzoyl chloride (0.85 ml, 7.3 mmol, 3.3 mol eq.) in chloroform (6 ml) was added over 5 min. The reaction mixture was allowed to stir overnight. The reaction was monitored by TLC [hexane – EtOAc, (5:1)], and upon completion methanol (excess) was added to destroy any unreacted benzoyl chloride. The reaction mixture was allowed to stir for a further 0.5 h. The resulting solution was concentrated and coevaporated with toluene to give a syrup. The reaction mixture was diluted with an equal volume of DCM and washed with 2 M aq. HCl (100 ml) and 10 % aq. Na_2CO_3 (100 ml) solutions. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. A small portion of *octyl 2,3,4-tri-O-benzoyl-6-O-tert-butyldimethylsilyl-β-D-glucopyranoside* **(4.18)** was kept aside for characterisation (see below).

mp 66 - 68°C (95% EtOH); (Found: C, 68.76; H, 7.49. $C_{41}H_{54}O_9Si$ Requires C, 68.49; H, 7.57%); $[\alpha]_D$ -15.5° (*c* 0.16, $CHCl_3$); δ_H ($CDCl_3$): 0.01 (6 H, s, 2 x CH_3 of TBDMS), 0.79 – 1.23 (22 H, m, 5 x CH_2, 3 x CH_3 of TBDMS and 1 x CH_3 of C_8H_{17}), 1.42 – 1.59 (2 H, m, O CH_2CH_2), 3.50 – 3.53, 3.86 – 3.92 (2 H, 2 x m, OCH_2), 3.81 (3 H, br s, H-5, H-6 and H-6'), 4.75 (1H, d, $J_{1,2}$ 7.8, H-1), 5.44 (1H, t, $J_{3,4}$ 9.7, H-4), 5.44 (1H, dd, $J_{1,2}$, $J_{2,3}$ 9.7, H-2), 5.83 (1H, t, $J_{2,3}$, $J_{3,4}$ H-3), 7.23 – 7.52, 7.78 – 7.95 (15 H, 2 x m, Ar); δ_C ($CDCl_3$): -5.5 (2 C, 2 x CH_3 of TBDMS), 14.0, 18.2, 22.5, 25.7 (3 C), 29.1 (2 C), 29.4, 31.7 (10C, 3 x CH_3 of TBDMS, 1 x CH_3 of C_8H_{17} and 6 x CH_2), 62.9, 69.9, 70.0, 72.1, 73.3, 75.3 (C-2 – C-6 and OCH_2), 101.1 (C-1), 128.3 (4 C), 128.4 (3 C), 129.2, 129.3, 129.7, 129.9 (5 C) (15 C, Ar), 133.2 (2 C), 133.3 (3 C, 3 x quat. Ar C), 165.3, 165.4, 166.1 (3 x *C*O).

The remaining silylated sugar (1.34 g, 1.9 mmol) **(4.18)** was treated with 80 % aqueous acetic acid (40 ml). The reaction was allowed to stir until TLC [hexane – EtOAc, (5:1)]

showed the reaction to be complete. The resulting solution was concentrated and coevaporated with toluene to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 5:1 – 2:1) gave *octyl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside* **(4.20)** as an oil (0.99 g, 74 %); (Found: C, 69.20; H, 6.74. $C_{35}H_{40}O_9$ Requires C, 69.52; H, 6.67%); $[\alpha]_D$ -16.4° (*c* 1.0, $CHCl_3$); δ_H ($CDCl_3$): 0.75 (3 H, t, *J* 6.7, CH_3), 0.99 – 1.18 (10 H, m, 5 x CH_2), 1.41 – 1.47 (2 H, m, OCH_2CH_2), 2.47 (1 H, br s, *OH*), 3.42 – 3.49, 3.81 – 3.89 (2 H, 2 x m, OCH_2), 3.62 – 3.77 (3 H, m, H-5, H-6 and H-6'), 4.75 (1 H, d, $J_{1,2}$ 8.0, H-1), 5.42 (1 H, t, *J* 9.8, H-4), 5.43 (1 H, dd, $J_{1,2}$, $J_{2,3}$ 9.8, H-2), 5.86 (1 H, *J* 9.8, H-3), 7.15 – 7.45, 7.75 – 7.90 (15 H, 2 x m, Ar); δ_C ($CDCl_3$) 14.0 (CH_3), 22.6, 25.8, 29.1, 29.2, 29.4, 31.7 (6 x CH_2), 61.5, 69.7, 70.4, 72.0, 73.0, 74.7, (OCH_2, C-2, C-3, C-4, C-5 and C-6), 101.4 (C-1), 128.4 (4 C), 128.6 (2 C), 128.8, 129.1, 129.6, 129.9 (4 C), 130.0 (2 C) (15 C, Ar), 133.3, 133.3, 133.8 (3 x quat. Ar C), 165.2, 166.1, 166.2 (3 x *C*O); MALDI-TOF (+ve): *m/z* 627.3 $(M+Na)^+$, ($C_{35}H_{40}O_9Na$ requires *m/z* 627.3).

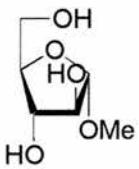
Methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (4.22)[20,21]



A suspension of D-arabinose (10 g, 67 mmol) in anhydrous methanol (200 ml) was treated with methanolic HCl [prepared by the *careful* addition of acetyl chloride (5.2 g, 67 mmol) to anhydrous methanol (53.4 ml) at 0°C]. The mixture was allowed to stir overnight at 0 - 5°C . Upon dissolution of the sugar, pyridine (35 ml) was added. The resulting solution was concentrated and coevaporated with toluene to give a yellow syrup. The residue was then dissolved in anhydrous pyridine (68 ml), cooled and benzoyl chloride (56.2 g, 400 mmol) was added (0°C). The reaction mixture was then left to warm to room temperature until TLC [toluene – EtOAc, (5:1)] indicated completion (approx. 48 h.). The reaction mixture was diluted with an equal volume of DCM and washed with 10 % aq. $NaHCO_3$ solution (2 x 200 ml) and water (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Crystallisation gave the title compound **(4.22)** as a white solid (22.03 g, 69 %); mp 99 - 100°C (95 % EtOH), (lit., [20] 98 - 101°C); $[\alpha]_D$ –20.7° (*c*

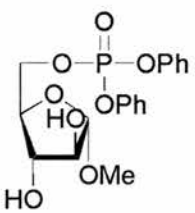
0.528, $CHCl_3$), (lit., [20] $[\alpha]_D$ −19.1° (*c* 2.05, $CHCl_3$)); δ_H ($CDCl_3$): 3.49 (3 H, s, OCH_3), 4.54 – 4.60 (1 H, m, H-4), 4.70 (1H, dd, $J_{4,5A}$ 2.4, $J_{5A,5B}$ 8.0, H-5_A), 4.85 (1 H, dd, $J_{4,5B}$ 2.4, $J_{5A,5B}$, H-5_B), 5.19 (1 H, br s, H-1), 5.52 (1 H, d, $J_{2,3}$ 1.4, H-2), 5.59 (1 H, d, $J_{3,4}$ 4.7, H-3), 7.26 – 7.49 (15 H, m, Ar), 8.00 – 8.10 (5 H, m, Ar); δ_C ($CDCl_3$) 55.0 (OCH_3), 63.8 (C-5), 78.0 (C-4), 80.9 (C-3), 82.3 (C-2), 107.0 (C-1), 128.5 (2 C), 128.6 (3 C), 129.2, 129.9 (3 C), 130.0 (3 C), 130.1 (3 C) (15 C, Ar), 133.2, 133.6 (2 C) (3 x quat. Ar C), 165.7, 166.0, 166.4 (3 x *C*O).

Methyl α-D-arabinofuranoside (4.83)[20,22,23]



To a solution of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(4.22)** (500 mg, 1.05 mmol) in anhydrous methanol (2 ml), was added a stock solution of NaOMe/MeOH (10 mg/ml) (2 ml). The reaction was allowed to stir until TLC [DCM– methanol, (5:1)] showed that the reaction had gone to completion (within 1 h.). The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo* to yield the title compound, methyl α-D-arabinofuranoside **(4.83)** as an oil (160 mg, 93 %); $[\alpha]_D$ +119° (*c* 1.0, H_2O), (lit., [23] $[\alpha]_D$ +123° (*c* 1.2, H_2O)); δ_H (CD_3OD): 3.28 (3 H, s, OCH_3), 3.52 - 3.86 (5 H, m, H-2, H-3, H-4, H-5_A and H-5_B), 4.75 (1 H, br s, H-1); δ_C (CD_3OD): 54.2 (OCH_3), 61.9 (C-5), 77.6, 82.2, 84.4 (C-2, C-3, C-4), 109.4 (C-1).

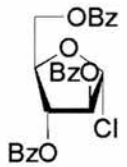
Methyl 5-diphenylphosphoro-α-D-arabinfuranoside (4.28)



To an ice cold solution of methyl α-D-arabinofuranoside **(4.83)** (302 mg, 1.8 mmol) in pyridine (3 ml), was added diphenyl chlorophosphate (560 µl, 2.7 mmol, 1.5 eq). The reaction was monitored by TLC [DCM– methanol, (8.5:1)], and upon completion methanol (excess) was added to destroy any remaining diphenyl chlorophosphate. The resulting

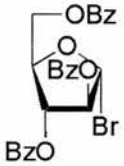
solution was concentrated and coevaporated with toluene to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 2:3) gave *the title compound* **(4.28)** as an oil (428 mg, 60 %); $[\alpha]_D$ +63.2° (*c* 0.85, $CHCl_3$); δ_H (CD_3OD): 3.25 (3 H, s, OCH_3), 3.63 (2 H, br s, 2 x O*H*), 3.83 (1 H, m, H-3), 3.96 (1 H, m, H-2), 4.07 (1 H, m, H-4), 4.26 – 4.33 (2 H, m, H-5 and H-5'), 4.74 (1 H, s, H-1), 7.07 – 7.27 (10 H, m, Ar); δ_C ($CDCl_3$) 54.5 (OCH_3), 67.9 (1 C, d, $J_{C,P}$ 6.3, C-5), 76.7, 80.3 (C-2 and C-3), 82.1 (1 C, d, $J_{C,P}$ 6.9, C-4), 108.3 (C-1), 119.5 (3 C), 119.6, 125.0 (2 C), 129.3 (2 C), 129.3 (2 C) (10 C, Ar), 149.7, 149.8 (2 x quat. Ar C); δ_P ($CDCl_3$): -6.4 (1 P, s, *P*=O); *m/z* (ES) 397 ($M+H^+$, 100%); HRMS: Found: 397.1038. $C_{18}H_{21}O_8P$ ($M+H^+$) Requires 397.1052.

2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl chloride (4.23)



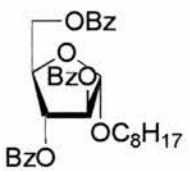
To a solution of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(4.22)** (1.0 g, 2.1 mmol) in anhydrous DCM (10 ml), was added DCMME (3.8 ml, 42 mmol). $SnCl_4$ (0.33 ml, 3.2 mmol) was added dropwise and the reaction was allowed to stir under nitrogen until TLC [toluene – EtOAc, (5:1)] indicated completion (approx. 0.5 h). The reaction mixture was then diluted with equal volumes of ice-cold water and DCM. The organic layer was then washed several times 10 % aq. $NaHCO_3$ solution (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give *the title compound* as a foam **(4.23)** (937 mg, 91 %); $[\alpha]_D$ +34.1° (*c* 1.0, $(CH_3)_2CO$), (lit., [24] $[\alpha]_D$ -28.0° for the L-isomer (*c* 0.99, $(CH_3)_2CO$)); δ_H ($CDCl_3$): 4.80 – 5.10 (3 H, m, H-4, H-5 and H-5'), 5.76 (1 H, br s, H-3), 5.79 (1 H, s, H-2), 6.39 (1 H, s, H-1), 7.28 – 7.96, 7.99 – 8.24 (15 H, 2 x m, Ar); δ_C ($CDCl_3$) 63.1 (C-5), 76.9, 84.1, 85.1 (C-2, C-3 and C-4), 95.6 (C-1), 128.5 (2 C), 128.8 (3 C), 129.7, 128.0 (3 C), 130.1 (3 C), 130.2 (3 C) (15 C, Ar), 133.4, 134.0 (2 C) (3 C, 3 x quat. C), 165.4, 165.9, 166.3 (3 C, 3 x *C*O); *m/z* (ES) 445 (M-Cl, 100%); HRMS: Found: 445.1287 $C_{26}H_{21}O_7$ (M-Cl) Requires 445.1287.

2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide (4.24)[20,25]



To a solution of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(4.22)** (1.0 g, 2.1 mmol) in glacial acetic acid (5 ml), was added 45 % HBr in glacial acetic acid (5 ml). The reaction was allowed to stir under nitrogen until TLC [toluene – EtOAc, (5:1)] indicated completion (approx. 0.5 h). The reaction mixture was then diluted with equal volumes of ice-cold water and DCM. The organic layer was then washed with 10 % aq. $NaHCO_3$ solution. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a foam **(4.24)** (774 mg, 70 %); $[\alpha]_D$ +82.9° (*c* 0.5, $CHCl_3$), (lit., [20] $[\alpha]_D$ +84.8° (*c* 1.15, $CHCl_3$)); δ_H ($CDCl_3$): 4.75 – 4.95 (3 H, m, H-4, H-5 and H-5'), 5.66 (1 H, t, $J_{3,4}$ 4.7, H-3), 5.98 (1 H, s, H-2), 6.66 (1 H, s, H-1), 7.25 – 7.60 (15 H, m, Ar), 7.98 – 8.16 (6 H, m, Ar); δ_C ($CDCl_3$) 62.7 (C-5), 76.8, 84.8, 85.8 (C-2, C-3 and C-4), 88.9 (C-1), 128.5 (2 C), 128.8 (3 C), 128.9,129.6, 129.9 (3 C), 130.1 (3 C), 130.2 (2 C) (15 C, Ar), 133.4, 134.0 (2 C) (3 C, 3 x quat. Ar C), 165.3, 165.9, 166.2 (3 x *C*O).

Octyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (4.25)[26,27,28]

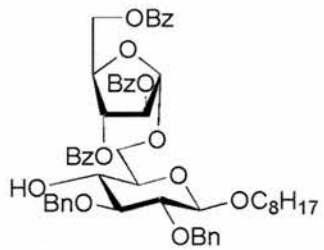


To a solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl chloride **(4.23)** (300 mg, 0.63 mmol) in anhydrous acetonitrile (3 ml), was added molecular iodine (360 mg, 1.42 mmol) and anhydrous octanol (0.2 ml, 1.26 mmol). The solution was allowed to stir until TLC [hexane – EtOAc, (6:1)] indicated completion. The reaction mixture was then diluted in an equal volume of DCM and washed with 10 % aq. $Na_2S_2O_3$ solution (2 x 200 ml) and 10 % aq. Na_2CO_3 solution (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 6:1) gave *octyl 2,3,5-tri-O-benzoyl-α-D-arabinofuranoside* **(4.25)** as an oil (250 mg, 69 %); (Found: C, 70.78; H, 6.85. $C_{34}H_{38}O_8$ Requires C, 71.06; H, 6.67%); $[\alpha]_D$ -16.0° (*c* 0.35,

$CHCl_3$)*; δ_H ($CDCl_3$): 0.87 (3 H, t, *J* 6.3, OCH_3), 1.11 – 1.42 (10 H, m, 5 x CH_2), 1.63 – 1.70 (2 H, m, OCH_2CH_2), 3.60, 3.80 (2 H, 2 x m, OCH_2CH_2), 4.60 (1 H, m, H-4), 4.73 (1 H, dd, $J_{4,5}$ 5.2, $J_{5,5'}$ 11.8, H-5), 4.86 (1 H, dd, $J_{4,5'}$ 3.6, $J_{5,5'}$, H-5'), 5.31 (1 H, s, H-1), 5.54 (1 H, m, H-2), 5.60 (1 H, d, $J_{3,4}$ 4.7, H-3), 7.26 – 7.61, 8.00 – 8.11 (15 H, 2 x m, Ar); δ_C ($CDCl_3$) 14.1 (CH_3), 22.6, 26.2, 29.3, 29.4, 29.6, 31.8 (6 x CH_2), 63.9, 67.7 (C-5 and OCH_2), 78.1, 81.1, 82.2 (C-2, C-3 and C-4),105.8 (C-1), 128.5 (2 C), 128.6 (3 C), 129.3, 129.4, 129.9 (2 C), 130.0 (3 C), 130.1 (3 C) (15 C, Ar), 133.2, 133.6 (2 C) (3 x quat Ar. C) 165.6, 166.0, 166.4 (3 x *C*O).

*Although there are several references[26,27,28] to this compound in the literature no optical rotation, ^1H and ^{13}C data have been recorded.

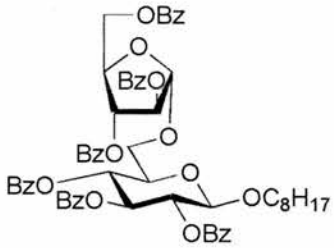
Octyl 6-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-β-D-glucopyranoside (4.26)



To a mixture of octyl 2,3-di-*O*-benzyl-β-D-glucopyranoside **(4.21)** (198 mg, 0.35 mmol), molecular sieves (4 Å) (500 mg), and anhydrous DCM (2 ml) was added a solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide **(4.24)** (369 mg, 0.70 mmol) in anhydrous DCM (3 ml). The mixture was cooled (-30°C) and stirred for 1 h under nitrogen, then silver trifluoromethanesulfonate (455 mg, 3.51 mmol) was quickly added. Once TLC [hexane – EtOAc, (3:1)] showed that the reaction had gone to completion (within 10 min.), 2,4,6-collidine (0.02 ml, 0.35 mmol, 0.5 eq) was added and stirring was continued for approx. 0.5 h. The reaction mixture was then diluted with DCM (50 ml), filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 2 M HCl (200 ml) and extracted with DCM (2 x 200 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ solution (2 x 200 ml) and water (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 4.5:1) gave *octyl 6-O-(2,3,5-tri-O-*

benzoyl-α-D-arabinofuranosyl)-2,3-di-O-benzyl-β-D-glucopyranoside **(4.26)** as a clear oil (258 mg, 73 %); (Found: C, 70.35; H, 6.59. $C_{54}H_{60}O_{13}$ Requires C, 70.72; H, 6.59%); $[\alpha]_D$ – 31.9° (*c* 0.28, $CHCl_3$); δ_H ($CDCl_3$): 1.05 (3 H, t, *J* 6.9, CH_2CH_3), 1.38 – 1.59 (10 H, m, 5 x CH_2), 1.62 – 1.80 (2 H, m, OCH_2CH_2), 3.61 – 3.69 (4 H, m, H-2, H-3, H-5 and 1 H of OCH_2), 3.88 (1 H, t, $J_{3,4}$ 9.1, $J_{4,5}$ 9.1, H-4), 4.06 - 4.11 (2 H, m, H-6' and 1 H of OCH_2), 4.25 (1 H, dd, $J_{5,6}$ 5.2, $J_{6,6'}$ 11.0, H-6), 4.61 (1 H, d, $J_{1,2}$ 7.4, H-1), 4.82 – 4.84 (1 H, m, H-4'), 4.86 – 4.94 (3 H, m, H-5'$_A$ and 1 H each of 2 x CH_2Ph), 5.02 (1 H, dd, $J_{5'A,5'B}$ 8.6, $J_{4',5'B}$ 5.0, H-5'$_B$), 5.13 (1 H, d, *J* 7.2, 1 H of CH_2Ph), 5.15 (1 H, d, *J* 6.6, 1 H of CH_2Ph), 5.56 (1 H, s, H-1'), 5.78 (1 H, d, $J_{3',4'}$ 6.3, H-3'), 5.79 (1 H, s, H-2'), 7.42 – 8.35 (25 H, Ar)); δ_C ($CDCl_3$): 14.0 (CH_3), 22.6, 26.1, 19.2, 19.4, 29.7, 31.8 (6 x CH_2), 63.8 (C-5'), 66.5 (C-6), 70.2 (OCH_2), 70.4 (C-4), 74.0 (C-5), 74.7 (OCH_2Ph), 75.2 (OCH_2Ph), 77.9 (C-3'), 81.4 (C-4'), 81.8 (C-2), 81.9 (C-2'), 84.0 (C-3), 103.9 (C-1), 105.7 (C-1'), 127.8, 128.0, 128.1 (2 C), 128.3 (2 C), 128.4 (3 C), 128.5 (2 C), 128.7 (4 C), 129.2, 129.9 (3 C), 130.0 (3 C), 130.1 (3 C), (25 C, Ar), 133.1, 133.6 (2 C), 138.6, 138.8 (5 C, 5 x quat. Ar C), 165.5, 165.9, 166.4 (3 x CO).

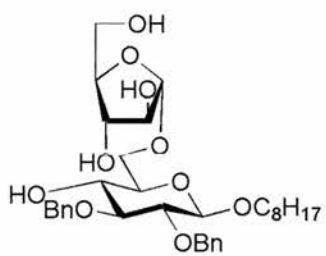
Octyl 6-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.27)



To a mixture of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)** (225 mg, 0.37 mmol), molecular sieves (4 Å) (600 mg), and anhydrous DCM (2.5 ml) was added a solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide **(4.24)** (391 mg, 0.74 mmol) in anhydrous DCM (3.5 ml). The mixture was cooled (-30°C) and stirred for 1 h under nitrogen, then silver trifluoromethanesulfonate (285 mg, 1.11 mmol, 3 eq) was quickly added. Once TLC [hexane – EtOAc, (3:1)] had shown that the reaction had gone to completion (approx. 5 - 10 min), 2,4,6-collidine (0.02 ml, 0.18 mmol, 0.5 eq) was added and stirring was continued for approx. 0.5 h. The reaction mixture was then diluted with DCM (50 ml), filtered through

Celite and concentrated under reduced pressure. The resulting syrup was diluted with 2 M HCl (200 ml) and extracted with DCM (2 x 200 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ solution (2 x 200 ml) and water (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 4.5:1) gave *octyl 6-O-(2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside* **(4.27)** as a white solid (272 mg, 70 %); mp 66 - 68°C (95% EtOH); (Found: C, 70.06; H, 5.76. $C_{61}H_{60}O_{16}$ Requires C, 69.84; H, 5.66%); $[\alpha]_D$ -18.9° (*c* 0.095, $CHCl_3$); δ_H ($CDCl_3$): 0.82 (3 H, t, *J* 7.2, OCH_3), 0.97 – 1.22 (10 H, m, 5 x CH_2), 1.32 – 1.50 (2 H, m, OCH_2CH_2), 3.45 (1 H, m, 1 H of OCH_2), 3.81 – 4.10 (4 H, m, H-5, H-6, H-6' and 1 H of OCH_2), 4.60 – 4.77 (3 H, m, H-5'$_A$, H-5'$_B$ and H-4'), 4.79 (1 H, d, $J_{1,2}$ 7.9, H-1), 5.32 (1 H, s, H-1'), 5.49 – 5.55 (3 H, m, H-2, H-2' and H-3'), 5.67 (1 H, t, $J_{2,3}$ 9.7, $J_{3,4}$ 9.7/$J_{3,4}$ 9.7, $J_{4,5}$ 9.7, H-3/H-4), 5.88 (1 H, t, $J_{2,3}$ 9.7, $J_{3,4}$ 9.7/$J_{3,4}$ 9.7, $J_{4,5}$ 9.7, H-3/H-4) 7.19 – 8.22 (30 H, m, Ar); δ_C ($CDCl_3$): 14.0 (CH_3), 22.6, 25.7, 29.0, 29.2, 29.3, 31.7 (6 x CH_2), 63.7, 65.6, 70.2 (OCH_2, C-6 and C-5'), 69.5, 71.9, 73.1, 73.2, 77.7, 81.4, 81.9 (C-2, C-3, C-4, C-5, C-2', C-3' and C-4'), 101.3 (C-1), 105.5 (C-1'), 128.1 (2 C), 128.2 (3 C), 128.4 (3 C), 128.5 (2 C), 128.8 (2 C), 128.9 (2 C), 129.0 (2 C), 129.3 (2 C), 129.4 (3 C), 129.7 (4 C), 129.8 (3 C), 130.0 (2 C) (30 C, Ar), 132.9, 133.0 (2 C), 133.3, 133.4, 133.5 (6 C, 6 x quat. Ar C), 165.0, 165.0, 165.2, 165.8 (2 C), 166.1 (6 C, 6 x CO); MALDI-TOF (+ve): *m/z* 1071.4 ($M+Na^+$), ($C_{61}H_{60}O_{16}Na$ requires *m/z* 1071.4).

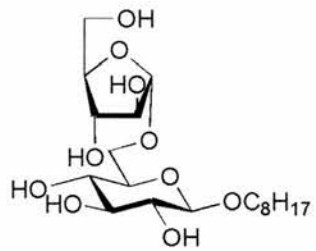
Octyl 6-*O*-(α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-β-D-glucopyranoside (4.29)



To a solution of octyl 6-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-β-D-glucopyranoside **(4.26)** (261 mg, 0.26 mmol) in anhydrous methanol (5 ml), was added a stock solution of NaOMe/MeOH (10 mg/ml) (0.5 ml). The reaction was allowed to stir until TLC [DCM– methanol, (10:1)] showed that the reaction had gone to completion (within 1

h.). The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo*. Column chromatography (silica gel; DCM– methanol, 20:1) gave *octyl 6-O-(α-D-arabinofuranosyl)-2,3-di-O-benzoyl-β-D-glucopyranoside* **(4.29)** as an oil (142 mg, 90 %); (Found: C, 65.21; H, 7.98. $C_{33}H_{48}O_{10}$ Requires C, 65.54; H, 8.00%); $[\alpha]_D$ +8.1° (*c* 0.38, $CHCl_3$); δ_H (CD_3OD): 0.87 (3 H, t, *J* 7.1, OCH_3), 1.26 - 1.39 (10 H, m, 5 x CH_2), 1.58 – 1.60 (2 H, m, OCH_2CH_2), 3.27 – 3.99 (13 H, m, H-2, H-3, H-4, H-5, H-6_A, H-6_B, H-2', H-3', H-4', H-5'$_A$, H-5'$_B$ and OCH_2), 4.42 (1 H, d, $J_{1,2}$ 7.7, H-1), 4.66 (1 H, d, *J* 11.3, 1 H of CH_2Ph), 4.69 – 4.96 (4 H, 3 H of 2 x CH_2Ph and H-1'), 7.25 – 7.35 (10 H, Ar); δ_C (CD_3OD):13.0 (CH_3), 22.3, 25.9, 29.0, 29.1, 29.5, 31.5 (6 x CH_2), 61.7 (C-5'), 65.9, 69.6, 70.2, 74.3, 74.9, 75.1, 77.5, 81.8 (2 C), 84.5, 84.6 (11 C, 2 x OCH_2Ph, OCH_2, C-2, C-3, C-4, C-5, C-6, C-2', C-3' and C-4'), 103.5 (C-1), 108.3 (C-1'), 127.2 (2 C), 127.7 (4 C), 127.9 (4 C) (10 C, Ar), 138.8, 139.0 (2 x quat. Ar C); MALDI-TOF (+ve): *m/z* 627.3 ($M+Na^+$), ($C_{33}H_{48}O_{10}Na$ requires *m/z* 627.3)

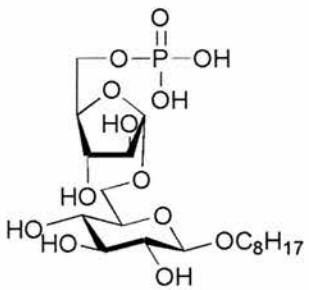
Octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside (4.12)[29]



To a solution of octyl 6-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.27)** (179 mg, 0.17 mmol) in anhydrous methanol (4 ml), was added a stock solution of NaOMe/MeOH (10 mg/ml) (0.2 ml). The reaction was allowed to stir until TLC [DCM– methanol, (10:1)] showed that the reaction had gone to completion (within 1 h.). The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo* to yield the title compound, octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** as a foam (60 mg, 83 %); $[\alpha]_D$ +25.7° (*c* 0.3, CH_3OH), (lit., [29] $[\alpha]_D$ -75.0° (*c* 0.6, CH_3OH)); δ_H (CD_3OD): 0.89 (3 H, t, *J* 6.9, CH_2CH_3), 1.28 - 1.40 (10 H, m, 5 x CH_2), 1.59 – 1.61 (2 H, m, OCH_2CH_2), 3.16 – 4.00 (13 H, m, H-2, H-3, H-4, H-5, H-6_A, H-6_B, H-2', H-3', H-4', H-5'$_A$, H-5'$_B$ and OCH_2), 4.24 (1 H, d, $J_{1,2}$ 8.0, H-1), 4.94 (1 H, d, $J_{1',2'}$ 0.8, H-1'); δ_C (CD_3OD):14.5 (CH_3), 23.8, 27.2, 30.5, 30.7, 30.9, 33.1 (6 x CH_2), 63.3 (C-5'),

67.6 (C-6), 71.2 (OCH_2), 71.6, 75.3, 76.6, 78.2, 79.0, 83.2, 86.1 (C-2, C-3, C-4, C-5, C-2', C-3', C-4'), 104.7 (C-1), 109.8 (C-1'); MALDI-TOF (+ve): *m/z* 447.2 ($M+Na^+$), ($C_{19}H_{36}O_{10}Na$ requires *m/z* 447.2); *m/z* (ES) 442 ($M+NH_4^+$, 100%); HRMS: Found: 442.2653. $C_{19}H_{40}NO_{10}$ ($M+NH_4^+$) Requires 442.2652.

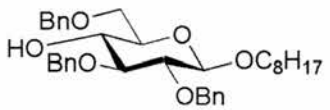
Octyl 6-*O*-(5-*O*-phospho-α-D-arabinofuranosyl)-β-D-glucopyranoside (4.11)



To a solution of octyl 6-*O*-(α-D-arabinofuranosyl)-β-D-glucopyranoside **(4.12)** (230 mg, 0.2 mmol) in anhydrous pyridine (2 ml) at 0°C, was added diphenyl chlorophosphate (118 µl, 0.57 mmol, 1.1 mol eq.). The mixture was stirred with the temperature being allowed to rise to ambient over a 12 h. period. The progress of the reaction was followed by TLC [DCM – methanol, (8:2)]. The resulting solution was concentrated and coevaporated with toluene to give a syrup. Column chromatography (silica gel; DCM – methanol, 8:2) gave *octyl 6-O-(5-O-diphenylphosphoro-α-D-arabinofuranosyl)-β-D-glucopyranoside* as a syrup (178 mg, 52 %); $[\alpha]_D$ +12.9° (*c* 1.78, CH_3OH); δ_H (CD_3OD): 1.35 (3 H, t, *J* 6.3, CH_3), 1.62 – 1.82 (10 H, m, 5 x CH_2), 2.05 – 2.10 (2 H, m, OCH_2CH_2), 3.68 (1 H, m, 1 H of OCH_2), 3.98 (1 H, m, 1 H of OCH_2), 3.76 – 3.90, 4.09 – 4.70, 4.81 – 5.00 (11 H, 3 x m, H-2 – H-6_A, H-6_B, H-2' – H-5'$_A$ and H-5'$_B$), 4.72 (1 H, d, $J_{1,2}$ 7.8, H-1), 5.44 (1 H, s, H-1'), 7.68 – 7.89 (10 H, Ar); δ_C (CD_3OD): 13.9 (CH_3), 23.1, 26.5, 29.8, 30.0, 30.3, 32.4 (6 x CH_2), 69.5 (1 C, d, $J_{C,P}$ 6.3, C-5'), 82.7 (1 C, d, $J_{C,P}$ 6.9, C-4'), 67.3, 70.6, 71.0, 74.6, 75.9, 77.5, 78.0, 82.6 (C-2, C-3, C-4, C-5, C-6, C-2', C-3' and OCH_2), 104.0 (C-1), 109.3 (C-1'), 120.8 (2 C), 120.9 (3 C), 126.5, 130.7 (4 C) (10 C, Ar), 151.4, 151.5 (2 x quat. Ar C); *m/z* (ES) 674 ($M+NH_4^+$, 100%); HRMS: Found: 674.2935. $C_{31}H_{49}NO_{13}P$ ($M+NH_4^+$) Requires: 674.2942. To a solution of octyl 6-*O*-(5-*O*-diphenylphosphoro-α-D-arabinofuranosyl)-β-D-glucopyranoside (178 mg, 0.27 mmol) in acetic acid (2 ml), was added 10% Pt on C (10 mg) and PtO_2 (10 mg). The reaction mixture was allowed to stir under a hydrogen atmosphere until TLC [DCM –

methanol, (7:3)] showed that the reaction had gone to completion (overnight). The reaction mixture was then diluted with methanol (100 ml), filtered through Celite and concentrated and coevaporated with toluene under reduced pressure. The crude material was then dissolved in 9:1 H_2O/methanol and loaded on to a Sephadex LH-20 gel filtration column (2.8 x 73 cm). Elution with 9:1 H_2O/methanol at a rate of 12 ml per hour gave *octyl 6-O-(5-O-phospho-α-D-arabinofuranosyl)-β-D-glucopyranoside* **(4.11)** as a solid (135 mg, 100 %); $[\alpha]_D$ +9.3° (*c* 0.4, CH_3OH); δ_H (CD_3OD): 1.36 (3 H, t, *J* 7.1, CH_3), 1.69 – 1.76 (10 H, m, 5 x CH_2), 2.03 – 2.10 (2 H, m, OCH_2CH_2), 3.65 – 4.69 (13 H, m, H-2 – H-6_A, H-6_B, H-2' – H-$5'_A$ and H-$5'_B$ and OCH_2), 4.71 (1 H, d, $J_{1,2}$ 7.8, H-1), 5.43 (1 H, s, H-1'); δ_C (CD_3OD): 12.4 (CH_3), 21.6, 25.0, 28.3, 28.5, 28.7, 30.9 (6 x CH_2), 65.5 (1 C, d, $J_{C,P}$ 4.7, C-5'), 81.9 (1 C, d, $J_{C,P}$ 5.2, C-4'), 65.2, 69.0, 69.4, 73.0, 74.3, 75.9, 76.7, 81.0 (C-2, C-3, C-4, C-5, C-6, C-2', C-3' and OCH_2), 102.4 (C-1), 107.6 (C-1'); δ_P (CD_3OD): 1.6 (1 P, s, *P*=O); *m/z* (ES) 522 ($M+NH_4^+$, 100%); HRMS: Found: 522.2312. $C_{19}H_{41}NO_{13}P$ ($M+NH_4^+$) Requires: 522.2316.

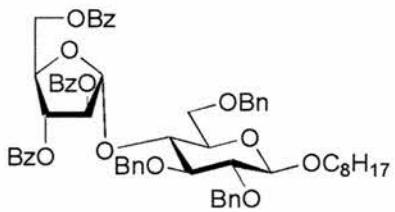
Octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4.30)[18]



Compound **(4.30)** was prepared using a selective reductive acetal ring opening procedure.[30] A solution of octyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside **(4.19)** (1.04 g, 1.85 mmol) was dissolved in anhydrous DCM (11 ml) and stirred before triethylsilane (1.08 g, 9.3 mmol, 5 mol eq.) and TFA (0.72 ml, 9.3 mmol) were added. Stirring was continued stirred until TLC [hexane– EtOAc, (5:1)] showed the reaction had gone to completion approx. 1 h). The reaction mixture was diluted in DCM and washed sequentially with 10 % aq. K_2SO_3 solution, 10 % aq. Na_2CO_3 solution and a saturated aq. NaCl solution. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 5:1 – 1:1) gave octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(4.30)** as an oil (0.85 g, 82 %); $[\alpha]_D$ -21.8° (*c* 1.0, $CHCl_3$); (lit., [18] $[\alpha]_D$ -22.6° (*c* 0.8, $CHCl_3$)); δ_H ($CDCl_3$): 0.88 (3 H, t, *J* 6.9, CH_3), 1.24 - 1.29 (10 H, m, 5 x CH_2), 1.63 - 1.70 (2 H, m, OCH_2CH_2), 3.38 – 3.62 (5 H, m, H-2, H-3, H-4, H-5 and 1 H of OCH_2), 3.66 – 3.82 (2 H, m, H-6 and H-6'), 3.93 – 3.98(1 H, m, 1 H of OCH_2), 4.42 (1 H, d,

$J_{1,2}$ 7.4, H-1), 4.57 (1 H, d, *J* 11.0, 1 H of CH_2Ph) 4.62 (1 H, d, *J* 11.0, 1 H of CH_2Ph), 4.72 (1 H, d, *J* 11.6, 1 H of CH_2Ph), 4.73 (1 H, d, *J* 10.9, 1 H of CH_2Ph), 4.94 (1 H, d, *J* 11.4, 1 H of CH_2Ph), 4.96 (1 H, d, *J* 11.0, 1 H of CH_2Ph), 7.27 – 7.36 (15 H, m, 3 x C_6H_5); δ_C ($CDCl_3$) 14.1 (CH_3), 22.7 26.2, 29.3, 29.4, 29.8, 31.8 (6 x CH_2), 70.2, 70.4, 71.7, 73.7, 73.9, 74.7, 75.3, 81.7, 84.0 (3 x CH_2Ph, 1 x OCH_2, C-2, C-3, C-4, C-5 and C-6), 103.7 (C-1), 127.7 (3 C), 127.8, 128.0 (2 C), 128.1 (2 C), 128.3 (2 C), 128.4 (3 C), 128.5 (2 C) (15 C, Ar), 137.9, 138.4, 138.6 (3 x quat. Ar C).

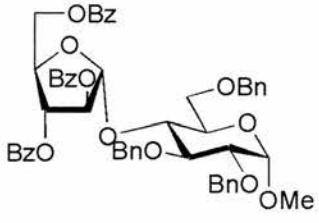
Octyl 4-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4.32)



To a mixture of octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(4.30)** (100 mg, 0.18 mmol), molecular sieves (4 Å) (300 mg), and anhydrous DCM (1.0 ml) was added a solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide **(4.24)** (187 mg, 0.36 mmol) in anhydrous DCM (2.0 ml). The mixture was cooled (-30°C) and stirred for 1 h under nitrogen, then silver trifluoromethanesulfonate (231 mg, 0.90 mmol) was quickly added. Once TLC [hexane – EtOAc, (3:1)] showed that the reaction had gone to completion (approx. 1 h.), 2,4,6-collidine (0.01 ml, 0.09 mmol, 0.5 eq) was added and stirring was continued for approx. 0.5 h. The reaction mixture was then diluted with DCM (50 ml), filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 2 M HCl (200 ml) and extracted with DCM (2 x 200 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ solution (200 ml) and water (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 5:1) gave *octyl 4-O-(2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside* **(4.32)** as a foam (64 mg, 35 %); (Found: C, 72.54; H, 7.15. $C_{61}H_{66}O_{13}.H_2O$ Requires: C, 72.60; H, 6.79%); $[\alpha]_D$ -4.8° (*c* 0.02, $CHCl_3$); δ_H ($CDCl_3$): 0.87 (3 H, t, *J* 6.9, CH_2CH_3), 1.27 – 1.40 (10 H, m, 5 x CH_2), 1.62 – 1.69 (2 H, m, OCH_2CH_2), 3.46 – 3.84 (7 H, m, H-2, H-3, H-4, H-5, , H-6_A, H-

6_B and 1 H of OCH_2), 3.98 (1 H, m, 1 H of OCH_2), 4.29 (1 H, m, H-4'), 4.38 – 4.60 (5 H, m, CH_2Ph, H-1, H-5'$_A$, H-5'$_B$), 4.69 (1 H, d, *J* 10.9, 1 H of CH_2Ph), 4.72 (1 H, d, *J* 9.6, 1 H of CH_2Ph), 4.95 (1 H, d, *J* 10.9, 1 H of CH_2Ph), 4.97 (1 H, d, *J* 10.9, 1 H of CH_2Ph), 5.46 (1 H, d, $J_{3',4'}$ 4.4, H-3'), 5.52 (1 H, s, H-1'), 5.78 (1 H, s, H-2'), 6.96 – 8.08 (30 H, Ar); δ_C ($CDCl_3$): 14.1 (CH_3), 22.6, 26.2, 29.2, 29.4, 29.8, 31.8 (6 x CH_2), 63.9 (C-5'), 69.7, 70.3, 73.5, 74.0, 74.4, 74.7, 75.4, 78.0, 81.7, 82.0, 82.3, 84.6 (3 x OCH_2Ph, C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4'and OCH_2), 103.6 (C-1), 106.7 (C-1'), 127.4, 127.6 (2 C), 127.7 (3 C), 127.8, 128.2 (3 C), 128.4 (4 C), 128.5 (4 C), 128.7 (3 C), 129.0 (2 C), 129.3, 129.6, 129.9 (2 C), 130.1 (3 C) (30 C, Ar), 133.2, 133.6, 133.7 (3 x quat. Ar C'), 138.3 (2 C), 138.5 (3 C, 3 x quat. Ar C), 165.3, 165.7, 166.3 (3 x *C*O); *m/z* (ES) 1024 ($M+NH_4^+$, 100%); HRMS: Found: 1024.4844. $C_{61}H_{70}NO_{13}$ ($M+NH_4^+$) Requires 1024.4847.

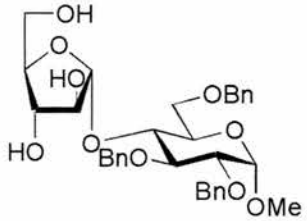
Methyl 4-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (4.33)



To a mixture of methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[31] **(4.31)** (which was kindly donated by Dr. P. McGurk, University of St. Andrews, 2000) (146 mg, 0.31 mmol), molecular sieves (4 Å) (450 mg), and anhydrous DCM (1.5 ml) was added a solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide **(4.24)** (329 mg, 0.63 mmol) in anhydrous DCM (3.5 ml). The mixture was cooled (-30°C) and stirred for 1 h under nitrogen, then silver trifluoromethanesulfonate (239 mg, 0.93 mmol) was quickly added. Once TLC [hexane – EtOAc, (3:1)] showed that the reaction had gone to completion (approx. 40 min.), 2,4,6-collidine (0.01 ml, 0.18 mmol, 0.5 eq) was added and stirring was continued for approx. 0.5 h. The reaction mixture was then diluted with DCM (50 ml), filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 2 M HCl (200 ml) and extracted with DCM (2 x 200 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ solution (2 x 200 ml) and water (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a

syrup. Column chromatography (silica gel; Hexane – EtOAc, 6:1) gave *methyl 4-O-(2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside* **(4.33)** as a foam (90 mg, 32 %); (Found: C, 71.10; H, 5.80. $C_{54}H_{52}O_{13}$ Requires C, 71.35; H, 5.77%); $[\alpha]_D$ -4.8° (*c* 0.02, $CHCl_3$); δ_H ($CDCl_3$): 3.44 (3 H, s, OCH_3), 3.57 (1 H, dd, $J_{2,3}$ 9.6, $J_{1,2}$ 3.6, H-2), 3.63 – 3.76 (2 H, m, H-3, H-4), 3.85 (2 H, m, H-6_A and H-6_B), 4.05 (1 H, m, H-5), 4.27 (1 H, m, H-4'), 4.41 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.75 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.78 (1 H, d, *J* 10.7, 1 H of CH_2Ph), 5.00 (1 H, d, *J* 10.7, 1 H of CH_2Ph), 4.46 – 4.65 (5 H, m, CH_2Ph, H-1, H-5'$_A$, H-5'$_B$), 5.47 (1 H, d, $J_{3',4'}$ 4.1, H-3'), 5.55 (1 H, s, H-1'), 5.82 (1 H, s, H-2'), 6.99 – 8.10 (30 H, Ar); δ_C ($CDCl_3$): 55.4 (*C*H_3), 63.9, 69.2, 69.7, 73.4, 73.5, 74.1, 75.5, 78.1, 80.1, 81.7 (2 C), 82.0 (12 C, 3 x O*C*H_2Ph, C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5'), 98.1 (C-1), 106.9 (C-1'), 127.4, 127.7 (3 C), 128.1, 128.3 (4 C), 128.4 (5 C), 128.5 (2 C), 128.6 (3 C), 128.7 (2 C), 129.1, 129.3, 129.9 (3 C), 130.1 (4 C) (30 C, Ar), 133.1, 133.5, 133.7 (2 C), 138.2, 138.5 (6 C, 6 x quat. Ar C), 165.3, 165.7, 166.3 (3 x *C*O); MALDI-TOF (+ve): *m/z* 931.3 ($M+Na^+$), ($C_{54}H_{52}O_{13}Na$ requires *m/z* 931.3).

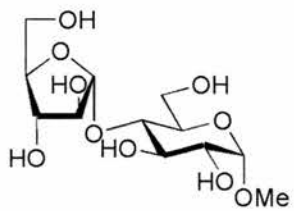
Methyl 4-*O*-(α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (4.34)



To a solution of methyl 4-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(4.33)** (475 mg, 0.52 mmol) in anhydrous methanol (6 ml), was added a stock solution of NaOMe/MeOH (10 mg/ml) (0.6 ml). The reaction was allowed to stir until TLC [DCM– methanol, (9.7:0.3)] showed that the reaction had gone to completion (within 1 h.). The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo*. Column chromatography (silica gel; hexane – EtOAc, 3:1 — 1:2) gave *methyl 4-O-(α-D-arabinofuranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside* **(4.34)** as a solid (296 mg, 95 %); (Found: C, 66.41; H, 6.80. $C_{33}H_{40}O_{10}$ Requires C, 66.43; H, 6.76 %); $[\alpha]_D$ +106.5° (*c* 3.2, CH_3OH); δ_H (CD_3OD): 3.37 (3 H, s, OCH_3), 3.49 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 9.6, H-2), 3.57 – 3.90 (9 H, m, H-3, H-4, H-5, H-6_A, H-6_B, H-2', H-3', H-5'$_A$, H-

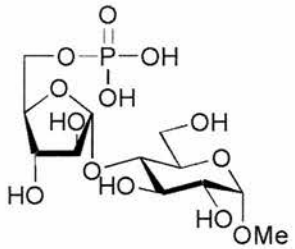
5'$_B$), 3.99 (1 H, m, H-4'), 4.52 (1 H, d, *J* 11.8, 1 H of OCH_2Ph), 4.60 (1 H, d, *J* 11.8, 1 H of OCH_2Ph), 4.61 (1 H, d, *J* 12.1, 1 H of OCH_2Ph), 4.66 (1 H, d, *J* 12.1, 1 H of OCH_2Ph), 4.77 (1 H, d, *J* 12.1, 1 H of OCH_2Ph), 4.85 (1 H, d, *J* 12.1, 1 H of OCH_2Ph), 4.70 (1 H, d, $J_{1,2}$, H-1), 5.37 (1 H, d, $J_{1',2'}$ 1.7, H-1'), 7.24 – 7.39 (15 H, Ar); δ_C (CD_3OD): 54.2 (CH_3), 61.4 (C-5'), 69.5, 69.8, 72.6, 73.1, 75.0, 75.2, 77.2, 80.1, 81.5, 82.3, 84.6 (3 x OCH_2Ph, C-2, C-3, C-4, C-5, C-6, C-2', C-3' and C-4'), 97.6 (C-1), 109.2 (C-1'), 127.4 (2 C), 127.7 (2 C), 127.9 (2 C), 128.0 (2 C), 128.1 (3 C), 128.2 (4 C) (15 C, Ar), 138.4, 138.5, 138.6 (3 x quat. Ar C); MALDI-TOF (+ve): *m/z* 619.1 ($M+Na^+$), ($C_{33}H_{40}O_{10}Na$ requires *m/z* 619.3).

Methyl 4-*O*-(-α-D-arabinofuranosyl)-α-D-glucopyranoside (4.82)



The title compound was prepared using a standard debenzylation procedure.[32] To a solution of methyl 4-*O*-(α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(4.34)** (60 mg, 0.1 mmol) in HPLC methanol (2.5 ml), was added 10 % Pd-C (10 mg). The reaction was allowed to stir until TLC [DCM– methanol, (4:1)] showed that the reaction had gone to completion (overnight). The reaction mixture was then diluted with methanol (50 ml), filtered through Celite and concentrated under reduced pressure to give *methyl 4-O-(α-D-arabinofuranosyl)-α-D-glucopyranoside* as a solid **(4.82)** (37 mg, 87 %); $[\alpha]_D$ +145.0° (*c* 0.4, CH_3OH); δ_H (CD_3OD): 3.39 (3 H, s, OCH_3), 3.41 – 4.02 (11 H, m, H-2 – H-6_A, H-6_B, H-2' - H-5'$_A$ and H-5'$_B$), 4.66 (1 H, d, $J_{1,2}$ 3.6, H-1), 5.33 (1 H, s, H-1'); δ_C (CD_3OD): 54.2 (OCH_3), 61.4 (C-5'), 61.8 (C-6), 70.9, 72.2, 74.0, 75.6, 77.0, 81.9, 84.7 (C-2 - C-5, C-2' - C-4'), 99.8 (C-1), 109.1 (C-1'); MALDI-TOF (+ve): *m/z* 349.2 ($M+Na^+$), ($C_{12}H_{22}O_{10}Na$ requires *m/z* 349.2); *m/z* (ES) 327 ($M+H^+$, 100%); HRMS: Found: 327.1289. $C_{12}H_{22}O_{10}$ ($M+H^+$) Requires 327.1291.

Methyl 4-*O*-(5-phospho-α-D-arabinofuranosyl)-α-D-glucopyranoside (4.9)

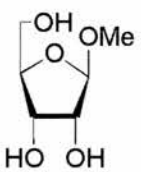


To a solution of methyl 4-*O*-(α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(4.34)** (50 mg, 0.084 mmol) in anhydrous pyridine (500 µl) at 0°C, was added diphenyl chlorophosphate (19 µl, 0.09 mmol, 1.1 mol eq.). The mixture was stirred with the temperature being allowed to rise to ambient over a 12 h. period. The progress of the reaction was followed by TLC [Hexane – EtOAc, (2:3)]. The resulting solution was concentrated and coevaporated with toluene to give a syrup. The reaction mixture was then diluted with DCM (200 ml) and washed with 2M HCl (2 x 100 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a white solid. Column chromatography (silica gel; Hexane – EtOAc, 1:1) gave *methyl 4-O-(5-O-diphenylphosphoro-α-D-arabinofuranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside* **(4.35)** as a white foam (45 mg, 65 %); δ_H ($CDCl_3$): 3.30 (3 H, s, OCH_3), 3.40 – 3.60, 3.80 – 3.88, 4.11 – 4.15 (11 H, 3 x m, H-2 – H-6_A, H-6_B, H-2' – H-5'$_A$ and H-5'$_B$), 4.51 (1 H, d, $J_{1,2}$ 3.3, H-1), 4.38 (1 H, d, *J* 12.2, 1 H of CH_2Ph), 4.47 (1 H, d, *J* 12.2, 1 H of CH_2Ph), 4.56 (1 H, d, *J* 12.2, 1 H of CH_2Ph), 4.63 (1 H, d, *J* 11.0, 1 H of CH_2Ph), 4.69 (1 H, d, *J* 11.7, 1 H of CH_2Ph), 4.89 (1 H, d, *J* 11.0, 1 H of CH_2Ph), 5.20 (1 H, s, H-1'), 6.88 – 7.69 (25 H, Ar); (due to the instability of this compound. The carbon spectrum was only run for a short period of time. The diagnostic signals we can see are); δ_C ($CDCl_3$): 55.2 (CH_3), 68.1, 69.4 (1 C, d, $J_{C,P}$ 10.3, C-5'), 73.3 (1 C, d, $J_{C,P}$ 11.5, C-4'), 75.9 (2 C), 77.2 (2 C), 79.9, 80.8, 81.8 (12 C, 3 x OCH_2Ph, C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4' and C-5'), 97.9 (C-1), 109.4 (C-1'), 120.1 (4 C), 120.2 (2 C), 125.6 (2 C), 127.7 (2 C), 127.9 (2 C), 128.1 (2 C), 128.2 (2 C), 128.4 (3 C), 128.6 (3 C), 129.9 (3 C) (25 C, Ar), 138.1 (2 C), 138.7, (3 C, 3 x quat. Ar C of Bn), 150.4 (2 C, 2 x quat. Ar C of Ph); *m/z* (ES) 846 ($M+NH_4^+$, 100%); HRMS: Found: 846.3222. $C_{45}H_{53}NO_{13}P$ ($M+NH_4^+$) Requires 846.3255.

The title compound was prepared using a standard dephenylation procedure.[32] To a solution of methyl 4-*O*-(5-*O*-diphenylphosphoro-α-D-arabinofuranosyl)-2,3,6-tri-*O*-benzyl-α-D-

glucopyranoside **(4.35)** (45 mg, 0.05 mmol) in acetic acid (1 ml), was added 10 % Pd-C (10 mg). The reaction was allowed to stir under a hydrogen atmosphere until TLC [DCM – methanol, (7:1)] showed that the reaction had gone to completion (overnight). The reaction mixture was then diluted with methanol (100 ml), filtered through Celite and concentrated under reduced pressure to give *methyl 4-O-(5-O-diphenylphosphoro-α-D-arabinofuranosyl)-α-D-glucopyranoside* as a solid (26 mg, 87 %). This material was redissolved in acetic acid (1 ml), was added 10% Pt on C (10 mg) and PtO_2 (10 mg). The reaction mixture was allowed to stir under a hydrogen atmosphere until TLC [DCM – methanol, (8:2)] showed that the reaction had gone to completion (overnight). The reaction mixture was then diluted with methanol (100 ml), filtered through Celite and concentrated under reduced pressure to give *methyl 4-O-(5-phospho-α-D-arabinofuranosyl)-α-D-glucopyranoside* **(4.9)** as a solid (11 mg, 57%); $[\alpha]_D$ +79.3° (*c* 0.1, H_2O); δ_H (D_2O): 3.24 (3 H, s, OCH_3), 3.37 – 3.43, 3.45 – 3.69, 3.72 – 3.89, 3.97 – 4.03 (11 H, 4 x m, H-2 – H-6_A, H-6_B, H-2' – H-5'$_A$ and H-5'$_B$), 4.64 (2 H, br s, H-1 and *H*OD), 5.20 (1 H, s, H-1'); δ_C (D_2O): 55.5 (CH_3), 61.0 (C-6), 64.9 (1 C, d, $J_{C,P}$ 5.2, C-5'), 70.7, 71.6, 73.8, 76.2, 76.6, 81.6 (C-2, C-3, C-4, C-5, C-2' and C-3'), 83.5 (1 C, d, $J_{C,P}$ 8.0, C-4'), 99.6 (C-1), 109.3 (C-1'); δ_P (D_2O): 0.7 (1 P, s, *P*=O); *m/z* (ES) 405 ($M+H^+$, 100%); HRMS: Found: 405.0793. $C_{12}H_{21}O_{13}P$ ($M+H^+$) Requires 405.0798.

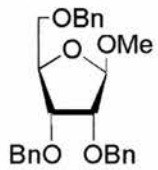
Methyl β-D-ribofuranoside (4.39)[33,34]



To a solution of D-ribose (5 g, 33.3 mmol) in anhydrous methanol (100 ml) at 0°C was added concentrated H_2SO_4 (0.5 ml). The mixture was allowed to stir at 4°C until TLC [DCM – MeOH, (8.5:1)] indicated completion (approx. 12 h). The reaction mixture was neutralised with Amberlite IRA 400 ion exchange resin and decolourised with charcoal. The reaction mixture was then filtered through Celite, washed with water (50 ml) and concentrated to give a syrup. Crystallisation gave the known title compound **(4.39)** as a white solid (4.10 g, 75 %); mp 78 - 80°C (EtOAc), (lit., [33,34] 79 - 80°C); $[\alpha]_D$ -49.3° (*c* 1.0, H_2O), (lit., [33,34] $[\alpha]_D$ -50.0° (*c* 2.0, H_2O)); δ_H (CD_3OD): 3.26 (3 H, s, OCH_3), 3.44 (1 H, dd, $J_{4,5'}$ 6.5,

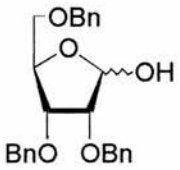
$J_{5,5'}$ 11.9, H-5'), 3.62 (1 H, dd, $J_{4,5}$ 3.4, $J_{5,5'}$, H-5), 3.77 (1 H, d, $J_{2,3}$ 4.5, H-2), 3.84 (1 H, m, H-4), 3.93 (1H, dd, $J_{2,3}$, $J_{3,4}$ 6.8, H-3), 4.65 (1H, s, H-1); δ_C (CD_3OD): 54.2 (CH_3), 63.8 (C-5), 71.5, 75.0, 83.7 (C-2, C-3, C-4), 105.8 (C-1).

Methyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranoside (4.40)[33]



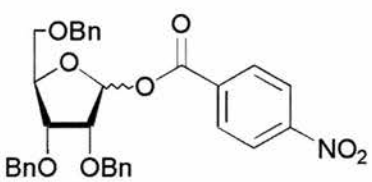
The title compound was prepared using a standard benzylation procedure.[19] A solution of methyl β-D-ribofuranoside **(4.39)** (100 mg, 0.61 mmol) was dissolved in anhydrous DMF (1 ml) and cooled (0°C) before sodium hydride (60 % w/w suspension in mineral oil) (80 mg, 2.01 mmol) was slowly added. After 0.5 h BnBr (0.24 ml, 2.01 mmol) was slowly added and the solution stirred until TLC [hexane– EtOAc, (4:1)] showed the reaction had gone to completion. The reaction mixture was cooled (0°C) before methanol (15 ml) was added. The solution was stirred 0.5 h. and concentrated under reduced pressure to a syrup. The syrup was diluted with an equal volume of DCM and washed with water (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 6:1) gave the known title compound **(4.40)** as a syrup (193 mg, 73 %); $[\alpha]_D$ +21.4° (*c* 1.9, dioxane); (lit., [33] $[\alpha]_D$ +22.4° (*c* 3.6, dioxane)); δ_H ($CDCl_3$): 3.24 (3 H, s, OCH_3), 3.44 (1 H, dd, $J_{4,5'}$ 5.8, $J_{5,5'}$ 10.6, H-5'), 3.54 (1 H, dd, $J_{4,5}$ 3.7, $J_{5,5'}$, H-5) 3.76 (1 H, d, $J_{2,3}$ 4.5, H-2), 3.94 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 7.0, H-3), 4.27 (1 H, m, H-4), 4.37 (1 H, d, *J* 11.9, 1 H of CH_2Ph), 4.46 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.48 (1 H, d, *J* 11.9, 1 H of CH_2Ph), 4.51 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.53 (1 H, d, *J* 12.3, 1 H of CH_2Ph), 4.59 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.85 (1 H, s, H-1), 7.18 – 7.30 (15 H, Ar); δ_C ($CDCl_3$): 54.4 (CH_3), 70.7, 71.7, 71.8, 72.5 (4 x CH_2), 77.8, 79.1, 79.8 (7 C, 3 x CH_2Ph, 3 x *C*H and 1 x CH_2), 105.8 (C-1), 127.0 (3 C), 127.3 (4 C), 127.4 (2 C), 127.8 (4 C), 127.9 (2 C) (15 C, Ar), 137.3 (2 C), 137.8 (3 C, 3 x quat. Ar C).

2,3,5-tri-*O*-benzyl-D-ribofuranose (4.41)[33]



To a solution of methyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranoside **(4.40)** (100 mg, 0.23 mmol) in DCM (1 ml), was added 80 % aqueous TFA solution (1 ml). The solution was stirred until TLC [hexane– EtOAc, (4:1)] showed the reaction had gone to completion (24 hours). The resulting solution was concentrated and coevaporated with isopropanol to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 3.5:1) gave the title compound **(4.41)** as an oil (52 mg, 54 %); $[\alpha]_D$ +31.5° (*c* 0.7, dioxane); (lit., [33] $[\alpha]_D$ +37.0° (*c* 4.0, dioxane)); δ_H ($CDCl_3$): 3.33 – 3.92 (7 H, H-2 (α and β), H-3 (β), H-5_A (α and β), H-5_B (α and β)), 4.12 – 4.65 (16 H, m, 3 x CH_2Ph (α and β), H-1 (β), H-3 (α), H-4 (α and β)), 5.23 (1 H, s, H-1 (α)), 7.14 – 7.31 (30 H, m, Ar); δ_C ($CDCl_3$): 68.8, 69.3, 71.6, 71.8 (2 C), 72.1, 72.8 (2 C) (8 C, 3 x CH_2Ph (α and β), C-5 (α and β)), 76.6, 77.0, 77.1, 80.1, 80.1, 80.4 (C-2 (α and β), C-3 (α and β), C-4 (α and β)), 95.6, 99.7 (C-1 (α and β)), 127.0 (3 C), 127.2 (2 C), 127.3 (5 C), 127.4 (6 C), 127.5 (2 C), 127.9 (12 C), (30 C, Ar), 136.8, 136.9 (2 C), 137.2, 137.3, 137.3 (6 C, 6 x Quat. Ar C); MALDI-TOF (+ve): *m/z* 443.2 $(M+Na)^+$, ($C_{26}H_{28}O_5Na$ requires *m/z* 443.2).

2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-ribofuranose (4.42)[35,36]

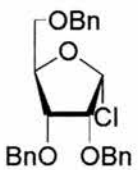


To an ice cold solution of 2,3,5-tri-*O*-benzyl-D-ribofuranose **(4.41)** (170 mg, 0.4 mmol) in anhydrous DCM (2 ml), was added a solution of *p*-nitrobenzoyl chloride (83 mg, 0.44 mmol) in anhydrous DCM (250 µl) and anhydrous pyridine (100 µl). The solution was stirred until TLC [hexane – EtOAc, (3:1)] showed the reaction had gone to completion (24 hours). The resulting solution was diluted with DCM (100 ml) and washed with 2 M aq. HCl (100 ml), saturated aq. $NaHCO_3$ (100 ml) and water (100 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column

chromatography (silica gel; Hexane – EtOAc, 5:1) gave the title compound **(4.42)*** as an oil (150 mg, 66 %); δ_H ($CDCl_3$): 3.45 (2 H, d, $J_{4,5A}$ 3.7, H-5 (α/β)), 3.56, 3.62 (2 H, 2 x dd, $J_{4,5A}$ 3.0, $J_{5A,5B}$ 10.0, H-5 (α/β), 3.97 – 4.77 (18 H, m, 3 x CH_2Ph (α and β), H-2 (α and β), H-3 (α and β), H-4 (α and β)), 6.37 (1 H, s, H-1 (α/β)), 6.48 (1 H, d, $J_{1,2}$ 4.0, H-1 (α/β)), 7.06 – 8.11 (38 H, m, Ar); δ_C ($CDCl_3$): 69.0, 70.0, 72.3, 72.6, 72.9, 73.1, 73.4, 73.6, 76.1 (2 C), 78.8, 79.0, 82.0, 85.1 (14 C, 3 x CH_2Ph (α and β), C-2 - C-5 (α and β)), 96.1, 100.3 (C-1 (α and β)), 123.4, 123.5 (2 C), 123.6, 130.9 (2 C), 131.3 (2 C) (8 C, 4 x Ar C of *p*-nitrobenzoate (α and β)), 127.7 (5 C), 127.8 (2 C), 127.9, 128.0 (3 C), 128.1 (2 C), 128.2 (3 C), 128.3 (2 C), 128.4 (2 C), 128.5, 128.6 (9 C) (30 C, Ar), 135.1, 135.9, 150.7 (2 C) (4 C, 4 x Quat. Ar C of *p*-nitrobenzoate (α and β)), 137.4, 137.6 (2 C), 137.9, 138.2 (2 C) (6 C, 6 x Quat. Ar C of CH_2Ph (α and β)), 163.5, 164.2 (*C*O (α and β)); *m/z* (ES) 587 ($M+NH_4^+$, 100%); HRMS: Found: 587.2393. $C_{33}H_{35}N_2O_8$ ($M+NH_4^+$) Requires 587.2393.

*Reports in the literature[35] suggest that this preparation yields the β-glycoside. An α/β mixture of products was obtained in our hands.

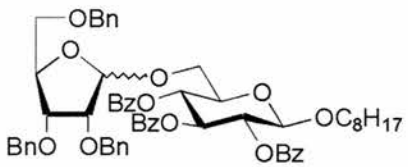
2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl chloride (4.43)[35]



A stream of dry hydrogen chloride was passed in to a solution of 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-ribofuranose **(4.42)** (150 mg, 0.26 mmol) in anhydrous DCM (10 ml). The reaction was followed closely by TLC [hexane – EtOAc, (4:1)]. Upon completion (45 min), the precipitated *p*-nitrobenzoic acid was removed by filtration and washed with anhydrous DCM. The combined filtrate and washings were then concentrated *in vacuo* to yield the title compound as a syrup (80 mg, 69%)*. δ_H ($CDCl_3$): 3.60 (1 H, m, H-5), 4.06 (1 H, d, $J_{3,4}$ 4.1, H-4), 4.28 – 4.61 (8 H, m, H-2, H-3 and 3 x CH_2Ph), 5.98 (1 H, s, H-1), 7.12 – 7.28 (15 H, Ar); δ_C ($CDCl_3$): 70.2, 72.7, 72.8, 73.3, 73.5, 83.1, 83.2 (C-2 – C-5 and 3 x CH_2Ph), 95.6 (C-1), 127.7 (3 C), 127.9, 128.1 (3 C), 128.2 (2 C), 128.3, 128.4 (2 C), 128.6 (2 C), 128.7 (15 C, Ar), 137.3, 137.5, 138.2 (3 x Quat. Ar C).

*Reports in the literature suggest that this procedure yields an anomeric mixture.[35] Only one stereoisomer was obtained in our hands. Although there are references[35] to this compound in the literature no optical rotation, ^{1}H and ^{13}C data have been recorded. Due to it's instability this compound was reacted without further characterization.

Octyl 6-*O*-(2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.45)

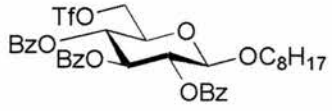


To a mixture of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)** (95 mg, 0.16 mmol), molecular sieves (4 Å) (300 mg), and anhydrous DCM (1 ml) was added a solution of 2,3,5-tri-*O*-benzyl-α-D-ribofuranosyl chloride **(4.43)** (208 mg, 0.47 mmol, 3 mol eq.) in anhydrous DCM (2 ml). The mixture was cooled (-30°C) and stirred for 1 h under nitrogen, then silver trifluoromethanesulfonate (120 mg, 0.47 mmol, 3 eq) was quickly added. Once TLC [hexane – EtOAc, (3:1)] had shown that the reaction had gone to completion (approx. 5 - 10 min), 2,4,6-collidine (0.02 ml, 0.18 mmol, 0.5 eq) was added and stirring was continued for approx. 0.5 h. The reaction mixture was then diluted with DCM (50 ml), filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 2 M HCl (200 ml) and extracted with DCM (2 x 200 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ solution (2 x 200 ml) and water (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 10:1) gave *octyl 6-O-(2,3,5-tri-O-benzyl-α-D-ribofuranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside* **(4.45)*** as yellow syrup (42 mg, 26 %); and *octyl 6-O-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside* as yellow syrup (10 mg, 6 %), **(4.45)**, α-anomer: $[\alpha]_D$ +33.2° (*c* 0.25, $CHCl_3$); δ_H ($CDCl_3$): 0.78 (3 H, t, *J* 7.0, OCH_3), 1.07 – 1.24 (10 H, m, 5 x CH_2), 1.40 – 1.44 (2 H, m, OCH_2CH_2), 3.36 - 3.49 (3 H, m, 1 H of OCH_2 and H-5'$_2$), 3.73 – 3.98 (5 H, m, H-2', H-3', H-6$_2$ and 1 H of OCH_2), 4.10 (1 H, m, H-5), 4.28 (1 H, m, H-4'), 4.38, 4.46, 4.54, 4.64 (4 H, 4 x d, *J* 12.1 and 12.4, 2 x CH_2Ph), 4.59 (2 H, s, CH_2Ph), 4.75 (1 H, d, $J_{1,2}$

7.8, H-1), 4.99 (1 H, d, $J_{1',2'}$ 4.0, H-1'), 5.41 – 5.47 (2 H, m, H-2 and H-4), 5.86 (1 H, t, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6, H-3), 7.19 – 7.86 (30 H, m, Ar); δ_C ($CDCl_3$): 14.0 (CH_3), 22.6, 25.7, 29.3, 29.6, 31.6, 31.8 (6 x CH_2), 69.8, 70.0, 70.2, 72.0, 72.1, 72.6, 73.2, 73.4, 73.8, 75.1, 77.1, 77.6, 81.7 (C-2 - C-6, C-2' - C-5', OCH_2 and 3 x CH_2Ph), 100.9 (C-1), 101.4 (C-1'), 126.4, 127.3, 127.7 (5 C), 127.9 (2 C), 128.1 (3 C), 128.2 (3 C), 128.3 (4 C), 128.4 (5 C), 129.1, 129.8 (3 C), 129.9 (2 C) (30 C, Ar), 133.1, 133.2, 133.4 (3 x quat. Ar C), 137.7, 138.0, 138.3 (3 x quat. Ar C) 165.2, 165.5, 166.0 (3 x CO); *m/z* (ES) 1024 ($M+NH_4^+$, 100%); HRMS: Found: 1024.4849. $C_{61}H_{70}NO_{13}$ ($M+NH_4^+$) Requires 1024.4847, β-anomer: $[\alpha]_D$ +25.0° (*c* 0.1, $CHCl_3$); δ_H ($CDCl_3$): 0.75 (3 H, t, *J* 7.0, OCH_3), 0.81 – 1.18 (10 H, m, 5 x CH_2), 1.36 – 1.42 (2 H, m, OCH_2CH_2), 3.40 - 3.53 (3 H, m, 1 H of OCH_2 and H-5'$_2$), 3.55 – 3.67 (2 H, m, H-6$_2$), 3.76 – 3.92 (3 H, H-2', H-5 and 1 H of OCH_2), 4.01 (1 H, m, H-3'), 4.29 (1 H, m, H-4'), 4.36, 4.48, 4.50, 4.56, 4.59, 4.62 (6 H, 6 x d, *J* 12.0 3 x CH_2Ph), 4.63 (1 H, d, $J_{1,2}$ 8.0, H-1), 4.98 (1 H, s, H-1'), 5.35 (1 H, dd, $J_{1,2}$, $J_{2,3}$ 9.6, H-2), 5.42 (1 H, t, $J_{3,4}$ 9.6, $J_{4,5}$ 9.6, H-4), 5.72 (1 H, t, $J_{2,3}$, $J_{3,4}$, H-3), 7.12 – 8.04 (30 H, m, Ar); δ_C ($CDCl_3$): 13.9 (CH_3), 22.5, 25.7, 29.0, 29.2, 29.3, 31.6 (6 x CH_2), 66.5, 69.7, 70.1, 71.4, 71.9, 72.2, 72.3, 73.1, 73.3, 77.2, 78.4, 79.5, 80.7 (C-2 - C-6, C-2' - C-5', OCH_2 and 3 x CH_2Ph), 101.1 (C-1), 105.9 (C-1'), 127.5, 127.6 (2 C), 127.7 (3 C), 127.8 (5 C), 128.0 (2 C), 128.3 (3 C), 128.4 (8 C), 128.5, 129.1, 129.6, 129.8 (3 C) (30 C, Ar), 133.2 (2 C), 133.5 (3 C, 3 x quat. Ar C), 138.0 (2 C), 138.5 (3 C, 3 x quat. Ar C) 165.3, 166.0 (2 C) (3 x CO); *m/z* (ES) 1024 ($M+NH_4^+$, 100%); HRMS: Found: 1024.4855. $C_{61}H_{70}NO_{13}$ ($M+NH_4^+$) Requires 1024.4847.

*NMR data reported[37] for closely related ribosides allowed us to distinguish between our alpha and beta-linked disaccharides.

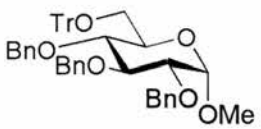
Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside (4.58)



A solution of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)** (420 mg, 0.69 mmol) and 2,6-di-*tert*.butyl-4-methylpyridine (178 mg, 0.87 mmol) in anhydrous DCM (4 ml) was cooled to -40°C. Triflic anhydride (129 µl, 0.76 mmol) was then added, and the reaction was stirred until TLC [hexane – EtOAc, (3:1)] showed the reaction had gone to completion

(approx. 6 h). A 10 % aq. $NaHCO_3$ solution (2 ml) and Et_2O (3 ml) were then added and the reaction allowed to stir for a further 0.5 h. The resulting solution was extracted with Et_2O (2 x 20 ml), and the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give *octyl 2,3,4-tri-O-benzoyl-6-O-trifluoromethanesulfonyl-β-D-glucopyranoside* **(4.58)** as an oil (173 mg, 34 %); $[\alpha]_D$ -8.5° (*c* 1.7, $CHCl_3$); δ_H ($CDCl_3$): 0.75 (3 H, t, *J* 7.0, CH_3), 0.80 – 1.18 (10 H, m, 5 x CH_2), 1.45 – 1.49 (2 H, m, OCH_2CH_2), 3.47 (1 H, m, 1 H of OCH_2), 3.86 (1 H, m, H-5), 4.07 (1 H, m, 1 H of OCH_2), 4.52 – 4.65 (2 H, m, H-6_2), 4.77 (1 H, $J_{1,2}$ 7.8, H-1), 5.31 – 5.47 (2 H, m, H-2 and H-4), 5.85 (1 H, t, $J_{2,3}$ 9.6, H-3), 7.17 – 7.89 (15 H, Ar)); δ_C ($CDCl_3$) 13.9 (CH_3), 22.5, 25.7, 29.0, 29.1, 29.2, 31.6 (6 x CH_2), 69.3, 70.4, 71.5, 71.9, 72.4, 74.0, (OCH_2, C-2, C-3, C-4, C-5 and C-6), 101.2 (C-1), 119.1 (1 C, q, $J_{C,F}$ 157.5, CF_3), 128.3, 128.4 (4 C), 128.7 (3 C), 129.3, 129.8 (4 C), 130.0 (2 C) (15 C, Ar), 133.4, 133.5, 134.0 (3 x quat. Ar C), 165.1, 165.6, 165.9 (3 x CO); *m/z* (ES) 754 ($M+NH_4^+$, 100%); HRMS: Found: 754.2509. $C_{36}H_{43}NF_3O_{11}S$ ($M+NH_4^+$) Requires 754.2509.

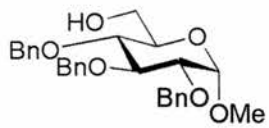
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (4.60)[38]



The title compound was prepared using a standard benzylation procedure.[19] A solution of methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside[39] (which was kindly donated by Dr. J. D. Hall, University of East Anglia) **(4.59)** (2.0 g, 4.6 mmol) was dissolved in anhydrous DMF (20 ml) and cooled (0° C) before sodium hydride (60 % w/w suspension in mineral oil) (605 mg, 15.1 mmol) was slowly added. After 0.5 h BnBr (1.8 ml, 15.1 mmol) was slowly added and the solution was stirred until TLC [hexane – EtOAc, (7:1)] showed the reaction had gone to completion. The reaction mixture was cooled (0°C) before triethylamine (640 µl, 4.6 mmol) was added (to quench the benzyl bromide). The solution was stirred 0.5 h. and concentrated under reduced pressure to a syrup. The syrup was diluted with an equal volume of DCM and washed with water (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 7:1) gave methyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside **(4.60)** as a white foam (2.53 g, 78 %); $[\alpha]_D$ +16.6° (*c* 7.3, $CHCl_3$); (lit., [38]

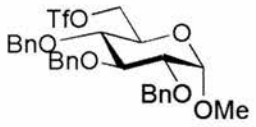
$[\alpha]_D$ +17.9° (*c* 2.96, $CHCl_3$)); δ_H ($CDCl_3$): 3.10 (1 H, dd, $J_{5,6}$ 4.5, $J_{6,6'}$ 10.0, H-6'), 3.33 (3 H, s, OCH_3), 3.41 (1 H, d, $J_{6,6'}$, H-6), 3.51 - 3.57 (2 H, m, H-4 and H-5), 3.72 (1 H, dd, $J_{1,2}$ 3.2, $J_{2,3}$ 9.4, H-2), 3.89 (1 H, t, $J_{2,3}$, $J_{3,4}$ 9.4, H-3), 4.19 (1 H, d, *J* 10.4, 1 H of CH_2Ph), 4.60 (1 H, d, *J* 10.4, 1 H of CH_2Ph), 4.61 (1 H, d, *J* 12.3, 1 H of CH_2Ph), 4.71 (1 H, d, *J* 10.4, 1 H of CH_2Ph), 4.72 (1 H, d, *J* 12.3, 1 H of CH_2Ph), 4.86 (1 H, d, *J* 10.7, 1 H of CH_2Ph), 4.66 (1 H, d, $J_{1,2}$, H-1), 6.75 - 6.77, 7.03 - 7.38 (30 H, 2 x m, Ar); δ_C ($CDCl_3$) 54.4 (CH_3), 62.1 (C-6), 72.8, 74.5, 76.2 (3 x CH_2Ph), 69.8, 77.6, 79.8, 81.8 (C-2, C-3, C-4, C-5), 85.8 ($C(Ph)_3$), 97.4, (C-1), 126.5 (3 C), 127.1, 127.3, 127.4 (8 C), 127.6 (5 C), 127.7 (2 C), 127.8 (3 C), 128.0 (4 C), 128.4 (3 C) (30 C, Ar), 137.6, 137.9, 138.3 (3 x quat. C of Bn), 143.6 (3C, 3 x quat. C of Tr).

Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (4.61)[39-42]



A solution of methyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside **(4.60)** 1.8 g, 2.6 mmol) in 80 % aqueous trifluoroacetic acid (18 ml) and DCM (18 ml) was stirred until TLC [hexane– EtOAc, (5:1)] showed the reaction had gone to completion approx. 2 h. The resulting solution was concentrated and coevaporated with isopropanol to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 5:1 - 2:1) gave methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.61)** as a white foam (858 mg, 71 %); $[\alpha]_D$ +25.3° (*c* 1.9, $CHCl_3$); (lit., [39] $[\alpha]_D$ +23.5° (*c* 1.0, $CHCl_3$)); δ_H ($CDCl_3$): 3.26 (3 H, s, OCH_3), 3.40 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 9.6, H-2), 3.43 (1 H, t, $J_{2,3}$, $J_{3,4}$,/$J_{3,4}$, $J_{4,5}$, H-3/H-4), 3.92 (1 H, t, $J_{2,3}$, $J_{3,4}$,/$J_{3,4}$, $J_{4,5}$, H-3/H-4), 3.53 - 3.68 (3 H, m, H-5 and H-6 and H-6'), 4.47 (1 H, d, $J_{1,2}$, H-1), 4.54 (1 H, d, *J* 11.0, 1 H of CH_2Ph), 4.55 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.69 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.74 (1 H, d, *J* 11.0, 1 H of CH_2Ph), 4.78 (1 H, d, *J* 11.3, 1 H of CH_2Ph), 4.89 (1 H, d, *J* 11.0, 1 H of CH_2Ph), 7.16 - 7.28 (15 H, m, Ar); δ_C ($CDCl_3$) 55.0 (CH_3), 61.6 (C-6), 73.2, 74.9, 75.6 (3 x CH_2Ph), 70.6, 77.3, 79.9, 81.8 (C-2, C-3, C-4, C-5), 98.1, (C-1), 127.6, 127.8, 127.9 (2 C), 128.0 (2 C), 128.1 (2 C), 128.4 (2 C), 128.5 (5 C), (15 C, Ar), 138.1, 138.2, 138.7 (3 x quat. Ar C).

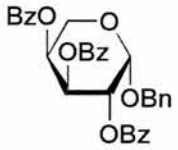
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside (4.62)[43-48]



A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.61)** (80 mg, 0.172 mmol) and 2,6-di-*tert*.butyl-4-methylpyridine (44 mg, 0.215 mmol) in anhydrous DCM (2 ml) was cooled to -40°C. Triflic anhydride (31 μl, 0.1892 mmol) was then added, and the reaction was stirred until TLC [hexane – EtOAc, (3:1)] showed the reaction had gone to completion (approx. 6 h). A 10 % aq. $NaHCO_3$ solution (2 ml) and Et_2O (3 ml) were then added and the reaction allowed to stir for a further 0.5 h. The resulting solution was extracted with Et_2O (2 x 20 ml), and the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give methyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside **(4.62)** as an oil (72 mg, 70 %); $[\alpha]_D$ +91.3° (*c* 0.7, $CHCl_3$)*; δ_H ($CDCl_3$); 3.29 (3 H, s, OCH_3), 3.34 (1 H, t, $J_{3,4}$ 9.4, $J_{4,5}$ 9.4, H-4), 3.94 (1 H, t, $J_{2,3}$ 9.4, $J_{3,4}$ H-3), 3.45 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$, H-2), 3.78 (1 H, m, H-5), 4.37, 4.46 (2 H, 2 x dd, $J_{5,6}$ 5.2, $J_{6,6'}$ 10.7, H-6 and H-6'), 4.48 (1 H, d, *J* 10.8, 1 H of CH_2Ph), 4.57 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.70 (1 H, d, *J* 12.1, 1 H of CH_2Ph), 4.73 (1 H, d, *J* 10.8, 1 H of CH_2Ph), 4.84 (1 H, d, *J* 11.1, 1 H of CH_2Ph), 4.94 (1 H, d, *J* 10.8, 1 H of CH_2Ph), 4.52 (1 H, d, $J_{1,2}$, H-1), 7.16 – 7.27 (15 H, Ar); δ_C ($CDCl_3$); 55.5 (CH_3), 68.2 (C-6), 73.5, 74.9, 75.1, 75.8, 76.5, 79.8, 81.8 (3 x CH_2Ph, C-2, C-3, C-4, C-5), 98.1, (C-1), 116.6 (CF_3) (signals due to coupling with F are not observed), 127.9, 128.0 (2 C), 128.2 (5 C), 128.3, 128.6 (2 C), 128.6 (2 C), 128.7 (2 C) (15 C, Ar), 137.6, 138.0, 138.5 (3 x quat. Ar C); *m/z* (ES) 614 ($M+NH_4^+$, 100%); HRMS: Found: 614.2032. $C_{29}H_{35}NF_3O_8S$ ($M+NH_4^+$) Requires 614.2035.

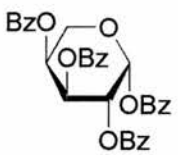
*Although there are several references[43-48] to this compound in the literature no optical rotation value has been recorded. ^1H and ^{13}C spectral data are in agreement with the literature.[46,48]

Benzyl 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranoside (4.71)[49]



To an ice cold solution of benzyl β-L-arabinopyranoside[50,51] **(4.70)** (which was kindly donated by Dr. A. H. Haines, University of East Anglia) (1.0 g, 4.5 mmol) in pyridine (10 ml), was added benzoyl chloride (2.3 ml, 20.1 mmol). The reaction allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed that the reaction had gone to completion (within 1 h.). Methanol (excess) was added and the reaction mixture was allowed to stir for a further 0.5 h. The reaction mixture was then diluted in DCM (200 ml) and washed with 2 M HCl (2 x 200 ml) and 10 % aq. Na_2CO_3 solution (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 5:1) gave benzyl 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranoside **(4.71)** as a foam (2.15 g, 87 %); $[\alpha]_D$ +263.2° (*c* 21.5, $CHCl_3$), (lit., [49] $[\alpha]_D$ +277° (*c* 1.0, $CHCl_3$)); δ_H ($CDCl_3$): 4.09 (1 H, d, $J_{5A,5B}$ 12.3, H-5_A), 4.34 (1 H, d, $J_{5A,5B}$, H-5_B), 4.66 (1 H, d, *J* 12.4, 1 H of CH_2Ph), 4.90 (1 H, d, *J* 12.4, 1 H of CH_2Ph), 5.55 (1 H, d, $J_{1,2}$ 3.4, H-1), 5.95 (1 H, dd, $J_{2,3}$ 11.5, $J_{1,2}$, H-2), 5.95 (1 H, br s, H-4), 6.19 (1 H, dd, $J_{2,3}$, $J_{3,4}$ 3.3, H-3), 7.22 – 7.60, 7.96 – 8.22 (20 H, 2 x m, Ar); δ_C ($CDCl_3$): 60.9 (C-5), 68.3, 69.5, 70.4 (C-2, C-3, C-4), 69.8 (*C*H_2Ph), 96.2 (C-1), 127.8 (3 C), 128.1 (2 C), 128.5 (2 C), 128.6 (4 C), 128.7 (2 C), 129.8 (2 C), 129.9 (3 C), 130.0 (2 C) (20 C, Ar), 133.3, 133.5 (2 C), 137.2 (4 C, 4 x quat. Ar. C), 165.8, 166.0, 166.1 (3 x *C*=O); *m/z* (ES) 570 ($M+NH_4^+$, 100%); HRMS: Found: 570.2118. $C_{33}H_{32}NO_8$ ($M+NH_4^+$) Requires 570.2128.

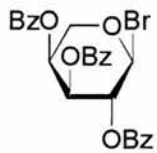
1,2,3,4-Tetra-*O*-benzoyl-β-L-arabinopyranose (4.84)[52-56]



L-Arabinose (2 g, 13.3 mmol), was suspended in anhydrous pyridine (12 ml). The reaction mixture was cooled to 0°C and benzoyl chloride (9.6 ml, 82.7 mmol) was added dropwise. The rate of addition of the acid halide was adjusted so that the temperature of the reaction mixture did not rise above 4°C. After 3.5 hr. the slurry was left overnight at 4°C. The

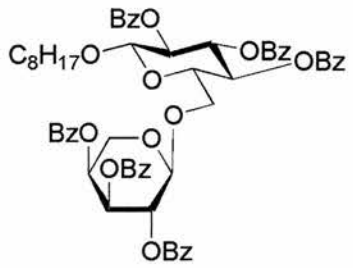
reaction mixture was then left for 5 hr. at room temperature, H_2O (2 ml) was added and the reaction mixture allowed to stir for 0.5 hr. The reaction mixture was then diluted in DCM (200 ml) and washed with 2 M HCl (200 ml) and sat. aq. $NaHCO_3$ (200 ml) and H_2O (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to a syrup. The crude material was then dissolved in EtOH (20 ml) and reconcentrated, giving a white solid (6.3 g, 84 %); mp 157 - 158°C (EtOH), (lit., [52] 160 - 161°C); $[\alpha]_D$ +324.6° (*c* 0.25, $CHCl_3$), (lit., [52] $[\alpha]_D$ +322.7° (*c* 0.981, $CHCl_3$)); δ_H ($CDCl_3$): 4.11 (1 H, dd, $J_{5A,5B}$ 13.4, $J_{4,5A}$ 2.2, H-5_A), 4.33 (1 H, d, $J_{4,5B}$, H-5_B), 5.82 (1 H, s, H-4), 5.96 – 6.00 (2 H, m, H-2 and H-3), 6.79 (1 H, d, $J_{1,2}$ 1.5, H-1), 7.17 – 8.07 (20 H, 2 x m, Ar); δ_C ($CDCl_3$): 63.0 (C-5), 67.8, 68.2, 69.5 (C-2, C-3, C-4), 91.1 (C-1), 128.4 (2 C), 128.6 (2 C), 128.7 (2 C), 128.9 (2 C), 129.1 (2 C), 129.3 (2 C), 129.7 (4 C), 129.9 (4 C), (20 C, Ar), 133.4 (2 C), 133.5, 133.8 (4 C, 4 x Quat. Ar. C), 164.7, 165.6, 165.7 (2 C) (4 C, 4 x *C*=O);

2,3,4 Tri-*O*-benzoyl-α-L-arabinopyranosyl bromide (4.72)[52,57]



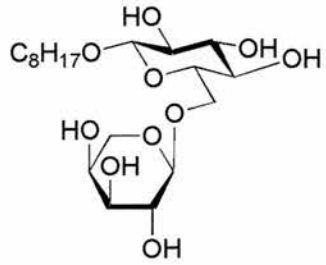
To a solution of benzyl 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranoside **(4.71)** (220 mg, 0.39 mmol) in glacial acetic acid (0.5 ml), was added 45 % HBr in glacial acetic acid (1.5 ml). The reaction was allowed to stir under nitrogen until TLC [Hexane – EtOAc, (4:1)] indicated completion (approx. 3 h). The reaction mixture was then diluted with ice-cold water (50 ml) and DCM (50 ml). The organic layer was then washed with 10 % aq. $NaHCO_3$ solution (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give an oil **(4.72)** (238 mg, 100 %); (Found: C, 59.44; H, 4.09; Br, 15.13. $C_{26}H_{21}BrO_7$ Requires: C, 59.44; H, 4.03; Br, 15.21%); $[\alpha]_D$ +207.1° (*c* 2.3, $CHCl_3$), (lit., [52] $[\alpha]_D$ +203° (*c* 1.7, $CHCl_3$)); δ_H ($CDCl_3$): 4.13 (1 H, dd, $J_{5A,5B}$ 13.5, $J_{4,5A}$ 1.8, H-5_A), 4.36 (1 H, d, $J_{5A,5B}$, H-5_B), 5.62 (1 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.5, H-2), 5.74 (1 H, m, H-4), 5.91 (1 H, dd, $J_{3,4}$ 3.4, $J_{2,3}$, H-3), 6.84 (1 H, d, $J_{1,2}$, H-1), 7.16 – 8.01 (15 H, Ar); δ_C ($CDCl_3$): 64.9 (C-5), 68.5, 68.6, 68.8, (C-2, C-3, C-4), 89.8 (C-1), 128.4, 128.6 (2 C), 128.7 (2 C), 128.8, 128.9, 129.0, 129.2, 129.7 (2 C), 129.9 (2 C), 130.0 (2 C) (15 C, Ar), 133.4, 133.7, 133.8 (3 x quat. Ar. C), 165.5, 165.6, 165.7 (3 x *C*=O).

Octyl 6-*O*-(2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.73)



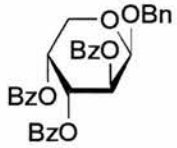
To a mixture of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)** (77 mg, 0.127 mmol) in anhydrous acetonitile (1 ml) was added a solution of 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl bromide **(4.72)** (200 mg, 0.38 mmol) in anhydrous acetonitrile (2 ml). The mixture was cooled (-20°C) and stirred for 1 h under nitrogen, then iodine monobromide (320 μl, 0.32 mmol, 2.5 eq) was quickly added. Once TLC [hexane – EtOAc, (4:1)] had shown that the reaction had gone to completion (approx.6 hr.), the reaction mixture was then diluted with DCM (200 ml) and washed with 10 % aq. $Na_2S_2O_3$ solution (3 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 6:1 – 5:1) gave *octyl 6-O-(2,3,4-tri-O-benzoyl-α-L-arabinopyranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside* **(4.73)** as a white foam (64 mg, 48 %); $[\alpha]_D$ +56.6° (*c* 0.6, $CHCl_3$); δ_H ($CDCl_3$): 0.74 (3 H, t, *J* 7.0, CH_3), 0.81 – 0.94, 0.94 – 1.18 (12 H, 2 x m, 6 x CH_2), 3.12, 3.51, 3.76, 3.99 (5 H, 4 x m, H-5, H-6_A, H-6_B and OCH_2), 4.08 (1 H, dd, $J_{4',5'}$ 4.1, $J_{5'A,5'B}$ 12.8, H-5'$_A$), 4.18 (1 H, dd, $J_{4',5'}$, $J_{5'A,5'B}$, H-5'$_B$), 4.57 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.78 (1 H, d, $J_{1',2'}$ 5.9, H-1'), 5.28 – 5.35, 5.52 – 5.69 (5 H, 2 x m, H-2, H-4, H-2', H-3' and H-4'), 5.76 (1 H, t, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6, H-3), 7.14 – 7.95 (30 H, Ar); δ_C ($CDCl_3$): 13.9 (CH_3), 22.5, 25.6, 29.0, 29.1, 29.2, 31.6 (6 x CH_2), 62.2 (C-5'), 68.2, 68.3, 69.8, 69.9, 70.3, 71.9, 72.9, 73.9, 76.6, (C-2 – C-6, C-2' – C-4' and OCH_2), 100.9, 101.1 (C-1 and C-1'), 128.4 (3 C), 128.5 (6 C), 128.8 (2 C), 128.9 (2 C), 129.2 (2 C), 129.4 (2 C), 129.5 (2 C), 129.8 (3 C), 129.9 (8 C) (30 C, Ar), 133.2 (2 C), 133.4 (2 C), 133.5 (2 C) (6 x quat. Ar C), 165.1, 165.3, 165.5, 165.7, 165.8, 165.9 (6 x *C*O); *m/z* (ES) 1066 (M, 100%); HRMS: Found: 1066.4194. $C_{61}H_{64}NO_{16}$ ($M+NH_4^+$) Requires 1066.4225.

Octyl 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranoside (4.69)[58-61]



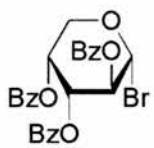
To a solution of octyl 6-*O*-(2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.73)** (64 mg, 0.06 mmol) in anhydrous methanol (2 ml), was added a stock solution of NaOMe/MeOH (10 mg/ml) (0.5 ml). The reaction was allowed to stir until TLC [DCM– methanol, (8:2)] showed that the reaction had gone to completion (within 1 h). The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo*. The crude material was then dissolved in 9:1 H_2O/methanol and loaded on to a Sephadex LH-20 gel filtration column (2.8 x 73 cm). Elution with 9:1 H_2O/methanol at a rate of 12 ml per hour gave octyl 6-*O*-(-α-L-arabinopyranosyl)-β-D-glucopyranoside **(4.69)** as a foam (15 mg, 59 %); $[\alpha]_D$ -22.9° (*c* 0.1, CH_3OH), (lit., [61] $[\alpha]_D$ -29.2° (CH_3OH)); δ_H (CD_3OD): 0.89 (3 H, t, *J* 7.2, OCH_3), 1.29 – 1.37 (10 H, m, 5 x CH_2), 1.57 – 1.64 (2 H, m, OCH_2CH_2), 3.17 (1 H, m, H-2), 3.32 – 3.35 (2 H, m, H-3 and H-4), 3.42 (1 H, m, H-5), 3.49 – 3.56 (3 H, m, H-3', H-5'$_B$ and 1 H of OCH_2), 3.59 (1 H, dd, $J_{1',2'}$ 6.6, $J_{2',3'}$ 8.8, H-2'), 3.72 (1 H, dd, $J_{6A,6B}$ 11.5, $J_{5,6}$ 5.4, H-6$_B$), 3.79 (1 H, m, H-4'), 3.83 – 3.89 (2 H, m, H-5'$_A$ and 1 H of OCH_2), 4.08 (1 H, dd, $J_{6A,6B}$, $J_{5,6}$, H-6$_A$), 4.24 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.30 (1 H, d, $J_{1',2'}$ 6.8, H-1'); δ_C (CD_3OD): 14.0 (CH_3), 23.5, 26.7, 26.9, 30.1, 30.6, 33.0 (6 x CH_2), 66.4, 69.1, 69.4, 70.9, 71.0, 72.1, 74.0, 75.0, 76.5, 77.5 (C-2 – C-6, C-2' – C-5' and OCH_2), 104.0, 104.8 (C-1 and C-1'); *m/z* (ES) 442 ($M+NH_4^+$, 100%); HRMS: Found: 442.2651. $C_{19}H_{40}NO_{10}$ ($M+NH_4^+$) Requires 442.2652.

Benzyl 2,3,4-tri-*O*-benzoyl-β-D-arabinopyranoside (4.75)



To an ice cold solution of benzyl β-D-arabinopyranoside[62] **(4.74)** (which was kindly donated by Dr. A. H. Haines, University of East Anglia) (1.0 g, 4.5 mmol) in pyridine (10 ml), was added benzoyl chloride (2.3 ml, 20.1 mmol). The reaction allowed to stir until TLC [Hexane – EtOAc, (4:1)] showed that the reaction had gone to completion (within 1 h.). Methanol (excess) was added and the reaction mixture was allowed to stir for a further 0.5 h. The reaction mixture was then diluted in DCM (200 ml) and washed with 2 M HCl (2 x 200 ml) and 10 % aq. Na_2CO_3 solution (2 x 200 ml) The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica gel; Hexane – EtOAc, 5:1) gave *benzyl 2,3,4-tri-O-benzoyl-β-D-arabinopyranoside* **(4.75)** as a foam (2.10 g, 85 %); $[\alpha]_D$ –286° (*c* 12.5, $CHCl_3$); δ_H ($CDCl_3$): 4.05 (1 H, d, $J_{5A,5B}$ 13.0, H-5_B), 4.31 (1 H, d, $J_{5A,5B}$, H-5_A), 4.63 (1 H, d, *J* 12.4, 1 H of CH_2Ph), 4.86 (1 H, d, *J* 12.5, 1 H of CH_2Ph), 5.49 (1 H, d, 1 H, d, $J_{1,2}$ 3.4, H-1), 5.85 - 5.90 (2 H, m, H-2 and H-4), 6.12 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.4, H-3), 7.21 - 7.58, 7.90 - 8.17 (20 H, Ar); δ_C ($CDCl_3$): 60.8 (C-5), 68.2, 69.4, 69.8, 70.3 (C-2, C-3, C-4 and CH_2Ph), 96.1 (C-1), 127.8 (2 C), 128.0, 128.4 (2 C), 128.5 (3 C), 128.7 (2 C), 129.4, 129.8 (2 C), 130.0 (6 C), 130.2 (20 C, Ar), 133.3, 133.5 (2 C), 137.2 (4 C, 4 x quat. Ar C), 165.8, 166.0, 166.1 (3 x *C*O); *m/z* (ES) 570 ($M+NH_4^+$, 100%); HRMS: Found: 570.2129. $C_{33}H_{32}NO_8$ ($M+NH_4^+$) Requires: 570.2128.

2,3,4-tri-*O*-benzoyl-α-D-arabinopyranosyl bromide (4.76)[63]

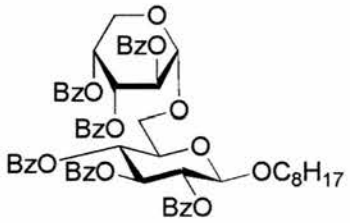


To a solution of benzyl 2,3,4-tri-*O*-benzoyl-β-D-arabinopyranoside **(4.75)** (330 mg, 0.59 mmol) in glacial acetic acid (0.5 ml), was added 45 % HBr in glacial acetic acid (1.5 ml). The reaction was allowed to stir under nitrogen until TLC [Hexane – EtOAc, (4:1)] indicated completion (approx. 3 h). The reaction mixture was then diluted with ice-cold water (50 ml) and DCM (150 ml). The organic layer was then washed with 10 % aq.

$NaHCO_3$ solution (2 x 200 ml) The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a white foam **(4.76)** (275 mg, 88 %); (Found: C, 59.58; H, 4.10; Br, 14.99. $C_{26}H_{21}BrO_7$ Requires: C, 59.44; H, 4.03; Br, 15.21%); $[\alpha]_D$ -330° (*c* 1.0,$CHCl_3$)*, (lit., [52] $[\alpha]_D$ +203° for the L-isomer (*c* 1.7, $CHCl_3$)); δ_H ($CDCl_3$): 4.14 (1 H, dd, $J_{5A,5B}$ 13.6, $J_{4,5A}$ 1.9, H-5_A), 4.38 (1 H, d, $J_{5A,5B}$, H-5_B), 5.63 (1 H, dd, $J_{1,2}$ 3.9, $J_{2,3}$ 10.5, H-2), 5.75 (1 H, m, H-4), 5.92 (1 H, dd, $J_{3,4}$ 3.4, $J_{2,3}$, H-3), 6.85 (1 H, d, $J_{1,2}$, H-1), 7.16 – 8.15 (15 H, Ar); δ_C ($CDCl_3$): 65.0 (C-5), 68.5, 68.6, 68.8, (C-2, C-3, C-4), 89.8 (C-1), 128.3 (2 C), 128.5 (2 C), 128.6 (2 C), 128.9 (2 C), 129.2, 129.7 (2 C), 129.8 (2 C), 130.0 (2 C) (15 C, Ar), 133.3, 133.6, 133.7 (3 x quat. Ar. C), 165.4, 165.5, 165.6 (3 x *C*=O).

* Although there are references[63] to compound **(4.76)** in the literature no optical rotation data has been detailed.

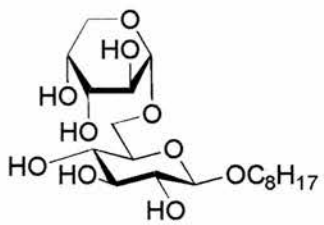
Octyl 6-*O*-(2,3,4-tri-*O*-benzoyl-α-D-arabinopyranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.77)



To a mixture of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)** (67 mg, 0.11 mmol) in anhydrous acetonitile (1 ml) was added a solution of 2,3,4-tri-*O*-benzoyl-β-D-arabinopyranosyl bromide **(4.76)** (173 mg, 0.33 mmol, 3 mol eq.) in anhydrous acetonitrile (2 ml). The mixture was cooled (-20°C) and stirred for 1 h under nitrogen, then iodine monobromide (275 µl, 0.275 mmol, 2.5 eq) was quickly added. Once TLC [hexane – EtOAc, (4:1)] had shown that the reaction had gone to completion (approx.6 hr.), the reaction mixture was then diluted with DCM (200 ml) and washed with 10 % aq. $Na_2S_2O_3$ solution (3 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 6:1 – 5:1) gave *octyl 6-O-(2,3,4-tri-O-benzoyl-α-D-arabinopyranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside* **(4.77)** as a white foam (82 mg, 45 %); $[\alpha]_D$ –53.6° (*c* 1.4, $CHCl_3$); δ_H ($CDCl_3$): 0.74 (3 H, t, *J* 7.1, OCH_3), 0.81 – 1.21, 1.21 – 1.35 (12 H, 2 x m, 6 x CH_2), 3.28 (1

H, m, 1 H of OCH_2), 3.71 – 4.04 (5 H, m, 1 H of OCH_2, H-5'$_B$, H-5, H-6$_A$ and H-6$_B$), 4.22 (1 H, dd, $J_{5'A,5'B}$ 12.3, $J_{4,5'A}$ 5.2, H-5'$_A$), 4.62 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.83 (1 H, d, $J_{1',2'}$ 5.1, H-1'), 5.33 (1 H, dd, $J_{2,3}$ 9.6, $J_{1,2}$, H-2), 5.40 (1 H, t, $J_{2,3}$, $J_{3,4}$, H-4), 5.53 – 5.61 (3 H, m, H-2', H-3' and H-4'), 5.75 (1 H, t, $J_{2,3}$, $J_{3,4}$, H-3), 7.16 – 8.02 (30 H, Ar); δ_C ($CDCl_3$): 13.9 (*C*H_3), 22.5, 25.7, 29.0, 29.1, 29.3, 31.6 (6 x *C*H_2), 61.4 (C-5'), 67.7, 67.8 (C-6 and O*C*H_2), 69.5, 69.9 (2 C), 70.0, 71.9, 73.1, 73.9 (7 C, C-2 – C-5 and C-2' – C-4'), 100.0, 101.2 (C-1 and C-1'), 128.3 (2 C), 128.4 (4 C), 128.5 (3 C), 128.6 (2 C), 129.0, 129.3, 129.4, 129.5, 129.6, 129.8 (5 C), 130.0 (9 C) (30 C, Ar), 133.2 (2 C), 133.4 (4 C) (6 x quat. Ar C), 165.1, 165.2 (2 C), 165.7, 165.8, 166.0 (6 C, 6 x *C*O); ***m/z*** (ES) 1066 ($M+NH_4^+$, 100%); HRMS: Found: 1066.4221. $C_{61}H_{64}NO_{16}$ ($M+NH_4^+$) Requires 1066.4225.

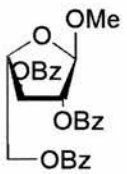
Octyl 6-*O*-(α-D-arabinopyranosyl)-β-D-glucopyranoside (4.68)



To a solution of octyl 6-*O*-(2,3,4-tri-*O*-benzoyl-α-D-arabinopyranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.77)** (135 mg, 0.13 mmol) in anhydrous methanol (2 ml), was added a stock solution of NaOMe/MeOH (10 mg/ml) (0.5 ml). The reaction was allowed to stir until TLC [DCM– methanol, (8:2)] showed that the reaction had gone to completion (within 1 h.). The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo*. The crude material was then dissolved in 9:1 H_2O/methanol and loaded on to a Sephadex LH-20 gel filtration column (2.8 x 73 cm). Elution with 9:1 H_2O/methanol at a rate of 12 ml per hour gave *octyl 6-O-(α-D-arabinopyranosyl)-β-D-glucopyranoside* **(4.68)** as a foam (49 mg, 90 %); $[\alpha]_D$ –25.6° (*c* 0.3, CH_3OH); δ_H (CD_3OD): 0.80 (3 H, t, *J* 7.0, OCH_3), 1.20 – 1.30 (10 H, m, 5 x CH_2), 1.50 – 1.55 (2 H, m, OCH_2CH_2), 3.08 (1 H, dd, $J_{2,3}$ 8.9, $J_{1,2}$ 7.8, H-2), 3.20 – 3.81 (11 H, m, H-3 – H-6$_2$, H-2' – H-5'$_B$ and OCH_2), 3.97 (1 H, dd, $J_{5'A,5'B}$ 10.8, $J_{4,5'A}$ 3.7, H-5'$_A$), 4.16 (1 H, d, $J_{1',2'}$ 6.4, H-1'), 4.17 (1 H, d, $J_{1,2}$, H-1); δ_C (CD_3OD): 13.8 (*C*H_3), 23.1, 26.5, 29.8, 30.0, 30.2, 32.4 (6 x *C*H_2), 66.3, 68.1, 70.7 (C-6, C-5' and O*C*H_2), 68.9, 70.6, 71.8, 73.5, 74.6, 76.0, 77.3 (C-2 – C-5, C-2' –

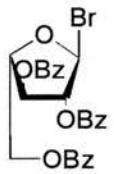
C-4'), 104.2 (2 C, C-1 and C-1'); *m/z* (ES) 447 ($M+Na^+$, 100%); HRMS: Found: 447.2200 $C_{19}H_{36}O_{10}Na$ ($M+Na^+$) Requires: 447.2206.

Methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside (4.78)[64,65]



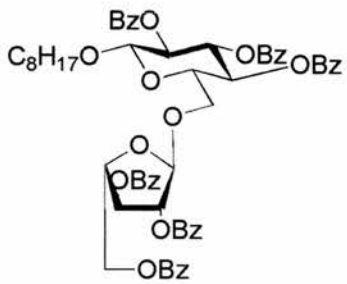
A suspension of L-arabinose (10 g, 67 mmol) in anhydrous methanol (200 ml) was treated with methanolic HCl [prepared by the addition of acetyl chloride (5.2 g, 67 mmol) to anhydrous methanol (53.4 ml) at 0°C]. The mixture was allowed to stir overnight at 0 - 5°C. Upon dissolution of the sugar, pyridine (35 ml) was added. The resulting solution was concentrated and coevaporated with toluene to give a yellow syrup. The residue was then dissolved in anhydrous pyridine (68 ml) and benzoyl chloride (46.4 ml, 400 mmol) was added with cooling (0°C). The reaction mixture was then left at room temperature until TLC [toluene – EtOAc, (5:1)] indicated completion (approx. 48 h.). The reaction mixture was then diluted with an equal volume of DCM and washed with 10 % aq. $NaHCO_3$ solution (2 x 200 ml) and water (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Crystallisation gave the known title compound **(4.78)** as a white solid (23.9 g, 75 %); mp 99 - 101°C (EtOH), (lit., [64] 101 - 102°C); $[\alpha]_D$ +20.3° (*c* 1.3, $CHCl_3$), (lit., [64] $[\alpha]_D$ +19.4° (*c* 2.05, $CHCl_3$)); δ_H ($CDCl_3$): 3.42 (3 H, s, OCH_3), 4.54 (1 H, m, H-4), 4.64 (1 H, dd, $J_{4,5}$ 4.0, $J_{5,5'}$ 11.9, H-5), 4.79 (1 H, dd, $J_{4,5'}$, $J_{5,5'}$, H-5') 5.11 (1 H, s, H-1), 5.44 (1 H, s, H-2), 5.51 (1 H, d, $J_{3,4}$ 5.1, H-3), 7.18 – 7.52, 7.92 – 8.02 (15 H, 2 x m, Ar); δ_C ($CDCl_3$): 54.5 (OCH_3), 63.2 (C-5), 77.5, 80.4, 81.8 (C-2, C-3, C-4), 106.5 (C-1), 127.9 (2 C), 128.1 (3 C), 128.1 (2 C), 128.7 (2 C), 129.4 (2 C), 129.5 (2 C), 129.6 (2 C) (15 C, Ar), 132.7, 133.1 (2 C) (3 C, 3 x quat. Ar C), 165.2, 165.5, 165.9 (3 x *C*O).

2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide (4.79)[66,67]



To a solution of methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside **(4.78)** (300 mg, 0.6 mmol) in glacial acetic acid (0.5 ml), was added 45 % HBr in glacial acetic acid (1.5 ml). The reaction was allowed to stir under nitrogen until TLC [toluene – EtOAc, (5:1)] indicated completion (approx. 0.5 h). The reaction mixture was then diluted with equal volumes of ice-cold water and DCM. The organic layer was then washed with 10 % aq. $NaHCO_3$ solution (2 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a foam **(4.79)** (271 mg, 82 %); $[\alpha]_D$ -45.2° (*c* 3.0, $CHCl_3$), (lit., [66,67] $[\alpha]_D$ -47.5° (*c* 0.57, $CHCl_3$)); δ_H ($CDCl_3$): 4.64 – 4.83 (3 H, m, H-4, H-5 and H-5'), 5.54 (1 H, d, $J_{3,4}$ 4.1, H-3), 5.86 (1 H, s, H-2), 6.54 (1 H, s, H-1), 7.14 – 7.52 (9 H, m, Ar), 7.83 – 8.04 (6 H, m, Ar); δ_C ($CDCl_3$) 62.5 (C-5), 76.0, 84.0, 85.1 (C-2, C-3 and C-4), 88.0 (C-1), 127.8 (3 C), 128.0, 128.1 (2 C), 128.2, 128.9, 129.2 (2 C), 129.3, 129.4 (2 C), 129.5 (2 C) (15 C, Ar), 132.7, 133.0, 133.3 (3 x quat. Ar C), 164.6, 165.2, 165.5 (3 x *C*O).

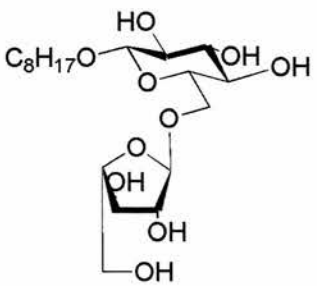
Octyl 6-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (4.80)



To a mixture of octyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.20)** (93 mg, 0.15 mmol), molecular sieves (4 Å) (400 mg), and DCM (1 ml) was added a solution of 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide **(4.79)** (323 mg, 0.61 mmol) in DCM (3 ml). The mixture was cooled (-30°C) and stirred for 1 h under nitrogen, then silver trifluoromethanesulfonate (116 mg, 0.45 mmol, 3 eq) was quickly added. Once TLC [hexane – EtOAc, (3:1)] had shown that the reaction had gone to completion (approx. 5-10 min),

2,4,6-collidine (0.01 ml, 0.09 mmol, 0.5 eq) was added and stirring was continued for approx. 0.5 h. The reaction mixture was then diluted with DCM (50 ml), filtered through Celite and concentrated under reduced pressure. The resulting syrup was diluted with 2 M HCl (200 ml) and extracted with DCM (2 x 200 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ solution (2 x 200 ml) and water (200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a syrup. Column chromatography (silica gel; Hexane – EtOAc, 5:1) gave *octyl 6-O-(2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside* **(4.80)** as a foam (91 mg, 58 %); (Found: C, 69.22; H, 5.97. $C_{61}H_{60}O_{16}.0.5\ H_2O$ Requires: C, 69.23; H, 5.81%); $[\alpha]_D$ +2.5° (*c* 3.2, CH_3OH); (*c* 0.2, $CHCl_3$); δ_H ($CDCl_3$): 0.81 (3 H, t, *J* 7.1, CH_2CH_3), 0.88 – 1.34 (12 H, m, 6 x CH_2), 3.47 (1 H, m, 1 H of OCH_2), 3.80 – 4.13 (5 H, m, H-4', H-5, H-6_A, H-6_B and 1 H of OCH_2), 4.57 – 4.66, 4.76 – 4.82 (3 H, 2 x m, H-5'$_A$, H-5'$_B$ and H-1), 5.42 – 5.58 (5 H, m, H-2, H-4, H-1', H-2' and H-3'), 5.89 (1 H, t, $J_{2,3}$ 9.8, $J_{3,4}$ 9.8, H-3), 7.25 – 7.63, 7.81 – 8.12 (30 H, 2 x m, Ar); δ_C ($CDCl_3$): 13.5 (CH_3), 22.0, 25.2, 28.5, 28.6, 28.8, 31.2 (6 x CH_2), 63.2, 66.1, 69.5 (OCH_2, C-6 and C-5'), 69.7, 71.5, 72.6, 73.6, 76.1, 80.6, 81.6 (C-2, C-3, C-4, C-5, C-2', C-3' and C-4'), 100.8 (C-1), 106.0 (C-1'), 127.9 (4 C), 128.0 (4 C), 128.1 (2 C), 128.2 (2 C), 128.5 (2 C), 128.7 (2 C), 128.8 (2 C), 129.1 (2 C), 129.4 (4 C), 129.5 (4 C), 129.6 (2 C) (30 C, Ar), 132.7 (2 C), 132.8, 133.0, 133.1, 133.2 (6 C, 6 x quat. C), 164.7, 164.9 (2 C), 165.5 (2 C), 165.9 (6 C, 6 x *C*O); *m/z* (ES) 1071 ($M+Na^+$, 100%); HRMS: Found: 1071.3796. $C_{61}H_{60}O_{16}Na$ ($M+Na^+$) Requires 1071.3779.

Octyl 6-*O*-(α-L-arabinofuranosyl)-β-D-glucopyranoside (4.81)



To a solution of octyl 6-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **(4.80)** (90 mg, 0.087 mmol) in anhydrous methanol (2 ml), was added a stock solution of NaOMe/MeOH (10 mg/ml) (0.2 ml). The reaction was allowed to stir

until TLC [DCM– methanol, (10:1)] showed that the reaction had gone to completion (within 1 h.). The solution was neutralised with Amberlite 120 (H^+) ion exchange resin, filtered and concentrated *in vacuo* to yield the title compound, *octyl 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranoside* **(4.80)** as an oil (36 mg, 100 %); $[\alpha]_D$ –98.6° (*c* 3.0, CH_3OH); δ_H (CD_3OD): 0.80 (3 H, t, *J* 6.7, CH_2CH_3), 1.21 (10 H, br s, 5 x CH_2), 1.48 – 1.55 (2 H, m, OCH_2CH_2), 3.04 – 3.94 (13 H, m, H-2 - H-6_2, H-2' - H-$5'_2$ and OCH_2), 4.15 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.86 (1 H, d, $J_{1',2'}$ 0.8 H-1'); δ_C (CD_3OD): 13.2 (CH_3), 22.5, 25.8, 29.2, 29.3, 29.6, 31.8 (6 x CH_2), 61.9, 66.9, 69.9 (C-5', C-6 and OCH_2), 70.8, 73.9, 75.5, 76.8, 77.7, 82.0, 84.7 (C-2 - C-5, C-2' - C-4'), 103.3 (C-1), 108.8 (C-1'); *m/z* (ES) 442 ($M+NH_4^+$, 100%); HRMS: Found: 442.2655. $C_{19}H_{40}NO_{10}$ ($M+NH_4^+$) Requires 442.2652.

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