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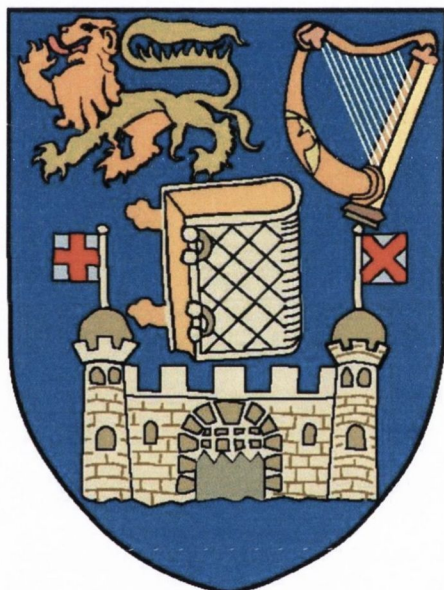
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THE SYNTHESIS AND BIOLOGICAL EVALUATION OF
MYCOBACTERIUM TUBERCULOSIS ASSOCIATED
GLYCAN DERIVATIVES



A thesis submitted to the University of Dublin
for the degree of Doctor of Philosophy

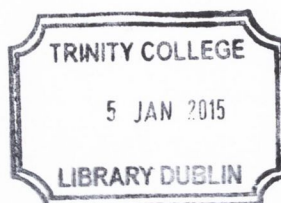
By
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June 2014

Trinity College Dublin

Contents

Declaration	i
Personal declaration	ii
Acknowledgements	iii
Abstract	iv
Abbreviations	v



Chapter 1: Introduction

1.1	Part 1: Tuberculosis	1
1.1.1	An introduction to tuberculosis	1
1.1.1.1	The Global burden of tuberculosis	1
1.1.1.2	Types of TB and their diagnosis	1
1.1.1.3	Challenges in TB treatment	2
1.1.1.4	Standard TB treatments	2
1.1.1.5	Drug resistant TB treatments	3
1.1.2	An overview of the immune responses to tuberculosis	4
1.1.2.1	The T _h 1/T _h 2 paradigm	4
1.1.2.3	Cytokine production during the disease state	6
1.1.2.3.1	Phagocytosis	6
1.1.2.3.2	The granulomatous response	7
1.1.2.3.3	The importance of IFN- γ	7
1.1.3	Mycobacteria and their characteristics	8
1.1.3.1	Extractable lipids	9
1.1.4	The need for chemically synthesised biomolecules for biological Investigations	11
1.1.4.1	The importance of the glycan region	12
1.1.5	The initial identification of PGLs: mycosides A, B, and G	13
1.1.5.1	The prevalence and function of PGLs in different mycobacteria	13
1.1.5.2	<i>M. kansasii</i> and mycoside A	14
1.1.5.3	<i>M. marinum</i> and mycoside G	14
1.1.5.4	<i>M. Bovis</i> and mycoside B	15
1.1.5.5	<i>M. leprae</i> and PGL-1	16

1.1.5.6	<i>M. tuberculosis</i> and PGL-tb1	17
1.1.5.7	A comparison between the immunological properties of mycosides A, B, and G and PGL-tb1	18
1.1.6	PGL production	20
1.1.7	<i>p</i> -HBAD structure and discovery	21
1.1.7.1	<i>p</i> -HBAD biosynthesis and relationship to PGLs	22
1.1.7.2	Research to date on the role of <i>p</i> -HBADs	23
1.1.8	Relevant chemical syntheses	25
1.1.8.1	Syntheses directed at the glycan of PGL-tb1	25
1.1.8.1.1	Synthesis of a PGL-tb1 fragment by Daffé <i>et al.</i>	25
1.1.8.1.2	Fujiwara's synthesis of the trisaccharide of PGL-tb1	28
1.1.8.2.1	Synthesis of <i>M. kansasii</i> glycan fragment by Gurjar <i>et al.</i>	32
1.1.8.2.2	Other PGL syntheses	35
1.1.8.3	Synthesis of PGL-tb1 by Barroso <i>et al.</i>	36
1.1.8.4	<i>p</i> -HBAD syntheses	39
1.1.8.5	A discussion of PGL glycan syntheses to date	40
1.2	Part two: The immobilisation of carbohydrates	41
1.2.1	Studying carbohydrate interactions	41
1.2.1.1	Immobilised carbohydrates as a tool for the investigation of carbohydrate interactions	42
1.2.2	Microarray technology	42
1.2.2.1	Carbohydrate microarrays	42
1.2.2.2	Noncovalently generated carbohydrate microarrays	43
1.2.2.2.1	Noncovalent attachment of unconjugated carbohydrates	43
1.2.2.2.2	Noncovalent attachment of conjugated carbohydrates	44
1.2.2.2.3	Assessment of the suitability of noncovalent attachment	46
1.2.2.3	Covalently generated carbohydrate microarrays	46
1.2.2.3.1	Covalent attachment of conjugated carbohydrates to functionalised surfaces	46
1.2.2.3.2	Covalent attachment of unconjugated carbohydrates to functionalised surfaces	47
1.2.2.3.3	Covalent attachment of conjugated carbohydrates to unfunctionalised surfaces	48
1.2.2.3.3.1	Advantages to carbohydrate functionalised gold nanoparticles (GNP)	49

1.2.3	Carbohydrate microarrays as a means for the investigation of carbohydrate biological properties	49
1.3	Aims	51

Chapter 2: The synthesis of tuberculosis associated *para* hydroxybenzoic acid derivatives and phenolic glycolipids

2.1	Rationale for the of the PGL/ <i>p</i> -HBAD project	52
2.1.1	Challenges to be considered before undertaking a synthesis.	53
2.1.2	Neighbouring group participation	54
2.1.2	Challenges highlighted by previously reported syntheses	55
2.1.3	Establishment of a methylation pattern	55
2.1.3.1	Diol protecting groups	56
2.1.3.2	Methodologies for distinguishing between the 2- and 3-hydroxyls of rhamnose	57
2.1.3.3	A brief discussion of possible protecting group strategies	59
2.1.4	The choice of donor	59
2.1.5	Lipid attachment	60
2.1.6	<i>p</i> -HBAD synthesis	61
2.2	Synthesis of functionalised rhamnosyl donors	64
2.3	Synthesis of fucosyl thioglycosides	75
2.4	Installation of a functionalised aromatic moiety and <i>p</i> -HBAD synthesis	76
2.5	Further development of the rhamnosyl thioglycoside library	85
2.6	Synthesis of a library of <i>p</i> -HBADs	90
2.7	PGL synthesis	94
2.8	Future work	105

Chapter 3: Immobilisation of phenolic carbohydrates on surfaces

3.1	The importance of immobilised carbohydrates	107
3.2	Diazonium grafting	108
3.2.1	Nitro aromatics as amine precursors	110
3.2.2	Reductions of an aromatic nitro to an amine	112
3.2.3	Non-rhamnose sugars	114
3.2.4	Protection and deprotection of anilines	116
3.2.5	Removal of phthalimide protecting group	118

3.2.6	Glycosylation of <i>N</i> -(4-hydroxyphenyl)phthalimide	120
3.2.7	Conclusion	121
3.3	Linker based immobilisation strategies	122
3.3.1	Linker choice and attachment	123
3.3.2	Synthesis of a thiol terminated linker	125
3.3.3	Synthesis of an azide terminated linker	126
3.3.4	Conclusion	129
3.4	Future work	129

Chapter 4: The biological evaluation of mycobacterial *p*-hydroxybenzoic acid derivatives (p-HBADs)

4.1	Pathogenic background	130
4.1.2	Rational for biological testing	130
4.1.3	Lipopolysaccharide effects	131
4.1.4	Design of experiments	132
4.1.5	Detection of cytokines	132
4.2	Splenocytes	133
4.2.1	Splenocyte experiments	133
4.2.2	T cell cytokine	134
4.2.2.1	IFN- γ	134
4.2.2.2	IL-17	137
4.2.2.3	IL-10	138
4.2.2.4	Overall cytokine response by splenocytes incubated in the presence of <i>p</i> -HBADS	140
4.2.3	Activation of T cells and analysis by flow cytometry	141
4.3	Macrophages	146
4.3.1	Macrophage experiments	146
4.3.2	Macrophage cytokines	148
4.3.2.1	IL-12p40	148
4.3.2.2	IL-10	150
4.3.2.3	IL-6	151
4.3.2.4	TNF- α	153
4.3.2.5	Overall cytokine response by macrophages incubated in the presence of <i>p</i> -HBADs	154

4.3.3	Aqua live/dead	155
4.3.4	Clusters of differentiation	157
4.4	Conclusions	160

Chapter 5: Conclusion

5.1	Concluded aims	161
5.2	Published works	162

Chapter 6: Experimental

6.1	Materials and methods	163
6.2	General experimental	164

7.0	Appendix A	279
8.0	References	289

DECLARATION

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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PERSONAL DECLARATION

I hereby declare that the work described in this thesis was performed by me
unless otherwise stated.

A handwritten signature in blue ink, appearing to read 'Jean Bourke', is written above a horizontal line.

Jean Bourke

Acknowledgements

I have now been a student for the vast majority of my life. For this, at times mixed blessing, I have to thank my parents for impressing upon me, from an early age, the importance of hard work and a good education. Their unwavering support has been invaluable.

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Abstract

Mycobacterium tuberculosis establishes chronic infection and causes disease through manipulation of the host's innate and adaptive immune response. The bacterial cell wall is highly complex and contains a rich variety of glycosylated compounds, some of which are secreted during infection and have been proposed as immunomodulatory molecules. Amongst the most important of these are the *p*-hydroxybenzoic acid derivatives (*p*-HBADs) and phenolic glycolipids (PGLs). Herein is reported a synthetic methodology for the synthesis of these important biomolecules and their unnatural analogues and also the first *in vitro* study of the immunomodulatory effects of *p*-HBADs in isolation from the host bacterium.

Native carbohydrates can often be complex and synthetically challenging to prepare. The preparation of significant quantities of free-sugars for biological evaluation is often not feasible. Carbohydrate microarrays offer a solution to the problems associated with limited quantities of oligosaccharides for biological screening as they require small quantities of the carbohydrate and allow for the efficient isolation and recovery of the carbohydrate after the required biological testing is concluded. Various methods have been reported for the immobilisation of carbohydrates, many of which require modified carbohydrate precursors. The synthesis of *p*-HBAD and PGL derivatives suitable for surface immobilisation is also described.

Abbreviations

Ac	-	Acetyl
aCD3	-	Anti-Cluster of Differentiation 3 antibodies
AFB	-	Acid Fast Bacteria
AIDS	-	Acquired Immunodeficiency Syndrome
AMF	-	Atomic Force Microscopy
BCG	-	Bacillus Calmette-Guérin
BMM	-	Bone marrow Derived Macrophages
Bn	-	Benzyl
Cat.	-	Catalytic
Boc	-	Tertbutyl Dicarbonate
BSA	-	Bovine Serum Albumin
CD	-	Cluster of Differentiation
CFSE	-	Carboxyl fluorescein Succinimidyl Ester
CSA	-	Camphor Sulfonic Acid
CuAAC	-	Copper(I)-Catalysed Azide-Alkyne Cycloaddition
D	-	Dimensional
d	-	Doublet
DABCO	-	1,4-Diazobiscyclo[2.2.2]octane
DBU	-	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	-	<i>N,N'</i> -Dicyclohexenylcarbodiimide
DCM	-	Dichloromethane
DIM	-	Phthiocerol Dimycocerosates
DMAP	-	4-Dimethylaminopyridine
DMF	-	Dimethyl Formamide

DMTST	-	Dimethyl-(Methylthio)-Sulfonium Triflate
DNA	-	Deoxyribonucleic Acid
DR	-	Drug Resistant
DST	-	Drug Susceptibility Testing
EDA	-	Ethylene Diamine
ELISA	-	Enzyme-Linked Immunosorbent Assay
EPTB	-	Extra Pulmonary Tuberculosis
Et	-	Ethyl
FGM	-	Functional Group Manipulation(s)
GNP	-	Gold Nanoparticles
GTPase	-	Guanosine Triphosphatase
HIV	-	Human Immunodeficiency Virus
HK	-	Heat Killed
HMDM	-	Human Monocyte Derived Macrophage
HR	-	An anti-tuberculosis drug regime consisting of isoniazid and rifampicin
HRZE	-	An anti-tuberculosis drug regime consisting of isoniazid, rifampicin, pyrazinamide, and ethambutol
IFN	-	Interferon
IL	-	Interleukin
iNOS	-	Inducible nitric oxide synthase
IR	-	Infrared
ITC	-	Isothermal Titration Calorimetry
LAM	-	Lipoarabinomannan
Lev	-	Levulinate Protecting Group
LevOH	-	Levulinic Acid

LM	-	Lipomannan
LPS	-	Lipopolysaccharide
M.	-	Mycobacterium
MCP	-	Monocyte Chemotactic Protein
M-CSF	-	Macrophage Colony Stimulating Factor
MDR	-	Multi Drug Resistant
Me	-	Methyl
MHC	-	Major Histocompatibility Complex
MPM	-	Mannosyl- β -1-Phosphomycoketides
Mtb	-	Mycobacterium Tuberculosis
MTBC	-	Mycobacterium Tuberculosis Complex
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate-Oxidase
NHS	-	<i>N</i> -Hydroxysuccinimide
NIS	-	<i>N</i> -Iodosuccinimide
NMR	-	Nuclear Magnetic Resonance
Pam3Cys	-	Tripalmitoyl Cysteine
PDIM	-	Phenolphthiocerol Dimycocerosate
PEG	-	Polyethylene Glycol
PGL	-	Phenolic Glycolipids
Ph	-	Phenyl
<i>p</i> -HBAD	-	<i>Para</i> -Hydroxybenzoic Acid
Phox	-	Phagosome Oxidase
PIM ₂	-	Phosphatidylinositol Dimannoside
PKS	-	Polyketide Synthase

PLGA	-	Poly(lactic-co-glycolic) Acid
PMA	-	Phorbol Myristate Acetate
PPD	-	Purified Protein Derivative
Pr	-	Propyl
PTB	-	Pulmonary Tuberculosis
<i>p</i> -TSA	-	<i>Para</i> -Toluene Sulfonic Acid
q	-	Quartet
RNA	-	Ribonucleic Acid
rt	-	Room temperature
s	-	Singlet
SAM	-	Self-Assembled Monolayer
SAR	-	Structure Activity Relationship
SPR	-	Surface Plasmon Resonance
STM	-	Scanning Tunnelling Microscopy
t	-	Triplet
TB	-	Tuberculosis
TBAB	-	Tetra-butylammonium Bromide
TBAI	-	Tetra-butylammonium Iodide
TCR	-	T Cell Receptor
TEA	-	Triethyl Amine
Tf	-	Triflyl
TFA	-	Trifluoroacetic Acid
THF	-	Tetrahydrofuran
TLC	-	Thin Layer Chromatography

TLR	-	Toll-Like Receptor
TMS	-	Trimethyl Silane
TNF	-	Tumour Necrosis Factor
TTBP	-	2,4,6-Tri-Tert-Butyl Pyrimidine
UM	-	Unmethylated
WHO	-	World Health Organisation
XDR	-	Extensively Drug Resistant Tuberculosis

1.0 Chapter 1: Introduction

1.1 Part 1: Tuberculosis

1.1.1 An introduction to tuberculosis

Tuberculosis (TB) is an infectious, mainly pulmonary, disease whose primary causative agent is *Mycobacterium tuberculosis* (*Mtb*).

1.1.1.1 The global burden of tuberculosis

The World Health Organisation (WHO) declared TB a global health emergency in 1993. At present it is one of the major causes of mortality and morbidity facing humanity. In 2011 there were nine million new cases and 1.4 million deaths attributable to TB of which 430 000 were in human immunodeficiency virus (HIV) positive individuals. At present there are twelve million prevalent cases of TB. The rates of TB incident, prevalence, and mortality have been declining since 1990. However, the combined threat of drug-resistant TB (DR-TB) and multidrug-resistant TB (MDR-TB) is becoming a major concern; in 2011 there were approximately 630, 000 cases of MDR-TB.

TB infection is acquired through the inhalation of aerosols containing the infective bacilli. The disease may take one of two courses; which is taken is predominantly dictated by host factors such as the condition of the host immune system and the strength of the immune response. Approximately 10% of individuals infected will develop the disease proper whereas the other 90% can develop a latent form. *Mycobacterium tuberculosis* (*Mtb*) has evolved to persist effectively in host macrophages; in the latent form of the disease, granulomas develop containing mainly infected macrophages and lymphocytes which are essentially walled off from the rest of the host. Bacterial dormancy can be viewed as mutually beneficial to both host and bacterium though it is not indefinite and there may be a resurgence of disease, e.g. if the host's immune system is in some way compromised. It is estimated that one third of the world's population is latently infected in this way.¹⁻³

1.1.1.2 Types of TB and their diagnosis

Tuberculosis typically infects the lungs (pulmonary tuberculosis: PTB) or can be extra-pulmonary (EPTB). Typically, EPTB is found in the pleura, lymph nodes, abdomen,

genitourinary tract, skin, joints, bones, and meninges though a patient may have both PTB and EPTB.

If a patient is suspected to have PTB they must submit a minimum of two sputum samples for microscopic investigation. These are checked for acid fast bacteria (AFB) allowing the case to be classified as smear-positive (highly infectious) or smear-negative (common with HIV infected patients and with a high mortality in such patients). A sputum culture is also carried out to confirm the diagnosis; a case can be smear-negative but culture-positive. In the case of EPTB a histopathological examination of the tissue in question is required in addition to a chest X-ray and sputum check. After the initial diagnosis, once treatment has begun patients need to be monitored with regular sputum checks.²

Coinfection with HIV greatly complicates TB cases, often resulting in an atypical disease phenotype.⁴ Such co-infected patients often have other mycobacterial infections making an accurate diagnosis problematic and resulting in a high proportion of smear-negative patients;^{5, 6} as a result it is recommended that such patients are diagnosed via culture methods which can be a lengthy process.⁷ Early diagnosis and prompt treatment is vital in all cases but particularly in HIV/AIDS (acquired immunodeficiency syndrome) patients who display a particularly high mortality rate of 45% (vs. 19% in healthy controls).^{8, 9}

1.1.1.3 Challenges in TB treatment

There are several challenges to be overcome when treating TB. One of the major difficulties is that *Mtb* is an intracellular parasite that has evolved to survive within macrophages. This means that the disease remains latent and hidden re-emerging when treatment is discontinued; as a result treatment needs to be long term.¹⁰ Once a drug has been administered it must reach its target to be effective and here there is another difficulty to be overcome: the cell wall acts as a barrier preventing anti-tuberculosis drugs reaching their targets.^{11, 12}

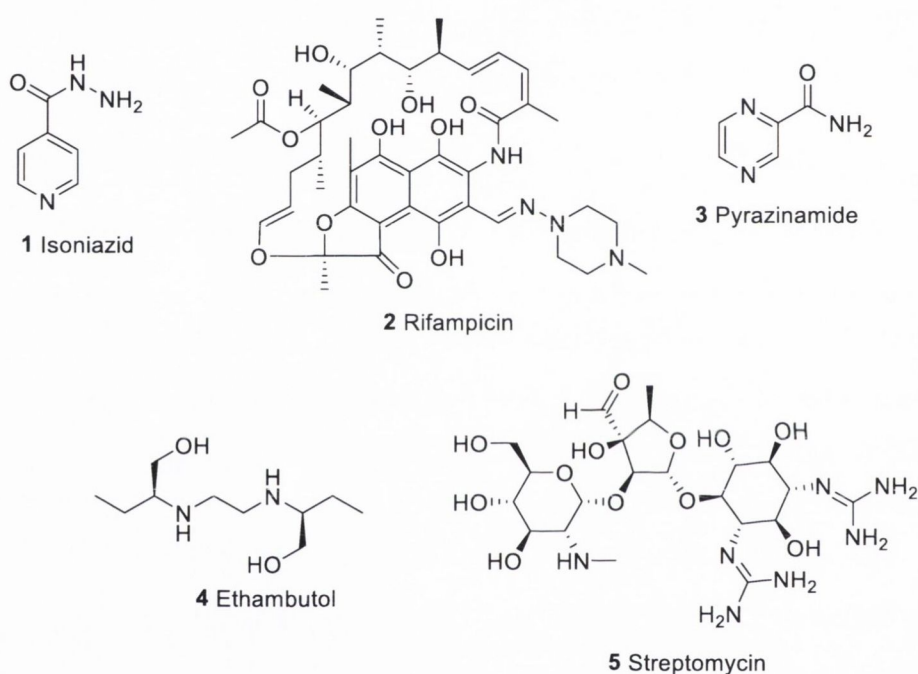
Despite these challenges in 2010 the treatment success rate was 85% for new cases.²

1.1.1.4 Standard TB treatments

Treatment of tuberculosis typically consists of an initial intensive phase with daily administration of first-line anti-TB drugs for two months, followed by a continuation phase during which drugs are administered three times a week for four months. First-line drugs for

the treatment of TB are isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin (Figure 1.1). The standard regime for new patients is a two month combination treatment of isoniazid, rifampicin, pyrazinamide, and ethambutol (collectively referred to as HRZE) followed by four months of isoniazid and rifampicin (together referred to as HR); the complete treatment regime is called 2HRZE/4HR. There is some variation in the treatment used: for example, if there is a high incidence of isoniazid resistance in the population in question then ethambutol is included in the continuation phase.

Figure 1.1 First line anti-TB drugs



1.1.1.5 Drug resistant TB treatments

As previously mentioned drug-resistant TB (DR-TB) and multidrug-resistant TB (MDR-TB) are becoming more prevalent; consequently, if a patient has been previously treated for tuberculosis then specimens of the mycobacteria should be obtained for drug susceptibility testing (DST). DST can facilitate the identification of DR-TB or MDR-TB. Recently, extensively drug-resistant TB strains (XDR-TB) have also been identified. At present 3.7% of new cases and 20% of previously treated cases are estimated to be MDR-TB. XDR-TB is currently in eighty-four countries and 9% of new MDR-TB cases are XDR-TB.¹³ Perhaps unsurprisingly a MDR-TB case is more common in relapses, failed treatments (TB still present), and people who default on treatment. WHO guidelines for MDR-TB treatment specify a longer eight month intensive phase and a significantly extended total treatment time of twenty

months. Drugs used to treat MDR-TB are categorised based on efficacy, time of use, and drug class. A MDR-TB regime will consist of a minimum of four drugs, including one from each of the first four groups outlined below.¹³

Group 1 drugs are front-line oral drugs. They are regarded as potent and best tolerated.

Group 2 is the injectables and they are considered second-line reserved drugs (except streptomycin): kanamycin, amikacin, capreomycin, and streptomycin.

Group 3 is made up of the fluoroquinolones: levofloxacin, moxifloxacin, and ofloxacin.

Group 4 consists of oral bacteriostatic second-line drugs: *p*-aminosalicylic acid, cycloserine, terizidone, ethionamide, and prothionamide. Ethionamide is quite inexpensive though the more expensive *p*-aminosalicylic acid is preferred.

Group 5 drugs are classed as having an unclear role in treatment of DR-TB and are not recommended by WHO for routine use but can be used in XDR-TB cases. Group 5 includes clofazimine, linezolid, amoxicillin/clavulanate, thioacetazone, imipenem/cilastatin, high dose isoniazid, and clarithromycin.

1.1.2 An overview of the immune responses to tuberculosis

As previously stated, not all people who come in contact with infective TB bacilli will develop TB. The possibility of developing TB is linked to both host and environmental factors. One of the biggest host factors in disease progression is the immune response of the host to the invading pathogen. Communication between the different cellular components of the immune system is achieved by means of cytokines (cell signalling molecules) which are secreted by cells in response to infection. Different cytokines can result in different modes of immune system activation and this plays a vital role in determining host survival. Helper T cells (T_h cells) play a crucial role in regulating the immune system and the release of certain cytokines by T_h cells can lead to certain stereotyped patterns of immune response. Ideally for the host a strong bactericidal response, referred to as T_h1 , is elicited; on the other hand, the bacterium benefits from an allergic type response, termed T_h2 .¹⁴ More virulent strains seem to elicit a weaker T_h1 response allowing the less effective T_h2 response to predominate.

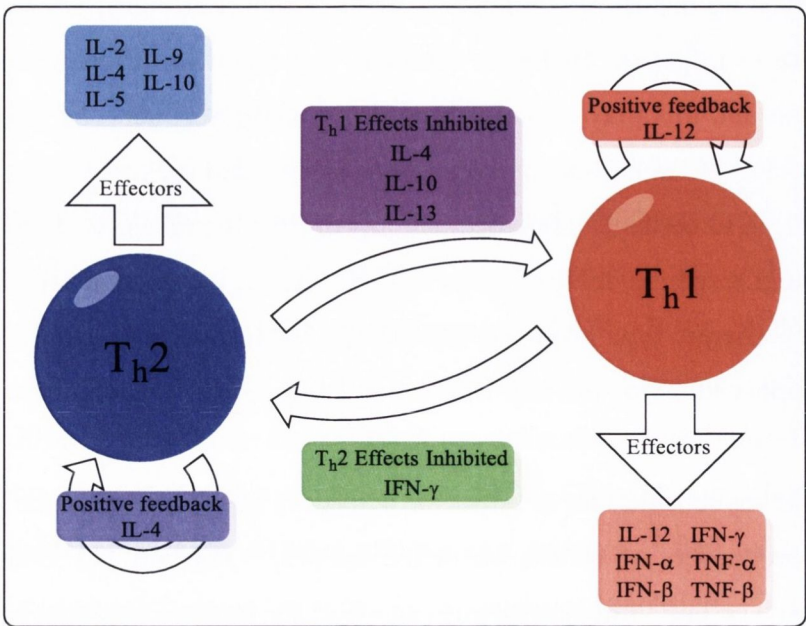
The ability of the mycobacterium to effect the activation of the host immune system has been linked to the production of certain virulence factors.¹⁵ This project aims to further our knowledge of how certain virulence factors can affect the immune response of the host during infection. By examining the cytokine response of immune cells exposed to mycobacterial

biomolecules we can gain an understanding of the role of these molecules in disease progression.

1.1.2.1 The T_h1/T_h2 paradigm

In order to investigate the effects of the compounds discussed in this report on the immune system, a rudimentary understanding of the complex immune factors involved is necessary. As discussed above there certain cytokine profiles that result in different kinds of overall immune response i.e. T_h1 and T_h2. Both of these responses are a result of T cells differentiating in response to cytokines produced by front line immune cells such as macrophages and dendritic cells.¹⁴ Once differentiated T_h1/ T_h2 cells produce cytokines to promote their type of immune response while simultaneously down regulating the other. T_h1 cells have differentiated to produce interferon- γ (IFN- γ) which inhibits T_h2 effects and induces T_h1 effects. (Figure 1.2). It is a very important cytokine during TB infection and is discussed more fully below. Similarly, T_h2 cells produce interleukin-4 (IL-4) but not IFN- γ . IL-4 is a T_h2 effector and Th1 suppressor.¹⁶ An understanding of the key cytokines is important in helping to decide which cytokines to monitor during biological testing of compounds that are suspected of having immune effects. In examining the response of cells to bacterial or molecules, detecting high levels of IFN- γ would indicated a strong T_h1 (bactericidal) response. Conversely, low levels would indicated a weak, less effective response to bacterial infections.

Figure 1.2 Key cytokines produced by T_h1 and T_h2 cells.



IFN- γ and IL-4 are not the only cytokines involved (Figure 1.2). IL-12, IFN- α , IFN- β , tumour necrosis factor α (TNF- α), and IL-18 are associated with a bactericidal T_h1 response.^{17, 18} The corresponding T_h2 inducing cytokines include IL-4, IL-5, IL-9, and IL-10.^{18, 19, 20}

The mouse (murine) model of the immune system has been extensively used to study cytokine responses and is quite similar to the human model.²¹ Though there are some differences, for example human T cells are less restricted in their cytokine profile than murine cells, murine cells are ideal for initial investigations of the immune effects of molecules.

1.1.2.3 Cytokine production during the disease state

To fully comprehend the role of molecules that act as virulence factors, it is necessary to understand the implications of altered cytokine profiles on disease progression. Understanding the role different cytokines play during infection means allow us to speculate about how a virulence factor effects bacterial and host survival. For example, if a bacterium releases a virulence factor that can inhibit the early immune response it has more time to gain a foothold before coming under attack by the host immune system. There are several stages in TB progression and these are discussed below.

1.1.2.3.1 Phagocytosis

Once mycobacteria have gained entry to the lungs, they are typically taken up by immune cells. The process by which this occurs is termed phagocytosis and involves a cell fully encapsulating molecules or pathogens. The engulfed articles are packaged into small lipid bilayer enclosed vesicles that, when generated through phagocytosis, are termed phagosomes. In the lungs, bacteria are typically phagocytosed by alveolar macrophages, epithelial type II pneumocytes and dendritic cells. Macrophages are the main cells infected by *Mtb* and are a major source of the cytokines required to modulate host immune responses.¹⁵ Macrophages are activated by IFN- γ (secreted by T cells) and TNF- α (secreted by other macrophages). Activated macrophages express IL-10 and IL-12 which compete and have opposing functions: IL-12 promotes INF- γ release and hence macrophage activation; whereas IL-10 down-regulates macrophage activation.²² Following from this, if an invading pathogen could alter the ratio of IL-10 to IL -12 produces it might be able to skew the immune response in its favour allowing it to evade the host immune system. A PGL produced by *M. Marinum* called mycoside G has been linked to an inhibition of phagosome maturation (Section 1.1.4.2).²³

Mtb has evolved to persist inside macrophages and inhibits the maturation of phagosomes and the resulting digestion.²⁴ Components of the *Mtb* cell wall have been associated with the inhibition of both lysosome fusion and the acidification of the phagosome. These component could be regarded as vital virulence factors since they enhance mycobacterial survival. However, the exact mechanism by which *Mtb* avoids digestions and is able to proliferate and cause infection is not yet fully understood.

1.1.2.3.2 The granulomatous response

At the site of infection, both macrophages and T cells accumulate due to relaxation of the vasculature and the cytokine environment. In this manner a granuloma, within which infected cells are contained by a barrier of epithelioid macrophages, is generated. The size of the resultant granuloma is proportional to the amount of TNF- α secreted by macrophages, although how the size affects the host is uncertain. TNF- α is essential for early granuloma formation and integrity, however, if production remains high caseous necrosis and cavitation can result. Such pathology is observed later in disease progression.

Initial bacterial infection can be controlled without the formation of granulomas. However, in the chronic form of the disease the granulomatous response helps contain the infection and will last almost the entire life of the host.²⁵ Any bacteria that survive or escape the initial inflammatory response enter an indefinite chronic disease state within the granuloma. While in this state, the bacterial load does not increase, but there is a continuous inflammatory host response.

1.1.2.3.3 The importance of IFN- γ

As discussed above TNF- α is important in the formation of granulomas. Another crucial cytokine is IFN- γ . As the infection proceeds, a protective T cell response is mounted and IL-12 secreted by macrophages induces IFN- γ secretion by sensitised T cell. These IFN- γ secreting T cells are vital for an effective immune response to a mycobacterial infection. IFN- γ plays a role in several bactericidal processes including the production of toxic molecules. The secreted toxic molecules can damage host tissues as well as bacteria and consequently feedback is necessary to curtail their production. As bacteria are killed, less and less antigens are present to activate the immune response allowing for a gradual winding down of IFN- γ secreting T cells.

Mycobacterial cells have a high lipid content many of which are structurally unusual and unique to mycobacteria.³⁹ Mycolic acids are a hallmark of the mycobacterial cell envelope and account for an estimated 60%. They are long chain (C70-90) α -alkyl, β -hydroxylated fatty acids that can vary in length, branching and modification.^{40, 41} The cell envelope plays a vital role in mycobacterial survival in the host consists of three superimposed parts: a plasma membrane, the cell wall skeleton and the capsule (Figure 1.3).^{42, 43} Components of the cell envelope are virulence factors that can aid mycobacterial survival by altering the host cytokine response and hence the immune response. As well as components of the cell envelope, secreted molecules, such as extractable lipids, can also alter the host cytokine response.^{44, 45} It is these molecules that are the subject of this work.

1.1.3.1 Extractable lipids

Beyond the cell wall and the capsule there are species-specific, noncovalently associated lipids termed extractable lipids. In pathogenic mycobacteria there are two classes: trehalose and phthiocerol lipids. Trehalose itself is made up of two α -(1 $\rightarrow$ 1) linked glucoses **6** esterified with fatty acids to form a trehalose lipid (Figure 1.4).⁴³ Phthiocerol lipids are a class of molecules consisting of phthiocerol dimycocerosates **7** (DIM), phenolphthiocerol dimycocerosates **8** (PDIM) and phenolic glycolipids **9** (PGL). PGLs consist of a PDIM lipid core that is ω terminated by an aromatic nucleus glycosylated through the hydroxyl of the phenyl moiety by a species specific mono-, tri- or tetrasaccharide.⁴⁶

Three IFN- γ associated pathways in particular are associated with combatting *Mtb* infection: inducible nitric oxide synthase (iNOS) which generates reactive nitrogen species;²⁶ phagosome oxidase (phox) which generates reactive oxygen species; and the phagosome associated guanosine triphosphatase (GTPase) LRG-47 which is linked to vesicular trafficking.²⁷⁻²⁹ The vital role these processes play in controlling infection has been demonstrated using mice that are deficient in one or more of them. Mice deficient in iNOS²⁶ and LRG-47²⁷ cannot control mycobacterial replication and are particularly vulnerable to mycobacterial infection though phox deficient mice are not as vulnerable.³⁰⁻³³ IFN- γ deficient mice display an increased susceptibility to mycobacterial infections³⁴ and are more vulnerable than iNOS deficient mice indicating that IFN- γ has several protective functions.²⁷ Mycobacteria can evade the host immune response by inhibiting IFN- γ induced gene expression.³⁵ Additionally they can induce the production of IL-12³⁶ which down-regulates the antimicrobial activities of T-cells.^{37, 38} There is a clear link between the induced cytokine response and bacterial survival and molecules that mediate the immune response can help further our understanding of the disease mechanism and are consequently of therapeutic interest.

1.1.3 Mycobacteria and their characteristics

The Mtb complex (MTBC) is a group of genetically similar mycobacterial species that cause TB: *M. tuberculosis*, *M. canetti*, *M. africanum*, *M. microti*, *M. bovis*, *M. caprae*, *M. pinnipedii*. Mycobacteria are characterised by a unique and highly complex cell envelope containing long chain α -alkyl, β -hydroxy fatty acids and exhibit acid-fast staining if the Zhiel-Neelson technique is employed.

Figure 1.3 Mycobacterial cell envelope.

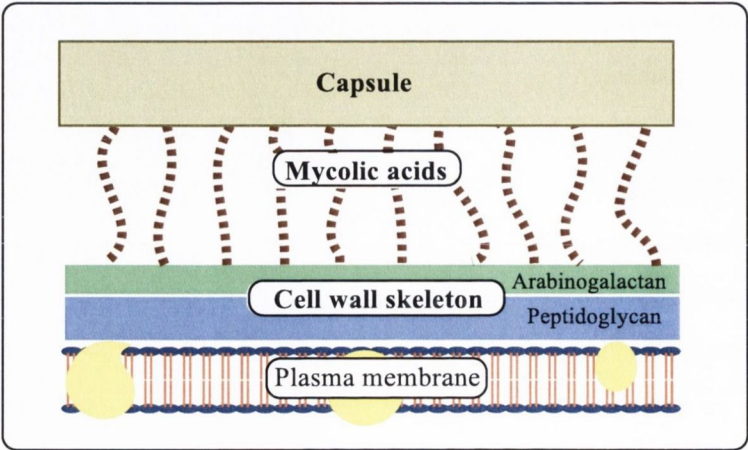
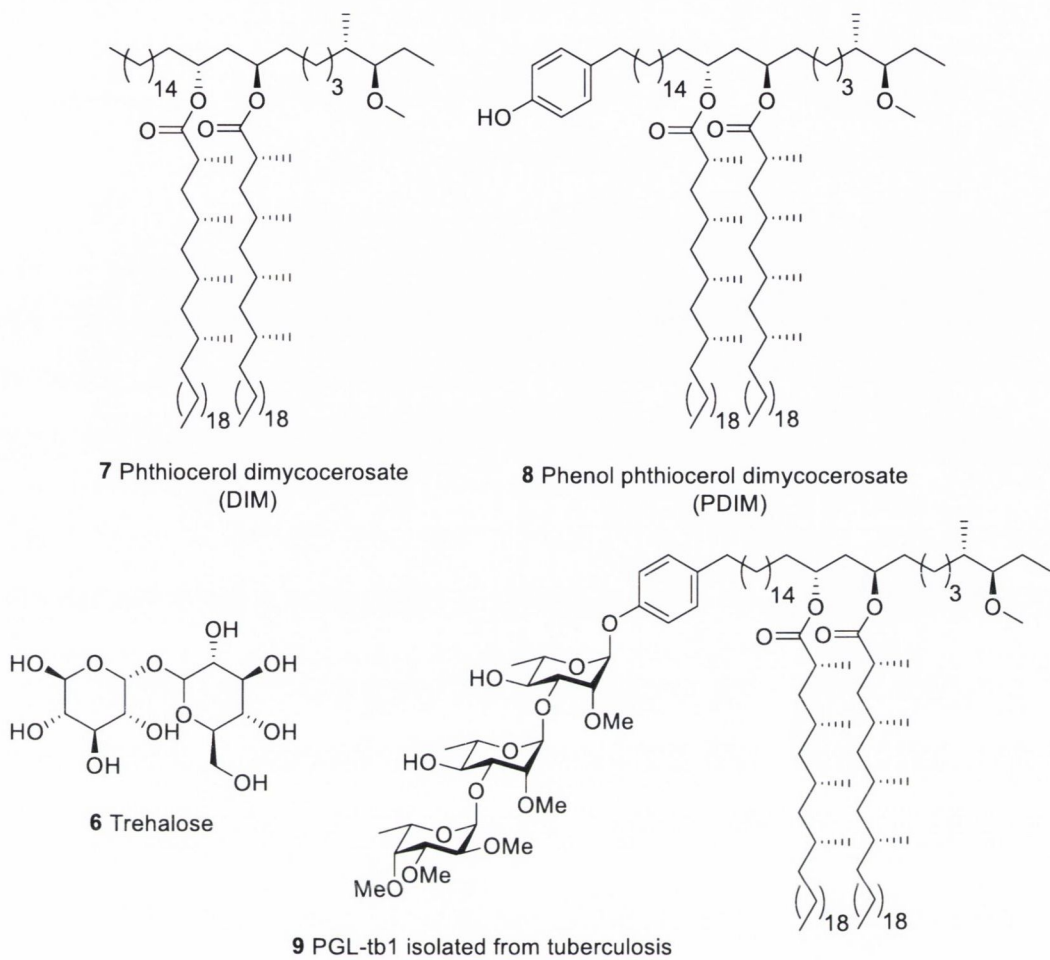


Figure 1.4 Structure of trehalose and a typical PGL.



The link between extractable lipids and virulence has been demonstrated by Manca *et al.* who infected monocytes from healthy donors with two different strains of *Mtb* that are characterised by different clinical outcomes (pathogenicity).⁴⁷ The first strain, CDC1551, causes an early and vigorous cytokine response⁴⁸ with a high antibody response and few cases of active disease.⁴⁹ The second strain, HN878, does not induce a strong early T_h1 type response, results in a high proportion of active cases and is often associated with extrapulmonary TB.^{45, 50} When human peripheral monocytes were infected, both strains resulted in the up-regulation of cytokines. When the levels of induction by each strain were compared it was found that CDC1551, the less pathogenic strain, induced much higher levels of proinflammatory molecules than did the more aggressive HN878. The more pathogenic HN878 induced significantly higher levels of the macrophage deactivating cytokines. Inducing an immune response of the wrong type is advantageous to the bacterium and adversely impacts the host.

When the lipid extracts of both strains were used instead of whole bacteria, comparable results were obtained. Since the lipid-induced gene profile closely resembled that of the mycobacterial infection the authors concluded that the lipids extracted from the mycobacteria were responsible for the observed differential activation by different strains.

Following from these initial results, additional strains were investigated: three strains of the highly pathogenic W/Beijing family (W4, W10, and 210) and the non-Beijing strain NHN5. Beijing strains are often associated with higher pathogenicity;⁵¹ of the original strains, HN878 is a Beijing strain and CDC1551 is a non-Beijing strain.⁴⁷ The Beijing strains resulted in significantly lower levels of two key proinflammatory cytokines, IL-12 and TNF- α , compared to the non-Beijing strains, despite similar levels of intracellular replication. These observations led to the postulate that some members of the W/Beijing family produce certain lipids that fail to induce a bactericidal T_h1 type immune response leading to a more severe disease state. As discussed above, the T_h1 response is linked to TNF- α and IFN- γ production and it requires higher levels of IL-12 production. This work suggests that the virulent phenotype of the W/Beijing family is associated with lipid-mediated subversion of the host's protective immune response.

The extractable lipids that are thought to be responsible for this effect are the subject of this thesis. As discussed above, there are several classes of extractable lipids, however, for a molecule associated with variations in strain pathogenicity it must also show variation in its structure and/or production between strains. Of the extractable lipids, DIM, PDIM and trehalose are common to pathogenic bacteria and show very minimal strain specific variation; PGLs, however, are highly strain specific.

1.1.4 The need for chemically synthesised biomolecules for biological investigations

When samples from a biological source are used, a major challenge is the reproducibility of both results and experiments. Samples isolated from different sources, or different batches of the same source, will likely contain different amounts of the target molecule. Without knowing the purity it is difficult to assess the concentration of an active compound or the ratio of several such compounds.

Certain contaminants, such as lipopolysaccharides (LPS), are common in biological samples; though experiments can be designed to incorporate controls to exclude the effects of LPS. LPS

are so ubiquitous as contaminants that even when synthetically prepared samples are used, LPS controls must still be used in assay to discount any LPS contaminants that may find their way into the experiment from any number of sources. Pure samples are not only required to examine the activity of a molecule but also to accurately assess its concentration

Although samples of both PGLs and related molecules can be accessed by purifying the relevant cell extracts the results are consistent in neither their concentration nor purity and are often contaminated with other biomolecules including LPS. Difficulties in purification and analysis make the use of molecules from biological sources less than ideal. Access to chemically prepared samples would help overcome many of the challenges faces when using biologically isolated compounds. Additionally, chemical syntheses would allow for the preparation of non-native analogues whose testing could greatly further our understanding of the structure activity relationship of the molecules in question.

1.1.4.1 The importance of the glycan region

The main focus of this thesis is the role of the glycan region as it is the main area of distinction between strains and species. Another reason for the focus on the glycan is that the seroreactive properties of the PGLs examined to date were generally attributed to the glycan portion of PGLs.^{52, 53} Anti-PGL antibodies have been shown to bind to the glycan but not the aglycan portion of PGLs.⁵²⁻⁵⁴ Furthermore the biological activity of PGLs is disrupted when the glycan portion is degraded.⁵⁵ Overall it seems reasonable to conclude that the evidence presented supports the theory that the glycan is responsible for the seroreactive properties and the immune-modulatory effects.

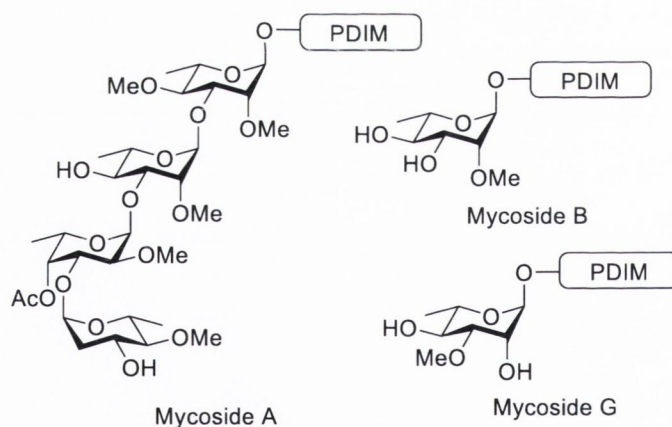
Research to date has focused on the applications of PGLs with relatively little work documented on their roles in disease pathogenesis. The work that has been carried out indicates that they are virulence factors and associates their loss with an attenuation of the mycobacterial strain. Our understanding of their function is still incomplete at best and it is this deficit that this work intends to address. The synthesis of both native and non-native PGL analogues and the investigation of their immunomodulatory and immunoreactive properties is the proposed to further our understanding of their role. Since certain carbohydrate patterns are maintained in many PGLs a link may be inferred between their structure and activities observed.

Since the glycan seems to be the source of the biological properties so it is the focus of this work. The lipid portion is simplified so as to shorten the synthesis and keep the glycan at the fore. Some linear syntheses of the carbohydrate portion of various PGLs have been carried out and these are relevant and serve to direct these efforts to address problems they encountered and to expand the number of glycans available for investigation. These are discussed further below (Section 1.8).

1.1.5 The initial identification of PGLs: mycosides A, B, and G

PGLs were initially identified by Smith *et al.* who used IR analysis of immunising fractions to identify the molecules that would come to be called mycoside A⁵⁶ in 1954, and mycoside B⁵⁷ in 1957, found in *M. kansasii* and *M. bovis* respectively (Figure 1.5). The term “mycoside” refers to type-specific glycolipids of mycobacteria.⁵⁸ In 1965, Navalkar *et al.*⁵⁹ reported their discovery of a specific glycolipid, mycoside G, in *M. marinum* and *M. balnei*. The carbohydrate portions of all three PGLs were found to be different though they all had the same PDIM core.

Figure 1.5 Mycosides A, B and G; All 3 mycosides are phenolic glycolipids (PGL)



1.1.5.1 The prevalence and function of PGLs in different mycobacteria

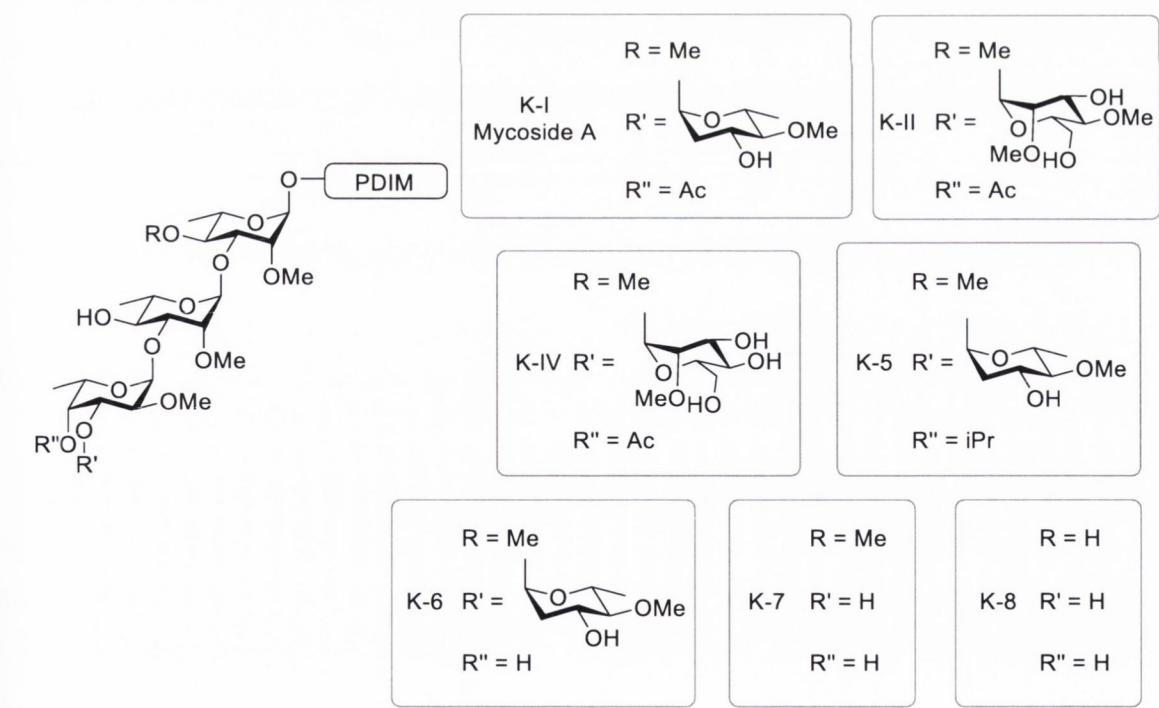
Molecules associated with tuberculosis and hence *Mtb* are the subject of this work. However, PGLs are produced by many mycobacteria including members of the *Mtb* complex (MTBC). When investigating the function of PGLs associated with *Mtb*, an understanding of the prevalence, characteristics and function of PGLs in other related mycobacterial species can lead to a better understanding of *Mtb* PGLs and related molecules. Overall, it has been clearly demonstrated that PGLs induce a cytokine response. Additionally, different species produce different PGLs that differ mainly in the carbohydrate region, though minor differences in the

lipid core have been demonstrated.^{60, 61} Below is a brief discussion of what is currently known about PGLs and their properties.

1.1.5.2 *M. kansasii* and mycoside A

Working with mycoside A of *M. kansasii* Papa *et al.* demonstrated that antibodies specific to mycoside A did not cross react with extracts from twenty-one other mycobacterial species.⁶² Since the aglycan portion is the same or very similar between species this suggests that anti-PGL antibodies are specific to the glycan portion which is itself specific to the strain. Further investigation of *M. kansasii* and the closely related *M. gastri* has led to the identification of seven PGLs associated with the species (Figure 1.6).^{53, 63-66} These results demonstrate that within a species or strain sever PGLs differing in their glycan region can be produced. Each PGL may have a different function, or they may work in concert to enhance bacterial survival. The synthesis of these PGLs would allow for the investigation of their properties in isolation and as mixtures.

Figure 1.6 Variations observed in *M. kansasii* phenolic glycolipids (PGLs)



1.1.4.2 *M. marinum* and mycoside G

Mmarinum is the closest living relative of *Mtb* and they share several virulence determinants.⁶⁷ As such, it has been proposed that *M marinum* can serve as a model for some aspects of *Mtb*

pathogenesis. Following from this, the behaviour of *M. marinum* derived PGLs could be an indicator of potential properties of *Mtb* PGLs.

Mycoside G has been linked to the inhibition of phagosome maturation and is thus an important virulence factor.²³ As previously discussed mycobacteria persist in phagosomes and the ability to inhibit their maturation is of benefit to the mycobacterium. Using human monocyte-derived macrophages (HMDM) it has been demonstrated that a coating of mycoside G on other microbes such as *Staphylococcus aureus* as well as polystyrene beads is enough to inhibit maturation. In mycobacterial mutants that have lost the ability to inhibit phagosome maturation, the additions of mycoside G is sufficient to complement the loss.

In experiments with human monocyte-derived macrophages (HMDMs) mycoside G has been linked with reduced production of proinflammatory cytokines TNF- α and IL-12 (IL-12p40). Heat-killed *M. bovis* BCG induces high levels of the pro-inflammatory cytokines. A coating of mycoside G was sufficient to drastically reduce cytokine secretion. The production of key proinflammatory cytokine TNF- α in particular was decreased by a factor of 125. This inhibition of proinflammatory cytokine release is related to the previously discussed phagosome maturation as it is induced by IFN- γ which is itself induced by TNF- α .⁶⁸ These findings, together with the close relationship between *M. marinum* and *Mtb* gives us an insight into the potential role of PGLs in tuberculosis pathogenesis.

1.1.4.3 *M. Bovis* and mycoside B

In *M. bovis* four PGLs were identified, differing in both the glycan (Figure 1.7) and aglycan (Figure 1.8).^{60,61}

The variation observed in the aglycan is not limited to *M. bovis*, and is not exclusive to the functional groups at the end of the lipid chain. The lipid chain can be of variable length as can the mycocerosic acid side chains. The variation in length is a result of repeated enzyme-based elongation cycles discussed below. The disparity seen in the lipid moiety is thought to be of less significance as it is not species specific but instead is based on slight differences in the activity of certain enzymes.

Figure 1.7 Phenolic glycolipids (PGLs) isolated from *M. bovis*

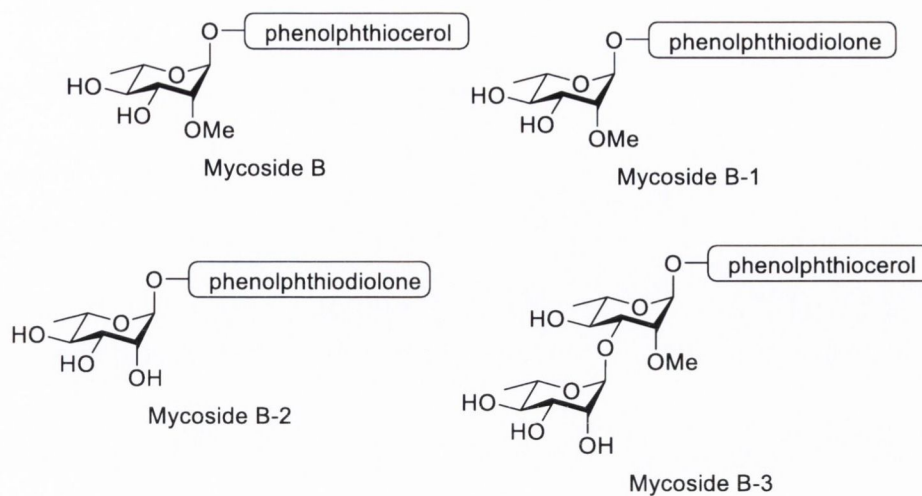
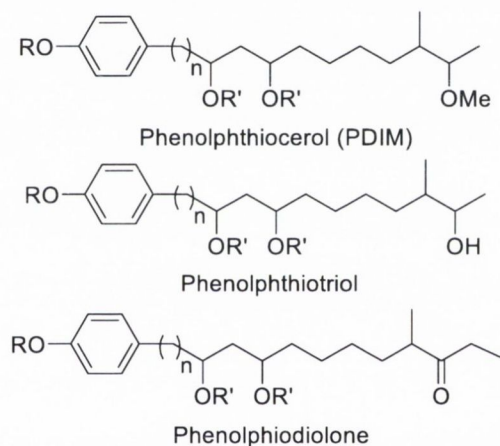


Figure 1.8 Variations in aglycan, R is a glycan, R' is a mycocerosic acid.

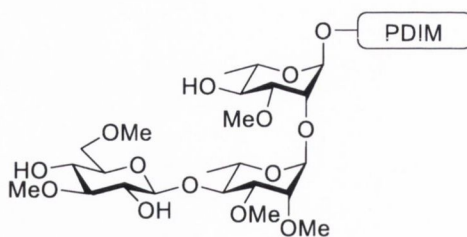


As discussed above, *M. bovis* BCG results in a strong proinflammatory cytokine response. Mycoside B is the major strain specific constituent of the glycolipids released by *M. bovis* BCG into host macrophage and as such a degree of the response can be attributed to it.⁶⁹

1.1.5.5 *M. leprae* and PGL-1

In 1981, Hunter and Brennan⁷⁰ identified a PGL exclusive to *M. leprae* (Figure 1.9). Building on this, at present three PGLs have been characterised and attributed to *M. leprae*.⁷¹⁻⁷³ The original *M. leprae* PGL was named PGL-1 and sparked a lot of interest due to its immunological properties and potential as a diagnostic tool.^{52, 74, 75}

Figure 1.9 Glycan portion of PGL-1



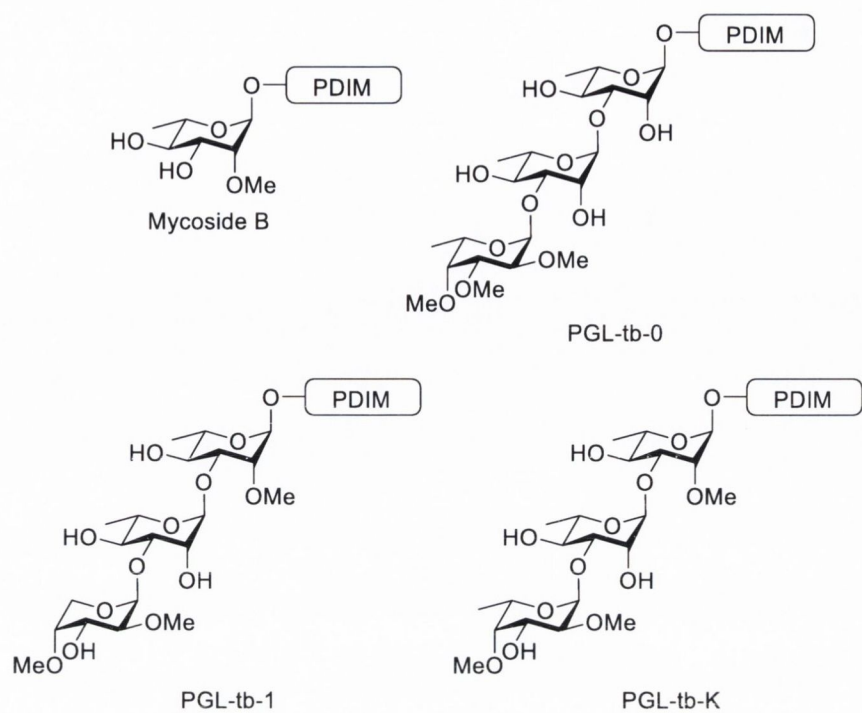
PGL-1 has been associated with a general resistance to intracellular degradation,⁷⁶ the tropism of leprosy to the peripheral nervous system (PNS).⁷⁷ Additionally, PGL-1 inhibits the monocyte oxidative response thus modulating the phagocytic cell function.⁷⁸ PGL-1 has also been linked to decreased production of proinflammatory cytokines (TNF- α , IL- β , and IL-10) and increased production of anti-inflammatory cytokines.^{79, 80} *M. bovis* BCG induces a strong TNF- α response and it has been demonstrated that PGL-1 expression by recombinant *M. bovis* BCG suppressed this.⁸¹ Since PGL-1 not only fails to induce but also inhibits a strong TNF- α response it appears to modulate the release of proinflammatory cytokines⁸² and in this manner may play a vital role in determining disease progression.⁸⁰

These findings all indicate that PGL-1 is a vital virulence factor in *M. leprae*. This is by no means the full extent of the research carried out on *M. leprae* associated PGLs, however, an exhaustive discussion of the research in question is beyond the scope of this work, as such only certain relevant works are highlighted. PGL-1 is key to the pathogenicity of *M. leprae*. Though PGL-1 is produced by all strains of *M. leprae* the same cannot necessarily be said of all strain specific PLG production by mycobacteria. The production of PGLs by *Mtb* is more complex and the biological activity less studied, as discussed below.

1.1.5.6 *M. tuberculosis* and PGL-tb1

In 1987, Daffé *et al.*⁴⁶ reported the isolation and characterisation of a PGL from the *Mtb* strain Canetti (Figure 1.10). They noted that there was some variation in the aglycan moiety with two homologs of phenolphthiocerol being observed. The mycoserosic acids also exhibited some variation in chain length. Small amounts of a PGL similar to mycoside B were also observed indicating that mycoside B is not as strain specific as certain other PGLs. Over time several PGLs were isolated from *Mtb* including mycoside B, the rhamnosyl functionalised PDIM, PGL-tb-0 which is a version of PGL-tb1 without a methyl group at the 2-position, and another PGL-tb1 variant lacking the methyl at the fucosyl 3-position termed PGL-tb-K.^{73, 83}

Figure 1.10 Structure of the glycan of PGL-tb1



In 2004, Reet *et al.*⁴⁴ infected mice with wild type PLG producing strain HN858 and an attenuated mutant HN858 that could not produce PGLs. A ninety day difference in survival rates of infected mice was noted despite no significant difference in bacterial load or survival. Following from these results, bone marrow derived macrophages (BMM) were infected with H37Rv (a non PGL-tb1 producer), the attenuated HN878 strain and the attenuated strain with PGL production restored through a plasmid. An inverse relationship between PGL production and the secretion of pro-inflammatory cytokines including TNF- α , IL-6, IL-12 and monocyte chemotactic protein 1 (MCP-1) was observed. The BMMs were incubated with lipid extracts from an attenuated strain in the presence of a series of PGL concentrations and dose dependent inhibition of pro-inflammatory cytokines was observed. When the experiment was repeated with mycoside B from *M. bovis* BCG a differential TNF- α and IL-6 secretion profile was observed whereas PDIM was not inhibitory. These results imply a link between PGL-tb1 production and the induction of a less acute proinflammatory host response. This biological activity could potentially benefit the bacterium and would suggest that a more thorough investigation of these molecules could be beneficial to our understanding of both the TB disease models and the cause of different clinical outcomes observed with different strains.

Like that of PGL-1, PGL-tb1 antiserum was also found to be specific and highly reactive.^{84, 85} However PGL-tb1 was not ultimately applicable to the serodiagnosis of *Mtb* as it is not produced by the majority of clinical isolates.⁸⁶⁻⁸⁸

1.1.5.7 A comparison between the immunological properties of mycosides A, B and G and PGL-tb1

The variation in PGLs can account for the diverse biological activities observed and the ability of each PGL to alter cytokine production has been discussed. Work by Fäldt *et al.*⁵⁵ aimed to directly compare the affect each PGL has on bactericidal activity in the same system. They investigated how mycoside B, mycoside A, mycoside G, and PGL-tb1 altered the production of reactive oxygen species (a pathogen damaging response) by neutrophils. Neutrophils were studied *in lieu* of macrophages as neutrophils are phagocytic in an analogous manner to macrophages. The authors reasoned that neutrophils are quite likely to be the immune cells that first encounter the mycobacteria and hence their response could bias the host immune system. The oxidative activity of neutrophils is mediated by cytokines and differential responses would be in keeping with the previously observed differential cytokine responses.

Overall the mycoside A tetrasaccharide was a stronger inducer than the PGL-tb1 trisaccharide whereas the monosaccharides of mycosides B and G were inhibitory. Since the main variable between the PGLs is the glycan the activity could reasonably be attributed to this portion of the molecule. When the carbohydrate portions were degraded the associated burst of oxidative activity was reduced. The inhibitory PGLs, mycoside B and G, differ only in the location of a single methoxy yet still had different activity profiles. This indicates that the methylation pattern is important in directing the biological activity.

Fucose and rhamnose monosaccharides alone were unable to inhibit the oxidative activity likely due to either suboptimal methylation patterns or the binding site being more compatible with an oligosaccharide.

An additional finding was that the neutrophil oxidative response was mainly extracellular with very little being detected intracellularly which is in keeping with the observation that mycobacteria inhibit the normal degradation processes within phagosomes. So though some PGLs result in a burst of oxidative activity this does not necessarily correlate with effective purging of intracellular mycobacteria.

1.1.6 PLG production

Though the PGLs generated vary between species and even between strains within a species, they are nonetheless quite common amongst mycobacteria. Since their production is preserved in many species it follows that they must serve a biological purpose that can effect mycobacterial survival to an extent that compensates for the energy and resources required to synthesise these large molecules. Overall PGLs have been demonstrated to be virulence factors for several mycobacterial species. The variation in the glycan region is species and strain specific with host antibodies generally displaying specificity for the glycan with no cross reactivity between PGL producers. This raises the question of why and how only some strains of *Mtb* produce PGL-tb1.

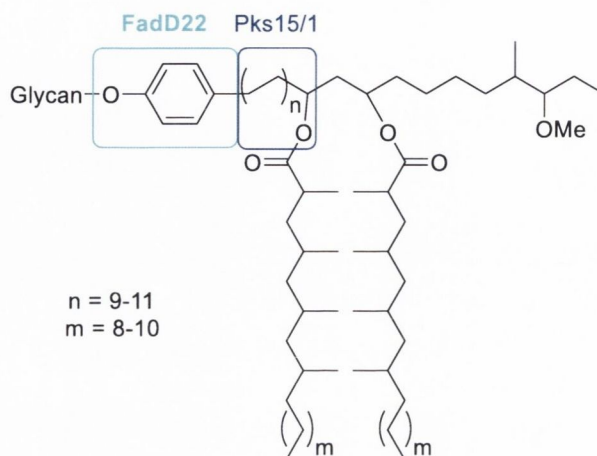
As discussed above a hypervirulent phenotype appears linked to the lipid profile of the mycobacterium. Reed *et al.*⁴⁴ attempted to characterise the differences between the hypervirulent W/Beijing strain HN878 and the non-Beijing non-hypervirulent CDC1551.⁴⁴ They found that the hypervirulent strain produced PGLs whereas the other strain did not. Disruption of PGLs synthesis resulted in loss of hyperlethality and increased proinflammatory cytokine production without affective bacterial growth. Purified PGL alone resulted in a decrease of proinflammatory cytokines. They found that PGL deficient mutants contained a disrupted *pks15/1* gene cluster. The *pks15/1* gene codes for a six-domain reducing iterative type I polyketide synthase already associated with PGLs.³⁹ These findings tie in with earlier work by Lee *et al.* who found that PLG-tb1 producers possessed a 13 Kb DNA fragment absent in non-producers.⁸⁹

NHN5, a non-PGL producing strain, did not have the same frame-shift mutation in the *pks15/1* gene indicating that multiple independent mutations can result in the loss of PGL production. It is important to note that more than twenty-five enzymes have been linked with PGL synthesis so the loss of PGL production is a complex issue.⁹⁰ Mutation in various glycosyl transferases and other related enzymes account for the variation in the glycan region.

As previously discussed the length of the PDIM core of PGLs can vary. The biological mechanism for this is linked to *pks15/1*. A *p*-hydroxyphenyl-polyketide PGL precursor is generated by the co-operation of Pks15/1 and another enzyme FadD22.⁹¹ FadD22 introduces the aromatic region and Pks15/1 elongating the lipid chain (Figure 1.11).⁹² Pks15/1 has a relaxed

control of the catalytic cycle iterations which would account for the observed variability in the PGL alkyl chain length.

Figure 1.11 Source of variable chain lengths in PDIM and related molecules

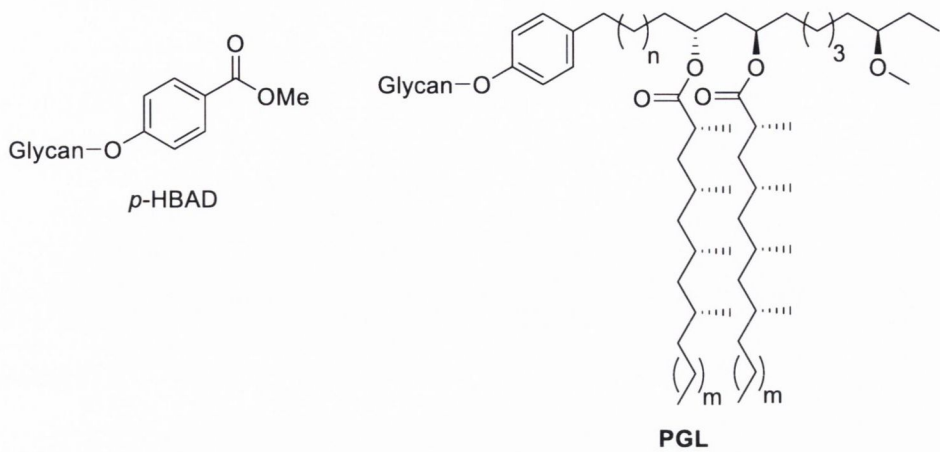


Recently, it has been shown that not all strains of the W/Beijing family produce PGLs and they have been associated with a variety of potential virulence factors.⁹³ It has been postulated that PGL-tb1 may work in concert with these other factor to induce the hypervirulent phenotype.⁹⁴ To fully understand the role of PGLs it may be necessary to not only investigate mixtures of PGLs but also mixtures of PGLs and other virulent factors.

1.1.7 *p*-HBAD structure and discovery

Other such related virulence factors include glycosylated derivatives of *p*-hydroxybenzoic acid methyl ester (*p*-HBADs). These were originally discovered when Constant *et al.*³⁹ examined eight reference strains and ten clinical isolates of *Mtb* and found that they all produced these *p*-HBADs (Figure 1.12). The glycan portion of these molecules was found to be related to the glycan of the type specific PGL. These findings suggest that strains deficient in PGL synthesis may be unable to elongate a postulated *p*-hydroxybenzoic acid PGL precursor.

Figure 1.12 Structure of *p*-HBADs and PGLs

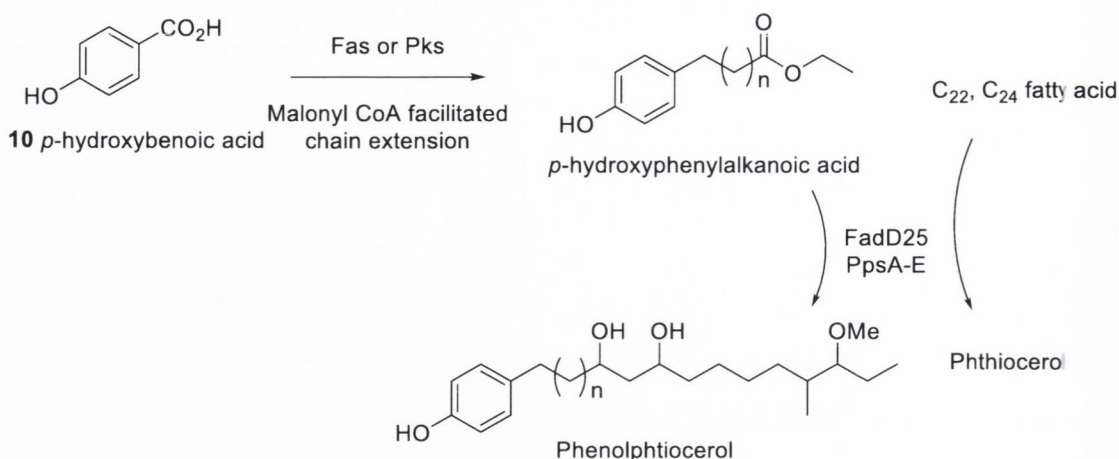


1.1.7.1 *p*-HBAD biosynthesis and relationship to PGLs

As previously discussed non-PGL producers have a frameshift mutation in *pks15/1* whereas PGL producers such as *M. leprae*, *M. bovis*, and PGL-producing *Mtb* strains lack this mutation. It is postulated that Pks15/1 elongates *p*-hydroxybenzoic acid to *p*-hydroxyphenyl alkanoates which are likely further elongated to phenolphthiocerol derivatives by PpsA-E synthase using malonyl-CoA (Figure 1.12).³⁹

When the non-PGL producing *Mtb* H37Rv was complemented with an intact version of the *pks15/1* gene it continued to synthesise *p*-hydroxybenzoic acid derivatives (*p*-HBADs) and while also synthesising PGLs. In known fatty acid synthase or polyketide synthase processes, the acid functionality is esterified before the substrate is transferred to the enzyme meaning that the methyl ester of *p*-HBADs could prevent them being substrates for Pks15/1. In light of these facts it is probable that *p*-HBADs are not intermediates in the synthesis of PGLs but rather are a distinct class of molecules that share a common precursor with PGLs.

Figure 1.13 Postulated biosynthetic route of phenolphthiocerol, $n = 9-11$

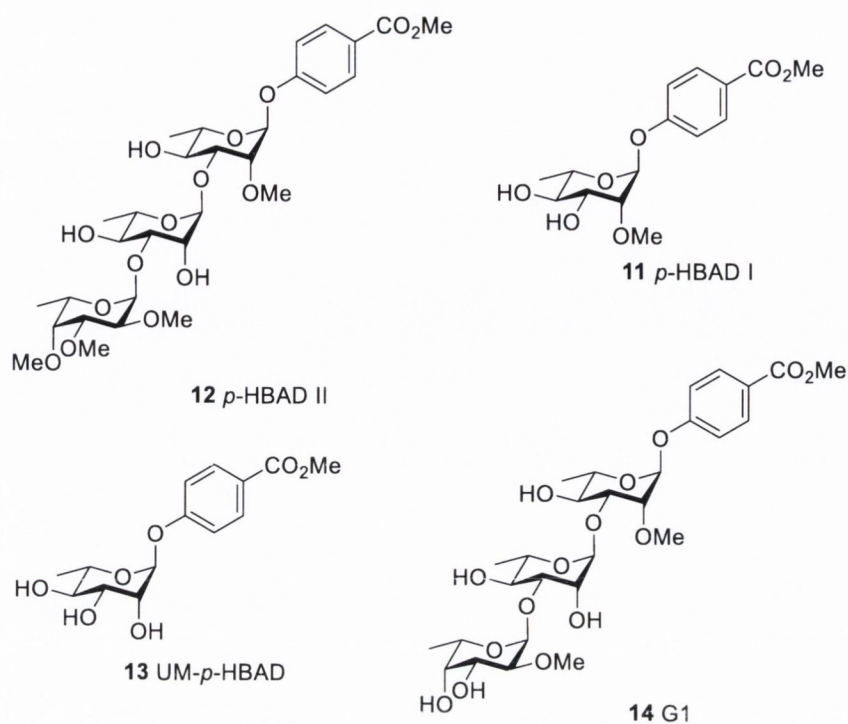


1.1.7.2 Research to date on the role of *p*-HBADs

As they are biosynthetically and structurally related to PGLs, with the region of similarity being the critical glycan, the biological effect of PGLs and *p*-HBADs is likely similar. Following from this assertion, Stadthagen *et al.*⁹⁵ investigated the contribution of *p*-HBADs to *Mtb* pathogenicity. They accomplished this by comparing the pathology and immunology of *p*-HBAD deficient mutants of a non-PGL producing strain. *p*-HBAD production was disrupted by knocking out genes that code for the enzymes involved in the biosynthesis of *p*-HBADs, and infecting mice with these knockout strains. Post-mortem investigations showed that the bacillary loads were comparable but the resultant pulmonary lesions, and inflammation were different.

Four strains in particular were looked at. The original wild type strain (Mt103) had the *pks15/1* mutation common to non-PGL-producers and was able to synthesis *p*-HBAD I **11**, *p*-HBAD II **12**, UM-*p*-HBAD I **13** and G1 **14** (Figure 1.13). In the other three strains were in some way deficient in *p*-HBAD synthesis: strain 7B7 was able to produce only the monosaccharide *p*-HBADs **11** and **13**; strain 29D6 generated just UM-*p*-HBAD **3**; strain 66C7 produced no *p*-HBADs at all.

Figure 1.14 Native *Mtb* *p*-HBADs



The wild type Mt103 resulted in well organised lesions with mature granuloma-like structures, no extension to the pleural membrane and no vascular lesions. The UM-*p*-HBAD I producer, 29D6 gave similar results to the wild type strain. Though all strains resulted in chronic lesions 7B7 (the monosaccharide producer) resulted in the most severe lesions. Several histopathological differences which indicated acute inflammation were observed. The non *p*-HBAD producing 66C7 had an intermediate phenotype with slightly more intense and diffuse inflammatory reaction than in wild type Mt103 infected mice but less acute than 7B7.

In order to understand the basis for the different *in vivo* virulence profiles of each strains the cytokine response by murine bone marrow derived macrophages infect with the different strains was measured. As before, 29D6 (the UM-*p*-HBAD I producer) had a similar effect to the wild type strain. Both other mutant strains (7B7 that produced monosaccharides and the non *p*-HBAD producing 66C7) resulted in much higher levels of the T_H1 promoting cytokines IL-6 and TNF- α than the wild type. The monosaccharide producing 7B7 also raised the levels of the T_H2 inhibiting IL-12 produced.

It is particularly interesting to note that when the mutant strains were complemented with intact version of their damaged genes the complemented 7B7 behaved peculiarly. Complemented 66C7 (the non *p*-HBAD producer) so that it could once again synthesise *p*-HBADs a cytokine

response similar to the wild type resulted. On the other hand, complemented 7B7 (monosaccharide *p*-HBAD producer) resulted in a more extreme version of the wild type response. This is theorised to be due to overexpression of the trisaccharide *p*-HBAD II to such an extent that the ratio of *p*-HBADs is disturbed; this postulate is in keeping with previous assertions that the makeup of the virulence factor cocktail is vital.

There is not a lot of data available on the biological properties of *p*-HBADs and what is available is limited to the work discussed above. All clinical isolates examined to date, including the non-PGL producers, synthesis these molecules via their own biosynthetic route and not merely as intermediates or by products; as such it seems likely that they serve an important biological function. This is substantiated by the above findings that link the production of *p*-HBADs to virulence.⁹⁵ The synthesis of pure *p*-HBADs would allow their investigation in isolation and could provide data on their roles in disease pathogenesis. Cocktails of compounds where the ratios of the individual *p*-HBADs can be precisely controlled (to give a more accurate representation of the extracellular medium) could also be prepared and assayed.

1.1.8 Relevant chemical syntheses

Some chemical syntheses have been carried out that are relevant to this work. Due to the complex nature of carbohydrates the field of carbohydrate chemistry, though a constant over many years, has burgeoned in recent years due to many advances, particularly in characterisation techniques.

1.1.8.1 Syntheses directed at the glycan of PGL-tb1

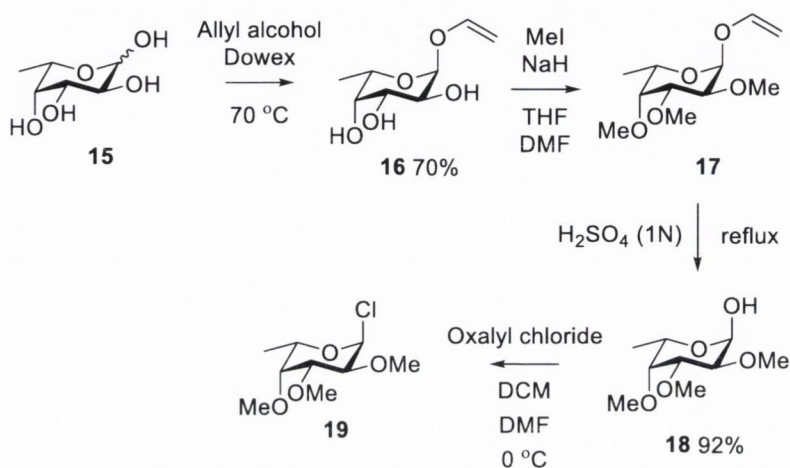
At the onset of this project three syntheses of either a segment or the entire glycan of PGL-tb1 had been reported. These strategies are discussed in the following section.

1.1.8.1.1 Synthesis of a PGL-tb1 fragment by Daffé *et al.*

In 1991 Daffé *et al.*⁸⁶ reported the first chemical synthesis of a portion of the PGL-tb1 glycan. They describe the synthesis of the disaccharide portion from the reducing end of the trisaccharide: a fucosylated rhamnose. The first part of the synthesis produces the methylated fucosyl donor and is relatively facile (Scheme 1.1). The anomeric position was initially protected by an allyl group to give compound **16**.⁹⁶ The orientation of the allyl group was not crucial as it would later be hydrolysed. Compound **16** was methylated to give the protected

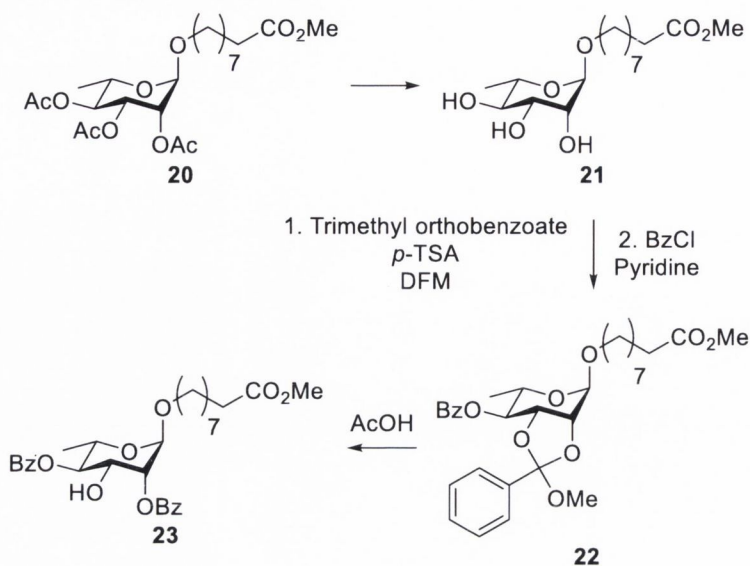
donor **17**. The temporary allyl group was removed by acid catalysed hydrolysis to give the hemiacetal **18** which was then converted to the chlorocarbohydrate donor **19**. Due to the reactive nature of the chlorofucose donor **19** it was used in the subsequent glycosylation reaction without being fully purified.

Scheme 1.1 Synthesis of fucosyl donor reported by Daffé *et al.*



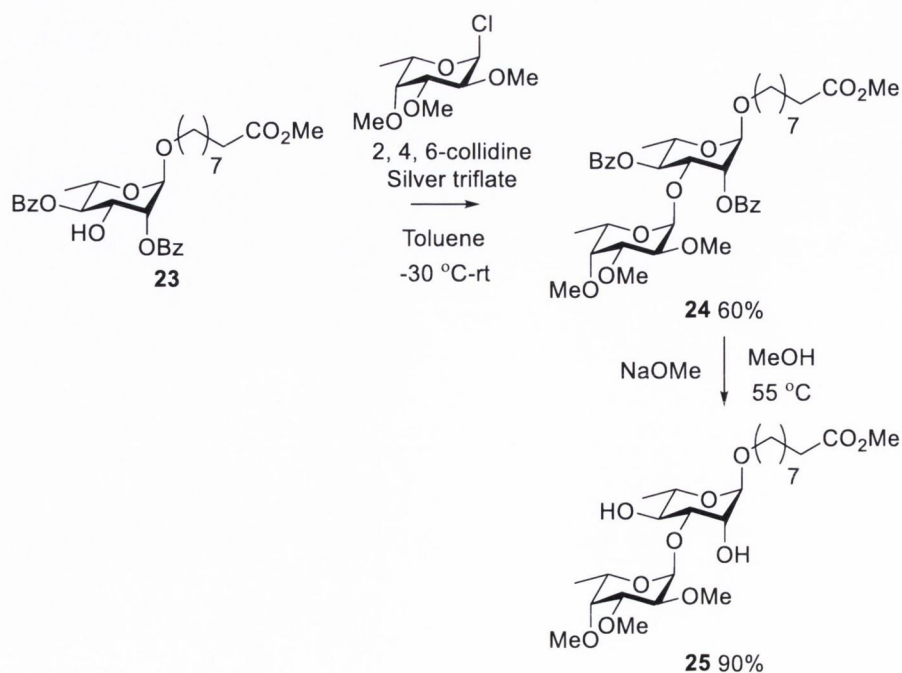
Since a specific substitution pattern is required on the rhamnosyl acceptor, its synthesis was more complex. The synthetic route reported was based on the use of acetyl groups as temporary protecting groups with the advantage of acting as participating groups in the functionalization of the anomeric position (Scheme 1.2). The acetyl groups on compound **20** were removed and replaced with benzoyl protecting groups; this substitution pattern was achieved through four synthetic steps. The first step was the removal of the acetyls followed by the benzylation of the C-4 positions and the introduction of the 2,3-ortho-benzoate as a temporary protecting group over both the C-2 and C-3 positions of compound **22**. When the orthoester was opened it furnished the 2, 4 substituted rhamnosyl acceptor **23**.

Scheme 1.2 Synthesis of the rhamnosyl acceptor by Daffé *et al.*



The glycosylation reaction between glycosyl donor **19** and acceptor **23** gave the desired disaccharide **24** (Scheme 1.3). This glycosylation gave the α disaccharide as the major product despite a lack of anchimeric groups. It is possible that bulk around the acceptor site may influence the result of the glycosylation. Following the base catalysed deprotection of disaccharide **24** to give disaccharide **25** the methyl ester was converted into the hydrazinocarbonyl functionality by reaction with hydrazine hydrate. The authors then conjugated this with bovine serum albumin (BSA) for ELIZA studies of patient sera.

Scheme 1.3 Assembly of the neoantigen by Daffé *et al.*

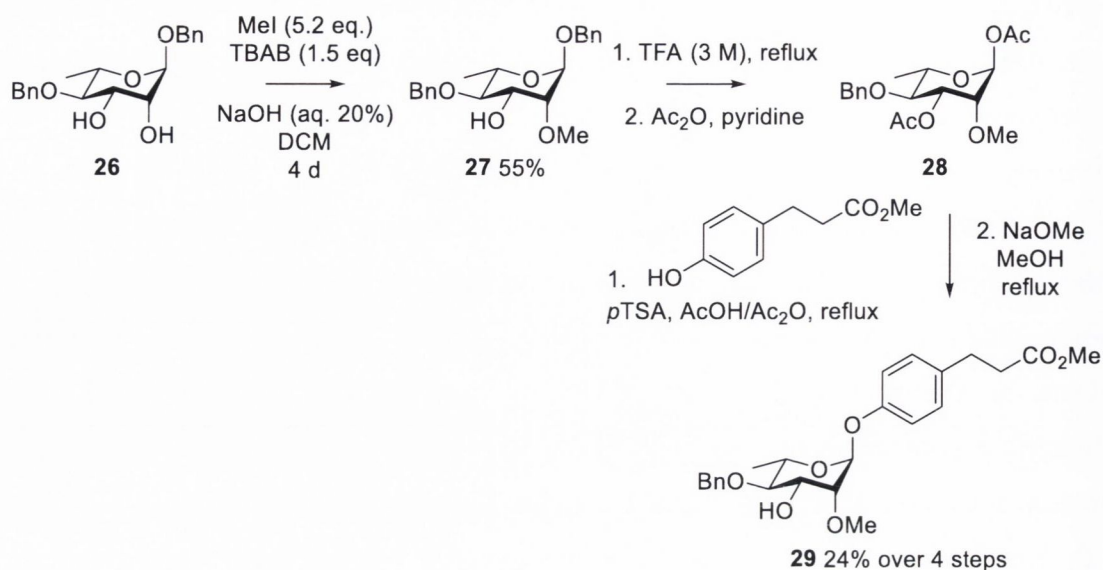


Daffé *et al.* describe the synthesis of the disaccharide from the non-reducing end of the PGL-tb1 trisaccharide. Since the first rhamnosyl is omitted the methylation pattern on rhamnose is not required and as a result, the synthesis is simpler than that of the full trisaccharide. One difficulty associated with this route is the use of the chloride donor without full purification, most likely due to the relative instability of this donor. This is likely to be a major contribution to the relatively low yield of disaccharide **24** obtained. Another difficulty is not directly related to the synthesis but rather the spectroscopic technology available at the time: difficulty characterising the stereochemistry of the glycosyl linkages. The initial glycosylation should generate the α anomer of compound **20** either exclusively or in a large excess due to the anchimeric assistance of the acetyls. The fucosyl donor, however, does not promote the formation of an α linkage, rather, due to the absence of participating groups, it fails to promote the β . The resulting orientation is not ensured, despite the authors' careful planning, and difficult to analyse given the methods available at the time.

1.1.8.1.2 Fujiwara's synthesis of the trisaccharide of PGL-tb1

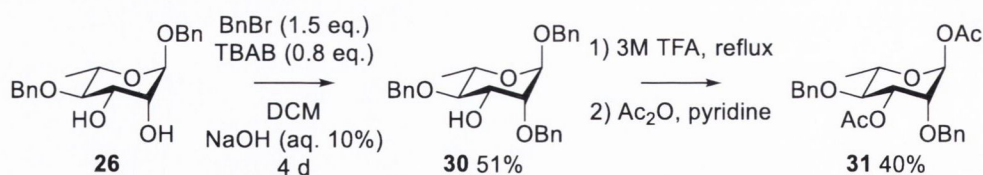
In 1991, Fujiwara⁹⁷ synthesised conjugate of the PGL-tb1 trisaccharide coupled to bovine serum albumin (BSA). This synthesis was carried out in an attempt to avoid the low solubility and poor reproducibility encountered when using PGL-tb1 itself for the serodiagnosis of TB. The synthesis reported involved the stepwise condensation of chlorocarbohydrates either generated *in situ* or previously prepared. Benzyl groups were employed as more stable temporary protecting groups and acetyl groups as more short term protecting groups. Regioselective functionalization of the C-2 over the C-3 position on rhamnose is achieved through phase transfer reaction conditions as seen in the preparation of compound **27** (Scheme 1.4).

Scheme 1.4 Synthesis of functionalised rhamnosyl conjugate by Fujiwara



Though successful, this regioselective phase transfer reaction was slow and not very high yielding. The methylation of the 2-hydroxyl required >5 eq. of the toxic MeI and after four days reaction gave the desired product in 55% yield. The donor **28** was generated over two steps: the anomeric position of compound **27** was first hydrolysed with TFA and then converted into the acetyl while concurrently acetylating the C-3 position. Donor **28** was used to glycosylate methyl *p*-hydroxyphenyl propionate; deacetylation of the intermediate gave the rhamnosyl acceptor **29**.

Scheme 1.5 Synthesis of the second rhamnosyl donor by Fujiwara

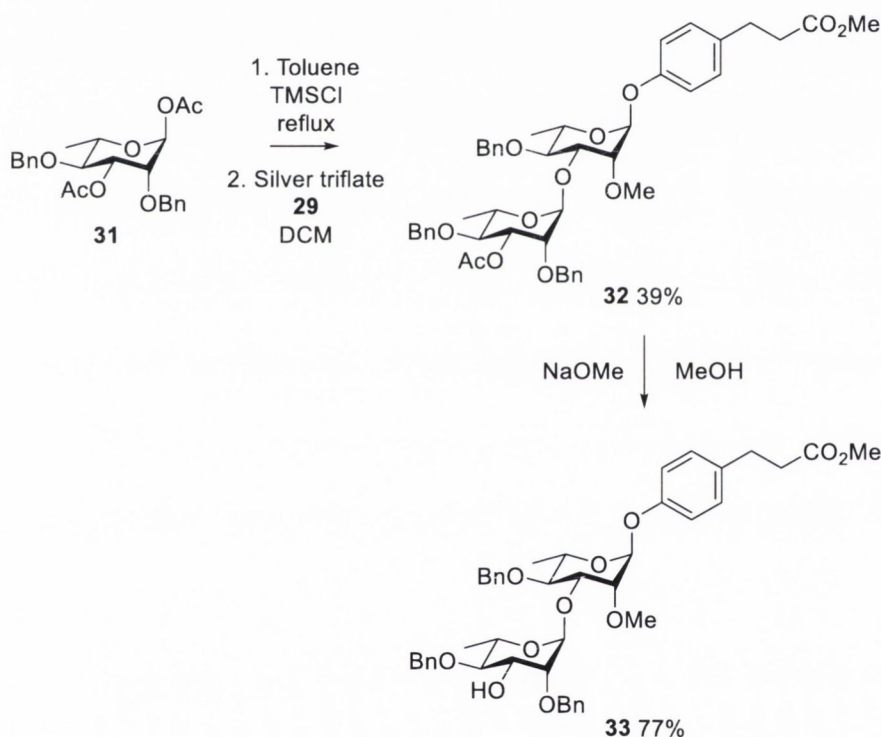


The regioselective benzylation of the C- 2 position of compound **26** did not necessitate the use of large quantities of BnBr, in fact a very slight excess is utilised and gave compound **30** in 51% yield after four days. Due to the free hydroxyl at the 3 position and its inactivity compound **30** had to be converted into a fully protected donor before it could be used in a glycosylation reaction. A similar strategy to that employed for the generation of compound **28** was used to give donor **31**: hydrolysis of the anomeric position and acetylation of both the anomeric

position and the C-3. The low yield of **31** may be attributable to the lack of selectivity for the α anomer and difficulties which may have been encountered during its isolation.

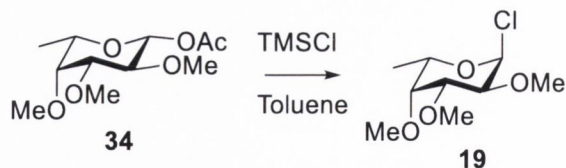
The glycosylation of acceptor **29** needed a more reactive donor than the acetylated donor **31**, thus **31** was converted to the reactive chlorocarbohydrate donor *in situ* prior to the addition of the acceptor **29** for the glycosylation reaction (Scheme 1.7). The yield of **32** was quite low at 38.8%, possibly owing to the hindered nature of the rhamnose C-3 position and to difficulties in isolating only one anomer. Steric hindrance is likely the reason for the relatively low yield achieved in deacetylating the 3 position (76.7%) to give the acceptor **33**.

Scheme 1.7 Rhamnosyl glycosylation reported by Fujiwara



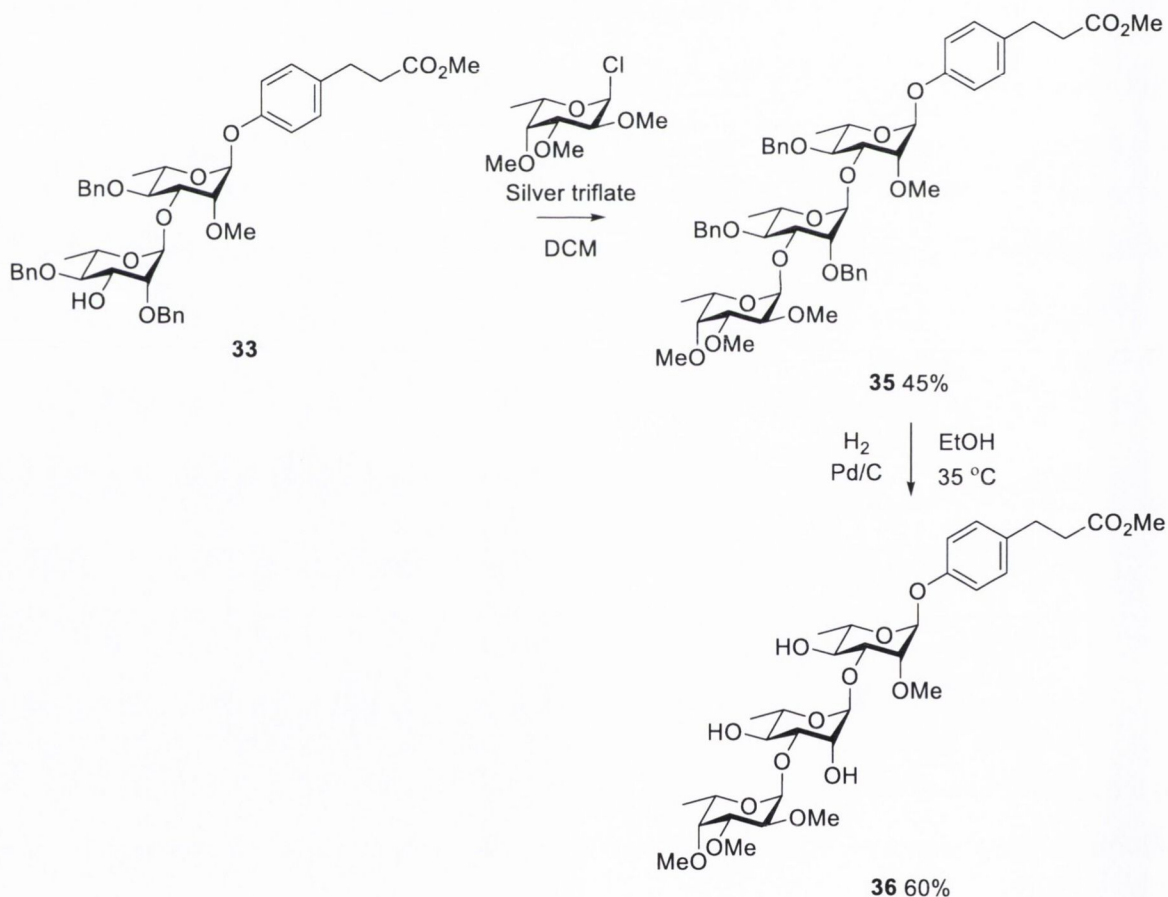
In the case of fucose, the glycosyl chloride was generated prior to the glycosylation, unlike the chlororhamnosyl donor. This may have been due to the “armed” nature of benzylated donors, such as the chlorocarbohydrate version of **30**, which makes them more reactive than a methylated donor such as **17**. The fucosyl donor **17** was prepared from donor **33** and isolated prior to the glycosylation reaction; though it was cleaned only crudely (Scheme 1.8).

Scheme 1.8 Synthesis of fucosyl donor by Fujiwara



The second glycan-glycan coupling to give trisaccharide **35** was low yielding (Scheme 1.9). This result was likely due to the flanking benzyl groups of disaccharide **33** making the already hindered C-3 position even more inaccessible. The global debenzoylation of trisaccharide **35** was facile and relatively high yielding, giving the target trisaccharide **36** in 60% yield.

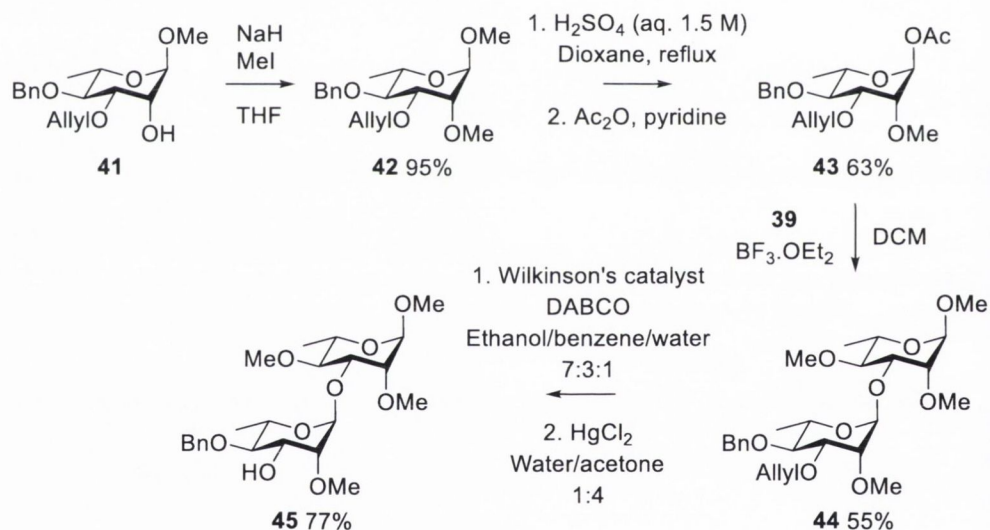
Scheme 1.9 Assembly of Fujiwara's trisaccharide



This synthesis demonstrates that the hindered nature of the C-3 position of rhamnose can greatly affect the synthesis of these molecules. It can allow for rather effective mono-functionalisation under phase transfer conditions but can also hinder glycosylation of the C-3 position. Phase transfer alkylation allows for regioselective functionalisation of the more

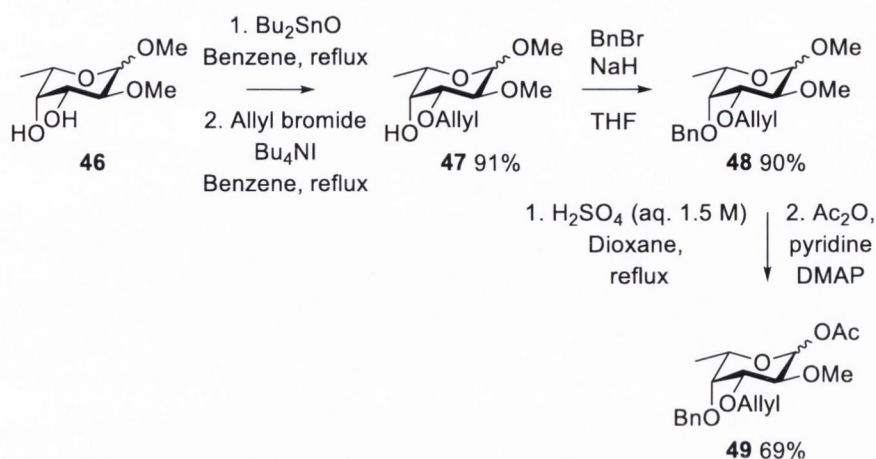
The early synthetic steps of the second rhamnoside which was to be the donor were based on work by Pozgay *et al.* to give compound **41**.¹⁰⁰ The second part of the synthesis of the rhamnosyl donor involved the methylation of the remaining free hydroxyl at the C-2 position to give compound **42**. This was followed by the hydrolysis and subsequent acetylation of the anomeric position to generate donor **43**. The Lewis acid boron trifluoride facilitated the activation of donor **43** to glycosylate the previously prepared acceptor **39** to give disaccharide **44** in 55% yield. This too was a relatively low yielding glycosylation as previously seen with attempts to glycosylate at the hindered rhamnosyl C-3. Another possible contributory factor is the lack of anchimeric groups to promote the desired α anomer. The two step removal of the allyl protecting group at the C-3 position of the reducing end rhamnosyl moiety gave the acceptor **45**.

Scheme 1.11 Disaccharide synthesis reported by Gurjar *et al.*



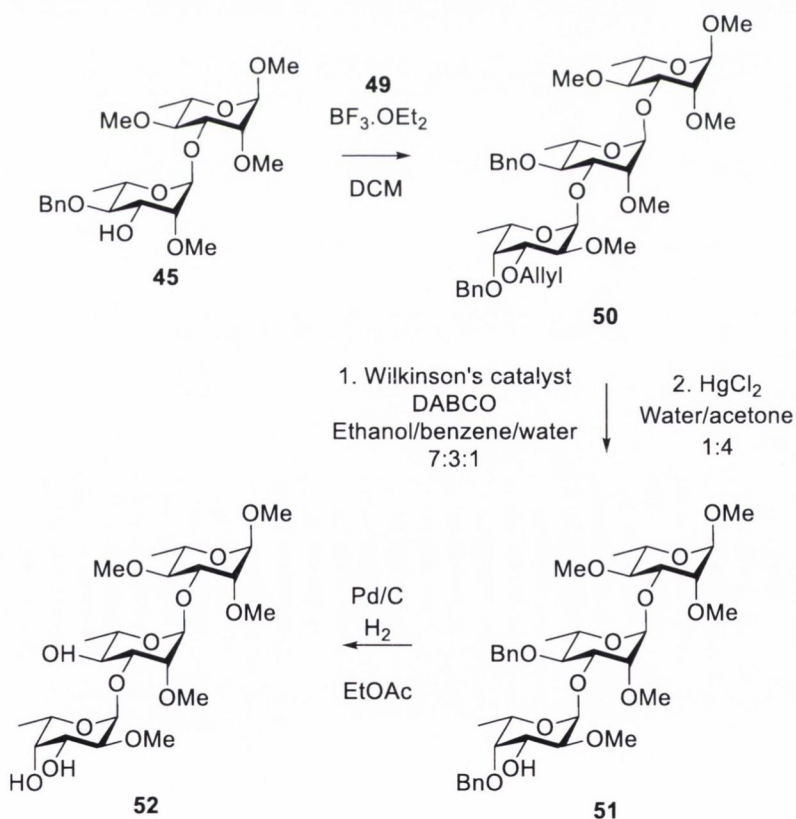
The fucosyl donor **49** was generated through the use of dibutyl tin oxide and allyl bromide (Scheme 1.12).¹⁰¹ This allowed for the regioselective protection of the fucosyl C-2 position in an analogous manner to the protection of the rhamnose C-2 to give compound **47**. In both cases the stannylenes form on adjacent axial and equatorial hydroxyls and the equatorial position is subsequently functionalised followed by hydrolysis of the stannylenes. The free hydroxyl at the C-4 position of compound **47** was benzylated to give compound **48**. The anomeric methoxy of compound **48** was converted to an acetyl in the same manner employed for rhamnosyl donors described above to give the donor **49**.

Scheme 1.12 Generation of the fucosyl donor by Gurjar *et al.*



The second glycosylation step, the condensation of acceptor **45** and donor **49**, was also facilitated by boron trifluoride (Scheme 1.13).

Scheme 1.13 Trisaccharide assembly reported by Gurjar *et al.*



The trisaccharide **50** was generated in 50% yield although NMR analysis showed it was contaminated with approximately 10% of disaccharide starting material **45**. The first

deprotection step, removal of the allyl group, gave a clean trisaccharide **51** allowing an accurate yield to be recorded for subsequent hydrogenation (90%) to give the final target **52**.

Gurjar *et al.* used allyl groups as a temporary protecting groups in the C-3 position for both rhamnose donors, removing it in a two-step process utilising Wilkinson's catalyst and DABCO followed by cleavage with mercuric chloride-mercuric oxide.

This synthesis incorporated several reactions that are potentially of great use; most notably the use of a stannylene ether for the regioselective functionalization of C-2 position over the C-3 position. This method is higher yielding and more selective than the phase transfer reactions described above; though it too is somewhat limited in the protecting groups that can be used. The use of the more stable acetyl glycosides as donors means that they could be fully purified allowing for both characterisation, accurate yields and more accurate calculations. A drawback to using acetyls at the anomeric is that they cannot then be used at other regions as temporary protecting groups and participating groups. It appears that Gurjar *et al.* had experienced the same difficulties that Fujiwara and Daffé *et al.* did in glycosylating the C-3 position of rhamnose. This challenge indicates that this glycosylation reaction in particular needs to be carefully considered when undertaking the synthesis of the PGL-tb1 trisaccharide.

1.1.8.2.2 Other PGL syntheses

Zegelaar-Jaarsveld *et al.* synthesised the carbohydrate portions of PGLs from *M. kansasii* serovars I,¹⁰² II, and IV.¹⁰³ Though these syntheses did not aim to produce the carbohydrates that are the subject of this thesis, they are rather elegant syntheses that employ some reactions in common with the work described herein. Due to its diagnostic utility and the interest generated by its biological properties significant work has been carried out in synthesising and characterising PGL-1 from *M. leprae*. A full discussion of these syntheses is outside the scope of this report.

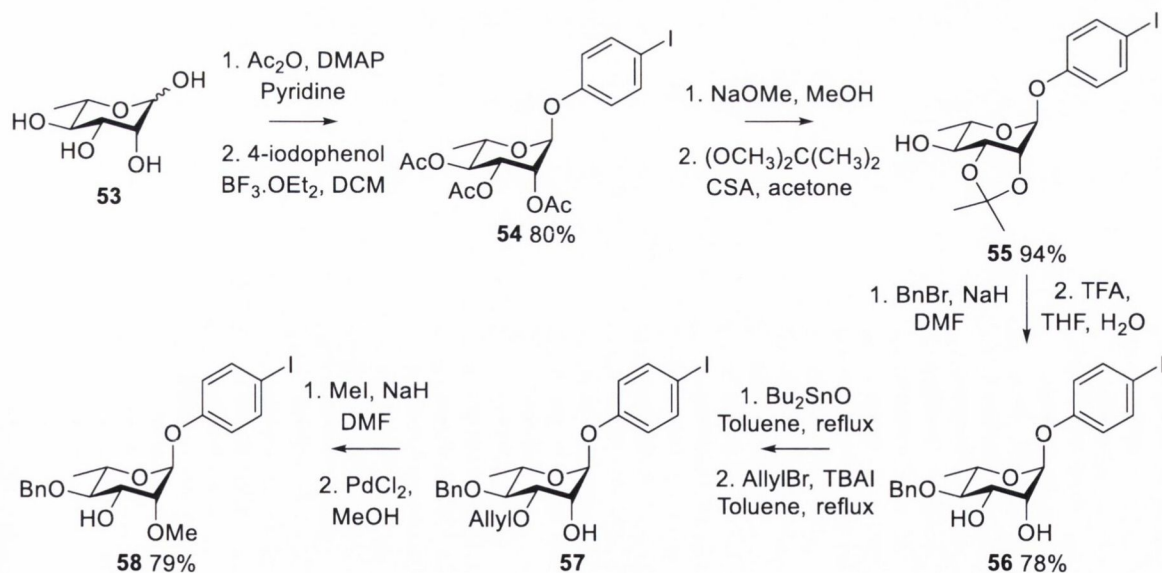
PGL-tb1 did not generate a comparable level of interest and when this project was conceptualised, besides Fujiwara's trisaccharide synthesis and Daffé *et al.*'s disaccharide, no other synthesis of PGL-tb1 or any portion of it had been attempted.

1.1.8.3 Synthesis of PGL-tb1 by Barroso *et al.*

Recently, a full synthesis of PGL-tb1 has been carried out by Barroso *et al.*¹⁰⁴ Their main point of interest was the lipid portion. The lipid was attached to the glycosylated phenolic moiety via a Sonogashira reaction between the alkyne functionalised lipid portion and the *para*-iodophenyl trisaccharide. The catalyst utilised was $\text{PdCl}_2(\text{PPh}_3)_2$ and the desired product was obtained in 51% yield with 35% of the starting material being recovered. Since their synthesis was lipid centred that section will not be discussed in detail. It is important to note that their synthesis of the glycan was not influential in the development of this project as it was reported after the design and implementation of the synthesis that is the subject of this thesis.

The synthesis of the carbohydrate appears similar to that reported by Gurjar *et al.*⁹⁸ as the protecting group strategy is the same and as such will not be discussed in detail (Scheme 1.14). Rhamnose **53** was acetylated and used to glycosylate 4-iodophenol to give compound **54**. A series of protecting group manipulations were performed starting with the deacetylation of compound **54** and eventually leading to the rhamnosyl acceptor **58**.

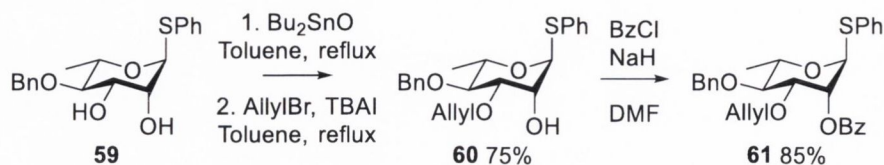
Scheme 1.14 Glycosylation of 4-iodophenol



The second rhamnosyl monosaccharide donor was functionalised through previously described functional group manipulations (Scheme 1.15). The donor chosen by Barroso *et al.* was a thioglycoside which has several advantages over the chloro donor used by Gurjar *et al.* These advantages include the stability of the thioglycoside under a variety of reaction conditions and its facile activation. An additional difference between Barroso *et al.* and Gurjar *et al.*'s

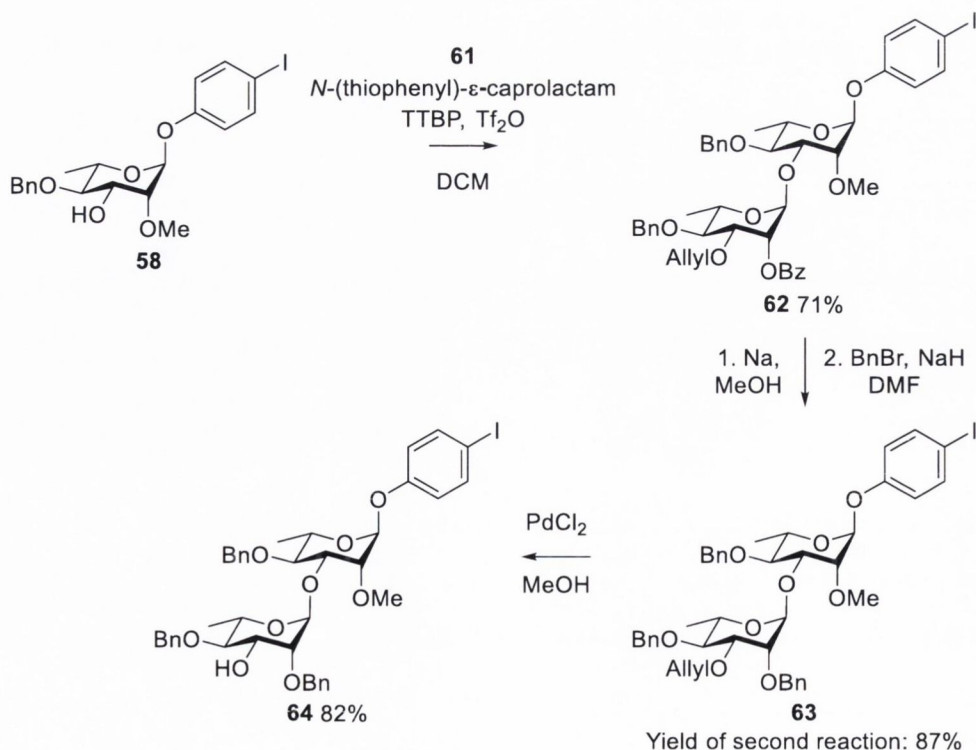
syntheses is in the use of benzoyl chloride to protect the C-2 to give donor **61** (Scheme 1.15). This may have been incorporated to allow for some neighbouring group participation in generating the glycosidic bond.

Scheme 1.15 Synthesis of the second rhamnosyl donor by Barroso *et al.*



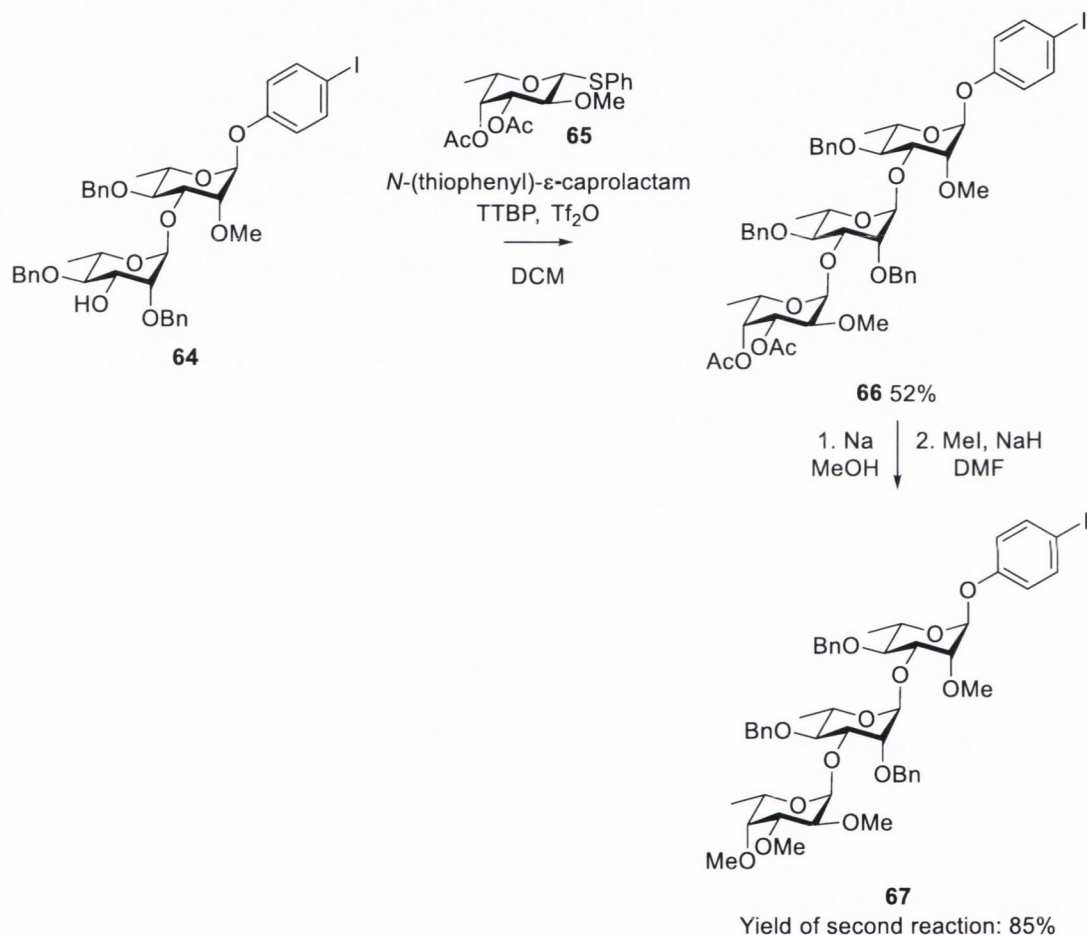
The glycosylation of acceptor **58** by donor **61** was facilitated by *N*-(thiophenyl)- ϵ -caprolactam and 2,4,6-tri-*tert*-butyl pyrimidine (TTBP) and gave the disaccharide **62** in 71% yield (Scheme 1.16). This is a significant improvement over previously described similar glycosylation and may be due to a more stable thioglycoside donor in conjunction with generally purer starting materials. Several functional group manipulations were performed on the disaccharide in order to convert the participating benzoyl group into to the benzyl group seen in disaccharide **64**. The benzyl groups are temporary protecting groups and this conversion means fewer deprotection reactions to be carried out on the trisaccharide.

Scheme 1.16 Disaccharide synthesis described by Barroso *et al.*



The second glycosylation reaction was carried out analogously to first but gave a lower yield of trisaccharide **66** than could be expected, likely due to the steric conditions around the hydroxyl of compound **64** (Scheme 1.17). Following the glycosylation the final facile protecting group manipulations were performed to convert the fucose acetyls to the required methoxies in trisaccharide **67**.

Scheme 1.17 Trisaccharide synthesis by Barroso *et al.*



As previously stated the glycan was not the region being investigated by Barroso *et al.* and as such they did not make significant changes to previously discussed syntheses aside from a few key points: the benzoyl group and the thioglycoside donor. The activation of the thiophenyl glycoside is less facile than the activation of simpler thioglycoside though it does offer enhanced stability. The advantage the incorporation of a benzoyl group at the C-2 position of compound **54** is that it can participate in the glycosylation reaction and promotes the formation of the desired α anomeric linkage. The disadvantage is that this necessitates the use of several protecting group manipulations on the disaccharide to convert the protecting group at the C-2

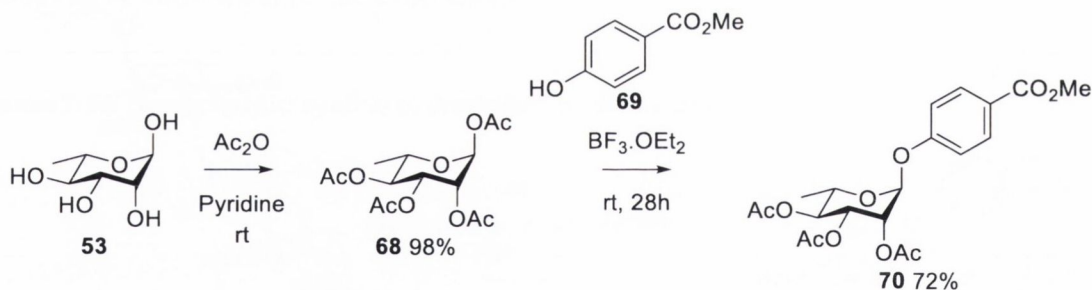
of compound **55** to one that matches the other temporary protecting groups used (benzyl). This was done so that a global debenzylation could be carried out after the trisaccharide had been generated. There is a trade-off: more protecting group manipulations performed on the disaccharide means fewer need to be performed on the trisaccharide. Despite these measures several steps still needed to be carried out on the trisaccharide to establish the desired methylation pattern on the fucose. It is generally preferable to minimise the number of reactions to be carried out on the rather precious trisaccharide generated.

1.1.8.4 *p*-HBAD syntheses

At the outset of this work, and indeed until quite recently, a chemical synthesis of either native or non-native *p*-HBADs had not been reported; however, in 2013 Barroso *et al.*¹⁰⁵ reported the first chemical preparation of a *p*-HBAD I along with the first synthesis of mycoside B.

Barroso *et al.* prepared a *p*-HBAD I precursor **70** by glycosylation methyl-4-hydroxybenzoate **69** with peracetylated rhamnose **68** (Scheme 1.18).

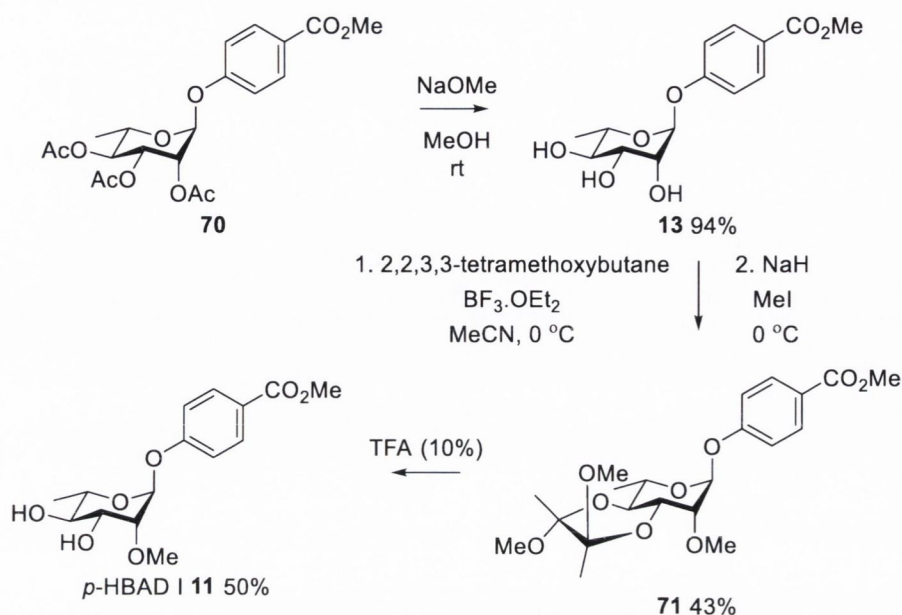
Scheme 1.18 Glycosylation of methyl-4-hydroxybenzoate.



The *p*-HBAD precursor **70** was deprotected under Zemplén conditions to give the deprotected *p*-HBAD **13** (Scheme 1.19). 2,2,3,3-tetramethoxybutane was utilised to protect the rhamnosyl 3,4 *trans* diol allowing the rhamnosyl 2-hydroxyl to be methylated to give *p*-HBAD **71**. The diol protecting groups was removed to give the target *p*-HBAD I **11**.

Though the initial glycosylation reaction was high yielding the subsequent protecting group manipulations were relatively low yielding. However, the route employed ensured a short and facile synthesis of *p*-HBAD I.

Scheme 1.19 Synthesis of *p*-HBAD I



1.1.8.5 A critical discussion of PGL glycan syntheses to date

The syntheses described to date have been linear with a single target whereas this thesis aims to describe a more modular approach that would allow for the preparation of a variety of donors that can be used to generate mono- and oligosaccharides with complete control of the methylation pattern in the product.

Undeniably there is potential for using more stable donors that could be stored instead of having to be generated when needed as seen in the syntheses discussed above. The protecting group strategy used by Fujiwara was well suited to their purposes but this thesis aims to describe the development of a more robust and versatile route that would allow for more variation and ideally reduce the quantities of highly toxic reagents required. It also aims to increase the overall yield for trisaccharide assembly.

1.2 Part two: The immobilisation of carbohydrates

1.2.1 Studying carbohydrate interactions

Carbohydrates are inherently complex as they can form a virtually limitless number of compounds from a few monomers connected in different way and with a plethora of substitution patterns. Due to this complexity they can act as information carriers and as such participate in many vital cellular processes such as cell adhesion, signalling and trafficking through protein-carbohydrate interactions. Furthermore, carbohydrates have the potential to be antigenic and effect the immune response, as discussed above. A better understanding of protein-carbohydrate, DNA-carbohydrate and carbohydrate-carbohydrate interactions is vital to better comprehend biological processes including the activation of the immune system.¹⁰⁶⁻

108

The information carried by the carbohydrates discussed in Section 1.1 is what triggers the biological responses discussed therein. This work aims to further understanding of how the structure of PGLs and *p*-HBADs affects their biological activity, i.e. what information the methylation and glycosylation pattern carries. To achieve this their interactions with cells of the host immune system and associated molecules such as antibodies need to be examined and the responses analysed.

The putative receptors for these molecules have not yet been identified though their existence can be inferred from the responses observed. Understanding how their structure affect their activity can also give us information on the possible structure and location of such receptors.

Traditional approaches for investigating interactions with carbohydrates comprise enzyme-linked lectin (carbohydrate binding protein) assays,¹⁰⁹ surface plasmon resonance (SPR),¹¹⁰ isothermal titration calorimetry (ITC)¹¹¹ and the hemagglutination inhibition assay.¹¹² Though effective, they are quite labour intensive and tend to require large samples of carbohydrate. Due to their inherent complexity, carbohydrates can be both challenging and labour intensive to synthesise chemically; this is compounded by the fact that they can be very difficult to purify from biological sources. Regardless of the method used they are costly and difficult to obtain. Consequently consuming large quantities to investigate potential interactions, though necessary, is inefficient and unappealing. More sensitive and facile methods to analyse carbohydrate interactions are required.

1.2.1.1 Immobilised carbohydrates as a tool for the investigation of carbohydrate interactions

Carbohydrate microarrays and functionalised particles, essentially immobilised carbohydrates, offer a potential solution. They can be used for high throughput screening of carbohydrates in a variety of systems allowing for more facile investigation of carbohydrate recognitions events. Such an approach would allow for the identification of cells that interact with the mycobacterial molecules discussed above in a fast, efficient manner..

Additionally, the immobilisations of carbohydrates can help reduce the quantities required for *in vitro* testing as microarrays can be washed and reused and carbohydrate functionalised particles can be recovered; this is, of course, assuming modification of the carbohydrates does not take place during the *in vitro* experiment. It was this latter advantage that particularly appealed as the carbohydrates discussed in this report have very precise and complex structures making their production challenging. Investigating the application of previously described immobilisations principles to the subject carbohydrates would allow for the development of synthetic strategies to immobilise them and thus reduce the need for their labour intensive production. In order to propose potential syntheses and immobilisations it is necessary to examine some of the work carried out to date in the area.

1.2.2 Microarray technology

Microarrays are not a new concept and both DNA and protein microarray technologies have advanced greatly in the past decade and found many applications in genomics, transcriptomics and proteomics in high throughput analysis.¹¹³⁻¹¹⁵

1.2.2.1 Carbohydrate microarrays

Carbohydrate microarray technology is more recent, having been first reported in 2002 by several groups.¹¹⁶⁻¹¹⁹ Carbohydrate microarrays are finding many applications including the detection of pathogens and antibodies, high throughput analysis of carbohydrate-protein interactions, the screening of binding inhibitors, the study of cell recognition events.¹²⁰ Another advantage of immobilised carbohydrates is that though protein-carbohydrate interactions in solution are often weak they are enhanced in solid surface attached carbohydrates due to the cluster effect.¹²¹ This effect results in more accurate results through better mimicking of native conditions. The concentration of immobilised carbohydrate, and therefore the distance between molecules, can influence binding as can the orientation of the carbohydrates. Irrespective of

the methodology used the carbohydrate must retain all recognition characteristics. To facilitate analysis and biological testing the carbohydrate microarray produced must be highly stable, water soluble and easily handled. Advances are constantly being made with a lot of research based on developing new linking chemistries. These are needed in order to minimise the requirement for synthetic manipulations as they are labour intensive and time consuming. This research can also serve to expand the number of carbohydrates that can be arrayed and consequently the applications of carbohydrate microarrays.

Many of the techniques developed are for the preparation of two dimensional arrays with some recent research into two dimensional arrays including the production of carbohydrate functionalised gels.¹²² Three dimensional arrays can be advantageous as a higher density of surface carbohydrates can be achieved and resulting in a more accurate model of biological conditions.¹²³ Over the years many methods have been developed and a full discussion would be beyond the scope of this text.^{123, 124}

There are two major strategies for the generations of carbohydrate functionalised surfaces based on the method for attachment: covalent and noncovalent. The attachment can be site specific or site nonspecific. Each method has advantages and disadvantages to its use.

1.2.2.2 Noncovalently generated carbohydrate microarrays

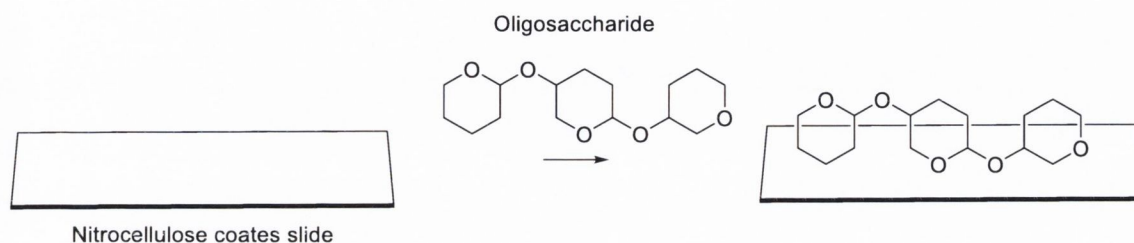
The noncovalent interactions that affix a molecule to a surface tend to be weak individually but strong cumulatively. This means that the efficiency of noncovalent attachment is influenced by molecule size whereby larger ones tend to have more interactions resulting in stronger binding.¹¹⁶ The interactions in question tend to be site-nonspecific and an associated drawback with noncovalent immobilisation is that the orientation of the glycan is thus not controlled. Another potential disadvantage is the potential for lateral migration of noncovalently attached carbohydrates. There are two subsets of noncovalent techniques using conjugated and unconjugated carbohydrates.

1.2.2.2.1 Noncovalent attachment of unconjugated carbohydrates

A major advantage to the use of unconjugated sugars is that it cuts down on the need for synthetic manipulations; it requires relatively large carbohydrate molecules. A facile method for the nonspecific noncovalent immobilisation of unconjugated carbohydrates involves spotting the carbohydrates onto nitrocellulose coated glass slides (Scheme 1.20). Wang *et al.*

were able to immobilise the 3.3 kDa inulin and dextran preparations ranging from 20 to 20000 kDa.¹¹⁶

Scheme 1.20 Noncovalent attachment of an unconjugated oligosaccharide to a nitrocellulose coated glass slide

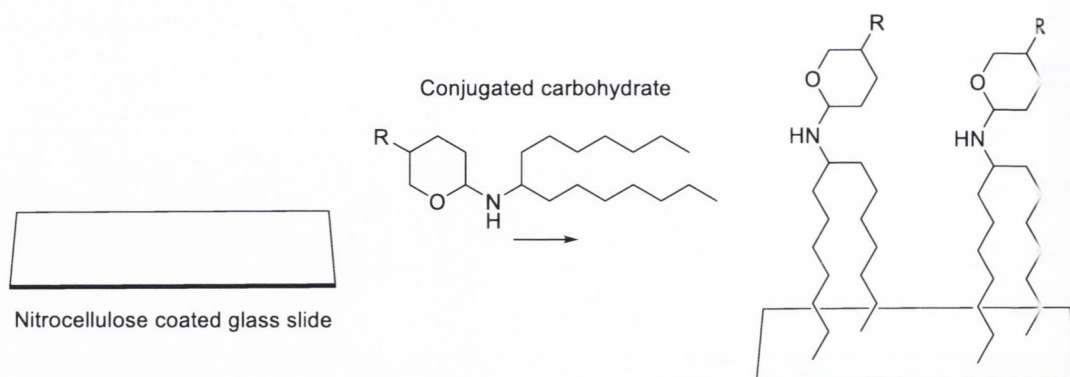


1.2.2.2.2 Noncovalent attachment of conjugated carbohydrates

The use of noncovalently attached conjugates (e.g. a lipid) is common when the carbohydrate portions are small. The conjugated aglycan can be large enough to form cumulative strong non-covalent interactions with the surface. This can be somewhat disadvantageous as there is also a risk that regions of the aglycan conjugate are reactive or antigenic. It is vital that the compounds involved bind strongly enough to withstand the washing, sometimes with detergent, used in assays.^{123, 124}

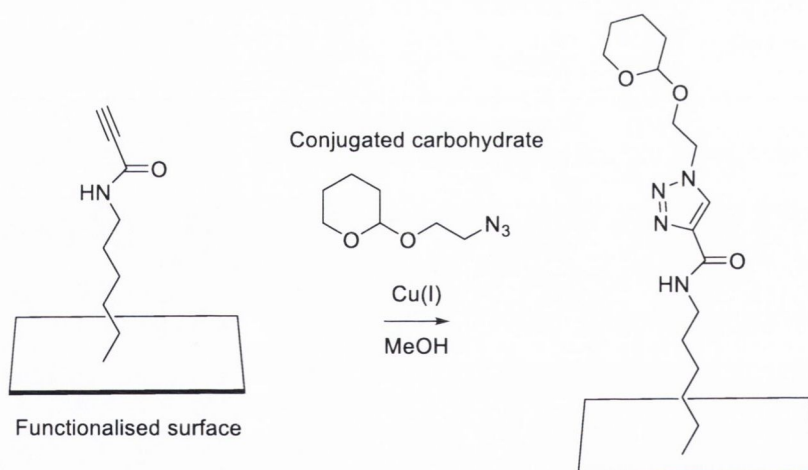
Conjugated carbohydrates are not used solely to enhance noncovalent binding; they can also serve to aid visualisation for example fluorescently tagged carbohydrates can be spotted onto Teflon/epoxy treated glass slides.¹²⁵ Both unconjugated carbohydrates (Scheme 1.20) and conjugated carbohydrates can be immobilised on nitrocellulose coated glass slides (Scheme 1.21).¹¹⁷ The conjugated carbohydrates seen below (Scheme 1.21) resemble PGLs and this method may be applicable to synthetically generated PGLs.

Scheme 1.21 Noncovalent attachment of conjugated carbohydrates to a nitrocellulose coated glass slide. R is a carbohydrate or lipid.



The conjugation reaction to attach the carbohydrate can take place before or after binding to the surface has been achieved. For example, a [1,2,3]-triazole ring can be simply generated via the 1,3 dipolar cycloadditions between an azide functionalised carbohydrate and an alkyne noncovalently immobilised on a surface (Scheme 1.22).¹¹⁸

Scheme 1.22 Noncovalent immobilised aglycan conjugated with a carbohydrate



These conjugated carbohydrate generated arrays offer greater variety and flexibility with better defined orientation. However, the issue of lateral migration is not addressed and it is still necessary to ensure that the association can withstand the required washing steps.

1.2.2.2.3 Assessment of the suitability of noncovalent attachment

The carbohydrates that are the subject of this thesis are not likely to be large enough to effectively associate with a surface. Additionally this would likely prevent effective interaction with putative receptors.

It may be possible to use noncovalent interactions to bind PGLs to a surface using the hydrophobic interactions of the lipid chains. The potential for lateral migration and the strength of the binding would still need to be addressed so this strategy is not appealing at present though it may become of interest at a later date. As stability is important covalent methods are more appealing.

1.2.2.3 Covalently generated carbohydrate microarrays

Covalent attachment is more synthetically challenging and labour intensive as it requires modified carbohydrates and/or derivatised surfaces. The major challenges are the selection and incorporation of an appropriate linker and ligation reaction. However, covalently immobilised carbohydrates offer enhanced stability, no risk of lateral migration and can potentially be applied to a wider range of carbohydrates.

The length of the linker used can influence binding and as a general guideline linkers of three or more atoms ensure better recognition and binding.¹¹⁹ The nature of the conjugated linker can affect the interaction of the carbohydrate with a lectin since, in general, the use of hydrophilic linkers such as those based on polyethylene glycol (PEG) results in improved binding compared to the incorporation of hydrophobic linkers. The linker used must not be antigenic in itself and it must be chosen to minimise nonspecific protein interactions.

There are several options for establishing the covalent linkage: the carbohydrate and the surface can both be functionalised or just one can be functionalised. As such a very large variety of linkers and linkages are available.

1.2.2.3.1 Covalent attachment of conjugated carbohydrates to functionalised surfaces

Many methods have been developed for the immobilisation of conjugated carbohydrates onto functionalised surfaces. This is a relatively straightforward strategy as well known and established coupling reactions can be used ensuring high yield though it is synthetically intensive.

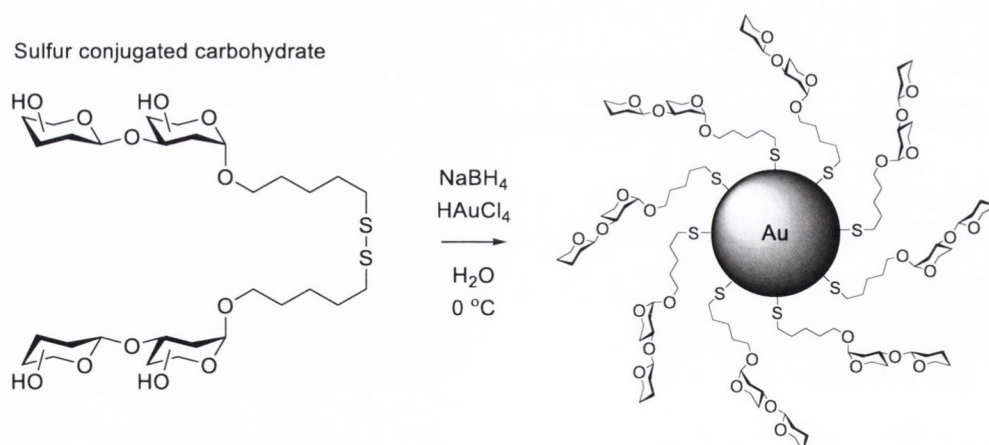
the already large synthetic burden. A strategy that uses unfunctionalised surfaces are more appealing. Additionally, since an aromatic moiety must be installed as part of the carbohydrate synthesis this could be used to tether the linker potentially further reducing the synthetic steps involved.

1.2.2.3.3 Covalent attachment of conjugated carbohydrates to unfunctionalised surfaces

Functionalising the carbohydrate prior to immobilisation meant that it can be unambiguously characterised prior to attachment. For example, in the PGL and *p*-HBAD glycans examined above, the aromatic group is attached at the anomeric position through an α -linkage and this can be put in place prior to immobilisation.

The affinity of sulphur for gold offers a solution to the challenge of generating covalent bonds to unfunctionalised surfaces. Carbohydrates bearing sulphur moieties will form what is termed a self-assembled monolayer (SAM) on a gold surface. The preparation of carbohydrate encapsulated gold nanoparticles (GNP) is well documented, a representative synthesis is depicted in Scheme 1.25.¹²⁹ On a surface a 2D-SAM will form but when nanoparticles are used the result is a 3D-SAM making this methodology quite versatile. Linker length can be varied through the incorporation of well-known groups such as polyethylene glycol (PEG) chains as spacers.¹³⁰

Scheme 1.25 Monolayer self-assembly on a gold nanoparticle



The use of sulphur-gold bonds is not limited to carbohydrates with thioalkyl linkers. Glycopeptides whose peptide regions contain sulphur bearing amino acids (cysteine) or have

been functionalised with sulphur containing linkers can also be used. This alternative can be advantageous as the peptide region can potentially enhance both solubility and lectin binding.

Gold surfaces, colloidal particles and microarrays have all been functionalised with carbohydrates¹³¹ and it has been shown that PEG thiol linkers can help to optimise the presentations of carbohydrates.^{132, 133} Short chain alkanethiols can encourage the formation of disordered SAMs so the use of longer and/or more rigid linkers may improve the structure of the SAM.^{134, 135} Integrity of monolayers can be determined by cyclic voltammetry (CV): if a similar result to bare gold is obtained a poorly ordered monolayer with many 'holes' is inferred. More modified surfaces are more insulated (less penetrations of ferricyanide in solution giving a decreased current response) which is consistent with a more ordered surface. Recognition by ligands may even be prevented by sub optimal ligand density (as a linear response of carbohydrate presenting surfaces to lectins is typically only seen at ligand coverage >5%).¹³⁶ It is important to note that enhanced order is linked to enhanced stability and hence to the generation of a more versatile and robust multivalent platform.

1.2.2.3.3.1 Advantages to carbohydrate functionalised gold nanoparticles (GNP)

Advantages of gold based glyconanoparticles include their biocompatibility and water solubility. GNPs can be very versatile as the density and presentation of the carbohydrates can be controlled as well as the size and shape of the particle itself.¹²³

GNPs are characterised by a discrete surface plasmon absorption band in the visible electronic spectrum.¹³⁷ The band is dependent on several parameters including the size of the nanoparticles which can be determined by light scattering and transmission electron microscopy.¹³⁸ The advantage itself is that, unlike most techniques, surface plasmon resonance (SPR) can be used for real time label free analysis. SPR can detect modification of carbohydrate on the surface. Recently a SPR imaging technology has been developed for the rapid analysis of bimolecular interactions.¹²³

1.2.4 Carbohydrate microarrays as a means for the investigation of carbohydrate biological properties

The carbohydrates discussed in this thesis appear to act on the immune system and consequently *in vitro* studies will generate insight but may not be sufficient for a complete

understanding of the activity. The microarrays discussed above have applications in *in vitro* screening of carbohydrate, however, GNPs can also be used for *in vivo* studies.

As discussed above carbohydrate microarrays can be used to reduce the need for synthetically generated carbohydrates. As the glycan targets of this thesis are highly complex and synthetically challenging to produce a means of reducing the quantity needed for biological testing is greatly appealing. When generating a microarray of PGL/*p*-HBAD carbohydrates it is likely that the aromatic moiety must be conserved as it is a constant in these biomolecules and is likely to be of biological importance. As such the methods employed for carbohydrate attachment would need to be based on the immobilisation of functionalised carbohydrates.

1.3 Aims

PGLs have diverse biological roles in mycobacteria and are often linked to virulence. In *Mtb* the exact function of PGL-tb-1 is not known and, though it is not expressed by the majority of clinical isolates, it appears to be a virulence factor. On the other hand, *p*-HBADs are expressed by all clinical isolates of *Mtb* examined to date. Though they are linked to virulence, the activity of *p*-HBADs is not fully understood. The chemical synthesis of these molecules would allow for the investigation of their activities and their interaction with the host immune system. A synthesis of native carbohydrates with an intricate and specific methylation pattern is expected to be complex making the production of significant quantities challenging. The ability to immobilise these molecules on surfaces would allow for the investigation of their properties using small quantities of recoverable carbohydrates.

- Develop a protecting group strategy to control the methylation pattern on rhamnose
- Prepare a library of monosaccharide building blocks for the synthesis of native and non-native molecules
- Using the above monosaccharide building blocks generate a library of *p*-HBADs
- Design and execute a synthetic route for the preparation of PGL or PGL-like molecules
- Investigate the biological function of *p*-HBADs
- Explore strategies for the immobilisation of carbohydrates functionalised with aromatic groups at the anomeric position, with a view to using immobilised carbohydrates to investigate biological activity

2.0 Chapter 2: The synthesis of tuberculosis associated *para* hydroxybenzoic acid derivatives and phenolic glycolipids

2.1 Rationale for the PGL/*p*-HBAD project

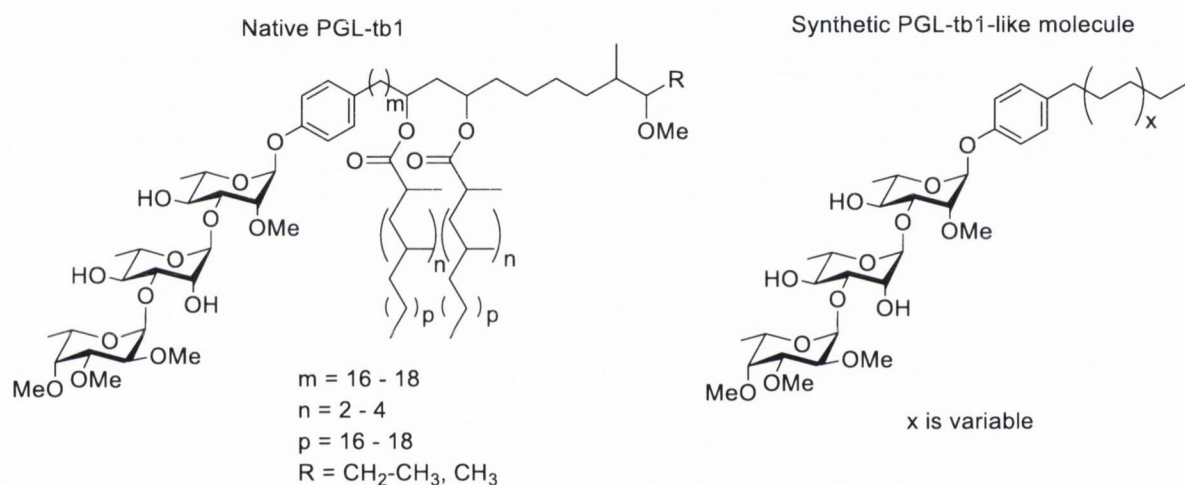
The majority of research in PGLs to date has focused on their diagnostic applications with little work published on their roles in disease pathogenesis. There has been relatively little research on *p*-HBADs with the exception of that discussed in the introduction. As previously discussed, some work has been carried out that would indicate that both classes of molecules are virulence factors, associating their loss with an attenuation of the mycobacterial strain. Our understanding of their function is still incomplete at best and it is this shortcoming that this work intends to address.

The evidence accumulated to date appears to support the theory that the carbohydrate region of both PGLs and *p*-HBADs is responsible for the seroreactive properties and the immunomodulatory effects observed.⁵²⁻⁵⁴ The synthesis of both native and non-native analogues would allow for investigation of their immuno-modulatory and immuno-reactive properties. Since the glycan regions of *p*-HBADs and PGLs are identical, their syntheses would only vary in the attachment of the aglycan portion. Certain carbohydrate patterns appear conserved in most PGLs possibly implicating them in the function of these molecules. This observation raises several questions about the relationship between the PGL structure and their function; for example the possible importance of the carbohydrate's length and methylation pattern as well as the role of hydrogen-bonding free hydroxyls.

Though some linear syntheses of PGL-tb1 and PLG-tb1-like glycans had previously been carried out at the outset of this work, the synthesis of a PGL or a *p*-HBAD had not been attempted. It was anticipated that the preparation of these compounds and libraries of their analogues would require the establishment of versatile syntheses, ideally from a common pool of simple precursors that could be generated in high yields and quantities. Such libraries would facilitate the investigation of the biological properties of both native and non-native compounds. This could help foster an understanding of their role in disease pathogenesis which would be of both prognostic and diagnostic value. The synthesis and investigation of non-native molecules could improve understanding of the structure-activity relationship (SAR) in *p*-HBADs and PGLs. This improved understanding could allow for the design and synthesis of molecules with desired activities.

Since the carbohydrate moiety appears to be integral to the immune response of these compounds it is the focus of this thesis. In the case of *p*-HBADs a total synthesis of the exact molecule found in nature could be carried out since the glycan moiety is functionalised with a relatively simple conjugated methyl benzoate at the reducing terminus. On the other hand, PGLs are significantly more complex and thus pose a much greater synthetic challenge. The lipid portion was simplified to an alkyl chain to shorten the synthesis and keep the glycan at the fore (Figure 2.1). This is justified by the work of various groups who demonstrated that variations in the lipid moiety are observed *in vivo* and chemical alterations of the lipid do not appear to effect *in vitro* activity.⁵²⁻⁵⁴

Figure 2.1 Simplification of PGL aglycan moiety



2.1.1 Challenges to be considered before undertaking synthesis.

As with the vast majority of biomolecules, stereochemistry is crucial and must be installed and unambiguously defined during the chemical synthesis. In carbohydrate chemistry most of the stereocentres are predefined in the monosaccharide starting material, however, control of the anomeric linkage is crucial and must be carefully considered. Many factors can influence the stereochemical outcome of a glycosylation and efficient stereoselective anomeric bond formation can be challenging. Major factors include the structure of the donor and acceptor, the glycosylation promoter and the solvent. Other factors include temperature, reaction concentration, and the order in which the reagents are added.

2.1.2 Neighbouring group participation

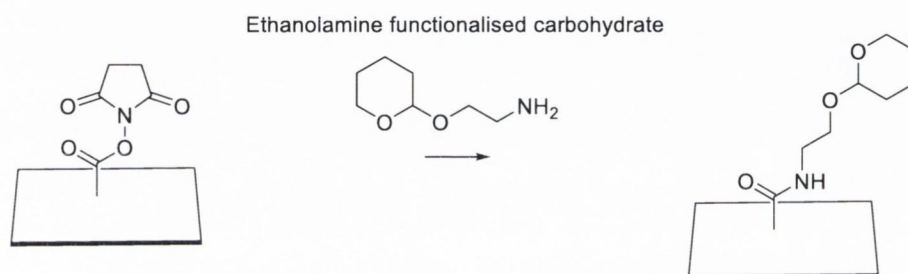
A common strategy in carbohydrate chemistry is the use of neighbouring group participation to influence the orientation of the anomeric linkage during a glycosylation. With the departure of the protecting group at the anomeric position the carbocation that results is converted into a reactive oxocarbenium ion through electron donation by the ring oxygen. The elimination of the leaving group and the formation of the oxocarbenium ion occur in concerted manner. The presence of a carbonyl containing participating group at the C-2 position allows the generation of an acyloxonium ion which can then undergo attack by the acceptor to form the glycosidic linkage (Scheme 2.1). The acyloxonium has one hindered face unlike the oxocarbenium ion which can undergo nucleophilic attack from either face.

If a glycosylation reaction involves a rhamnosyl donor with a participating group at the 2-hydroxyl position the α -linked product is expected to predominate. When the rhamnosyl donor **68** is converted into an oxocarbenium ion **72** then the neighbouring acetyl can participate in the glycosylation mechanism to furnish the acyloxonium ion intermediate **73** (Scheme 2.1). The reactive intermediate **73** has one face of the ring essentially blocked by the participating neighbouring group. This helps ensure 1, 2-*trans* selectivity and results in the observed α -selectivity. In the carbohydrate portion of PGL-tb-1, the anomeric linkages are α and therefore using rhamnosyl donors with anchimeric groups would be beneficial.

The generation of an α linkage is less facile when using a fucosyl donor since neighbouring group participation will result in increased β anomeric linkages. This can be rationalised by examining the structure of the acyloxonium ion **76** (Scheme 2.1). Compound **76** is formed from an appropriately functionalised fucosyl donor **74** via oxocarbenium **75**. The acyloxonium ion **76** has a blocked lower face and the 1, 2-*trans* selectivity results in the predominance of the β -linked product.

An example of such a method is the use of quinone functionalised surfaces. Quinones can undergo rapid Diels Alder reactions with carbohydrates bearing cyclopentadiene moieties, a well-known reaction. Both the carbohydrate conjugates and the surface modification can have several PEG linkers incorporated thus tuning the linker length.^{124, 126} Another such methodology is based on *N*-hydroxysuccinimide (NHS) ester coated slides which are commercially available. These can be reacted with amine derivatised carbohydrates such as ethanolamine functionalised carbohydrates (Scheme 1.23).¹²⁷ The generation of such amine derived carbohydrates can be achieved using a masked amine functionality and glycosylation

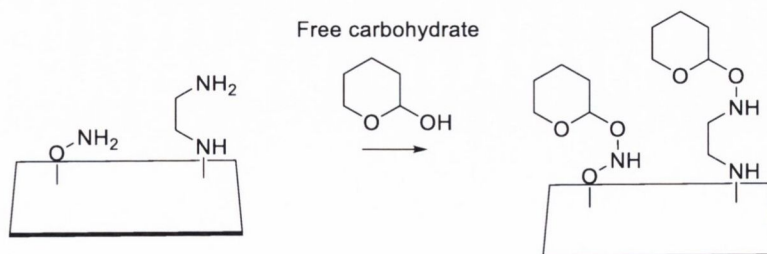
Scheme 1.23 Immobilisation of ethanolamine functionalised carbohydrates using *N*-hydroxysuccinimide ester coated slides



1.2.2.3.2 Covalent attachment of unconjugated carbohydrates to functionalised surfaces

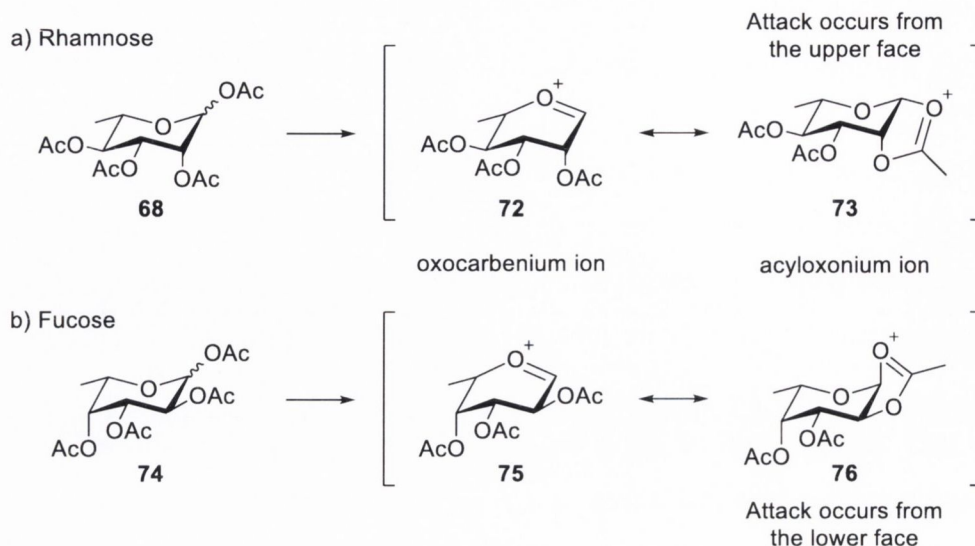
An alternative strategy for covalent immobilisation dispenses with the need for functionalised carbohydrates and instead focuses on surface functionalisation. For example a surface can either already have free amine groups or can be functionalised with nitrogen based groups such as amino-oxy or hydrazine which can react with free carbohydrates (Scheme 1.24).¹²⁸ This methodology also gives site specific covalent attachment.

Scheme 1.24 Immobilisation of free carbohydrates with amine functionalised surfaces



However due to the nature of the carbohydrates being studied, carbohydrate functionalisation is necessary to install the aromatic moiety. Strategies that involve functionalise surfaces add to

Scheme 2.1 Neighbouring group participation in (a) rhamnose and (b) fucose



2.1.2 Challenges highlighted by previously reported syntheses

Some linear syntheses of the carbohydrate portion of various PGLs have previously been carried out and serve to illustrate some of the potential challenges that may be encountered in developing a more versatile and robust synthesis.

When examining the syntheses of Daffé *et al.*, Fujiwara *et al.* and Gurjar *et al.* it is clear that the glycan-glycan bond forming glycosylations are lower yielding than would be expected.^{86, 97, 98} When using a rhamnosyl donor and acceptor, yields of 39%, 55% and 60% were obtained; when a rhamnosyl acceptor and fucosyl donor were used yields of 45% and a contaminated 50% were observed. These are quite low yields for such glycosylation reactions and can perhaps be attributed to the hindered nature of the rhamnosyl 3-hydroxyl group and the protecting groups employed at the flanking 2- and 4-hydroxyl groups. Since the trisaccharide target contains $\alpha 1 \rightarrow 3$ linkages, the steric challenge of performing a glycosylation at the 3-hydroxyl could adversely affect the overall yield for the trisaccharide assembly.

2.1.3 Establishment of a methylation pattern

Another important consideration is the establishment of the highly specific methylation pattern observed in the natural products. The targets discussed in this report are not only the native compounds but also non-native analogues bearing different methylation patterns. The synthesis developed here must be reproducible and also robust and versatile. Ideally, a few simple precursors that are easily produced on a large scale can undergo minimal manipulations to

generate a wide variety of native and non-native structures. The preparation of specific methylation patterns and the appropriate glycosidic linkages necessitates carefully considered regioselective protection.

If the anomeric position is discounted, in both rhamnose and fucose there are three hydroxyls whose functionalization must be precisely controlled. If two of these can be simultaneously protected, only one position would remain free to react therefore allowing control over the regioselectivity of the methylation pattern.

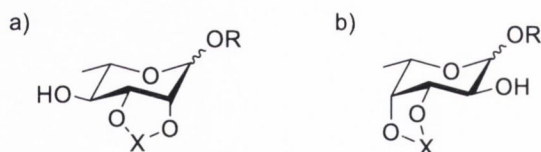
2.1.3.1 Diol protecting groups

Several classes of molecules are capable of the simultaneous protection of a diol including cyclic acetals and some silyl protecting groups.¹³⁹ The protecting group chosen depends on the stereochemical relationship between the diols to be protected. Benzyldienes tend to form six-membered dioxane type acetals. Isopropylidenes prefer to form as five-membered dioxolane acetals on vicinal *cis* diols (i.e. adjacent diols where one is axial and the other equatorial). However, this selectivity is not complete and some deviations can be expected. It is worth noting, although it is not of direct relevance to this work, that cyclic acetals that select for vicinal *trans* diols have been developed, e.g. dispiroketal, cyclohexane-1,2-diacetal and butane-1,2-diacetal.^{139, 140}

Cyclic acetals have the added advantage that they can often be regioselectively opened so that one of the protected hydroxyls is still protected whereas the other is free to react. This is commonly used for benzyldiene acetals and their derivatives though it can also be used with acrolein acetals. In the case of a 2,3 benzyldiene ether, the application of various conditions always gives the 3-*O*-benzyl product.^{141, 142}

In rhamnose, a cyclic acetal or similar cyclic protecting group could be employed for the simultaneous protection of the 2- and 3-hydroxyls, assuming the anomeric position is suitably protected, and would allow the 4-hydroxyl to be manipulated (Figure 2.1). In the case of fucose, an analogous reaction would selectively protect the 3- and 4-hydroxyls leaving the 2-hydroxyl free to be functionalised.

Figure 2.1 5-membered ring protecting group formation in (a) rhamnose and (b) fucose. X = alkyl or coordinating metal



2.1.3.2 Methodologies for distinguishing between the 2- and 3-hydroxyls of rhamnose

A major challenge in the regioselective protection of rhamnose lies in distinguishing between the 2- and 3-hydroxyls. Axial secondary hydroxyls are generally more reactive than equatorial secondary hydroxyls though this variation in reactivity is quite subtle and difficult to exploit synthetically.¹⁴³ Since the rhamnose 2-hydroxyl is axial whereas the 3-hydroxyl is equatorial differentiating between them can be based on their orientation and hence reactivity.

Certain strategies have been developed to introduce groups regioselectively onto rhamnose, such as stannyl activation (used by Gurjar *et al.*).⁹⁸ The stannylene ether or acetal can regioselectively enhance the nucleophilicity of the hydroxyls involved allowing regioselective acylation or alkylation. Two types of reagent are commonly used: bis(trialkyltin) oxides which generate trialkylstannyl ethers; and dialkyltin oxides which forms dialkylstannylene acetals. The resulting carbohydrate stannylene compounds can then be functionalised at the activated position. The addition of nucleophilic halide ions can help increase the reaction rate. A wide variety of electrophiles are compatible with the use of the stannyl activation including acyl chlorides, silyl chlorides and alkyl halides.^{144, 145}

An alternative method of distinguishing between the 2- and 3-hydroxyl groups of rhamnose was demonstrated by Fujiwara *et al.*⁹⁷ who used phase transfer conditions. The basis for this is that the monoalkylated product is less likely to drift into the aqueous phase to be deprotonated and then alkylated a second time as it is too lipophilic. The 2-hydroxyl group is closer to the ring oxygen and hence more acidic which often makes it more reactive under phase transfer conditions. Though this method is effective it does not offer the precise control desired for the synthesis of a library of compounds. In addition, it is wasteful of reagents and requires long reaction times with a low yield payoff. More recent work by Zegelaar-Jaarsveld *et al.*¹⁴⁶ demonstrated that the regioselectivity obtained under phase transfer conditions is far from ideal and the positional isomers are not separable. For these reasons, the syntheses described herein

will focus on differentiating between the C-2 and C-3 of rhamnose through the considered use of appropriate protecting groups rather than phase transfer conditions.

Another metal based method for regioselective functionalisation is based on generating a dianion then utilising a copper salt forming a carbohydrate-copper complex. In the case of rhamnose this method typically generated the 3-*O*-functionalised product. However, there is an associated drawback that although a little disubstituted product is formed, the methodology is not as selective as tin based methods.¹⁴⁷ As a result, some generation of the 2-*O*-substituted product would be anticipated. The separation of two such structural isomers would be exceedingly problematic and thus is best avoided in synthetic strategies. In conclusion, for similar reasons to those given for discounting phase transfer conditions, copper-carbohydrate salt formation was also discounted in favour of protecting group manipulation.

As mentioned above, acetals can be regioselectively opened as can orthoesters, which are acid labile. Typically, mild acid treatment of a vicinal *cis* diol protecting orthoester will furnish the protected axial hydroxyl.¹³⁹ A difficulty associated with applying this strategy is the well-known tendency of acyls to migrate.

Of the above discussed strategies, some are more applicable to rhamnose (and fucose) than others. For the reasons discussed above, phase transfer conditions are unappealing and can be discounted, at least during the initial planning stages. Since benzylidene acetals tend to favour six-membered rings they are not as easily introduced into these 6-deoxy sugars despite their advantages in selective opening. An orthoester approach would generate a rhamnosyl with a protected 2-hydroxyl. As mentioned above copper based complexes are not as selective as tin based one and so the latter is more appealing as a strategy. The application of a tin based strategy to the 2- and 3-hydroxyls of rhamnose should generate 3-*O*-substituted derivatives.

When synthesising carbohydrates with a complex and varied methylation pattern, it is vital to plan for the regioselective introduction of each methyl group. However, even a well thought out synthesis can involve a reaction that is found to be difficult or low yielding. As such, it is important to have considered several different ways to generate the target compound to quickly and efficiently adapt if and when challenges are encountered.

2.1.3.3 A brief discussion of possible protecting group strategies

When examining possible protecting group strategies, it was necessary to identify temporary, intermediate and permanent protecting groups. A wide range of protecting groups and conditions for their introduction/removal have been reported.¹³⁹ As such, the optimisation and adaptation of both synthetic routes and individual reactions should be achievable. From the onset orthogonality must be considered, though a full discussion of all the possible protecting groups for hydroxyl moieties is beyond the scope of this thesis.¹³⁹

Some protecting groups are commonly employed in carbohydrate syntheses due to their properties and the added advantage of being well studied. Acetyl groups for example are convenient temporary groups that can also be anchimeric. The use of benzyl protecting groups as a more permanent protecting groups appeals due the relatively mild conditions associated with their introduction and removal. Another possible advantage is that benzyl groups can be regioselectively introduced using the different methods discussed above.

A large number of protecting groups and synthetic steps may be required in order to obtain full control of each position within the sugar moiety. Consequently, the generation of a library of donors is quite a complex synthetic work. It is virtually impossible to unambiguously predict all the effects due to the protecting group pattern on the reactivity of the donors generated and alternative strategies may have to be employed at various points.

2.1.4 The choice of donor

The major synthetic approach taken in this project is modular and the synthetic route to the oligosaccharide targets described is convergent. Since several glycosylation steps are required the type of donor(s) to be used must be considered.

Thioglycosides are an ideal choice as glycosyl donors being both robust and stable under a large variety of functional group manipulation conditions. They are readily prepared and have the additional advantage that they can be interconverted into more reactive donors such as glycosyl trichloroacetimidates and glycosyl halides. Thioglycoside activation results in the loss of a sulfonium ion which is typically achieved by reaction with soft electrophiles. Different types of thioglycoside glycans can have different reactivities allowing for selective activation. Simple alkyl and aryl thioglycosides are less reactive than heteroaromatic thioglycosides.¹³⁹ Simple thioglycosides are thus less likely to be activated by mild alkylating agents, e.g. MeI,

and consequently would be better suited to lengthy syntheses with several functional groups manipulations. One foreseeable incompatibility with thioglycosides would be with the palladium required for the catalytic removal of benzyl groups. Since that would be one of the last steps in the synthesis and the thioglycoside would already have been consumed, it was not considered a problem for this strategy.

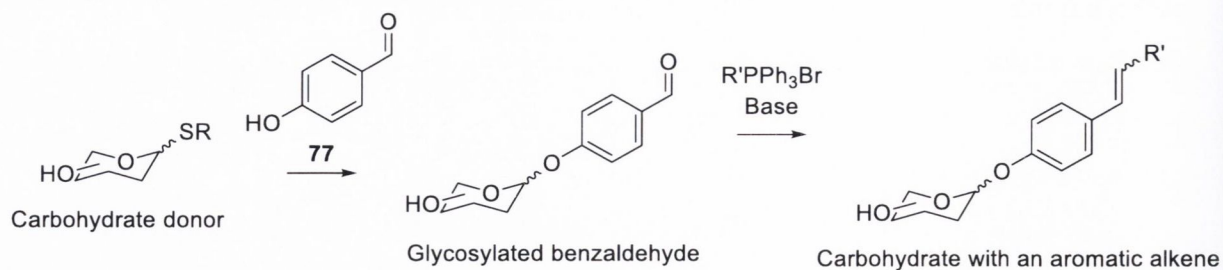
2.1.5 Lipid attachment

In PGLs, the carbohydrate moiety is attached to the aromatic nucleus *via* an oxygen-carbon bond and the lipid is attached to the aromatic nucleus through a carbon-carbon bond. The attachment of the carbohydrate can be accomplished by glycosylating a *para* hydroxylated aromatic ring with an appropriate glycosyl donor. The lipid moiety is attached to the aromatic nucleus through a direct C-C bond and the generation of this C-C bond needs to be carefully considered. A common method for the generation of C-C bonds involves the use of metal catalysts such as palladium and tin for Suzuki-Miyaura and Stille coupling reactions.¹⁴⁸ The removal of the metal catalyst prior to biological testing is crucial and can be somewhat problematic. As such this did not seem ideal as the coupling reaction is one of the last reactions to be performed in the synthetic pathway.

An ideal methodology would be mild and mediated by easily removable reagents that are relatively nontoxic. Since the lipid portion is not a synthetic target a method that can utilise readily available commercial alkyl chains would be advantageous.

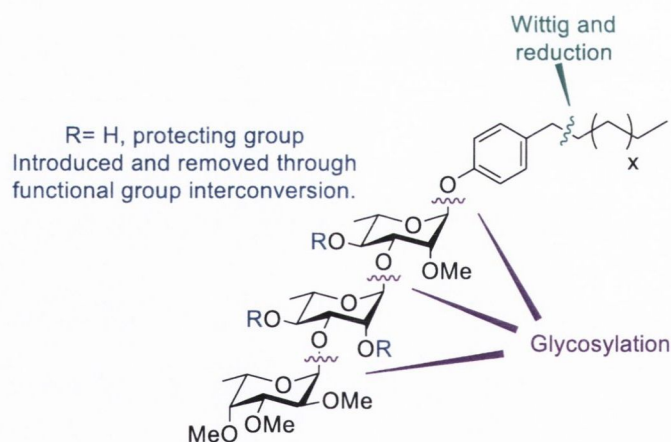
A Wittig reaction between an aromatic aldehyde and an appropriate alkyl phosphonium salt would generate a double bond that could be concurrently reduced during debenzylation. Since the double bond would be subsequently reduced, the isomer or mixture of isomers formed would be inconsequential. The Wittig reaction strategy requires the presence of an aldehyde on the aromatic ring. The required aldehyde moiety could theoretically be installed on an established aromatic ring through a Vilsmeier-Haack formylation.¹⁴⁸ However, this would involve potentially very harsh conditions. Alternatively a glycosylation performed on *para*-hydroxy benzaldehyde **77** should give the benzaldehyde functionalised carbohydrate directly (Scheme 2.2).^{149, 150} The partner for the Wittig coupling reaction can be easily generated by reacting an alkyl halide with triphenyl phosphine. Such alkyl halides are commercially available and cost effective. A Wittig reaction generates triphenyl phosphine oxide which can be removed by a combination of precipitation and column chromatography.

Scheme 2.2 Glycosylation and subsequent Wittig reaction



Overall the PGL-tb1-like molecule will be assembled in a stepwise manner from thioglycoside donors, an aromatic nucleus with an aldehyde moiety and an alkyl chain (Figure 2.2). The aromatic nucleus will be glycosylated first and the trisaccharide assembled *via* a series of glycosylations and deprotections using conditions that will leave the aldehyde unaltered. The Wittig reaction can be performed either pre or post the removal of the protections groups on the trisaccharide. The final reaction will be the reduction of the alkene double bond generated during the Wittig reaction to give the target alkyl functionalised phenolic carbohydrate.

Figure 2.2 Disconnection of a PGL-tb1-like molecule



2.1.6 *p*-HBAD synthesis

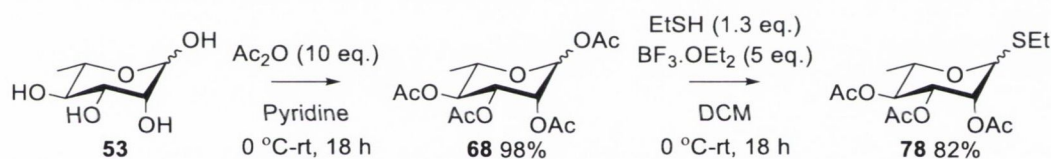
The family of molecules known *p*-HBADs consist of a glycosylated methyl benzoate. This groups of molecules can theoretically be prepared from the benzaldehyde derivatives described above by oxidation and esterification. It is the aim of this project to create a pool of common precursors for the synthesis of PGL mimics and *p*-HBADs so it would be more appropriate to generate *p*-HBADs directly. However, since the aim is to have a flexible and robust synthetic route the possibility of interconverting the PGL precursors and *p*-HBAD may be beneficial.

The simplest synthetic route is to glycosylate methyl-4-hydroxybenzoate with the appropriate glycosyl donors.

2.2 Synthesis of functionalised rhamnosyl donors

As discussed previously, thioglycosides were selected as the donor of choice for this synthesis project and a rhamnosyl thioglycoside donor was prepared starting from free rhamnose **53** over two steps (Scheme 2.3). Rhamnose **53** was quantitatively acetylated by pyridine mediated reaction with acetic anhydride to give peracetylated rhamnose **68**. Per-acetylated rhamnose **68** was activated by an excess of Lewis acid and used as the donor in the subsequent glycosylation reaction with ethane thiol acting as nucleophilic acceptor. The solvent used was nonparticipating and the acetyl group at the rhamnosyl 2-hydroxyl helped ensure the formation of the α 1,2 *trans*, anomer of compound **78** as the major product. The α anomer was the major product making up approximately 70% of the thioglycoside product.

Scheme 2.3 Rhamnosyl thioglycoside synthesis

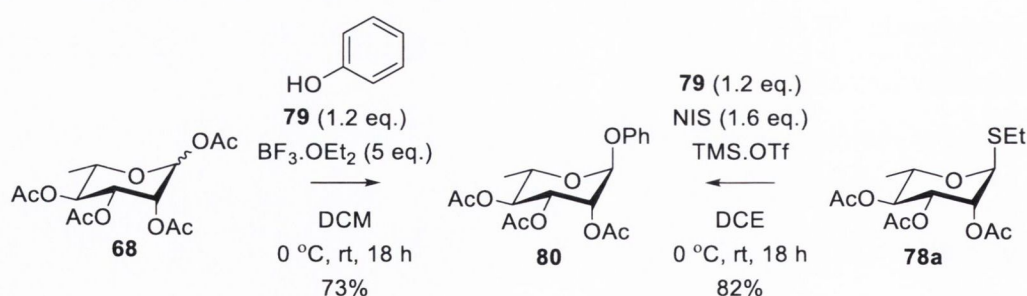


Due to the difficulties associated with characterising a mixture of anomers, only one thioglycoside anomer was used for the ensuing reactions and it was decided to exclusively use the α thioglycoside. The H1-H2 coupling in the ^1H NMR spectra of the resulting compounds should thus stay virtually constant. Both α and β anomers were isolated and characterised. The NMR spectra of each were completely different in both the anomeric H and C shifts as well as the H1-H2 coupling. Any significant deviation in the coupling value or shifts observed following a glycosylation could be attributable to a change in anomeric configuration. The overall yield quoted is for the combined total of both anomers of **78**. The yield of the α anomer **78a**, obtained after one separation by column chromatography, was 61%. Though the thioglycoside could be purified by recrystallization, this did not allow for complete separation of the individual anomers as slight, but still unacceptable, contamination by the other anomer was observed following recrystallization.

At the initial stages of the synthetic route, it was attempted to replicate some previously described synthetic steps from the literature. This would give an indication of yields and might help to identify any problems with the protecting groups in question. In order to have an anomeric group that could mimic the steric effects observed in the native compounds, the glycosylation of an aryl group was initially probed with phenol. This had the additional advantage of allowing for facile identification of products by TLC analysis and ultraviolet light. The resulting phenyl compounds were anticipated to serve as simplified versions of *p*-HBADs as they would be identical except for the absence of a *para* methyl ester. As mentioned above the phenyl ring could be functionalised using a Vilsmeier-Haack reaction. Once the biological activity of free *p*-HBADs is satisfactorily elucidated analogues can be investigated for tuneable reactivity. Such analogues would include alternative esters, immobilised aromatic glycans and the phenyl glycosides described herein.

The phenyl moiety can be installed in a similar manner to the preparation of the thioglycoside **78** (Scheme 2.3) from the acetylated rhamnosyl donor **68** (Scheme 2.4). The thioglycoside **78** can also be used as a donor to give compound **80**. Both methods include neighbouring group participation ensuring that the desired α anomer **80** is formed exclusively. Glycosylating with the acetylated donor **68** was most facile and high yielding. Since the thioglycoside can be used, functional group manipulations can be performed both pre- or post-glycosylation.

Scheme 2.4 Synthesis of a phenyl functionalised rhamnoside

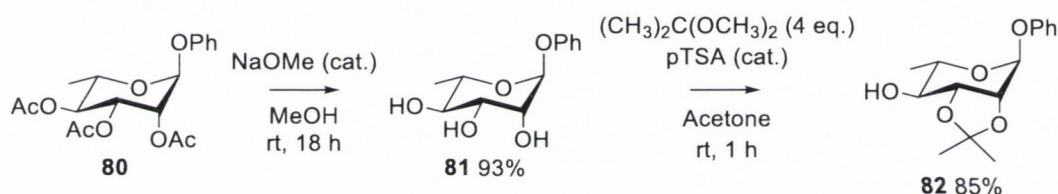


The first few reactions to be investigated involved the use of a benzyl protecting group. The removal of the benzyl protecting groups involves the use of palladium as a catalyst and as such this deprotection reaction cannot be performed on a thioglycoside. The thioglycoside **78a** was converted to the phenyl functionalised rhamnose **80** prior to the protecting group manipulation reactions. The protecting group manipulations described by Gurjar *et al.*⁹⁸ in their *M. kansasii* glycan synthesis were based on work by Yoshimura *et al.*⁹⁹ and Nashed *et al.*¹⁵¹ The slightly

modified series of protecting group manipulations performed on rhamnose by Zuurmond *et al.*¹⁵² is more applicable and circumvents the need for carcinogenic benzene. Overall, these reactions allow for accessing the 4-hydroxyl and differentiating between the 2- and 3-hydroxyls using a stannylene ether intermediate.¹⁵³ These reactions were performed on the phenyl functionalised rhamnose **80**.

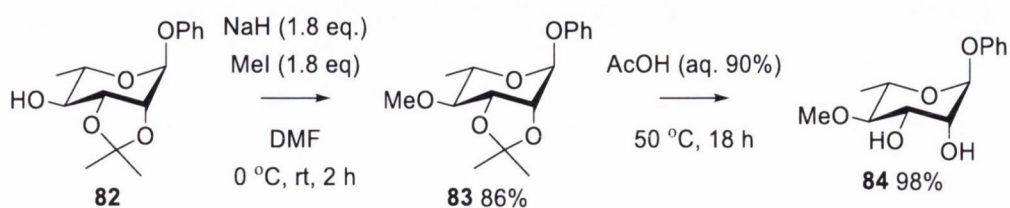
The Zemplén deacetylation of compound **80** gave compound **81** in high yield without the need for further purification (Scheme 2.5). The isopropylidene diol protecting group was regioselectively introduced to the 2- and 3-hydroxyls as it preferentially forms five-member rings with vicinal *cis* diols as discussed above. The product **82** was sufficiently pure to be used directly.

Scheme 2.5 Use of isopropylidene to target the rhamnosyl 4-hydroxyl



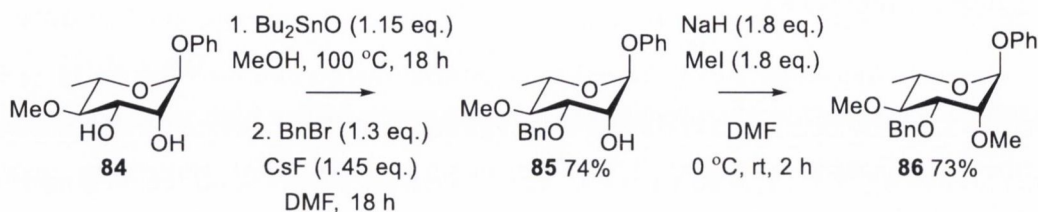
Continuing with the reported synthetic route, the free 4-hydroxyl of compound **82** can be freely functionalised, but the resulting molecule must be stable to the acidic conditions required to remove the isopropylidene (Scheme 2.6). Compound **82** was methylated in high yield and refluxing the resulting rhamnoside **83** in aqueous protic acid cleanly removed the isopropylidene to give the monomethylated rhamnoside **84**. The removal of acetic acid and residual water was accomplished *in vacuo* and facilitated by co-evaporation with toluene. These initial clean and high yielding reactions demonstrated that the isopropylidene group was an ideal way of targeting the 4-hydroxyl of rhamnose.

Scheme 2.6 Removal of isopropylidene post methylation



The use of a stannylene ether for the regioselective benzylation of the rhamnosyl 3-hydroxyl was accomplished over two steps (Scheme 2.7).

Scheme 2.7 Stannylene ether mediated regioselective functionalisation for rhamnose

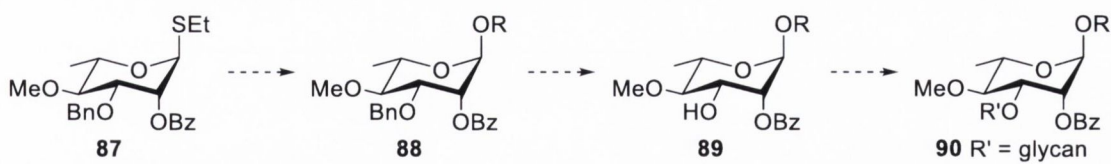


The first step generated the stannylene ether intermediate by refluxing compound **84** with dibutyltin oxide in methanol. The intermediate was not isolated, rather the reaction mixture was concentrated *in vacuo* and water traces removed by azeotroping with toluene. The resulting syrup was resuspended in anhydrous DMF with CsF and BnBr. The regioselectivity of the process was efficient and the desired monobenzylated product **85** required minimal purification by column chromatography.¹⁵² The remaining free hydroxyl of compound **85** could be functionalised as desired. In this case compound **85** was methylated to give the 2,3-dimethylated product **86**.

Although benzyl groups are not often regarded as temporary protecting groups, their removal can be accomplished under relatively mild conditions by palladium catalysed hydrogenation. Assuming facile removal of the 3-*O*-benzyl, then benzoyl groups can be used as permanent protecting groups for the 2- and 4-hydroxyls in a similar manner to that reported by Daffé *et al.*⁸⁶ The benzoyl group is anchimeric meaning that it can help to ensure the desired anomeric configuration. Additionally, a 2,3-*O*-benzylidene cyclic acetal on rhamnose can be selectively opened onto the 2-hydroxyl group potentially eliminating the need for a toxic tin-based reagent.

If the literature procedure¹⁵² could be successfully applied, the orthogonally protected thioglycoside **87** could hypothetically be generated from compound **85** prepared above. Following from this, compound **87** could be used as an α -directing donor to give the fully protected **88** (Scheme 2.8). Acceptor **89** could be generated from compound **88** by catalytic hydrogenation to remove the 3-*O*-benzyl. The 3-hydroxyl of compound **89** could theoretically be glycosylated to generate a disaccharide **90** (Scheme 2.8).

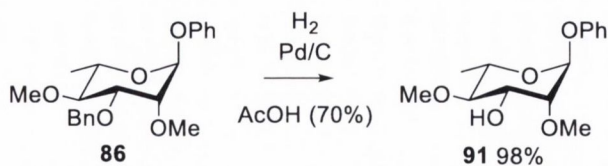
Scheme 2.8 Benzyl and benzoyl orthogonal protection of rhamnose 2- and 3-hydroxyls



After compound **86** was synthesised, the remaining step, the removal of the 3-*O*-benzyl, was not as facile as would be expected though several test reactions were carried out. Initially the hydrogenation was attempted in methanol with 10% palladium overnight but the unaltered starting material was recovered. The reaction was repeated in a 1:1 v/v THF/MeOH mixture to improve solubility of both the starting material and the product. Both the THF/MeOH mixture, pure THF and 1:1 v/v isopropanol/water were unsuccessful despite the application of higher pressures of hydrogen and significantly extending the reaction time to several days. The difficulties encountered in deprotecting the C-3 position may be attributable to its hindered nature though the flanking methoxy groups at C-4 and C-2 should not significantly contribute to the surrounding steric bulk. In the reported synthesis, acetic acid was used as solvent and when this was employed the reaction proceeded in a comparatively short time (Scheme 2.9).

However, these acidic reaction conditions would facilitate the migration of any acyl groups such as benzoyls and acetyls, and be incompatible with acid labile protecting groups. An alternative synthetic route to the previously reported one was deemed necessary. As mentioned above, the need to investigate alternative strategies was not entirely unexpected. The benzyl groups were reassigned as more permanent protecting groups.

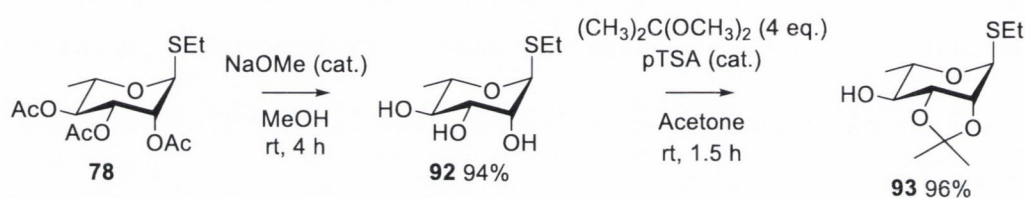
Scheme 2.9 Removal of 3-*O*-benzyl on phenolic rhamnosyl derivative



A similar sequence of reactions to that described above (Schemes 2.5, 2.6, and 2.7) was performed on the α rhamnosyl thioglycoside **78**. The types of reactions and their sequence were kept largely unaltered.

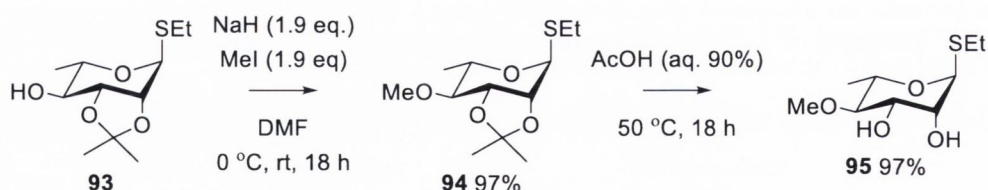
The deacetylation of compound **78** to the deprotected **92** and the following isopropylidene protection to give compound **93** were quite high yielding and sufficiently clean to render any further purification unnecessary (Scheme 2.10). Zemplén deacetylation can be quite fast though, as it is quite mild, it can be left for longer periods of time, assuming no base sensitive moieties are present. Progress can be monitored by TLC when appropriate or convenient. Thioglycoside **78** could be functionalised with various protecting groups at the 4-hydroxyl position and as such could be regarded as a key synthetic intermediate.

Scheme 2.10 Regioselective protection of the 2- and 3-hydroxyls on a rhamnosyl thioglycoside



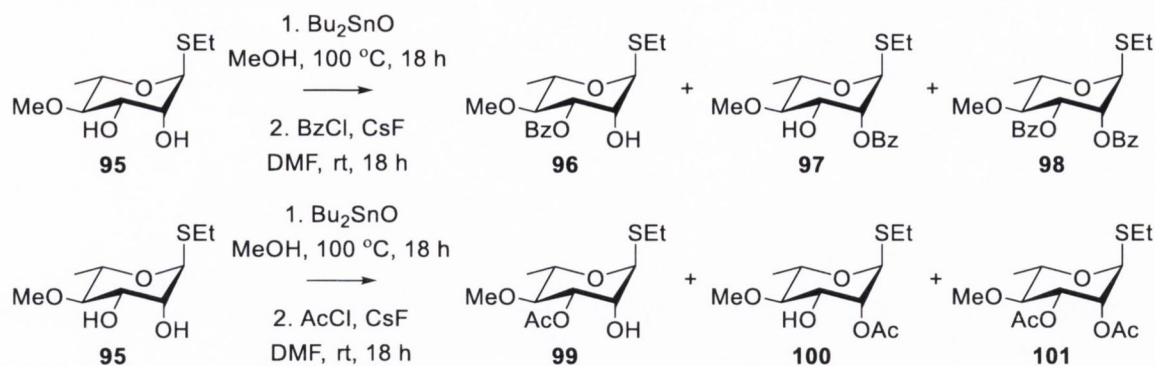
The methylation reaction to give compound **94** could potentially be problematic because, as discussed above, some more reactive thioglycosides could potentially be activated by methylating agents. The ethyl thioglycoside, however, is more stable and was able to withstand the conditions used; this was confirmed by the high yield of compound **94**. The acid catalysed removal of the isopropylidene moiety to yield compound **95** was both as high yielding and clean as that observed for the deprotection of phenolic glycoside **83** described above (Scheme 2.11).

Scheme 2.11 Protection of the rhamnosyl 2- and 3-hydroxyl by isopropylidene and its removal



Since the use of stannylene activation allowed for highly regioselective functionalisation it was decided to apply it for the regioselective benzylation and also the regioselective acylation of **95** at C-3.

Scheme 2.12 Stannylene activation of a rhamnosyl thioglycoside prior to acylation

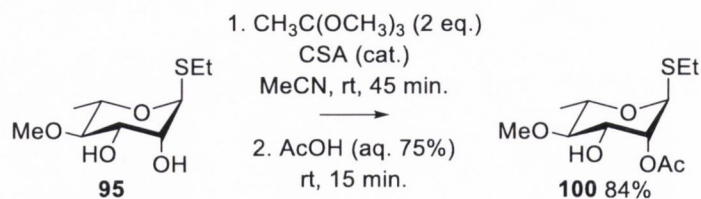


Unfortunately, stannylene activation was found to be incompatible with both benzoyl chloride and acetyl chloride (Scheme 2.12). In both cases mixtures of both regioisomers and the diacylated compounds were obtained. Separation of the diacylated compounds **98** and **101** from their monoacylated counterparts was relatively facile. However, pairs of regioisomers **96** and **97**, and **99** and **100**, were very difficult to separate to an appreciable extent.

Another difficulty associated with the use of benzoyl groups is that their aqueous base-catalysed removal may adversely affect the methyl ester of *p*-HBADs. As stated above the benzyl group could be used as a permanent protecting groups to be removed during the final stages of the synthesis. The conditions required to remove benzyl groups are relatively mild and should leave methyl ester and most other protecting groups unaltered. The disadvantage to replacing the benzoyl with a benzyl is the loss of neighbouring group participation but the effect of this on reaction selectivity could only be determined experimentally.

Another possible resolution to the problem of 2,3 regioselectivity for rhamnose was the use of an orthoester intermediate that could be opened regioselectively. It would be possible to generate a benzoyl through the use of a benzylidene, however since the usually straightforward removal of a benzyl at the rhamnosyl 3-hydroxyl was troublesome it was decided to use a more labile temporary protecting group. The use of trimethyl orthoacetate would be expected to lead to an acylated axial position but acyl migration would favour the equatorial and must be minimised to prevent the formation of an inseparable mixture of regioisomers from forming.

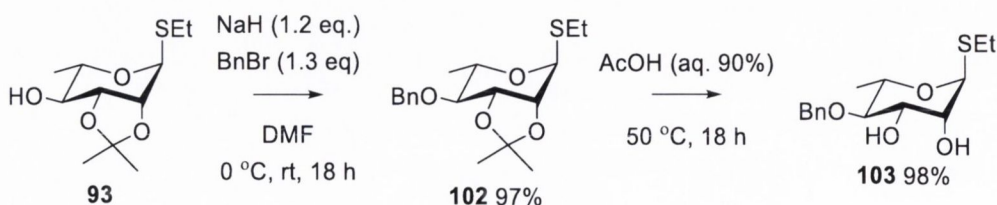
Scheme 2.13 Use of an orthoester to regioselectively protect the 2-hydroxyl of rhamnose



The reaction on thioglycoside **95** to introduce the orthoester was rapid and clean (Scheme 2.13).¹⁰³ The opening of the rhamnosyl 2,3-orthoester was accomplished in the same reaction vessel by the addition of aqueous acetic acid. Column chromatography was necessary to isolate the clean compound but was relatively facile. The product **100** was kept cold to prevent acetyl group migration; thought without the presence of an acid or base catalyst this migration was likely to be slow.

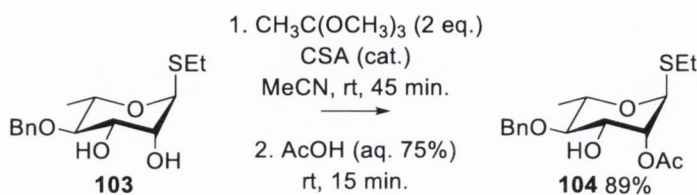
The same series of steps was repeated with a slight alteration in order to prepare thioglycoside donor **104**: the installation of the permanent benzyl protecting group was used rather than a methyl on the rhamnosyl 4-hydroxyl position (Scheme 2.14). As previously stated, compound **93** can be regarded as a key synthetic intermediate for p-HBAD synthesis since the functionality at the rhamnosyl 4-hydroxyl is defined and fixed at this point in the synthetic route. In this synthetic route the 4-hydroxyl is either methylated or protected with a benzyl group that can be removed later in the synthesis as part of a global deprotection. Both types of alkylation reaction were accomplished in 97% yield with little purification necessary (Scheme 2.14). After facilitating the benzylation of the 4-hydroxyl of compound **93** to give compound **102**, the isopropylidene was removed by refluxing in acetic acid to give the rhamnoside diol **103**.

Scheme 2.14 Benzylation of the 4-hydroxyl on an isopropylidene protected rhamnoside



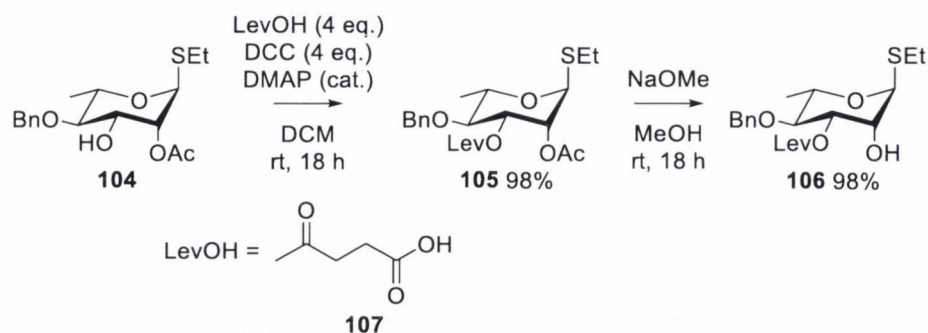
The benzylated compound **103** was converted to the acetylated **104** in higher yield than the 4-*O*-methylated system (Scheme 2.15). That the regioselective opening of the orthoester was more efficient with a 4-*O*-benzylated rhamnose was likely due to the steric effects of the benzyl.

Scheme 2.15 Orthoester installation and regioselective opening on a rhamnosyl thioglycoside



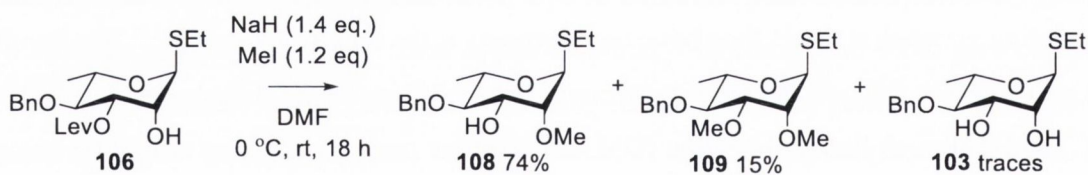
Since the application of an orthoester appeared to have solved the 2- and 3-hydroxyl regioselectivity issue, the protecting group strategy had to be reassessed. The acetyl group is anchimeric but it is highly labile and tends to migrate. Overall it is unappealing as an even semi-permanent rhamnosyl 2-hydroxyl protecting group and it was consequently assigned temporary, even transient status. This called for a protecting group that could be installed at the rhamnosyl 3-hydroxyl using conditions that both leave the 2-*O*-acetyl unaffected and do not induce its migration. The rhamnosyl 2-*O*-acetyl could then be removed and replaced with a more appropriate group: for example, a methyl or benzyl group. The orthogonal protecting group at the rhamnosyl 3-hydroxyl would have to be more labile than a benzyl and capable of withstanding the mild basic conditions used to remove the acetyl. It should be noted that alternative methods for acetyl removal have been reported and could be employed if necessary. A protecting group that meets the above requirements is the levulinoyl group.¹⁵⁴ The levulinoyl (lev) group can be introduced using mild coupling conditions: *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). The lev protecting group would be located at the hindered rhamnosyl 3-hydroxyl position and is much less base labile than an acetyl. Theoretically it should remain intact even after the acetyl is removed. However, the levulinoyl group would most likely be removed under the conditions required to install a methyl or benzyl groups at the 2-hydroxyl which typically involves the strong base sodium hydride. This would be advantageous as the levulinoyl group could be replaced with either a methoxy or a more labile group to protect the 3-hydroxyl during the subsequent glycosylation.

Scheme 2.16 Installation of a levulinoyl at the rhamnosyl 3-hydroxyl



The reaction to install the levulinoyl functionality is typically performed using two or fewer equivalents of levulinic acid **107**. When these conditions were used to install a levulinoyl on the 3-hydroxyl of compound **104** the target compound **105** was obtained in only 85% yield. This result is likely due to the steric hindrance around the rhamnosyl 3-*O*-hydroxyl. The moderate yield was greatly increased to 98% by altering the number of equivalents of acid used (Scheme 2.16). Zemplén deacetylation of compound **105** gave thioglycoside **106** in good yield. The presence of the levulinoyl group was confirmed by both mass spectroscopy and NMR analysis of compound **106**. Thioglycoside **106** can be regarded as another key synthetic intermediate for *p*-HBAD synthesis as at this point the functionality of the 2-hydroxyl is set as either a methoxy or a benzyl protecting group.

Scheme 2.17 Methylation of the 2-hydroxyl on a rhamnosyl thioglycoside

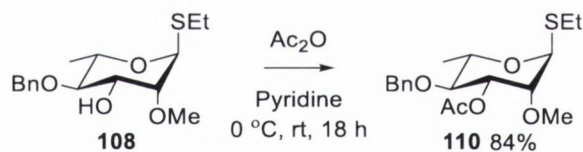


This methylation reaction to produce compound **108** required some optimisation as it generated three compounds: the dimethylated compound **109**, the monomethylated target compound **108** and the deprotected compound **103** (Scheme 2.17). As expected the levulinoyl group of compound **106** was removed under the alkylation conditions. The first reaction was performed using 1.3 equivalents of NaH and 1.1 of methyl iodide. A slight increase to 1.2 equivalents of methyl iodide increased the yield of the target thioglycoside **108** without significantly increasing the quantity of dimethylated **109**. Since starting material was recovered the quantity of sodium hydride used was increased to 1.4 equivalents. These modifications served to

increase the yield of compound **108** from 61% to a consistent 74%. The yield of dimethylated compound **109** could be increased by simply increasing both reagents to at least two equivalents. Though not necessary for the synthesis of the native glycan, thioglycoside **109** could be used for the generation of non-native glycan and forms part of the target thioglycoside library for the preparation of unnatural *p*-HBAD analogues. The isolation of both the monomethylated **108** and the dimethylated **109** from the one reaction circumvented the need for an additional reaction to prepare compound **109**. As the column conditions were optimised for the isolation of the target monoalkylated compound the yield of the side products are approximate. If the column conditions were varied it would be possible to isolate more of the side products and obtain an accurate yield.

Since the 2-*O*-methyl rhamnosyl thioglycoside **108** was to be employed as donor in a glycosylation reaction, the free 3-hydroxyl needed to be protected to prevent compound **108** simultaneously acting as an acceptor with itself. The protecting group at the 3-hydroxyl was only needed for the duration of the glycosylation so the labile acetyl was used.

Scheme 2.18 Acetylation of the 3-hydroxyl of a rhamnosyl thioglycoside prior to glycosylation



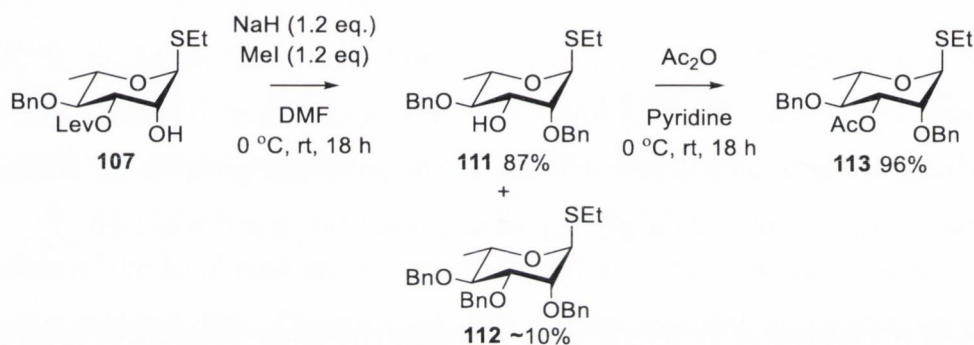
The acetylation of compound **109** to give compound **110** was not as high yielding as would be expected a result that was likely due to the hindered nature of the rhamnosyl 3-hydroxyl (Scheme 2.18). Once the glycosylation reaction was achieved the acetyl protecting group could readily be removed to yield an acceptor molecule for disaccharide synthesis.

As discussed above (Section 2.1.3), one of the initial aims of the project was to prepare a library of thioglycosides that could be used to generate a variety of mono- and oligosaccharides that are both natural and unnatural analogues of *p*-HBADs and PGLs. These carbohydrates would be based on the structure of the glycan portions of PGLs, mainly PGL-tb 1 and mycoside B, as both of these are produced by clinical isolates of *Mtb*. To accomplish this it was necessary to establish complete control of the methylation pattern of rhamnose. The synthetic strategy described above, based on functional group manipulations, allows for the installation of

orthogonal groups at each position thus giving complete control of the methylation pattern in rhamnose.

To complete the generation of a thioglycoside library necessary for the synthesis of the native glycans, several more thioglycosides had to be synthesised. The final thioglycoside necessary for the native glycan total-synthesis was prepared from a previously prepared key synthetic intermediate, thioglycoside **107**. Precursor **107** was benzylated at the 2-hydroxyl to give **110** (Scheme 2.19).

Scheme 2.19 Generation of the second rhamnosyl donor for PGL-tb1 glycan

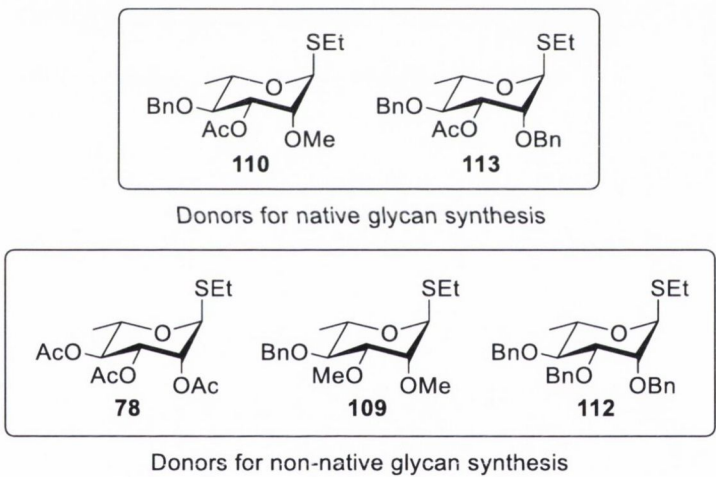


The benzylation of compound **107** to give compound **111** was high yielding with little tri-benzylated **112** generated from the outset. This is quite likely due to the steric bulk associated with benzylys: the 2-hydroxyl would be benzylated first thus making the 3-hydroxyl relatively inaccessible. This is supported by the isolation of minimal amounts of tribenzylated **112**. It is important to note that, though minimal, the dialkylation reaction did take place. In the case of the methylation reaction the dimethylated **109** accounted for approximately 15% of the products formed; with the benzylation reaction compound **112** tended to be below 10%. In both cases approximately equal amounts of dialkylated compound and deprotected **104** were recovered. As before, the free rhamnosyl 3-hydroxyl needed to be protected before thioglycoside **111** could act as a donor in a glycosylation reaction. Acetylated of the rhamnosyl 3-hydroxyl gave the fully protected rhamnosyl thioglycoside donor **113**. As with the dimethylated thioglycoside **109** the perbenzylated **112** also forms part of the proposed library of thioglycosides and as before the column conditions were not tailored to the isolation of the perbenzylated compound and its yield is consequently approximate.

The yields of these side products were not always accurately recorded due to incomplete purification. The column purification was tailored to the target monoalkylated compound and consequently the side products were often isolated as slightly contaminated fractions. These impure fractions were collected and cumulatively purified to provide highly pure usable thioglycosides with alternative alkylation patterns for biological study.

At this stage, the rhamnosyl thioglycoside donors required for the preparation of the target trisaccharide had been synthesised. In addition several other thioglycosides (including functionalised intermediates in the above syntheses) that could be used for the synthesis of non-native *p*-HBADs and PGLs were obtained. Together these thioglycosides formed a small library of rhamnosyl thioglycoside donors (Figure 2.3).

Figure 2.3 Thioglycosides isolated in the course of synthesising the native glycans

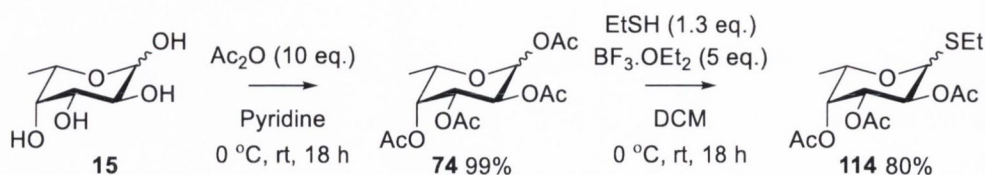


The preparation of a library of thioglycosides was addressed to allow for the synthesis of unnatural analogues of PGLs and *p*-HBADs. These non-native compounds would facilitate the study of the effect of unnatural methylations patterns on the immunological response and help determine how important the defined methylation pattern is for their activity. Initial results from biological testing will help direct future syntheses. With a previously prepared library of thioglycosides available non-native PGLs and *p*-HBADs can be readily prepared when biological testing indicates that they are necessary.

2.3 Synthesis of fucosyl thioglycosides

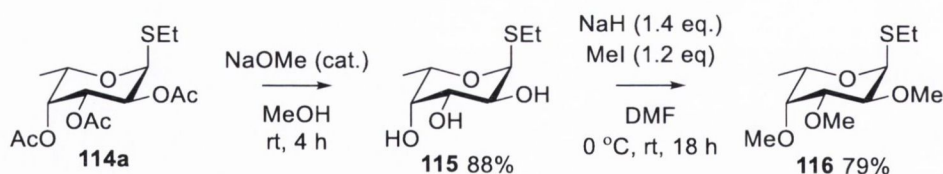
With the appropriately protected glycosyl donors and acceptors in hand the synthesis of the native p-HBADs was addressed.

Scheme 2.20 Synthesis of a fucosyl thioglycoside



The acetylation of free fucose **15** to give compound **74** was high yielding as expected, as was the subsequent glycosylation reaction with ethanethiol to give the thioglycoside **114** (Scheme 2.20). The yield of thioglycoside **114** quoted in Scheme 2.20 is for the mixture of anomers although neighbouring group participation means that major product was the β anomer accounting for approximately 70% of the thioglycoside product. Despite this, since only two additional reactions were required to generate the methylated fucosyl thioglycoside target compound **116**, it was decided that for the initial reactions the α anomer, isolated in 33%, would be used. This decision was taken for similar reasons to the decision to exclusively use the α anomer of rhamnose: knowing the orientation of the anomeric position from the onset. As with rhamnose both anomers were isolated and fully characterised.

Scheme 2.21 Methylation of the fucosyl thioglycoside



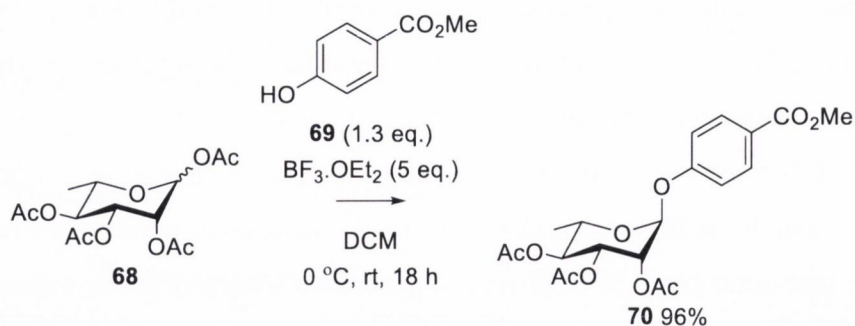
The deacetylation of thioglycoside **114** under Zemplén conditions was facile and compound **115** did not require any purification by column chromatography (Scheme 2.21). The methylation of the fucosyl thioglycoside **115** to give the target methylated fucosyl thioglycoside **116** was high yielding and necessitated only minimal purification. Methoxy groups are not participating groups meaning glycosylations using thioglycoside **116** as donor does not necessarily favour formation of the β product.

The orientation of the rhamnosyl hydroxyls (excluding the anomeric) is axial at the C-2, and equatorial at the C-3 and C-4; in fucose it is equatorial at C-2 and C-3, and axial at C-4. The stereochemistry of the hydroxyls in fucose can be regarded as being related to rhamnose but in reverse order. Consequently, though several fucosyl donors were not required at the time it should be noted that the protecting group strategy applied to rhamnose would also serve to regioselectively functionalise fucose. Theoretically the same route utilising the same protecting groups in the same order could be employed to generate a library of fucosyl thioglycosides. As with rhamnose, key synthetic intermediates compounds would be used to establish the methylation pattern at key points. Theoretically the methodology depicted would allow complete control over the methylation pattern of fucose with the same benzyl permanent protecting group as used for rhamnose. It would seem reasonable to assume that, since the two carbohydrates are so similar in their orientation, the steric environment around the 3-hydroxyl of fucose may be similar in nature to that of rhamnose. However, its effect on the chemistry at that position can only be determined experimentally.

2.4 Installation of a functionalised aromatic moiety and *p*-HBAD synthesis

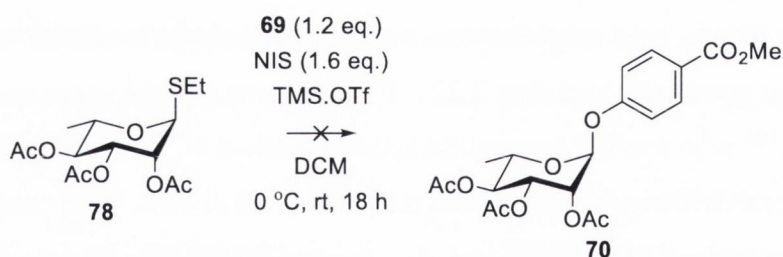
To synthesise the target *p*-HBADs an aromatic ring with a *para* methylbenzoate needed to be installed at the anomeric of a rhamnoside. As demonstrated above peracetylated rhamnose **68** can act as a donor and is activated by a strong Lewis acid such as boron trifluoride. The rhamnosyl donor **68** was used to glycosylate methyl-4-hydroxybenzoate **69** to give the desired α anomer of **70** in good yield (Scheme 2.22). This is essentially the same reaction carried out by Barroso *et al*¹⁰⁵ who applied two equivalents of methyl-4-hydroxybenzoate **69** and three equivalents of boron trifluoride and isolated the product **70** in 72% yield containing 4% of the β product. In carrying out this glycosylate, the quantity of acceptor **69** was decreased and the quantity of activator increased so that the product **70** was obtained in significantly higher yield than those previously reported and with none of the β product observed due to the neighbouring acetyl group.

Scheme 2.22 Glycosylation of methyl-4-hydroxybenzoate with peracetylated rhamnose



As previously stated, this project proposes to generate various motifs from a common library of donors, the preparation of which was discussed above. To this end, reaction conditions must be established for the use of thioglycoside donors to glycosylate methyl-4-hydroxy benzoate. Initially, the glycosylation was attempted using hitherto successfully applied conditions for thioglycoside activation: NIS enhanced with TMS.OTf (Scheme 2.23). Unfortunately, the application of these conditions to this system was not successful. This may be attributable to the electron withdrawing effects of the aromatic carbonyl which render methyl-4-hydroxybenzoate a much poorer acceptor than phenol.

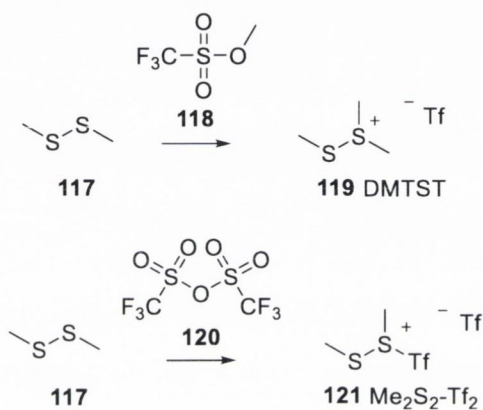
Scheme 2.23 Unsuccessful conditions for the glycosylation of methyl-4-hydroxybenzoate



The effect of the carbonyl group can be seen in the different pK_a values of methyl-4-hydroxybenzoate and phenol: 8.3 and 9.95 respectively. Phenol is a much better nucleophile capable of reacting with the carboxonium intermediate (or its orthoester variant). The poor reactivity of the acceptor may be overcome by the use of a more reactive donor such as a trichloroacetimidates. This solution is not ideal as it would necessitate two reactions to convert the thioglycoside **78** into a trichloroacetimidates. An alternative methodology is the use of different thioglycoside activation conditions. Various methods have been reported for the activation of thioglycosides using, amongst other reagents, organosulfur compounds. The powerful alkylsulfenylating agent dimethyl(methylthio)sulfonium triflate (DMTST) was first

reported in 1986 and several more reagents followed.¹⁵⁵⁻¹⁵⁷ In the preparation of DMTST, dimethyl disulphide **117** is reacted with methyl triflate **118** to generate the promoter **119** (Scheme 2.24). Tatai and Fügedi aimed to generate a powerful activator that is active at low temperatures, with commercially available and inexpensive reagents.¹⁵⁷ They reported that a related activator **121** can be prepared by the reaction of dimethyl disulphide **136** with triflic anhydride **120**. The resulting compound **121** is more reactive than DMTST **119** as a much more electron-withdrawing trifluoromethanesulfonyl replaces one of the methyls in compound **119**.

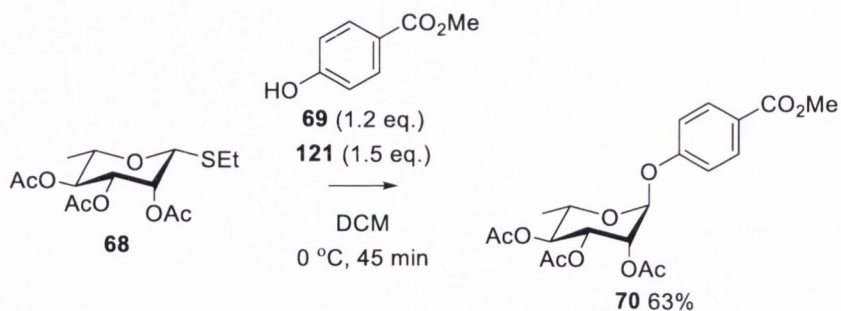
Scheme 2.24 Synthesis of two related thioglycoside activators



Reactions promoted by $\text{Me}_2\text{S}_2\text{-Tf}_2$ **121** are typically fast and require a stoichiometric quantity of freshly prepared promoter **121**, though in practice, an excess of 1.5 equivalents typically gave the best results. When tested $\text{Me}_2\text{S}_2\text{-Tf}_2$ **121** was found to be able to overcome the low reactivity of acceptors and was compatible with many common hydroxyl protecting groups.

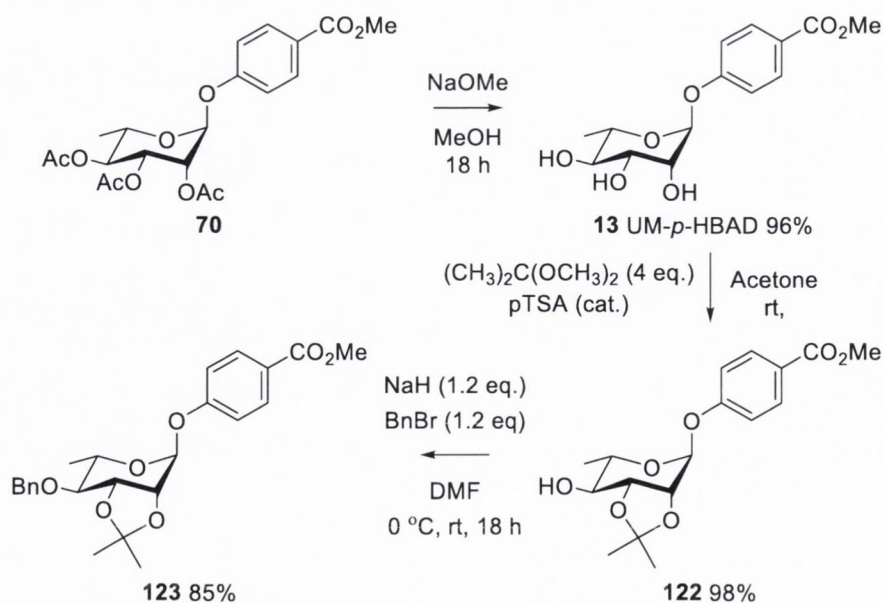
When compound **121** was used to promote the glycosylation of methyl-4-hydroxybenzoate **69** by the rhamnosyl thioglycoside **78** the reaction proceeded very quickly to give the product **70** in an improved yield of 63% in 45 minutes (Scheme 2.25). The reaction was quite clean and exclusively gave the α -anomer of compound **70**. The major challenge in purifying *p*-HBAD **70** was in removing the methyl-4-hydroxybenzoate which had a similar R_f to the product. This reaction also determined that both the α and β rhamnosyl thioglycosides give the same product and hence both donors could be used.

Scheme 2.25 Glycosylation of methyl-4-hydroxybenzoate with a rhamnosyl thioglycoside



Since it was successful for promoting glycosylation of methyl-4-hydroxybenzoate, the activator **121** could potentially be applied to previously prepared thioglycosides to generate a large variety of *p*-HBADs. A large quantity of the first *p*-HBAD acceptor would be required for the synthesis of both *p*-HBAD II and mycoside B to allow for biological testing. As such it was decided to carry out the protecting group manipulations post- rather than pre-glycosylation. A large quantity of the peracetylated *p*-HBAD **70** was prepared by the use of the peracetylated rhamnosyl donor **69** promoted by the Lewis acid boron trifluoride. This *p*-HBAD **70** served as starting material for a series of protecting group manipulations that were carried out to generate the desired *p*-HBADs (Scheme 2.26).

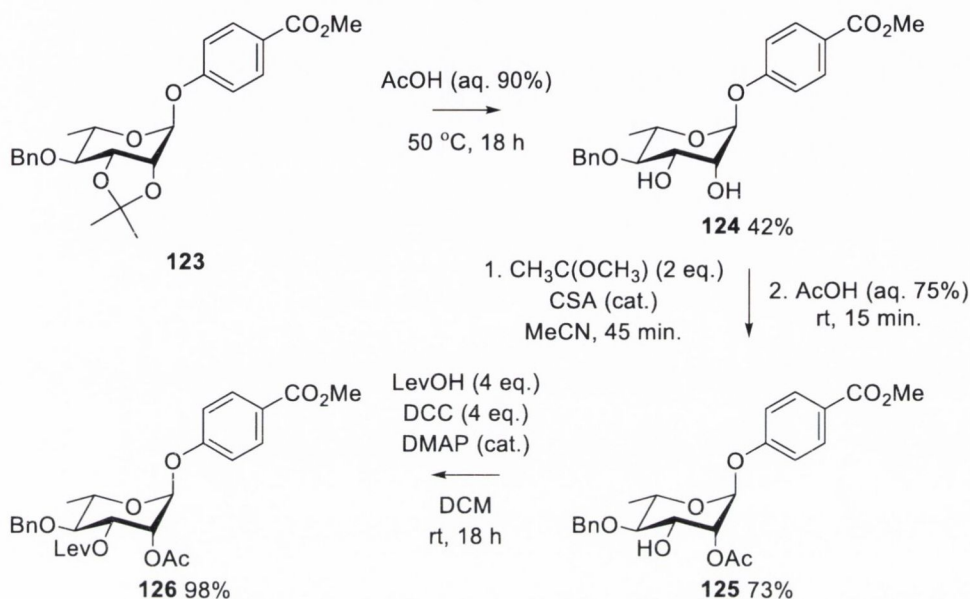
Scheme 2.26 Functional group manipulations to generate a series *p*-HBADs



The *p*-HBAD **70** was deacetylated to give *p*-HBAD **13** which was itself previously isolated from cells and was designated UM-*p*-HBAD.⁹⁵ The protecting group strategy employed was

much like the one described above for rhamnosyl thioglycosides and it gave comparable yields of the isopropylidene protected, *p*-HBAD **122** and the benzylated **123**. The isopropylidene protected *p*-HBAD **123** is another key synthetic intermediate analogous to thioglycoside **102**.

Scheme 2.27 *p*-HBAD protecting group manipulations

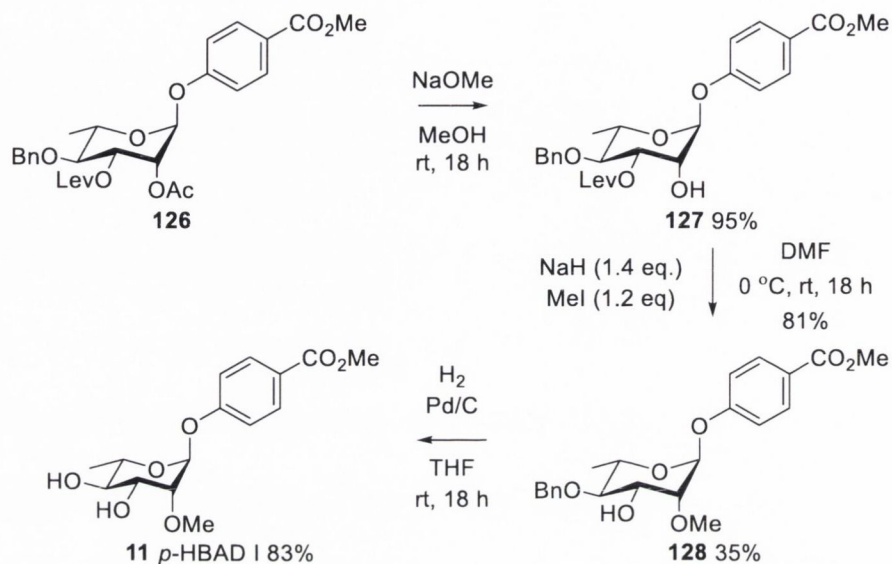


The isopropylidene was removed by refluxing compound **123** in acetic acid, however this deprotection reaction was lower yielding than would typically be expected (Scheme 2.27). This could be due to a reaction between the aqueous acid and the methyl ester. Though the acid is relatively weak the somewhat forcing conditions involved may have resulted in a degree of hydrolysis of the methyl ester. The orthoester formation and cleavage to give *p*-HBAD **125** were efficient and clean, as was the coupling reaction to install the lev group at the 3-hydroxyl to give the fully protected *p*-HBAD **126**.

Removal of the acetyl group on compound **126** resulted in compound **127** which is a *p*-HBAD analogue of the above described rhamnosyl thioglycoside **107** (Scheme 2.28). Since both *p*-HBAD I and *p*-HBAD II have a methyl at the 2-hydroxyl position on the first rhamnosyl residue, compound **127** was methylated with the concurrent removal of the lev group to give compound **128**. The yield of the monomethylated product was quite low possibly because the methylation reaction had not been optimised on the *p*-HBAD system. The Palladium catalysed hydrogenation of *p*-HBAD **128** generated *p*-HBAD I **11** in good yield. Alternatively,

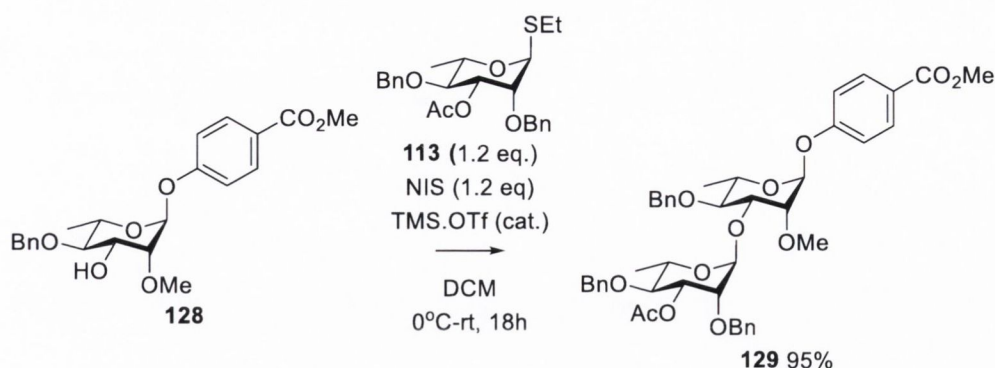
compound **128** could be used as an acceptor for the second glycosylation in the synthesis of *p*-HBAD II **9** or other non-native *p*-HBADs.

Scheme 2.28 Synthesis of *p*-HBAD I



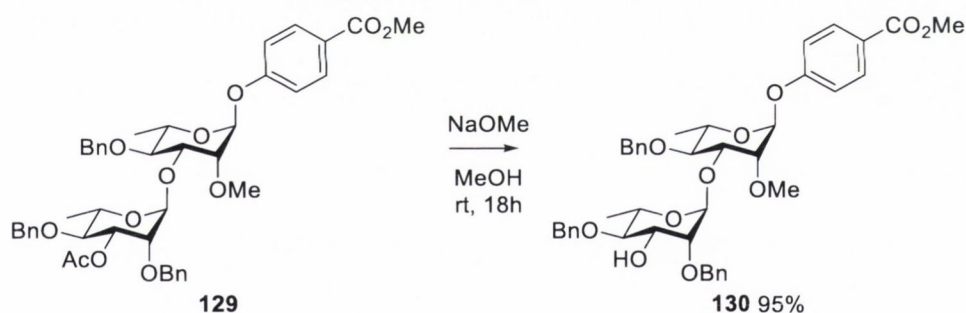
In the route to *p*-HBAD II, compound **128** was glycosylated with thioglycoside **113** whose preparation was described above (Scheme 2.28). Since *p*-HBAD **128** is not a poor acceptor the more usual thioglycoside activators, NIS and TMS.OTf were used as they are easier to handle and measure as well as being less toxic. The glycosylation of the *p*-HBAD acceptor **128** by rhamnosyl thioglycoside donor **113** was high yielding compared to previously described syntheses. The α disaccharide product **129** was formed exclusively despite the absence of anchimeric assistance. This may have been due to the large amount of steric bulk around both the donor **113** and the acceptor **128**. The 3-hydroxyl of *p*-HBAD **128** is hindered by the flanking alkyl groups, particularly the 4-*O*-benzyl, this limits how the acceptor can approach the donor. Additionally the donor **113** has a large 2-*O*-benzyl group and an acetyl blocking one of its faces.

Scheme 2.29 Glycosylation to form a disaccharide portion of *p*-HBAD II



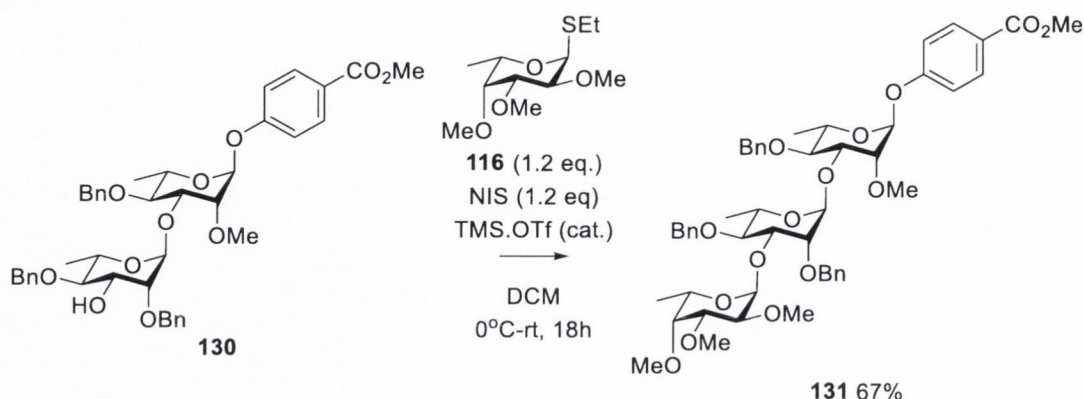
Disaccharide *p*-HBAD **129** was converted into an acceptor by the base catalysed hydrolysis of the 3-*O*-acetyl to give compound **130** (Scheme 2.30). Ordinarily this reaction is both fast and facile when carried out with commercially available sodium methoxide. However, when this deprotection was carried out the hindered nature of the rhamnosyl 3-hydroxyl position slowed the reaction, greatly increasing the time required for complete deacetylation to occur.

Scheme 2.30 Deacetylation of the *p*-HBAD disaccharide



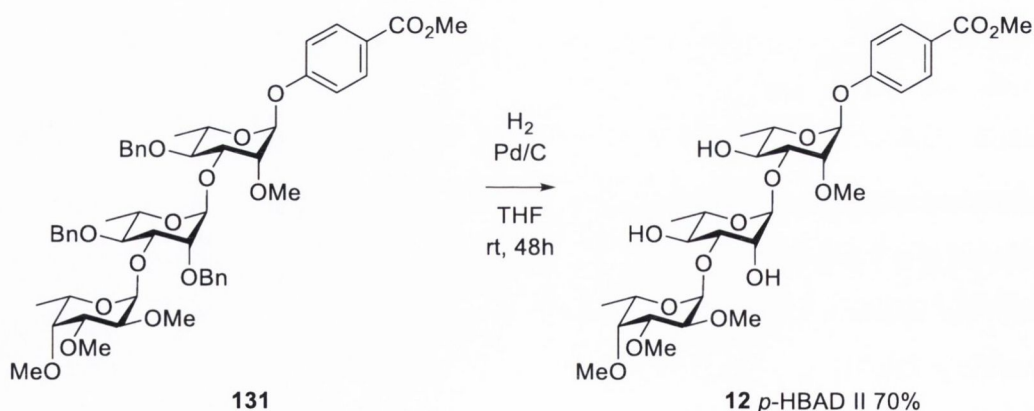
The utilisation of freshly prepared sodium methoxide, generated by the addition of sodium metal directly to methanol, resolved the problem of extended reaction times. With this alteration the reaction proceeded as would be expected for a deacetylation to give the disaccharide *p*-HBAD acceptor **130** in high yield. Although these deacetylation reactions are typically very clean the disaccharide *p*-HBAD **130** was purified by column chromatography to ensure the purity of the acceptor for the subsequent glycosylation.

Scheme 2.31 Synthesis of the *p*-HBAD II trisaccharide



In previously reported literature syntheses, this final glycosylation reaction is typically low yielding at 45% and 52%.^{98, 104} Though a higher yield was obtained, the glycosylation of the disaccharide *p*-HBAD **130** to give the trisaccharide *p*-HBAD **131** was still not very high yielding (Scheme 2.31). The disaccharide *p*-HBAD acceptor is the most synthetically challenging of the starting materials and fortunately the unreacted portion can be efficiently recovered and the reaction repeated if required. The absence of anchimeric assistance would promote the formation of the α linked mannose product. It is possible that a small quantity of the α linked product was formed but none was isolated or observed.

Scheme 2.32 *p*-HBAD II synthesis



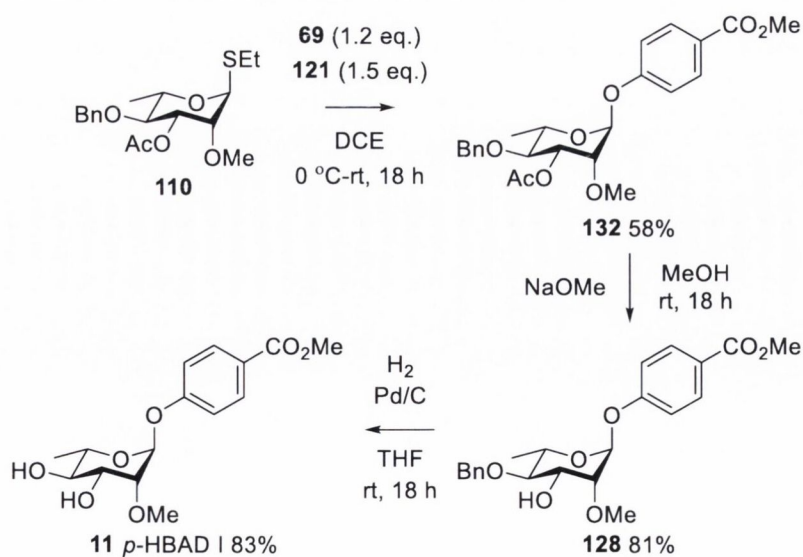
The global debenzylation of compound **131** was somewhat slow when carried out under an atmosphere of hydrogen. When hydrogen was bubbled through the reaction mixture to saturate the solvent the reaction rate was greatly enhanced (Scheme 2.32). The resulting native trisaccharide *p*-HBAD II **12** was freeze-dried prior to being used for biological testing. This concluded the synthesis of the challenging *p*-HBAD II trisaccharide. The stereochemistry of

each of the glycosyl linkages was confirmed by NMR analysis. The regiochemistry of the glycosylation steps and the methylation pattern was carefully controlled by rigorous selection of orthogonal protecting groups.

As previously discussed, *p*-HBAD I **11** was produced from an intermediate generated during the synthesis of *p*-HBAD II **12**. The native *p*-HBAD **11** can also be generated by the glycosylation of methyl-4-hydroxybenzoate using the appropriate thioglycoside donor from the library of thioglycoside (Scheme 2.33).

The glycosylation of methyl-4-hydroxybenzoate **69** proceeded with a comparable yield to that described for the acetylated thioglycoside **78** but with a noticeable difference in the purity of product. As before, the alternative thioglycoside activation conditions of Me₂S₂-Tf₂ **121** were used. The purification of the product *p*-HBAD **132** was best achieved in two steps. First a crude silica gel column to remove most of the impurities followed by a second slower and more thorough column. The biggest challenge in purifying compound **132** was the removal of the acceptor **69** which ran very close to the product regardless of the conditions employed. This difficulty in purifying the product may have contributed to the relatively low yield of compound **132** isolated. None of the β anomer was isolated though it may have been formed its presence was not detected during the initial or the second column.

Scheme 2.33 Glycosylation for *p*-HBAD I synthesis



The deacetylation of *p*-HBAD **132** to give compound **128** also required freshly prepared sodium methoxide to proceed at an appreciable rate and was quite high yielding (Scheme 2.33). Shorter reaction times for the deacetylation of *p*-HBADs are preferable in order to prevent base catalysed hydrolysis of the methyl ester.

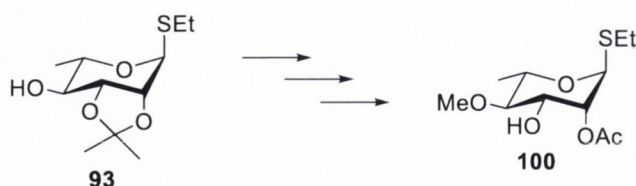
The debenzylation reaction to form the native *p*-HBAD I **11** was fast with the application of a hydrogen bubbler and relatively clean. The product *p*-HBAD I **11** was freeze-dried prior to biological testing.

At this point, all three native *p*-HBADs had been synthesised with both high efficiency and high purity as had a library of rhamnosyl thioglycosides. The synthesis whose development is described above allows for complete control of the methylation pattern of rhamnose and theoretically of fucose. At this stage several of the initial aims of this project had been accomplished.

2.5 Further development of the rhamnosyl thioglycoside library

The rhamnosyl thioglycoside library, however, was not complete, nor was the *p*-HBAD library. Since these two aims were related, the further development of the rhamnosyl thioglycoside library was addressed first. The synthesis of several rhamnosyl thioglycosides that can be thought of key synthetic intermediates have already been described. One such intermediate is thioglycoside **93**. In the majority of the work described above the rhamnosyl 4-hydroxyl was benzylated, however, the preparation of 4-*O*-methyl analogues was necessary for the development of the thioglycoside library. The conversion of thioglycoside **93** to the acetylated thioglycoside **100** has already been discussed (Scheme 2.34).

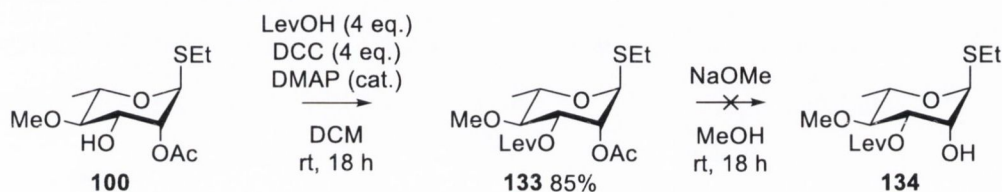
Scheme 2.34 Synthesis of 4-*O*-methyl rhamnosyl thioglycosides



The 2-*O*-acetyl of **100** could potentially migrate, making **100** a somewhat unstable intermediate and after being prepared it was immediately coupled with levulinic acid to give the fully protected thioglycoside **133** in good yield (Scheme 2.35). When the 4-*O*-benzyl rhamnosyl

thioglycoside equivalent of **133** was deacetylated the lev group at the 3-hydroxyl remained intact. However with the 4-*O*-methyl compound **133** both the acetyl and the lev were removed. The reaction was monitored by TLC and only two spots were ever observed (the starting material **133** and the deprotected thioglycoside **95**). This result indicates that both groups were simultaneously removed.

Scheme 2.35 Further functionalization of a 4-*O*-methyl rhamnosyl thioglycoside



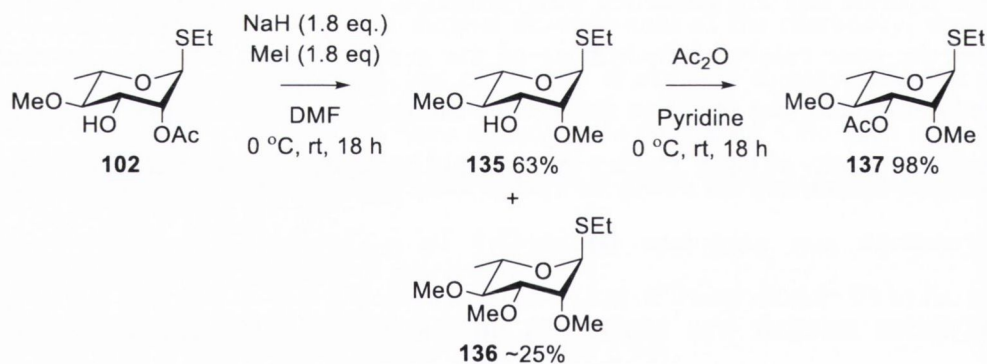
At this stage several options were available for proceeding with the synthesis and it was decided to methylate thioglycoside **100** using a limited quantity of methyl iodide in order to observe the result. It was postulated that one position could be alkylated preferentially since for the acetyl to be removed water must be present and the methylation reaction is performed under anhydrous conditions. The methylation reaction was performed in DMF using methyl iodide and sodium hydride and was quenched with methanol. The methylation reaction should occur faster than the base catalysed hydrolysis of the acetyl. Should the main product be the dialkylated compound, the selective removal of the acetyl could be readdressed by adding a stoichiometric quantity of base. The lev group could be replaced with an alternative protecting group.

The methylation reaction was carried out on thioglycoside **102** and the monoalkylated compound **135** was obtained as the major product along with the dialkylated **136** and some deprotected **95** (Scheme 2.36). As in the case of the benzyl equivalent **108** the column was optimised for the isolation of the target thioglycoside **135** meaning that the yields of the dialkylated **136** were inaccurate. The side product, the permethylated **136**, also formed part of the rhamnosyl thioglycoside library. It can be rationalised that with limited quantities of methyl iodide present the mono alkylate product should predominate as there is not enough methyl iodide to give a majority of the permethylated product. The regioselectivity of the reaction is not as would have been initially expected as the 2-hydroxyl was selectively alkylated over the 3-hydroxyl. In characterising the acetylated starting material **102** it was noted that, if left in deuterated chloroform solution overnight, **102** would equilibrate to form a mixture of the 2-*O*-

acetyl and 3-*O*-acetyl compound. This migration of the acetyl groups is expected and is catalysed by acidic or basic conditions. It was attempted to limit this by keeping **102** in very cold conditions. As discussed above, the benzyl version **104** was reacted as soon as possible to limit this migration. When alkylating **102** the sodium hydride was added prior to the methyl iodide meaning that the base catalysed migration would have time to occur before methylation. Additionally it may be possible that some very small quantity of water was present and if this was the case it would have further promoted the migration of the acetyl group. This migration would have favoured an equatorial position for the acetyl leaving the axial 2-hydroxyl free to be methylated. Additionally the 2-hydroxyl group is closer to the ring oxygen and hence more acidic which would make it more reactive under these alkylation conditions.

Overall the methylation of **102** was less selective than the alkylation of the lev functionalised thioglycoside **107**. This is likely due to the use of the more labile acetyl group, together with the observed migration. For thioglycoside **135** to act as a donor it was acetylated to give the fully protected thioglycoside **137**. The acetylation was accomplished almost quantitatively.

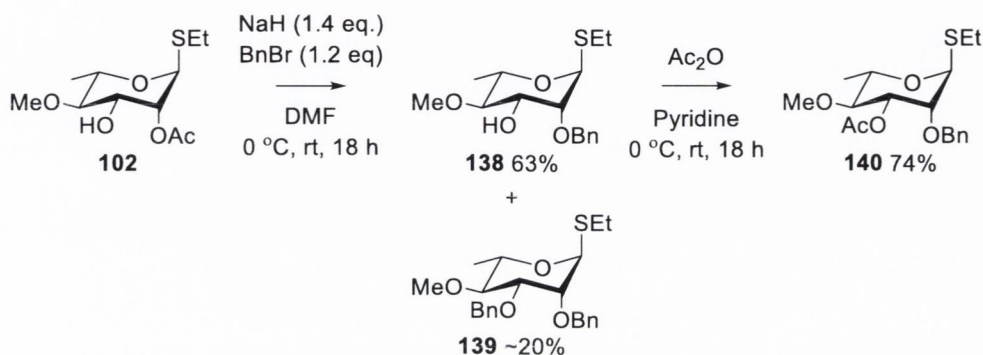
Scheme 2.36 Methylated derivatives of a 4-*O*-methyl rhamnosyl thioglycoside



Since the methylation reaction was reasonably successful, thioglycoside **102** was also benzylated (Scheme 2.37). The benzylation of **102** proceeded in much the same manner as the methylation (Scheme 2.36) and as with **136**, the dialkylated compound **139** was formed as a side product. As with other similar alkylation reactions the dialkylated side product was part of the thioglycoside library and thus a synthetic target in its own right. As with the methylation reaction the 2-hydroxyl was selectively functionalised and the above rationalisation can also be put forward for this reaction.

The acetylation of **138** to give the fully protected thioglycoside **140** was not as high yielding as the equivalent reaction with the methylated compound (Scheme 2.37). This was likely due to the steric bulk of the 2-*O*-benzyl hindering the rhamnosyl 3-hydroxyl.

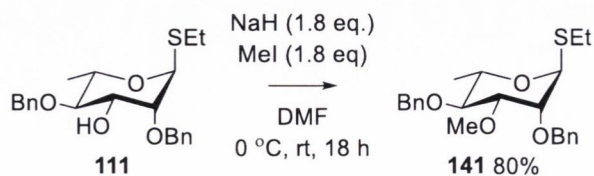
Scheme 2.37 Benzylated derivatives of a 4-*O*-methyl rhamnosyl thioglycoside



Though successful, the above reaction scheme was not very high yielding. At the time, only small quantities of the thioglycosides were required so the route sufficed. However, if, at a later date, larger quantities are required the route can be reassessed and if necessary modified.

Although many rhamnosyl thioglycosides had been synthesised the library did not contain all the possible methylated variations. Many of these variants could be readily synthesised from thioglycoside intermediates already prepared. One such compound was the thioglycoside necessary to generate a *p*-HBAD or similar derivative with a 3-*O*-methyl group. The relevant thioglycoside **141** was generated from one of the intermediates discussed above, the dibenzylated compound **111**, by a single methylation reaction (Scheme 2.38). The yield was not as high as with other rhamnosyl thioglycoside methylation reactions likely due to the steric bulk of the flanking benzyls.

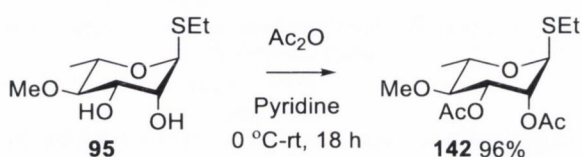
Scheme 2.38 Synthesis of a 3-*O*-methyl rhamnosyl thioglycoside



Though 4-*O*-methyl derivatives could be prepared from thioglycoside **139** it may be considered more convenient to use more labile protection groups. The isopropylidene protected

compound **94** could also be used but the aqueous acid catalysed deprotection can be incompatible with functionalised aromatic anomeric groups. The 4-*O*-methyl thioglycoside **95** was acetylated to give compound **142** in an efficient and high yielding reaction (Scheme 2.39). The product was sufficiently pure to require only minimal purification in order to remove all traces of pyridine. Besides being readily labile, acetyl protecting groups are anchimeric and can help to promote the formation of the α production when compound **142** is used as a donor in a glycosylation.

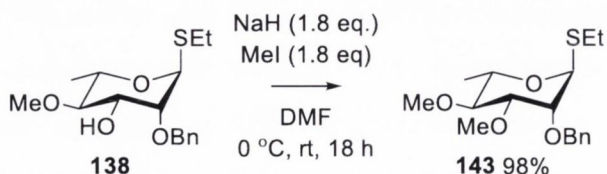
Scheme 2.39 Synthesis of a 4-*O*-methyl rhamnosyl thioglycoside



The 2,3-di-*O*-methylated derivatives could be accessed as 2,3-di-*O*-methylated rhamnosyl thioglycoside **109**, had already been synthesised as a side product of the methylation to generate the monomethylated **108**. The similar 3,4-di-*O*-methylated rhamnosyl derivatives could be synthesised by the rather lengthy process of deacetylation after glycosylation with compound **140** as the donor followed by methylation. In this route several protecting group manipulations would be performed after a relatively low yielding glycosylation. It would be more prudent to introduce the methylation pattern prior to carrying out a glycosylation.

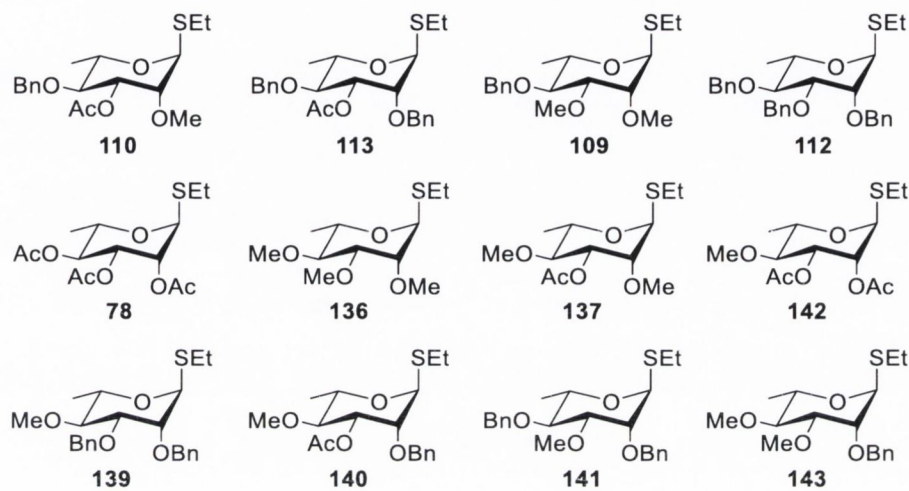
The previously prepared thioglycoside **138** was methylated to compound **143** in an almost stoichiometric reaction (Scheme 2.40). The 3-hydroxyl of compound **138** is less hindered than that of the 2,4-di-*O*-dibenzylated **11** and as a result the methylation of compound **138** was significantly higher yielding.

Scheme 2.40 Synthesis of a 3,4-di-*O*-methylated rhamnosyl thioglycoside



At this stage the library of rhamnosyl thioglycosides could be regarded as being almost complete (Figure 2.5). The methodology developed in the preparation of this thioglycoside library could allow for any necessary alternative rhamnosyl thioglycosides to be prepared.

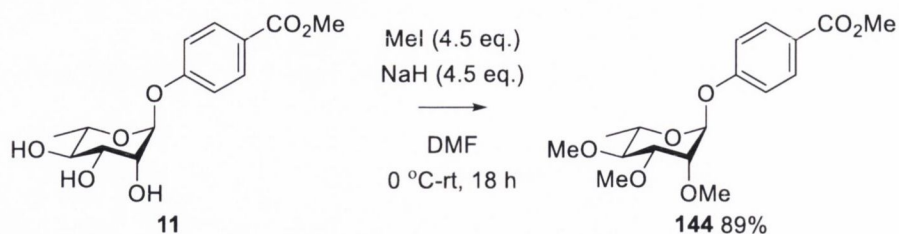
Figure 2.5 Library of rhamnosyl thioglycosides prepared



2.6 Synthesis of a library of *p*-HBADs

Since the library of rhamnosyl thioglycoside donors was now complete it was necessary to address the synthesis of a variety of *p*-HBADs. A major aim of this project was to investigate the immunological activity of the native *p*-HBADs: UM- *p*-HBAD, *p*-HBAD I and *p*-HBAD II. During this investigation one of the most interesting results would be determining the difference in observed activity between UM- *p*-HBAD and *p*-HBAD I. The reason for this is that the only difference between the two compounds in the presence or absence of a methoxy at the 2-position. These investigations are described and discussed separately in Chapter 4. In the native *p*-HBADs and PGLs the methylation pattern is conserved and it may be vital for the biological activity of the compounds in question. It now remains to determine the importance of the methylation pattern, for example is there some significance to each position and does the presence of an increased number of methoxy groups increase or decrease the observed activity? In order to investigate these compounds and compare their activities *p*-HBADs with various methylation patterns need to be synthesised. The thioglycoside library whose preparation is discussed above allows for the preparation of a library of *p*-HBADs with the required variation.

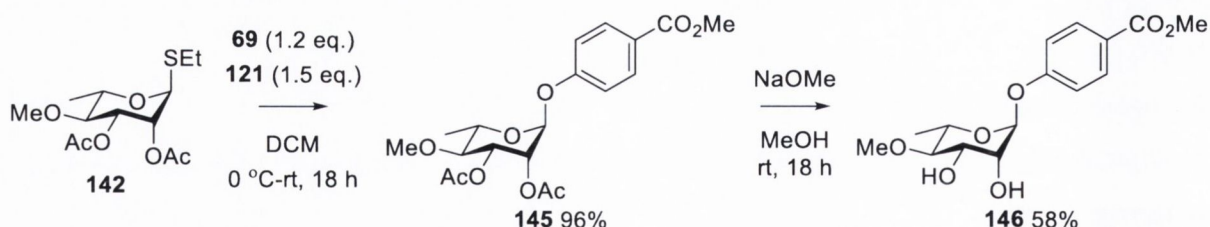
Scheme 2.41 Synthesis of permethylated rhamnosyl *p*-HBAD



The methylated *p*-HBAD **144** could be synthesised by glycosylating methyl-4-hydroxybenzoate **69** with thioglycoside **146**. However, since the target molecule did not have a specific methylation pattern, it was decided to methylate the fully deprotected UM-*p*-HBAD **11** (Scheme 2.41). The methylation proceeded efficiently and gave the target *p*-HBAD **144** in good yield with facile purification. The *p*-HBAD **144** is essentially the fully methylated version of UM-*p*-HBAD so its biological activity is of interest.

In *p*-HBAD I and mycoside B the carbohydrate moiety is the same and consists of a 2-*O*-methylated rhamnosyl residue. The activity of *p*-HBAD I and UM-*p*-HBAD only provide some information on the role of the 2-*O*-methyl. For a more in depth understanding of the effect of the 2-*O*-methyl, the activity of *p*-HBADs whose only difference is the location of the methoxy can be compared. The synthesis of *p*-HBAD I has already been discussed and it now remains to discuss the synthesis of *p*-HBADs with methoxy groups at varying positions.

Scheme 2.42 Synthesis of 4-*O*-methyl rhamnosyl *p*-HBAD



The 4-*O*-methyl rhamnosyl *p*-HBAD **146** was generated from the diacetylated thioglycoside **142** (Scheme 2.42). Possibly due to the aid of the 2-*O*-acetyl anchimeric group of compound **142**, the glycosylation proceeded quite cleanly and in very high yield to give the α compound **145**. A facile Zemplén deacetylation gave the target *p*-HBAD **146**.

Scheme 2.43 Synthesis of 3-*O*-methyl *p*-HBAD



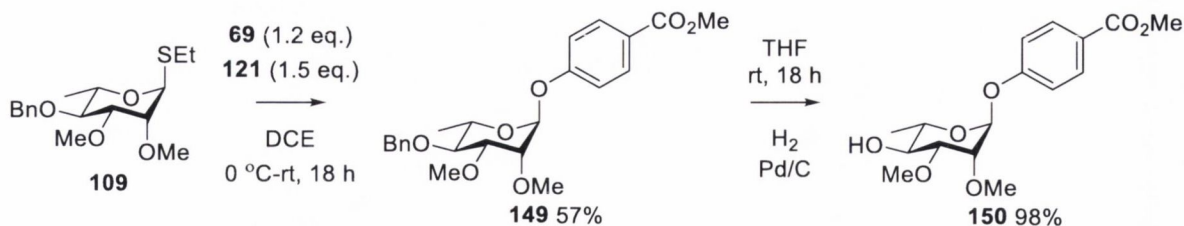
The 3-*O*-methyl rhamnosyl *p*-HBAD **148** was prepared from the previously prepared thioglycoside **141** (Scheme 2.43). The yield of *p*-HBAD **147** was lower than would be expected and this was due in part to problems with purification. Despite clear separation of the glycosylation reaction products by TLC analysis, column chromatography gave relatively impure fractions with poor separation. This difficulty in purification may be the reason why none of the β anomer was observed though it may have been formed. However, in the initial TLC there appeared to be only one anomer as the β -rhamnose typically has a slightly lower R_f than the corresponding α anomer.

The three remaining rhamnosyl monosaccharides *p*-HBADs, the dimethylated ones, could all be readily synthesised from previously generated thioglycosides.

The 2,3-di-*O*-methylated rhamnosyl *p*-HBAD **150** is of synthetic interest as it has a somewhat similar substitution pattern to *p*-HBAD II except that the glycosyl moiety at the 3-hydroxyl is replaced with a methyl. Although less sterically bulky, the methyl may mimic the electronic effects of a glycosylated 2-hydroxyl. As a result the activity of *p*-HBAD R110 may be similar or related to the activity of *p*-HBAD I and is consequently of synthetic interest.

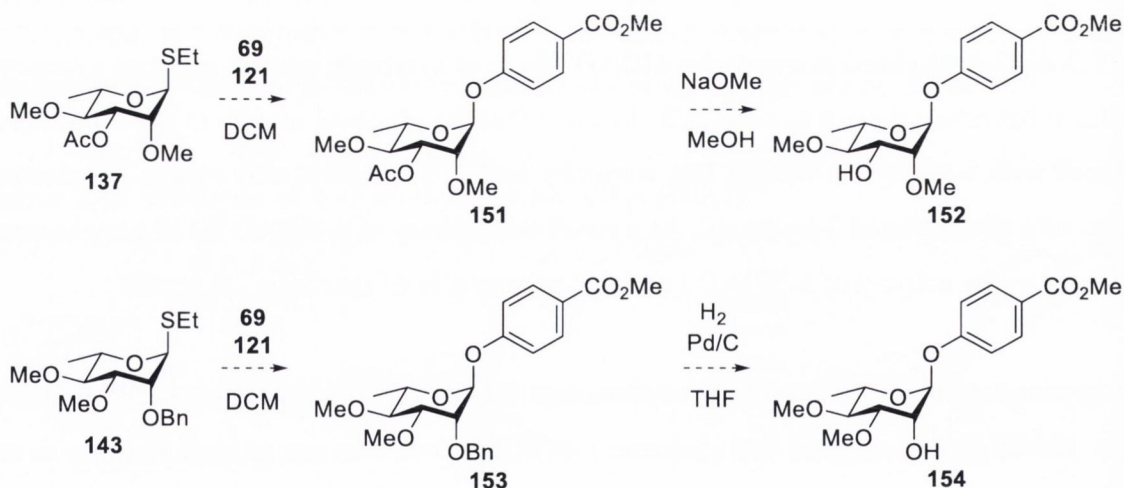
The glycosylation of methyl-4-hydroxybenzoate **69** with 2,3-di-*O*-methylated thioglycoside donor **109** to give compound **149** (Scheme 2.44). The yield was not as high yielding as some similar glycosylations and this may be due to the absence of an anchimeric group at the rhamnosyl 2-hydroxyl. Without anchimeric assistance the glycosylation proceeded less selectively thus lowering the yield of the target α compound. However, the debenzoylation was highly efficient and gave *p*-HBAD **150** in high yield.

Scheme 2.44 Synthesis of rhamnosyl 2,3-di-*O*-methylated *p*-HBAD



At present the remaining two dimethylated rhamnosyl monosaccharide *p*-HBADs have not been prepared. Despite this, should the need arise, they may be readily be prepared from two thioglycoside donors whose synthesis has been described above (Scheme 2.45). The glycosylation of methyl-4-hydroxybenzoate **69** with 2,4-di-*O*-methyl rhamnosyl thioglycoside **137** would give *p*-HBAD **151**. The deacetylation of *p*-HBAD **151** should give the target *p*-HBAD **152**. The Zemplén deacetylation of the rhamnosyl 3-hydroxyl would likely require freshly prepared sodium methoxide to proceed efficiently. Similarly glycosylation of methyl-4-hydroxybenzoate **69** by 3,4-di-*O*-methyl rhamnosyl thioglycoside **143** would give *p*-HBAD **153** and palladium catalysed hydrogenolysis would provide *p*-HBAD **154**.

Scheme 2.45 Proposed synthesis of dimethylated *p*-HBADs



Thus the thioglycoside library prepared can furnish *p*-HBADs as required. A library of both native and non-native *p*-HBADs has been developed from the thioglycoside library. This collection of *p*-HBADs should help to further our understanding of the biological function of this class of molecules. Though mainly monosaccharide *p*-HBADs have been prepared, the synthesis of *p*-HBAD II **9** demonstrates that oligosaccharides can also be accessed using the same library. The biological evaluation of the generated monosaccharide *p*-HBADs should

provide information on the effect of the methylation pattern on the immune-reactivity. Once a better understanding of the monosaccharides is established, a detailed investigation of oligosaccharide containing *p*-HBADs can be carried out.

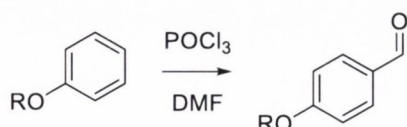
2.7 PGL synthesis

The glycan portions of *p*-HBADs and PGLs tend to be the same and there is evidence that the glycan is the source of the observed biological activity. However, this does not necessarily mean that their activities are the same but they may be related. It would be beneficial to directly compare the activities of *p*-HBADs to both other *p*-HBADs and their PGL equivalents.

As discussed in Section 2.1.5 the native lipid is attached to the carbohydrate moiety through a direct C-C bond. If regarded as a phenolic lipid then the carbohydrate is attached through the more common C-O motif to the aglycan. In the synthesis described herein the molecule was assembled in a stepwise manner with the aromatic moiety being installed prior to the extension of the carbohydrate and later functionalised with the lipid functionality. The proposed method to achieve this attachment was through the use of a Wittig reaction to install the lipid moiety through a C=C bond. The double bond would be reduced during the hydrogenation that would achieve global debenzylation thus the use of a Wittig reaction would not increase the number of synthetic steps in the route. The use of a Wittig reaction as the coupling reaction for lipid attachment necessitates the functionalisation of the aromatic moiety with an aldehyde at the 4-position which can be carried out either pre- or post-glycosylation.

If the aldehyde functionality were to be installed post-glycosylation a Vilsmeier-Haack formylation may be carried out on a phenyl functionalised carbohydrate to give a benzaldehyde functionalised derivative (Scheme 2.46).

Scheme 2.46 Vilsmeier-Haack reaction post glycosylation of phenol

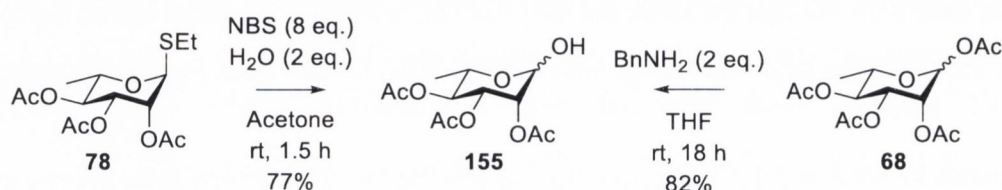


Where R is a glycan

A more facile synthesis of glycosylated benzaldehyde derivatives would be the glycosylation of 4-hydroxybenzaldehyde directly. 4-Hydroxybenzaldehyde is less electron rich than a phenol

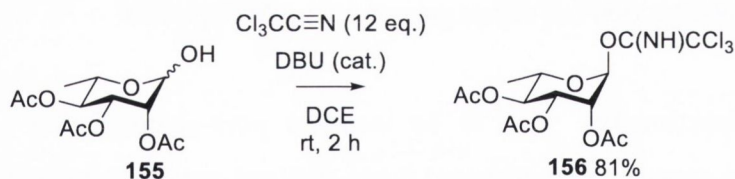
and methyl-4-hydroxybenzoate and is consequently a poor acceptor. The use of a stronger promoter was explored in the synthesis of methylated benzoic acid derivatives described above and another option was also explored here: the use of a trichloroacetimidate donor. A trichloroacetimidate can be generated from other donors by first generating the hemiacetal (Scheme 2.47) then installing the trichloroacetimidates moiety (Scheme 2.48).

Scheme 2.47 Synthesis of a rhamnosyl hemiacetal



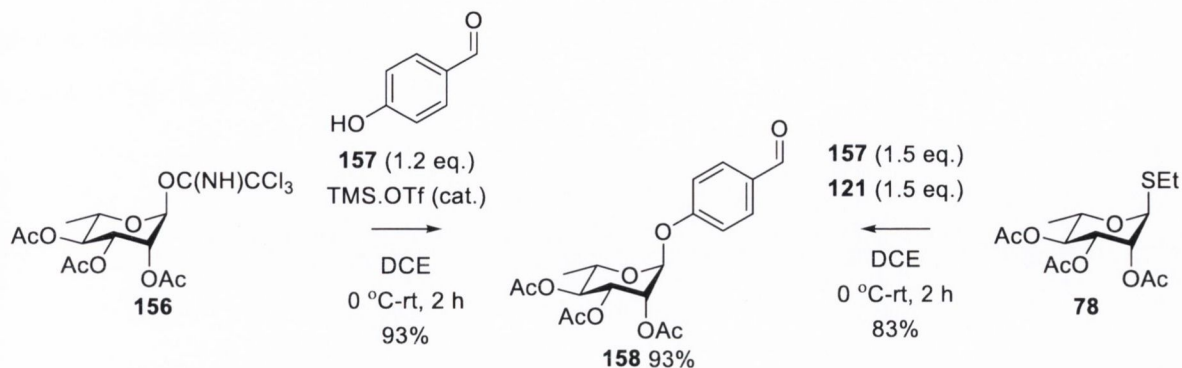
Conversion of thioglycoside **78** into the hemiacetal **155** was initially quite low yielding at 50% when carried out with 90% acetone in water and 4.3 equivalents of *N*-bromosuccinimide (NBS) (Scheme 2.47). When the amount of water was reduced to two equivalents and NBS increased to eight equivalents the yield was increased to 77%. Benzyl amine facilitated hydrolysis of the peracetylated **68** was slightly higher yielding at 82%. The hemiacetal is unstable due to the ability of the acetyls to migrate and was stored cold.

Scheme 2.48 Generation of a rhamnosyl trichloroacetimidate



The trichloroacetimidate donor **156** was generated by reacting the hemiacetal **155** with trichloroacetonitrile in the presence of a base (Scheme 2.48). The identity of the base helps determine the anomer formed, in this case the strongly basic 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was used and gave the α anomer exclusively.¹⁵⁸

Scheme 2.49 Glycosylation of 4-hydroxybenzaldehyde with a rhamnosyl trichloroacetimidate donor and a rhamnosyl thioglycoside donor



The peracetylated rhamnosyl trichloroacetimidate **156** was an effective donor for the glycosylation of 4-hydroxybenzaldehyde **157** to give the benzaldehyde derivative **158** in high yield (Scheme 2.49). The analogous reaction with the acetylated rhamnosyl thioglycoside **78** required the use of the more reactive promoter $\text{Me}_2\text{S}_2\text{-TF}_2\text{O}$ **121** to give the benzaldehyde derivative **158** in good yield. In both cases the α anomer was formed exclusively with none of the β anomer being formed.

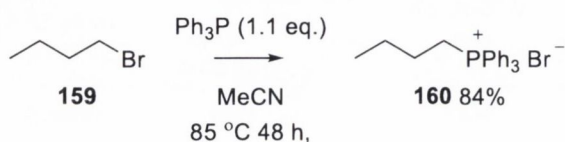
When the more common, NIS/TMS.OTf activator system was employed the yield of the benzaldehyde derivative **158** was extremely low. The more powerful activator allows for the use of the thioglycoside library without the need to convert thioglycosides to the trichloroacetimidate equivalent, though this too can be achieved if necessary.

Since the introduction of the aldehyde ‘handle’ had been successfully addressed, the application of the Wittig reaction for the attachment of the lipid could be explored. For the reaction to be carried out a phosphonium salt of the alkyl group to be attached must be generated. Alkyl phosphine ylides are unstabilised and tend to give the *Z*-alkene product. Since the double bond will be subsequently reduced, the nature of the alkene formed is of little consequence and separation of anomers would not be required. Phosphonium salts are synthesized by reacting an alkyl halide with triphenylphosphine to give the alkyltriphenylphosphonium salt directly.

Initially, a Wittig reaction on the glycosylated benzaldehyde derivative was attempted using a shorter alkyl chain based on the relatively inexpensive and readily available 1-bromobutane

159 (Scheme 2.50). The formation of the phosphonium salt **160** was accomplished in toluene but the reaction was quite low yielding at 28%.¹⁴⁹ However switching to the significantly more polar acetonitrile as solvent increased the yield to 84%.¹⁵⁰ Purification was facile and consisted of concentrating the mixture and washing the residue with diethyl ether in order to remove unreacted starting materials.

Scheme 2.50 Phosphonium salt of 1-bromobutane

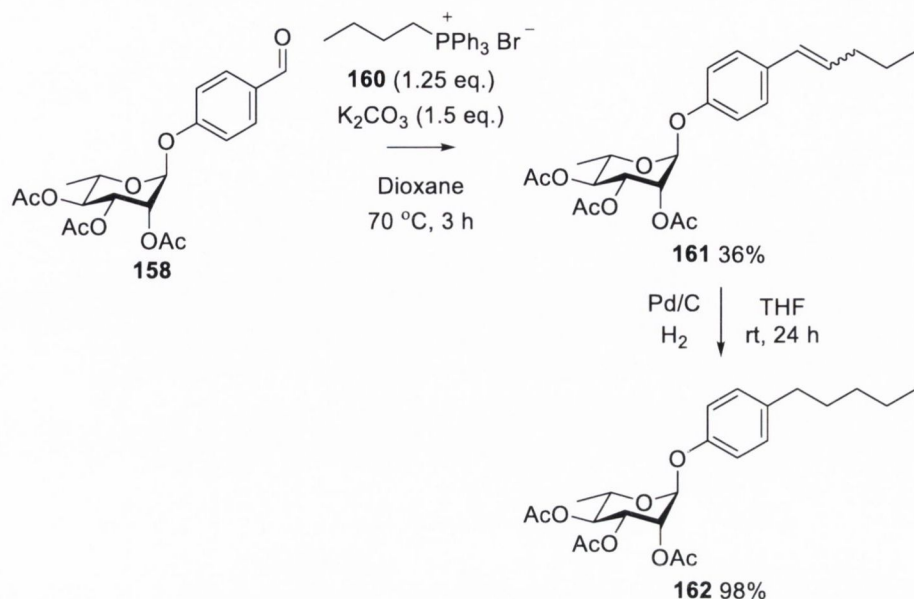


The Wittig reaction itself is more complex and requires more careful consideration of factors such as solvent, temperature and base. To begin with, a simple system of one equivalent of each of the starting materials in dioxane with an excess of sodium hydroxide was used.¹⁴⁹ This was quite low yielding at 15%.

An examination of the literature revealed some general observation on Wittig reactions that could help to increase the yield.¹⁵⁰ Firstly, solvents with a higher dielectric constant tend to give higher yields as they enhance the strength of the base used. Water based reactions tend to be slow due to poorly soluble aldehyde molecules and stabilisation of the reaction intermediates through hydrogen bonding. The addition of small quantities of water to a reaction run in dioxane can actually enhance the yield. As regards the choice of base, hydroxide bases tend to give lower yields than carbonate bases. These reduced yields can be attributed to the formation of secondary products *via* the Cannizzaro reaction and the hydrolysis of the ylide. The nature of the cation also affects the yield as larger cations result in more easily solubilised carbonate bases.

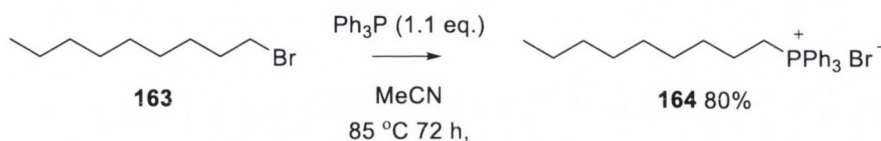
Overall dioxane was retained as solvent and sodium hydroxide exchanged for potassium carbonate (Scheme 2.51). A moderate yield increase was observed at 36% of the alkene **161** and 58% of recovered starting material **158**. The alkene was isolated as a mixture of E and Z isomers at an approximate ratio of 3:2 respectively. The alkene **161** was reduced to the corresponding alkane **162** in almost quantitative yield.

Scheme 2.51 Wittig reaction to generate a five-carbon lipid chain



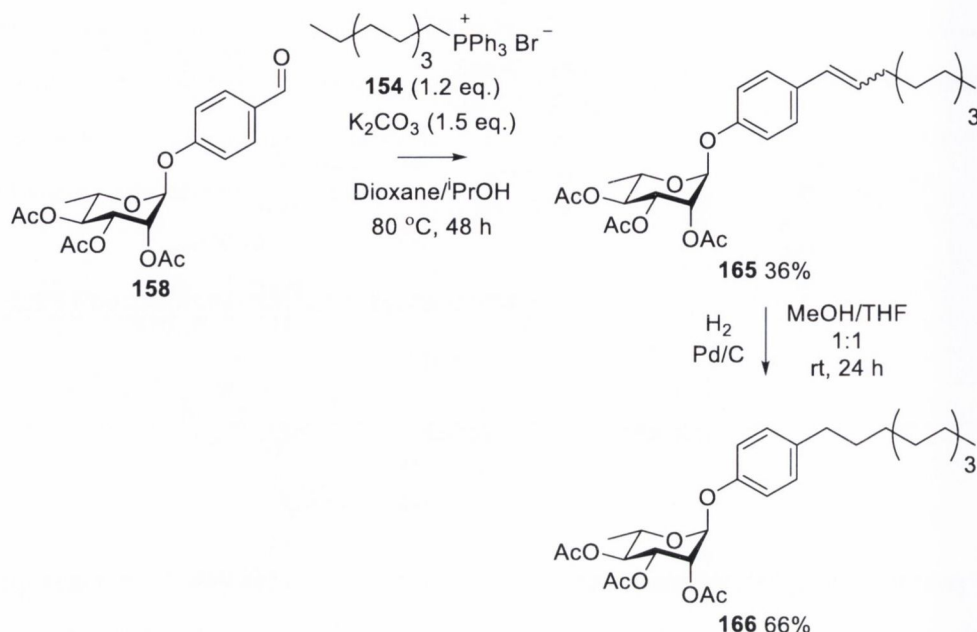
Since this test reaction demonstrated that the synthetic strategy was viable, it was decided to proceed and attempt the reaction with a longer alkyl phosphonium salt. It was suggested that the yield could be improved by longer reaction times. To this end, a nine-carbon alkyl chain **163** was converted to a phosphonium ylide **164** (Scheme 2.52). Possibly due to the length of the alkyl chain, longer reaction times were necessary to achieve comparable yields to those obtained for the four-carbon alkyl chain.

Scheme 2.52 Phosphonium salt of 1-bromononane



The phosphonium salt **164** was reacted with rhamnosyl benzaldehyde **158** in dioxane but there were solubility issues (Scheme 2.53). It was decided that in order to encourage the dissolution of **164**, polar protic isopropanol should be added. The mixture of alkene products **165** was approximately 1:1 E/Z. The ration was of no consequence as the mixture was reduced to the alkane **166** in order to aid characterisation. The attachment of the lipid portion was confirmed by NMR spectroscopic analysis.

Scheme 2.53 Wittig reaction to generate a ten-carbon lipid chain

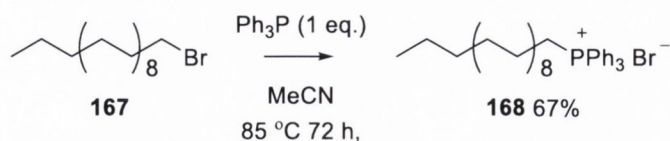


The yield of the alkene product **165** was not very high giving 36%. The mild conditions used allowed for the recovery of 26% of the starting material **158**. The low yield of compound **165** may be increased by decreasing the reaction times and thus reducing the occurrence of side reactions.

Since this route was reasonably successful the principle was applied to an even longer alkyl chain. The native alkyl chain in PGLs is twenty-six to thirty-two carbons in length. The twenty carbon chain bromoalkyl chain seems to be the upper limit for commercially available bromoalkyl chains. Once the carbonyl carbon is taken into account the total length of the alkyl chain would be twenty-one carbons in length which is of comparable length to the native PGL. As previously discussed the alkyl chain is simplified approximation of the native lipid to mimic the native lipid *in vitro* while simplifying the synthesis.

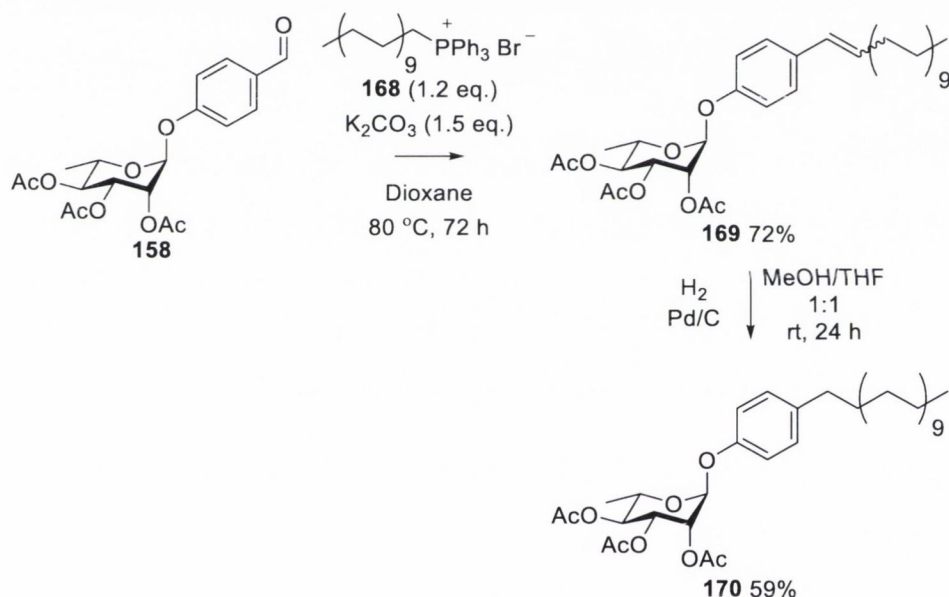
The twenty carbon chain alkyl phosphonium salt **168** was prepared from 1-bromoeicosane **167** (Scheme 2.54). As with the previously prepared phosphonium ylides, minimal purification was required however there was a general trend for lower yields of phosphonium salt with increasing alkyl chain length.

Scheme 2.54 Phosphonium salt of 1-bromoeicosane



The Wittig reaction of the twenty-carbon chain alkyl phosphonium salt **168** with the rhamnosyl functionalised aldehyde **158** did not encounter the same solubility problems as the nine-carbon equivalent (Scheme 2.55). The yield was enhanced by longer reaction times to 72% of a 1:1 mixture of the E and Z isomers. The reduction of the alkene **169** to the alkane **170** was somewhat low yielding at 59 % but was sufficient to allow access to the desired PGL analogue **170**.

Scheme 2.55 Wittig reaction to generate a twenty-one-carbon lipid chain

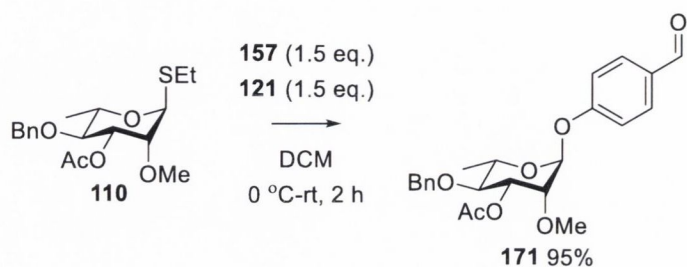


The above reactions served as a proof of principle, demonstrating the utility of the Wittig reaction for the synthesis of PGL mimics. The yields were not high but the additional purpose of these test reactions was to identify potential problems and improve yields. Further optimisation could be carried out prior to scale up.

The two main PGL targets were the native compound: mycoside B and PGL-tb1. Mycoside B is the simpler of the two and it was the first synthetic target once the synthetic route had been developed. Thioglycoside **110** was used to glycosylate benzaldehyde **157** using Me_2S_2 - Tf_2O **121** as the activator (Scheme 2.56). The rhamnosyl functionalised benzaldehyde **171** was

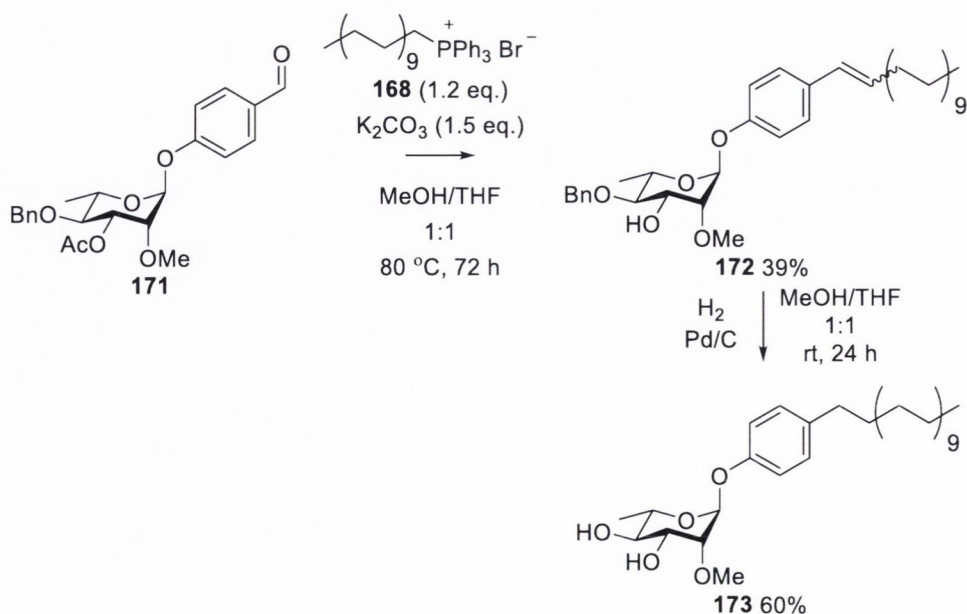
obtained in good yield with no β anomer being observed. The bulk of the acceptor and donor together with the relatively low temperature used may have contributed to the predominance of the 1,2-*trans* α product.

Scheme 2.56 Preparation of a functionalised rhamnosyl benzaldehyde derivative



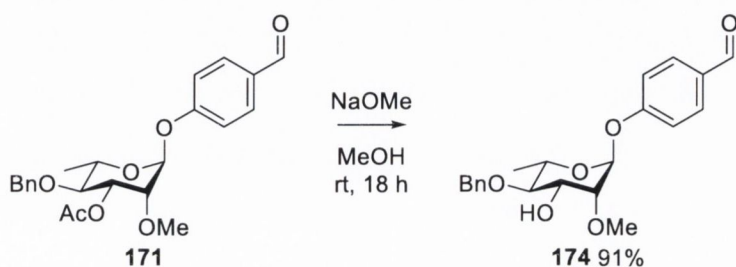
In the synthesis of mycoside B it was not necessary to remove the benzyl protecting group from compound **171** prior to the Wittig reaction due to the basic conditions employed to attach the lipid (Scheme 2.57). Initially, dioxane was used as the solvent for the Wittig reaction but proved ineffective possibly due to poor solubility of the phosphonium salt. Though methanol was also investigated as a solvent it was found that a 1:1 mixture of methanol and tetrahydrofuran (THF) gave the highest yield at 39%. This yield can potentially be improved further by longer reaction times and more forcing conditions. The reduction of the alkene **172** simultaneously removed the 4-*O*-benzyl to give the mycoside B mimic **173**.

Scheme 2.57 Synthesis of a mycoside B mimic



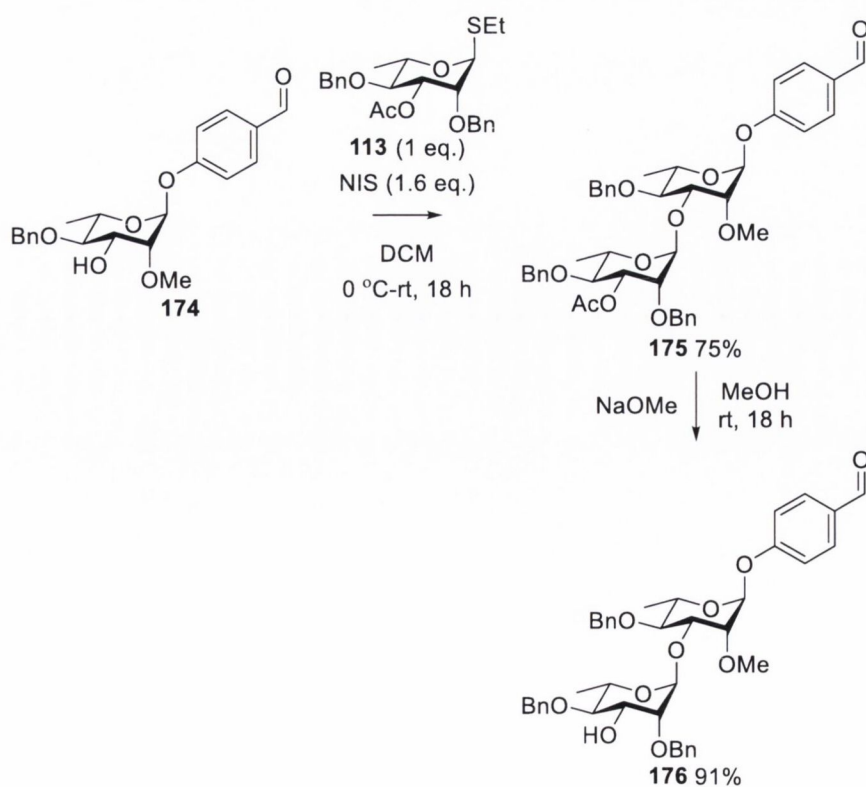
A PGL-tb1 mimic was the next synthetic target and this required the assembly of the trisaccharide glycan portion. The previously prepared glycosylated benzaldehyde derivative **171** was deacetylated using freshly prepared sodium methoxide to give the acceptor **174** (Scheme 2.58).

Scheme 2.58 Deacetylation of a rhamnosyl glycosylated benzaldehyde derivative



For the glycosylation of acceptor **174** by thioglycoside donor **113**, as with the synthesis of *p*-HBAD II, the activator used was NIS aided by TMS.OTf (Scheme 2.59). The yield for the glycosylation to product **165** at 75% was higher than those reported previously though lower than for the *p*-HBAD molecule **129** (91%). A similar rationalisation for the absence of the β anomer, based on the steric bulk around both the acceptor and the donor, also applies here.

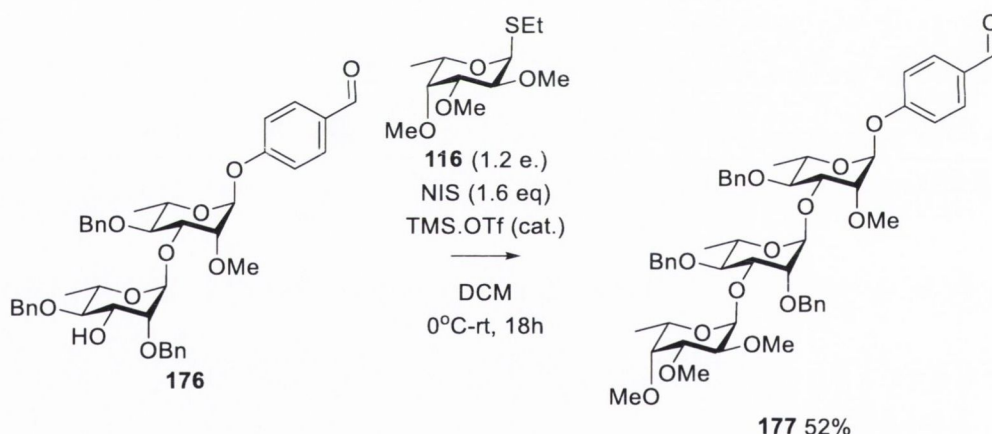
Scheme 2.59 Generation of a disaccharide functionalised benzaldehyde



Freshly prepared sodium methoxide was used to deacetylate the disaccharide **175** to form the disaccharide acceptor **176** in 91% yield. This was a similar yield to the analogous *p*-HBAD reaction which gave compound **130** in 95% yield. The freshly prepared sodium methoxide shortens the reaction time compared to commercially available sodium methoxide.

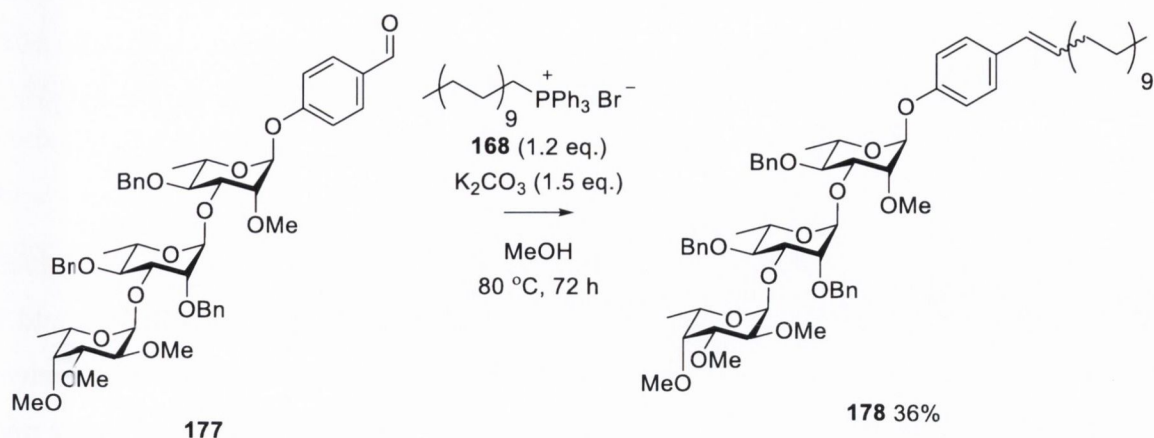
The final glycosylation reaction in the preparation of the trisaccharide was achieved using NIS/TMS.OTf activating conditions (Scheme 2.60). The disaccharide acceptor **176** was glycosylated with the methylated fucosyl thioglycoside **116** to give the protected trisaccharide **177**. The yield of trisaccharide **177** (52%) was slightly lower than for the *p*-HBAD analogue **131** (62%). However this was a difficult glycosylation due to the hindered rhamnosyl 2-hydroxyl of compound **176** with flanking benzyls. As with the *p*-HBAD equivalent, the absence of anchimeric assistance would have helped promote the formation of the α anomer and may be why there was no β observed.

Scheme 2.60 Preparation of the PGL-tb1 trisaccharide



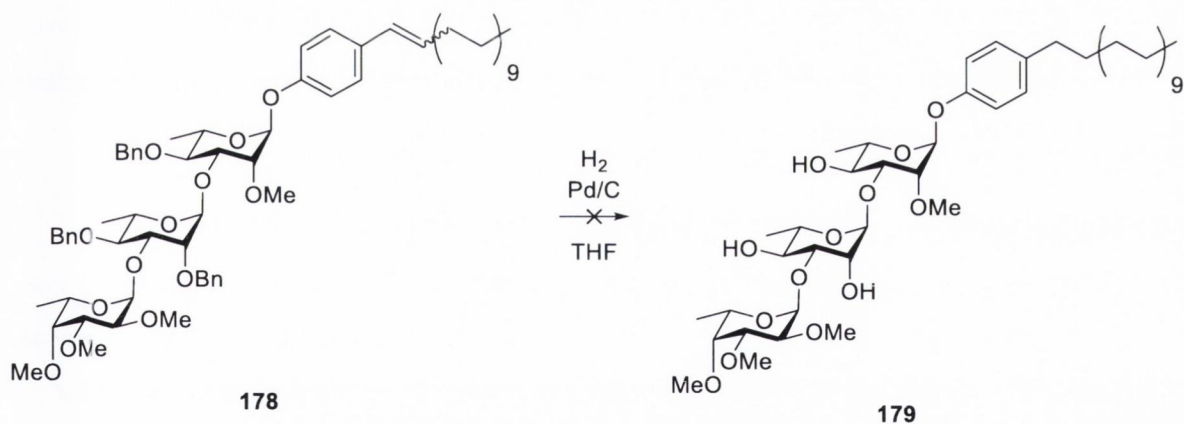
The Wittig reaction of PGL-tb-1 trisaccharide **177** with the twenty-carbon chain alkyl phosphonium salt **168** was not expected to be facile given that two large compounds were being coupled (Scheme 2.61). Though no solubility issues were observed it may be possible to increase the yield of compound **178** from the observed 36%. Though the reaction was repeated a similar result was obtained and in both cases a large quantity of the starting material was recovered and the product was isolated as an approximately 1:1 mixture of anomers. Since starting material was isolated more forcing conditions with higher temperatures and longer reaction times may help increase to yield of compound **178**.

Scheme 2.61 Wittig reaction to generate a PGL-tb1 mimic alkene precursor



The double bond generated during the Wittig reaction to form alkene **178** was reduced during the global deprotection (Scheme 2.63). Part way through the reaction, after TLC analysis was inconclusive, the reaction was worked up by filtering off the palladium and concentrating the reaction mixture.

Scheme 2.63 Global deprotection and reduction of alkene double bond



1H NMR spectrum indicated that the double bond had been reduced during the hydrogenation, however the debenzyl reaction had only partially progressed as a strong benzyl signal was observed in the aromatic region of the 1H -NMR. The reaction was repeated with more forcing conditions involving bubbling hydrogen through the reaction solvent for six hours. After TLC analysis indicated that the benzyl groups had been removed the reaction was again worked up and purified by column chromatography. Several fractions were isolated although 1H NMR analysis did not detect carbohydrate functionalities in any of the fractions. Unfortunately this result indicated that the conditions used were too harsh.

Though the preparation of the PGL-tb1 mimic was not successful, the difficulties were only encountered at the last reaction and this reaction could be repeated with alternative debenzylolation conditions to give the target compound **168**. Alternatively the benzyl groups could be removed prior to the Wittig reaction.

The glycosylation of 4-hydroxy benzaldehyde **157** was accomplished with several thioglycosides indicating that a library of glycosylated benzaldehyde derivatives could be prepared. A Wittig reaction was successfully applied to several glycosylated benzaldehyde derivatives. A mycoside B mimic was successfully prepared indicating that the synthetic route developed could be applied successfully to a variety of glycosylated benzaldehyde precursors.

As with the library of *p*-HBADs, the immunological properties of mimics of native PGLs could be assessed *in vitro* however it would perhaps be appropriate to determine whether or not the simplified lipid portion introduced is appropriate. Potentially commercial mycoside B could be obtained and compared directly to the mycoside B mimic prepared above. The results of such an investigation would indicate whether or not the lipid chain used in the PGL mimics is appropriate and if it alters the observed biological activity. Should the PGL mimics prove an acceptable approximation of the rather complex native compounds a library could be prepared and their activities examined.

2.8 Future work

A large library of rhamnose thioglycosides has been prepared. Though a lot of progress has been, made the library of thioglycosides will not be truly completed until a library of fucose thioglycosides is prepared. This would be a large undertaking but could employ a similar strategy to that used for rhamnose.

A library of rhamnose thioglycosides was been prepared and this allowed for the synthesis of a library of *p*-HBADs. The library of *p*-HBADs is not complete, however, biological investigations will aid in directing these future syntheses. The results of preliminary biological investigations are presented in Chapter 4 though more work still remains to be done in building an understanding of the structure-activity relationship of *p*-HBADs.

The deprotection of the PGL-tb1 mimic could be repeated in order to obtain compound **179**. The library of thioglycosides can be used to prepare a library of PGLs allowing for the investigation of their biological properties. If a sample of native mycoside B, both it and the synthetic mycoside B mimic can be tested and the results compared. If comparable results are obtained it would be reasonable to conclude that the alkyl chain is a sufficiently accurate approximation.

3.0 Chapter 3: Immobilisation of phenolic carbohydrates on surfaces

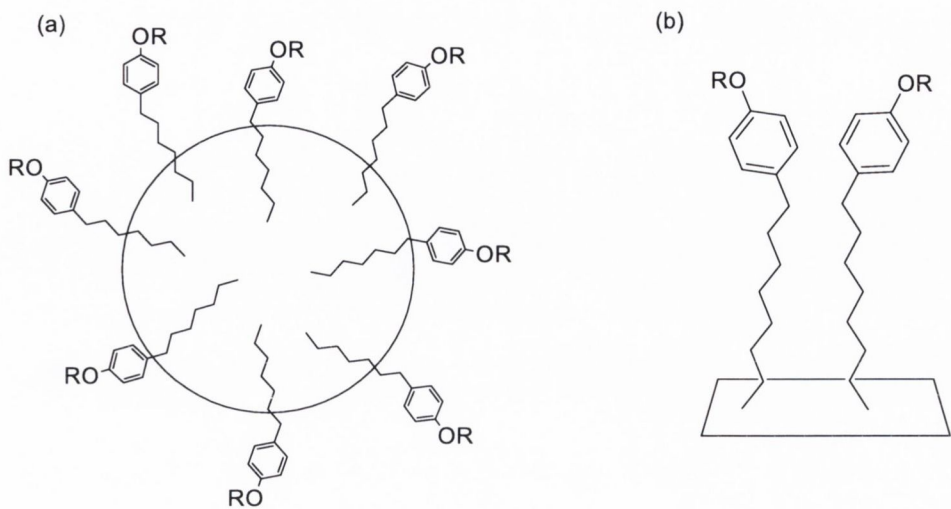
3.1 The importance of immobilised carbohydrates

As previously discussed in Chapter 1, the immobilisation of carbohydrates can allow for more rapid and facile investigation of their properties. It also reduces the quantities of carbohydrate required for such investigations which can often be a limiting factor when the carbohydrate under investigation is a complex oligosaccharide. These advantages mean that the development of immobilisation methods for phenolic carbohydrates, could allow for more facile investigation of PGL and *p*-HBAD biological properties.

Carbohydrate functionalised surfaces can be used for *in vivo* investigations, although carbohydrate functionalised particles may be more versatile as they can theoretically be used both *in vitro* and *in vivo*. Initially *in vitro* studies are necessary to gain some basic knowledge of the biological properties of the prepared compounds. For a better understanding of their properties and mode of function *in vivo* studies may be required. Since these kinds of studies require rather large quantities of the synthetically complex sugars it is for *in vivo* studies that immobilised carbohydrates may be particularly advantageous.

The most facile method to immobilise PGL like molecules would be through noncovalent interactions with the lipid moiety (Figure 3.1). Immobilisation in this manner is not exclusive to surfaces and can be applied to nanoparticles as well. However, such methods have associated disadvantages as discussed in Chapter 1. Additionally the preparation of PGL-like molecules is lengthy; and shorter, more facile routes to immobilised carbohydrates would be preferable.

Figure 3.1 Noncovalent immobilisation of PGL-like molecules on (a) a nanoparticle and (b) an appropriate surface. R = carbohydrate



Covalent immobilisation techniques are more appealing for many reasons, mainly the overall stability of the system. Several methodologies for covalent immobilisation of *p*-HBADs and PGLs were investigated, all of which have a functionalised aromatic moiety at their core. The aromatic ring is important for emulating the native carbohydrate environment. Additionally, the aromatic linker serves as a rigid support for the carbohydrate moiety keeping it suspended above the surface. This presentation may allow for better interaction with receptor proteins

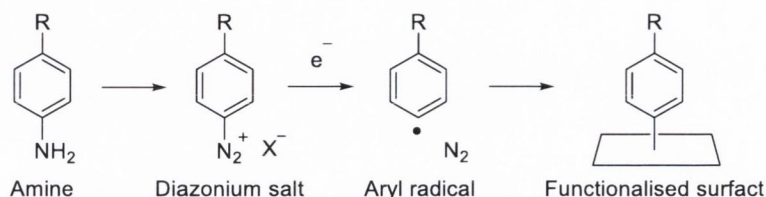
3.2 Diazonium grafting

Initially, diazonium grafting was developed as an electrochemical technique by Delamar *et al* who used aryl diazonium salts to functionalise electrodes.¹⁵⁹ A diazonium salt was dissolved in an aprotic medium with an electrolyte or in aqueous acid and reduced using the target surface as the cathode.¹⁶⁰ The reaction proceeds quickly and requires low potentials that leave other reducible groups untouched. The strong electron-withdrawing effect of the diazonium group contributes to the ease of reduction of the compound. The diazonium starting materials are facile to synthesise from amine precursors *via* a variety of well-established methods. Diazonium grafting has been employed to functionalise many materials including carbon,¹⁶¹ semiconductors¹⁶² and metals.¹⁶³

The overall reaction that takes place in diazonium mediated functionalisation of a surface material is electron transfer from the surface or reagents to the diazonium to generate a radical resulting in dinitrogen cleavage (Scheme 3.1). The aryl radical is generated at the cathode

surface which it then reacts with. The absence of the N_2^+ stretch at $2300\text{--}2130\text{ cm}^{-1}$ in the IR spectrum of a surfaces after diazonium grafting indicates that diazonium salt are not just adsorbed onto a surface.

Scheme 3.1 Diazonium grafting



The possibility of a radical anion mediated reaction is excluded as diazonium salts that could not form radical anions can be grafted. Furthermore, radical-anions have been generated from aromatic and haloaromatic compounds with no resultant grafting. The bonds formed by diazonium grafting are not disrupted by temperatures that would affect hydrogen bonding or van der Waals interactions indicating that they are covalent. Ultrasonic cleaning in various solvents is also ineffective. The aryl layer can, however, be removed mechanically by polishing or scratching the surface. The nature and orientation of surface bonded groups can be characterised by various method. Voltammetry and IR spectroscopy can provide information on the surface groups including their orientation. Atomic force microscopy (AFM) and scanning tunnelling microscopy (STM) can show the layer on a surface as changes in height and texture.

It has been demonstrated that electrochemistry is not a prerequisite for surface functionalisation using diazonium salts. The reaction can occur spontaneously and simple immersion in a diazonium solution is often adequate.¹⁶⁴⁻¹⁶⁶ Due to the difficulty associated with isolating diazoniums the amine group can be converted to a diazonium *in situ*.

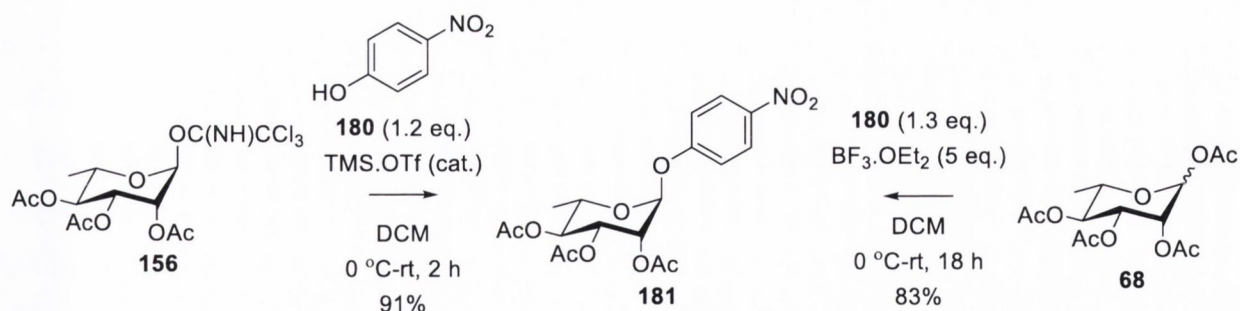
The main synthetic challenge in the proposed methodology is the preparation of the aromatic amine functionalised carbohydrate starting material for the diazonium reaction. Two main routes to the amine were explored: the first was based on functional group interconversion from the aromatic nitro post glycosylation; the second was centred on generating protected aniline derivatives that could be glycosylated then unmasked. The advantage of using the nitro group is that it is quite inert to many reaction conditions allowing for various chemical manipulations to be carried out on the carbohydrate post glycosylation. If the latter strategy is employed the

protecting group used must fully disguise the amines reactivity for a glycosylation to be carried out.

3.2.1 Nitro aromatics as aniline precursors

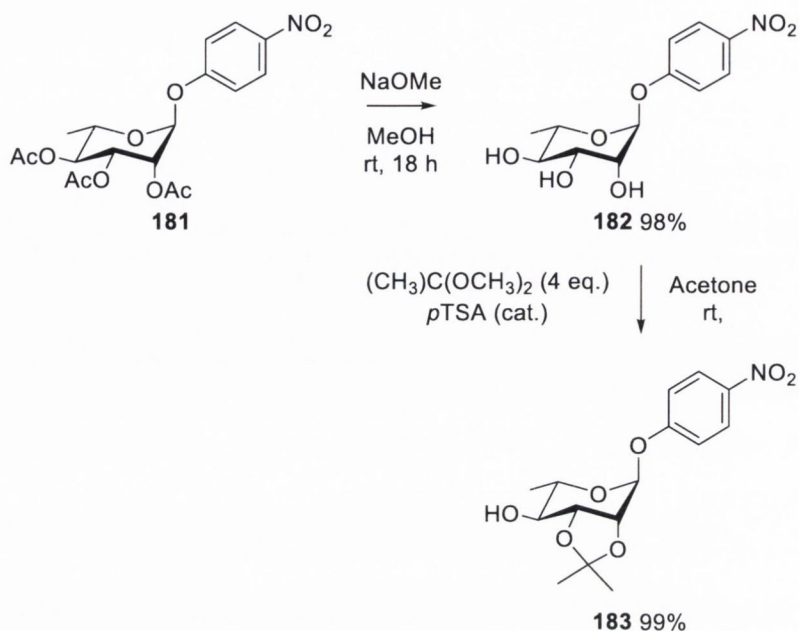
The first challenge to be addressed was the glycosylation of an aromatic group bearing a nitro moiety. Several methods are available for the glycosylation, however it would be preferable to use thioglycosides to take advantage of the pre-prepared library of donors discussed in Chapter 2 of this thesis. Due to the electron withdrawing nitro moiety, 4-nitrophenol **180** is a poor acceptor. Several glycosylations were carried out using a variety of rhamnosyl donors. The acetylated rhamnosyl thioglycoside **78** proved an ineffective donor for the glycosylation of 4-nitrophenol **180** when NIS/TMS.OTf was used as the activator system. However, the peracetylated rhamnosyl donor **68** together with $\text{BF}_3 \cdot \text{OEt}_2$ as activator gave the target glycosylated nitrophenol **181** in good yield. The α anomer was produced exclusively due to the anomeric effect in conjunction with the participating neighbouring acetyl (Scheme 3.2). It was postulated that the yield could be increased further by using the more reactive rhamnosyl trichloroacetimidate donor **156**. The glycosylation using donor **156**, though higher yielding, was not sufficiently high to compensate for the additional reactions necessary to generate the trichloroacetimidate. With both donors the glycosylation were high yielding with none of the β anomer of **181** observed through anchimeric assistance. The purification of α anomer was facile.

Scheme 3.2 Glycosylation of 4-nitrophenol by two different rhamnosyl donors



Due to the unreactive nature of the nitro group it should be inert under most protecting group manipulation conditions. It was postulated that a protecting group on one of the rhamnose hydroxyls may help to identify the orientation of the carbohydrate residue once on a surface. The rhamnosyl 4-hydroxyl can be easily accessed through the use of an isopropylidene protecting group.

Scheme 3.3 Protection of the 2- and 3-hydroxyls of rhamnose to allow for the regioselective functionalization of the 4-hydroxyl

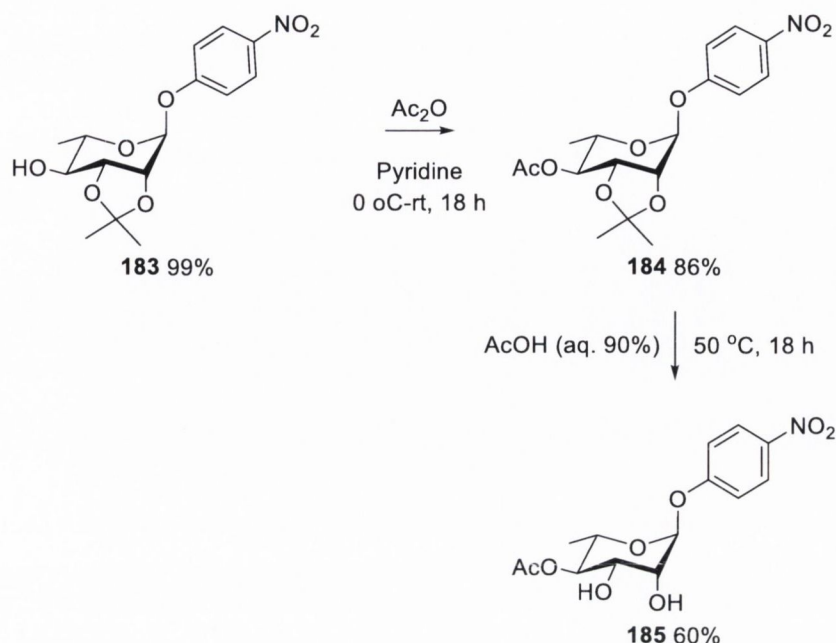


Some basic protection, deprotection steps were performed on the glycosylated nitrophenol **181** (Scheme 3.3). The first reaction was a Zemplén deacetylation to give the deprotected rhamnosyl product **182**. The isopropylidene was introduced with ease and in high yield to give the partially protected **183**. In this manner the rhamnosyl 4-hydroxyl of the rhamnosyl glycosylated nitrophenol **183** was left free to be functionalised.

There is a wide variety of protecting groups to choose from and it was decided to use a protecting group that is simple to introduce but clearly visible by spectroscopic techniques. The acetyl is easily introduced and should be stable under the conditions required to generate the nitrogen leaving group. Though acetyl migration is promoted by acidic and basic conditions, it should not occur in the target compound **185** due to the orientation of the rhamnosyl hydroxyls. The rhamnosyl 4-hydroxyl is equatorial, as is its 3-hydroxyl neighbour. An acetyl group migrates through a series of orthoester formations and openings. For the orthoester to form, vicinal *cis* diols are required to form the orthoester and as such the rhamnosyl 4-*O*-acetyl is not able to migrate.

The acetyl was introduced under standard acetylation conditions to give the fully protected rhamnosyl functionalised 4-nitrophenol **184** (Scheme 3.4). Having served its purpose, the isopropylidene of **184** removed by refluxing in aqueous acid to give the acetylated target **185**.

Scheme 3.4 Regioselective acetylation of the rhamnosyl 4-hydroxyl



Though the thioglycoside activation conditions described above were not successful, the previously described alternative activation conditions $\text{Me}_2\text{S}_2\text{-OTf}$ may offer an alternative synthetic route. This would allow for the use of the thioglycoside library discussed in Chapter 2. If a thioglycoside was used to glycosylate the 4-nitrophenol the functional group manipulations could be performed prior to the glycosylation reaction. Besides generating the acetylated target compound **185**, the synthetic route discussed above also served to demonstrate the stability of the aromatic nitro moiety under several reaction conditions. Thus, the protecting group manipulations could alternatively be performed post glycosylation.

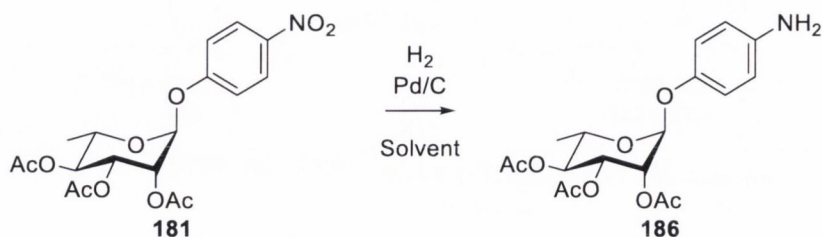
3.2.2 Reduction of an aromatic nitro to an amine

The next challenge was the reduction of the nitro group to an amine and this was more difficult to achieve. Initially palladium on carbon was used together with an atmosphere of hydrogen (Scheme 3.5). The reaction did not proceed to an appreciable extent despite the use of several solvent systems, including methanol, THF and mixed solvent systems of methanol with THF. The pressure of H_2 used was also varied between one and three atmospheres but this too did not improve the reaction.

Overall, the Pd catalysed hydrogenations appeared to give some product but the reaction was non-selective for the desired amine and generated many side products that were difficult to

separate from the amine target **186**. NMR analysis indicated the synthesis of the amino compound **186** though TLC analysis indicated that the isolated compound was impure.

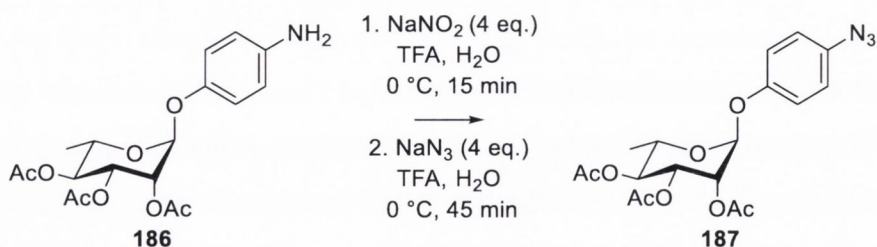
Scheme 3.5 Reduction of an acetylated rhamnosyl-functionalised aromatic nitro group



Despite the lack of pure rhamnosyl glycosylated amine compound **186** the crude product was used to generate the azido compound **187** through a substitution reaction (Scheme 3.6). The presence of the amine was inferred by the production of the azide product **187** though the side products from the preparation of the amine starting material **186** meant that the reaction to generate compound **187** was not clean or high yielding.

The presence of the azido compound **187** was determined by a number of methods including NMR spectroscopy, mass spectroscopy and IR spectroscopy. However, full characterisation was not possible due to difficulties associated with purification of the product **187** due to starting material impurities.

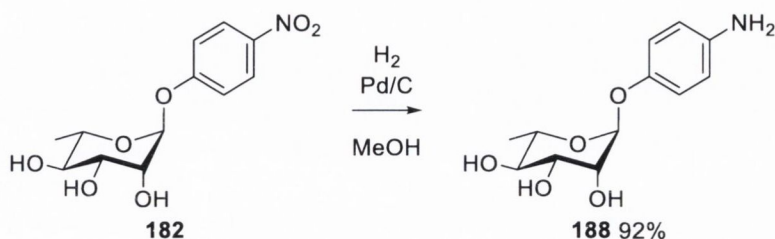
Scheme 3.6 Inference of an amine functionality by the synthesis of an azide



Since several solvent conditions were studied it is unlikely that solubility issues caused the observed poor conversion of the nitro derivative **181** to the amine compound **186**. It was decided to repeat the nitro reduction reaction but on the deprotected rhamnosyl compound **182** to exclude any interference by the acetyl protecting groups (Scheme 3.7). The reaction proceeded quickly and cleanly to give the fully deprotected amino compound **186** in high yield. The reaction was sufficiently clean that after compound **188** was separated from the palladium

catalyst by filtration and the removal of the solvent *in vacuo* no other purification was required. This was fortunate as the extremely polar nature of the deprotected glycosylated aniline derivative **188** means that its purification by column chromatography would be difficult.

Scheme 3.7 Reduction of the deprotected rhamnosyl-functionalised aromatic nitro group



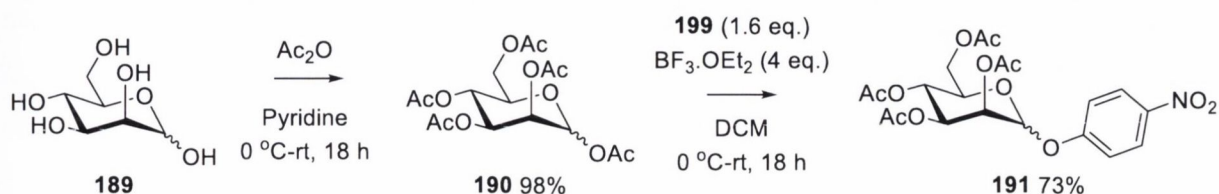
Theoretically, the acetyl protecting groups could be replaced with protecting groups that are more stable under the hydrogenation conditions employed. Partially deprotected and methylated glycosylated nitro aromatic derivatives could theoretically be reduced using the conditions discussed above.

3.2.3 Non-rhamnose sugars

The principle of using a nitro aromatic as a precursor to the amine had been successfully demonstrated with rhamnose. It was decided to examine the glycosylation of 4-nitrophenol with a variety of carbohydrates to examine the flexibility of the synthetic route.

The related carbohydrate D-mannose **189** was converted into a donor and used to glycosylate 4-nitrophenol **180** (Scheme 3.8). The acetylation of D-mannose **189** gave the peracetylated donor **190** in good yield and high purity. The glycosylation of 4-nitrophenol by the acetylated mannosyl donor **190** gave compound **191** in reasonable yield but as a mixture of anomers despite the anchimeric effect of the acetyl groups. Overall the α anomer was in excess accounting for just over 60% of the product formed.

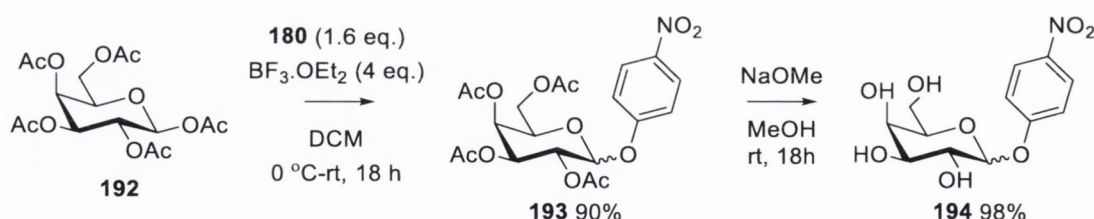
Scheme 3.8 Glycosylation of 4-nitrophenol by a peracetyled mannosyl donor



The separation of the anomers of compound **191** by column chromatography proved somewhat problematic giving 20% of the α anomer, 6% of the β and the remaining 47% as a mixture of anomers. The mixture of anomers obtained may have been due to the orientation of the mannosyl 2-hydroxyl groups which is axial. A participating group at the axial mannosyl 2-hydroxyl promotes the formation of the axial α product which is in keeping with the anomeric effect. These two effects are in conflict with steric effects that promote an equatorial orientation for bulky groups. If a carbohydrate with an equatorial 2-*O*-acetyl was used, the steric effects and the anchimeric effect would reinforce each other and promote the formation of the equatorial β product.

The glycosylation of 4-nitrophenol **180** was attempted using a different carbohydrate, one where the anchimeric effect promotes the equatorial β compound. D-galactose is commercially available as the peracetylated β compound **192** and this was used as a donor to glycosylate 4-nitrophenol **180** (Scheme 3.9). Unfortunately, though high yielding, the glycosylation of compound **180** with the acetylated galactosyl donor **192** gave the glycosylated nitrophenol derivative **193** as a mixture of anomers with the β predominating at approximately 70% of the product.

Scheme 3.9 Glycosylation of 4-nitrophenol by a peracetylated galactosyl donor



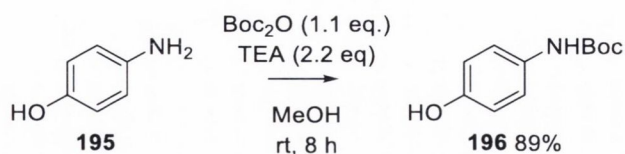
Purification of compound **193** by column chromatography gave only 5% of the α anomer, 4% of the β and 81% as a mixture. As with the mannosyl derivative, the anomers proved extremely difficult to separate. The reaction was repeated using 1.1 equivalents of **192** and 1.1 equivalents of SnCl_4 as activator in an attempt to promote formation of only one anomer. This strategy was also ineffective, possibly due 4-nitrophenol **180** being a poor acceptor. The deacetylation necessary to generate the deprotected compound **194** for the reduction reaction was high yielding and clean as would be expected. Prior to reduction to the amine **194** could be functionalised to generate a specific methylation pattern as desired.

Since the generation of a library of amino functionalised sugars was not the aim of this work this was the extent that this synthetic strategy was applied. This work aimed to investigate different methods for the production of aniline functionalised carbohydrates that could be used for surface functionalization via diazonium chemistry. One such method was *via* functional group interconversion of a nitro moiety to an amino. The glycosylation necessary for the preparation of nitro aromatic derivatives was achieved using several carbohydrates and the reduction successfully performed. The work to date indicates that acetyls are not appropriate protecting groups for the reduction of the nitro aromatic to an amine. However, the results demonstrate that the nitro is stable under a variety of conditions meaning that a specific methylation pattern can be established post glycosylation and pre-reduction. Despite the relative success of the above syntheses alternative strategies could be necessary and to that end the alternative methodology of glycosylation a protected amine was also investigated.

3.2.4 Protection and deprotection of anilines

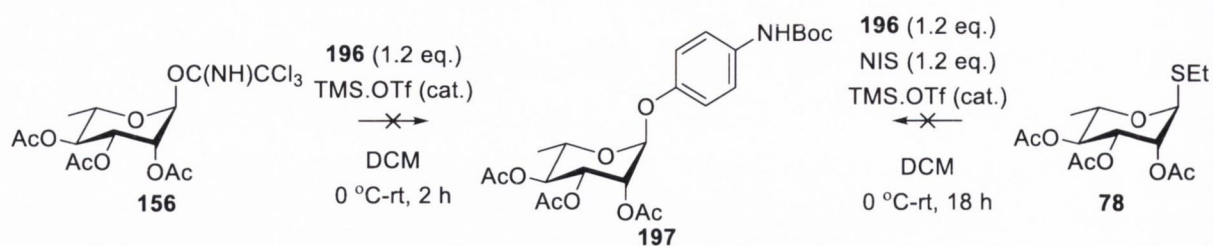
An alternative strategy to functional group interconversion is to have the target functional group present from the onset but disguise its reactivity. When protecting the amine of 4-aminophenol the hydroxyl group can compete with the amine. However the amine is more reactive and thus more easily protected. A common protecting group that is introduced under mild conditions is *tert* butyl dicarbonate (boc). It is introduced under basic conditions and removed with acid. The mono-*N*-boc aromatic compound **196** was generated from 4-aminophenol **195** in good yield (Scheme 3.10).

Scheme 3.10 Protection of 4-aminophenol with one boc group



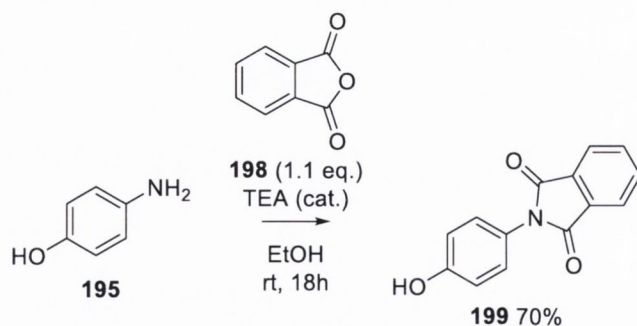
The glycosylation of the resulting mono-*N*-boc compound **196** was not successful although several different donors were screened for this reaction (Scheme 3.11). This effect may have been due to the reactivity of the mono-protected amine or that of the protecting group itself interfering with the activation of the donor.

Scheme 3.11 Failed glycosylations of *N*-boc-4-aminophenol



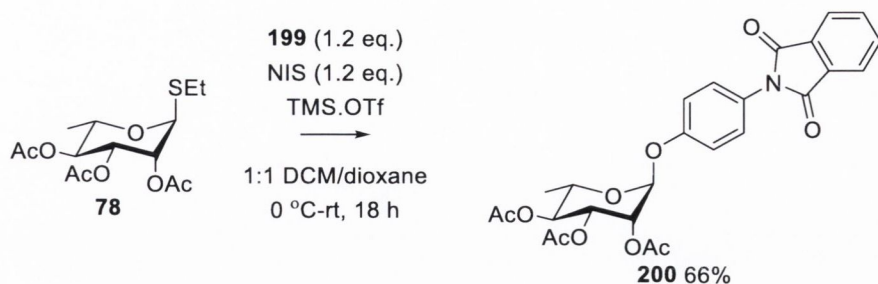
To pursue this strategy a fully protected amine was deemed necessary. It was possible to pursue the formation of the *N,N*-di boc compound or to investigate the possibility of applying a different amine protecting group. Since more forcing conditions would be required to generate the di-boc compound it would be more likely to produce a mixture of compounds, therefore alternative amine protecting groups were investigated. A protecting group that can bond twice to the nitrogen of the amine, such as a phthalimide, was investigated as an alternative. Phthalic anhydride **198** was reacted with 4-aminophenol **197** in the presence of TEA to give *N*-(4-hydroxyphenyl)phthalimide **199** (Scheme 3.12). Purification of compound **199** was accomplished by dissolving it in EtOAc and washing with dilute aqueous acid to remove the excess amine.

Scheme 3.12 Preparation of *N*-(4-hydroxyphenyl)phthalimide



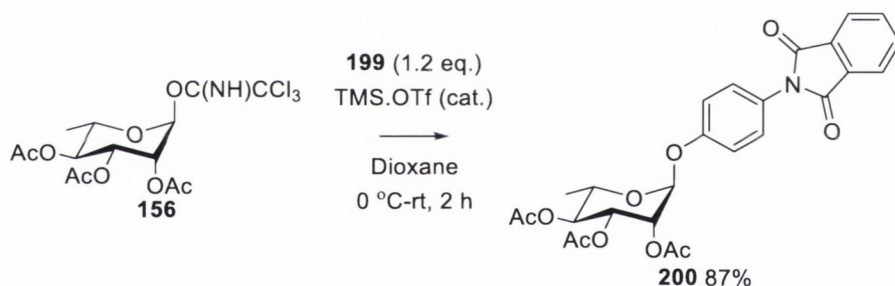
The glycosylation of *N*-(4-hydroxyphenyl)phthalimide **199** by the thioglycoside **78** was performed in a mixed solvent system as compound **199** was not soluble in DCM and the NIS activator was not soluble in dioxane (Scheme 3.13). The glycosylation reaction to produce the glycosylated derivative **200** was not very high yielding though the acetyls ensured that no β product was formed.

Scheme 3.13 Glycosylation of *N*-(4-hydroxyphenyl)phthalimide by a rhamnosyl thioglycoside donor



The glycosylation was repeated using the trichloroacetimidate donor **156** and dioxane as solvent since the dioxane insoluble NIS was not required (Scheme 3.14). The functionalised rhamnosyl product **200** was obtained as the α anomer in good yield due to the anchimeric effect.

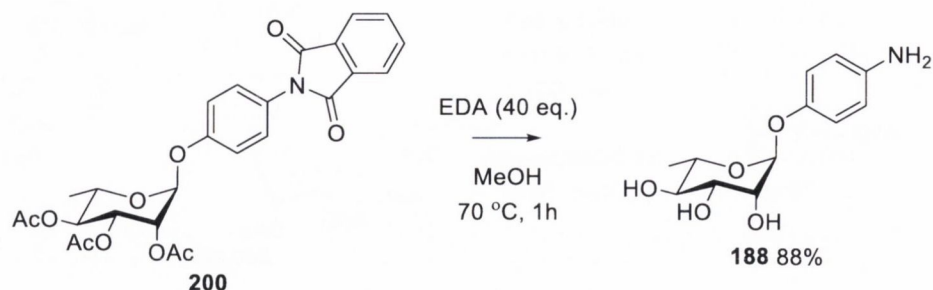
Scheme 3.14 Glycosylation of *N*-(4-hydroxyphenyl)phthalimide by a rhamnosyl trichloroacetimidate donor



3.2.5 Removal of phthalimide protecting group

For this methodology to be complete the phthalimide protecting group had to be successfully removed to reveal the free amine. This was accomplished by refluxing the glycosylated *N*-(4-hydroxyphenyl)phthalimide **200** in a large excess of ethylenediamine (Scheme 3.15). Under such forcing basic conditions the acetyl groups of compound **200** were concurrently removed and the product was the aniline functionalised deprotected rhamnosyl product **201**.

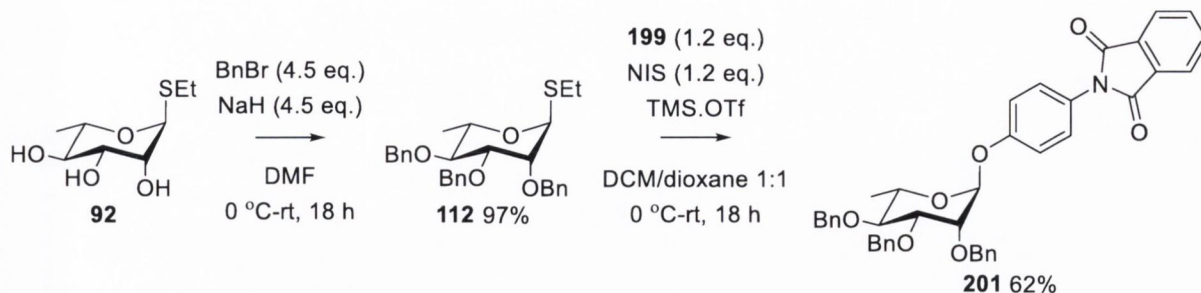
Scheme 3.15 EDA facilitated removal of the phthalimide amine protecting group with concurrent deacetylate



Due to its highly polar nature, the purification of compound **188** was difficult and it was suggested that it would be preferable to have more control over when the carbohydrate was deprotected. Base stable protecting groups such as benzyls should remain on the carbohydrate during the ethylenediamine reaction. Additionally, their apolar nature can compensate for the polarity of the amine aiding purification of the product by flash chromatography.

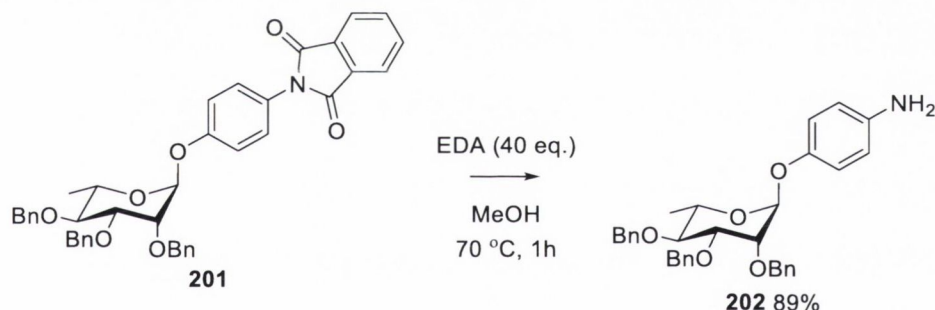
To demonstrate this effect the benzylated glycosyl derivative of *N*-(4-hydroxyphenyl)phthalimide **201** was prepared. The glycosylation of *N*-(4-hydroxyphenyl)phthalimide **199** was accomplished using the benzylated rhamnosyl thioglycoside donor **112**. The thioglycoside was used despite better yields having been obtained with the trichloroacetimidate due to the additional reactions necessary to generate the trichloroacetimidate from the thioglycoside. Since a functionalised carbohydrate donor was required, the protecting group manipulations were performed on the thioglycoside prior to glycosylation with the phenolic acceptor. To generate the trichloroacetimidate from the thioglycoside requires two steps and the yield of these must also be taken into account when examining the overall route. The higher yield obtained when using the trichloroacetimidate donor was not high enough to compensate for the longer synthetic route. The deprotected rhamnosyl donor **92** was perbenzylated to give the thioglycoside donor **112** in high yield which was used to glycosylate **199** to give compound **201** in fair yield (Scheme 3.16). The absence of neighbouring group participation would have made the reaction less selective for the α anomer but the large benzyl groups effectively block one face of the donor ensure the apparently exclusive formation of the α product.

Scheme 3.16 Glycosylation of *N*-(4-hydroxyphenyl)phthalimide by a benzylated rhamnosyl thioglycoside donor



The removal of the phthalimide protecting group on compound **201** was accomplished without any effect on the benzyls (Scheme 3.17). The yield of the deprotection of the benzylated **201** to form compound **202** was similar to that of the deprotection of the acetylated **200** to form compound **188**. The purification of compound **201** was more facile than that of compound **188** due to its significantly more apolar nature.

Scheme 3.17 EDA facilitated removal of the phthalimide amine protecting group

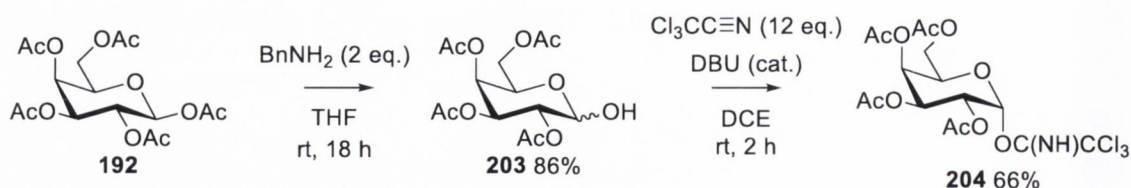


3.2.6 Glycosylation *N*-(4-hydroxyphenyl)phthalimide

As with the nitro-amine interconversion the same reaction sequence was repeated with other carbohydrates to demonstrate the versatility of the route. The reactions were repeated with galactose. It was decided to carry out the glycosylation reaction using a galactosyl trichloroacetimidate as the donor to ensure higher yields. The trichloroacetimidate could be prepared directly from the acetylated compound without having to go through the thioglycoside meaning that the synthetic route to the donor was only lengthened by one step.

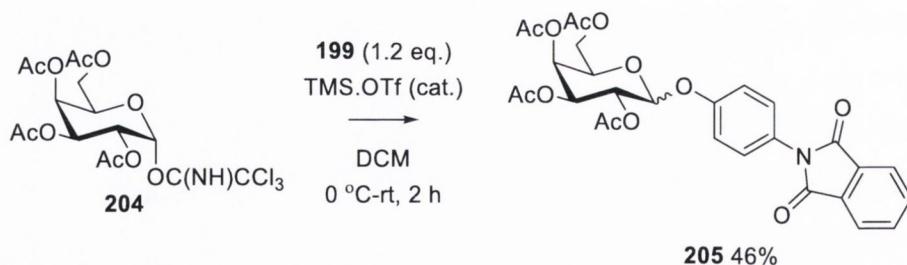
The commercially available acetylated galactose **192** was converted to the corresponding hemiacetal **204** (Scheme 3.18). The hemiacetal **203** was used to prepare the galactosyl trichloroacetimidate donor **204**.

Scheme 3.18 Preparation of a galactosyl trichloroacetimidate donor



The glycosylation of *N*-(4-hydroxyphenyl)phthalimide **199** by the galactose trichloroacetimidate donor **204** was not as clean as the corresponding reaction with the rhamnosyl trichloroacetimidate donor **156**. The glycosylated product **205** was obtained in 46% overall yield as a mixture of anomers that were separated by column chromatography. The β anomer was the major product at 30% and the α was obtained in 12% yield. The higher β yield was in keeping with the anchimeric effect and the preference for having equatorial bulky groups. The selectivity and separation was better than that observed with glycosylated 4-nitro phenol derivatives possibly due to the greater steric bulk of the *N*-(4-hydroxyphenyl)phthalimide **199**. Though some fractions were obtained that contained both anomer these only accounted for an additional 4% yield.

Scheme 3.19 Glycosylation of *N*-(4-hydroxyphenyl)phthalimide by a galactosyl trichloroacetimidate donor

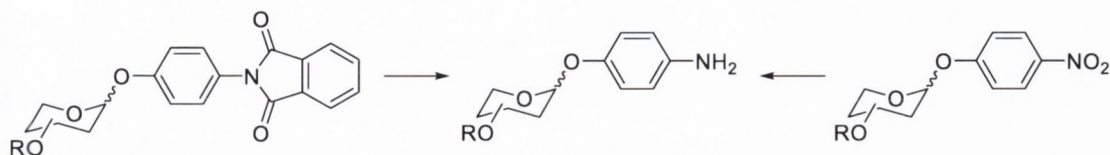


3.2.7 Conclusion

Several methods have been investigated for the preparation of aniline functionalised carbohydrates (Scheme 3.20). Two methods that were found to be successful are the use of phthalimide protected amines and the direct reduction of a glycosylated aromatic nitro group to an amine. Both methods have successfully been applied to a number of monosaccharides indicating that the methodology can be generally applied. As discussed above, specific methylation patterns can be installed pre- or post-glycosylation. Though the use of the diazonium grafting reaction for covalently modifying materials is known it has not been applied

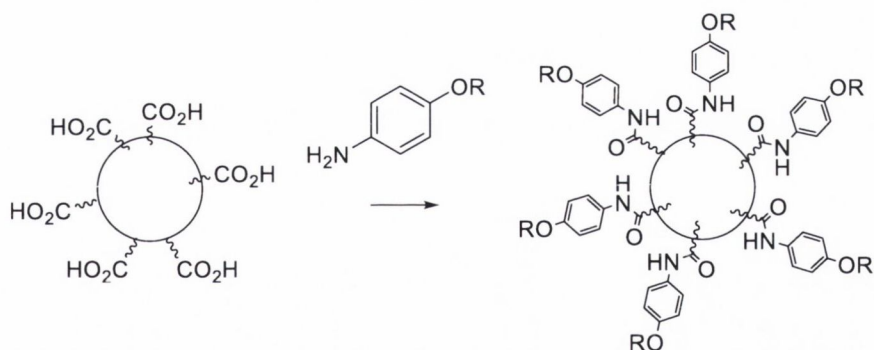
to carbohydrates before. Through the application of the methods described herein, diazonium grafting can potentially be used to generate a variety of carbohydrate arrays, and functionalised surfaces and particles.

Scheme 3.20 Routes for amine functionalised carbohydrate synthesis



The amine functionalised carbohydrates were investigated for the purpose of using them as starting materials in diazonium grafting however this is not the limit of their potential for surface functionalisation. Amine functionalised carbohydrate can be used for alternative immobilisation methods based on coupling reactions. For example PLGA (poly(lactic-co-glycolic) acid) nanoparticles have terminal carboxylic acid moieties that can be used to form amide linkages.¹⁶⁷ Such carboxylic acid moieties can be functionalised by coupling with glycosylated aromatic amine derivatives to generate stable amide linkages (Scheme 3.21).

Scheme 3.21 Covalent immobilisation through an amide bond R = carbohydrate



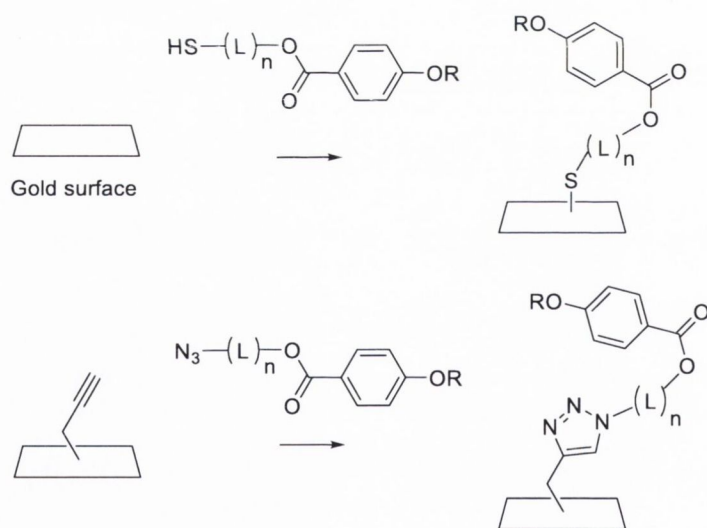
3.3 Linker based immobilisation strategies

The amine precursor to the diazonium salt needs to be directly attached to the aromatic ring. Alternative strategies could allow for the incorporation of a linker to introduce flexibility and tailor the properties of the resulting surface. Since the compounds to be immobilised are based on *p*-HBADs it may be important to preserve the aromatic methyl ester moiety. The carbonyl moiety can serve as a handle for the attachment of a linker. An alternative ester can be generated whose *O*-alkyl is a linker molecule. The alkyl chain length is important to prevent intramolecular reactions at the carbonyl. If the initial linker used contains a good leaving group

it can be converted into a variety of functional groups allowing access to a range of immobilisation reactions. The choice of linker can thus influence the flexibility of the synthetic route. An alkyl linker that terminates in a halogen can undergo nucleophilic substitution and can thus serve as a common precursor for a variety of functionalities.

Ideally the immobilisation reaction should be either spontaneous or facile so as to leave other functionalities untouched. The carbonyl of the ester is particularly vulnerable under acid or basic conditions. An immobilisation reaction that occurs spontaneously is the formation of self-assembled monolayer on gold surfaces by thiols which can easily be prepared from alkyl halides. There are several methods for the conversion of an alkyl halide to a thiol, many of which are quite mild. The halide can be easily displaced by a number of functionalities including an azide. An alkyl azide could be used for coupling reactions, for example, though not spontaneous, the copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) can be performed under quite mild conditions (Scheme 3.22).

Scheme 3.22 Functionalization of surfaces using amine derivatives. R = carbohydrate

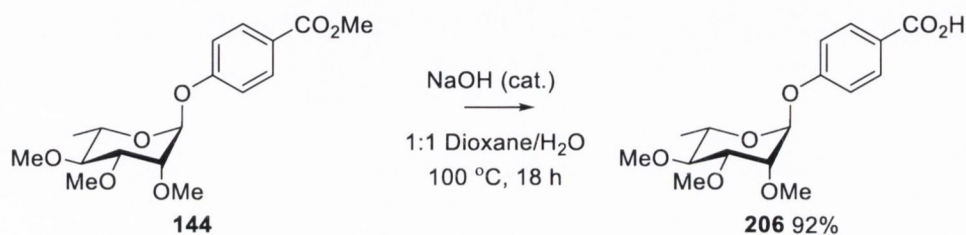


3.3.1 Linker choice and attachment

For the purposes of this project a short alkyl linker was chosen. Either a quite short or a quite long linker would be best suited to avoid intramolecular reactions at the carbonyl. Longer linkers can aid lectin binding by making the carbohydrate more accessible. The aromatic moiety may itself serve as part of the linker, adding both rigidity and length thus making the carbohydrate moiety easily accessible for binding. As this work aimed to establish a methodology and because of the assistance afforded by the aromatic group a short linker was

chosen. For the initial investigative reaction it was decided to use a simple *p*-HBAD. Neither the deprotected UM-*p*-HBAD **11** nor its acetylated precursor **75** could be used as free hydroxyls or acetyls might interfere in later reactions. The deprotected UM-*p*-HBAD **11** was previously methylated to give the simple *p*-HBAD **144**.

Scheme 3.23 Hydrolysis of the *p*-HBAD methyl ester

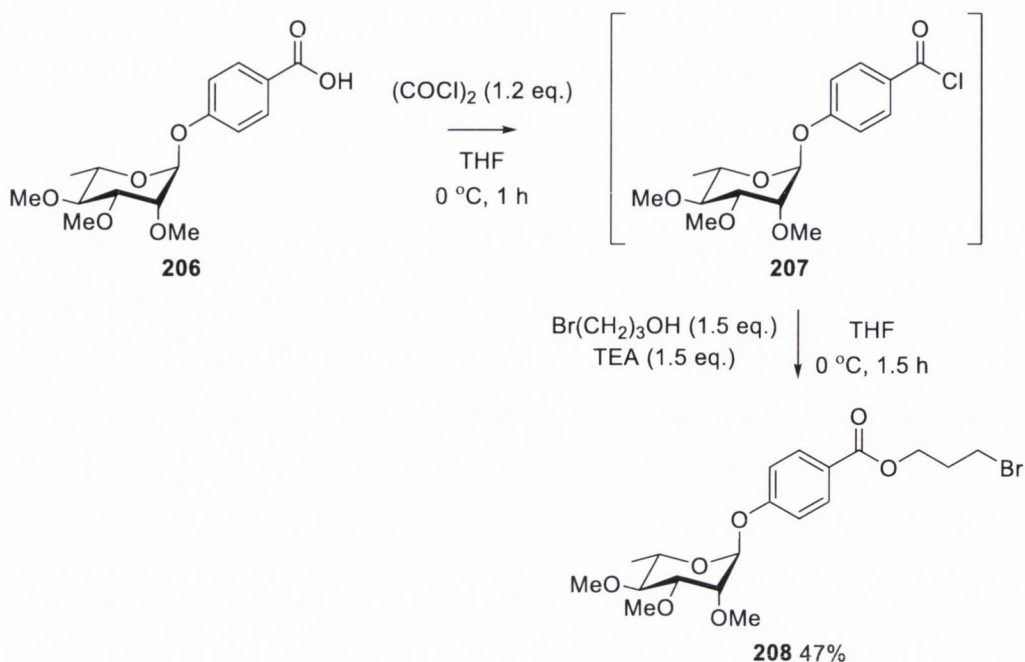


The aromatic methyl ester of *p*-HBAD **144** was hydrolysed using a strong aqueous base and refluxing conditions to force the reaction. The starting material **144** is not soluble in water which is required both for the reaction and to solubilise the NaOH. A 1:1 mixture of dioxane and water was used which allowed both reagent to be in solution without the formation of a bi-phasic system. The hydrolysis of *p*-HBAD **144** gave *p*-HBAD **206** in almost quantitative yield. The carboxylic acid moiety of *p*-HBAD **206** could be used as a handle to attach the desired linker.

There are several methods available for the attachment of a linker through a C-O bond using a linker with an alcohol moiety. Two common methods are a coupling reaction with an alcohol or conversion of the carboxylic acid to an acyl chloride followed by nucleophilic attack by an alcohol. Due to the high reactivity of acyl chlorides it may be more appropriate to generate the acyl chloride *in situ* and then add the alcohol. Both methods were applied and their yields and efficiencies compared.

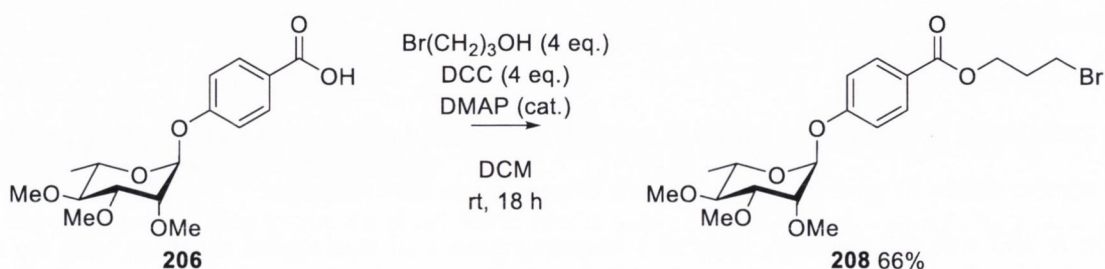
The carboxylic of compound **206** was reacted with oxalyl chloride in freshly distilled THF at low temperatures to give the acyl chloride intermediate **207** (Scheme 3.24). The reactive acyl chloride **207** was not isolated, instead 3-bromopropan-1-ol was added together with the non-nucleophilic base TEA to give the bromoalkyl ester **208**.

Scheme 3.24 Preparation and reaction of the acyl chloride



Since the acyl chloride route was not very high yielding it was decided to carry out the DCC mediated coupling reaction to generate compound **208** (Scheme 3.25). Two equivalent each of DCC and 3-bromopropan-1-ol with a catalytic quantity of DMAP gave the target bromoalkyl ester **208** in a higher yielding and cleaner reaction. Increasing the quantities of DCC and 3-bromo-propan-1-ol to four increased the yield of **208** from 53% to 66%. The glycosylated aromatic bromoalkyl ester could then be used as a precursor for functional group interconversion at the bromine to introduce suitable functional groups for surface ligation reactions.

Scheme 3.25 Linker coupling reaction

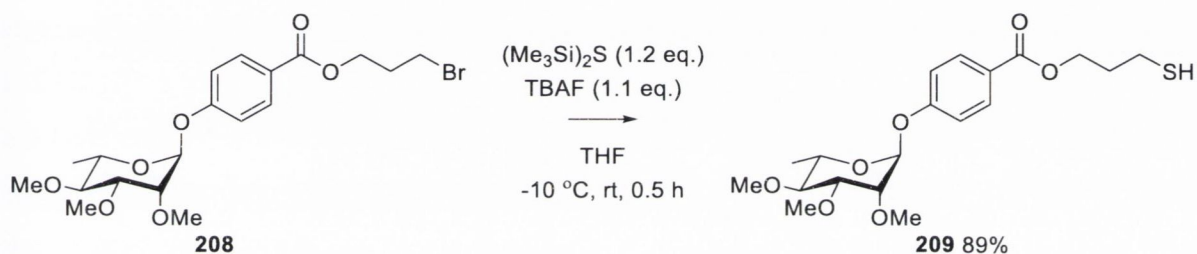


3.3.2 Synthesis of a thiol terminated linker

The bromine is a good leaving group and can be converted to a variety of functional groups and in this case it was converted to a thiol. Though a range of reactions have been reported for

the preparations of alkenethiolates from alkylhalide precursors a mild method was required. The mild method was necessary to ensure that the ester remained unaltered. Tetrabutyl ammonium fluoride (TBAF) and hexamethyldisilathiane was used to generate tetrabutylammonium trimethylsilylthiolate ($\text{Me}_3\text{Si}^-\text{Bu}_4\text{N}^+$) *in situ* and which reacted with the alkylhalide **208**.¹⁶⁸ An aqueous work up ensured removal of the trimethylsilyl group to give the free thiol **209** (Scheme 3.26). NMR and IR analysis confirmed that the ester was intact and the thioester isomer had not formed. The free thiol could potentially be used to directly prepare gold-nanoparticles displaying *p*-HBADs. It is envisaged that this cluster effect may enhance the biological activity of these sugars.

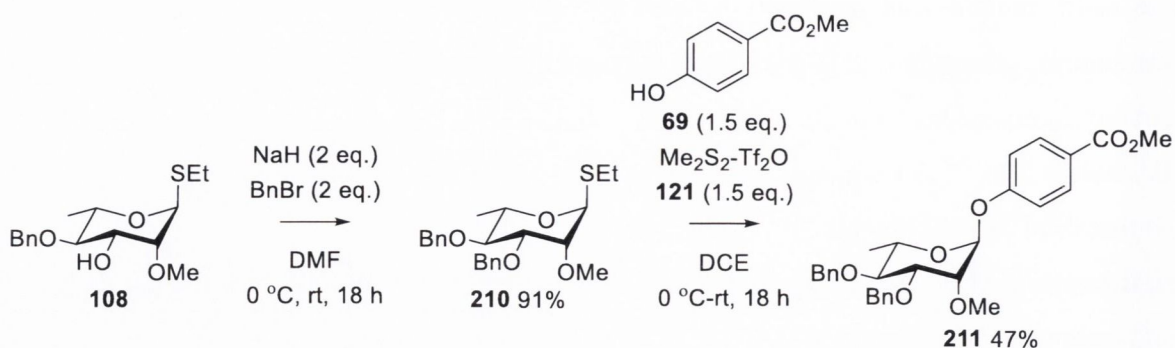
Scheme 3.26 Conversion of an alkylbromide to an alkylthiol



3.3.3 Synthesis of an azide terminated linker

A simple *p*-HBAD was used to demonstrate the application of the synthetic route to the bromoalkyl ester and subsequently the route was repeated with a more complex *p*-HBAD with a specific substitution pattern installed. As previously discussed, native *p*-HBAD I **10** has a methyl at the rhamnosyl 2-hydroxyl and it was decided to prepare a compound with a 2-*O*-methyl rhamnosyl carbohydrate suitable for surface immobilisation. In the synthesis of the mycoside B mimic **173** and *p*-HBAD I **10** an acetyl protected the 3-hydroxyl of the rhamnosyl and a benzyl the 4-hydroxyl. This substitution pattern is not compatible with the current synthetic route as acetyls are base labile. However, benzyl protecting groups should be stable under such conditions and can be used to protect both the 3- and 4-hydroxyl of the rhamnosyl moiety.

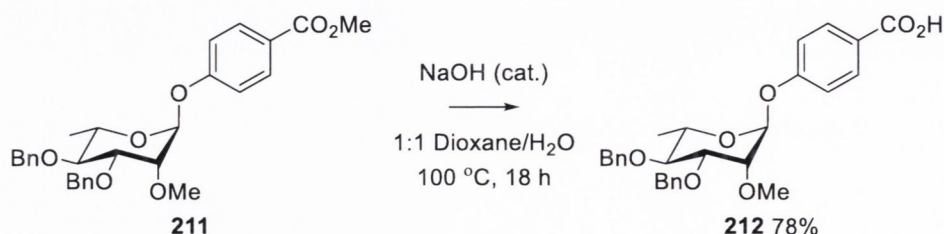
Scheme 3.27 Preparation of dibenzylated *p*-HBAD



The free 3-hydroxyl of the partially protected rhamnosyl thioglycoside **108** was benzylated to give the fully protected thioglycoside **210** in good yield despite any potential steric issues (Scheme 3.27). Thioglycoside donor **210** was used to glycosylate methyl-4-hydroxybenzoate **69** using $\text{Me}_2\text{S}_2\text{-OTf}_2$ **121** as the activator to give *p*-HBAD **211**. The low yield of *p*-HBAD **211** obtained was most likely due to difficulties associated with its purification rather than the glycosylation reaction itself. In particular, the separation of methyl-4-hydroxy benzoate **69** from *p*-HBAD **211** was challenging. When TLC analysis was performed the compounds displayed reasonable separation but were eluted together when flash chromatography was performed. Despite the use of a variety of mobile phases separation was still poor giving a correspondingly poor yield of *p*-HBAD **211**. The bulk around the bottom face of the donor would have promoted the formation of the α product as the bulky acceptor would have been limited in how it would approach. This, together with the difficulties in purifying the product meant that no β was observed.

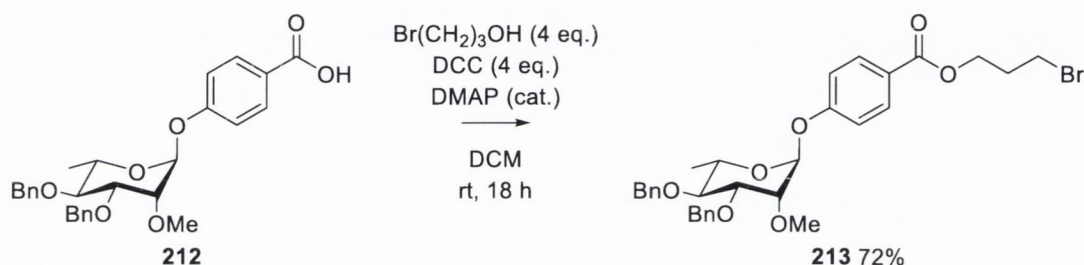
The hydrolysis of *p*-HBAD **211** was facile and though not high yielding allowed for the isolation of pure *p*-HBAD **212** (Scheme 3.28).

Scheme 3.28 Base mediated ester hydrolysis



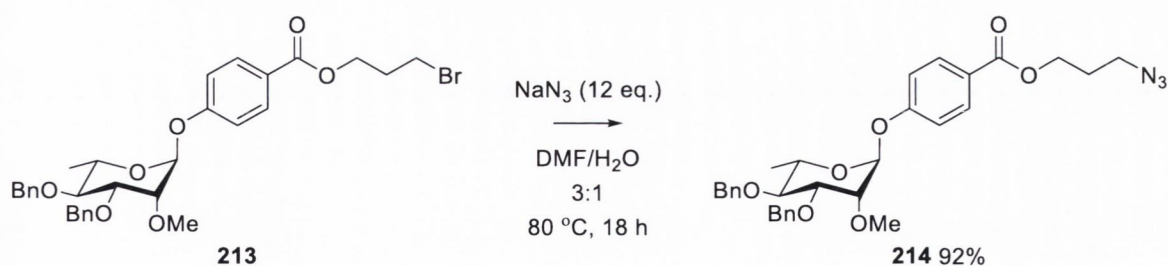
The DCC mediated coupling of the acid with the alcohol moiety was previously demonstrated to be higher yielding than the use of an acyl chloride intermediate. The coupling reaction to generate the bromoalkyl ester **213** from the carboxylic acid **212** was accomplished using the increased equivalent as before and was both clean and high yielding (Scheme 3.30). Overall, the DCC coupling reaction appears to be a more facile method for linker attachment and should be used for this purpose for all future syntheses of these compounds.

Scheme 3.29 DCC mediated linker coupling



Since the synthesis of the thiol had already been demonstrated it was decided to prepare a compound for an alternative ligation reaction: the copper (I) catalysed click reaction. Sodium azide is a convenient source of nucleophilic N_3^- which can displace Br^- of **213** to form the alkyl azide **214** (Scheme 3.31). The solubility of sodium azide in the DMF solvent was low and to facilitate the reaction, water had to be added to solubilise the sodium azide. The reaction did not proceed at room temperature though when forcing reflux conditions were utilised the reaction proceeded in good yield.

Scheme 3.30 Conversion of an alkyl bromide to an alkyl azide



The azide **214** can be used directly in solid surface attachment by reacting with an appropriately functionalised surface. Alternatively, the azide can be reduced to the corresponding amine (with the simultaneous removal of the benzyl protecting groups) and used in coupling reaction, also with an appropriately functionalised surface.

3.3.4 Conclusion

A facile method for linker attachment to monosaccharides and *p*-HBADs has been successfully demonstrated. The linker used can be varied to modify the properties of the resulting compound. The 3-bromopropan-1-ol used herein demonstrated that an alkylhalide makes for a very versatile linker. Functional group interconversion, based mainly on nucleophilic displacement, can be used to generate a variety of compounds from the alkyl halide

3.4 Future work

The syntheses discussed above do not represent an exhaustive study of the potential of the alkylhalide linker. A more thorough investigation is beyond the scope of this project. The work herein lays the foundations for further investigations as this linker has potential to be used for the immobilisation of carbohydrates and the potential enhancement of their immunological effects both *in vitro* and *in vivo*.

4.0 Chapter 4 The biological evaluation of mycobacterial *p*-hydroxybenzoic acid derivatives (*p*-HBADs)

4.1 Pathogenic background

As previously discussed in Chapter 1, once present in the lungs, bacteria are phagocytosed and kept in phagosomes by macrophages, which then release proinflammatory cytokines. Mycobacteria in the phagosome will proliferate and secrete compounds into the phagosome. Some of these compounds are removed into the endosomal intracellular pathway allowing them to be displayed and used to sensitise T cells. This sensitisation gives rise to acquired specific resistance. Macrophages and T cells accumulate at the site of infection due to the local chemokine environment and adhesion molecules on endothelial cells.

4.1.2 Rational for biological testing

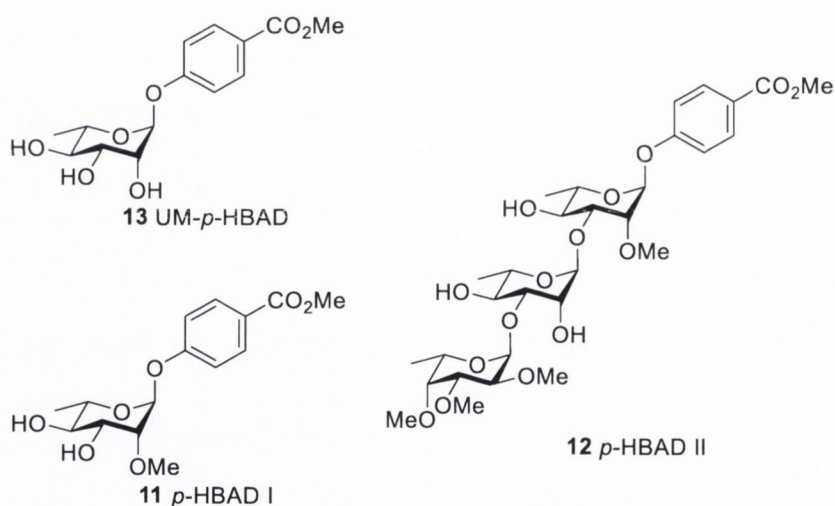
PGL consist of a dimycocerosates (DIM) phthiocerol (PDIM) lipid core that is ω terminated by a glycosylated phenyl. The glycosylation is through the OH of the phenyl and the carbohydrate groups are species and strain specific. The *p*-HBADs are a group of small molecules related to PGLs. Though the biosynthesis of *p*-HBADs proceeds *via* its own biosynthetic route, the glycosyl portion of *Mtb* *p*-HBADs is the same as that found in *Mtb* PGLs. For example, *p*-HBAD I and *p*-HBAD II have the same carbohydrate regions as mycoside B and PGL-tb-1 respectively. As such, the synthesis of the carbohydrate regions is thought to be *via* the same pathway in *p*-HBADs and PGLs. Some of the enzymes involved in the biosynthesis of the carbohydrate region have been identified: *Rv2957*, a glycosyl transferase, adds the fucose monomer; *Rv2958c*, a glycosyl transferase, adds the second rhamnose monomer; *Rv2959c*, a methyl transferase, acts on the 2-hydroxyl of the first rhamnose monosaccharide; *Rv2962c*, a glycosyl transferase, adds the first rhamnose monomer.

Both PGLs and *p*-HBADs have been shown to play a role in disease pathogenesis. Since *p*-HBADs, unlike, PGLs, are excreted by all clinical isolates of *Mtb* examined to date, it was decided to focus on them as substrates for preliminary biological studies. As previously discussed, there is evidence to suggest that the carbohydrate portion is responsible for the observed immunological effects of PGLs.⁵³ Since this region is conserved in both *p*-HBADs and PGLs, determining the nature of the lectins that bind these carbohydrates would be of significant interest in TB research and may allow for the development of new carbohydrate based therapeutics.

Although work has been carried out on the effect of *p*-HBADs both *in vivo* and *in vitro* using strains of *Mtb* in which *p*-HBAD production had been disrupted, they have not been investigated in isolation.⁹⁵ The chemical synthesis of these compound allows for probing their biological effects in isolation from the parent bacterium. Using chemically prepared molecules for biological testing helps ensure that the observed effects are due exclusively to the compound being examined. Should the properties of a mixture of molecules be of interest access to high purity material ensures that ratios can be precisely controlled.

Several *p*-HBADs, both native and non-native have been synthesised, however, initial biological testing focused on native *p*-HBADs (Figure 4.1). The analogues could serve to assist further understanding of the role of the methylation pattern once some basic information about the activity had been established.

Figure 4.1 Native *p*-HBADs synthesised for biological testing



4.1.3 Lipopolysaccharide effects

Lipopolysaccharides (LPS) are a common contaminant in samples and can be problematic for immunological studies since LPS can activate innate immune cells.¹⁶⁹ Though LPS contamination is expected to be low due to the use of organic solvents and clean glassware, it cannot be discounted. As such, controls were put in place to exclude any effects due to LPS.

Toll-like receptors (TLRs) are an important family of pathogen recognising receptors that aid in establishing a host response to infection. The outer membrane of gram negative bacteria has

a high LPS content and TLR4 responds to this family of compounds. Once activated TLRs induce the production of proinflammatory cytokines.¹⁷⁰

The cells used for these *in vitro* tests were derived from two different strains of mice. The first strain used was wild type CH3/HeN mice. Their cells express TLR4 and consequently are sensitive to LPS. The other strain used was CH3/HeJ and these are TLR defective and so do not express TLR4 on their cell. LPS will not activate the CH3/HeJ TLR defective immune cells. The responses of HeJ cells can be compared to those of HeN cells giving an indication of the level of LPS contamination and allowing effects due to LPS to be discounted.

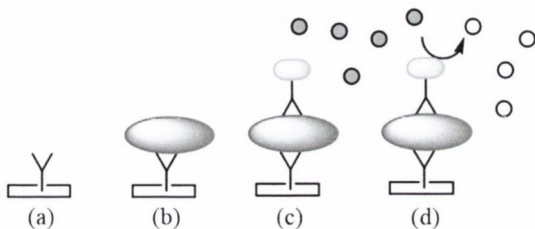
4.1.4 Design of experiments

Mouse spleen cells (splenocytes) and bone marrow derived macrophages (BMM) were employed for the *in vitro* studies. Three sets of experiments were performed and on each occasion cells were incubated with each of the three native *p*-HBADs at various concentrations, either alone or together with an appropriate stimulus. This meant that the ability of the *p*-HBADs to both induce cytokine production on their own and to modulate the response to a stimulus was assessed. Since each experiment was performed in triplicate, error bars are present in the resultant data. Positive controls were used to ensure that the cells were alive and responsive and consisted of incubating the cells together with the appropriate stimulus.

4.1.5 Detection of cytokines

The cytokines produced were quantified by enzyme-linked immunosorbent assay (ELISA), more specifically sandwich ELISA (Figure 4.2). In a sandwich ELISA the surface has a capture antibody for the antigen of interest (a). The sample being examined for the antigen in question is administered and unbound antigen is washed away (b). Then a specific antibody for the antigen is added (c). This last antibody is linked to an enzyme and when the substrate for this enzyme is added a colour signal that can easily be read results (d).

Figure 4.2 Sandwich ELISA.



4.2 Splenocytes

The mouse spleen is particularly useful for adjuvant research as it contains a wide variety of cell types including many immune cells. Splenocytes are a mixed population including many T cells and play a key role in immune responses.¹⁷¹ Splenocytes were plated as a single cell solution and stimulated with anti-CD3e. Anti-CD3e (aCD3) is a T cell activator and promotes lymphocyte proliferation and cytokine production.¹⁷² CD3 (cluster of differentiation 3) is a T cell co-receptor.¹⁷³ When activated, CD3 associates with the T cell receptor (TCR) and the ζ -chain to form the TCR complex resulting in a T cell activation signal. The TCR is a receptor on T cells that recognises antigens presented by major histocompatibility complex (MHC) molecules. CD3 is an accessory molecule and can help convey the external signal to the inside of the cell: it is essentially the signalling component of the TCR complex.

For the splenocyte population these consisted of aCD3 together with aCD28. Cluster of differentiation 28 (CD28) is a costimulatory molecule present on the outside of T-cells.¹⁷⁴ It provides a potent costimulatory signal for the production of cytokines and the activation of the T-cell.

4.2.1 Splenocyte experiments

In the first set of experiments two different concentrations of aCD3 were used to stimulate the cells: 0.25 $\mu\text{g/mL}$ and 0.5 $\mu\text{g/mL}$. The higher concentration appeared to strongly stimulate the cells, perhaps slightly overshadowing the responses to the quantities of *p*-HBADs applied: 1 μmol , 10 μmol , and 100 μmol .

In the second set of experiments lower concentrations of aCD3 were used in conjunction with higher concentrations of *p*-HBADs. AntiCD3e was applied in 0.1 $\mu\text{g/mL}$ and 0.4 $\mu\text{g/mL}$ concentrations. 20 μmol , 100 μmol , and 500 μmol of each of the *p*-HBADs were used. The 0.4 $\mu\text{g/mL}$ concentration of aCD3 together with 100 μmol of *p*-HBAD was found to be most effective in visualising the response.

In the third set of experiments 0.4 $\mu\text{g/mL}$ of aCD3 was again used, as was 100 μmol of *p*-HBAD and the much higher 500 μmol dose of *p*-HBAD. As *p*-HBAD II is quite difficult to synthesise, the higher dose was not administered in experiment 3 as it was deemed potentially wasteful.

The *p*-HBADs could induce an immune response, either proinflammatory or anti-inflammatory, in their own right. The type of response induced can be determined by types of cytokines produced in response to an antigen. Alternatively, or additionally, *p*-HBADs could alter an induced immune response, either enhancing or suppressing it. The experiments performed give an indication of the activity of the different native *p*-HBADs.

4.2.2 T cell cytokines

As discussed in chapter 1 there are several kinds of T cells, of which helper T cells (T_h cells) play a key role in mediating the immune response. These T_h cells can polarise to mediate different kinds of immune responses: T_h1 and T_h2 .¹⁴

T_h1 cells secrete IFN- γ which is vital for host survival and plays a role in several bactericidal processes. IFN- γ activates macrophages whereas IL-10, also secreted by T cells, downregulates macrophages.²² IL-10 is produced by T_h2 cells. IL-17 is linked to the recruitment of immune cells and promotes the production of proinflammatory cytokines.¹⁴

As bacteria are killed, less and less antigens are present to activate the immune response allowing a gradual downregulation of IFN- γ secreting T cells.

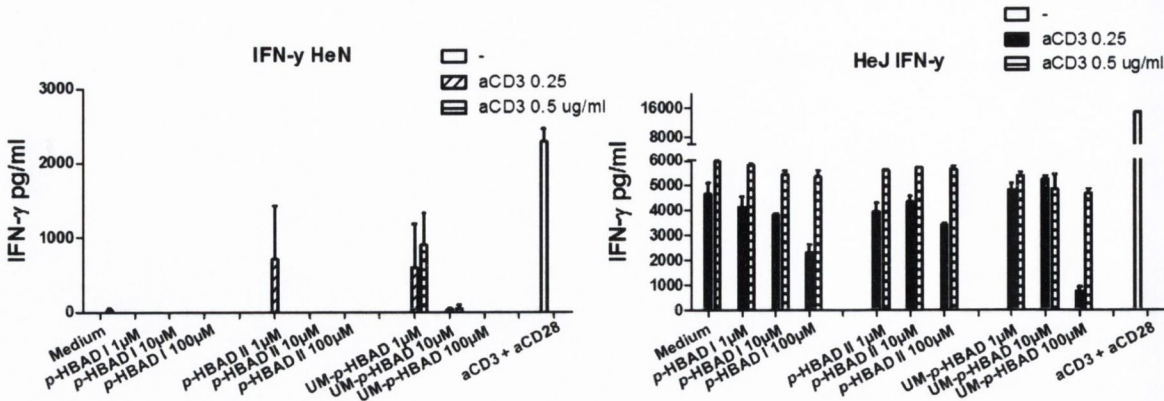
The response of both cell lines under the various conditions will be discussed in the context of each cytokine examined in turn. The levels of IFN- γ , IL-17, IL-10, and IL-4 produced were analysed by ELISA. The former three cytokines are discussed below. No IL-4 was detected so it will not be discussed further in the context of ELISA.

4.2.2.1 INF- γ

An increase in the production of IFN- γ would result in an enhanced T_h1 proinflammatory immune response. This would be detrimental to mycobacterial survival. As *p*-HBADs are thought to be virulence factors they are expected to decrease the proinflammatory response, potentially by inhibiting the production of IFN- γ .

The HeN cells in experiment 1 failed to produce any appreciable quantity of INF- γ except in the case of the positive control (Figure 4.3).

Figure 4.3 IFN- γ production by HeN and HeJ splenocytes in experiment 1.

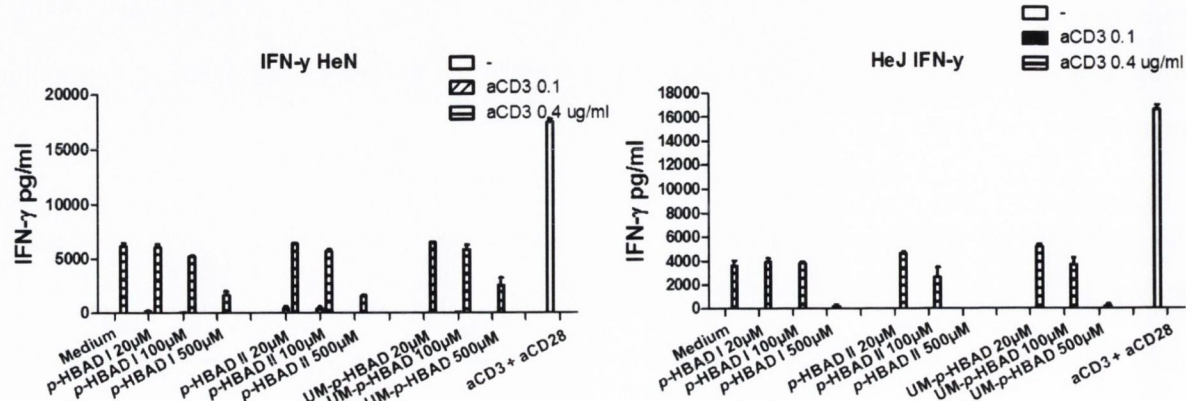


The HeJ cells in comparison, and contrary to expectation, did produce IFN- γ (Figure 4.3). Those stimulated by 0.5 μ g/mL of aCD3 did not seem affected by any of the *p*-HBADs (*p*-HBAD I, *p*-HBAD II, and UM-*p*-HBAD), however, production of IFN- γ by HeJ cells stimulated with the lower concentration of aCD3 (0.25 μ g/mL) appeared diminished in the presence of *p*-HBAD I.

It was decided to repeat the experiment but with slightly altered conditions. The HeN response was not particularly informative but the HeJ response seemed to indicate that the 0.5 μ g/mL concentration of aCD3 used may have been too high for the applied quantity of *p*-HBADs.

In experiment 2, 0.1 μ g/mL of aCD3 did not stimulate the cells sufficiently for an appreciable quantity of IFN- γ to be formed though the 0.4 μ g/mL dose was sufficient. The production of IFN- γ by HeN cells was inhibited by all three *p*-HBADs as a clear downward trend was observed with increasing *p*-HBAD concentration (Figure 4.4)

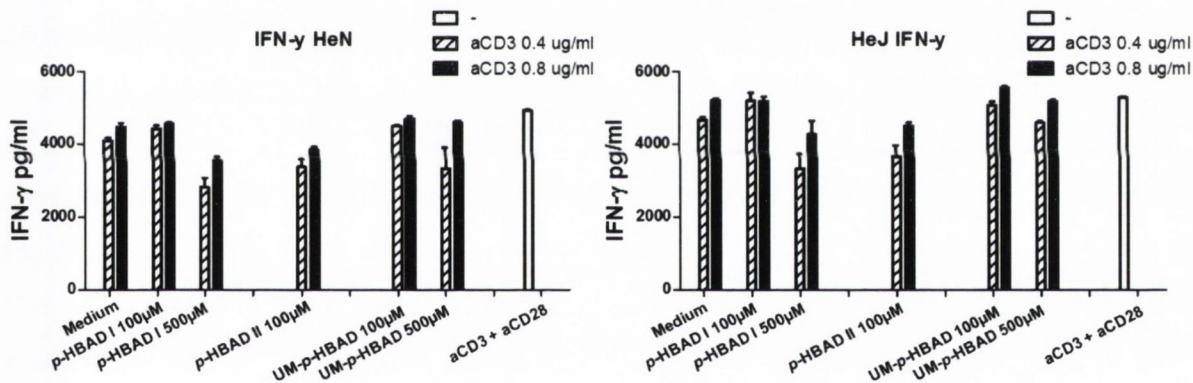
Figure 4.4 IFN- γ production by HeN and HeJ splenocytes in experiment 2



The results for the HeJ cells were less clear but did show a similar trend towards inhibition (Figure 4.4). While *p*-HBAD I did not elicit much of a response both *p*-HBAD II and UM-*p*-HBAD had some inhibitory properties. The HeN and HeJ IFN- γ production was of a similar scale in all cases including the positive control (aCD3 and aCD28). This is encouraging as it implies a lack of any LPS contamination.

Since the 0.1 $\mu\text{g/mL}$ concentration of aCD3 stimulus appeared too mild and the 0.4 $\mu\text{g/mL}$ was successful it was decided to keep the latter concentration in the next experiment. An increased concentration of the potentially inhibitory *p*-HBADs was used. As higher concentration of *p*-HBADs were being used the quantity of aCD03 was also increased. In experiment 3, both HeN and HeJ cells behaved virtually identically with all 3 *p*-HBADs inhibiting production of IFN- γ (Figure 4.5).

Figure 4.5 IFN- γ production by HeN and HeJ splenocytes in experiment 3.



By comparing the data for all of the IFN- γ experiments, an overall inhibitory trend can be observed with the *p*-HBADs failing to induce IFN- γ production by themselves but inhibiting IFN- γ production in response to an externally applied stimulus. This is the first time that the immunomodulatory behaviour of synthetic *p*-HBADs was investigated.

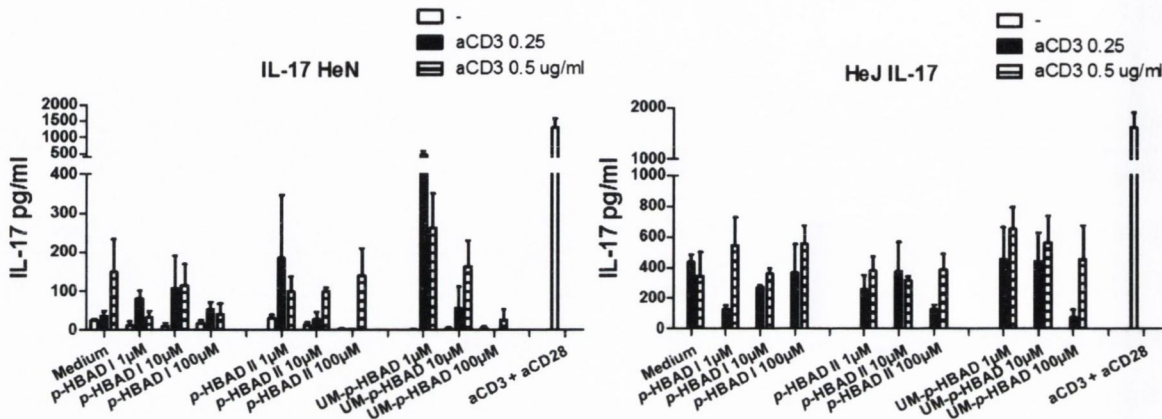
4.2.2.2 IL-17

IL-17 is a proinflammatory cytokine that helps draw immune cells to the site of infection.¹⁴ As with IFN- γ , increased levels of IL-17 would be detrimental to mycobacterial survival and the *p*-HBADs were not expected to enhance its production.

In the first of the three sets of experiments performed, both HeN and HeJ cells responded to the aCD3 stimulus and released IL-17. However, an overall pattern was difficult to discern

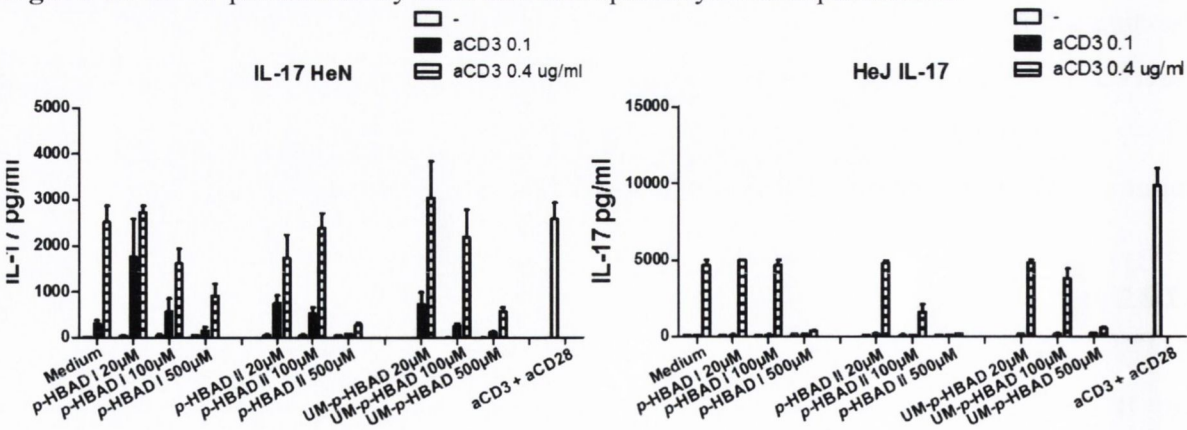
(Figure 4.6). There was potentially some inhibitory activity by increasing concentrations of UM-*p*-HBAD.

Figure 4.6 IL-17 production by HeN and HeJ splenocytes in experiment 1



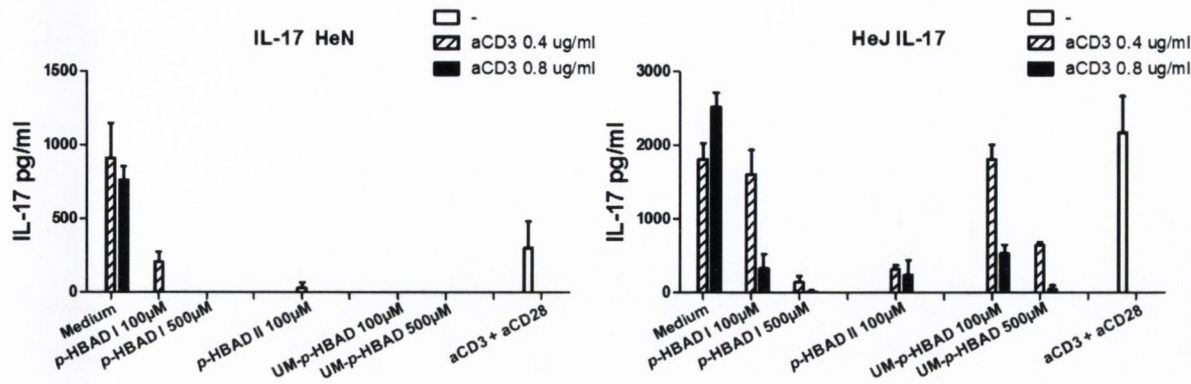
In experiment 2, the HeN cells showed inhibited IL-17 production in the presence of *p*-HBAD I, however, these results were not reflected in the HeJ cells (Figure 4.7). With UM *p*-HBAD and *p*-HBAD II, both HeN and HeJ cells showed inhibited production of IL-17. The downward trend for *p*-HBAD II was less distinct in the HeN population as was the downward trend in response to UM-*p*-HBAD in the HeJ population. Despite the small discrepancies it appears that all three *p*-HBADs, while failing to induce IL-17 production, can act to inhibit IL-17 production by splenocytes in response to a stimulus.

Figure 4.7 IL-17 production by HeN and HeJ splenocytes in experiment 2.



The HeN population in experiment 3 largely failed to produce any IL-17 (Figure 4.8). The HeJ population indicated a decrease in IL-17 levels in response to increasing *p*-HBAD concentrations though the trend was not as distinct as it might have been.

Figure 4.8 IL-17 production by HeN and HeJ splenocytes in experiment 3.

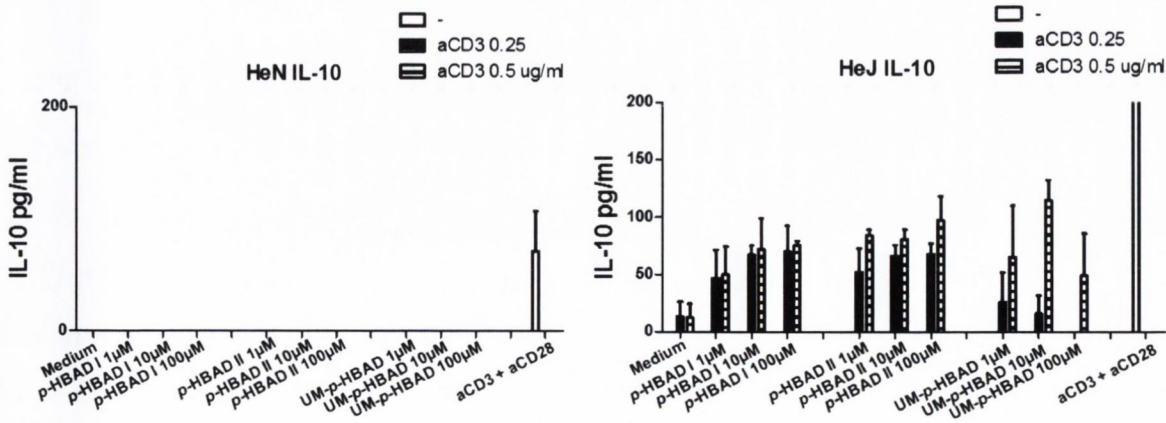


4.2.2.3 IL-10

IL-10 is produced by T_h2 cells. The T_h2 immune response is not effective at clearing intracellular parasites such as mycobacteria. The production of cytokines, such as IL-10, that promote a T_h2 immune response would be beneficial to the mycobacterium. Virulence factors that enhance mycobacterial survival may enhance the production of IL-10 or they may not affect its production.

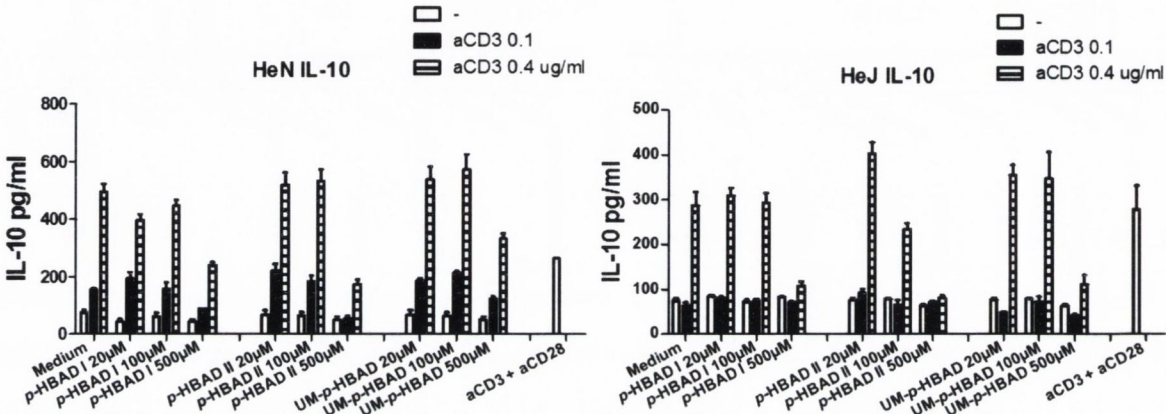
In experiment 1, HeN cells failed to produce IL-10 despite the positive control behaving as expected (Figure 4.9). This is similar to the result obtained when this population was examined for IFN- γ . The HeJ cells did respond but peculiarly as they appeared to produce more IL-10 in the presence of the p-HBADs than in their absence. No general trends could be extracted from the data.

Figure 4.9 IL-10 production by HeN and HeJ splenocytes in experiment 1.



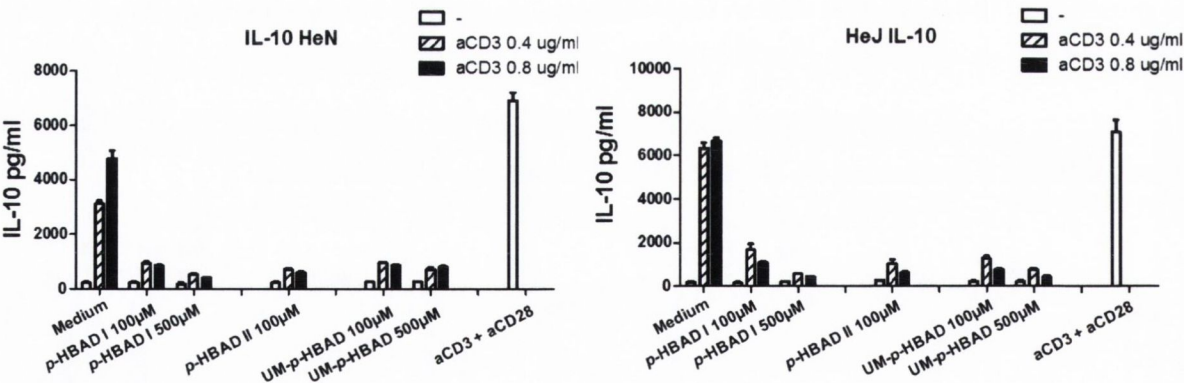
In experiment 2 an inhibitory effect in response to the three *p*-HBADs appeared to be emerging, this was particularly true of the higher doses (Figure 4.10). However the overall levels of IL-10 produced were relatively low.

Figure 4.10 IL-10 production by HeN and HeJ splenocytes in experiment 2.



In experiment 3, the levels of IL-10 produced were more significant and an inhibitory effect could be discerned in response to the *p*-HBADs (Figure 4.11). This inhibition increased with increasing *p*-HBAD concentration.

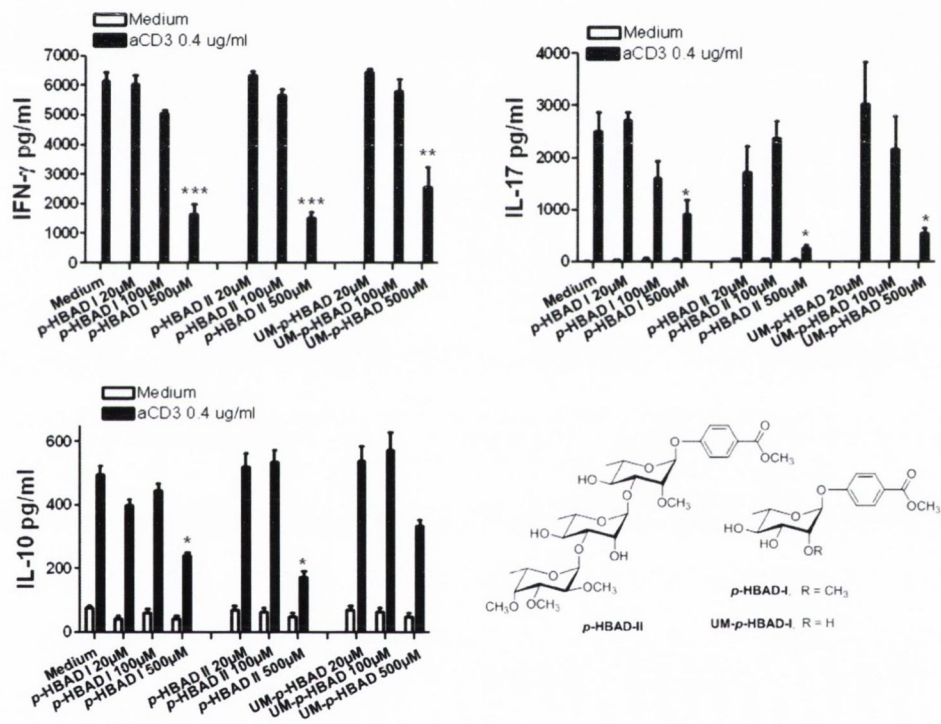
Figure 4.11 IL-10 production by HeN and HeJ splenocytes in experiment 3.



4.2.2.4 Overall cytokine response by splenocytes incubated in the presence of *p*-HBADs
 All three *p*-HBADs inhibited the secretion of IFN- γ , IL-17 and IL-10 by anti-CD3e-stimulated splenocytes in a dose dependent manner (Figure 4.12). Though this reduction was most apparent at higher concentrations of *p*-HBADs, it was still observable at lower concentrations. The results indicated that these three *p*-HBADs themselves do not have immunostimulatory properties as they failed to induce cytokine production in the absence of anti-CD3.

IFN- γ and IL-17 are both proinflammatory cytokines that prime the immune system to respond aggressively to an intracellular parasite *via* a T_h1 type response.¹⁷ If *p*-HBADs serve to reduce the defence mounted by the host immune response they would be expected to inhibit such proinflammatory response mediators. IL-10 on the other hand is anti-inflammatory and more typical of a T_h2 response. By the same reasoning, *p*-HBADs would not necessarily be expected to affect the production of anti-inflammatory cytokines such as IL-10. Nonetheless the experiments above indicate that the *p*-HBADs examined inhibit the production cytokines in general, both proinflammatory and anti-inflammatory cytokines.

Figure 4.12 Demonstrative results for the inhibition of cytokine production by *p*-HBADs. The *p*-HBADs utilised are also depicted.



4.2.3 Activation of T cells and analysis by flow cytometry

Flow cytometry was used to assess the condition of the cells in the experiments. Flow cytometry is a laser-based technique, which can analyse physical and chemical characteristics of cells suspended in a flow of liquid. The light can be scattered by particle and optional fluorescent dyes can emit light of a different wavelength to the original beam. Multiple laser sources allow for the analysis of several attributes at once. Detectors are placed to pick up the light output and analysed.

Flow cytometry was used to examine intracellular IFN- γ in both HeN and HeJ cells incubated in the presence of *p*-HBADs. Two population of cells were examines: CD3⁺CD4⁺ and CD3⁺CD4⁻ cells. All T cells express the CD3 receptor on their surface but CD4 is mainly expressed by just T helper cells (T_h cells). T_h cells are not cytotoxic or phagocytic, they activate and direct other immune cells. Amongst other functions, they activate cytotoxic T cells, and prime the bactericidal activity of macrophages. T_h cells express CD4 when mature and hence are referred to as CD4⁺ cells.¹⁷⁵

The cells were treated with CFSE (carboxyfluorescein succinimidyl ester) a fluorescent stain. Once in the cell CFSE attaches to intracellular molecules. The CSFE in cells that divide is split between the two daughter cells and thus the proliferation of a cell population can be detected using the fluorescence.

Both HeN and HeJ cells were examined by flow cytometry. The cells were incubated in the presence of three *p*-HBADs both with and without the T cell stimulant aCD3. Controls of medium with and without aCD3 were used as comparisons. Flow cytometry provided information on the activity of splenocytes, specifically on proliferation and the production of IFN- γ . The numbers in the corners indicate the percentage of the cells examined in each sample (Figure 4.13). The percentage in the top left corner represents the cells that are neither dividing (proliferating) nor actively producing IFN- γ . The top right number is the portion of the cells that are producing IFN- γ but not proliferating. The bottom left number represents the proliferating portion of the population that are not producing IFN- γ . The number in the bottom right is the portion of cells that are both proliferating and producing IFN- γ . The numbers are not essential as once the format of the graphic is understood the results can be seen in the shapes obtained.

Figure 4.13 Interpretation of flow cytometry data.

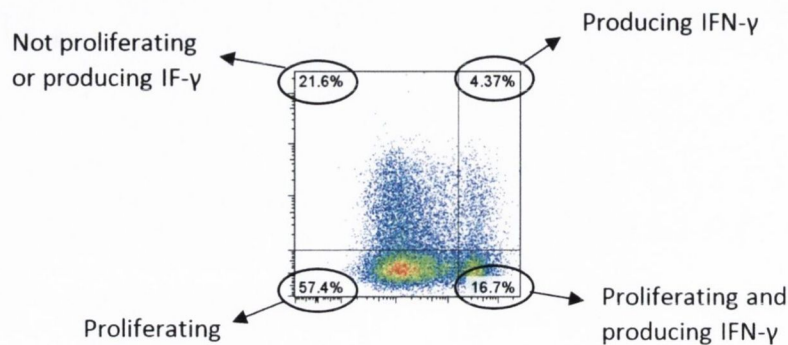
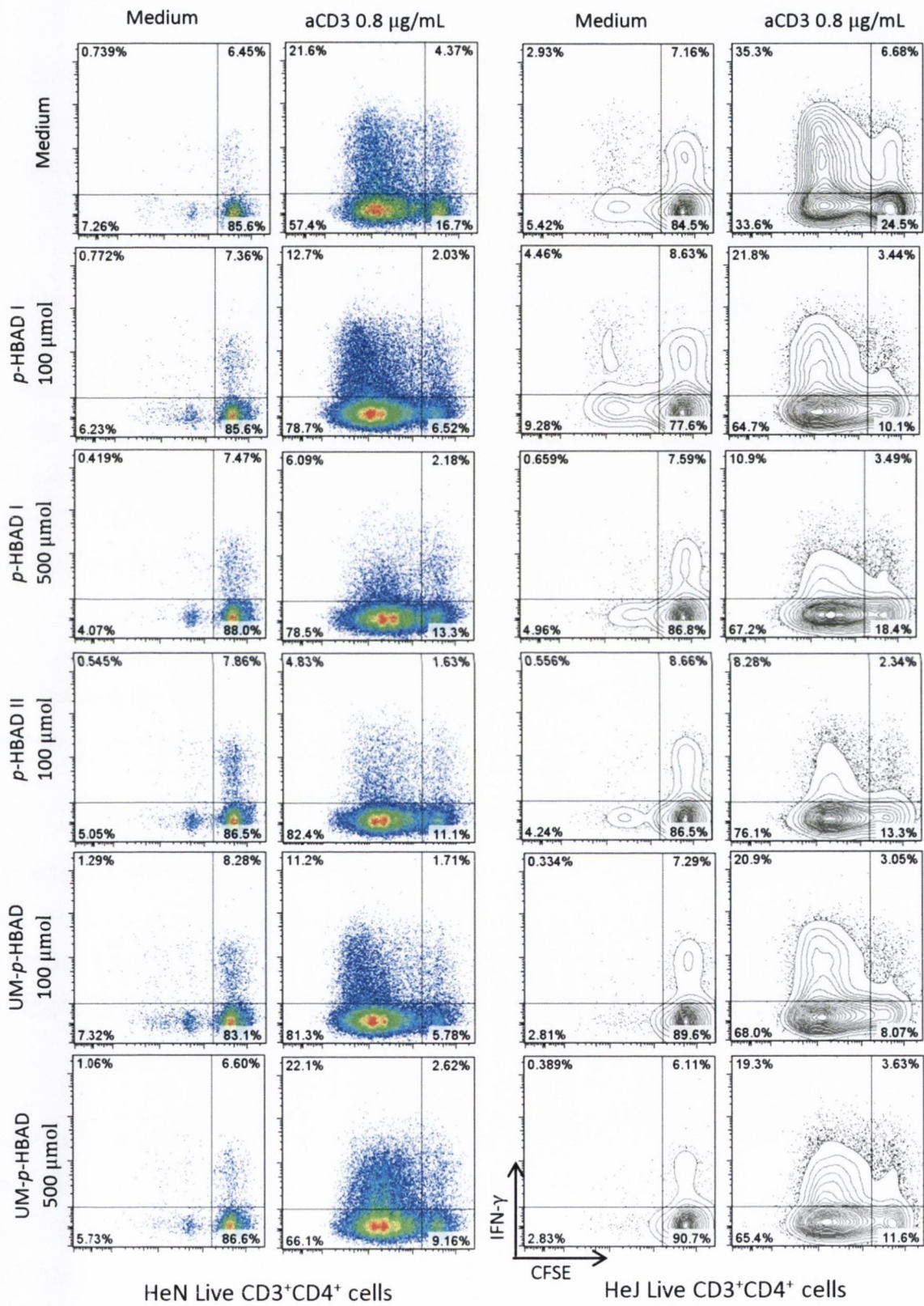


Figure 4.14 Flow cytometry of T_h cells incubated in the presence of various *p*-HBAD concentrations and the necessary controls.



The results obtained from both populations of cells are very similar which indicates that LPS contamination can be discounted as the source of the observed activity (Figure 4.14).

In the absence of both *p*-HBADs and aCD3 the cells appeared to mainly proliferate and produce IFN- γ . Even when *p*-HBADs were added to the mix there was only a slight increase in the proportion of cells producing IFN- γ . On their own, none of the *p*-HBADs examined caused a significant response.

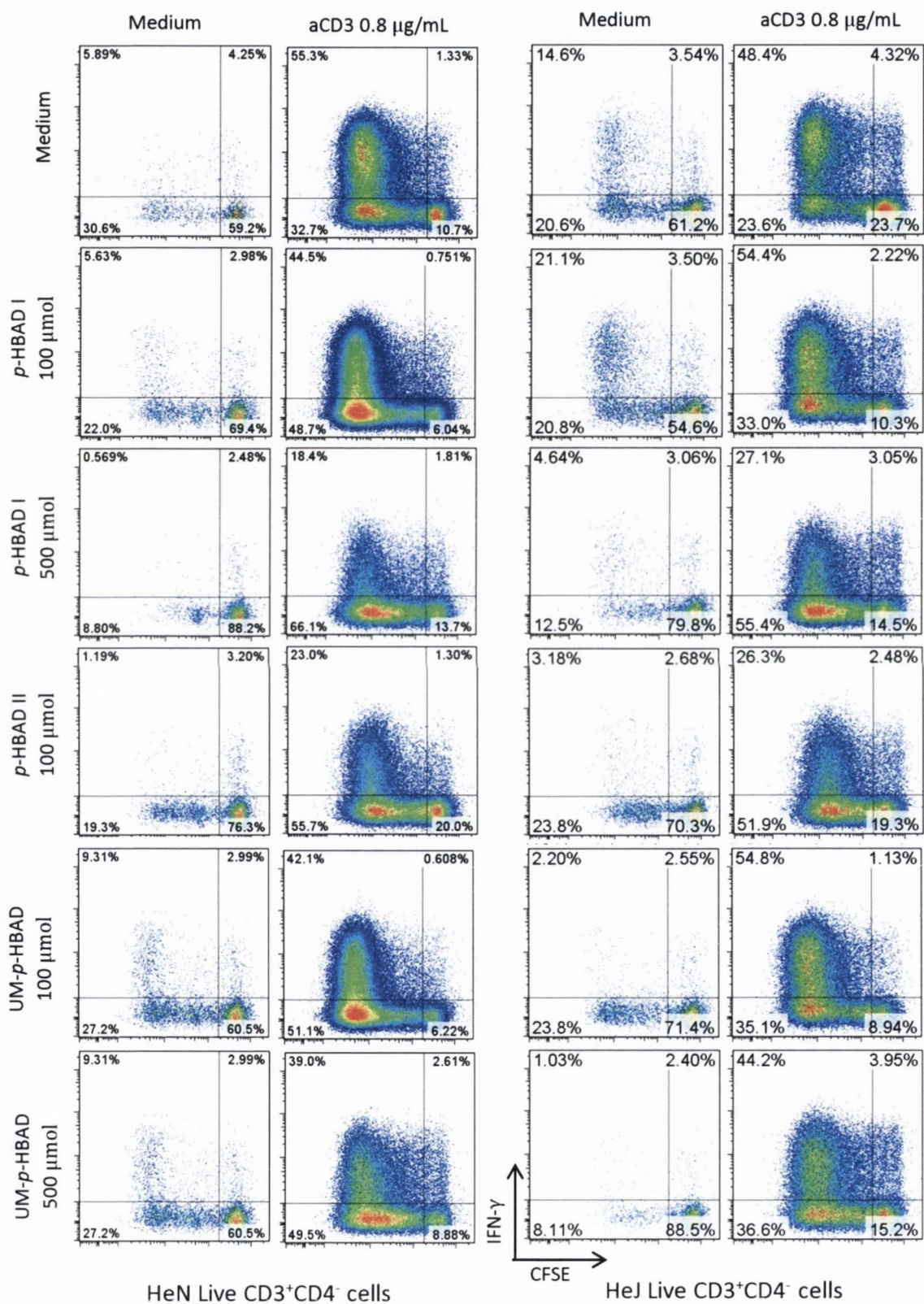
When aCD3 was added to the cells without any *p*-HBADs there was a clear surge of activity with the majority of cells proliferating but not producing IFN- γ . Once *p*-HBADs were added to the mixture the effect of the aCD3 appeared to be enhanced; there was a significant increase in the number of cells proliferating and a noticeable and consistent decrease in IFN- γ production. This is in keeping with the previously described ELISA results that indicate the inhibition of IFN- γ production by immune cells in response to the three *p*-HBADs. Though these cells are proliferating and can thus be regarded as being active, further work is required to determine which, if any, cytokines they are producing and at what levels.

Of the three *p*-HBADs examined, *p*-HBAD II had the strongest effect on both cell populations. The next most potent *p*-HBAD appeared to be *p*-HBAD I with UM *p*-HBAD having a similar but weaker effect.

From these data it can be concluded that the *p*-HBADs examined antagonise the stimulatory properties of aCD3 on IFN- γ production but enhance its effect on T_h cell proliferation. However, *p*-HBADs alone do not induce proliferation without aCD3. It appears that *p*-HBADs inhibit the production of proinflammatory cytokines by means of decreased IFN- γ production but without adversely affecting T_h cell proliferation.

The observations described above are true for T_h cells, but further work was necessary to determine if T cells in general are affected in a similar manner. Similar work was carried out using both HeN and HeJ CD3⁺CD4⁻ cells (Figure 4.15). As previously discussed, all T cells express CD3 and are thus known as CD3⁺ cells. The CD4⁻ indicates that the cells do not express the CD4 receptor on their surface and hence are not T_h cells.

Figure 4.15 Flow cytometry of T cells incubated in the presence of various *p*-HBAD concentrations and the necessary controls.



The three *p*-HBADs examined appear to have similar effects on both CD3⁺CD4⁺ and CD3⁺CD4⁻ cells. As in the case of the CD3⁺CD4⁺ cells, there was little difference between the HeN and HeJ cell populations. In the presence of the *p*-HBADs, but without any added aCD3 stimulus, there was some IFN- γ production with a small increase in proliferation over medium alone. Both proliferation and production with a small increase in proliferation over medium alone. Both proliferation and IFN- γ production increased significantly on addition of the aCD3 stimulus in both HeN and HeJ cell populations. The application of *p*-HBADs caused the proliferation to increase further while at the same time decreasing the IFN- γ production. This leads us to conclude that the cells are proliferating but doing something other than producing IFN- γ . It still remains to be determined what the T cells are doing instead of producing IFN- γ . For CD3⁺CD4⁻ cells *p*-HBAD II had the most significant effect on activated T cells followed by *p*-HBAD I. UM *p*-HBAD had the least, if any effect on the production of IFN- γ by CD3⁺CD4⁻ cells unlike in CD3⁺CD4⁺ cells on which it appeared to exert some effect.

These results are in keeping with those obtained for the CD3⁺CD4⁺ T_h cells and seem to imply the same conclusion: *p*-HBADs modulate of the immune response to a stimulus but are unable to induce a strong immune response on their own. Since IFN- γ production is crucial for the mediation of host bactericidal effects^{28, 176} these results indicate that *p*-HBADs may help the bacterium evade the host immune system.

4.3 Macrophages

Macrophages are an important component of the host immune system and are often the first immune cells to encounter the invading pathogen. Macrophages phagocytose the *Mtb* bacilli and package them into phagosomes. *Mtb* has evolved to persist in these phagosomes and thus macrophages are the main host cell directly affected, along with other phagocytic immune cells.¹⁰ Macrophages are also important as antigen presenting cells and can induce antigen specific T cell responses which have been implicated in protective immunity.¹⁷⁷ If *p*-HBADs can modulate the macrophage cytokine response this could have knock on effects in the cells that are recruited to the site of infection and the type of immune response elicited.

4.3.1 Macrophage experiments

The macrophages used were bone marrow derived macrophages (BMM) which were isolated from the femurs of both HeN and HeJ mice. Both HeN and HeJ cells were used in order to exclude LPS effects. When isolated, the cells were not fully mature and were incubated with

macrophage colony stimulating factor (M-CSF) to obtain fully differentiated mature macrophages.

Macrophages express toll-like receptors (TLRs) and these recognise antigens from microbes to stimulate the macrophage to secrete cytokines and respond to infection. In order to stimulate cytokine production TLR agonists are applied in an analogous manner to the splenocyte experiments. Irradiated *Mtb* H37Rv cells have many TLR agonists on their cell surface but are dead and unable to divide and consequently unable to cause infection and these can be added to the extra cellular medium to stimulate the macrophages.¹⁷⁸ The degree of stimulation depend on the number of cells added.

In the first set of experiments a ratio of ten bacterial cells to each macrophage was used. Tripalmitoyl cysteine (Pam3Cys), a TLR-2 agonist, was used as an alternative stimulus.¹⁷⁹ The two positive controls used were LPS (lipopolysaccharide) and CpG. As for splenocytes, HeN macrophages are sensitive to LPS. Where a cytosine nucleotide is next to a guanine on DNA strand this is called CpG. CpG sites are significantly more common in bacterial DNA than in animal DNA, and are TLR-9 agonists.¹⁸⁰ Pam3Cys did not induce a strong cytokine response, however, the irradiated H37Rv cells did. As such it was decided to continue using the H37Rv in the later experiments but to discontinue the use of Pam3Cys. The same *p*-HBAD concentrations that were used in the first splenocyte experiment were used in the first macrophage experiment: 1 μmol , 10 μmol , and 100 μmol .

In the second set of experiments carried out, a lower ratio of 1:1 bacilli to macrophages was used as well as the original successful 10:1 ratio. The rationale behind this was that a lower ratio could potentially result in a more sensitive response. The lower ratio appeared to be too weak a stimulus and failed to elicit a strong cytokine response. As with the splenocytes, the *p*-HBAD quantities were increased to 20 μmol , 100 μmol , and 500 μmol of each.

In the third experiment the successful combinations of 10:1 and 100 μmol were maintained as was the higher 500 μmol dose of *p*-HBADs. A higher 20:1 ratio of H37Rv cells to each macrophage was used as a stronger stimulus to determine if the *p*-HBADs could still exert an effect even in the presence of such a potent stimulus.

4.3.2 Macrophage cytokines

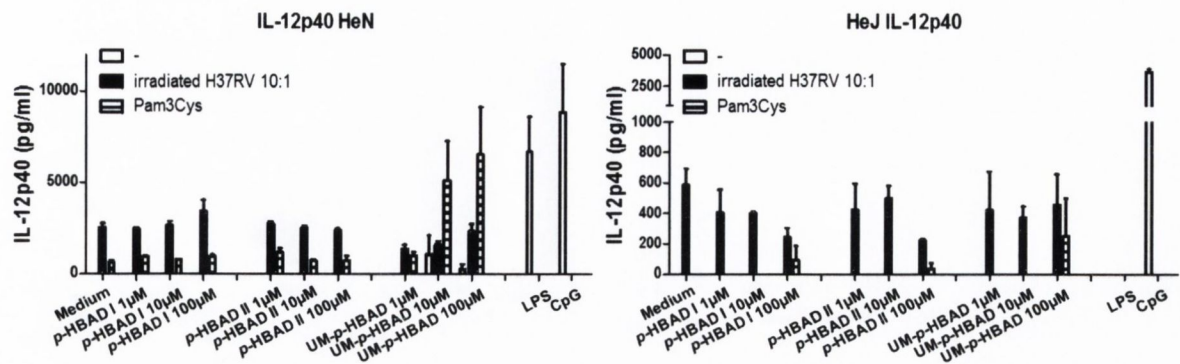
Macrophages are activated by IFN- γ secreted by natural killer T cells and TNF- α secreted by macrophages. Activated macrophages also express the anti-inflammatory IL-10 and the proinflammatory IL-12 which compete.²² Overall, a large variety of cytokines is produced by macrophages and some are of particular interest in tuberculosis infection. TNF- α released by infected macrophages induces the secretion of chemokines by epithelia and endothelial cells. TNF- α is vital for granuloma formation though how this is related to disease pathogenesis is uncertain. IL-6 and IL-1 encourage differentiation of T cells, and induces expression of IL-2 receptors by T cells. IL-12 and IL-8 produced by macrophages are proinflammatory and linked to cell influx.^{18, 181} IL-12 is associated with granuloma formation and induces IFN- γ secretion by sensitised T cells.^{14, 25} IL-12 and TNF- α produced by macrophages promote a T_h1 immune response and IL-12 also acts to downregulate the T_h2 response. IL-10 downregulates the T_h1 response.^{19, 20}

4.3.2.1 IL-12p40

IL-12 is a proinflammatory cytokine that promotes a strong immune response against invading mycobacteria. The ability to reduce the production of IL-12 would be of benefit to mycobacteria as a suboptimal immune response would enhance bacterial survival.

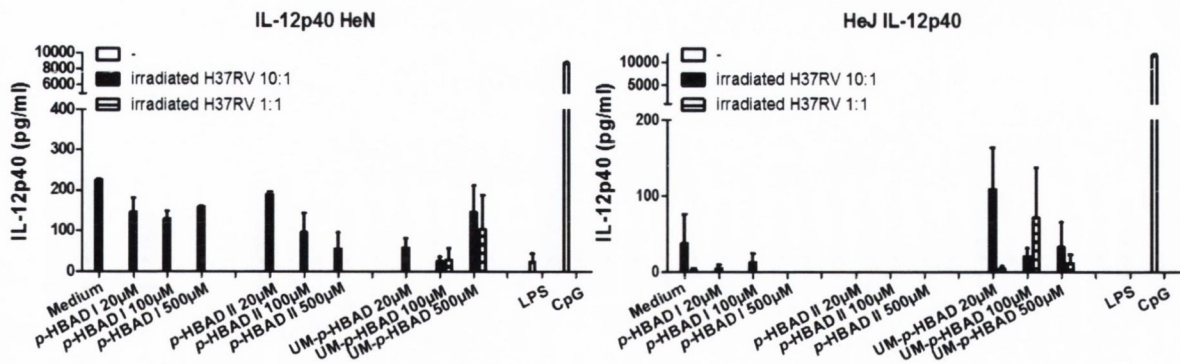
IL-12p40 is a subunit of IL-12 and this was tested for *in lieu* of IL-12. In the first set of experiments the HeN cells failed to produce a significant amount of IL-12p40 that could allow for trends to be observed (Figure 4.16). The HeJ cells responded more vigorously and showed a general decrease in IL-12p40 in response to *p*-HBAD I and also to *p*-HBAD II. No trend was observed in response to UM-*p*-HBAD but since these were initial experiments the possibility was not discounted. Neither population responded to the Pam3Cys stimulus. As expected both HeN and HeJ cells responded strongly to CpG and only the HeN cells responded to LPS.

Figure 4.16 IL-12p40 production by HeN and HeJ macrophages in experiment 1.



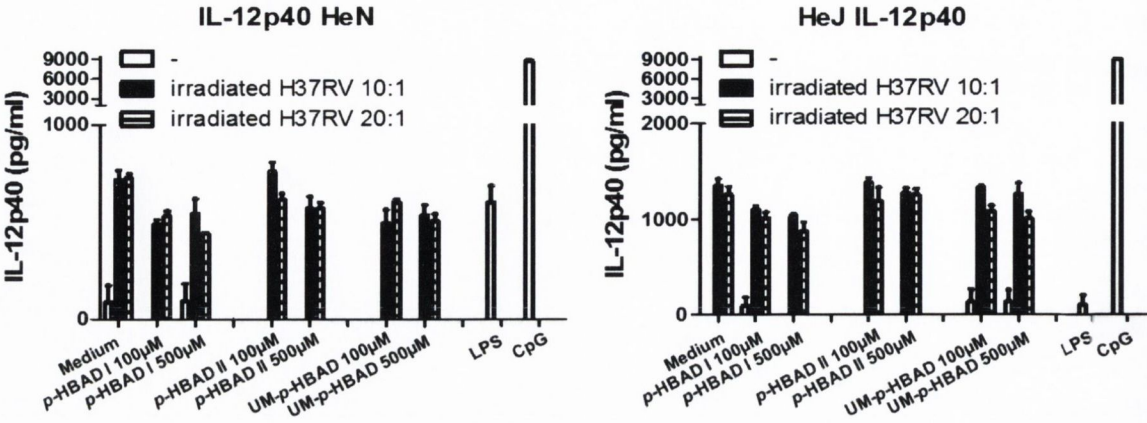
In the second set of experiments the HeN cells produced little IL-12p40, and they exhibited a very weak reaction to LPS (Figure 4.17). Nonetheless, the previously observed trends were clearly replicated in the HeN population. Both *p*-HBAD I and *p*-HBAD II inhibited the production of IL-12 by activated macrophages in a dose dependent manner. The HeJ cells failed to produce significant quantities of IL-12p40. Though there appeared to be a pattern in response to UM *p*-HBAD, this cannot be reasonably analysed in the absence of a standard response for comparison. Though the HeJ cells did not respond this does not necessarily imply LPS contamination; rather, it appears that the HeJ cells failed to respond to the applied H37Rv stimulus. Additionally LPS did not result in significant cytokine production by HeN cells.

Figure 4.17 IL-12p40 production by HeN and HeJ macrophages in experiment 2.



In experiment 3 both sets of cells responded similarly to all three *p*-HBADs (Figure 4.18). UM *p*-HBAD did not appear to inhibit IL-12 production and *p*-HBAD II was somewhat inhibitory. A clear effect on IL-12 production was observed in response to *p*-HBAD I.

Figure 4.18 IL-12p40 production by HeN and HeJ macrophages in experiment 3.

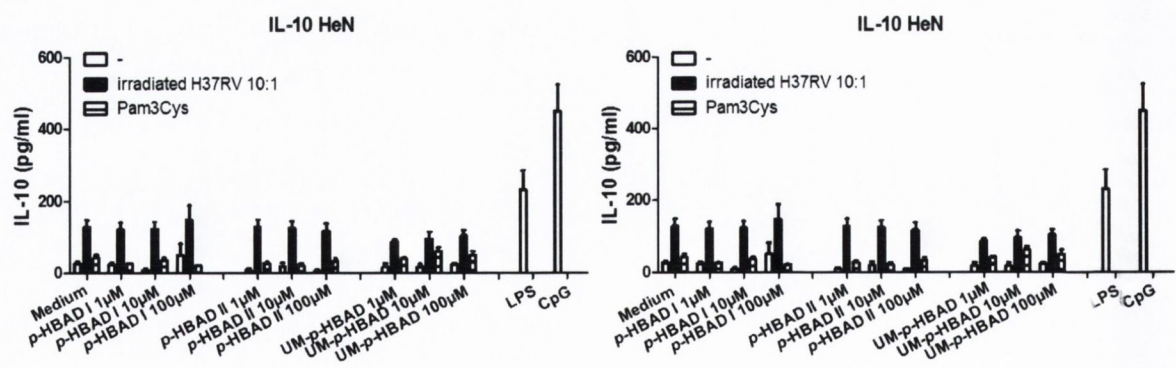


4.3.2.2 IL-10

As discussed above, IL-10 production is linked to a suboptimal immune response and enhanced mycobacterial survival. The induction of IL-10 or its unaltered production could both be viewed as potentially beneficial to the mycobacterium.

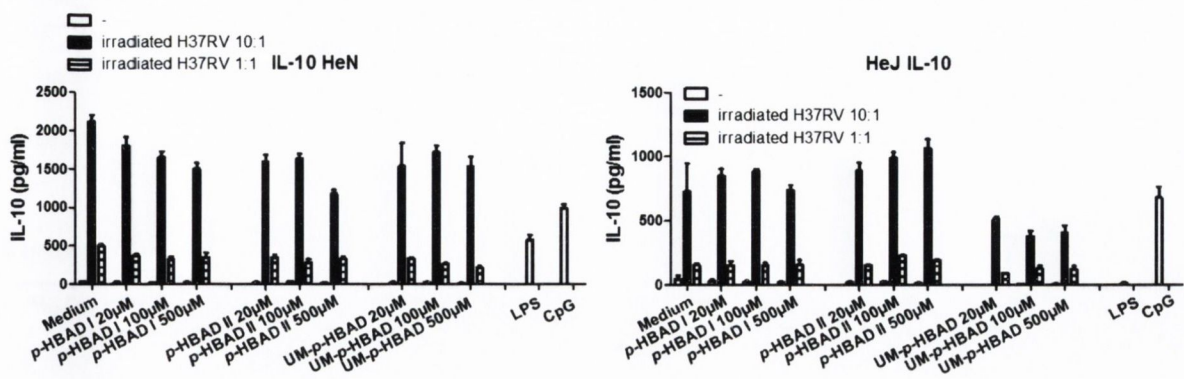
In experiments 1 the levels of IL-10 produced by both HeN and HeJ cells were very low (Figure 4.19). As for IL-12p40, Pam3Cys failed to induce the production of high levels of IL-10. The production of IL-10 in the positive controls was relatively low though some was produced. Production of IL-10 by both HeN and HeJ appeared unaffected by any of the *p*-HBADs.

Figure 4.19 IL-10 production by HeN and HeJ macrophages in experiment 1.



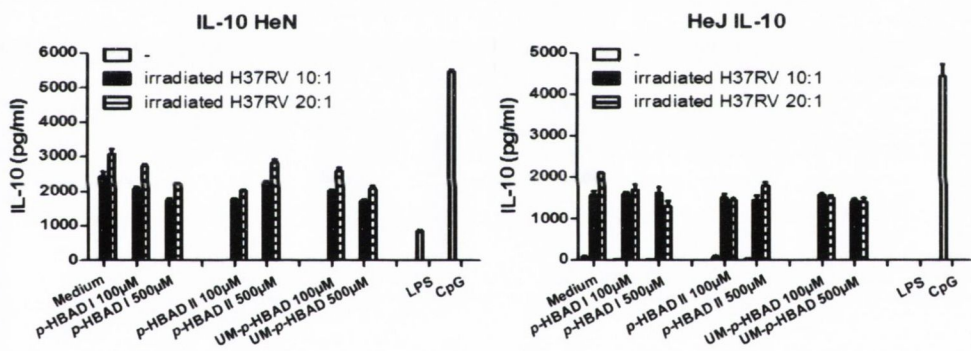
In experiment 2 both HeN and HeJ cells responded well to the higher ratio of H37Rvbacilli to macrophages but not the lower ratio (Figure 4.20). No real trend could be discerned in IL-10 production in response to *p*-HBADs.

Figure 4.20 IL-10 production by HeN and HeJ macrophages in experiment 2.



In examining the results of experiments 3 there was still no evidence of the *p*-HBADs examined inhibiting the production of IL-10 (Figure 4.21). In both experiment 2 and 3 *p*-HBAD I appears somewhat inhibitory but the results are not consistent or significant enough to be noteworthy.

Figure 4.21 IL-10 production by HeN and HeJ macrophages in experiment 3.



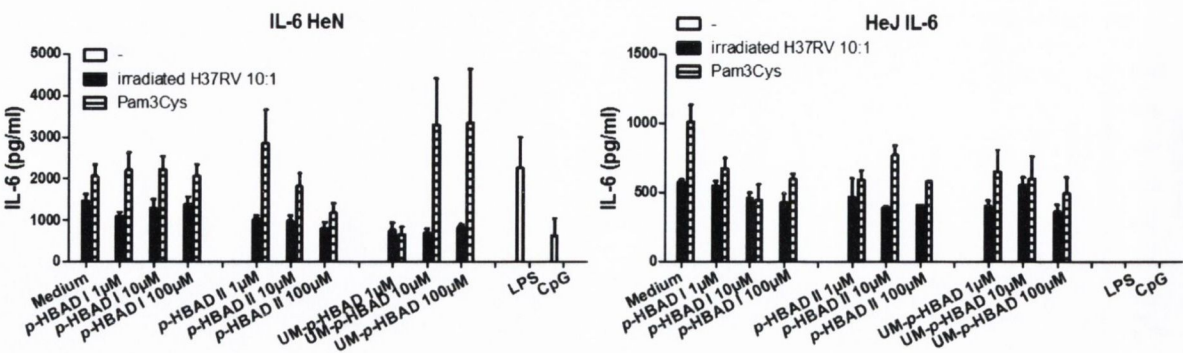
One could reasonable conclude that the *p*-HBADs examined did not have a large and consistent effect on the production of IL-10 by murine bone marrow derived macrophages.

4.3.2.3 IL-6

IL-6 is proinflammatory and associated with decreased mycobacterial survival. As virulence factors, *p*-HBADs might be expected to reduce its production and thus aid mycobacterial survival.

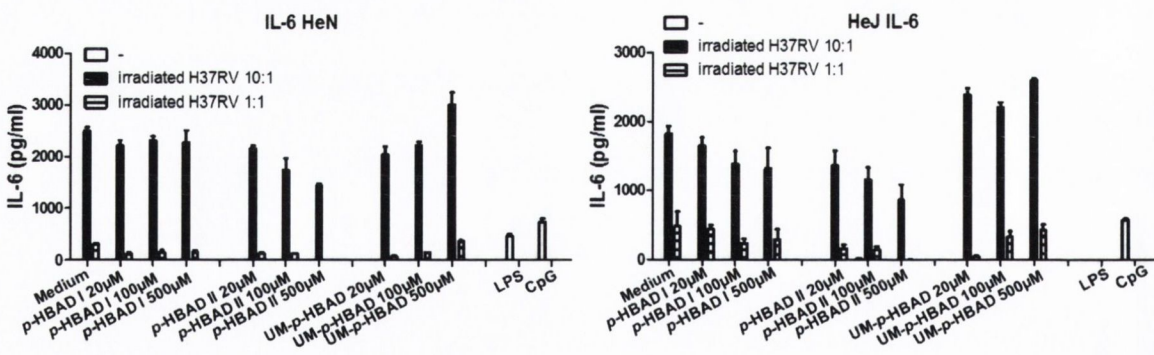
In experiment 1, both methods of stimulation applied to both HeN and HeJ cells resulted in the production of appreciable levels of IL-6 (Figure 4.22). It would seem that Pam3Cys was a stronger inducer of IL-6 that the other two cytokines discussed above. Despite being effective in the context of IL-6 production Pam3Cys was not a potent inducer of cytokine production overall.

Figure 4.22 IL-6 production by HeN and HeJ macrophages in experiment 1.



In experiment 2, the response of the HeN cells to CpG was weak and the HeJ cells failed to respond to CpG at all (Figure 4.23). No general, consistent trends could be observed. With HeN cells, *p*-HBAD II seemed to reduce IL-6 levels but since the levels, even in the presence of 100 µmol of *p*-HBAD II, are comparable to those produced in the absence of any *p*-HBADs this is most likely not an indication of any biological activity. With HeJ cells, *p*-HBAD I, and maybe UM *p*-HBAD, might be seen to inhibit IL-6 but this was not a clear pattern and so, unless replicated, must be discounted. The failure of the CpG positive control also detracted from any potential patterns that may have been observed with the HeJ cells.

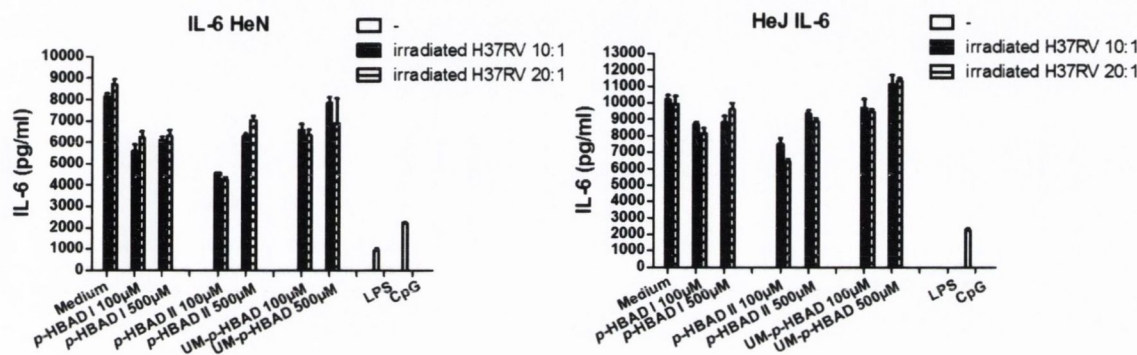
Figure 4.23 IL-6 production by HeN and HeJ macrophages in experiment 2.



In experiment 2 the lower ratio of bacilli to macrophages failed to induce any significant IL-6 production (Figure 4.23). As before, the 10:1 ratio provided adequate stimulus for a clearly observable IL-6 response. This time however, the positive controls were more consistent, with both populations of cells responding to CpG and the HeN cells responding to LPS. The only potential trend replicated by both HeN and HeJ is a slight decrease in IL-6 production in the presence of *p*-HBAD II.

Experiment 3 failed to confirm any of the potential trends detected in the first 2 experiments (Figure 4.24). As such, the results for IL-6 are somewhat debatable but if further investigations were to be carried out it might be prudent to focus on *p*-HBAD II as it is the *p*-HBAD most likely to demonstrate an effect based on the previous data acquired.

Figure 4.24 IL-6 production by HeN and HeJ macrophages in experiment 3.

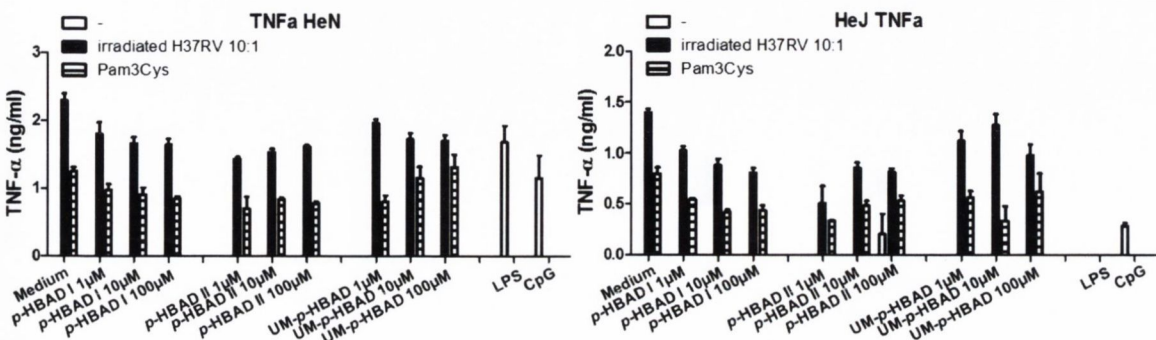


4.3.2.4 TNF-α

As one of the major proinflammatory cytokines, the production of TNF-α plays an important role in mycobacterial survival. The ability to curtail its production would be greatly beneficial to the bacterium.

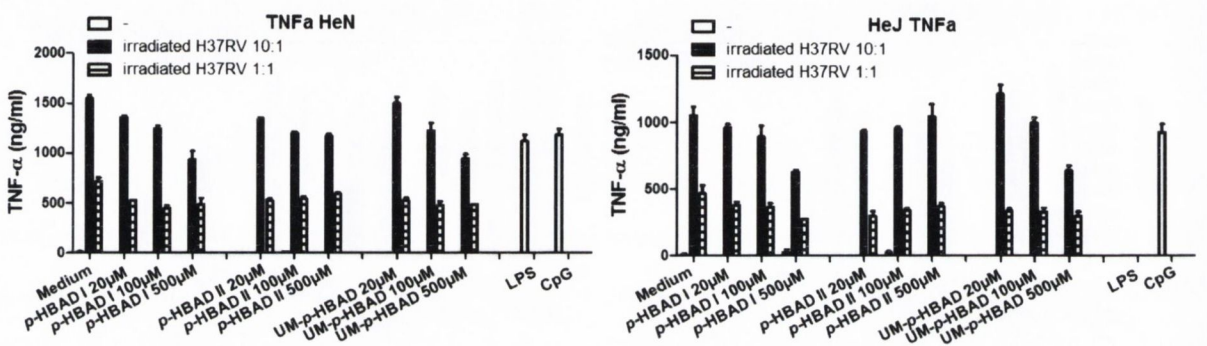
Significant quantities of TNF-α were produced in all three experiments. In experiment 1 both HeN and HeJ cells responded to both stimuli by producing TNF-α (Figure 4.25). However, the response to Pam3Cys was significantly lower and HeJ produced less TNF-α than the HeN cells. Both populations of cells respond similarly, showing significant inhibition in response to *p*-HBAD I in a dose dependent manner.

Figure 4.25 TNF-α production by HeN and HeJ macrophages in experiment 1.



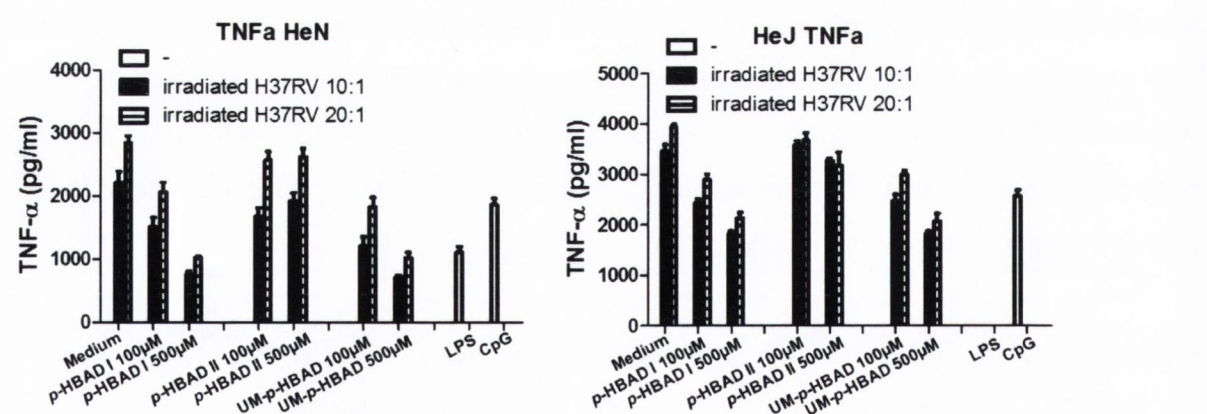
In experiment 2, 10:1 bacilli to macrophages induced a strong response, and the 1:1 ratio a weaker one (Figure 4.26). As in experiment 1, the application of *p*-HBAD I reduced TNF- α production in proportion to the dose applied. Though no pattern is obvious in TNF- α in the presence of *p*-HBAD II, UM *p*-HBAD was inhibitory in a similar manner to *p*-HBAD I.

Figure 4.26 TNF- α production by HeN and HeJ macrophages in experiment 2.



Experiment 3 showed results consistent with the previous two experiments (Figure 4.27). Both HeN and HeJ cells responded almost identically. Both *p*-HBAD I and UM-*p*-HBAD inhibited the production of TNF- α by activated macrophages despite the strong external stimulus applied. Over the three experiments *p*-HBAD II did not appear to be similarly inhibitory.

Figure 4.27 TNF- α production by HeN and HeJ macrophages in experiment 3.

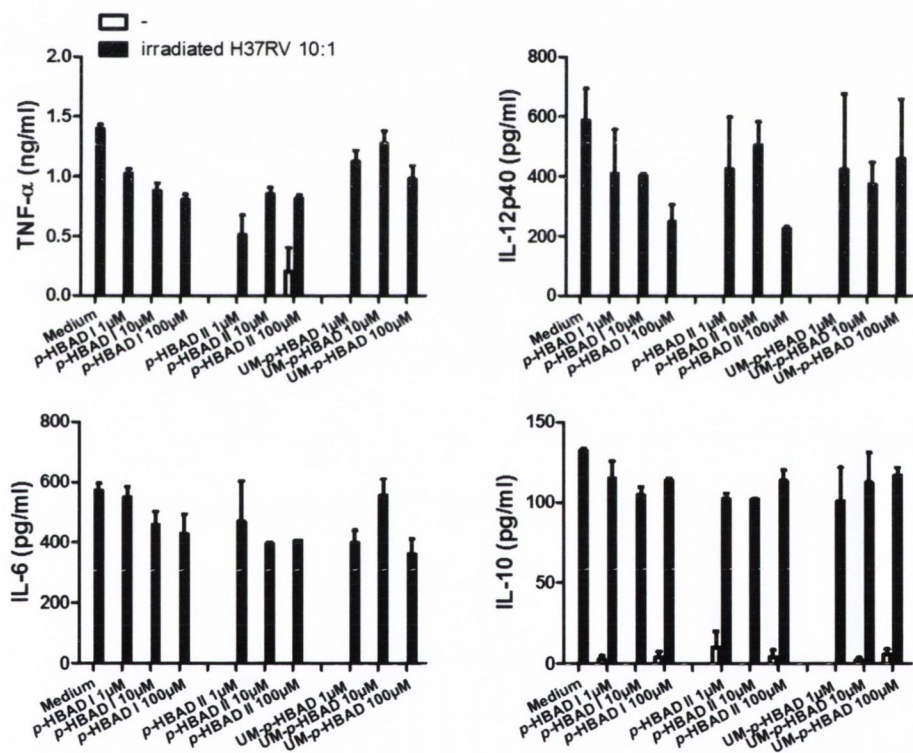


4.3.2.5 Overall cytokine response by macrophages incubated in the presence of *p*-HBADs

The response to *p*-HBADs by macrophages was related to that observed with splenocytes. Stimulation in the presence of *p*-HBAD-I significantly decreased TNF- α and IL-12 production by the activated macrophages (Figure 4.28). A similar but much weaker effect was observed on IL-6 and there was no discernible effect on IL-10 production. UM-*p*-HBAD had a similar effect and significantly reduced the production of TNF- α and also had no effect on IL-10

production in response to stimulation with H37Rv. Though *p*-HBAD II appeared to affect cytokine production by activated splenocytes it did not appear to affect cytokine production by BMMs. TNF- α , IL-12 and IL-6 are all proinflammatory cytokines that upregulate the immune response towards invading pathogens.¹⁴ IL-10 is an anti-inflammatory cytokine that downregulates the response to such pathogens. The *p*-HBADs appear to reduce the production of proinflammatory cytokines but not anti-inflammatory cytokines by macrophages. This inhibition could potentially improve bacterial survival by delaying the activation of the immune system by the first line macrophages.

Figure 4.28 Demonstrative results for the inhibition of cytokine production by activated macrophages in the presence of *p*-HBADs.



4.3.3 Aqua live/dead

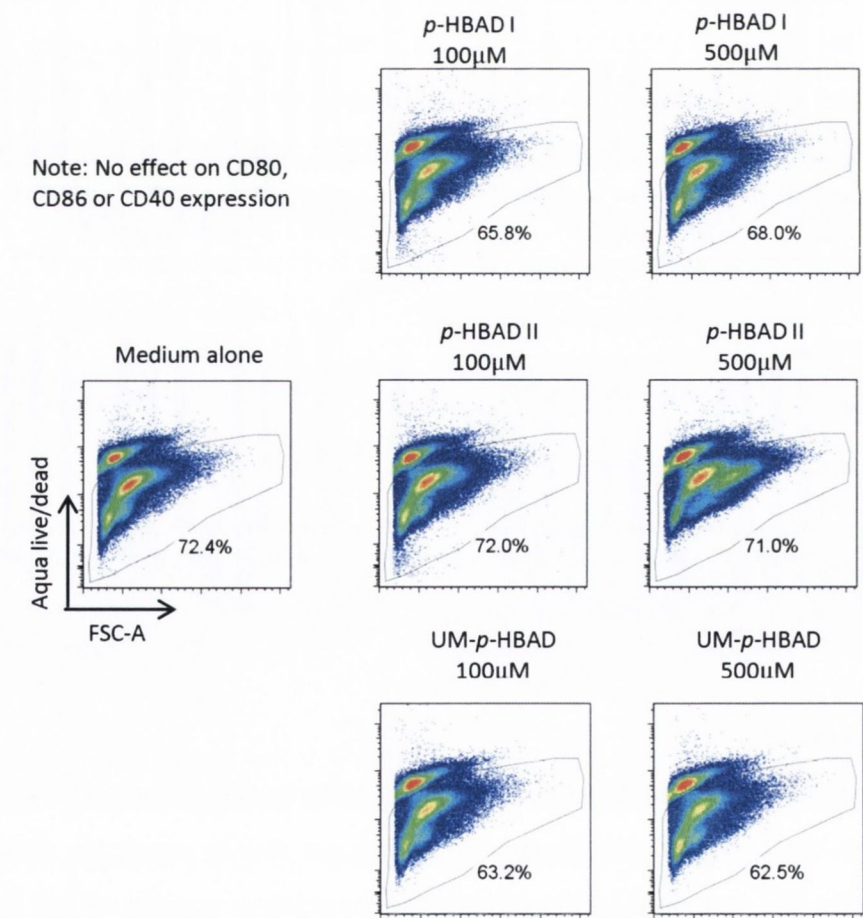
In order to conclude that the *p*-HBADs examined modify the secretion of cytokines it must be established that the observed inhibitory effects are not due to cytotoxic properties. Aqua live/dead staining is a technique used to determine the relative number of still living cells. In aqua live/dead staining an amine reactive fluorescent dye is applied to cells. If a cell has a compromised membrane, the dye reacts with free amines in the cytoplasm and the cell surface resulting in fluorescent staining. If the membrane is intact, the dye can only react with amines

on the surface generating greatly reduced fluorescence. A flow cytometer can be used to analyse the cells and determine what proportion of the population is still alive.

HeJ macrophages were treated with aqua live/dead stain then incubated in the presence of various concentrations of *p*-HBADs. While *p*-HBAD II had no adverse effect on cell survival *p*-HBAD I had a negligible effect (Figure 4.29). UM *p*-HBAD had a very slight impact but not enough for the impact of UM-*p*-HBAD on cytokines to be attributed to a decrease in cell numbers.

From this it can be concluded that the immunomodulatory properties of *p*-HBADs observed were not due to cytotoxicity.

Figure 4.29 Aqua live/dead staining of macrophages.

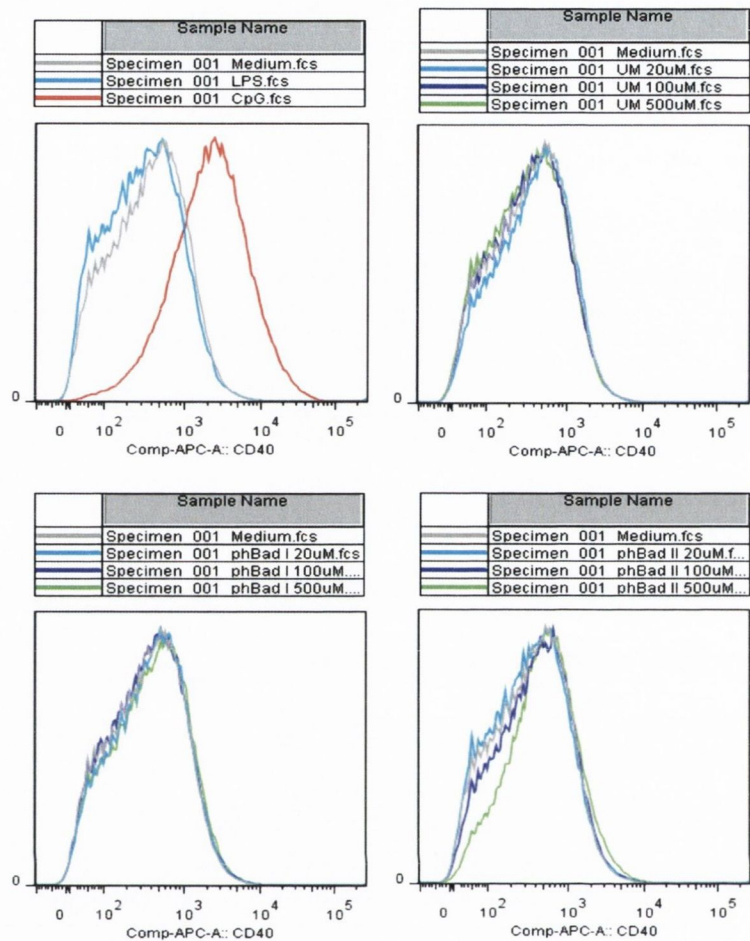


4.3.4 Clusters of differentiation

Flow cytometry was used to determine if the applied cytokines had an effect on the expression of certain proteins required for the activation of T cells found on the cell surface. CD40 is a costimulatory protein found on antigen presenting cells such as macrophages and is necessary for their activation. Additionally CD40 is important in many immune responses including humoral immunity and B cell proliferation.¹⁸² CD80 and CD86 are sometimes also referred to as B7.1 and B7.2 respectively. CD80 and CD86 both bind to CD28 found on T cells and are costimulatory; they induce the up regulation of a high affinity IL-2 receptor allowing T cells to proliferate.¹⁸³ These proteins are particularly important in T cell activation and their expression indicates what kind of signals are being produced and what types of immune cells are recruited and how they are activated at the site of infection.

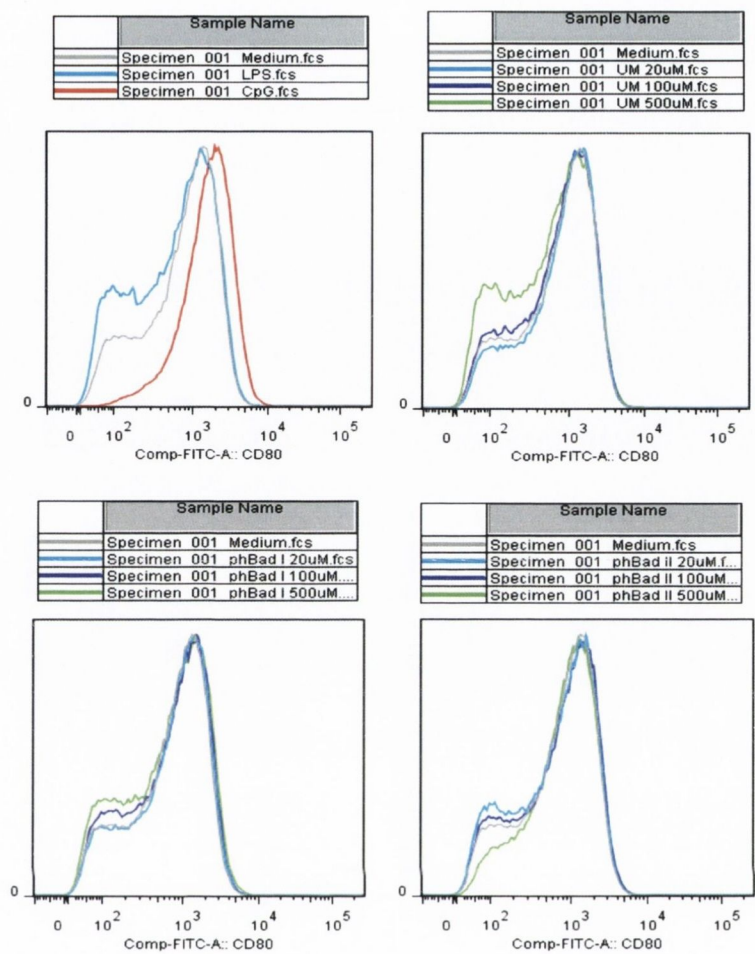
In response to stimulation by CpG the expression of CD40 is unregulated but not so with the three *p*-HBADs applied (Figure 4.30). Since CD40 is important for the activation of macrophages and other immune cells the up regulation of its expression in response to *p*-HBADS would indicate that the *p*-HBADs are activating the host immune system.

Figure 4.30 CD40 expression in response to *p*-HBADs.



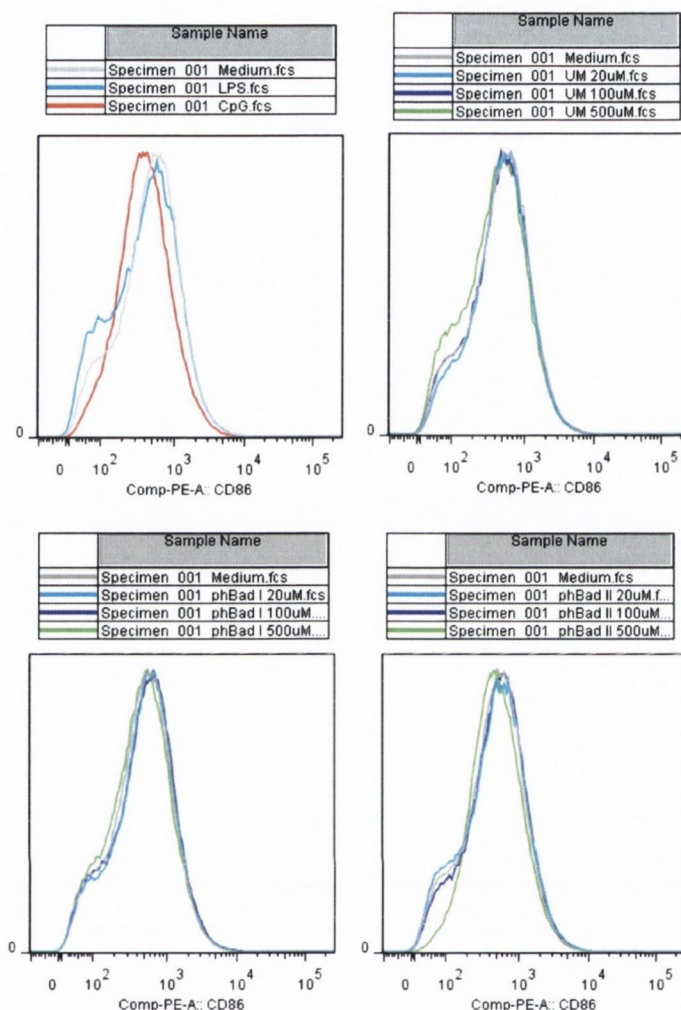
When macrophages were examined for CD80 similar results were observed (Figure 4.31). The expression of both CD80 and CD86 remains unaltered indicating being unregulated since these are stimulatory markers required for their activation.

Figure 4.31 CD80 production in response to *p*-HBADs.



CD80 works with CD86 to activate T cells and the macrophages were examined for CD86 expression as well as CD80 (Figure 4.32). As with both CD40 and CD80 the response by the cells is closer to that of unstimulated cell than stimulated cells. These results back up the observations discussed above that *p*-HBADs themselves do not activate the host immune system.

Figure 4.32 CD86 production in response to *p*-HBADs.

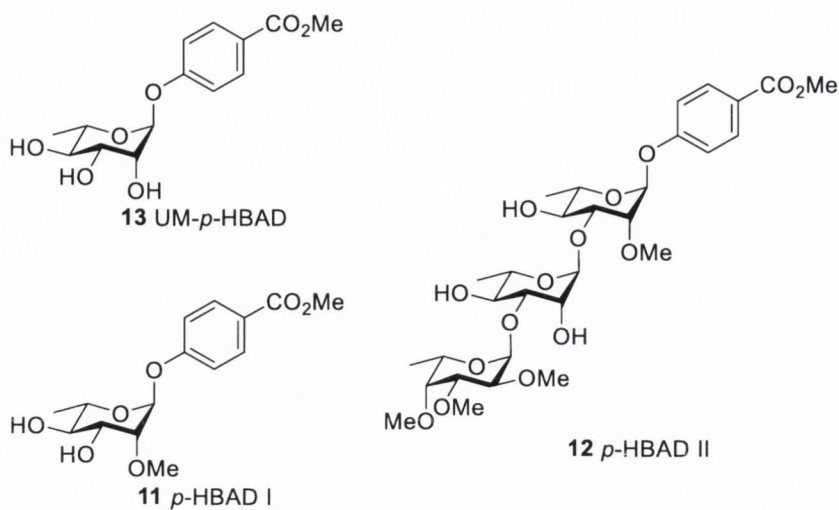


4.4 Conclusions

From a chemical point of view, the findings discussed above are particularly interesting due to the disparity between the effects of *p*-HBAD I and UM *p*-HBAD. These two *p*-HBADs differ structurally only in the presence of a methoxy group at the C-2 position of *p*-HBAD I. This methyl appears to have a crucial role to play in facilitating the biological activity of the molecules, such as it might be.

Compared to *p*-HBAD I, *p*-HBAD II is larger and more complex but it does not exert as strong an effect on the production of the cytokines examined as *p*-HBAD I. Since mycoside B, the PGL equivalent of *p*-HBAD I does not seem to affect virulence in a similar manner to PGL-tb-1, the PGL equivalent of *p*-HBAD II, it could be argued that the two different carbohydrate regions interact with different loci on or in cells of the immune system.

Figure 4.33 Native *p*-HBADs synthesised for biological testing



From a biological perspective, the above data suggests that *p*-HBADs in general have an effect on the secretion of certain cytokines by both T cells and macrophages. Further research may be needed to draw more definite conclusions as to the effect of *p*-HBADs on cytokine secretion, however, some general conclusions can be drawn. The *p*-HBADs examined inhibited the production of proinflammatory cytokines but did not similarly affect the production of anti-inflammatory cytokines. These *p*-HBADs do not activate macrophages directly and T cells either directly or indirectly are not cytotoxic.

5.0 Chapter 5: Conclusion

5.1 Concluded aims

The aims below have been achieved thus paving the way for future work in the area to be carried out in a directed manner

- Develop a protecting group strategy to control the methylation pattern on rhamnose
 - A strategy that achieves this has been developed and applied. The strategy used for rhamnose has paved the way for a similar strategy to be developed for fucose. This may be important in future syntheses of non-native PGLs and *p*-HBADs.
- Prepare a library of monosaccharide building blocks for the synthesis of native and non-native molecules
 - A library of rhamnosyl thioglycosides has been prepared. These donors have been activated using different methods and their conversion to alternative donors has been demonstrated.
- Using the above monosaccharide building blocks generate a library of *p*-HBADs
 - Both native and non-native *p*-HBADs has been prepared. It has been shown that protecting group manipulations may be carried out prior to or post the glycosylation of the methyl-4-hydroxy benzoate.
- Design and execute a synthetic route for the preparation of PGL or PGL-like molecules
 - The attachment of a long alkyl chain that could mimic the lipophilic properties of the native PGL lipid portion has been achieved using mild conditions that do not call for the use of metal catalysis.
- Investigate the biological function of *p*-HBADs
 - The effect of synthetically prepared native *p*-HBADs on murine immune cells, both in isolation and together with a stimulus, has been investigated. Overall it has been established that they can alter the response of both T cells and macrophages in response to an applied stimulus and do not induce a response on their own.

- Explore strategies for the immobilisation of carbohydrates functionalised with aromatic groups at the anomeric position, with a view to using immobilised carbohydrates to investigate biological activity
 - Several methods have been investigated through the production of appropriate precursor molecules. The diazonium grafting technique was successfully applied by collaborators Drs. Deidre M. Murphy and Dilushan R. Jayasundara supervised by Prof. Paula E. Colavita.

5.2 Published works

E. M. Scanlan, P. E. Colavita, D. Murphy, J. Bourke, J. "Carbohydrate Functionalised Surfaces". Application Number: 11184038.5 - 2112, 2011.

D. R. Jayasundara, T. Duff, M. D. Angione, J. Bourke, D. M. Murphy, E. M. Scanlan and P. E. Colavita, *Chem. Mater.*, 2013, 4122-4128.

J. Bourke, C. F. Brereton, S. V. Gordon, E. C. Lavelle and E. M. Scanlan, *Org. Biomol. Chem.*, 1114-1123, 2014.

6.0 Chapter 6: Experimental

6.1 Materials and methods

6.1.1 Mice

C3H/HeJ mice were purchased from Harlan UK. All mice were maintained according to EU regulations and experiments were performed under licence from the Department of Health and Children and with approval from the Trinity College Dublin Bioresources Ethics Committee.

6.1.2 Spleen cells

Cells were isolated from the spleens of C3H/HeJ mice. 1.5×10^6 cells/ml were activated on 96-well flat-bottomed tissue-culture plates (Greiner) that had been coated with $0.4 \mu\text{g}$ anti-CD3e (BD Pharmingen) for 3 h at 37°C , and then washed 3 times with sterile PBS. Spleen cells were cultured in medium alone or with pHBad I, pHBad II or UMphBad ($100 \mu\text{M}$). Supernatants were collected after 4 d and IFN γ (BD Pharmingen), IL-17 and IL-10 (R&D Systems) concentrations were determined by ELISA. Spleen cells were stimulated for 5 h with PMA ($0.1 \mu\text{g}/\text{ml}$) and ionomycin ($0.5 \mu\text{g}/\text{ml}$) and for the final 4 h with brefeldin A ($10 \mu\text{g}/\text{ml}$). Cells were washed, and blocked with Fc γ blocker (BD Pharmingen $1 \mu\text{g}/\text{ml}$) before staining with aqua Live/Dead stain (Invitrogen). Extracellular staining for surface CD3, CD4 and CD8 (eBioscience) was carried out and then spleen cells were fixed and permeabilized (Intracellular Fixation and Permeabilization kit (eBioscience)) and stained intracellularly for IFN γ (eBioscience). Flow cytometric analysis was performed using a BD LSRFortessa cell analyser (BD Biosciences).

6.1.3 Bone-marrow derived macrophages

Bone marrow-derived macrophages were prepared by culturing bone marrow cells from C3H/HeJ mice in medium supplemented with 15% macrophage colony-stimulating factor (M-CSF). M-CSF was collected in the supernatant of L929 cells in culture. On day 4, fresh medium containing 20% MCSF was added to the cell cultures. On day 7 cells were removed from the culture flasks and re-suspended in fresh medium containing 15% M-CSF. BMDMs were cultured with medium alone or irradiated H37RV (10:1), with or without pHBad I, pHBad II or UM pHBad (1, 10 or $100 \mu\text{M}$). Supernatants were recovered after 24 h and IL-12p40 (BD Pharmingen) and TNF- α (R&D Systems) concentrations were determined by ELISA.

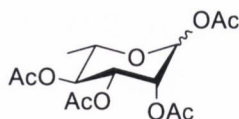
6.2 General experimental

Proton nuclear magnetic resonance (NMR) spectra were recorded on: Bruker Avance III 400 MHz, Bruker Avance II 600 MHz and Bruker DPX400 400 MHz. Chemical shifts are reported in ppm and coupling constants (*J*) are quoted in Hertz (Hz). Carbon NMR spectra were recorded on the previously mentioned instruments with total proton decoupling. HSQC, HMBC and TOCSY NMR experiments were used to aid assignment of NMR peaks when required. Spectra recorded in CDCl₃ were referenced to residual CHCl₃ at 7.26 ppm for ¹H and 77.0 ppm for ¹³C. Infrared spectra were obtained on a Perkin Elmer spectrophotometer. A Waters micromass LCT-TOF mass spectrometer was used in ESI positive and ESI negative modes for electrospray ionization mass spectrometry. Electron Impact mass spectra were recorded on the same machine in EI mode. Flash chromatography was carried out using silica gel, particle size 0.04-0.063 mm correlated with TLC mobility. TLC analysis was performed on precoated 60F254 slides, and visualised by either UV irradiation or molybdenum staining (5 g ammonium molybdate and 5.3 mL concentrated H₂SO₄ in 100 mL water). Optical rotation measurements were made on a Rudolph Research Analytical Autopol IV instrument, and are quoted in units of 10⁻¹ deg cm² g⁻¹. Anhydrous solvents were obtained by drying the relevant solvent with activated 3 Å and 4 Å molecular sieves. Unless otherwise stated, all chemicals were obtained from commercial sources and used as received. Where fresh sodium methoxide was required it was prepared by the dissolution of 100 mg of methanol washed sodium metal to 50 mL of methanol. Approximately 0.1 mL of the resulting sodium methoxide solution were used per 100 mg of substrate. The sodium hydride used was wet with mineral oil with 60% w/w sodium hydride.

Me₂S₂-Tf₂ activating agent **121**

Trifluoromethanesulfonic anhydride **120** (0.075 mL, 0.45 mmol) was added to a solution of dimethyl disulfide **117** (0.045 mL, 0.51 mmol) in anhydrous DCM (1 mL) at 0 °C and reacted for 15 min at 0 °C to give a yellow brown solution. This solution was prepared freshly and used as an activator in some glycosylations.

1,2,3,4-Tetra-*O*-acetyl-L-rhamnopyranoside (68)



L-Rhamnose **53** (2.0 g, 12.2 mmol) was dissolved in pyridine (10 mL, 124 mmol) under N₂ at 0 °C. Ac₂O (10 mL, 106 mmol) was added in increments. After 18 hours the mixture was concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product, a white solid, as a mixture of anomers in 98% yield (4.0 g).

Alternative purification: after 18 hours the mixture was quenched with iced water, diluted with EtOAc (30 mL), washed with water (20 mL), dil. aq. CuSO₄ (6 x 20 mL), and water (20 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The product was isolated in 91% yield.

Data for the α anomer:

δ_{H} (400 MHz, CDCl₃): 5.86 (1H, d, $J_{1,2} = 2.0$ Hz, H1), 5.16 (1H, m, H3), 5.14 (1H, m, H2), 5.09 (1H, dd, $J_{2,1} = 2.0$ Hz, $J_{2,3} = 3.5$ Hz, H2), 3.78 (1H, m, H5), 2.01 (3H, s, CH₃), 2.00 (3H, s, CH₃), 1.91, 1.85 (3H, s, CH₃), 1.08 (3H, d, $J_{6,5} = 6.4$ Hz, H6).

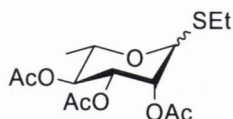
δ_{C} (100 MHz, CDCl₃): 170 (C=O), 169.87 (C=O), 169.8 (C=O), 168.42 (C=O), 90.64 (C1), 70.46 (C5), 68.77 (CH), 68.72 (CH), 68.64 (CH), 20.93 (CH₃), 20.81 (CH₃), 20.78 (CH₃), 20.71 (CH₃), 17.45 (C6).

IR: 2987.04, 1743.01, 1368.67, 1210, 1146.81, 1051.54, 969.84 cm⁻¹.

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₄H₂₀O₉Na 355.1005; found 355.0990.

The data are consistent with those in the current literature.¹⁸⁴

Ethyl-2,3,4-tri-*O*-acetyl-1-thio- β -rhamnopyranoside (78)



1,2,3,4-Tetra-*O*-acetyl- β -rhamnopyranoside **68** (4.8 g, 14.7 mmol) was dissolved in anhydrous DCM (40 mL) at 0 °C under N₂. Ethane thiol (1.2 mL, 19.0 mmol) was added followed by BF₃·OEt₂ (9.1 mL, 73.5 mmol) in increments. The stirred solution was allowed to come to room temperature. After 18 hours the reaction was quenched by treatment with sat. aq. NaHCO₃ solution (ca. 20 mL) and solid NaHCO₃. The organic layer was separated, dried with MgSO₄, filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as white solid with an overall yield of 82% (4.01 g) and the α anomer in 61% (3.01 g).

Data for the major α anomer:

δ_{H} (400 MHz, CDCl₃): 5.36 (1H, dd, $J_{2,1} = 1.6$ Hz $J_{2,3} = 3.4$ Hz, H2), 5.26 (1H, dd, $J_{3,2} = 3.4$ Hz $J_{3,4} = 10.1$ Hz, H3), 5.22 (1H, d, $J = 1.5$ Hz, H1), 5.12 (1H, app t, H4), 4.26 (1H, dq, $J_{5,6} = 6.2$ Hz, $J_{5,4} = 9.7$ Hz, H5), 2.67 (2H, m, CH₂), 2.13(3H, s, CH₃), 2.08(3H, s, CH₃), 2.01 (3H, s, CH₃), 1.32 (3H, t, $J = 7.5$ Hz, CH₂CH₃), 1.26 (3H, d, $J_{6,5} = 6.2$ Hz, H6).

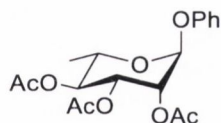
δ_{C} (100 MHz, CDCl₃): 170.10 (C=O), 170.03 (C=O), 169.89 (C=O), 82 (C1), 71.5 (C2), 71.3 (C4), 69.5 (C3), 66.7 (C5), 25.4 (CH₂CH₃), 21.0 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 17.4 (C6), 14.9 (CH₂CH₃).

IR: 2934, 1739, 1368, 1220, 1105 cm⁻¹.

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₄H₂₂O₇SNa 357.0984; found 357.0973.

The data are consistent with those in the current literature.¹⁸⁵

2,3,4-Tri-*O*-acetyl-1-phenol- α -L-rhamnopyranoside (80)



1. 2,3,4-Tri-*O*-acetyl-L-rhamnopyranoside **68** (4.00 g, 12.1 mmol) was dissolved in anhydrous DCM (20 mL) at 0 °C under N₂. Phenol (1.365 g, 14.5 mmol) was added followed by BF₃.OEt₂ (7.5 mL, 60.4 mmol) in increments. After 18 hours the reaction was quenched by treatment with sat. aq. NaHCO₃ solution (ca. 50 mL) and solid NaHCO₃. The organic layer was filtered, dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a white solid in 73% yield (3.22 g). R_f = 0.6 (hexane/EtOAc 60:40).
2. Ethyl-2,3,4-tri-*O*-acetyl-1-thio- α -L-rhamnopyranoside **78** (3.0 g, 9.0 mmol) was dissolved in anhydrous DCE (20 mL) at 0 °C under N₂. Phenol (1.016 g, 10.8 mmol), NIS (3.239 g, 14.4 mmol) and 10 μ L of TMS.OTf were added. After 18 hours the mixture was quenched with TEA (ca. 2 mL), washed with sat. aq. Na₂S₂O₃ solution (2 x 50 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/ EtOAc 80:20) gave the product as a white solid in 82% yield (2.7 g).

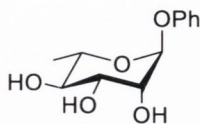
δ_{H} (400 MHz, CDCl₃): 7.31 (2H, m, Ar), 7.08 (3H, m, Ar), 5.54 (1H, dd, $J_{3,2} = 3.5$ Hz, $J_{3,4} = 10.1$ Hz H3), 5.48 (1H, d, $J_{1,2} = 1.2$ Hz, H1), 5.45 (1H, m, H2), 5.17 (1H, app t, H4), 4.02 (1H, m, H5), 2.21 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.05 (3H, s, CH₃), 1.22 (3H, d, $J_{6,5} = 6.4$ Hz, H6).

δ_{C} (100 MHz, CDCl₃): 170.09 (C=O), 170.07 (C=O), 170.03 (C=O), 155.9 (Cq), 129.7 (Cq), 122.8 (Cq), 116.4 (Ar), 95.6 (C1), 71.0 (C4), 69.7 (C2), 68.93 (C3), 67.1 (C5), 20.9 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 17.5 (C6).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₈H₂₂O₈Na 389.1216; found 389.1212.

The data are consistent with those in the current literature.^{186, 187}

1-Phenol- α -L-rhamnopyranoside (81)



2,3,4-Tri-*O*-acetyl-1-phenol- α -L-rhamnopyranoside **80** (245 mg, 0.67 mmol) was dissolved in MeOH (40 mL) with catalytic NaOMe. After 18 hours the reaction was quenched with DOWEX, filtered and concentrated *in vacuo* to give the product as a white solid in 93% yield (150 mg).

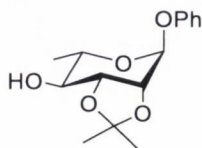
δ_{H} (400 MHz, CDCl_3): 7.31 (2H, t, $J = 7.3$ Hz, Ar-H meta), 7.08 (2H, d, $J = 7.8$ Hz, Ar-H ortho), 7.02 (1H, t, $J = 7.3$ Hz, Ar-H para), 5.44 (1H, d $J = 1.6$ Hz, H1), 4.02 (1H, dd, $J_{2,1} = 1.6$ Hz, $J_{2,3} = 3.4$ Hz, H2), 3.87 (1H, dd, $J_{3,2} = 3.4$ Hz, $J_{3,4} = 9.5$ Hz, H3), 3.67 (1H, m, H-5), 3.48 (1H, app t, H4), 1.25 (3H, d, $J_{6,5} = 6.3$ Hz, H6).

δ_{C} (100 MHz, CDCl_3): 156.0 (Cq), 129.5 (Ar-m), 122.2 (Ar-p), 116.5 (Ar-o), 98.8 (C1), 72.8 (C4), 71.2 (C3), 71.1 (C2), 69.6 (C5), 17.0 (C6).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{12}\text{H}_{16}\text{O}_5\text{Na}$ 263.0887; found 263.0890.

The data are consistent with those in the current literature.¹⁸⁷

2,3-*O*-Isopropyl-1-phenol- α -L-rhamnopyranoside (**82**)



1-Phenol- α -L-rhamnopyranoside **81** (195 mg, 0.81 mmol) was dissolved in anhydrous acetone (10 mL) under N₂. 2, 2-dimethoxypropane (0.4 mL, 3.25 mmol) and catalytic *p*TSA were added. After 1 hour the reaction was quenched with sat. aq. NaHCO₃ solution (5 mL), filtered and concentrated *in vacuo* to give the products as colourless oil in 85% yield (193 mg). $[\alpha]_{\text{D}}^{20}$ -72.1° (c 0.115, CHCl₃).

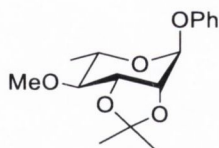
δ_{H} (400 MHz, CDCl₃): 7.34 (2H, m, Ar), 7.08 (3H, m, Ar), 5.75 (1H, app s, H1), 4.40 (1H, dd, $J_{2,1} = 0.6$ Hz, $J_{2,3} = 5.7$ Hz, H2), 4.28 (1H, m, H5), 3.38 (1H, m, H3), 3.51 (1H, dd, $J_{4,3} = 9.6$ Hz, $J_{4,5} = 7.3$ Hz, H4), 1.60 (3H, s, CH₃), 1.44 (3H, s, CH₃), 1.28 (3H, d, $J_{6,5} = 6.3$ Hz, H6)

δ_{C} (100 MHz, CDCl₃): 156.2 (Cq), 129.6 (Ar), 122.3 (Ar), 116.4 (Ar), 95.44 (C1), 78.4 (C5), 75.9 (C5), 74.7 (C4), 66.6 (C3), 28.1 (CH₃), 26.3 (CH₃), 17.4 (C6).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for C₁₅H₂₀O₅Na 303.1208; found 303.1215.

ν_{max} (thin film): 3486, 2915, 1600, 1497, 1387, 1214, 1137 cm⁻¹.

2,3-*O*-Isopropyl-4-methoxy-1-phenol- α -L-rhamnopyranoside (**83**)



2,3-*O*-Isopropyl-1-phenol- α -L-rhamnopyranoside **82** (500mg, 1.79 mmol) was dissolved in anhydrous DMF (7 mL) at 0 °C under N₂. NaH (129 mg, 3.2 mmol) and MeI (0.2 mL, 3.2 mmol) were added. After 2 hours the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (25 mL), washed with H₂O (2 x 25 mL) and sat. aq. NaHCO₃ solution (25 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. The product was isolated as a colourless oil in 86% yield (450 mg). $[\alpha]_D^{20}$ -60.5° (c 0.104, CHCl₃).

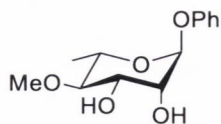
δ_H (400 MHz, CDCl₃): 7.31 (2H, m, Ar), 7.06 (3H, m, Ar), 5.74 (1H, s, H1), 4.37 (1H, dd, $J_{2,1} = 0.7$ Hz, $J_{2,3} = 5.7$ Hz, H2), 4.34 (1H, m H3), 3.77 (1H, m, H5), 3.59 (3H, s, OCH₃), 3.08 (1H, dd, $J_{4,3} = 9.8$ Hz, $J_{4,5} = 6.8$ Hz, H4), 1.62 (3H, s, CH₃), 1.44 (3H, s, CH₃), 1.25 (3H, d, $J = 6.3$ Hz, H6).

δ_C (100 MHz, CDCl₃): 156.2 (Cq), 129.5 (Ar), 122.2 (Ar), 116.4 (Ar), 109.5 (Cq), 95.3 (C1), 83.5 (C4), 78.3 (C3), 75.9 (C2), 65.6 (C5), 59.6 (OCH₃), 28.1, 26.4 (CH₃), 17.8 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₁₆H₂₂O₅Na 317.1365; found 317.1369.

$\nu_{\max}$ (thin film): 2937, 1600, 1499, 1385, 1301, 1218, 1140, 1079 cm⁻¹.

4-Methoxy-l-phenol- α -L-rhamnopyranoside (**84**)



2,3-*O*-Isopropyl-4-methoxy-l-phenol- α -L-rhamnopyranoside **83** (137 mg, 0.466 mmol) was dissolved in acetone/H₂O 8:1 (10 mL) and refluxed at 50 °C. After 18 hours the mixture was concentrated *in vacuo* and co-evaporated with toluene (2 x 5 mL) to give the product as a white solid in 98% yield (117 mg). $[\alpha]_{\text{D}}^{20}$ -51.5° (c 0.095, CHCl₃).

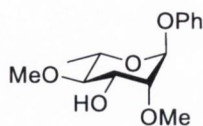
δ_{H} (400 MHz, CDCl₃): 7.32 (2H, m, Ar), 7.07 (3H, m, Ar). 5.53 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 4.19 (1H, dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H2), 4.10 (1H, dd, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 9.3 Hz, H3), 3.79 (1H, m, H5), 3.62 (3H, s, OCH₃), 3.19 (1H, app t, H4), 1.32, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_{C} (100 MHz, CDCl₃): 156.2 (Cq), 129.6 (Ar), 122.3 (Ar), 116.3 (Ar), 97.3 (C1), 83.2 (C4), 71.2 (C3), 71.0 (C2), 68.0 (C5), 61.0 (OCH₃), 18.0 (C6).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for C₁₃H₁₈O₅Na 277.1044; found 277.1052.

ν_{max} (thin film): 3375, 2971, 2934, 1380, 1289, 1222, 1030 cm⁻¹.

4-Methoxy-l-phenol- α -L-rhamnopyranoside (**91**)



3-Benzyloxy-4-methoxy-l-phenol- α -L-rhamnopyranoside **86** (100 mg, 0.279 mmol) was dissolved in aqueous acetic acid (70%, 10 mL) and a catalytic quantity of Pd/C was added. The solvent was degassed by bubbling through N₂. H₂ gas was bubbled through for 4 h and the reaction progress checked by TLC analysis. The catalyst was removed by filtration and the reaction mixture was concentrated *in vacuo* to give the product as a pale yellow oil in 98% yield (74 mg). $[\alpha]_D^{20}$ -72.3 ° (c 0.217, CHCl₃).

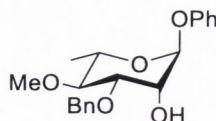
δ_H (400 MHz, CDCl₃): 7.32 (2H, m, Ar), 7.07 (3H, m, Ar), 5.57 (1H, app s, H1), 4.07 (1H, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.0$ Hz, H3), 3.72 (1H, m, H5), 3.69 (1H, m, H2), 3.62 (3H, s, OCH₃), 3.57 (3H, s, OCH₃), 3.08 (1H, app t, H4), 1.29 (3H, d, $J_{6,5} = 6.3$ Hz, H6).

δ_C (100 MHz, CDCl₃): 156.3 (Cq), 129.4, 122.1, 116.2 (Ar), 94.4 (C1), 83.5 (C4), 80.4 (C2), 71.0 (C3), 67.9 (C5), 60.8 (OCH₃), 58.9 (OCH₃), 17.8 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₁₄H₂₀O₅Na 291.1203; found 291.1202.

ν_{max} (thin film): 3481, 2929, 1591, 1495, 1227 cm⁻¹.

3-Benzyloxy-4-methoxy-l-phenol- α -L-rhamnopyranoside (85)



4-Methoxy-l-phenol- α -L-rhamnopyranoside **84** (310 mg, 1.22 mmol) was dissolved in anhydrous MeOH (7 mL) under N₂. Dibutyl tin oxide (348 mg, 1.40 mmol) was added and the mixture was refluxed at 100 °C. After 18 hours the mixture was concentrated *in vacuo* and co-evaporated twice with toluene (2 x 3 mL) to give a clear colourless oil. The residue was re-dissolved in anhydrous DMF (5 mL). Cesium fluoride (242 mg, 1.59 mmol) and benzyl bromide (0.21 mL, 1.77 mmol) were added. After 36 hours the mixture was concentrated and re-dissolved in diethyl ether (30 mL). The organic layer was washed with 1M aq. KF solution (25 mL) and H₂O (25 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20-70:30) gave the product as a colourless oil in 74% yield (310 mg). $[\alpha]_D^{20}$ 14.2° (c 0.035, CHCl₃).

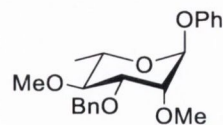
δ_H (400 MHz, CDCl₃): 7.42 (5H, m, Ar), 7.32 (2H, m, Ar), 7.06 (3H, m Ar), 5.57 (1H, d, $J_{1,2}$ = 1.7 Hz, H1), 4.81 (2H, dd, J = 11.51 Hz, J = 16.0 Hz, CH₂Bn), 4.22 (1H, dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 3.4 Hz, H2), 3.96 (1H, dd, $J_{3,2}$ = 3.4 Hz, $J_{3,4}$ = 9.2 Hz, H3), 3.77 (1H, m, H5), 3.63 (3H, s, OCH₃), 3.26 (1H, app t, H4), 1.30 (3H, d, $J_{6,5}$ = 1.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 156.2 (Cq), 138.0 (Cq), 130.0 (Ar), 128.5 (Ar), 128.0 (Ar), 127.8 (Ar), 122.2 (Ar), 116.4 (Ar), 97.0 (C1), 81.9 (C4), 79.6 (C3), 72.4 (CH₂Bn), 68.6 (C2), 68.1 (C5), 61.2 (OCH₃) 17.8 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₀H₂₄O₅Na 367.1521; found 367.1519.

ν_{max} (thin film): 3458, 2169, 1980, 1060, 799 cm⁻¹.

3-Benzyloxy-2,4-dimethoxy-1-phenol- α -L-rhamnopyranoside (86)



3-Benzyloxy-2,4-dimethoxy-1-phenol- α -L-rhamnopyranoside **85** (490 mg, 1.42 mmol) was dissolve in anhydrous DMF (7 mL) at 0 °C under N₂. NaH (100 mg, 2.56 mmol) and MeI (0.16 mL, 2.56 mmol) were added. After 2 hours the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (30 mL), washed with H₂O (2 x 25 mL), and sat. aq. NaHCO₃ solution (25 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a white solid in 73% yield (370 mg). $[\alpha]_D^{20}$ -98.1° (c 0.111, CHCl₃).

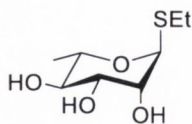
δ_H (400 MHz, CDCl₃): 7.47 (2H, m, Ar), 7.41 (2H, m, Ar), 7.33 (3H, m, Ar), 7.05 (3H, m, Ar), 5.53 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 4.81 (2H, dd, J = 11.9 Hz, J = 19.0 Hz, CH₂Bn), 3.97 (1H, dd, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H3) 3.72 (1H, m, H5), 3.69 (1H, m, H2), 3.63 (3H, s, OCH₃), 3.57 (3H, s, OCH₃), 3.31 (1H, app t, H4), 1.29 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 156.4 (Cq), 138.6 (Cq), 129.5(Cq), 128.4(Cq), 127.8(Cq), 122.7(Cq), 122.2(Ar), 116.3 (Ar), 95.5 (C1), 82.2 (C4), 79.4 (C3), 78.2 (C2), 72.6 (CH₂Bn), 68.7 (C5), 61.2 (OCH₃), 59.6 (OCH₃), 17.9 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₁H₂₆O₅Na 381.1678 found 381.1673.

ν_{max} (thin film): 3064, 2977, 1589, 1485, 1379, 1213, 1097, 975 cm⁻¹.

Ethyl-1-thio- α -L-rhamnopyranoside (92)



Ethyl-2,3,4-tri-*O*-acetyl-1-thio- α -L-rhamnopyranoside **78** (650 mg, 1.95 mmol) was dissolved in MeOH (25 mL) with catalytic NaOMe. After 3 hours the reaction was quenched with DOWEX, filtered and concentrated *in vacuo* to give the product as a colourless oil in 94% yield (410 mg).

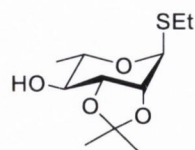
δ_{H} (400 MHz, CDCl_3): 5.29 (1H, s, H1), 4.07 (1H, m, H2), 4.05 (1H, m, H5), 3.77 (1H, dd, $J_{3,2} = 2.9$ Hz, $J_{3,4} = 9.4$ Hz, H3), 3.53 (1H, app t, H4), 2.65 (2H, m, CH_2CH_3), 1.36 (3H, d, $J_{6,5} = 6.2$ Hz, H6), 1.32 (3H, t, $J = 7.5$ Hz, CH_2CH_3).

δ_{C} (100 MHz, CDCl_3): 84.16 (C1), 73.49 (C4), 72.57 (C2), 72.27 (C3), 68.46 (C5), 25.21 (CH_2CH_3) 17.51 (C6), 14.95 (CH_2CH_3).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_8\text{H}_{16}\text{O}_4\text{NaS}$ 231.0667; found 231.0676.

The data are consistent with those in the current literature.¹⁸⁵

Ethyl-2,3-*O*-isopropyl-1-thio- α -L-rhamnopyranoside (**93**)



Ethyl-1-thio- α -L-rhamnopyranoside **92** (1.56 g, 7.50 mmol) was dissolved in anhydrous acetone (20 mL), 2,2-dimethoxy propane (3.67 mL, 30 mmol) was added along with catalytic para-toluene sulfonic acid. After 1.5 hours the reaction was quenched by the addition of sat. aq. NaHCO₃ solution (ca. 15 mL). The mixture was filtered, concentrated *in vacuo* and extracted with DCM. The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo* to give the product as a colourless oil in 96% yield (1.85 g).

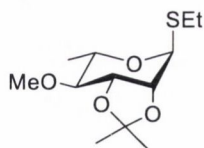
δ_H (400 MHz, CDCl₃): 5.19 (1H, s, H1), 4.17 (1H, d, $J_{2,3}$ = 5.5 Hz, H2), 4.04 (1H, dd, $J_{3,2}$ = 5.5 Hz, $J_{3,4}$ = 7.6 Hz, H3), 3.97 (1H, m, H5), 3.44 (1H, m, H4), 2.61 (2H, m, CH₂CH₃), 1.53 (3H, s, CH₃), 1.34 (3H, s, CH₃), 1.30 (3H, t, CH₂CH₃), 1.29 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 109.0 (Cq), 79.4 (C1), 78.3 (C3), 76.8 (C2), 75.4 (C4), 66.0 (C5), 28.2 (CH₃), 26.4 (CH₃), 24.4 (CH₂CH₃), 17.2 (C6), 14.6 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₁H₂₀O₄NaS 271.0980; found 271.0991.

The data are consistent with those in the current literature.¹⁸⁵

Ethyl-2,3-*O*-isopropyl-4-methoxy-1-thio- α -L-rhamnopyranoside (**94**)



Ethyl-2,3-*O*-isopropyl-1-thio- α -L-rhamnopyranoside **93** (3.288 g, 13.26 mmol) dissolved in DMF (15 mL) at 0 °C under N₂. NaH (955 mg, 23.87 mmol) and MeI (1.49 mL, 23.87 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (70 mL), washed with H₂O (2 x 50 mL) and sat. aq. NaHCO₃ solution (2 x 50 mL), dried with MgSO₄, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 95:5) gave the product as a colourless oil in 97% yield (3.371 g).

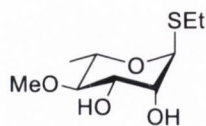
δ_{H} (400 MHz, CDCl₃): 5.51 (1H, app s, H1), 4.17 (1H, dd, $J_{2,1} = 0.7$ Hz, $J_{2,3} = 5.5$ Hz, H2), 4.14 (1H, dd, $J_{3,2} = 5.5$ Hz, $J_{3,4} = 7.1$ Hz, H3), 3.96 (1H, m, H5), 3.56 (3H, s, OCH₃), 3.04 (1H, dd, $J_{4,3} = 7.1$ Hz, $J_{4,5} = 9.8$ Hz, H4), 2.67 (1H, m, CH₂CH₃), 1.57 (3H, s, CH₃), 1.37 (3H, s, CH₃), 1.31 (3H, t, $J = 7.3$ Hz, CH₂CH₃), 1.29 (3H, d, $J_{6,5} = 6.3$ Hz, H6).

δ_{C} (100 MHz, CDCl₃): 109.2 (Cq), 84.0 (C4), 79.4 (C1), 78.1 (C3), 76.9 (C2), 65.3 (C5), 59.6 (OCH₃), 28.1 (CH₃), 26.4 (CH₃), 24.4 (CH₂CH₃), 17.7 (C6), 14.7 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₂H₂₂O₄NaS 285.1137; found 285.1136.

The data are consistent with those in the current literature.¹⁰²

Ethyl-4-methoxy-1- α -L-rhamnopyranoside (95)



Ethyl-4-methoxy-1-thio- α -L-rhamnopyranoside **94** (3.37 g, 12.86 mmol) was dissolved in acetone/H₂O 8:1 (30 mL) and refluxed at 50 °C. After 18 hours the mixture was concentrated *in vacuo* to give the product as a colourless oil in 97% yield (2.781 g).

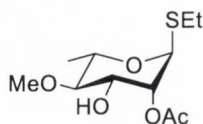
δ_{H} (400 MHz, CDCl₃): 5.25 (1H, d, $J_{1,2}$ = 0.7 Hz, H1), 4.05 (1H, m, H2), 4.00 (1H, m, H5), 3.83 (1H, dd, $J_{3,2}$ = 2.9 Hz, $J_{3,4}$ = 9.1 Hz, H3), 3.58 (3H, s, OCH₃), 3.15 (1H, app t, H4), 2.61 (2H, m, CH₂CH₃), 1.34 (3H, d, $J_{6,5}$ = 6.2 Hz, H6), 1.29 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 83.73 (C1), 83.56 (C4), 72.59 (C2), 71.71 (C3), 67.69 (C5), 60.73 (OCH₃), 25.06 (CH₂CH₃), 17.89 (C6), 14.94 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₉H₁₈O₄NaS 245.0824; found 245.0816.

The data are consistent with those in the current literature.¹⁰²

Ethyl-3-*O*-acetyl-4-methoxy-1-thio- α -L-rhamnopyranoside (102)



Ethyl-4-methoxy-1-thio- α -L-rhamnopyranoside **95** (100 mg, 0.45 mmol) was dissolved in acetonitrile (5 mL) under N₂. Trimethyl orthoacetate (108 mg, 0.9 mmol) and camphor sulfonic acid (10 mg, 0.045 mmol) were added. After 45 minutes, a mixture of acetic acid/H₂O 4:1 (5 mL) was added. After 15 minutes the mixture was diluted with DCM (30 mL). The organic layer was washed with H₂O (2 x 25 mL) and aq. sat. NaHCO₃ solution (2 x 25 mL), dried with MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a colourless oil in 84% yield (101 mg).). $[\alpha]_D^{20}$ -140.6° (*c* 0.172 CHCl₃).

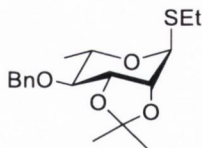
δ_H (400 MHz, CDCl₃): 5.21 (1H, app s, H1), 5.20 (1H, m, H2), 4.01 (1H, m, H5), 3.98 (1H, m, H3), 3.60 (3H, s, OCH₃), 3.12 (1H, app t, H4), 2.63 (2H, m, CH₂CH₃), 2.18 (3H, s, CH₃), 1.35 (3H, d, $J_{6,5}$ = 6.3 Hz, H6), 1.30 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 171.0 (C=O), 83.6 (C4), 82.1 (C1), 74.5 (C2), 70.5 (C3), 68.0 (C5), 60.9 (OCH₃), 25.6 (CH₂CH₃), 21.2 (CH₃), 17.9 (C6), 15.0 (CH₂CH₃).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₁₁H₂₀OS₅Na 287.0920; found 287.0932.

$\nu_{\max}$ (thin film): 3448, 2933, 1741, 1449, 1373, 1230, 1098 cm⁻¹.

Ethyl-4-benzyloxy-2,3-*O*-isopropyl-1-thio- α -l-rhamnopyranoside (103)



Ethyl-2,3-*O*-isopropyl-1-thio- α -l-rhamnopyranoside **93** (14.46 g, 58.30 mmol) was dissolved in anhydrous DMF (5 mL) at 0 °C under N₂. NaH (420 mg, 69.97 mmol) and BnBr (9.01 mL, 75.80 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (50 mL), washed with H₂O (2 x 40 mL) and sat. NaHCO₃ solution (2 x 40 mL), dried with MgSO₄, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a white solid in 97% yield (19.02 g). $[\alpha]_D^{20}$ -203.4 ° (c 0.116, CHCl₃).

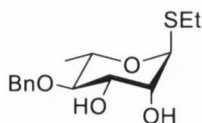
δ_H (400 MHz, CDCl₃): 7.36 (5H, m, Bn), 5.50 (1H, app s, H1), 4.74 (2H, dd, J = 11.4 Hz, J = 21.4 Hz, CH₂Bn), 4.24 (1H, dd, $J_{3,2}$ = 5.4 Hz, $J_{3,4}$ = 7.2 Hz, H3), 4.18 (1H, app d, J = 5.4 Hz, H2), 4.03 (1H, m, H5), 3.30 (1H, $J_{4,3}$ = 7.2 Hz, $J_{4,5}$ = 9.8 Hz, H4), 2.60 (2H, m, CH₂CH₃), 1.52 (3H, s, CH₃), 1.36 (3H, s, CH₃), 1.29 (3H, t, J = 7.3 Hz, CH₂CH₃), 1.28 (3H, d, $J_{6,5}$ = 6.1 Hz, H6).

δ_C (100 MHz, CDCl₃): 138.3 (Cq), 128.7 (Bn), 128.3 (Bn), 128.1 (Bn), 127.9 (Bn), 127.6 (Bn), 109.4 (Cq), 82.0 (C4), 79.5 (C1), 78.4 (C3), 77.0 (C2), 75.0 (CH₂Bn), 65.3 (C5), 28.1 (CH₃), 26.5 (CH₃), 24.4 (CH₂CH₃), 17.9 (C6), 14.9 (CH₂CH₃).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₁₈H₂₆O₄NaS 361.1450; found 361.1436.

ν_{max} (thin film): 2895, 1369, 1248, 1093 cm⁻¹.

Ethyl-4-benzyloxy-1-thio- α -L-rhamnopyranoside (**104**)



Ethyl-4-benzyloxy-2,3-*O*-isopropyl-1-thio- α -L-rhamnopyranoside **103** (585 mg, 1.73 mmol) was dissolved in 9:1 acetic acid/H₂O (5 mL). The mixture was reacted for 18 hours at 50 °C then concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a white solid in 98% yield (515 mg).

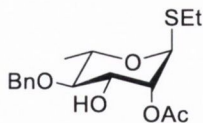
δ_{H} (400 MHz, CDCl₃): 7.40 (5H, m, Bn), 5.27 (1H, d, $J_{1,2} = 1.3$ Hz, H1), 4.77 (2H, dd, $J = 11.4$ Hz, $J = 19.8$ Hz, CH₂Bn), 4.13 (1H, m, H5), 4.06 (1H, dd, $J_{2,3} = 3.4$ Hz, $J_{2,1} = 1.4$ Hz, H2), 3.91 (1H, dd, $J_{3,2} = 3.4$ Hz, $J_{3,4} = 9.1$ Hz, H3), 3.41 (1H, app t, H4), 2.65 (2H, m, CH₂CH₃), 1.39 (3H, $J_{6,5} = 6.3$ Hz, H6), 1.32 (3H, t, $J = 7.4$ Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 138.2 (Cq), 128.7 (Bn), 128.1 (Bn), 127.9 (Bn), 83.6 (C1), 82.0 (C4), 75.0 (CH₂Bn), 72.6 (C2), 71.9 (C3), 67.7 (C5), 25.1 (CH₂CH₃), 18.0 (C6), 14.9 (CH₂CH₃).

HRMS-ESI (m/z): $[M + \text{Na}]^+$ calculated for C₁₅H₂₁O₄S 297.1161; found 297.1151.

The data are consistent with those in the current literature.¹⁴⁶

Ethyl-2-*O*-acetyl-4-*O*-benzyl-1-thio- α -l-rhamnopyranoside (**105**)



Ethyl-4-*O*-benzyl-1-thio- α -L-rhamnopyranoside **104** (420 mg, 1.41 mmol) was dissolved in acetonitrile (15 mL) under N₂. Trimethyl orthoacetate (0.36 mL, 2.82 mmol) and camphor sulfonic acid (catalytic) were added. After 45 minutes, acetic acid/H₂O 4:1 (5 mL) was added. After 15 minutes the mixture was diluted with DCM (30 mL) washed with H₂O (2 x 25 mL) and aq. sat NaHCO₃ solution (2 x 25 mL), dried with MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a colourless oil in 89% yield (427 mg).

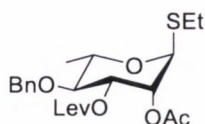
δ_{H} (400 MHz, CDCl₃): 7.36 (5H, m, Bn) 5.23 (1H, s, H1), 5.23 (1H, m, H2), 4.85 (1H, d, $J = 11.0$ Hz, CH₂Bn), 4.75 (1H, d, $J = 11.0$ Hz, CH₂Bn), 4.14 (1H, m, H5), 4.09 (1H, m, H3), 3.42 (1H, app t, H4), 2.65 (2H, m, CH₂CH₃), 2.19 (3H, s, CH₃), 1.39 (3H, d, $J_{6,5} = 6.2$ Hz, H6), 1.31 (3H, t, $J = 7.4$ Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 170.8 (C=O) 138.1 (Cq), 128.6 (Bn), 128.0 (Bn), 127.9 (Bn), 82.1 (C1), 82.0 (C4), 75.2 (CH₂), 74.6 (C2), 70.8 (C3), 68.0 (C5), 25.6 (CH₂CH₃), 21.2 (CH₃), 18.0 (C6), 15.0 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₇H₂₄O₅SNa 363.1242; found 363.1241.

The data are consistent with those in the current literature.^{146,188}

Ethyl-2-*O*-acetyl-4-benzyloxy-3-*O*-levulinoyl-1-thio- α -L-rhamnopyranoside (106)



Ethyl-2-*O*-acetyl-4-benzyloxy-1-thio- α -L-rhamnopyranoside **105** (12.50 g, 36.7 mmol) was dissolved in anhydrous DCM (30 mL) under N₂. Levulinic acid (17.25 g, 146.24 mmol), DCC (30.30 g, 146.24 mmol), and DMAP (catalytic) were added. After 18 hours, the mixture was filtered. The organic layer was washed with aq. sat. NaHCO₃ solution (50 mL), aq. sat. NaCl solution (50 mL), H₂O (50 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a colourless oil in 98% yield (16.00 g). $[\alpha]_D^{20}$ -163 ° (c 0.136, CHCl₃).

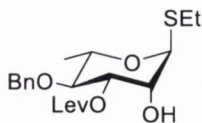
δ_H (400 MHz, CDCl₃): 7.34 (5H, m, Bn), 5.36 (1H, dd, $J_{2,1} = 1.6$ Hz, $J_{2,3} = 3.2$ Hz, H2), 5.27 (1H, dd, $J_{3,2} = 3.2$ Hz, $J_{3,4} = 9.7$ Hz, H3), 5.18 (1H, app s, H1), 4.76 (1H, d, $J = 11.3$ Hz, CH₂Bn), 4.66 (1H, d, $J = 11.3$ Hz, CH₂Bn), 4.20 (1H, m, H5), 3.57 (1H, app t, H4), 2.68 (4H, m, CH₂), 2.51 (2H, m, CH₂), 2.19 (3H, s, CH₃), 2.18 (3H, s, CH₃), 1.37 (3H, d, $J_{6,5} = 6.3$ Hz, H6), 1.30 (3H, t, $J = 7.3$ Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 206.1 (C=O), 171.5 (C=O), 169.9 (C=O), 137.9 (Cq), 128.3, 127.62, 127.59 (Bn), 81.1 (C1), 78.8 (C4), 74.8 (CH₂), 72.3 (C3), 71.8 (C2), 68.1 (C5), 37.7 (CH₂), 29.6 (CH₃), 27.7 (CH₂), 25.2 (CH₂CH₃), 20.8 (CH₃), 17.7 (C6), 14.7 (CH₂CH₃).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₂H₃₀O₇NaS 461.1610; found 461.1625.

ν_{max} (thin film): 2931, 1743, 1368, 1231, 1096 cm⁻¹.

Ethyl-4-benzyloxy-3-O-levulinoyl-1-thio- α -L-rhamnopyranoside (107)



Ethyl-2-O-acetyl-4-benzyloxy-3-O-levulinoyl-1-thio- α -L-rhamnopyranoside **106** (16.00 g, 36.49 mmol) was dissolved in MeOH (50 mL) with a catalytic amount of NaOMe. After 18 hours, the reaction was quenched with DOWEX, filtered and concentrated *in vacuo* to give the product as a white solid in 98% yield (14.33 g). $[\alpha]_D^{20}$ -156.8 ° (c 0.0051, CHCl₃).

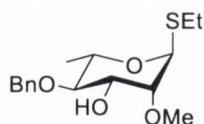
δ_H (400 MHz, CDCl₃): 7.39 (5H, m, Bn), 5.27 (1H, d, $J_{1,2}$ = 1.4 Hz, H1), 4.77 (2H, dd, J = 11.4 Hz, J = 15.6 Hz, CH₂Bn), 4.13 (1H, m, H5), 4.05 (1H, dd, $J_{2,1}$ = 1.4 Hz, $J_{2,3}$ = 3.4 Hz, H2), 3.91 (1H, dd, $J_{3,2}$ = 3.4 Hz, $J_{3,4}$ = 9.1 Hz, H3), 3.41 (1H, app t, H4), 2.79 (2H, t, J = 6.6 Hz, CH₂), 2.63 (2H, m, CH₂CH₃), 2.61 (2H, t, J = 6.6 Hz, CH₂), 2.22 (3H, s, CH₃), 1.38 (3H, d, $J_{6,5}$ = 6.3 Hz, H6), 1.31 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 206.7 (C=O), 173.3 (C=O), 138.2 (Cq), 128.7 (Bn), 128.0 (Bn), 127.9 (Bn), 83.6 (C1), 82.0 (C4), 75.0 (CH₂Bn), 72.6 (C2), 71.9 (C3), 67.7 (C5), 38.0 (CH₂), 29.9 (CH₃), 27.7 (CH₂), 25.1 (CH₂CH₃), 18.0 (C6), 14.9 (CH₂CH₃)..

HRMS-ESI (m/z): $[M + Na]^+$ calculate for C₂₀H₂₈O₆NaS 419.1499; found 419.1490.

ν_{max} (thin film): 3290, 2923, 1453, 1081 cm⁻¹

Ethyl-4-benzyloxy-2-methoxy-1-thio- α -L-rhamnopyranoside (**108**)



Ethyl-4-benzyloxy-3-*O*-levulinoyl-1-thio- α -L-rhamnopyranoside **107** (250 mg, 0.63 mmol) was dissolved in anhydrous DMF (4 mL) at 0 °C under N₂. NaH (35 mg, 0.88 mmol) and MeI (0.047 mL, 0.76 mmol) were added. After 18 hours, the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (20 mL), washed with H₂O (2 x 15 mL) and sat. aq. NaHCO₃ solution (2 x 15 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a yellow oil in 74% yield (146 mg) with the dimethylated compound **109** (characterisation overleaf), also a yellow oil, as a side product.

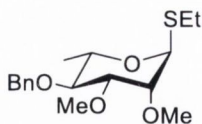
δ_{H} (400 MHz, CDCl₃): 7.38 (5H, m, Bn), 5.41 (1H, d, $J_{1,2} = 1.2$ Hz, H1), 4.93, 4.70 (1H, d, $J = 11.2$ Hz, CH₂Bn), 4.07 (1H, m, H5), 3.91 (1H, dd, $J_{3,2} = 3.7$ Hz, $J_{3,4} = 9.3$ Hz, H3), 3.61 (1H, dd, $J_{2,1} = 1.2$ Hz, $J_{2,3} = 3.7$ Hz, H2), 3.51 (3H, s, OCH₃), 3.33 (1H, app t, H4), 2.65 (2H, m, CH₂CH₃), 1.34 (3H, d, $J_{6,5} = 6.3$ Hz, H6), 1.32 (3H, t, $J = 7.4$ Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 138.5 (Cq), 128.4 (Bn), 128.0 (Bn), 127.7 (Bn), 82.5 (C4), 82.3 (C2), 80.1 (C1), 75.1 (CH₂Bn), 72.1 (C3), 67.6 (C5), 58.1 (CH₃), 25.2 (CH₂CH₃), 17.9 (C6), 15.0 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₆H₂₄O₄NaS 335.1293; found 335.1292.

The data are consistent with those in the current literature.¹⁴⁶

Ethyl-4-benzyloxy-2-dimethoxy-1-thio- α -L-rhamnopyranoside (**109**)



Compound **109** was isolated as a side product during the preparation of thioglycoside **108**.

Procedure on previous page.

$[\alpha]_D^{20}$ -101.6 ° (c 0.118, CHCl₃).

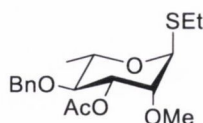
δ_H (400 MHz, CDCl₃): 8.02 (2H, m, Ar), 7.34 (5H, m, Bn), 7.14 (2H, m, Ar), 5.61 (1H, d, $J_{1,2}$ = 1.9 Hz, H1), 5.44 (1H, dd, $J_{3,2}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, H3), 4.72 (2H, dd, J = 11.2 Hz, J = 33.2 Hz, CH₂Bn), 3.91 (3H, s, OCH₃), 3.90 (1H, m, H2), 3.83 (1H, m, H5), 3.65 (1H, app t, H3), 3.55 (3H, s, OCH₃), 2.64 (2H, m, CH₂CH₃), 2.13 (3H, s, OCH₃), 1.32 (3H, d, $J_{6,5}$ = 6.2 Hz, H6), 1.30 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 138.8 (Cq), 128.4 (Bn), 127.9 (Bn), 127.6 (Bn), 82.0 (C3), 80.8 (C1), 80.7 (C4), 79.1 (C2), 75.3 (CH₂Bn), 68.1 (C5), 58.3 (OCH₃), 57.7 (OCH₃), 25.5 (CH₂CH₃), 17.8 (C6), 15.0 (CH₂CH₃)

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₄H₂₈O₈Na 467.1682; found 467.1689.

ν_{max} (thin film): 3452, 2927, 1457, 1095 cm⁻¹.

Ethyl-2-*O*-acetyl-4-benzyloxy-2-methoxy-1-thio- α -L-rhamnopyranoside (110)



Ethyl-4-benzyloxy-2-methoxy-1-thio- α -L-rhamnopyranoside **108** (320 mg, 1.026 mmol) was dissolved in pyridine (5 mL) at 0 °C under N₂. Ac₂O (5 mL) was added in increments. After 18 hours the mixture was quenched with iced H₂O and diluted with EtOAc. The organic layer washed with H₂O (20 mL), dilute aq. CuSO₄ solution (6 x 20 mL) and H₂O (20 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a colourless oil in 84% yield (307 mg).

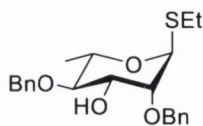
δ_{H} (400 MHz, CDCl₃): δ 7.34 (5H, m, Bn), 5.34 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 5.19 (1H, dd, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H3), 4.70 (2H, dd, J = 11.4 Hz, J = 33.7 Hz, CH₂Bn), 4.15 (1H, m, H5), 3.78 (1H, dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H2), 3.60 (1H, app t, H4), 3.47 (3H, s, OCH₃), 2.65 (2H, m, CH₂CH₃), 2.08 (3H, s, CH₃), 1.35 (3H, d, $J_{6,5}$ = 6.2 Hz, H6), 1.32 (3H, t, J = 7.5 Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 170.2 (C=O), 138.3 (Cq), 128.4 (Bn), 127.7 (Bn), 127.6 (Bn), 80.9 (C1), 80.2 (C2), 79.4 (C4), 75.0 (CH₂CH₃), 74.1 (C3), 68.1 (C5), 58.6 (OCH₃), 25.3 (CH₂), 21.1 (CH₃), 17.9 (C6), 14.9 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₈H₂₆O₅NaS 377.1399; found 377.1399.

The data are consistent with those in the current literature.¹⁴⁶

Ethyl-2,4-dibenzyloxy-1-thio- α -L-rhamnopyranoside (111)



Ethyl-4-benzyloxy-3-*O*-levulinoyl-1-thio- α -L-rhamnopyranoside **107** (1.00 g, 2.52 mmol) was dissolved in anhydrous DMF (10 mL) at 0 °C under N₂. NaH (121 mg, 3.03 mmol) and BnBr (0.375 mL, 3.03 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca 2 mL) and concentrated *in vacuo*. The residue was diluted with diethyl ether (75 mL), washed with H₂O (2 x 50 mL) and aq. sat. NaHCO₃ solution (2 x 50 mL), dried with MgSO₄, filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a colourless oil in 87% yield (849 mg) with the tribenzylated compound **112**, also a yellow oil, as a side product.

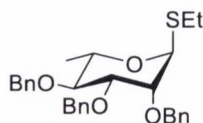
δ_H (400 MHz, CDCl₃): 7.39 (10H, m, Bn), 5.40 (1H, app s, H1), 4.95 (1H, d, J = 11.2 Hz, CH2), 4.81 (1H, d, J = 11.7 Hz, CH₂Bn), 4.70 (1H, d, J = 11.2 Hz, CH₂Bn), 4.60 (1H, d, J = 11.7 Hz, CH₂Bn), 4.10 (1H, m, H5), 3.97 (1H, dd, $J_{3,2}$ = 3.7 Hz, $J_{3,4}$ = 9.1 Hz, H3), 3.88 (1H, dd, $J_{2,1}$ = 1.4 Hz, $J_{2,3}$ = 3.7 Hz, H2), 3.41 (1H, app t, H4), 2.63 (2H, m, CH₂CH₃), 1.38 (3H, d, $J_{6,5}$ = 6.3 Hz, H6), 1.30 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 138.6 (Cq), 137.6 (Cq), 128.7 (Bn), 128.4 (Bn), 128.2 (Bn), 128.1 (Bn), 128.0 (Bn), 127.7 (Bn), 82.6 (C4), 81.0 (C1), 80.3 (C2), 75.1 (CH₂Bn), 72.5 (CH₂Bn), 72.2 (C3), 67.7 (C5), 25.2 (CH₂CH₃), 18. (C6), 15.0 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₂₂H₂₈O₄NaS 411.1606; found 411.1599.

The data are consistent with those in the current literature.¹⁸⁹

Ethyl-2,3,4-tribenzyloxy-1-thio- α -L-rhamnopyranoside (112)



Ethyl-1-thio- α -L-rhamnopyranoside **107** (620 mg, 2.77 mmol) was dissolved in anhydrous DMF (10 mL) at 0 °C under N₂. NaH (498 mg, 12.46 mmol) and BnBr (1.5 mL, 12.6 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca 2 mL) and concentrated *in vacuo*. The residue was diluted with diethyl ether (75 mL), washed with H₂O (2 x 50 mL) and aq. sat. NaHCO₃ solution (2 x 50 mL), dried with MgSO₄, filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 95:5) gave the product as a colourless oil in 97% yield (1.143 g).

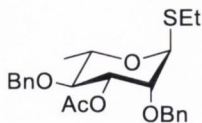
δ_{H} (400 MHz, CDCl₃): 7.36 (15H, m, Ar), 5.29 (1H, d, $J_{1,2} = 1.4$ Hz, H1), 4.98 (1H, d, $J = 10.8$ Hz, CH₂Bn), 4.74 (2H, dd, $J = 12.5$ Hz, $J = 17.5$ Hz, CH₂Bn), 4.67 (1H, d, $J = 10.8$ Hz, CH₂Bn), 4.60 (2H, dd, $J = 11.8$ Hz, $J = 14.2$ Hz, CH₂Bn), 4.06 (1H, m, H5), 3.86 (1H, dd, $J_{2,1} = 1.7$ Hz, $J_{2,3} = 3.1$ Hz, H2), 3.82 (1H, dd, $J_{3,2} = 3.2$ Hz, $J_{3,4} = 9.2$ Hz, H3), 3.67 (1H, app t, H4), 2.59 (2H, m, CH₂CH₃), 1.36 (3H, d, $J_{6,5} = 6.3$ Hz, H6), 1.26 (3H, t, $J = 7.3$ Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 138.7 (Cq), 138.3 (Cq), 138.1 (Cq), 128.4 (Bn), 128.38 (Bn), 128.36 (Bn), 127.99 (Bn), 127.98 (Bn), 127.8 (Bn), 127.7 (Bn), 127.63 (Bn), 127.61 (Bn), 82.0 (C1), 80.7 (C4), 80.3 (C3), 76.7 (C2), 75.4 (CH₂Bn), 72.2 (CH₂Bn), 72.1 (CH₂Bn), 64.4 (C5), 25.4 (CH₂CH₃), 17.9 (C6), 15.0 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₂₉H₃₄O₄NaS 501.2076 found 501.2060.

The data are consistent with those in the current literature.¹⁹⁰

Ethyl-3-*O*-acetyl-4-benzyloxy-3-*O*-levulinoyl-1-thio- α -L-rhamnopyranoside (**113**)



Ethyl-4-benzyloxy-3-*O*-levulinoyl-1-thio- α -L-rhamnopyranoside **111** (803 mg, 2.07 mmol) was dissolved in pyridine (5 mL) at 0 °C under N₂. Ac₂O (5 mL) was added in increment. After 18 hours the mixture was quenched with iced H₂O and diluted with EtOAc. The organic layer was washed with H₂O (20 mL), dilute aq. CuSO₄ solution (6 x 20 mL) and H₂O (20 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 90:10) gave the product as yellow oil 96% yield (859 mg).

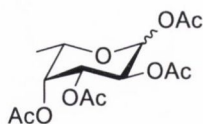
δ_{H} (400 MHz, CDCl₃): 7.36 (10H, m, Bn), 5.30 (1H, d, $J_{1,2}$ = 1.7 Hz, H1), 5.18 (1H, dd, $J_{3,2}$ = 3.4 Hz, $J_{3,4}$ = 9.5 Hz, H3), 4.72 (2H, dd, J = 11.7 Hz, J = 30.1 Hz, CH₂Bn), 4.71 (1H, d, J = 12.6 Hz, CH₂), 4.57 (1H, d, J = 12.2 Hz, CH₂), 4.17 (1H, m, H5), 4.00 (1H, dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 3.4 Hz, H2), 3.70 (1H, app t, H4), 2.62 (2H, m CH₂CH₃), 1.99 (3H, s, CH₃), 1.38 (3H, d, $J_{6,5}$ = 6.2 Hz, H6), 1.29 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 170.1 (C=O), 138.3 (Cq), 137.8 (Cq), 128.5 (Bn), 128.4 (Bn), 127.96 (Bn), 127.95 (Bn), 127.7 (Bn), 127.6 (Bn), 81.7 (C1), 79.4 (C4), 77.7 (C2), 74.9 (CH₂Bn), 74.0 (C3), 72.6 (CH₂Bn), 68.2 (C5), 25.3 (CH₂CH₃), 21.1 (CH₃), 18.0 (C6), 15.0 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₂₄H₃₀O₅NaS 453.1712; found 453.1714.

The data are consistent with those in the current literature.¹⁸⁹

1,2,3,4-Tetra-*O*-acetyl-L-fucopyranoside (74)



L-Fucose **13** (2.00 g, 12.18 mmol) was dissolved in pyridine (10 mL) at 0 °C under N₂. Ac₂O (10 mL, 106 mmol) was added. After 18 hours reaction the mixture was concentrated *in vacuo* and purification by column chromatography (hexane:EtOAc 80:20) gave the product, a sticky white solid, as a mixture of anomers in 99% yield (4.01 g).

Data for the major α anomer:

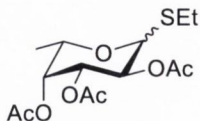
δ_{H} (400 MHz, CDCl₃): 6.36 (1H, d, $J_{1,2}$ = 2.6 Hz, H1), 5.35 (1H, m, H2), 5.33 (1H, m, H4), 5.10 (1H, dd, $J_{3,2}$ = 10.2 Hz, $J_{3,4}$ = 3.2 Hz, H3), 4.30 (1H, app q, H5), 2.21 (3H, s, CH₃), 2.18 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.03 (3H, s, CH₃), 1.19 (3H, d, $J_{6,5}$ = 6.5 Hz, H6).

δ_{C} (100 MHz, CDCl₃): 170.6 (C=O), 170.2 (C=O), 169.2 (C=O), 90.0 (C1), 70.6 (C2), 67.8 (C3), 67.3 (C5), 66.5 (C4), 21.0 (CH₃), 20.7 (CH₃), 20.7 (CH₃), 20.6 (CH₃), 15.9 (C6).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₄H₂₀O₉NaS 355.1005; found 355.1001.

The data are consistent with those in the current literature.¹⁹¹

Ethyl-2,3,4-*O*-triacetyl-1-thio-L-fucopyranoside (114)



1,2,3,4-Tetra-*O*-acetyl-L-fucopyranoside **74** (4.00 g, 12.0 mmol) was dissolved in anhydrous dichloromethane (40 mL) at 0 °C under N₂. Ethane thiol (1.15 mL, 15.66 mmol) was added followed by BF₃·OEt₂ (7.43 mL, 60.0 mmol) in increments. The stirred solution was allowed to come to room temperature. After 18 hours the reaction was quenched by treatment with sat. aq. NaHCO₃ solution (ca. 20 mL) and solid NaHCO₃. The organic layer was separated, dried with MgSO₄, filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane:EtOAc 80:20) gave the product, a sticky off white solid, as a mixture of anomers in 80% total yield (8.895 g); 33% α (3.922 mg) anomer.

Data for α anomer:

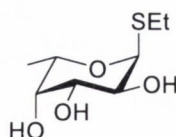
δ_H (400 MHz, CDCl₃): 5.72 (1H, d, *J*_{1,2} = 5.4 Hz, H1), 5.31 (1H, m, H3), 5.29 (1H, dd, *J*_{2,1} = 5.4 Hz, *J*_{2,3} = 10.7 Hz, H2), 5.23 (1H, m, H4), 4.51 (1H, app q, H5), 2.57 (2H, m, CH₂CH₃), 2.19 (CH₃), 2.09 (CH₃), 2.01 (CH₃), 1.28 (3H, app t, CH₂CH₃), 1.17 (3H, d, *J*_{6,5} = 6.5 Hz, H6).

δ_C (100 MHz, CDCl₃): 170.6 (C=O), 170.3 (C=O), 169.9 (C=O), 81.9 (C1), 71.0 (C3), 68.7 (C4), 68.0 (C2), 24.1 (CH₂CH₃), 20.9 (CH₃), 20.7 (CH₃), 20.7 (CH₃), 15.9 (C6), 14.8 (CH₂CH₃).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₁₄H₂₂O₇NaS 357.0984 found 357.0980.

The data are consistent with those in the current literature.¹⁹²

Ethyl-1-thio- α -L-fucopyranoside (115)



Ethyl-2,3,4-*O*-triacetyl-1-thio- α -L-fucopyranoside **114a** (750 mg, 2.25 mmol) was dissolved in MeOH (30 mL) with catalytic sodium methoxide. After 18 hours the reaction was quenched with DOWEX, filtered and concentrated *in vacuo* to give the product as a white solid in 88% yield (411 mg).

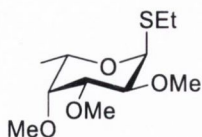
δ_{H} (400 MHz, CDCl_3): 5.37 (1H, d, $J_{1,2} = 5.4$ Hz, H1), 4.29 (1H, app t, H5), 4.05 (1H, m, H4), 3.80 (1H, app d, H3), 3.57 (1H, m, H2), 2.67 (2H, m, CH_2CH_3), 1.32 (3H, app t, CH_2CH_3), 1.31 (3H, d, $J_{6,5} = 6.4$ Hz, H6).

δ_{C} (100 MHz, CDCl_3): 87.4 (C1), 72.3 (C2), 71.5 (C3), 68.9 (C4), 67.0 (C5), 26.1 (CH_2CH_3), 16.1 (C6), 15.4 (CH_2CH_3).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_8\text{H}_{16}\text{O}_4\text{NaS}$ 231.0667; found 231.0665.

The data are consistent with those in the current literature.¹⁹²

Ethyl-2,3,4-trimethoxy-1-thio- α -L-fucopyranoside (**116**)



Ethyl-1-thio- α -L-fucopyranoside **115** (396 mg, 1.76 mmol) was dissolved in DMF (4 mL) under N₂. NaH (35 mg, 0.88 mmol) and MeI (0.047 mL, 0.76 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 5 mL) and concentrated *in vacuo*. The residue was redissolved in diethyl ether (20 mL), washed with water (2 x 15 mL) and sat. NaHCO₃ (2 x 15 mL), dried with MgSO₄, and concentrated *in vacuo*. Purification by column chromatography (hexane:EtOAc 60:40) gave the product as a yellow oil in 79% yield (250 mg).

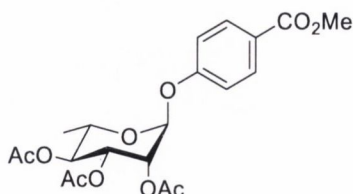
δ_{H} (400 MHz, CDCl₃): 5.56 (1H, d, $J_{1,2} = 5.5$ Hz, H1), 4.24 (1H, app q, H5), 3.96 (1H, dd, $J_{2,1} = 5.5$ Hz, $J_{2,3} = 9.7$ Hz, H2), 3.63 (OCH₃), 3.54 (OCH₃), 3.51 (OCH₃), 3.47 (1H, dd, $J_{4,3} = 3.1$ Hz, $J_{4,5} = 1.0$ Hz, H4), 3.44 (1H, dd, $J_{3,2} = 9.7$ Hz, $J_{3,4} = 3.1$ Hz, H3), 2.58 (2H, m, CH₂CH₃), 1.31 (3H, app t, CH₂CH₃), 1.29 (3H, d, $J_{6,5} = 6.5$ Hz, H6).

δ_{C} (100 MHz, CDCl₃): 82.9 (C1), 81.3 (C3), 79.2 (C4), 66.5 (C2), 61.9 (C5), 58.2 (OCH₃), 58.0 (OCH₃), 58.0 (OCH₃), 23.8 (CH₂CH₃), 16.4 (C6), 14.8 (CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₁H₂₂O₄NaS 273.1137; found 273.1139.

The data are consistent with those in the current literature.¹⁹³

2,3,4-Tri-*O*-acetyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (**70**)



- 1) 1,2,3,4-Tetra-*O*-acetyl-L-rhamnopyranoside **68** (2.00 g, 6.04 mmol) was dissolved in anhydrous DCM (20 mL) at 0 °C under N₂. Methyl-4-hydroxybenzoate (1.202 g, 7.85 mmol) was added followed by BF₃·OEt₂ (3.73 mL, 30.21 mmol) in increments. After 18 hours, the reaction was quenched by treatment with sat. aq. NaHCO₃ solution (ca. 50 mL) and solid NaHCO₃. The organic layer was filtered, dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a white solid in 96% yield (2.459 g).
- 2) Ethyl-2,3,4-tri-*O*-acetyl-1-thio- β -L-rhamnopyranoside **78** (100 mg, 0.30 mmol) was dissolved in anhydrous DCM (5 mL) at 0 °C under N₂. Methyl-4-benzoate (69 mg, 0.45 mmol) and 1.5 eq. of the activating Me₂S₂-Tf₂O solution were added. After 1 hour at 0 °C the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (15 mL), washed with 1M HCl solution (20 mL), sat. aq. NaHCO₃ solution (20 mL), H₂O (20 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a white solid in 63% yield (80 mg). R_f = 0.6 (hexane/EtOAc 60:40).

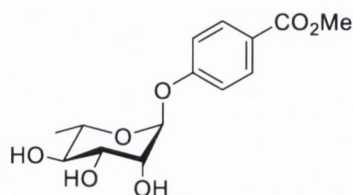
δ_H (400 MHz, CDCl₃): 8.02, 7.13 (2H, m, Ar), 5.55 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 5.52 (1H, dd, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 10.1 Hz, H3), 5.45 (1H, dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H2), 5.18 (1H, app t, H4), 3.95 (1H, m, H5), 3.91 (3H, s, CO₂CH₃), 2.22 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.05 (3H, s, CH₃), 1.21 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, CDCl₃): 170.0 (C=O), 166.6 (C=O), 159.3 (C=O), 131.6 (Ar), 124.5 (Cq), 115.8 (Ar), 95.3 (C1), 90.7 (Cq), 70.8 (H4), 69.5 (C2), 68.8 (C3), 67.5 (C5), 52.0 (CO₂CH₃), 20.9 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 17.4 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{20}H_{24}O_{10}Na$ 447.1267; found 447.1259.

The data are consistent with those in the current literature.¹⁰⁵

1-(4-(Methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (**11**)



2,3,4-Tri-*O*-acetyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **70** (1.066 mg, 2.51 mmol) was dissolved in MeOH (15 mL) with a catalytic amount of NaOMe. After 18 hours, the reaction was quenched with DOWEX, filtered and concentrated *in vacuo* to give the product as a sticky white solid in 96% yield (734 mg).

δ_H (400 MHz, CD_3OD): 7.96 (2H, m, Ar), 7.13 (2H, m, Ar), 5.52 (1H, d, $J_{1,2} = 1.2$ Hz, H1), 4.04 (1H, dd, $J_{2,1} = 1.2$ Hz, $J_{2,3} = 3.3$ Hz, H2), 3.86 (3H, s, CH_3), 3.83 (1H, dd, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.5$ Hz, H3), 3.57 (1H, m, H5), 3.46 (1H, app t, H4), 1.21 (3H, d, $J_{6,5} = 6.1$ Hz, H6).

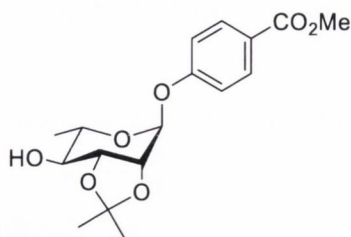
δ_C (100 MHz, CD_3OD): 167.2 (C=O), 160.9 (Cq), 131.6 (Ar), 124.0 (Cq), 116.1 (Ar), 98.7 (C1), 72.7 (C4), 71.1 (C3), 70.8 (C2), 70.0 (C5), 51.5 (CO_2CH_3), 17.0 (C6).

ν_{max} (thin film): 3365, 2937, 1700, 1606, 1436, 1238 cm^{-1} .

The NMR spectrum for this compound can be found in Appendix A.

The data are consistent with those in the current literature.¹⁰⁵

2,3-*O*-Isopropyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (**122**)



1-(4-(Methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **11** (745 mg, 2.50 mmol) was dissolved in anhydrous acetone (15 mL) under N₂. 2, 2-dimethoxypropane (1.22 mL, 10.00 mmol) and a catalytic amount of *p*TSA were added. After 3 hours, the reaction was quenched with sat. aq. NaHCO₃ solution (5 mL), filtered and concentrated *in vacuo* to give the product as a white solid in 98% yield (826 mg). [α]_D²⁰ -70.0 ° (c 0.18, CHCl₃).

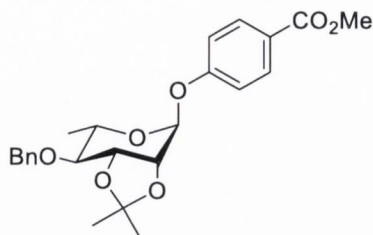
δ_{H} (400 MHz, CDCl₃): 8.00, 7.09 (2H, m, Ar), 5.79 (1H, app s, H1), 4.36 (1H, app d, H2), 4.23 (1H, dd, $J_{3,2} = 5.7$ Hz, $J_{3,4} = 7.4$ Hz, H3), 3.89 (3H, s, CO₂CH₃), 3.72 (1H, m, H5), 3.47 (1H, dd, $J_{4,3} = 7.4$ Hz, $J_{4,5} = 9.7$ Hz, H4), 1.57 (3H, s, CH₃), 1.43 (3H, s, CH₃), 1.23 (3H, d, $J_{6,5} = 6.3$ Hz, H6).

δ_{C} (100 MHz, CDCl₃): 166.6 (C=O), 159.7 (Cq), 131.5 (Ar), 123.9 (Cq), 116.0 (Ar), 109.9 (Cq), 95.3 (C1), 78.4 (C3), 75.7 (C2), 74.6 (C4), 66.9 (C5), 52.0 (CO₂CH₃), 28.1, 26.3 (CH₃), 17.3 (C6).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₇H₂₂O₇Na 361.1258; found 361.1257.

ν_{max} (thin film): 3465, 2938, 1718, 1606, 1509, 1239 cm⁻¹.

4-Benzoyloxy-2,3-*O*-isopropyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (123)



2,3-*O*-Isopropyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **123** (806 mg, 2.38 mmol) was dissolved in anhydrous DMF (7 mL) at 0 °C under N₂. NaH (114 mg, 2.86 mmol) and BnBr (0.34 mL, 2.86 mmol) were added. After 18 hours, the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (30 mL), washed with H₂O (2 x 25 mL) and sat. aq. NaHCO₃ solution (2 x 25 mL), dried with MgSO₄, filtered and concentrated. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a colourless oil in 85% yield (870 mg). [α]_D²⁰ -94.8° (*c* 0.117, CHCl₃).

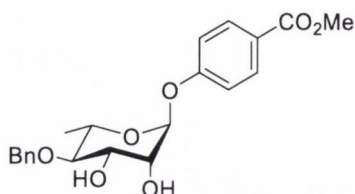
δ_H (400 MHz, CDCl₃): 7.99(2H, m, Ar), 7.33 (5H, m, Bn), 7.08 (2H, m, Ar), 5.78 (1H, app s, H1), 4.92, 4.65 (1H, d, *J* = 11.6 Hz, CH₂Bn), 4.44 (1H, m, H3), 4.37 (1H, app d, H2), 3.89 (3H, s, CO₂CH₃), 3.76 (1H, m, H5), 3.29 (1H, m, H4), 1.55 (3H, s, CH₃), 1.42 (3H, s, CH₃), 1.21 (3H, d, *J*_{6,5} = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 166.7 (C=O), 159.7, 138.3 (Cq), 131.6 (Ar), 128.3 (Bn), 128.0 (Bn), 127.7 (Bn), 124.0 (Cq), 115.8 (Ar), 109.7 (Cq), 95.2 (C1), 80.8 (C4), 78.5 (C3), 75.8 (C2), 73.0 (CH₂Bn), 65.9 (C5), 52.0 (CO₂CH₃), 28.0 (CH₃), 26.4 (CH₃), 17.9 (C6).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₂₄H₂₈O₇Na 451.1127; found 451.1727.

$\nu_{\max}$ (thin film): 2937, 1718, 1606, 1509, 1239 cm⁻¹.

4-Benzyloxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (124)



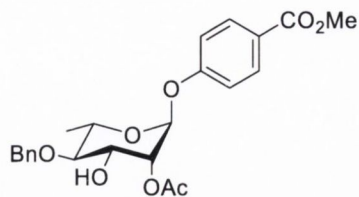
4-Benzyloxy-2,3-*O*-isopropyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **123** (850 mg, 1.98 mmol) was dissolved in acetic acid:H₂O 9:1 (10 mL) and refluxed at 50 °C. After 18 hours the reaction mixture was then concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a colourless oil in 42% yield (325 mg). $[\alpha]_D^{20}$ -102 ° (c 0.192, CHCl₃).

δ_H (400 MHz, CDCl₃): 8.02 (2H, m, Ar), 7.37 (5H, m, Bn), 7.10 (2H, m, Ar), 5.60 (1H, d, $J_{1,2}$ = 1.5 Hz, H1), 4.78 (2H, dd, J = 11.4 Hz, J = 19.4 Hz, CH₂Bn), 4.17 (1H, dd, $J_{2,1}$ = 1.5 Hz, $J_{2,3}$ = 3.3 Hz, H2), 4.13 (1H, dd, $J_{3,2}$ = 3.3 Hz, $J_{4,5}$ = 9.1 Hz, H3), 3.92 (3H, s, CO₂CH₃), 3.80 (1H, m, H5), 3.47 (1H, app t, H4), 1.34 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, CDCl₃): 166.7 (C=O), 159.7 (Cq), 138.1 (Cq), 131.6 (Ar), 128.7 (Bn), 128.6 (Bn), 128.2 (Bn), 128.0 (Bn), 124.0 (Cq), 115.8 (Ar), 97.0 (C1), 81.3 (C4), 75.2 (CH₂Bn), 71.2 (C3), 71.8 (C2), 68.4 (C5), 52.0 (CO₂CH₃), 18.0 (C6).

$\nu_{\max}$ (thin film): 3423, 2934, 1716, 1605, 1239 cm⁻¹.

2-*O*-Acetyl-4-benzyloxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (**125**)



4-Benzyloxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **124** (315 mg, 0.81 mmol) was dissolved in acetonitrile (5 mL) under N_2 . Trimethyl orthoacetate (0.2 mL, 1.6 mmol) and camphorsulfonic acid (catalytic) were added. The mixture was allowed to react for 45 minutes then 5 mL acetic acid:H₂O 4:1 were added. After 15 minutes the mixture was diluted with DCM (30 mL), washed with H₂O (50 mL) and aq. sat NaHCO₃ solution (50 mL), dried with MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (Hexane:EtOAc 70:30) gave the product as a colourless oil in 73% (256 mg). $[\alpha]_D^{20}$ -85.0 ° (c 0.08, CHCl₃).

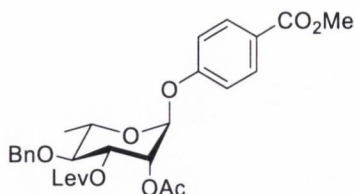
δ_H (400 MHz, CDCl₃): 8.01 (2H, m, Ar), 7.34 (5H, m, Bn), 7.12 (2H, m, Ar), 5.57 (2H, m, H1, H2), 4.87 (1H, d, J = 11.4 Hz, CH₂Bn), 4.76 (1H, d, J = 11.4 Hz, CH₂Bn), 4.33 (1H, dd, $J_{3,2}$ = 3.6 Hz, $J_{3,4}$ = 9.4 Hz, H3), 3.90 (3H, s, CO₂CH₃), 3.88 (1H, m, H5), 2.19 (3H, s, CH₃), 1.31 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 169.6 (C=O), 159.5 (Cq), 138.0 (Cq), 131.4 (Ar), 128.4 (Bn), 127.8 (Bn), 127.6 (Bn), 124.2 (Cq), 115.7 (Ar), 97.0 (C1), 95.2 (C2), 78.5 (C4), 75.1 (CH₂Bn), 69.2 (C3), 68.8 (C5), 51.8 (CO₂CH₃), 29.4 (CH₃), 17.9 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₃H₂₆O₈Na 453.1520; found 453.1533.

ν_{max} (thin film): 3472, 2952, 1719, 1605, 1980 cm⁻¹.

2-*O*-Acetyl-4-benzyloxy-3-*O*-levulinoyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (126)



2-*O*-Acetyl-4-benzyloxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **125** (246 mg, 0.57 mmol) was dissolved in anhydrous DCM (10 mL) under N₂. Levulinic acid (265 mg, 2.29 mmol), DCC (471 mg, 2.29 mmol), TEA (catalytic 2 mL) and DMAP (catalytic) were added. After 18 hours, the mixture was filtered, washed with sat. aq. NaHCO₃ solution (15 mL), aq. sat. NaCl solution (15 mL) and H₂O (15 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a colourless oil in 98% yield (295 mg). [α]_D²⁰ -24.3° (*c* 0.082, CHCl₃).

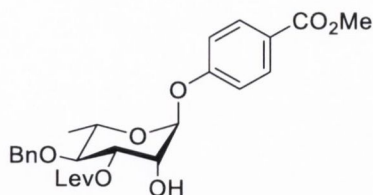
δ_{H} (400 MHz, CDCl₃): 7.98 (2H, m, Ar), 7.32 (5H, m, Bn), 7.07 (2H, m, Ar), 5.50 (1H, d, $J_{1,2}$ = 1.9 Hz, H1), 5.49 (1H, dd, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 9.5 Hz, H3), 5.43 (1H, dd, $J_{2,1}$ = 1.9 Hz, $J_{2,3}$ = 3.5 Hz, H2), 4.71 (2H, dd, J = 11.2 Hz, J = 45.6 Hz, CH₂Bn), 3.88 (3H, s, CO₂CH₃), 3.59 (1H, app t, H4), 2.74 (2H, m, CH₂), 2.54 (2H, m, CH₂), 2.19 (3H, s, CH₃), 2.18 (3H, s, CH₃), 1.30 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_{C} (100 MHz, CDCl₃): 206.3 (C=O), 172.0 (C=O), 170.2 (C=O), 162.2 (C=O), 159.5 (Cq), 138.0 (Cq), 131.7 (Ar), 128.6 (Bn), 128.0 (Bn), 127.9 (Bn), 124.4 (Cq), 116.0 (Ar), 95.4 (C1), 78.5 (C4), 75.2 (CH₂), 71.8 (C3), 69.9 (C2), 52.1 (CO₂CH₃), 38.0 (CH₂), 28.0 (CH₂), 29.2 (CH₃), 21.0 (CH₃), 18.0 (C6).

HRMS (EI) calculated for C₂₈H₃₂O₁₀Na 551.1893; found 551.1889.

ν_{max} (thin film): 2933, 1717, 1368, 1278, 983 cm⁻¹.

4-Benzoyloxy-3-O-levulinoyl-1-(4-(methoxycarbonyl)phenoxy)-α-L-rhamnopyranoside (127)



2-*O*-Acetyl-4-benzyloxy-3-*O*-levulinoyl-1-(4-(methoxycarbonyl)phenoxy)-α-L-rhamnopyranoside **126** (290 mg, 5.49 mmol) was dissolved in MeOH (20 mL) with a catalytic amount of NaOMe. After 18 hours the reaction was quenched with DOWEX, filtered and concentrated *in vacuo* to give the product as a pale yellow oil in 95% yield (253 mg). $[\alpha]_D^{20}$ -103.2 ° (c 0.091, CHCl₃).

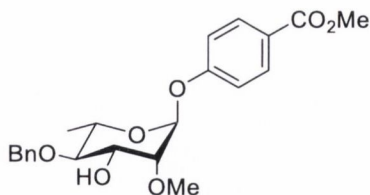
δ_H (400 MHz, CDCl₃): 7.99 (2H, m, Ar), 7.34 (5H, m, Bn), 7.07 (2H, m, Ar), 5.58 (1H, d, $J_{1,2}$ = 1.6 Hz, H1), 4.76 (2H, m, CH₂Bn), 4.15 (1H, dd, $J_{2,1}$ = 1.6 Hz, $J_{2,3}$ = 3.3 Hz, H2), 4.11 (1H, dd, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.0 Hz, H3), 3.89 (3H, s, CO₂CH₃), 3.76 (1H, m, H5), 3.45 (1H, app t, H4), 2.75 (2H, m, CH₂), 2.60 (2H, m, CH₂), 2.19 (CH₃), 1.30 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 206.5 (C=O), 168.0 (C=O), 166.9 (C=O), 160.0 (Cq), 137.9 (Cq), 131.6 (Ar), 128.7 (Bn), 128.1 (Bn), 128.0 (Bn), 124.0 (Cq), 115.8 (Ar), 97.02 (C1), 81.3 (C5), 75.2 (CH₂Bn), 71.2 (C3), 70.8 (C2), 68.4 (C5), 52.0 (CO₂CH₃), 49.4 (C4), 33.9 (CH₂), 25.6 (CH₂), 24.9 (CH₃), 18.2 (C6).

HRMS (EI) calculated for C₂₆H₃₀O₉Na 509.1782; found 509.1763.

IR: 3371, 2931, 1719, 1606, 1280, 981 cm⁻¹.

4-Benzyloxy-2-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (**128**)



- 1) 4-Benzyloxy-3-*O*-levulinoyl-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **127** (243 mg, 0.50 mmol) was dissolved in DMF (5 mL) at 0 °C under N₂. NaH (40 mg, 1.00 mmol) and MeI (0.06 mL, 1.00 mmol) were added. After 3 hours the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was redissolved in diethyl ether (20 mL), washed with water (2 x 15 mL) and sat. NaHCO₃ (2x15 mL), dried with MgSO₄, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a pale yellow oil in 35% yield (70 mg).
- 2) 3-*O*-Acetyl-4-benzyloxy-2-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (97.3 mg, 0.219 mmol) was dissolved in 7 mL MeOH along with a catalytic amount of NaOMe and allowed to react for 18 hr. The reaction was quenched with a catalytic amount of DOWEX, filtered and concentrated to give the product as a pale yellow oil in 81% yield (71.3 mg). $[\alpha]_D^{20}$ -79.3° (c 0.097, CHCl₃).

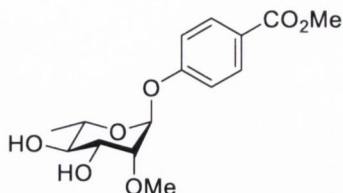
δ_H (400 MHz, CDCl₃): 8.01 (2H, m, Ar), 7.34 (5H, m, Bn), 7.10 (2H, m, Ar), 5.65 (1H, d, $J_{1,2}$ = 1.6 Hz, H1), 4.94 (1H, d, J = 10.9 Hz, CH₂Bn), 4.64 (1H, d, J = 10.9 Hz, CH₂), 3.90 (3H, s, CO₂CH₃), 3.82 (1H, dd, $J_{2,1}$ = 1.6 Hz, $J_{2,3}$ = 3.3 Hz, H2), 3.80 (1H, dd, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.1 Hz, H3), 3.69 (1H, m, H5), 3.61 (3H, s, OCH₃), 3.53 (1H, app t, H4), 1.27 (3H, d, $J_{6,5}$ = 6.1 Hz, H6).

δ_C (100 MHz, CDCl₃): 210.8 (C=O), 166.6 (C=O), 159.8 (Cq), 131.5 (Ar), 128.3 (Bn), 127.9 (Bn), 127.6 (Bn), 123.9 (Cq), 115.7 (Ar), 94.9 (C1), 81.2 (C3), 80.1 (C4), 77.0 (C2), 75.3 (CH₂Bn), 68.8 (C5), 59.4 (OCH₃), 51.8 (CO₂CH₃), 17.8 (C6).

$\nu_{\max}$ (thin film): 2931, 1716, 1605, 1278, 1098 cm⁻¹

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₂H₂₆O₇Na 425.1576; found 425.1565.

2-Methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (10)



4-Benzyloxy-2-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **128** (60.8 mg, 0.151 mmol) was dissolved in THF and a catalytic amount of palladium on carbon added. The mixture was degassed by bubbling N_2 through it for 1 hour then saturated with H_2 by bubbling H_2 through the mixture for 30 minutes. This saturation was repeated periodically over 2 days and the reaction kept under H_2 throughout. When TLC analysis indicated the consumption of the starting material N_2 was again bubbled through before exposing the mixture to air. The palladium was filtered off and the resulting solution concentrated. Purification by column chromatography (acetone/toluene 20:80) gave the product as a white solid in 83% yield (39.4 mg).

δ_H (400 MHz, $CDCl_3$): 8.01 (2H, d, $J = 8.7$ Hz, Ar), 7.11 (2H, d, $J = 8.7$ Hz, Ar), 5.67 (1H, d, $J_{1,2} = 1.4$ Hz, H1), 3.94 (1H, dd, $J_{3,2} = 3.7$ Hz, $J_{3,4} = 9.6$ Hz, H3), 3.90 (3H, s, CO_2CH_3), 3.70 (1H, dd, $J_{2,1} = 1.4$ Hz, $J_{2,3} = 3.7$ Hz, H2), 3.67 (1H, m, H5), 3.56 (3H, s, OCH_3), 3.47 (1H, app t, $J = 9.6$ Hz, H4), 1.27 (3H, d, $J_{6,5} = 6.5$ Hz, H6).

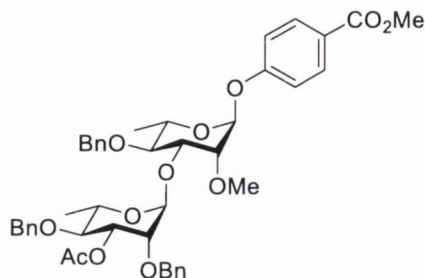
δ_C (100 MHz, $CDCl_3$): 166.7 (C=O), 159.9 (Cq), 131.6 (Ar), 124.2 (Cq), 115.8 (Ar), 94.3 (C1), 79.9 (C2), 73.8 (C4), 71.3 (C3), 68.9 (C5), 59.3 (OCH_3), 52.0 (CO_2CH_3), 17.6 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{15}H_{19}O_7Na$ 311.1131; found 311.1134.

The NMR spectrum for this compound can be found in Appendix A.

The data are consistent with those in the current literature.¹⁰⁵

3-*O*-Acetyl-2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-(methoxycarbonyl)phenoxy)-4-benzyloxy-2-methoxy- α -L-rhamnopyranoside (129)



4-Benzyloxy-2-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **128** (118 mg, 0.293 mmol) was dissolved in anhydrous DCM (5 mL) under N_2 at 0 °C. Ethyl-3-*O*-acetyl-2,4-dibenzyloxy-1-thio- α -L-rhamnopyranoside (152 mg, 0.352 mmol), NIS (106 mg, 0.352 mmol) and 10 μ L of TMS.OTf were added. After 18 hours at room temperature, the mixture was quenched with TEA (ca. 2 mL), washed with sat. aq. $Na_2S_2O_3$ solution (15 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a pale yellow oil in 95% yield (214 mg). R_f = 0.5 (hexane/EtOAc 70:30). $[\alpha]_D^{20}$ -34.9 ° (c 0.103, $CHCl_3$).

δ_H (400 MHz, $CDCl_3$): 8.03 (2H, m, Ar), 7.33 (15H, m, Bn), 7.12 (2H, m, Ar), 5.62 (1H, d, $J_{1,2}$ = 1.6 Hz, H1b), 5.32 (1H, dd, $J_{3,2}$ = 3.1 Hz, $J_{3,4}$ = 9.5 Hz, H3a), 5.17 (1H, d, $J_{1,2}$ = 1.5 Hz, H1a), 4.87 (1H, d, J = 11.5 Hz, CH_2 Bn), 4.77 (1H, d, J = 11.3 Hz, CH_2 Bn), 4.69 (2H, d, J = 11.7 Hz, CH_2 Bn), 4.47 (1H, d, J = 12.1 Hz, CH_2 Bn), 4.32 (1H, d, J = 12.1 Hz, CH_2 Bn), 4.25 (1H, dd, $J_{3,2}$ = 3.1 Hz, $J_{3,4}$ = 9.5 Hz, H3b), 4.06 (1H, m, H5b), 3.94 (1H, dd, $J_{2,1}$ = 1.9 Hz, $J_{2,3}$ = 1.5 Hz, H2a), 3.92 (3H, s, CO_2CH_3), 3.80 (1H, dd, $J_{2,1}$ = 1.9 Hz, $J_{2,3}$ = 3.0 Hz, H2b), 3.75 (1H, m, H5b), 3.71 (1H, app t, H4a), 3.62 (1H, app t, H4b), 3.60 (3H, s, OCH_3), 1.98 (3H, s, CH_3), 1.41 (3H, $J_{6,5}$ = 6.3 Hz, H6a), 1.27 (3H, d, $J_{6,5}$ = 6.2 Hz, H6b).

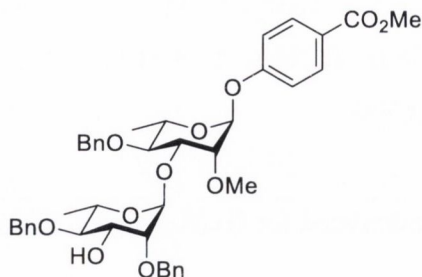
δ_C (100 MHz, $CDCl_3$): 170 (C=O), 166.5 (C=O), 159.8 (Cq), 138.3 (Bn), 138.1 (Bn), 137.8 (Bn), 137.1 (Cq), 128.3 (Bn), 128.24 (Bn), 128.23 (Bn), 128.12 (Bn), 128.08 (Bn), 128.0 (Bn), 127.9 (Bn), 127.85 (Bn),

127.82 (Bn), 127.6 (Bn), 127.59 (Bn), 127.55 (Bn), 127.54 (Bn), 127.51 (Bn), 127.4 (Bn), 115.8 (Ar), 100.0 (C1b), 94.7 (C1a), 79.9 (C2b), 79.7(C4b), 79.0 (C3b), 78.9 (C4a), 76.9 (C2a), 74.9, 74.8 (CH₂Bn), 73.4 (C3a), 72.8 (CH₂Bn), 69.1 (C5b), 68.4(C5a), 59.0 (OCH₃), 51.8 (CO₂CH₃), 20.9 (CH₃), 18.1 (C6a), 17.8 (C6b).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₄₄H₅₀O₁₂Na 793.3200; found 793.3180.

$\nu_{\max}$ (thin film): 2932, 1718, 1605, 1234, 1098 cm⁻¹.

2,4-Dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-(methoxycarbonyl)phenoxy)-4-benzyloxy-2-methoxy- α -L-rhamnopyranoside (130)



3-*O*-Acetyl-2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-(methoxycarbonyl)phenoxy)-4-benzyloxy-2-methoxy- α -L-rhamnopyranoside **129** (91.7 mg, 0.126 mmol) was dissolved in MeOH (10 mL) with a catalytic amount of NaOMe. After for 18 hours the reaction was quenched with DOWEX, filtered and concentrated *in vacuoto* give the product as a pale yellow oil in 95% yield (69.0 mg). $[\alpha]_D^{20}$ -50.9 ° (c 0.159, CHCl₃).

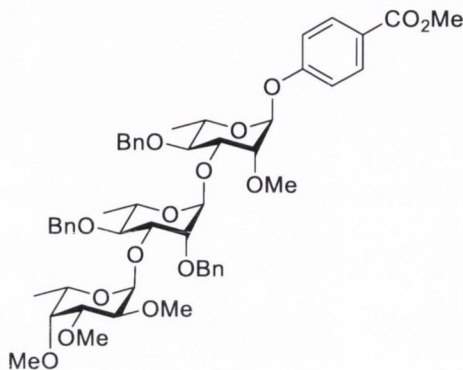
δ_H (400 MHz, CDCl₃): 8.04 (2H, m, Ar), 7.30 (15H, m, Bn), 7.14 (2H, m, Ar), 5.63 (1H, app s, H1b), 5.26 (1H, app s, H1a), 4.95 (1H, d, J = 10.6 Hz, CH₂Bn), 4.83 (1H, J = 11.1 Hz, CH₂Bn), 4.73 (2H, m, CH₂Bn), 4.45 (1H, d, J = 11.9 Hz, CH₂Bn), 4.29 (1H, dd, $J_{3,2}$ = 3.0 Hz, $J_{3,4}$ = 9.5 Hz, H3b), 4.24 (1H, d, J = 11.9 Hz, CH₂Bn), 4.06 (1H,dd, $J_{3,2}$ = 3.6 Hz, $J_{3,4}$ = 9.3 Hz, H3a), 3.95 (1H, m, H5a), 3.93 (3H, s, CO₂CH₃), 3.78 (3H, m, H2a, H2b, H5b), 3.61 (1H, app t, H4b), 3.59 (3H, s, OCH₃) 3.40 (1H, app t, H4a), 1.42 (3H, d, $J_{6,5}$ = 6.3 Hz, H6a), 1.29 (3H, d, $J_{6,5}$ = 5.8 Hz, H6b).

δ_C (100 MHz, CDCl₃): 166.7 (C=O), 159.9 (Cq), 138.5 (Cq), 138.3 (Cq), 137.7 (Cq), 131.7 (Ar), 128.5 (Bn), 128.46 (Bn), 128.4 (Bn), 128.0 (Bn), 127.9 (Bn), 127.8 (Bn), 127.7 (Bn), 127.6 (Bn), 127.0 (Bn), 124.1 (Cq), 115.9 (Ar), 99.4 (C1a), 94.9 (C1b), 82.1 (C4a), 80.3 (C4b), 80.1 (C2b), 79.2 (C2a), 78.4 (C3b), 75.1 (CH₂Bn), 75.0 (CH₂Bn), 72.7 (CH₂Bn), 71.6 (C3a), 69.3 (C5b), 68.0 (C5a), 59.2 (OCH₃), 52.0 (CO₂CH₃), 18.2 (C6b), 18.0 (C6a).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{43}H_{52}O_{10}Na$ 751.3458; found 751.3466.

$\nu_{\max}$ (thin film): 3376, 2926, 1717, 1606, 1279 cm^{-1} .

2,3,4-Trimethoxy-L-fucopyranosyl-(α 1-3)2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-(methoxycarbonyl)phenoxy)-2-methoxy-4-benzyloxy- α -L-rhamnopyranoside (131**)**



2,4-Dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-(methoxycarbonyl)phenoxy)-2-methoxy-4-benzyloxy- α -L-rhamnopyranoside **130** (91.7 mg, 0.1258) was dissolved in anhydrous DCM (5 mL) at 0 °C under N_2 . Ethyl-2,3,4-trimethoxy-1-thio- α -L-fucopyranoside **116** (28 mg, 0.112 mmol), NIS (40.0 mg, 0.1792 mmol) and a catalytic amount (ca. 10 μ L) of TMS.OTf were added. After 18 hours at room temperature the mixture was quenched with TEA (ca. 2 mL), washed with sat. aq. $Na_2S_2O_3$ solution (15 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a yellow oil in 67% yield (69.0 mg). R_f = 0.6 (hexane/EtOAc 60:40). $[\alpha]_D^{20}$ -82.0 ° (c 0.10, $CHCl_3$).

δ_H (400 MHz, $CDCl_3$): 8.02 (2H, m, Ar), 7.34 (15H, m, Bn), 7.12 (2H, m, Ar), 5.63 (1H, d, $J_{1,2}$ = 1.4 Hz, H1c), 5.26 (1H, d, $J_{1,2}$ = 1.3 Hz, H1b), 5.24 (1H, d, $J_{1,2}$ = 1.5 Hz, H1a), 5.21 (1H, d, J = 11.3 Hz, CH_2Bn), 4.89(1H, d, J = 11.6Hz, CH_2Bn), 4.70 (1H, d, J = 11.6 Hz, CH_2Bn), 4.64 (1H, d, J = 11.3 Hz, CH_2Bn), 4.59 (1H, d, J = 12.2 Hz, CH_2Bn), 4.28 (1H, d, J = 12.2 Hz, CH_2Bn), 4.27 (1H, dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.7 Hz, H3c), 4.12 (1H, dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, H3b), 3.98 (1H, m, H5b), 3.91 (3H, s, CO_2CH_3), 3.85

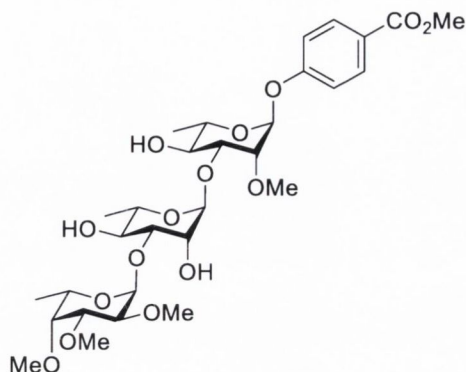
(1H, dd, $J_{2,1} = 1.8$ Hz, $J_{2,3} = 3.2$ Hz, H2b), 3.81 (1H, dd, $J_{2,1} = 1.8$ Hz, $J_{2,3} = 3.2$ Hz, H2c), 3.76 (1H, m, H5c), 3.71 (1H, m, H5a), 3.66 (1H, app t, $J = 9.4$ Hz, H4b), 3.60 (1H, app t, H4c), 3.59 (3H, s, OCH₃), 3.58 (2H, m, H2a, H3a), 3.56 (3H, s, OCH₃), 3.53 (3H, s, OCH₃), 3.36 (3H, s, OCH₃), 3.24 (1H, app s, H4a), 1.37 (3H, d, $J_{6,5} = 6.3$ Hz, H6b), 1.25 (3H, d, $J_{6,5} = 6.3$ Hz, H6c), 1.01 (3H, d, $J_{6,5} = 6.3$ Hz, H6a).

δ_C (100 MHz, CDCl₃): 166.7 (C=O), 156.0 (Cq), 139.1 (Cq), 138.4 (Cq), 138.3 (Cq), 131.6 (Ar), 128.4 (Bn), 128.3 (Bn), 128.2 (Bn), 127.7 (Bn), 127.5 (Bn), 127.4 (Bn), 127.3 (Bn), 127.2 (Bn), 127.1 (Bn), 124.1 (Cq), 115.8 (Ar), 99.6 (H1a), 99.4 (H1b), 94.7 (H1c), 80.4 (H2a), 80.1 (H2c), 79.77 (H4c), 79.75 (H4b), 79.4 (H3b, H3c), 79.2 (H4a), 78.9 (H2b), 77.9 (H3a), 75.0 (CH₂Bn), 74.7 (CH₂Bn), 71.5 (CH₂Bn), 69.2 (H5c), 68.9 (H5b), 67.4 (H5a), 61.7 (OCH₃), 59.2 (OCH₃), 59.0 (OCH₃), 58.0 (OCH₃), 52.0 (CO₂CH₃), 18.2 (H6b), 17.9 (H6c), 16.7 (H6a).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₅₁H₆₄O₁₅Na 939.4143; found 939.4139.

$\nu_{\max}$ (thin film): 2917, 1750, 1606, 1509, 1220 cm⁻¹.

2,3,4-Trimethoxy-L-fucopyranosyl-(α 1-3)-L-rhamnopyranosyl-(α 1-3)-1-(4-(methoxycarbonyl)phenoxy)-2-O-methyl-4- α -L-rhamnopyranoside (9)



2,3,4-Trimethoxy-L-fucopyranosyl-(α 1-3)-2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-(methoxycarbonyl)phenoxy)-2-methoxy-4-benzyloxy- α -L-rhamnopyranoside **131** (118 mg, 0.129 mmol) was dissolved in THF and a catalytic amount of palladium on carbon added. The mixture was degassed by suction then stirred vigorously under a hydrogen atmosphere until TLC analysis indicated the consumption of the starting material. A N_2 atmosphere was introduced before exposing the mixture to air. The palladium on carbon was filtered off and the resulting solution concentrated *in vacuo*. Purification by column chromatography (acetone/toluene 40:60) gave the product as a pale yellow oil in 70% yield (58.4 mg). $[\alpha]_D^{20}$ -89.3 ° (*c* 0.103, $CHCl_3$).

δ_H (600 MHz, $CDCl_3$): 8.03 (2H, m, Ar), 7.14 (2H, m, Ar), 5.65 (1H, d, $J_{1,2} = 1.6$ Hz, H1c), 5.16 (1H, d, $J_{1,2} = 3.3$ Hz, H1a), 5.162 (1H, d, $J_{1,2} = 1.4$ Hz, H1b), 4.13 (1H, dd, $J_{2,1} = 1.4$ Hz, H2b), 4.08 (1H, m, H4a), 4.05 (1H, dd, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.0$ Hz, H3c), 3.96 (1H, m, H5b), 3.92 (3H, s, CO_2CH_3), 3.82 (1H, dd, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.6$ Hz, H3b), 3.80 (1H, dd, $J_{2,1} = 1.6$ Hz, $J_{2,3} = 3.3$ Hz, H2c), 3.73 (1H, m, H5c), 3.72 (1H, m, H4c), 3.69 (1H, dd, $J_{2,1} = 3.3$ Hz, $J_{2,3} = 9.9$ Hz, H2a), 3.68 (1H, m, H4b), 3.66 (1H, m, H4a), 3.63 (3H, s, OCH_3), 3.61 (3H, s, OCH_3), 3.55 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 3.50 (1H, app d, $J = 2.5$ Hz, H3a), 1.40 (3H, d, $J_{6,5} = 6.4$ Hz, H6b), 1.304 (3H, d, $J_{6,5} = 5.6$ Hz, H6a), 1.299 (3H, d, $J_{6,5} = 6.2$ Hz, H6c).

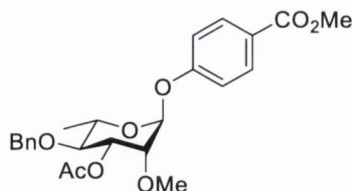
δ_C (150 MHz, $CDCl_3$): 166.8 (C=O), 160.0 (Cq), 131.6 (Ar), 124.2 (Cq), 115.9 (Ar), 102.2 (C1b), 100.9 (C1a), 94.5 (C1c), 83.2 (C3b), 81.0 (C4a), 79.8 (C3c), 79.0 (C3a), 78.8 (C2s), 71.6 (C4b), 71.5 (C5c), 71.2 (C2b), 69.5 (C4c), 68.7 (C5b), 67.6 (C4a), 61.9 (OCH₃), 60.3 (OCH₃), 58.7 (OCH₃), 57.7 (OCH₃), 52.0 (CO₂CH₃), 17.9 (C6b), 17.7 (C6c), 16.6 (C6a).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{30}H_{46}O_{15}Na$ 669.2734 found 669.2763.

ν_{max} (thin film): 3443, 2928, 1717, 1283, 1091 cm^{-1} .

The NMR spectrum for this compound can be found in Appendix A.

3-*O*-Acetyl-4-benzyloxy-2-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (132)



Ethyl-3-*O*-acetyl-4-benzyloxy-2-methoxy-1-thio- α -L-rhamnopyranoside **110** (520 mg, 1.29 mmol) was dissolved in anhydrous DCM (5 mL) at 0 °C under N₂. Methyl 4-hydroxybenzoate (296 mg, 1.94 mmol) and 1.5 eq of the activating Me₂S₂-Tf₂O solution were added. The mixture was reacted at 0 °C for 1 hour, quenched with TEA (ca. 2 mL), diluted with DCM (35 mL), washed with 1M HCl solution (25 mL), sat. aq. NaHCO₃ solution (25 mL), H₂O (25 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a pale yellow oil in 58% yield (333 mg). R_f = 0.5 (hexane/EtOAc 60:40). [α]_D²⁰ -92.8° (c 0.0070, CHCl₃).

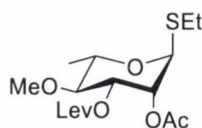
δ_H (400 MHz, CDCl₃): 8.02 (2H, m, Ar), 7.34 (5H, m, Bn), 7.14 (2H, m, Ar), 5.61 (1H, d, $J_{1,2}$ = 1.9 Hz, H1), 5.44 (1H, dd, $J_{3,2}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, H3), 4.72 (2H, dd, J = 11.2 Hz, J = 33.2 Hz, CH₂Bn), 3.91 (3H, s, OCH₃), 3.90 (1H, m, H2), 3.83 (1H, m, H5), 3.65 (1H, app t, H3), 3.55 (3H, s, OCH₃), 2.13 (3H, s, CH₃), 1.30 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, CDCl₃): 170.4 (C=O), 169.7 (Cq), 159.8 (Cq), 138.0 (Cq), 131.6 (Ar), 128.5 (Bn), 127.8 (Bn), 127.6 (Bn), 124.1 (Cq), 115.9 (Ar), 95.2 (C1), 78.9 (C4), 78.3 (C2), 75.1 (CH₂), 73.5 (C3), 68.8 (C5), 59.6, 52.0 (OCH₃), 21.2 (CH₃), 17.0 (C6).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₂₄H₂₈O₈Na 467.1682; found 467.1689.

ν_{max} (thin film): 2936, 1718, 1606, 1279, 1233 cm⁻¹.

Ethyl-2-*O*-acetyl-4-methoxy-3-*O*-levulinoyl-1-thio- α -L-rhamnopyranoside (**133**)



Ethyl-3-*O*-acetyl-4-methoxy-1-thio- α -L-rhamnopyranoside **102** (750 mg, 2.743 mmol) was dissolved in anhydrous DCM (30 mL) under N₂. Levulinic acid (1.274 g, 10.971 mmol), DCC (2.264 g, 10.971 mmol), and DMAP (catalytic) were added. After 18 hours, the mixture was filtered. The organic layer was washed with aq. sat. NaHCO₃ solution (50 mL), aq. sat. NaCl solution (50 mL), H₂O (50 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a colourless oil in 85% yield (2.681 g). $[\alpha]_D^{20}$ -192.4 ° (c 0.106, CHCl₃).

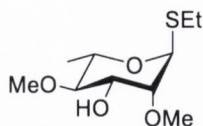
δ_H (400 MHz, CDCl₃): 5.31 (1H, dd, $J_{2,1} = 1.5$ Hz, $J_{2,3} = 3.3$ Hz, H2), 5.15 (2H, m, H1, H3), 4.09 (1H, m, H5), 3.51 (3H, s, OCH₃), 3.26 (1H, app t, H4), 2.83 (1H, m, CH₂), 2.72 (1H, m, CH₂), 2.62 (3H, m, CH₂CH₃, CH₂), 2.52 (2H, m, CH₂), 2.20 (3H, s, CH₃), 2.16 (3H, s, CH₃), 1.36 (3H, d, $J_{6,5} = 6.2$ Hz, H6), 1.29 (3H, t, $J = 7.4$ Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 206.8 (C=O), 171.7 (C=O), 170.1 (C=O), 81.8 (C1), 80.4 (C4), 72.1 (C3), 71.9 (C2), 68.2 (C5), 60.5 (OCH₃), 37.8 (CH₂Bn), 29.8 (CH₃), 27.9 (CH₂), 25.3 (CH₂CH₃), 20.9 (CH₃), 17.7 (CH₂CH₃), 14.8 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₁₆H₂₀O₇NaS 385.1297; found 385.1300.

$\nu_{\max}$ (thin film): 2927, 1745, 1372, 1234 cm⁻¹.

Ethyl-2,4-dimethoxy-1-thio- α -L-rhamnopyranoside (**135**)



Ethyl-3-*O*-acetyl-4-methoxy-1-thio- α -L-rhamnopyranoside **102** (71 mg, 0.270 mmol) dissolved in wet DMF (4 mL) at 0 °C under N₂. NaH (20 mg, 0.49 mmol) and MeI (0.030 mL, 0.49 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (25 mL), washed with H₂O (2 x 20 mL) and sat. aq. NaHCO₃ solution (2 x 20 mL), dried with MgSO₄, and concentrated. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a colourless oil in 63% yield (42 mg) with the permethylated compound **136** as a side product. $[\alpha]_D^{20}$ -177.7° (*c* 0.063, CHCl₃).

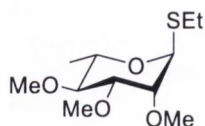
δ_H (400 MHz, CDCl₃): 5.38 (1H, app s, H1), 3.96 (1H, m, H5), 3.82 (1H, m, H3), 3.59 (3H, s, OCH₃), 3.58 (1H, m, H2), 3.49 (3H, s, OCH₃), 3.03 (1H, app t, H4), 2.63 (2H, m, CH₂CH₃), 1.32 (3H, m, H6), 1.31 (3H, m, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 84.1 (C4), 82.1 (C2), 80.1 (C1), 71.8 (C3), 67.6 (C5), 60.8 (OCH₃), 58.1 (OCH₃), 25.2 (CH₂CH₃), 17.8 (C6), 14.9 (CH₂CH₃).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₁₀H₂₀O₄NaS 259.0980 found 259.0993.

$\nu_{\max}$ (thin film): 3462, 2930, 1451, 1380, 1091 cm⁻¹.

Ethyl-2,3,4-trimethoxy-1-thio- α -L-rhamnopyranoside (136)



Compound **136** was isolated as a side product during the preparation of thioglycoside **135**.
Procedure on previous page.

$[\alpha]_{\text{D}}^{20} -100^{\circ}$ (c 0.077, CHCl_3).

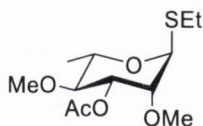
δ_{H} (400 MHz, CDCl_3): 5.38 (1H, app s, H1), 3.94 (1H, m, H5), 3.70 (1H, dd, $J_{2,1} = 1.5$ Hz, $J_{2,3} = 3.1$ Hz, H2), 3.57 (3H, s, OCH_3), 3.51 (3H, s, OCH_3), 3.51 (3H, s, OCH_3), 3.45 (1H, dd, $J_{3,2} = 3.2$ Hz, $J_{3,4} = 9.4$ Hz, H3), 3.17 (1H, app t, H4), 2.66 (2H, m, CH_2CH_3), 1.319 (3H, t, $J = 7.4$ Hz, CH_2CH_3), 1.317 (3H, d, $J_{6,5} = 6.2$ Hz, H6).

δ_{C} (100 MHz, CDCl_3): 82.4 (C4), 81.5 (C3), 80.7 (C1), 79.0 (C2), 68.1 (C5), 61.0 (OCH_3), 58.3 (OCH_3), 57.7 (OCH_3), 25.2 (CH_2CH_3), 17.7 (C6), 15.1 (CH_2CH_3).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{11}\text{H}_{22}\text{NaO}_4\text{S}$ 273.1131; found 273.1133.

ν_{max} (thin film): 2929, 1746, 1454, 1101 cm^{-1} .

Ethyl-3-*O*-acetyl-2, 4-dimethoxy-1-thio- α -L-rhamnopyranoside (**137**)



Ethyl-2,4-dimethoxy-1-thio- α -L-rhamnopyranoside **135** (238 mg, 1.01 mmol) was dissolved in pyridine (5 mL) at 0 °C under N₂. Ac₂O (5 mL) was added. After 18 hours the reaction was quenched by the addition of iced H₂O (10 mL), diluted with EtOAc (30 mL), washed with dil. aq. CuSO₄ solution (7 x 25 mL) and H₂O (25 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a colourless oil in 98% yield (274 mg). [α]_D²⁰ -148.0° (*c* 0.156, CHCl₃).

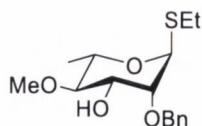
δ_{H} (400 MHz, CDCl₃): 5.29 (1H, app s, H1), 5.05 (1H, dd, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.6$ Hz, H3), 4.02 (1H, m, H5), 3.72 (1H, m, H2), 3.47 (3H, s, OCH₃), 3.43 (3H, s, OCH₃), 3.27 (1H, app t, H4), 2.60 (2H, m, CH₂CH₃), 2.13 (3H, s, CH₃), 1.31 (3H, d, $J_{6,5} = 6.2$ Hz, H6), 1.29 (3H, t, $J = 7.4$ Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 170.1 (C=O), 80.7 (C1), 80.5 (C4), 80.0 (C2), 73.8 (C3), 67.9 (C5), 60.4 (OCH₃), 58.4 (OCH₃), 25.1 (CH₂CH₃), 20.1 (CH₃), 17.6 (C6), 14.8 (CH₂CH₃).

HRMS-ESI (m/z): [M + H]⁺ calculated for C₁₂H₂₃O₅S 279.1266; found 279.1719.

ν_{max} (thin film): 2931, 1746, 1373, 1238, 1104 cm⁻¹.

Ethyl-2-benzyloxy-4-methoxy-1-thio- α -L-rhamnopyranoside (**138**)



Ethyl-3-*O*-acetyl-4-methoxy-1-thio- α -L-rhamnopyranoside **102** (36 mg, 0.134 mmol) was dissolved in wet DMF (4 mL) at 0 °C under N₂. NaH (7 mg, 0.16 mmol) and BnBr (0.019 mL, 0.16 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 2 mL), diluted with diethyl ether (25 mL), washed with H₂O (2 x 20 mL) and sat.aq. NaHCO₃ solution (2 x 20 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product in 63% yield (34.2 mg) as a pale yellow oil and the dibenzylated compound **139** (characterisation overleaf), also as a pale yellow oil, as a side product. $[\alpha]_{\text{D}}^{20}$ -95.7° (*c* 0.210, CHCl₃).

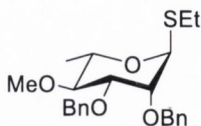
δ_{H} (400 MHz, CDCl₃): 7.38 (5H, m, Bn), 5.36 (1H, app s, H1), 4.78, 4.57 (1H, d, *J* = 11.7 Hz, CH₂Bn), 3.98 (1H, m, H5), 3.85 (2H, m, H2, H3), 3.60 (3H, s, OCH₃), 3.09 (1H, app t, H4), 2.61 (2H, m, CH₂CH₃), 1.34 (3H, d, *J*_{6,5} = 6.2 Hz, H6), 1.28 (3H, t, *J* = 7.3 Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 137.5 (Cq), 128.6 (Bn), 128.14 (Bn), 128.06 (Bn), 84.2 (C4), 80.9 (C1), 80.2 (C2), 72.4 (CH₂Bn), 71.8 (C3), 67.7 (C5), 60.9 (OCH₃), 25.2 (CH₂CH₃), 17.9 (C6), 15.0 (CH₂CH₃).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₁₆H₂₄O₄NaS 355.1288; found 335.1279.

ν_{max} (thin film): 3447, 2930, 1453, 1107 cm⁻¹.

Ethyl-2,3-dibenzyloxy-4-methoxy-1-thio- α -L-rhamnopyranoside (139)



Compound **139** was isolated as a side product during the preparation of thioglycoside **138**.

Procedure on previous page.

$[\alpha]_{\text{D}}^{20}$ -49.7° (c 0.385, CHCl_3).

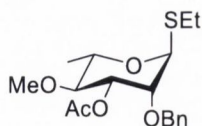
δ_{H} (400 MHz, CDCl_3): 7.36 (10H, m, Bn), 5.27 (1H, app s, H1), 4.72 (2H, dd, $J = 12.5$ Hz, $J = 18.0$ Hz, CH_2), 4.60 (2H, dd, $J = 11.9$ Hz, $J = 21.5$ Hz, CH_2), 3.95 (1H, m, H5), 3.83 (1H, m, H2), 3.70 (1H, dd, $J_{3,2} = 3.2$ Hz, $J_{3,4} = 9.5$ Hz, H3), 3.62 (3H, s, OCH_3), 3.37 (1H, app t, H4), 2.59 (2H, m, CH_2CH_3), 1.35 (3H, d, $J_{6,5} = 6.2$ Hz, H6), 1.26 (3H, t, $J = 7.3$ Hz, CH_2CH_3).

δ_{C} (100 MHz, CDCl_3): 138.5, 138.1 (Cq), 128.4 (Bn), 128.3 (Bn), 127.7 (Bn), 127.66 (Bn), 127.6 (Bn), 82.5 (C4), 81.9 (C1), 80.0 (C3), 76.7 (C2), 72.2 (2 x CH_2Bn), 68.4 (C5), 61.1 (CH_3), 25.4 (CH_2CH_3), 17.8 (C6), 15.0 (CH_2CH_3).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{23}\text{H}_{30}\text{O}_4\text{Na}$ 425.1763; found 425.1760.

ν_{max} (thin film): 2925, 2855, 1455, 1370, 1103 cm^{-1} .

Ethyl-3-*O*-acetyl-2-benzyloxy-4-methoxy-1-thio- α -L-rhamnopyranoside (140)



Ethyl-2-benzyloxy-4-methoxy-1-thio- α -L-rhamnopyranoside **138** (220 mg, 0.705 mmol) was dissolved in pyridine (5 mL) at 0 °C under N₂. Ac₂O (5 mL) was added. After 18 h the reaction was quenched by the addition of iced H₂O (10 mL), diluted with EtOAc (30 mL), washed with dil. aq. CuSO₄ solution (7 x 25 mL), H₂O (25 mL), dried with MgSO₄, filtered and concentrated. Purification by column chromatography (hexane/EtOAc 80:20) gave the product in 74% yield (186 mg). $[\alpha]_{\text{D}}^{20}$ -108.8° (*c* 0.386, CHCl₃).

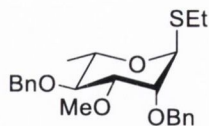
δ_{H} (400 MHz, CDCl₃): 7.35 (5H, m, Bn), 5.27 (1H, d $J_{1,2}$ = 1.5 Hz, H1), 5.05 (1H, dd, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H3), 4.63 (2H, dd, J = 12.3 Hz, J = 56.4 Hz, CH₂), 4.05 (1H, m, H5), 3.95 (1H, dd, $J_{2,1}$ = 1.5 Hz, $J_{2,3}$ = 3.3 Hz, H2), 3.51 (3H, s, OCH₃), 3.38 (1H, app t, H4), 2.61 (2H, m, CH₂CH₃), 2.07 (3H, s, CH₃), 1.36 (3H, d, $J_{6,5}$ = 6.2 Hz, H6), 1.28 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 170.1 (C=O), 137.8 (Cq), 128.4 (Bn), 127.94 (Bn), 127.92 (Bn), 81.6 (C1), 80.6 (C4), 77.6 (C2), 73.8 (C3), 72.5 (CH₂), 68.1 (C5), 60.5 (OCH₃), 25.3 (CH₂CH₃), 21.1 (CH₃), 17.8 (C6), 14.9 (CH₂CH₂).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₁₈H₂₆O₅NaS 377.1399; found 377.1400.

ν_{max} (thin film): 2933, 1740, 1368, 1236, 1104 cm⁻¹.

Ethyl-2,4-dibenzyloxy-3-methoxy-1-thio- α -L-rhamnopyranoside (**141**)



Ethyl-2,4-dibenzyloxy-1-thio- α -L-rhamnopyranoside **111** (158 mg, 0.407 mmol) was dissolved in DMF (4 mL) at 0 °C under N₂. NaH (24 mg, 0.61 mmol) and MeI (0.038 mL, 0.61 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 2 mL), concentrated, re-dissolved in diethyl ether (25 mL), washed with H₂O (2 x 20 mL) and sat. aq. NaHCO₃ solution (2 x 20 mL), dried with MgSO₄, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 90:10) gave the product in 80% yield (130.5 mg). $[\alpha]_D^{20}$ -95.8° (*c* 0.438, CHCl₃).

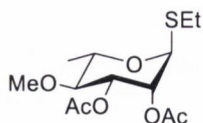
δ_H (400 MHz, CDCl₃): 7.40 (10H, m, Bn), 5.33 (1H, d, $J_{1,2}$ = 1.7 Hz, H1), 4.97 (1H, d, J = 11.0 Hz, CH₂Bn), 4.78 (2H, dd, J = 12.4 Hz, J = 15.9 Hz, CH₂), 4.67 (1H, d, J = 11.0 Hz, CH₂Bn), 4.07 (1H, m, H5), 3.94 (1H, dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, H2), 3.60 (1H, app t, H4), 3.56 (1H, dd, $J_{3,2}$ = 3.1 Hz, $J_{3,4}$ = 9.3 Hz, H3), 3.43 (3H, s, OCH₃), 2.62 (2H, m, CH₂CH₃), 1.37 (3H, d, $J_{6,5}$ = 6.1 Hz, H6), 1.28 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 138.8 (Cq), 138.1 (Cq), 128.43 (Bn), 128.37 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.6 (Bn), 82.2 (C3), 81.9 (c1), 80.7 (C4), 75.9 (C2), 75.3 (CH₂Bn), 72.2 (CH₂Bn), 68.2 (C5), 57.5 (OCH₃), 25.4 (CH₂CH₃), 18.0 (C6), 15.1 (CH₂CH₃).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₂₃H₃₀O₄NaS 425.1763 found 425.1776.

$\nu_{\max}$ (thin film): 2929, 1454, 1350, 1100, 736 cm⁻¹.

Ethyl-2,3-*O*-acetyl-4-methoxy-1-thio- α -L-rhamnopyranoside (**142**)



Ethyl-4-methoxy-1-thio- α -L-rhamnopyranoside **95** (200 mg, 0.90 mmol) was dissolved in pyridine (3 mL) at 0 °C under N₂. Ac₂O (3 mL) was added. After 18 hours the reaction was quenched by the addition of iced H₂O (10 mL), diluted with EtOAc (30 mL), washed with dil. aq. CuSO₄ solution (7 x 25 mL) and H₂O (25 mL). The organic layer was dried with MgSO₄, filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a pale yellow oil in 96% yield (265 mg). $[\alpha]_D^{20} -111.9^\circ$ (*c* 0.134, CHCl₃).

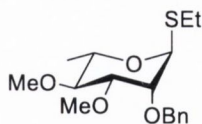
δ_H (400 MHz, CDCl₃): 5.31 (1H, dd, $J_{2,1} = 1.2$ Hz, $J_{2,3} = 3.2$ Hz, H2), 5.15 (1H, dd, $J_{3,2} = 3.2$ Hz, $J_{3,4} = 12.3$ Hz, H3), 5.14 (1H, app s, H1), 4.09 (1H, m, H5), 3.48 (3H, s, OCH₃), 3.24 (1H, app t, H4), 2.61 (2H, m, CH₂CH₃), 2.15 (3H, s, CH₃), 2.05 (3H, s, CH₃), 1.35 (3H, d, $J_{6,5} = 6.2$ Hz, H6), 1.28 (3H, t $J = 17.4$ Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 170.0, 169.8 (C=O), 81.8 (C1), 80.4 (C4), 72.0 (C2), 71.7 (C3), 68.2 (C5), 60.5 (OCH₃), 25.3 (CH₂), 21.0, 20.9 (3H, s, CH₃), 17.7 (C6), 14.8 (CH₂CH₃).

HRMS-ESI (*m/z*): $[M + Na]^+$ calculated for C₁₃H₂₂O₆NaS 329.1049; found 329.1035.

$\nu_{\max}$ (thin film): 2935, 1749, 1605, 1504, 1317, 1101 cm⁻¹.

Ethyl-2,4-dibenzyloxy-3-methoxy-1-thio- α -L-rhamnopyranoside (143)



Ethyl-2-benzyloxy-4-methoxy-1-thio- α -L-rhamnopyranoside **138** (74 mg, 0.238 mmol) was dissolved in DMF (4 mL) at 0 °C under N₂. NaH (19 mg, 0.475 mmol) and MeI (0.030 mL, 0.475 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 2 mL), concentrated, re-dissolved in diethyl ether (25 mL), washed with H₂O (2 x 20 mL) and sat. aq. NaHCO₃ solution (2 x 20 mL), dried with MgSO₄, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product in 98% yield (76 mg). [α]_D²⁰ -59.6° (*c* 0.483, CHCl₃).

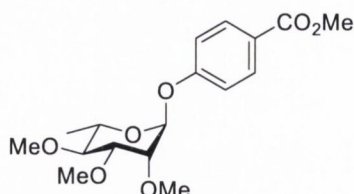
δ_H (400 MHz, CDCl₃): 7.42 (2H, m, Bn), 7.37 (2H, m, Bn), 7.31 (1H, m, Bn), 5.29 (1H, d, $J_{1,2}$ = 1.5 Hz, H1), 4.73 (2H, dd, J = 12.5 Hz, J = 17.2 Hz, CH₂Bn), 3.94 (1H, m, H5), 3.89 (1H, $J_{2,1}$ = 1.5 Hz, $J_{2,3}$ = 3.2 Hz, H2), 3.59 (3H, s, OCH₃), 3.43 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.4 Hz, H3), 3.40 (3H, s, OCH₃), 3.28 (1H, app t, H4), 2.60 (2H, m, CH₂CH₃), 1.34 (3H, d, $J_{6,5}$ = 6.2 Hz, H6), 1.27 (3H, t, J = 7.4 Hz, CH₂CH₃).

δ_C (100 MHz, CDCl₃): 138.0 (Cq), 128.2 (Bn), 127.8 (Bn), 127.6 (Bn), 82.2 (C4), 81.7 (C3, C1), 75.2 (C2), 72.0 (CH₂Bn), 68.1 (C5), 60.7 (OCH₃), 57.3 (OCH₃), 25.3 (CH₂CH₃), 17.6 (C6), 14.9 (CH₂CH₃).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₂₃H₃₀O₄NaS 425.1763 found 425.1776.

ν_{max} (thin film): 2925, 1455, 1166, 1103 cm⁻¹.

2,3,4-Trimethoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (144)



1-(4-(Methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **11** (500 mg, 1.676 mmol) was dissolved in anhydrous DMF (10 mL) at 0°C under N₂. NaH (302 mg, 7.543 mmol) and MeI (0.470 mL, 7.543 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 2 mL) and concentrated *in vacuo*. The residue was re-dissolved in diethyl ether (20 mL), washed with sat. aq. NaHCO₃ solution (2 x 20 mL), and H₂O (2 x 20 mL), dried with MgSO₄, filtered and concentrated. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a white solid in 89% yield (506 mg). R_f = 0.5 (hexane/EtOAc 60:40).

$[\alpha]_{\text{D}}^{20}$ - 103.9° (*c* 0.101, CHCl₃).

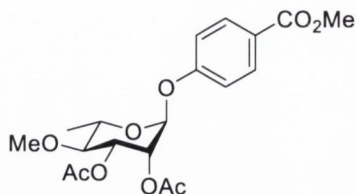
δ_{H} (400 MHz, CDCl₃): 8.00, 7.10 (2H, d, *J* = 8.7 Hz, Ar), 5.62 (1H, d, *J*_{1,2} = 1.4 Hz, H1), 3.89 (3H, s, CO₂CH₃), 3.78 (1H, dd, *J*_{2,1} = 1.4 Hz, *J*_{2,3} = 3.3 Hz, H2), 3.68 (1H, dd, *J*_{3,2} = 3.3 Hz, *J*_{3,4} = 9.4 Hz, H3), 3.60 (1H, m, H5), 3.57 (9H, s, OCH₃), 3.21 (1H, app t, H4), 1.26 (3H, d, *J*_{6,5} = 6.2 Hz, H6).

δ_{C} (100 MHz, CDCl₃): 166.7 (C=O), 160.0 (Cq), 131.6 (Ar), 124.0 (Cq), 115.8 (Ar), 95.0 (C1), 81.9 (C4), 80.8 (C3), 77.1 (C2), 68.9 (C5), 61.0 (OCH₃), 59.4 (OCH₃), 58.0 (OCH₃), 52.0 (CO₂CH₃), 17.8 (C6).

HRMS (ESI) calculated for C₁₇H₂₄O₇Na 363.1420; found 363.1436.

ν_{max} (thin film): 2934, 1720, 1606, 1279, 1103 cm⁻¹.

2,3-Di-*O*-acetyl-4-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (145)



4-Methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **142** (100 mg, 0.326 mmol) was dissolved in anhydrous DCM (10 mL) at 0 °C under N₂. Methyl-4-hydroxybenzoate (75 mg, 0.490 mmol) and 1.5 eq. of the activating Me₂S₂-Tf₂O solution were added. After 1 hour at 0 °C the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (15 mL), washed with 1M HCl solution (20 mL), sat. aq. NaHCO₃ solution (20 mL) and H₂O (20 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography gave the product as a pale yellow oil in 96% yield (125 mg). R_f = 0.5 (hexane/EtOAc 60:40). [α]_D²⁰ -108.6° (*c* 0.092, CHCl₃).

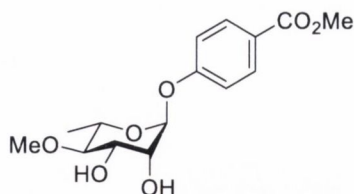
δ_{H} (400 MHz, CDCl₃): 8.00 (2H, m, Ar), 7.11 (2H, m, Ar), 5.50 (1H, d, $J_{1,2}$ = 1.5 Hz, H1), 5.43 (1H, m, H2), 5.41 (1H, m, H3), 3.90 (3H, s, OCH₃), 3.80 (1H, m, H5), 3.31 (1H, app t, H4), 3.52 (3H, s, OCH₃), 2.20 (3H, s, CH₃), 2.11 (3H, s, CH₃), 1.32 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_{C} (100 MHz, CDCl₃): 170.0 (C=O), 169.9 (C=O), 166.6 (C=O), 159.5 (Cq), 131.6 (Ar), 124.4 (Cq), 116.0 (Ar), 95.4 (C1), 80.0 (C4), 71.1 (C3), 70.0 (C2), 68.7 (C5), 60.8 (OCH₃), 52.0 (CO₂CH₃), 21.0 (CH₃), 20.9 (CH₃), 17.8 (C6).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₁₉H₂₄O₉Na 419.1328 found 419.1318.

ν_{max} (thin film): 2935, 1717, 1605, 1277, 1099 cm⁻¹.

4-Methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (146)



2,3-Di-*O*-acetyl-4-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **145** (125 mg, 0.323 mmol) was dissolved in MeOH along with a catalytic amount of NaOMe. After 18 h the reaction was quenched with DOWEX. The mixture was filtered and concentrated *in vacuo*. Purification by column chromatography furnished the product as a pale yellow oil in 58% yield (58 mg). $[\alpha_D]^{20} -108.5^\circ$ (*c* 0.188, CHCl₃).

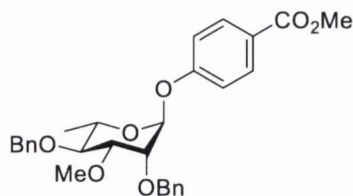
δ_H (400 MHz, CDCl₃): 8.01 (2H, d, $J = 8.6$ Hz, Ar), 7.09 (2H, d, $J = 8.6$ Hz, Ar), 5.60 (1H, d, $J_{1,2} = 1.4$ Hz, H1), 4.19 (1H, dd, $J_{2,1} = 1.4$ Hz, $J_{2,3} = 3.5$ Hz, H2), 4.08 (1H, dd, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.2$ Hz, H3), 3.91 (3H, s, CO₂CH₃), 3.70 (1H, m, H5), 3.61 (3H, s, OCH₃), 3.21 (1H, app t, $J = 9.4$ Hz, H4), 1.30 (3H, d, $J_{6,5} = 6.2$ Hz, H6).

δ_C (100 MHz, CDCl₃): 166.9 (C=O), 159.8 (Cq), 131.6 (Ar), 124.0 (Cq), 115.8 (Ar), 97.2 (C1), 83.1 (C4), 71.1 (C3), 70.7 (C2), 68.5 (C5), 61.0 (OCH₃), 51.2 (CO₂CH₃), 18.0 (C6).

HRMS (ESI) calculated for C₁₅H₂₀O₇Na 335.1101; found 335.1099.

$\nu_{\max}$ (thin film): 3443, 2920, 1717, 1279, 1110 cm⁻¹.

2,4-Dibenzyloxy-3-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (147)



Ethyl-2,4-benzyloxy-3-methoxy-1-thio- α -L-rhamnopyranoside **141** (70.0 mg, 0.174 mmol) was dissolved in anhydrous DCM (10 mL) at 0 °C under N₂. Methyl-4-hydroxy-benzoate (39.9 mg, 0.261 mmol) and 1.5 eq. of the activating Me₂S₂-Tf₂O solution were added. After 1 h at 0 °C the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (20 mL), washed with 1M HCl solution (15 mL), sat. aq. NaHCO₃ solution (15 mL) and H₂O (10 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography gave the product as a pale yellow oil in 36% yield (30.9 mg). R_f = 0.7 (hexane/EtOAc 60:40). [α]_D²⁰ -90.0° (*c* 0.121, CHCl₃).

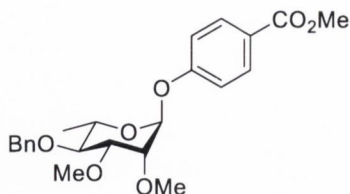
δ_H (400 MHz, CDCl₃): 7.95 (2H, m, Ar), 7.34 (5H, m, Bn), 7.00 (2H, m, Ar), 5.53 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 4.93 (1H, d, J = 11.0 Hz, CH₂), 4.85, 4.77 (1H, d, J = 12.4 Hz, CH₂Bn), 4.64 (1H, d, J = 11.0 Hz, CH₂Bn), 3.98 (1H, dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 9.1 Hz, H3), 3.90 (3H, s, CO₂CH₃), 3.69 (1H, m, H5), 3.60 (1H, app t, H4), 3.50 (3H, s, OCH₃), 1.26 (3H, d, $J_{6,5}$ = 6.0 Hz, H6).

δ_C (100 MHz, CDCl₃): 166.7 (C=O), 159.8 (Cq), 138.5 (Cq), 138.0 (Cq), 131.6 (Ar), 128.5 (Bn), 128.4 (Bn), 128.02 (Bn), 127.98 (Bn), 127.92 (Bn), 127.7 (Bn), 123.9 (Cq), 115.7 (Ar), 95.9 (C1), 81.6 (C3), 80.2 (C4), 75.4 (CH₂Bn), 74.0 (C2), 73.2 (CH₂Bn), 68.9 (C5), 58.0, 52.0 (CO₂CH₃), 21.6 (OCH₃), 18.1 (C6).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₃₁H₄₀Na O₇ 515.2041 found 515.2147.

$\nu_{\max}$ (thin film): 2929, 1454, 1350, 1100, 736 cm⁻¹.

4-Benzyloxy-2,3-dimethoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (149)



Ethyl-4-benzyloxy-2,3-dimethoxy-1-thio- α -L-rhamnopyranoside **109** (150 mg, 0.461 mmol) was dissolved in anhydrous DCM (10 mL) at 0 °C under N₂. Methyl-4-hydroxy-benzoate (84.4 mg, 0.691 mmol) and 1.5 eq. of the activating Me₂S₂-Tf₂O solution were added. After 1 h at 0 °C the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (15 mL), washed with 1M HCl solution (20 mL), sat. aq. NaHCO₃ solution (20 mL), H₂O (20 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography gave the product as a pale yellow oil in 57% yield (73.5 mg). R_f = 0.6 (hexane/EtOAc 60:40). [α]_D²⁰ -49.4 ° (*c* 0.194, CHCl₃).

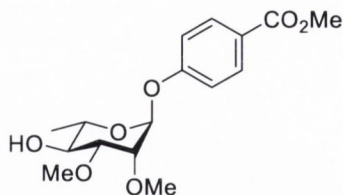
δ_H (400 MHz, CDCl₃): 8.02 (2H, m, Ar), 7.35 (5H, m, Bn), 7.11 (2H, m, Ar), 5.67 (1H, app s, H1), 4.96 (1H, d, *J* = 10.9 Hz, CH₂Bn), 4.65 (1H, d, *J* = 10.9 Hz, CH₂Bn), 3.92 (3H, s, CO₂CH₃), 3.84 (1H, m, H2), 3.82 (1H, m, H3), 3.72 (1H, m, H5), 3.62 (6H, s, OCH₃), 3.54 (1H, app t, *J* = 9.2 Hz, H4), 1.29 (3H, d, *J*_{6,5} = 6.1 Hz, H6).

δ_C (100 MHz, CDCl₃): 166.7 (C=O), 159.9 (Cq), 138.5 (Cq), 131.6 (Ar), 128.4 (Bn), 128.0 (Bn), 127.7 (Bn), 124.0 (Cq), 115.8 (Ar), 94.9 (C1), 81.2 (C3), 80.1 (C4), 77.1 (C2), 75.4 (CH₂Bn), 68.8 (C5), 59.5 (OCH₃), 59.1 (OCH₃), 52.0 (CO₂CH₃), 17.9 (C6).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₂₃H₂₈O₇Na 439.1722 found 439.1733.

$\nu_{\max}$ (thin film): 2935, 1717, 1605, 1278, 1092 cm⁻¹.

2,3-Dimethoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (150)



4-Benzyloxy-2,3-dimethoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **149**

(24 mg, 0.049 mmol) was dissolved in THF and a catalytic amount of palladium on carbon added. The mixture was degassed by bubbling N_2 through the solution for 1 hour then saturated with H_2 by bubbling H_2 through the mixture for 30 minutes. This saturation was repeated periodically over 2 days and the reaction kept under H_2 throughout. When TLC analysis indicated the consumption of the starting material N_2 was again bubbled through before exposing the mixture to air. The palladium was filtered off and the resulting solution concentrated. Purification by column chromatography (hexane/EtOAc 1:1) gave the product as a pale yellow oil in 61% yield (9.3 mg). $[\alpha]_D^{20} -167.1^\circ$ (c 0.128, $CHCl_3$).

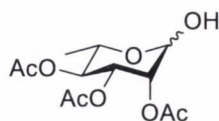
δ_H (400 MHz, $CDCl_3$): 8.04 (2H, m, Ar), 7.14 (2H, m, Ar), 5.69 (1H, d, $J_{1,2} = 1.6$ Hz, H1), 3.92 (3H, s, CO_2CH_3), 3.86 (1H, dd, $J_{2,1} = 1.6$ Hz, $J_{2,3} = 2.6$ Hz, H2), 3.70 (1H, m, H5), 3.68 (1H, m, H4), 3.66 (1H, m, H3), 3.60 (3H, s, OCH_3), 3.58 (3H, s, OCH_3), 1.30 (3H, d, $J_{6,5} = 5.7$ Hz, H6).

δ_C (100 MHz, $CDCl_3$): 166.7 (C=O), 159.9 (Cq), 131.7 (Ar), 124.2 (Cq), 115.8 (Ar), 95.2 (C1), 80.9 (C3), 75.7 (C2), 71.5 (C4), 69.3 (C5), 59.4 (OCH_3), 57.2 (OCH_3), 52.0 (CO_2CH_3), 17.8 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{16}H_{21}O_7$ 325.1287 found 325.1287.

ν_{max} (thin film): 3460, 2933, 1719, 1606, 1201, 1118 cm^{-1} .

2,3,4-Tri-*O*-acetyl-L-rhamnopyranoside (155)



- 1) Ethyl-2,3,4-tri-*O*-acetyl-1-thio- α -L-rhamnopyranoside **78** (100 mg, 0.3 mmol) was dissolved in anhydrous acetone (15 mL) with H₂O (11 μ L; 0.6 mmol) and *N*-bromosuccinimide (34.5 mg, 1.24 mmol). After 1.5 hours the reaction was quenched using NaHCO₃ dissolved in MeOH (ca. 5 mL) and concentrated *in vacuo*. The residue was re-suspended in DCM, washed with aq. sat. Na₂S₂O₃ solution (2 x 20 mL), and aq. sat. NaHCO₃ solution (2 x 20 mL), dried with MgSO₄, filtered and concentrated *in vacuo* to give the product as a white solid in 77% yield (67 mg).
- 2) 1,2,3,4-Tetra-*O*-acetyl-L-rhamnopyranose **68** (4.0 g, 12.0 mmol) was dissolved in THF and BnNH₂ (2.6 mL, 24.0 mmol) was added. After 18 hours the mixture was diluted with DCM, washed with ice cold 1M HCl, aq. sat. NaHCO₃ solution (2 x 50 mL), aq. sat. NaCl solution (2 x 50 mL), and H₂O (2 x 50 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30 – 60: 30) gave the product in 82% yield (2.846 g).

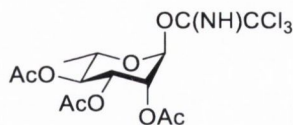
δ_{H} (400 MHz, CDCl₃): 5.40 (1H, dd, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 10.1$ Hz, H3), 5.30 (1H, dd, $J_{2,1} = 1.8$ Hz, $J_{2,3} = 3.3$ Hz, H2), 5.19 (1H, $J_{1,2} = 1.8$ Hz, H1), 5.11 (1H, app t, H4), 4.15 (1H, m, H5), 2.19 (CH₃), 2.09 (CH₃), 2.03 (CH₃), 1.25 (3H, d, $J_{6,5} = 6.2$ Hz, H6).

δ_{C} (100 MHz, CDCl₃): 170.3 (C=O), 170.12 (C=O), 170.09 (C=O), 92.2 (C1), 71.1 (C4), 70.2 (C2), 68.8 (C3), 66.4 (C5), 21.0 (CH₃), 20.9 (CH₃), 20.8 (CH₃), 17.5 (C6).

HRMS-ESI (m/z): $[M + \text{Na}]^+$ calculated for C₁₂H₁₈O₈Na 313.0899 found 313.0897.

The data are consistent with those in the current literature.^{194, 195}

2,3,4-Tri-*O*-acetyl-1-trichloroacetimidate- α -L-rhamnopyranoside (**156**)



2,3,4-Tri-*O*-acetyl- α -L-rhamnopyranoside **155** (2.80 g, 9.66 mmol) was dissolved in anhydrous DCE under N_2 . Trichloroacetonitrile (11.6 mL, 115.9 mmol) and sufficient 1,8-diazabicycloundec-7-ene to turn the solution black (~6 drops). After 2 hours the mixture was concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a colourless oil in 81% yield (3.395 g). $[\alpha]_D^{20}$ -65.1 ° (c 0.132, $CHCl_3$).

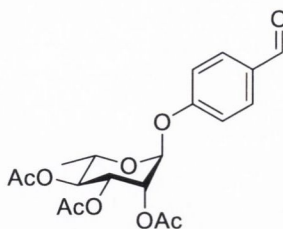
δ_H (400 MHz, $CDCl_3$): 8.76 (1H, s, NH), 6.23 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 5.49 (1H, dd, $J_{2,3}$ = 3.4 Hz, $J_{2,1}$ = 1.8 Hz, H2), 5.39 (1H, dd, $J_{3,4}$ = 10.0 Hz, H3), 5.20 (1H, app t, H4), 4.12 (1H, m, H5), 2.22 (3H, s, CH_3), 2.10 (3H, s, CH_3), 2.03 (3H, s, CH_3), 1.30 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, $CDCl_3$): 169.92 (C=O), 169.86 (C=O), 160.0 (C=O), 94.7 (C1), 70.3 (C4), 69.3 (C5), 68.8 (C3), 68.1 (C2), 20.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 17.5 (C6).

ν_{max} (thin film): 3308, 2979, 1741, 1673, 1451, 1367, 1220, 1036, 929 cm^{-1} .

The data are consistent with those in the current literature.¹⁸⁴

2,3,4-Tri-*O*-acetyl-1-(4-formylphenoxy)- α -L-rhamnopyranoside (158)



- 1) 2,3,4-Tri-*O*-acetyl-1-trichloroacetimidate- α -L-rhamnopyranoside **156** (200 mg; 0.46 mmol) was dissolved in anhydrous DCE under N₂ with 4-hydroxybenzaldehyde (67 mg; 0.55 mmol) and 10 μ L of TMS.OTf. After 2 hours at room temperature the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (25 mL), washed with sat. aq. Na₂S₂O₃ solution (25 mL), dried with MgSO₄, filtered and concentrated. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a white solid in 93% yield (168 mg).
- 2) Ethy-2,3,4-tri-*O*-acetyl-1-thio- β -L-rhamnopyranoside **78** (251 mg, 0.75 mmol) was dissolved in anhydrous DCM (10 mL) at 0 °C under N₂. 4-Hydroxybenzaldehyde (137 mg, 1.13 mmol) and 1.5 eq. of the activating Me₂S₂-Tf₂O solution were added. After 1 h at 0 °C the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (20 mL), washed with 1M HCl solution (20 mL), sat. aq. NaHCO₃ solution (20 mL), H₂O (20 mL), dried with MgSO₄, filtered and concentrated. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a white solid in 83% yield (245mg). R_f = 0.5 (hexane/EtOAc 60:40). [α]_D²⁰ -62.7° (c 0.145, CHCl₃).

δ_H (400 MHz, CDCl₃): 9.92 (1H, s, COH), 7.86 (2H, d, *J* = 8.8 Hz, Ar), 7.20 (2H, d, *J* = 8.7 Hz, Ar), 5.57 (1H, d, *J*_{1,2} = 1.8 Hz, H1), 5.49 (1H, dd, *J*_{3,2} = 3.5 Hz, *J*_{3,4} = 10.1 Hz, H3), 5.44 (1H, dd, *J*_{2,1} = 1.8 Hz, *J*_{2,3} = 3.5 Hz, H2), 5.17 (1H, app t, H4), 3.92, (1H, m H5), 2.21 (3H, s, CH₃), 2.06 (3H, s, CH₃), 2.04 (3H, s, CH₃), 1.20 (3H, d, *J*_{6,5} = 6.3 Hz, H6).

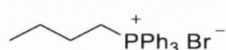
δ_C (100 MHz, CDCl₃): 190.8 (C=O), 170.1 (C=O), 170.0 (C=O), 160.5 (C=O), 131.9 (Ar), 131.4 (Cq), 116.5 (Ar), 95.3 (C1), 79.6 (C4), 69.4 (C2), 68.

7 (C3), 67.6 (C5), 20.9 (CH₃), 20.79 (CH₃), 20.76 (CH₃), 17.4 (C6).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₁₉H₂₂O₉K 433.0901; found 433.0894.

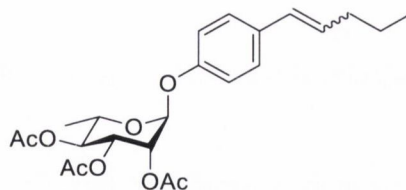
The data are consistent with those in the current literature.¹⁹⁶

(*n*-butyl)triphenylphosphonium bromide (**160**)



160 was prepared using a modified literature procedure.¹⁹⁷ Triphenyl phosphine (1 g, 3.7 mmol) and 1-bromo butane (0.45 mL, 4.2 mmol) dissolved in acetonitrile and refluxed at 85 °C. After 48 hours the mixture was concentrated *in vacuo*, and the resulting solid was washed with diethyether (5 x 20 mL) to give the product in 84% yield (1.243 g). The product was used without further purification or characterisation.

2,3,4-Tri-*O*-acetyl-1-*O*-(4-pent-1-en-1-ylbenzene)- α -L-rhamnopyranoside (161)



2,3,4-Tri-*O*-acetyl-1-(4-formylphenoxy)- α -L-rhamnopyranoside **158** (50.0 mg, 0.127 mmol) was dissolved in dioxane (5 mL). (n-butyl) triphenylphosphonium bromide (63 mg, 0.16 mmol) and K_2CO_3 (26 mg, 0.19 mmol) were added and the mixture was refluxed at 70 °C. After 3 hours the mixture was concentrated *in vacuo*, re-suspended in diethyl ether, stored at -18 °C. After 18 hours, the mixture was filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 75:25) gave the product as a pale yellow oil in 36% yield (20 mg) and the starting material in 58% yield (29 mg). $[\alpha]_D^{20}$ -1.6 ° (c 0.12, $CHCl_3$).

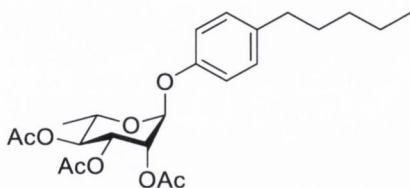
δ_H (400 MHz, $CDCl_3$): 7.24, 7.05 (2H, d, J = 8.5 Hz, Ar), 6.37 (1H, d, J = 11.5 Hz, PhCH=CHCH₂CH₂CH₃), 5.63 (1H, m, PhCH=CHCH₂CH₂CH₃), 5.54 (1H, dd, $J_{3,2}$ = 3.4 Hz, $J_{3,4}$ = 10.1 Hz, H3), 5.47 (1H, d, $J_{1,2}$ = 1.7 Hz, H1), 5.45 (1H, dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 3.4 Hz, H2), 5.18 (1H, app t, H4), 4.03 (1H, m, H5), 2.31 (2H, dd, J = 1.7 Hz, J = 7.2 Hz, PhCH=CHCH₂CH₂CH₃), 2.22 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.05 (3H, s, CH₃), 1.50 (2H, m, PhCH=CHCH₂CH₂CH₃), 1.23 (3H, d, $J_{6,5}$ = 6.3 Hz, H6), 0.96 (3H, t, J = 7.4 Hz, PhCH=CHCH₂CH₂CH₃).

δ_C (100 MHz, $CDCl_3$): 170.1 (C=O), 170.03 (C=O), 170.01 (C=O), 132.2 (PhCH=CHCH₂CH₂CH₃), 130.0 (Ar), 128.0 (PhCH=CHCH₂CH₂CH₃), 116.0 (Ar), 95.7 (C1), 71.0 (C4), 69.7 (C2), 68.9 (C3), 67.1 (C5), 30.7 (PhCH=CHCH₂CH₂CH₃), 22.8 (PhCH=CHCH₂CH₂CH₃), 20.9 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 17.4 (C6), 13.8 (PhCH=CHCH₂CH₂CH₃).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₃H₃₀O₈Na 457.1838; found 457.1845.

ν_{max} (thin film): 2927, 1747, 1605, 1508, 1213, 1032 cm⁻¹.

2,3,4-Tri-*O*-acetyl-1-*O*-(4-pentanylbenzene)- α -L-rhamnopyranoside (162)



2,3,4-Tri-*O*-acetyl-1-*O*-(4-pent-1-en-1-ylbenzene)- α -L-rhamnopyranoside **161** (15 mg, 0.037 mmol) was dissolved in THF and a catalytic amount of Pd/C added. The flask evacuated using a water pump, H₂ was introduced and the stirred reaction allowed to proceed under H₂ for 24 h; the H₂ was pumped out and air reintroduced. The Pd/C was filtered off and the mixture concentrated *in vacuo* to give the product as a colourless oil in 98% yield (15 mg). [α]_D²⁰ -148.6 ° (c 0.152, CHCl₃).

δ_{H} (400 MHz, CDCl₃): 7.12 (2H, m, Ar), 7.00 (2H, m, Ar), 5.54 (1H, dd, $J_{3,2} = 3.0$ Hz, $J_{3,4} = 10.1$ Hz, H3), 5.44 (1H, m H2), 5.43 (1H, app s, H1), 5.17 (1H, app t, H4), 4.03 (1H, m, H5), 2.56 (2H, t, $J = 7.7$ Hz), 2.25 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.00 (3H, s, CH₃), PhCH₂CH₂CH₂CH₂CH₃), 1.60 (2H, m, PhCH₂CH₂CH₂CH₂CH₃), 1.35 (2H, m, PhCH₂CH₂CH₂CH₂CH₃), 1.32 (2H, m, PhCH₂CH₂CH₂CH₂CH₃), 1.23 (3H, d, $J_{6,5} = 6.3$ Hz, H6), 0.90 (3H, t, $J = 7.0$ Hz, PhCH₂CH₂CH₂CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 170.13 (C=O), 170.10 (C=O), 170.09 (C=O), 153.9 (Cq), 137.3 (Cq), 129.4 (Ar), 116.2 (Ar), 95.9 (H1), 71.1 (H4), 69.8 (H2), 69.0 (H3), 67.0 (H5), 35.1 (PhCH₂CH₂CH₂CH₂CH₃), 31.5 (PhCH₂CH₂CH₂CH₂CH₃), 31.3 (PhCH₂CH₂CH₂CH₂CH₃), 22.5 (PhCH₂CH₂CH₂CH₂CH₃), 20.9 (CH₃), 20.8 (CH₃), 20.8 (CH₃), 17.5 (C6), 14.0 (PhCH₂CH₂CH₂CH₂CH₃).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₂₃H₃₂O₈Na 459.1995; found 459.2005.

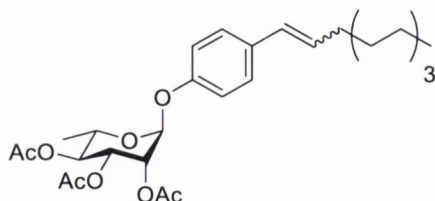
ν_{max} (thin film): 2928, 1745, 1511, 1377, 1209, 1030 cm⁻¹.

(n-Nonyl)Triphenyl phosphonium bromide (164)



164 was prepared using a modified literature procedure.¹⁹⁷ 1-bromononane (500 mg, 1.86 mmol) and triphenylphosphine (435 mg, 2.10 mmol) were dissolved in acetonitrile (20 mL) and refluxed at 85 °C for 72 hours. The mixture concentrated *in vacuo* and the residue was washed with ether to give the product as a white solid in 84% yield (700 mg). The product was used without further purification or characterisation.

2,3,4-Tri-*O*-acetyl-1-*O*-(4-dec-1-en-1-ylbenzene)- α -L-rhamnopyranoside (**165**)



2,3,4-Tri-*O*-acetyl-1-(4-formylphenoxy)- α -L-rhanopyranoside **158** (100 mg, 0.254 mmol) was dissolved in a dioxane (15 mL) and isopropanol (2 mL). Nonyltriphenyl phosphonium bromide (151 mg, 0.305 mmol) and potassium carbonate (53 mg, 0.381 mmol) were added and the mixture was refluxed at 80 °C. After 72 hours the mixture was concentrated *in vacuo*, re-suspended in diethyl ether and stored at -18 °C. After 18 hours the mixture was filtered then concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 90:10) gave the product as a pale yellow oil in 36% yield (45 mg).

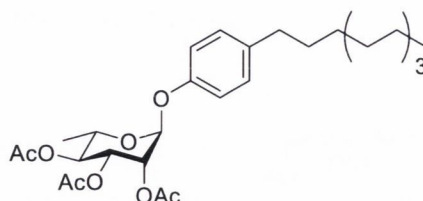
δ_{H} (400 MHz, CDCl_3): 7.30 (2H, m, Ar), 7.02 (2H, m, Ar), 6.35 (1H, d, $J = 15.7$ Hz, C=CH), 6.15 (1H, t, $J = 6.9$ Hz, C=CH), 5.55 (1H, dd, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.9$ Hz, H3), 5.47 (1H, d, $J_{1,2} = 1.7$ Hz, H1), 5.46 (1H, m, H2), 5.18 (1H, app t, H4), 4.04 (1H, m, H5), 2.22 (3H, s, CH_3), 2.20 (2H, m, CH_2), 2.09 (3H, s, CH_3), 2.06 (3H, s, CH_3), 1.48 (2H, m, CH_2), 1.32 (12H, m, CH_2), 1.23 (3H, d, $J_{6,5} = 6.0$ Hz, H6), 0.92 (3H, m, CH_3).

δ_{C} (100 MHz, CDCl_3): 170.2 (C=O), 154.7 (Cq), 132.8 (Cq), 130.0 (C=CH), 128.7 (C=CH), 127.0 (Ar), 116.4 (Ar), 95.7 (C1), 71.1 (C4), 69.7 (C2), 69.0 (C3), 67.1 (C5), 33.1 (CH_2), 31.9 (CH_2), 30.0 (CH_2), 29.5, 29.3 (CH_2), 28.6 (CH_2), 22.7 (CH_2), 20.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 17.4 (C6), 14.1 (CH_3).

HRMS (ESI) calculated for $\text{C}_{28}\text{H}_{40}\text{O}_8\text{Na}$ 527.2621 found 527.2636.

ν_{max} (thin film): 2924, 1749, 1508, 1215 cm^{-1} .

2,3,4-Tri-*O*-acetyl-1-*O*-(4-decylbenzene)- α -L-rhamnopyranoside (166)



2,3,4-Tri-*O*-acetyl-1-*O*-(4-dec-1-en-1-ylbenzene)- α -L-rhamnopyranoside **165** (45 mg, 0.089 mmol) was dissolved in a 1:1 mixture of MeOH and THF, and a catalytic amount of Pd/C added. The flask was evacuated using a water pump, H₂ was introduced and the stirred reaction was allowed to proceed under H₂ for 24 h then the H₂ was pumped out and air reintroduced. The Pd/C was filtered off and the mixture concentrated *in vacuo*. Purification by column chromatography (75:25 hexane:EtOAc) gave the product as a colourless oil in 66% yield (30 mg, 0.059 mmol). $[\alpha]_D^{20}$ -21.7° (c 0.023, CHCl₃).

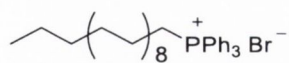
δ_H (400 MHz, CDCl₃): 7.12 (2H, d, J = 7.9 Hz, Ar), 7.00 (2H, d, J = 7.9 Hz, Ar), 5.54 (1H, dd, $J_{3,2}$ = 2.4 Hz, $J_{3,4}$ = 10.1 Hz, H3), 5.44 (1H, m, H2), 5.43 (1H, m, H1), 5.17 (1H, app t, H4), 4.03 (1H, m H5), 2.56 (2H, m, CH₂), 2.21 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.05 (3H, s, CH₃), 1.60 (2H, m, CH₂), 1.30 (14H, m, CH₂), 1.23 (3H, d, $J_{6,5}$ = 6.1 Hz, H6), 0.90 (3H, m CH₃).

δ_C (100 MHz, CDCl₃): 170.11 (C=O), 170.08 (C=O), 170.07 (C=O), 154.0 (Cq), 137.3 (Cq), 129.5 (Ar), 116.2 (Ar), 95.9 (C1), 71.1 (C4), 69.8 (C2), 68.98 (C3), 67.0 (C5), 35.1 (CH₂), 31.9 (CH₂), 31.6 (CH₂), 29.64 (CH₂), 29.61 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 22.7 (CH₂), 20.9 (CH₃), 28.8 (CH₃), 20.8 (CH₃), 17.5 (C6), 14.1 (CH₃).

HRMS (EI) calculated for C₂₈H₄₂O₈Na 529.2777 found 529.2779.

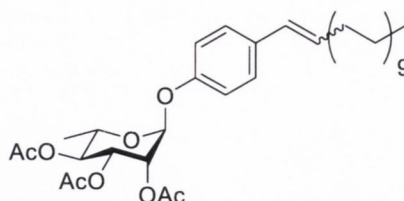
$\nu_{\max}$ (thin film): 2936, 1718, 1606, 1279, 1233 cm⁻¹.

Icosyltriphenyl phosphonium bromide (168)



168 was prepared using a modified literature procedure.¹⁹⁷ 1-bromoicosane (410 mg, 1.38 mmol) and triphenylphosphine (500 mg, 1.38 mmol) were dissolved in acetonitrile (20 mL) and refluxed at 85 °C. After 72 hours the mixture was concentrated *in vacuo* into a yellow oil. Diethyl ether (25 mL) was added and the product was isolated by filtration to give the a white powder in 99% yield (1.0 g). The product was use without further purification or characterisation.

2,3,4-Tri-*O*-acetyl-1-*O*-(4-henicos-1-en-1-ylbenzene)- α -L-rhamnopyranoside (**169**)



2,3,4-Tri-*O*-acetyl-1-(4-formylphenoxy)- α -L-rhanopyranoside **158** (50 mg, 0.127 mmol) was dissolved in dioxane (15 mL) along with icosyltriphenyl phosphonium bromide (112 mg, 0.152 mmol) and potassium carbonate (26 mg, 0.190 mmol) and refluxed at 80 °C. After 72 hours the mixture was concentrate *in vacuo* and the residue resuspended in diethyl ether (20 mL) and stored -18 °C. After 18 hours the mixture was filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 90:10) gave the product as a pale yellow oil in 72% yield (60 mg). $[\alpha]_D^{20}$ -19.0 ° (c 0.10, CHCl₃).

δ_H (400 MHz, CDCl₃): 7.24 (2H, m, Ar), 7.04 (2H, m, Ar), 6.35 (1H, m, C=CH), 5.55 (1H, m, C=CH), 5.55 (1H, dd, $J_{3,2} = 3.4$ Hz, $J_{3,4} = 9.9$ Hz, H3), 5.48 (1H, d, $J_{1,2} = 1.7$ Hz, H1), 5.46 (1H, dd, $J_{2,1} = 1.7$ Hz, $J_{2,3} = 3.4$ Hz, H3), 5.18 (1H, app t, H4), 4.03 (1H, m, H5), 2.32 (2H, m, CH₂), 2.22 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.06 (3H, s, CH₃), 1.46 (2H, m, CH₂), 1.29 (34H, m, CH₂), 1.23 (3H, d, $J_{6,5} = 6.2$ Hz, H6), 0.90 (3H, t, $J = 6.9$ Hz, CH₃).

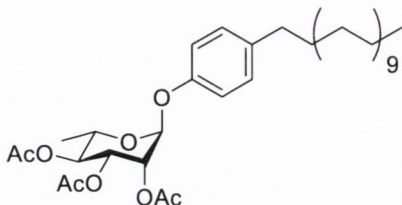
δ_C (100 MHz, CDCl₃): 170.12 (C=O), 170.09 (C=O), 170.06 (C=O), 154.4 (Cq), 132.6 (C=CH), 130.0 (Ar), 127.8 (C=CH), 116.4 (Ar), 95.9 (C1), 71.0 (C4), 69.8 (C2), 68.9 (C3), 67.1 (C5), 32.0 (CH₂), 29.7 (CH₂), 28.7 (CH₂), 22.7 (CH₂), 20.9 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 17.5 (C6), 14.2 (CH₃).

The carbon resonance at 29.7 ppm represents 14 carbon atoms as determined by HSQC and HMBC.

HRMS (ESI) calculated for C₃₉H₆₂O₈Na 681.4342 found 681.4342.

$\nu_{\max}$ (thin film): 2917, 1750, 1509, 1220 cm⁻¹.

2,3,4-Tri-*O*-acetyl-1-*O*-(4-henicosylbenzene)- α -L-rhamnopyranoside (170)



2,3,4-Tri-*O*-acetyl-1-*O*-(4-eicosane-1-en-1-ylbenzene)- α -L-rhamnopyranoside **169** (49 mg, 0.074 mmol) was dissolved in a 1:1 mixture of MeOH and THF, and a catalytic amount of Pd/C added. The flask was evacuated using a water pump, H₂ was introduced and the stirred reaction was allowed to proceed under H₂ for 24 hours then the H₂ was pumped out and air reintroduced. The Pd/C was filtered off and the mixture concentrated *in vacuo*. Purification by column chromatography (83:17 hexane:EtOAc) to give the product in 59% yield (29.2 mg, 0.044 mmol). $[\alpha]_D^{20}$ -146.6° (c 0.015, CHCl₃).

δ_H (400 MHz, CDCl₃): 7.12 (2H, d, J = 8.0 Hz, Ar), 7.00 (2H, d, J = 8.0 Hz, Ar), 5.54 (1H, dd, $J_{3,2}$ = 2.5 Hz, $J_{3,4}$ = 10.0 Hz, H3), 5.44 (1H, m, H2), 5.43 (1H, m, H1), 5.17 (1H, app t, H4), 4.03 (1H, m, H5), 2.56 (2H, m, CH₂), 2.21 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.05 (3H, s, CH₃), 1.59 (2H, m, CH₂), 1.29 (3H, m, CH₂), 1.22 (3H, d, $J_{6,5}$ = 6.3 Hz, H6), 0.90 (3H, m, CH₃).

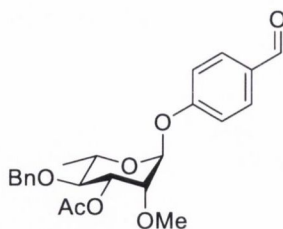
δ_C (100 MHz, CDCl₃): 170.1 (C=O), 170.07 (C=O), 170.06 (C=O), 153.9 (Cq), 137.3 (Cq), 129.4 (Ar), 116.2 (Ar), 95.9 (C1), 71.7 (C4), 69.8 (C2), 69.0 (C3), 67.0 (C5), 35.1 (CH₂), 31.9 (CH₂), 31.7 (CH₂), 29.7 (CH₂), 29.67 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 22.7 (CH₂), 20.9 (CH₃), 20.83 (CH₃), 20.78 (CH₃), 17.5 (C6), 14.1 (CH₃).

The carbon resonance at 29.7 ppm represents 11 carbon atoms as determined by HSQC and HMBC.

HRMS (EI) calculated for C₄₁H₄₆O₁₀Na 721.2989 found 721.2878.

ν_{max} (thin film): 2918, 2851, 1712, 1394, 1123 cm⁻¹.

3-*O*-Acetyl-4-benzyloxy-2-methyloxy-1-(4-formylphenoxy)- α -L-rhamnopyranoside (**171**)



Ethyl-3-*O*-acetyl-4-benzyloxy-2-methyloxy-1-thio- α -L-rhanopyranose **110** (307 mg, 0.866 mmol) was dissolved in anhydrous DCM (7 mL) at 0 °C under N₂. 4-hydroxybenzoate (159 mg, 1.3 mmol) and 1.5 eq of the activating Me₂S₂-Tf₂O solution was added. After 1 hour at 0 °C the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (20 mL), washed with 1M HCl solution (25 mL), sat. aq. NaHCO₃ solution (25 mL) and H₂O (25 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a pale yellow oil in 95% yield (341 mg). R_f = 0.5 (hexane/EtOAc 60:40). [α]_D²⁰ -104.1 ° (c 0.12, CHCl₃).

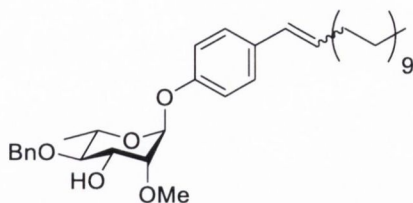
δ_H (400 MHz, CDCl₃): 9.92 (1H, s, COH), 7.86 (2H, m, Ar), 7.34 (5H, m, Bn), 7.23 (2H, m, Ar), 5.64 (1H, d, $J_{1,2}$ = 1.9 Hz, H1), 5.44 (1H, dd, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H3), 4.72 (2H, dd, J = 11.3 Hz, J = 34.3 Hz, CH₂Bn), 3.91 (1H, dd, $J_{2,1}$ = 1.9 Hz, $J_{2,3}$ = 3.3 Hz, H2), 3.82 (1H, m, H5), 3.66 (1H, app t, H4), 3.56 (3H, s, OCH₃), 2.13 (3H, s, CH₃), 1.31 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, CDCl₃): 190.9 (C=O), 170.4 (C=O), 161.0, (Cq), 138.0, (Cq), 131.9 (Ar), 131.1 (Cq), 128.5 (Bn), 127.9 (Bn), 127.7 (Bn), 116.5 (Ar), 95.3 (H1), 78.8 (H4), 78.2 (H2), 75.2 (CH₂Bn), 73.4 (H3), 69.0 (H5), 59.7 (OCH₃), 21.2 (CH₃), 18.0 (C6).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₂₃H₂₆O₇Na 437.1576 found 437.1579.

$\nu_{\max}$ (thin film): 2935, 1736, 1599, 1228 cm⁻¹.

4-Benzyloxy-2-methoxy-1-*O*-(4-henicos-1-en-1-ylbenzene)- α -L-rhamnopyranoside (172)



4-Benzyloxy-2-methoxy-1-(4-formylphenoxy)- α -L-rhanopyranoside **171** (96.7 mg, 0.234 mmol) was dissolved in a 1:1 methanol/THF (15 mL). Icosyltriphenyl phosphonium bromide (206 mg, 0.281 mmol) and potassium carbonate (46 mg, 0.351 mmol) were added and the mixture was refluxed at 80 °C. After 72 hours the mixture was concentrated and the residue was resuspended in diethyl ether and stored at -18 °C. After 18 hours the mixture was filtered the concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 75:25) gave the product as a colourless oil in 39% yield (58 mg). $[\alpha]_D^{20}$ -62.8 ° (*c* 0.199, CHCl₃).

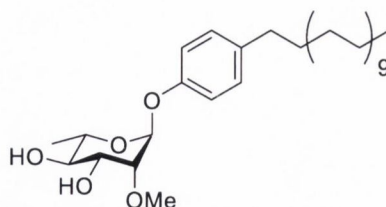
δ_H (400 MHz, CDCl₃): 7.39 (3H, m, Bn), 7.30 (3H, m, Bn, Ar), 7.00 (2H, m, Ar), 6.35 (1H, m, PhCH=CH), 6.14 (1H, m, PhCH=CH), 5.57 (1H, d, $J_{1,2}$ = 1.6 Hz, H1), 4.95 (1H, d, J = 11.2 Hz, CH₂Bn), 4.72 (1H, d, J = 11.2 Hz, CH₂Bn), 4.17 (1H, m, H3), 3.80 (1H, m, H5), 3.71 (1H, dd, $J_{2,1}$ = 1.6 Hz, $J_{2,3}$ = 3.6 Hz, H2), 3.58 (3H, s, OCH₃), 3.37 (1H, app t, J = 9.3 Hz, H4), 2.21 (2H, m, CH₂), 1.47 (2H, m, CH₂), 1.30 (3H, m, H6), 1.28 (34H, m, CH₂), 0.91 (3H, t, $J_{6,5}$ = 7.2 Hz, CH₃).

δ_C (100 MHz, CDCl₃): 155.2 (Cq), 138.4 (Cq), 132.3 (PhCH=CH), 132.0 (Cq), 129.9 (Ar), 128.8 (PhCH=CH), 128.4 (Bn), 127.9 (Bn), 126.9 (Bn), 116.3 (Ar), 94.4 (C1), 82.0 (C4), 80.5 (C2), 75.1 (CH₂Bn), 71.4 (C3), 67.9 (C5), 59.1 (OCH₃), 32.9 (CH₂), 32.0 (CH₂), 29.5 (CH₂), 28.7 (CH₂), 22.6 (CH₂), 18.0 (C6), 14.1 (CH₃). The carbon resonance at 29.5 ppm represents 14 carbon atoms as determined by HSQC and HMBC.

HRMS-ESI (*m/z*): [M - H]⁻ calculated for C₄₁H₆₃O₅ 635.4676; found 635.4666.

ν_{max} (thin film): 2918, 1470, 1228, 1101, 1016 cm⁻¹.

4-Benzyloxy-2-methoxy-1-*O*-(4-henicosylbenzene)- α -L-rhamnopyranoside (173)



4-Benzyloxy-2-methoxy-1-*O*-(4-henicos-1-en-1-ylbenzene)- α -L-rhamnopyranoside **191** (58 mg; 0.091 mmol) was dissolved in a 1:1 mixture of MeOH and THF, and a catalytic amount of Pd/C added. The flask was evacuated using a water pump, H₂ was introduced and the stirred reaction was allowed to proceed under H₂ for 24 h then the H₂ was pumped out and air reintroduced. The Pd/C was filtered off and the mixture concentrated *in vacuo*. Purification by column chromatography (95-85% toluene in acetone) gave the product in 60% yield (30 mg, 0.055 mmol). $[\alpha]_D^{20}$ -40.4° (*c* 0.042, CHCl₃).

δ_H (400 MHz, CDCl₃): 7.13 (2H, m, Ar), 7.01 (2H, m, Ar), 5.57 (1H, app s, H1), 3.95 (1H, m, H3), 3.78 (1H, m H5), 3.68 (1H, dd, $J_{2,1} = 1.4$ Hz, $J_{2,3} = 3.8$ Hz, H2), 3.56 (3H, s, OCH₃), 3.47 (1H, m, H4), 2.58 (2H, m, CH₂) 1.61 (4H, m, CH₂), 1.30 (3H, d, $J_{6,5} = 6.4$ Hz, H6), 1.28 (34H, m, CH₂), 0.91 (3H, t, $J = 7.1$ Hz, CH₃).

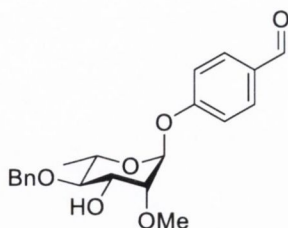
δ_C (100 MHz, CDCl₃): 154.3 (Cq), 136.8 (Cq), 128.9 (Ar), 115.8 (Ar), 94.6 (C1), 80.2 (C2), 73.9 (C4), 71.3 (C3), 68.3 (C5), 58.9 (CH₃), 35.0 (CH₂), 31.8 (CH₂), 31.5 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 17.4 (C6), 14.0 (CH₃).

The carbon resonance at 29.6 ppm represents 14 carbon atoms as determined by HSQC and HMBC.

HRMS-ESI (*m/z*): [M - H]⁻ calculated for C₃₄H₅₉O₅ 547.4363; found 547.4375.

$\nu_{\max}$ (thin film): 3405, 2922, 1727, 1460, 1018, 754 cm⁻¹.

4-Benzyloxy-2-methoxy-1-(4-formylphenoxy)- α -L-rhanopyranoside (**174**)



4-Benzyloxy-3-*O*-acetyl-2-methoxy-1-(4-formylphenoxy)- α -L-rhanopyranoside **171** (191 mg, 0.461 mmol) was dissolved in 15 mL MeOH along with a catalytic amount of NaOMe. After 18 hours the reaction was quenched with a catalytic amount of DOWEX, filtered and concentrated *in vacuo* to give the product as a pale yellow oil in 91% yield (156 mg). $[\alpha]_D^{20}$ -148.6° (c 0.115, CHCl₃).

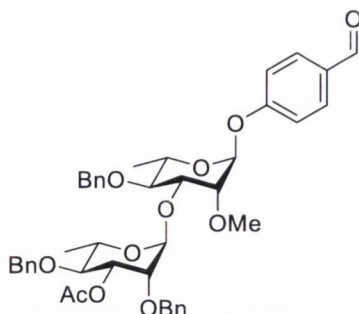
δ_H (400 MHz, CDCl₃): 9.93 (1H, s, COH), 7.87 (2H, d, J = 8.6 Hz, Ar), 7.35 (5H, m, Bn), 7.20 (2H, d, J = 8.6 Hz, Ar), 5.70 (1H, d, $J_{1,2}$ = 1.1 Hz, H1), 4.95 (1H, d, J = 11.1 Hz, CH₂Bn), 4.72 (1H, d, J = 11.1 Hz, CH₂Bn), 4.18 (1H, dd, $J_{3,2}$ = 3.7 Hz, $J_{3,4}$ = 9.3 Hz, H3), 3.74 (1H, m, H2), 3.73 (1H, m, H5), 3.61 (3H, s, OCH₃), 3.40 (1H, app t, H4), 1.31 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 190.9 (C=O), 161.1 (Cq), 138.2 (Cq), 131.9 (Ar), 131.0 (Cq), 128.5 (Bn), 128.0 (Bn), 127.9 (Bn), 116.4 (Ar), 94.3 (C1), 81.7 (C4), 80.2 (C2), 75.3 (CH₂Bn), 71.4 (C3), 68.5 (C5), 59.3 (OCH₃), 18.0 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₁H₂₄O₆Na 395.1465; found 395.1476.

$\nu_{\max}$ (thin film) 3454, 2936, 1600, 1239 cm⁻¹.

3-*O*-Acetyl-2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-4-benzyloxy-2-methoxy-1-(4-formylphenoxy)- α -L-rhamnopyranosyl (175)



4-Benzyloxy-2-methoxy-1-(4-formylphenoxy)- α -L-rhamnopyranoside **174** (109 mg, 0.254 mmol) was dissolved in anhydrous DCM (5 mL) under N₂ at 0 °C. Ethyl-2,3,4-trimethoxy-1-thio- α -L-fucopyranoside (100 mg, 0.264 mmol), NIS (76 mg, 0.339 mmol) and catalytic TMS.OTf (ca. 10 μ L) were added. After 18 hours at room temperature the reaction was quenched with TEA (ca. 2 mL), washed with sat. aq. NaHCO₃ solution (15 mL) and H₂O (10 mL). The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 75:25) gave the product in 75% yield (133 mg). R_f = 0.7 (hexane/EtOAc 60:40). $[\alpha]_D^{20}$ -62.3° (*c* 0.117, CHCl₃).

δ_H (400 MHz, CDCl₃): 9.94 (1H, s, COH), 7.88 (2H, m, Ar), 7.28 (17H, m, Bn, Ar), 5.66 (1H, d, $J_{1,2}$ = 1.7 Hz, H1b), 5.33 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, H3a), 5.19 (1H, d, $J_{1,2}$ = 1.6 Hz, H1a), 4.88 (1H, d, J = 11.7 Hz, CH₂Bn), 4.78 (1H, d, J = 11.2 Hz, CH₂Bn), 4.70 (2H, m, CH₂Bn), 4.48 (1H, d, J = 12.1 Hz, CH₂Bn), 4.32 (1H, d, J = 12.1 Hz, CH₂Bn), 4.26 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, H3b), 4.06 (1H, m, H5a), 3.95 (1H, dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.2 Hz, H2a), 3.82 (1H, dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, H2b), 3.76 (1H, m, H5b), 3.72 (1H, app t, H4a), 3.63 (1H, app t, H4b), 3.61 (3H, s, OCH₃), 1.99 (3H, s, CH₃), 1.43 (3H, d, $J_{6,5}$ = 6.3 Hz, H6a), 1.29 (3H, d, $J_{6,5}$ = 6.3 Hz, H6b).

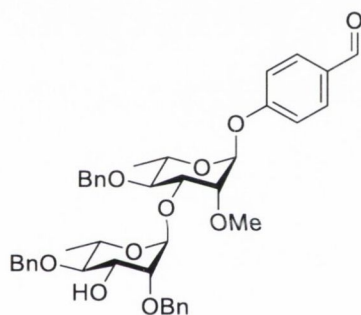
δ_C (100 MHz, CDCl₃): 190.9 (C=O), 170.2 (C=O), 161.2 (Cq), 138.4 (Cq), 138.3 (Cq), 137.9 (Cq), 131.9 (Ar), 131.1 (Cq), 128.45 (Bn), 128.44 (Bn),

128.4 (Bn), 127.82 (Bn), 127.79 (Bn), 127.76 (Bn), 127.66 (Bn), 127.65 (Bn), 127.33 (Bn), 116.5 (Ar), 100.1 (C1a), 94.9 (C1b), 79.9 (C2b), 79.8 (C4b), 79.1 (C3b), 79.0 (C4a), 76.8 (C2a), 75.1, 75.0 (CH₂), 73.6 (C3a), 72.9 (CH₂), 69.4 (C5b), 68.6 (C5a), 59.5 (OCH₃), 21.1 (CH₃), 18.3 (C6a), 18.0 (C6b).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₄₃H₄₈O₁₁Na 763.3094 found 763.3098.

ν_{max} (thin film): 2934, 1736, 1600, 1236, 1097 cm⁻¹.

2,4-Dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-4-benzyloxy-2-methoxy-1-(4-formylphenoxy)- α -L-rhamnopyranosyl (176)



3-*O*-Acetyl-2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-formylphenoxy)-4-benzyloxy-2-methoxy- α -L-rhamnopyranosyl **175** (156 mg, 0.216 mmol) was dissolved in MeOH (15 mL) and a catalytic amount of freshly prepared NaOMe added. After 18 hours the reaction was quenched with a catalytic amount of DOWEX, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 72:25) gave the product in 91% yield (137 mg). $[\alpha]_{\text{D}}^{20}$ -107.3° (c 0.096, CHCl₃).

δ_{H} (400 MHz, CDCl₃): 9.94 (1H, s, COH), 7.88 (2H, m, Ar), 7.38 (13H, m, Bn), 7.22 (4H, m, Ar, Bn), 5.66 (1H, d, $J_{1,2}$ = 1.8 Hz, H1b), 5.25 (1H, app, H1a), 4.94 (1H, d, J = 11.2 Hz, CH₂Bn), 4.82, 4.73 (1H, d, J = 11.6 Hz, CH₂), 4.71 (1H, d, J = 11.2 Hz, CH₂), 4.45 (1H, d, J = 11.7 Hz, CH₂), 4.28 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.6 Hz, H3b), 4.24 (1H, d, J = 11.7 Hz, CH₂), 4.06 (1H, dd, $J_{3,2}$ = 3.7 Hz, $J_{3,4}$ =

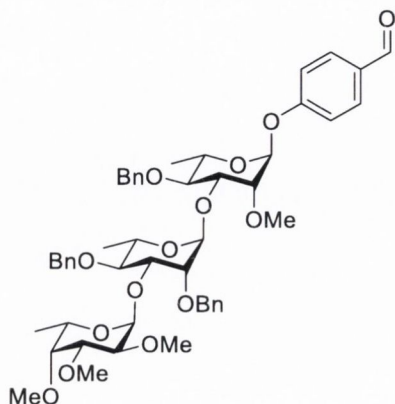
9.3 Hz, H3a), 3.94 (1H, m, H5b), 3.77 (3H, m, H2a, H2b, H5b), 3.62 (1H, app t, H4b), 3.59 (3H, s, OCH₃), 3.58 (3H, s, OCH₃), 3.40 (1H, app t, H4a), 1.42 (3H, d, $J_{6,5} = 6.2$ Hz, H6a), 1.29 (3H, d, $J_{6,5} = 6.1$ Hz, H6b).

δ_C (100 MHz, CDCl₃): 190.9 (C=O), 161.2 (Cq), 138.5 (Cq), 138.2 (Cq), 137.7 (Cq), 131.9 (Ar), 131.1 (Cq), 128.52 (Bn), 128.51 (Bn), 128.45 (Bn), 128.1 (Bn), 127.9 (Bn), 127.81 (Bn), 127.79 (Bn), 127.70 (Bn), 127.1 (Bn), 116.5 (Ar), 99.3 (C1b), 95.0 (C1a), 82.1 (C4a), 80.0 (C2b), 79.2 (C2a), 78.3 (C3b), 75.2, 75.0, 72.7 (CH₂), 71.6 (C3a), 69.5 (C5b), 68.0 (C5a), 59.3 (OCH₃), 18.2 (C6a), 18.0 (C6b).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₄₁H₄₆O₁₀Na 721.2989 found 721.2878.

$\nu_{\max}$ (thin film): 2930, 1695, 1239, 1136 cm⁻¹.

2,3,4-Trimethoxy-L-rhamnopyranosyl-(α 1-3)2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-4-benzyloxy-2-methoxy-1-(4-formylphenoxy)- α -L-rhamnopyranosyl (177**)**



2,4-Dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-formylphenoxy)-4-benzyloxy-2-methoxy- α -L-rhamnopyranosyl **176** (84.8 mg; 0.121 mmol) and Ethyl-2,3,4-trimethoxy-1- α -L-fucosose thioglycoside (36.5 mg, 0.146 mmol) were dissolved in anhydrous DMC at 0 °C under N₂. NIS (43.7 mg; 0.194 mmol) and a catalytic amount of TMS.OTf (10 μ L) were added sequentially and the reaction allowed to come to room temperature overnight. The reaction was quenched with TEA (ca. 2 mL), diluted with DCM, washed with sat. aq. Na₂S₂O₃ solution (2 x 20 mL) and H₂O (2 x 20 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 60:40) gave the product in 52% yield (56 mg). R_f = 0.5 (hexane/EtOAc 60:40). $[\alpha]_D^{20}$ -111.9° (*c* 0.151 CHCl₃).

δ_H (400 MHz, CDCl₃): 9.91 (1H, s, COH), 7.84 (2H, m, Ar), 7.31 (15H, m, Bn), 7.19 (2H, m, Ar), 5.63 (1H, d, $J_{1,2}$ = 1.4 Hz, H1c), 5.24 (1H, d, $J_{1,2}$ = 1.2 Hz, H1b), 5.21 (1H, d, H1a), 5.18 (1H, d, J = 11.4 Hz, CH₂Bn), 4.86 (1H, d, J = 11.6 Hz, CH₂Bn), 4.67 (1H, d, J = 11.6 Hz, CH₂Bn), 4.61 (1H, d, J = 11.4 Hz, CH₂Bn), 4.56 (1H, d, J = 12.3 Hz, CH₂Bn), 4.26 (1H, d, J = 12.3 Hz, CH₂Bn), 4.24 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.2 Hz, H3c), 4.09 (1H, dd, $J_{3,2}$ = 3.0 Hz, $J_{3,4}$ = 9.4 Hz, H3b), 3.95 (1H, m, H5b), 3.82 (1H, dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 2.7 Hz, H2b), 3.79 (1H, dd, $J_{2,1}$ = 2.0 Hz, $J_{2,3}$ = 2.9 Hz, H2c), 3.72 (1H, m, H5c), 3.69 (1H, m, H5a), 3.64 (1H, app t, H4b), 3.58 (1H, app t, H4c), 3.57 (3H, s, OCH₃), 3.55 (2H, m, H2a, H3a), 3.53 (3H, s, OCH₃), 3.51 (3H, s, OCH₃), 3.33 (3H, s,

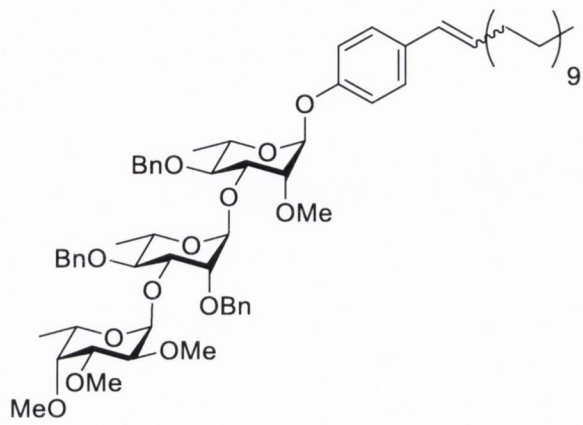
OCH₃), 3.21 (1H, app s, H4a), 1.34 (3H, d, $J_{6,5}$ = 6.3 Hz, H6b), 1.23(3H, d, $J_{6,5}$ = 6.1 Hz, H6c), 0.99 (3H, d, $J_{6,5}$ = 6.5 Hz, H6a).

δ_C (100 MHz, CDCl₃): 190.8 (C=O), 161.1 (Cq), 139.1 (Cq), 138.4 (Cq), 138.2 (Cq), 131.8 (Ar), 129.0 (Bn), 128.4 (Bn), 128.22 (Bn), 128.18 (Bn), 127.7 (Bn), 127.5 (Bn), 127.4 (Bn), 127.2 (Bn), 127.1 (Bn), 125.3 (Cq), 116.4 (Ar) 99.5 (C1a), 99.4 (C1b), 94.8 (C1c), 80.4 (C2a/C3a), 80.0 (C2c), 79.8 (C4b), 79.7 (C4c), 79.3 (C3b), 79.19 (C3c), 79.17 (C4a), 78.8 (C2b), 77.8 (C2a/C3a), 75.0 (CH₂Bn), 74.7 (CH₂Bn), 71.4 (CH₂Bn), 69.3 (C5c), 68.8 (C5b), 66.3 (C5a), 61.7 (OCH₃), 59.1 (OCH₃), 59.0 (OCH₃), 57.9 (OCH₃), 18.2 (C6b), 17.9 (C6c), 16.5 (C6a).

HRMS-ESI (m/z): [M + Na]⁺ calculated for C₅₀H₆₂O₁₄Na 909.3997 found 909.4037.

$\nu_{\max}$ (thin film): 2932, 1697, 1601, 1097 cm⁻¹.

2,3,4-Trimethoxy-L-fucopyranosyl-(α 1-3)2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-1-(4-henicos-1-en-1-ylbenzene)-4-benzyloxy-2-methoxy- α -L-rhamnopyranosyl (178)



2,3,4-Trimethoxy-L-fucopyranosyl-(α 1-3)2,4-dibenzyloxy-L-rhamnopyranosyl-(α 1-3)-4-benzyloxy-2-methoxy-1-(4-formylphenoxy)- α -L-rhamnopyranosyl **177** (35 mg; 0.039 mmol) was dissolved in anhydrous methanol (7 mL) under N₂. Potassium carbonate (8 mg; 0.059 mmol) and icosyltriphenyl phosphonium bromide (35 mg; 0.47 mmol) were added and the mixture refluxed at 70 °C. After 168 hours the mixture was cooled and concentrated *in vacuo*. The residue was resuspended in diethyl ether (25 mL) and stored at -18 °C for 18 to precipitate out the triphenyl phosphine oxide byproduct, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product as a colourless oil in 36% yield (16 mg). $[\alpha]_D^{20}$ -125.0° (*c* 0.048, CHCl₃).

δ_H (600 MHz, CDCl₃): 7.34 (15H, m, Bn), 7.24 (2H, m, Ar), 7.05 (2H, m, Ar), 6.35 (1H, m, CH=CH), 6.14 (1H, m, CH=CH), 5.55 (1H, d, $J_{1,2}$ = 1.7 Hz, H1c), 5.26 (1H, m, H1b), 5.24 (1H, m, H1a), 5.21 (1H, d, J = 11.4 Hz, CH₂), 4.88 (1H, d, J = 11.7 Hz, CH₂Bn), 4.70 (1H, d, J = 11.7 Hz, CH₂Bn), 4.64 (1H, d, J = 11.4 Hz, CH₂Bn), 4.59 (1H, d, J = 12.2 Hz, CH₂Bn), 4.27 (1H, m, H3c), 4.26 (1H, d, J = 12.2 Hz, CH₂Bn), 4.12 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.3 Hz, H3b), 3.98 (1H, m, H5b), 3.84 (1H, m, H2b), 3.82 (1H, m, H2c), 3.79 (2H, m, H2a, H5a), 3.71 (1H, m, H4b), 3.66 (1H, m, H4c), 3.58 (2H, m, H3a, H5c), 3.57 (3H, s, OCH₃), 3.56 (3H, s, OCH₃), 3.53 (3H, s, OCH₃), 3.35 (3H, s, OCH₃), 3.23 (1H, app s, H4a), 2.33

(2H, m, CH₂), 1.38 (34H, m, CH₂), 1.36 (3H, m, H6b), 1.25 (3H, m, H6c), 1.00 (3H, m, H6a), 0.91 (3H, m, CH₃).

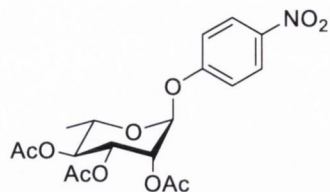
δ_C (150 MHz, CDCl₃): 155.4 (Cq), 138.9 (Cq), 138.2 (Cq), 132.2 (CH), 132.1 (Cq), 129.8 (Ar), 128.7 (Cq), 128.3 (Bn), 128.2 (CH), 128.07 (Bn), 128.04 (Bn), 128.00 (Bn), 127.98 (Bn), 127.86 (Bn), 127.73 (Bn), 127.44 (Bn), 127.33 (Bn), 127.24 (Bn), 127.15 (Bn), 127.03 (Bn), 127.00 (Bn), 126.97 (Bn), 126.80 (Bn), 115.81 (Ar), 99.4 (C1a), 99.2 (C1b), 94.8 (C1c), 80.5 (C2c), 80.3 (C5a), 80.2 (C5c), 80.1 (C3b), 79.9 (C4c), 79.7 (C5b), 79.4 (C3b), 79.1 (C4a), 78.8 (C2a), 77.7 (C3a), 74.8 (CH₂), 74.5 (CH₂), 71.3 (CH₂), 68.6 (C2b), 66.2 (C4b), 58.9 (OCH₃), 58.8 (OCH₃), 58.6 (OCH₃), 57.8 (OCH₃), 18.0 (C6b), 16.4 (C6c), 13.9 (C6a), 0.82 (CH₃).

The carbon resonance at 29.5 ppm represents 17 carbon atoms as determined by HSQC and HMBC.

HRMS (EI) calculated for C₇₀H₁₀₂O₁₃Na 1173.7218; found 1173.7246.

$\nu_{\max}$ (thin film): 2923, 1707, 1509, 1457, 1098 cm⁻¹.

2,3,4-Tri-*O*-acetyl-1-(4-nitrophenoxy)- α -L-rhamnopyranoside (181)



- 1) 1,2,3,4-Tetra-*O*-acetyl-L-rhamnopyranoside **68** (1.830 g, 5.3 mmol) was dissolved in anhydrous DCM at 0 °C under N₂. 4-nitrophenol (1.076 g; 7.74 mmol) was added followed by BF₃·OEt₂ (3.41 mL, 27.6 mmol) in increments. The stirred solution was allowed to come to room temperature. After 18 hours the reaction was quenched by treatment with saturated sodium bicarbonate solution (ca. 20 mL) and solid NaHCO₃. The organic layer was separated, dried with MgSO₄, filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 75:25) gave the product as a white solid in 83% yield (1.88 g).
- 2) 2,3,4-Tri-*O*-acetyl-1-trichloroacetimidate- α -L-rhamnose **156** (1.0 g, 2.30 mmol) was dissolved in anhydrous DCM (10 mL) at 0 °C under N₂. 4-nitrophenol (385 mg, 2.76 mmol) and 10 μ L of TMS.OTf were added. After 2 hours the reaction was quenched by the addition of TEA (ca. 2 mL), diluted with DCM, washed with sat. aq. Na₂S₂O₃ solution (2 x 20 mL), dried with MgSO₄, filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 75:25) gave the product as a white solid in 91% yield (863 mg). R_f = 0.4 (hexane/EtOAc 80:20). [α]_D²⁰ -108.2° (c 0.097, CHCl₃).

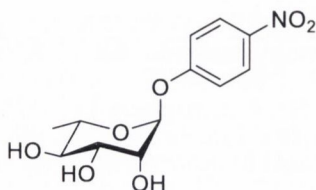
δ_H (400 MHz, CDCl₃): 8.25 (2H, d, J = 9.2 Hz, Ar), 7.21 (2H, d, J = 9.2 Hz, Ar), 5.59 (1H, d, $J_{1,2}$ = 1.7 Hz, H1), 5.51 (1H, dd, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 9.9 Hz, H3), 5.47 (1H, dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H2), 5.21 (1H, app t, H4), 3.93 (1H, m, H5), 2.24 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.07 (3H, s, CH₃), 1.24 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, CDCl₃): 170.1 (C=O), 160.4 (C=O), 142.9 (C=O), 125.9 (Ar), 116.3 (Ar), 115.6 (Cq), 95.6 (C1), 70.5 (C4), 69.2 (C2), 68.6 (C3), 67.8 (C5), 20.9 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 17.4 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{18}H_{21}NO_{10}Na$ 434.1063; found 434.1050.

$\nu_{\max}$ (thin film): 3087, 1750, 1514, 1425, 1220, 1043 cm^{-1} .

1-(4-Nitrophenoxy)- α -L-Rhamnopyranoside (**182**)



2,3,4-Tri-*O*-acetyl-1-(4-nitrophenoxy)- α -L-rhamnopyranoside **181** (500 mg, 1.22 mmol) was dissolved in methanol (20 mL) with catalytic sodium methoxide. After 18 hours the reaction was quenched with DOWEX, filtered and concentrated *in vacuo* to give the product as a white solid in 98% yield (332 mg). $[\alpha]_D^{20}$ -147.7° (c 0.153, MeOH).

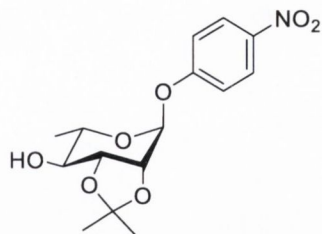
δ_H (400 MHz, $CDCl_3$): 8.25 (2H, m, Ar), 2.27 (2H, m, Ar), 5.62 (1H, d, $J_{1,2} = 1.7$ Hz, H1), 4.06 (1H, d, $J_{2,1} = 1.7$ Hz, $J_{2,3} = 3.4$ Hz, H2), 3.87 (1H, dd, $J_{3,2} = 3.4$ Hz, $J_{3,4} = 9.2$ Hz, H3), 3.57 (1H, m, H5), 3.51 (1H, app t, H4), 1.25 (3H, d, $J_{6,5} = 5.8$ Hz, H6).

δ_C (100 MHz, $CDCl_3$): 161.4 (Cq), 142.4 (Cq), 125.3 (Ar), 116.1 (Ar), 98.5 (C1), 72.1 (C4), 70.7 (C3), 70.3 (C2), 69.9 (C5), 16.6 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{12}H_{15}NO_7Na$ 308.0746; found 308.0738.

$\nu_{\max}$ (thin film): 3329, 2938, 2475, 1593, 1353, 1245, 1066 cm^{-1} .

2, 3-*O*-Isopropyl-1-(4-nitrophenoxy)- α -L-rhamnopyranoside (183)



1-(4-Nitrophenoxy)- α -L-Rhamnopyranoside **182** (330 mg, 1.16 mmol) was dissolved in anhydrous acetone (15 mL) under N₂. 2,2 dimethoxy propane (482 mg, 4.63 mmol) and catalytic para-toluene sulfonic acid were added. After 2 hours the reaction was quenched by the addition of sat. aq. NaHCO₃ (ca. 15 mL). The mixture was filtered and extracted with DCM. The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo* to give the product as a colourless oil in 99% yield (375 mg). $[\alpha]_D^{20}$ -100° (c 0.100, MeOH).

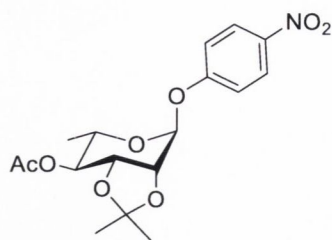
δ_H (400 MHz, CDCl₃): 8.24 (2H, m, Ar), 7.18 (2H, m, Ar), 5.85 (1H, app s, H1), 4.41 (1H, app d, H2), 4.27 (1H, dd, $J_{3,2} = 5.6$ Hz, $J_{3,4} = 7.4$ Hz, H3), 3.70 (1H, m, H5), 3.50 (1H, m, H4), 1.60 (3H, s, CH₃), 1.44 (3H, s, CH₃), 1.26 (3H, d, $J_{6,5} = 6.1$ Hz, H6).

δ_C (100 MHz, CDCl₃): 160.9 (Cq), 142.6 (Cq), 125.9 (Ar), 116.2 (Ar), 95.6 (C1), 78.3 (C3), 75.5 (C2), 74.4 (C4), 67.2 (C5), 28.1 (CH₃), 26.3 (CH₃), 17.2 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₁₅H₁₉NO₇Na 348.1059; found 348.1050.

ν_{max} (thin film): 3470, 2999, 1594, 1514, 1347, 1249 cm⁻¹.

4-*O*-Acetyl-2, 3-*O*-isopropyl-1-(4-nitrophenoxy)- α -L-rhamnopyranoside (184)



2,3-*O*-Isopropyl-1-(4-nitrophenoxy)- α -L-rhamnopyranoside **183** (330 mg, 1.02 mmol) was dissolved in pyridine (4 mL) under N₂ and acetic anhydride (4 mL) added. After 18 hours the mixture was concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a colourless oil in 86% yield (322 mg). $[\alpha]_D^{20}$ -101.6° (c 0.118, CHCl₃).

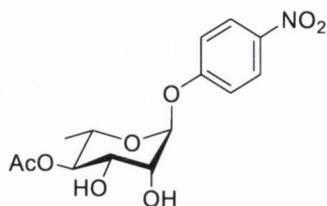
δ_H (400 MHz, CDCl₃): 8.25 (2H, m, Ar), 7.18 (2H, m, Ar), 5.87 (1H, s, H1), 4.97 (1H, dd, $J_{4,3}$ = 7.6 Hz, $J_{4,5}$ = 10.1 Hz, H4), 4.42 (1H, d, $J_{2,3}$ = 5.5 Hz, H2), 4.38 (1H, dd, $J_{3,2}$ = 5.5 Hz, $J_{3,4}$ = 7.6 Hz, H3), 3.79 (1H, m, H5), 2.14 (3H, s, CH₃), 1.64 (3H, s, CH₃), 1.42 (3H, s, CH₃), 1.14 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 170.0 (C=O), 160.8 (Cq), 142.6 (Cq), 125.9 (Ar), 116.2 (Ar), 110.4 (C=O), 95.5 (C1), 75.5 (C2), 75.5 (C3), 73.7 (C4), 65.8 (C5), 27.6 (CH₃), 26.5 (CH₃), 20.9 (CH₃), 17.0 (C6).

HRMS (EI) calculated for C₁₇H₂₁NO₈Na 390.1159; found 390.1154.

$\nu_{\max}$ (thin film): 2987, 1735 1593, 1512, 1358, 1233 cm⁻¹.

4-*O*-Acetyl-1-(4-nitrophenoxy)- α -L-rhamnopyranoside (**185**)



4-*O*-Acetyl-2,3-*O*-isopropyl-1-(4-nitrophenoxy)- α -L-rhamnopyranoside **184** (300 mg, 0.82 mmol) was dissolved in 9:1 acetic acid/H₂O (5 mL) and refluxed at 50 °C. After 18 hours the mixture was concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 20:80) gave the product as a colourless oil in 60% yield (160 mg). $[\alpha]_{\text{D}}^{20}$ -143.5° (c 0.104, CHCl₃).

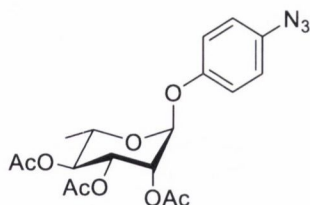
δ_{H} (400 MHz, CDCl₃): 8.26 (2H, m, Ar), 7.20 (2H, m, Ar), 5.70 (1H, d, $J_{1,2}$ = 1.6 Hz, H1), 4.91 (1H, app t, H4), 4.22 (1H, dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 7.1 Hz, H2), 4.13 (1H, m, H3), 3.84 (1H, m, H5), 3.18 (1H, d, J = 5.8 Hz, OH), 2.76 (1H, d, J = 3.5 Hz, OH), 2.18 (3H, s, CH₃), 1.24 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_{C} (100 MHz, CDCl₃): 172.4 (C=O), 160.8 (Cq), 142.7 (Cq), 125.9 (Ar), 116.2 (Ar), 97.2 (C1), 75.3 (C4), 70.3 (C2), 70.1 (C3), 66.9 (C5), 21.0 (CH₃), 17.5 (C6).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for C₁₄H₁₇NO₈Na 350.0855; found 350.0852.

ν_{max} (thin film): 3357, 2938, 1725, 1514, 1345, 1238, 1054 cm⁻¹.

2,3,4-Tri-*O*-acetyl-1-(4-azidophenoxy)- α -L-rhamnopyranoside (**187**)



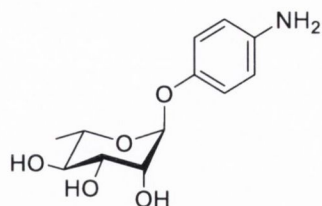
Crude 2,3,4-tri-*O*-acetyl-1-(4-aminophenoxy)- α -L-rhamnopyranoside **186** (200 mg 0.52 mmol) was dissolved in 3 mL TFA at 0 °C under N₂. NaNO₂ (192 mg, 2.8 mmol) dissolved in distilled water (5 mL) was added drop wise. After 10 minutes at 0 °C, NaN₃ dissolved in distilled water (5 mL) was added. After 45 minutes the mixture was diluted with DCM (20 mL), washed with sat. aq. NaHCO₃ solution (2 x 20 mL), dried with MgSO₄, filtered and concentrated *in vacuo* to give the impure product as an off-white oil.

δ_{H} (400 MHz, CDCl₃): 7.10 (2H, m, Ar), 6.98 (2H, m, Ar), 5.52 (1H, dd, $J_{3,2} = 3.4$ Hz, $J_{3,4} = 10.0$ Hz, H3), 5.44 (1H, overlapping dd, $J_{2,1} = 1.9$ Hz, $J_{2,3} = 3.3$ Hz, H2), 5.42 (1H, overlapping d, $J_{1,2} = 1.4$ Hz, H1), 5.18 (1H, app t, H4), 3.99 (1H, m, H5), 2.22 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.06 (3H, s, CH₃), 1.23 (3H, d, $J_{6,5} = 6.3$ Hz, H6).

HRMS-ESI (m/z): $[M + \text{Na}]^+$ calculated for C₁₈H₂₁N₃O₈Na 430.1226; found 430.1221.

ν_{max} (thin film): 3398, 2984, 2441, 2125, 1677, 1369, 1113, 1033 cm⁻¹.

1-(4-Nitrophenoxy)- α -L-Rhamnopyranoside (**188**)



- 1) 4-*O*-Acetyl-1-(4-aminophenoxy)- α -L-rhamnopyranoside **182** (80 mg; 0.280 mmol) was dissolved in methanol (10 mL) and a catalytic amount of Pd/C added. The flask was evacuated using a water pump, H₂ was introduced and the stirred reaction was allowed to proceed under H₂ for 4 hours then the H₂ was pumped out and air reintroduced. The Pd/C was filtered off and the mixture concentrated *in vacuo* to give the product as an off white solid in 92% yield (66 mg, 0.258 mmol).
- 2) 2,3,4-Tri-*O*-acetyl-1-(4-phthalimido phenoxy)- α -L-rhamnopyranoside **200** (350 mg, 0.685 mmol) was dissolved in MeOH (15 mL) and ethylene diamine (1.83 mL, 27.40 mmol) was added. The mixture was refluxed for 1 hour at 70 °C then concentrated. Purification by column chromatography (hexane/EtOAc/MeOH 40:60:10) gave the product as an off white solid in 88% yield (153 mg). $[\alpha]_D^{24}$ -0.136° (c 0.111, MeOH).

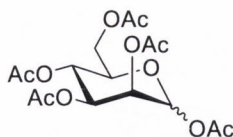
δ_H (400 MHz, CDCl₃): 6.87 (2H, m, Ar), 6.72 (2H, m, Ar), 5.25 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 3.99 (1H, dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H2), 3.84 (1H, dd, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 9.5 Hz, H3), 3.73 (1H, m, H5), 3.46 (1H, app t, H3), 1.26 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, CDCl₃): 149.5 (Cq), 142.0 (Cq), 117.5 (Ar), 116.5 (Ar), 99.6 (C1), 72.6 (C4), 70.9 (C3), 70.8 (C2), 69.0 (C5), 16.6 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₁₂H₁₈NO₅ 256.1185; found 256.1190.

ν_{max} (thin film): 3552, 3459, 3372, 1626, 1513 cm⁻¹.

1,2,3,4,6-Penta-*O*-acetyl-D-mannopyranoside (**190**)



D-Mannose **189** (1.0 g, 5.5 mmol) was dissolved in pyridine (5 mL) and acetic anhydride (5 mL, 53 mmol) under N₂. After 18 hours the mixture was concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a colourless oil in 98% yield (2.426 g).

Data for the α anomer:

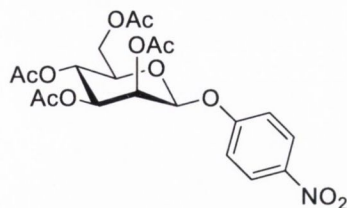
δ_{H} (400 MHz, CDCl₃): 6.11 (1H, d, $J_{1,2} = 1.9$ Hz, H1), 5.38 (2H, m, H3, H4), 5.30 (1H, m, H2), 4.31 (1H, dd, $J = 4.9$ Hz, $J = 12.5$ Hz, H6), 4.13 (1H, dd, $J = 2.5$ Hz, $J = 12.5$ Hz, H6), 4.08 (1H, m, H5).

δ_{C} (100 MHz, CDCl₃): 170.7 (C=O), 170.0 (C=O), 169.8 (C=O), 169.6 (C=O), 168.1 (C=O), 90.6 (C1), 70.6 (C5), 68.8 (C3), 68.4 (C2), 65.5 (C4), 62.1 (C6), 20.9 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 20.6 (CH₃).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for C₁₆H₂₂O₁₁Na 413.1060; found 413.1068.

The data are consistent with those in the current literature.¹⁹⁸

2,3,4,6-Penta-*O*-acetyl-1-(4-nitrophenoxy)- β -D-mannopyranoside (**191**)



1,2,3,4,6-Penta-*O*-acetyl- β -D-galactose **190** (1.0g, 2.56 mmol) was dissolved in anhydrous DCE (20 mL) at 0 °C under N₂. 4-nitrophenol (379 mg, 2.70 mmol) was added followed by BF₃·OEt₂ (1.3 mL, 10.5 mmol) in increments. The stirred solution was allowed to come to room temperature and reacted for 18h then quenched by treatment with saturated sodium bicarbonate solution (ca. 20 mL) and solid NaHCO₃. The organic layer was separated, dried with MgSO₄, filtered, and concentrated. Purification by column chromatography (hexane/EtOAc 75:25) gave the product as a colourless oil in 73% (870 mg) total yield. The α anomer was isolated in 20% yield and the β anomer was isolated in 6% yield. R_f = 0.4 (hexane/EtOAc 80:20). Data for the β anomer: $[\alpha]_{\text{D}}^{20}$ 104.4° (c 0.113, CHCl₃).

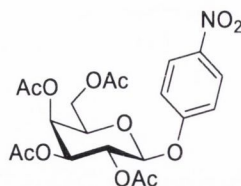
δ_{H} (400 MHz, CDCl₃): 8.20 (2H, m, Ar), 6.93 (2H, m, Ar), 5.92 (1H, d, $J_{1,2}$ = 3.5 Hz, H1), 5.59 (1H, dd, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.7 Hz, H2), 5.56 (1H, dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.2 Hz, H4), 5.35 (1H, dd, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$ = 3.5 Hz, H3), 4.29 (1H, app t, H5), 4.15 (1H, dd, J = 6.1, J = 11.4 Hz, CH₂), 4.09 (1H, dd, J = 7.2, J = 11.3 Hz, CH₂), 2.21 (3H, s, CH₃), 2.11 (3H, s, CH₃), 2.07 (3H, s, CH₃), 1.97 (3H, s, CH₃).

δ_{C} (100 MHz, CDCl₃): 170.4 (Cq), 170.3 (Cq), 170.1 (Cq), 170.1 (Cq), 168.9 (Cq), 161.4 (Cq), 126.3 (Ar), 115.7 (Ar), 99.8 (C1), 67.8 (C5), 67.6 (C4), 67.4 (C3), 67.2 (C2), 61.3 (CH₂), 20.7 (CH₃), 20.6 (CH₃), 20.6 (CH₃), 20.5 (CH₃).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for C₂₀H₂₃NO₁₂Na 492.1118; found 492.1118.

ν_{max} (thin film): 3345, 3118, 1745, 1592, 1338, 1112, 1048 cm⁻¹.

2,3,4,6-Penta-*O*-acetyl-1-(4-nitrophenoxy)- β -D-galactopyranoside (**193**)



1,2,3,4,6-Penta-*O*-acetyl- β -D-galactose **192** (1.0 g, 2.56 mmol) was dissolved in anhydrous DCE at 0 °C under N₂. 4-nitrophenol (139 mg, 2.72 mmol) was added followed by BF₃·OEt₂ (1.3 mL, 10.5 mmol) in increments. After 18 hours at room temperature the reaction was quenched by treatment with sat. aq. NaHCO₃ solution (ca. 20 mL) and solid NaHCO₃. The mixture was diluted with DCM, separated, dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a white solid in 90% overall yield. The α anomer was isolated in 5% yield and the β anomer in 4.4% yield (53 mg). R_f = 0.4 (hexane/EtOAc 80:20). Data for the β anomer: $[\alpha]_{\text{D}}^{20}$ 104.4° (c 0.113, CHCl₃).

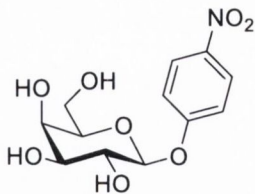
δ_{H} (400 MHz, CDCl₃): 8.20 (2H, m, Ar), 6.93 (2H, m, Ar), 5.92 (1H, d, $J_{1,2}$ = 3.6, H1), 5.59 (1H, dd, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 10.7 Hz, H2), 5.56 (1H, dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.2 Hz, H4), 5.35 (1H, dd, $J_{3,2}$ = 10.8 Hz, $J_{3,4}$ = 3.5 Hz, H3), 4.29 (1H, app t, H5), 4.15 (1H, dd, J = 6.1, 11.4 Hz, CH₂), 4.09 (1H, dd, J = 7.2, 11.3 Hz, CH₂), 2.21 (3H, s, CH₃), 2.11 (3H, s, CH₃), 2.07 (3H, s, CH₃), 1.97 (3H, s, CH₃).

δ_{C} (100 MHz, CDCl₃): 170.4 (Cq), 170.3 (Cq), 170.1 (Cq), 170.1 (Cq), 168.9 (Cq), 161.4 (Cq), 126.3 (Ar), 115.7 (Ar), 99.8 (C1), 67.8 (C5), 67.6 (C4), 67.4 (C3), 67.2 (C2), 61.3 (CH₂), 20.7 (CH₃), 20.6 (CH₃), 20.6 (CH₃), 20.5 (CH₃).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for C₂₀H₂₃NO₁₂Na 492.1118; found 492.1118.

ν_{max} (thin film): 3345, 3118, 1745, 1490, 1338, 1112 cm⁻¹.

1-(4-Nitrophenoxy)- β -D-Galactopyranoside



2,3,4,6-Tetra-*O*-acetyl-1-(4-nitrophenoxy)- β -D-galactopyranoside (110 mg, 0.23 mmol) was dissolved in MeOH with a catalytic amount of NaOMe. After 18 h the reaction was quenched with a catalytic amount of DOWEX, filtered and concentrated to give the product as a yellow solid in 98% yield (69 mg). Data for the β anomer: $[\alpha]_{\text{D}}^{20}$ 57.5° (c 0.04, MeOH).

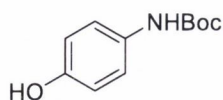
δ_{H} (400 MHz, CD_3OD): 8.24 (2H, m, Ar), 7.35 (2H, m, Ar), 5.71 (1H, d, $J_{1,2}$ = 3.3 Hz, H1), 4.03 (1H, dd, $J_{2,1}$ = 3.3 Hz, $J_{2,3}$ = 9.8 Hz, H2), 4.01 (1H, m, H4), 3.99 (1H, m, H3), 3.85 (1H, app t, H5), 3.71 (2H, d, $J_{6,5}$ = 6.0 Hz, H6).

δ_{C} (100 MHz, CD_3OD): 169.1 (Cq), 162.0 (Cq), 125.2 (Ar), 116.6 (Ar), 98.1 (C1), 72.5 (C5), 69.8 (C4), 69.3 (C3), 69.2 (C2), 62.0 (C6).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{12}\text{H}_{15}\text{NO}_8\text{Na}$ 324.0695; found 324.0709.

ν_{max} (thin film): 3250, 2932, 2168, 1592, 1345 cm^{-1} .

***N*-Boc-4-hydroxy aniline (196)**



4-Hydroxy aniline (500 mg, 4.58 mmol) was dissolved in MeOH (10 mL) and TEA (1.4 mL, 10 mmol) under N₂. After 18 hours, the mixture was concentrated *in vacuo* and resuspended in EtOAc. The organic layer was washed with 0.25N HCl solution (10 mL), NH₄Cl solution (10 mL), dried with MgSO₄ and concentrated *in vacuo* to give the product as an off-white solid in 89% yield (840 mg).

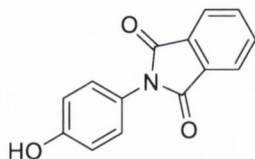
δ_{H} (400 MHz, CDCl₃): 7.47 (2H, s, Ar), 6.78 (2H, m, Ar), 1.53 (9H, s, CH₃).

HRMS (EI) calculated for C₁₁H₁₅NO₃Na 232.0950 found 232.0943.

ν_{max} (thin film): 3360, 2981, 1695, 1513, 1436, 1387, 1227 cm⁻¹.

The data are consistent with those in the current literature.¹⁹⁹

***N*-(4-Hydroxyphenyl)Phthalimide (199)**



4-Aminophenol (1.0 g, 9.18 mmol) and TEA (0.5 mL) were added to a solution of phthalic anhydride (1.5 g, 10.13 mmol) in ethanol (50 mL). After 18 hours at room temperature the mixture was concentrated *in vacuo*. The resulting oil was resuspended in distilled water (50 mL) and refluxed for 30 minutes then concentrated *in vacuo*. The crude product was dissolved in EtOAc (250 mL), washed with 0.1N HCl (15 mL) and H₂O (15 mL), dried with MgSO₄ and concentrated *in vacuo* to give the product as a white solid in 70% yield (3.05 g).

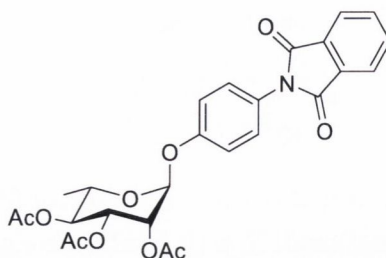
mp: 293-295 °C. Literature value 291-293 °C.

δ_{H} (400 MHz, DMSO): 9.80 (1H, s, OH), 7.92 (4H, m, Ar), 7.21 (2H, d, $J = 8.7$ Hz, Ar), 6.87 (2H, d, $J = 8.6$ Hz, Ar).

ν_{max} (thin film): 3405, 1706, 1515, 1117 cm⁻¹.

The data are consistent with those in the current literature.²⁰⁰

2,3,4-Tri-*O*-acetyl-1-(4-phthalimido phenoxy)- α -L-rhamnopyranoside (**200**)



- 1) Ethyl-2,3,4-tri-*O*-acetyl-1-thio- α -L-rhamnopyranoside **78** (560 mg, 1.68 mmol) was dissolved in 1:1 anhydrous dioxane:DCM (10 mL) at 0 °C under N₂. *N*-(4-hydroxyphenyl) phthalimide (482 mg, 2.02 mmol), NIS (603 mg, 2.68 mmol) and a catalytic amount (ca. 10 μ L) of TMS.OTf. After 18 hours at room temperature the reaction was quenched with TEA (ca. 2 mL), washed with sat. NaHCO₃ solution (15 mL), dried with MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (toluene:acetone 95:5) gave the product as a pale yellow solid in 66% yield (565 mg).
- 2) 2,3,4-Tri-*O*-acetyl-1- α -L-rhamnose trichloroacetimidate **156** (450 mg, 1.04 mmol) was dissolved in anhydrous dioxane (10 mL) at 0 °C under N₂. *N*-(4-hydroxyphenyl) phthalimide (297 mg, 1.24 mmol) and 10 μ L of TMS.OTf were added. After 3 hours the reaction was quenched with TEA (ca. 20 mL) and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a pale yellow solid in 87% yield (464 mg). R_f = 0.4 (hexane/EtOAc 60:40). $[\alpha]_D^{20}$ -0.094° (c 0.139, CHCl₃).

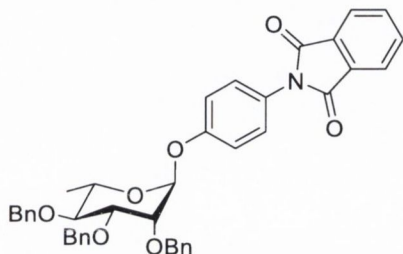
δ_H (400 MHz, CDCl₃): 7.99 (2H, m, Ar), 7.83 (2H, m, Ar), 7.40 (2H, m, Ar), 7.24 (2H, m, Ar), 5.55 (1H, dd, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 10.0 Hz, H3), 5.53 (1H, d, $J_{1,2}$ = 1.9 Hz, H1), 5.48 (1H, dd, $J_{2,1}$ = 1.9 Hz, $J_{2,3}$ = 3.5 Hz, H2), 5.20 (1H, app t, H4), 4.02 (1H, m, H5), 2.23, (3H, s, CH₃), 2.10 (3H, s, CH₃), 2.07 (3H, s, CH₃), 1.26 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 170.1 (Cq), 167.4 (Cq), 155.4 (Cq), 134.5 (Ar), 131.8 (Cq), 129.1 (Cq), 128.1 (Ar), 126.2 (Cq), 125.3 (Cq), 123.8, 116.9 (Ar), 95.7 (C1), 70.9 (C4), 69.6 (C2), 68.9 (C3), 67.3 (C5), 20.9 (CH₃), 20.8 (CH₃), 20.8 (CH₃), 17.5 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for $C_{26}H_{25}NO_{10}Na$ 534.1376; found 534.1368.

$\nu_{\max}$ (thin film): 2983, 1717, 1509, 1375, 1210, 1030 cm^{-1} .

1-(4-(1,3-Dioxoisindolin-2-yl)phenoxy)-2,3,4-Tri-benzyloxy- α -L-rhamnopyranoside (201)



Ethyl-2,3,4-tri-benzyloxy-1-thio- α -L-rhamnopyranoside **112** (571 mg, 1.19 mmol) was dissolved in 1:1 anhydrous dioxane/DCM (15 mL) at 0 °C under N_2 . *N*-(4-hydroxyphenyl) phthalimide (342 mg, 1.4 mmol), NIS (429 mg, 1.91 mmol) and a catalytic amount (ca. 10 μ L) of TMS.OTf. After 18 hours at room temperature the reaction was quenched with TEA (ca. 2 mL), washed with sat. aq. $NaHCO_3$ solution (50 mL), dried with $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (petroleum ether/EtOAc 90:10 increasing to 60:40) gave product as an colourless oil in 62% yield (485 mg). R_f = 0.8 (hexane/EtOAc 60:40). $[\alpha]_D^{20}$ -95.4° (c 0.111, $CHCl_3$).

δ_H (400 MHz, $CDCl_3$): 7.98 (2H, m, Phth), 7.82 (2H, m, Phth), 7.38 (17H, m, Ar, Bn), 7.13 (2H, m, Ar), 5.52 (1H, d, $J_{1,2}$ = 1.9 Hz, H1), 5.00 (1H, d, J = 10.8 Hz, CH_2Bn), 4.83 (3H, m, CH_2Bn), 4.74 (1H, d, J = 5.1 Hz, CH_2Bn), 4.71 (1H, d, J = 10.8 Hz, CH_2Bn), 4.10 (1H, dd, $J_{3,2}$ = 3.1 Hz, $J_{3,4}$ = 9.2 Hz, H3), 3.98 (1H, dd, $J_{2,1}$ = 1.9 Hz, $J_{2,3}$ = 3.1 Hz, H2), 3.83 (1H, m, H5), 3.74 (1H, app t, H4), 1.36 (3H, d, $J_{6,5}$ = 6.1 Hz, H6).

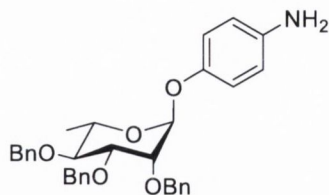
δ_C (100 MHz, $CDCl_3$): 167.5 (Cq), 156.0 (Cq), 138.5 (Cq), 138.0 (Cq), 134.4 (CH, Phth), 131.8 (Cq), 128.5 (Ar), 128.43 (Ar), 128.40, (Ar), 128.09 (Ar), 128.02 (Ar), 127.94 (Ar), 127.88 (Ar), 127.7 (Ar), 125.5 (Cq), 123.7 (CH, Phth), 116.8 (Ar), 96.5 (C1), 80.4 (C4), 79.8 (C3),

75.5 (CH₂), 74.5 (C2), 73.0 (CH₂Bn), 72.5 (CH₂Bn), 69.0 (C5),
18.1 (C6).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₄₁H₃₇NO₇Na 678.2468; found 678.2459.

$\nu_{\max}$ (thin film): 3030, 2894, 1712, 1513, 1235 cm⁻¹.

1-(4-Aminophenoxy)-2,3,4-Tri-benzyloxy- α -L-rhamnopyranoside (202)



1-(4-(1,3-Dioxoisindolin-2-yl)phenoxy)-2,3,4-Tri-benzyloxy- α -L-rhamnopyranoside **201** (632 mg, 0.96 mmol) was dissolved in MeOH (15 mL) and ethylene diamine (2.58 mL, 36.4 mmol) was added. The mixture was refluxed for 1 hour at 70 °C then concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a pale brown oil in 89% yield (450 mg). $[\alpha]_D^{20}$ -73.7° (c 0.099, CHCl₃).

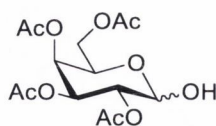
δ_H (400 MHz, CDCl₃): 7.36 (15H, m, Bn), 6.82 (2H, m, Ar), 6.63 (2H, m, Ar), 5.36 (1H, d, $J_{1,2}$ = 1.9 Hz, H1), 4.90 (1H, d, J = 10.8 Hz, CH₂), 4.81 (2H, dd, J = 12.5 Hz, J = 19.8 Hz, CH₂Bn), 4.71 (2H, dd, J = 14.3 Hz, J = 11.8 Hz, CH₂Bn), 4.69 (1H, d, J = 10.8 Hz, CH₂Bn), 4.07 (1H, dd, $J_{3,2}$ = 3.1 Hz, $J_{3,4}$ = 9.3 Hz, H3), 3.97 (1H, dd, $J_{2,1}$ = 1.9 Hz, $J_{2,3}$ = 3.1 Hz, H2), 3.86 (1H, m, H5), 3.71 (1H, app t, H4), 1.33 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 138.6 (Cq), 138.6 (Cq), 138.5 (Cq), 138.2 (Cq), 128.4 (Bn), 128.4 (Bn), 128.0 (Bn), 128.0 (Bn), 127.8 (Bn), 127.7 (Bn), 127.6 (Bn), 117.7 (Ar), 116.2 (Ar), 97.1 (C1), 80.5 (C4), 80.0 (C3), 75.4 (CH₂Bn), 74.8 (C2), 72.9 (CH₂Bn), 72.3 (CH₂Bn), 68.6 (C5), 18.0 (C6).

HRMS (EI) calculated for C₃₃H₃₆NO₅Na 526.2593 found 526.2603.

$\nu_{\max}$ (thin film): 3351, 2974, 1642, 1508, 1018 cm⁻¹.

2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranoside (**203**)



1,2,3,4,6-Penta-*O*-acetyl- β -D-galactose **192** (3.0 g, 7.69 mmol) was dissolved in THF (30 mL) and BnNH_2 (1.68 mL, 15.38 mmol) was added. After 3 hours the mixture was diluted with DCM (30 mL), washed with ice cold 1M HCl (20 mL), aq. sat. NaHCO_3 solution (20 mL), aq. sat. NaCl solution (20 mL) and H_2O (20 mL), dried with MgSO_4 , filtered and concentrated *in vacuo*. Column chromatography (hexane/EtOAc 70:30-50:50) furnished the product as a colourless oil in 86% yield (2.29 g).

Data for the major β anomer:

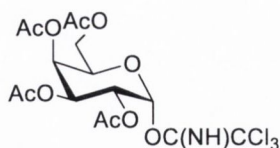
δ_{H} (400 MHz, CDCl_3): 5.53 (1H, d, $J_{1,2} = 3.6$ Hz, H1), 5.49 (1H, dd, $J_{4,3} = 3.3$ Hz, $J_{4,5} = 1.3$ Hz, H4), 5.43 (1H, dd, $J_{3,2} = 10.8$ Hz, $J_{3,4} = 3.3$ Hz, H3), 5.17 (1H, dd, $J_{2,1} = 3.6$ Hz, $J_{2,3} = 3.3$ Hz, H2), 4.49 (1H, app t, H5), 4.12 (2H, m, H6), 2.17 (CH_3), 2.12 (CH_3), 2.07 (CH_3), 2.01 (CH_3).

δ_{C} (100 MHz, CDCl_3): 170.6 (C=O), 170.5 (C=O), 170.3 (C=O), 170.2 (C=O), 90.6 (C1), 68.4 (C2), 68.2 (C4), 67.3 (C3), 66.2 (C5), 61.8 (CH_2), 20.8 (CH_3), 20.71 (CH_3), 20.65 (CH_3), 20.6 (CH_3).

HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{14}\text{H}_{20}\text{O}_{10}\text{Na}$ 371.0954; found 371.0952.

The data are consistent with those in the current literature.²⁰¹

2,3,4,6-Tetra-*O*-acetyl-1-trichloroacetimidate- α -D-galactopyranoside (204)



2,3,4,6-Tetra-*O*-acetyl-D-galactose **203** (1.0 g, 2.87 mmol) was dissolved in anhydrous DCM (15 mL) under N₂. Trichloroacetonitrile (3.46 mL, 34.5 mmol) and catalytic DBU (0.6 mL) were added. After 2 hours the mixture was concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a yellow oil in 66% yield (931 mg).

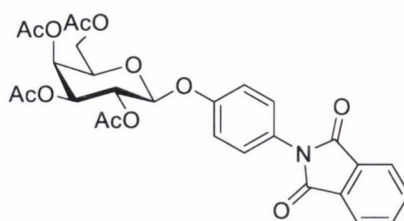
δ_{H} (400 MHz, CDCl₃): 8.70 (1H, s, COH), 6.64 (1H, d, $J_{1,2} = 3.5$ Hz, H1), 5.60 (1H, dd, $J_{4,3} = 3.2$ Hz, $J_{4,5} = 1.2$ Hz, H4), 5.46 (1H, dd, $J_{3,2} = 10.9$ Hz, $J_{3,4} = 3.2$ Hz, H3), 5.40 (1H, dd, $J_{2,1} = 3.4$ Hz, $J_{2,3} = 10.9$ Hz, H2), 4.47 (1H, m, H5), 4.16 (2H, m, H6), 2.20 (3H, s, CH₃), 2.06 (3H, s, CH₃), 2.05 (3H, s, CH₃), 2.05 (3H, s, CH₃).

δ_{C} (100 MHz, CDCl₃): 170.3 (C=O), 170.1 (C=O), 170.0 (C=O), 161.0 (C=O), 93.5 (C1), 90.9 (C=NH), 69.0 (C5), 67.5 (C3), 67.4 (C4), 66.9 (C2), 61.3 (C6), 31.0 (CCl₃), 20.7(CH₃), 20.65(CH₃), 20.61(CH₃), 20.56 (CH₃).

ν_{max} (thin film): 1741, 1677, 1371, 1217 cm⁻¹.

The data are consistent with those in the current literature.²⁰²

2,3,4,6-Tetra-*O*-acetyl-1-(4-phthalimido phenoxy)- β -D- galactopyranoside (205)



2,3,4,6-Tetra-*O*-acetyl-1- β -D-galactose trichloroacetimidate **204** (900 mg, 1.83 mmol) was dissolved in anhydrous dioxane (15 mL) under N₂. *N*-(4-hydroxyphenyl) phthalimide (416 mg, 1.74 mmol) and 10 μ L of TMS.OTf were added. After 3 hours the mixture was quenched with TEA (ca. 2mL) and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 70:30) gave the product in 46% total yield (477 mg). The α anomer was isolated in 12% yield (129 mg) and the β anomer in 30% yield (315 mg). R_f = 0.4 (hexane/EtOAc 60:40). Data for the β anomer: $[\alpha]_D^{20}$ 32.1 ° (c 0.115, CHCl₃).

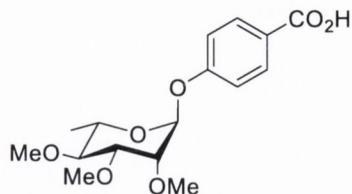
δ_H (400 MHz, CDCl₃): 7.99 (2H, m, Phth), 7.83 (2H, m, Phth), 7.40 (2H, m, Ar), 7.16 (2H, m, Ar), 5.55 (1H, dd, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 10.4 Hz, H2), 5.50 (1H, dd, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 0.7 Hz, H4), 5.15 (1H, dd, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 3.4 Hz, H3), 5.11 (1H, d, $J_{1,2}$ = 7.9 Hz, H1), 4.23 (2H, m, H6), 4.11 (1H, app t, Hz, H5), 2.23 (CH₃), 2.12 (CH₃), 2.10 (CH₃), 2.06 (CH₃).

δ_C (100 MHz, CDCl₃): 170.4 (C=O), 170.3 (C=O), 170.1 (C=O), 169.4 (C=O), 167.4, 156.4 (Cq), 134.5 (Cq Phth), 131.7 (Cq), 128.0 (Ar), 126.7 (Cq), 123.8 (Phth), 167.5 (Ar), 99.6 (C1), 71.1 (C5), 70.8 (C3), 68.5 (C2), 66.9 (C4), 61.4 (C6), 20.8 (CH₃), 20.7 (CH₃), 20.7 (CH₃), 20.6 (CH₃).

HRMS-ESI (m/z): $[M + Na]^+$ calculated for C₂₈H₂₇NO₁₂Na 592.1431; found 592.1426.

ν_{max} (thin film): 1742, 1712, 1610, 1511 cm⁻¹.

2,3,4-Tri-methoxy-1-(4-(oxycarbonyl)phenoxy)- α -L-rhamnopyranoside (206)



2,3,4-Tri-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **144** (503 mg, 1.48 mmol) was dissolved in dioxane/H₂O 2:1 (20 mL) along with a catalytic amount of NaOH. The mixture was refluxed at 105 °C. After 24 hours the reaction was quenched with DOWEX, filtered and diluted with EtOAc (35 mL). The organic layer was washed with H₂O, dried with MgSO₄, filtered, and concentrated *in vacuo* to give the product as a colourless oil in 92% yield (445 mg). $[\alpha]_{\text{D}}^{20}$ -119.5° (*c* 0.271, CHCl₃).

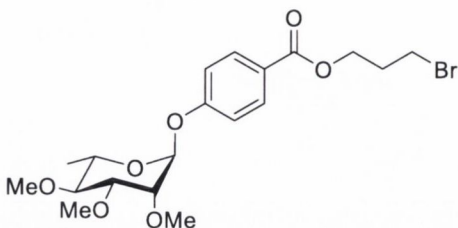
δ_{H} (400 MHz, CDCl₃): 8.10 (2H, d, J = 8.8 Hz, Ar), 7.16 (2H, d, J = 8.8 Hz, Ar), 5.66 (1H, app s, H1), 3.81 (1H, m, H2), 3.71 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.2 Hz, H3), 3.63 (1H, m, H5), 3.61 (3H, s, OCH₃), 3.60 (6H, s, OCH₃), 3.25 (1H, app t, H4), 1.29 (3H, d, $J_{6,5}$ = 6.3 Hz, H6).

δ_{C} (100 MHz, CDCl₃): 170.3 (C=O), 160.6 (Cq), 132.2 (Ar), 122.8 (Cq), 115.8 (Ar), 94.9 (C1), 81.8 (C4), 80.7 (C3), 77.2 (C2), 68.9 (C5), 60.9 (OCH₃), 59.3 (OCH₃), 57.9 (OCH₃), 17.7 (C6).

HRMS-ESI (m/z): $[\text{M} - \text{H}]^-$ calculated for C₁₆H₂₁O₇ 325.1287; found 325.1288.

ν_{max} (thin film): 2936, 1679, 1426, 1234, 1099 cm⁻¹.

2,3,4-Tri-methoxy-1-(4-(3-bromopropoxy)carbonyl)phenoxy)- α -L-rhamnopyranoside (208)



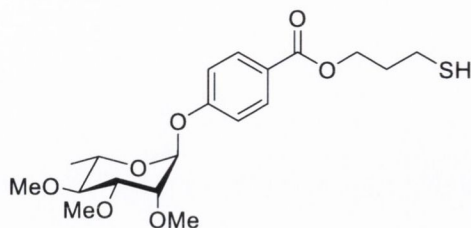
2,3,4-Tri-methoxy-1-(4-(oxycarbonyl)phenoxy)- α -L-rhamnopyranoside **206** (57 mg; 0.174 mmol) was dissolved in anhydrous DCM (7 mL). 3-bromopropan-1-ol (63 μ L; 0.696 mmol), DCC (143.6 mg; 0.696 mmol) and a catalytic amount of DMAP were added. After 18 hours the reaction mixture was filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 60:40) gave the product as a colourless oil in 66% yield (51 mg). $[\alpha]_D^{20}$ -119.5° (*c* 0.271, CHCl₃).

δ_H (400 MHz, CDCl₃): 7.95 (2H, m, Ar), 7.07 (2H, m, Ar), 5.81 (1H, app s, H1), 4.46 (2H, m, CH₂), 3.80 (1H, m, H2), 3.69 (1H, dd, $J_{3,2} = 3.4$ Hz, $J_{3,4} = 9.3$ Hz, H3), 3.60 (1H, m, H5) 3.56 (6H, s, OCH₃), 3.52 (6H, s, OCH₃), 3.50 (6H, s, OCH₃), 3.56 (2H, m, CH₂), 3.23 (1H, app t, H4), 2.33 (2H, m, CH₂), 1.27 (3H, d, $J_{6,5} = 6.3$ Hz, H6).

δ_C (100 MHz, CDCl₃): 165.9 (Cq), 160.0 (Cq), 131.8 (Ar), 123.9 (Cq), 116.0 (Ar), 95.0 (C1), 82.0 (C4), 80.9 (C3), 77.1 (C2), 68.9 (C5), 62.5 (CH₂), 61.0 (OCH₃), 59.4 (OCH₃), 58.0 (OCH₃), 31.9 (CH₂), 29.5 (CH₂), 17.8 (C6).

$\nu_{\max}$ (thin film): 2953, 1745, 1440, 1310, 1175 cm⁻¹.

2,3,4-Tri-methoxy-1-(4-(3-thiopropylloxycarbonyl)phenoxy)- α -L-rhamnopyranoside (209)



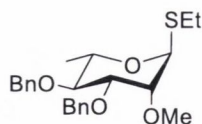
2,3,4-Tri-methoxy-1-(4-(3-bromopropylloxycarbonyl)phenoxy)- α -L-rhamnopyranoside **208** (51.3 mg; 0.114 mmol) was dissolved in anhydrous THF under N_2 at $-10\text{ }^{\circ}\text{C}$. Hexamethyl-disilyl-disulfide (29 μL ; 0.137 mmol) and tetrabutyl ammonium fluoride (1M solution in THF) (126 μL ; 0.126 mmol) were added at $-10\text{ }^{\circ}\text{C}$ and the reaction allowed to come to room temperature. After 45 minutes the reaction was check by TLC and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 73:30) gave the product as a pale yellow oil in 89% yield (41 mg). $[\alpha]_D^{20} -45.8^{\circ}$ (c 0.227, CHCl_3).

δ_{H} (400 MHz, CDCl_3): 8.00 (2H, m, Ar), 7.11 (2H, m, Ar), 5.63 (1H, app s, H1), 4.41 (2H, m, CH_2), 3.79 (1H, m, H2), 3.68 (1H, dd, $J_{3,2} = 2.9\text{ Hz}$, $J_{3,4} = 9.1\text{ Hz}$, H3), 3.59 (10 H, m, H5, OCH_3), 3.22 (1H, app t, H4), 2.85 (2H, m, CH_2), 2.18 (2H, m, CH_2), 1.26 (3H, d, $J_{6,5} = 6.3\text{ Hz}$, H6).

δ_{C} (100 MHz, CDCl_3): 165.9 (C=O), 159.9 (Cq), 131.4 (Ar), 123.8 (Cq), 115.7 (Ar), 94.9 (C1), 81.8 (C4), 80.7 (C3), 77.0 (C2), 68.7 (C5), 62.9 (CH_2), 60.8 (OCH_3), 59.2 (OCH_3), 57.8 (OCH_3), 35.1 (CH_2), 28.3 (CH_2), 17.6 (C6).

ν_{max} (thin film): 2926, 1715, 1605, 1242, 1103 cm^{-1} .

Ethyl-3,4-dibenzyloxy-2-methoxy-1-thio- α -L-rhamnopyranoside (**210**)



Ethyl-4-benzyloxy-2-methoxy-1-thio- α -L-rhamnopyranoside **108** (106 mg; 0.342 mmol) was dissolved in anhydrous DMF (5 mL) at 0°C under N₂. NaH (27 mg; 0.683 mmol) and BnBr (0.08 mL; 0.683 mmol) were added. After 18 hours the reaction was quenched with MeOH (ca. 2 mL) and diluted with diethyl ether. The organic layer was washed with sat. aq. NaHCO₃ solution (15 mL) and water (3 x 20 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc 80:20) gave the product as a colourless oil in 91% yield (125 mg). [α]_D²⁰ -124.1° (*c* 0.207, CHCl₃)

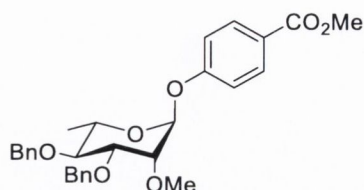
δ_{H} (400 MHz, CDCl₃): 7.34 (10H, m, Bn), 5.34 (1H, app s, H1), 4.96 (1H, d, *J* = 11.2 Hz, CH₂Bn), 4.71 (2H, dd, *J* = 11.7 Hz, *J* = 16.7 Hz, CH₂Bn), 4.64 (1H, d, *J* = 11.2 Hz, CH₂Bn), 4.05 (1H, m, H5), 3.82 (1H, dd, *J*_{3,2} = 3.2 Hz, *J*_{3,4} = 9.4 Hz, H3), 3.59 (1H, m, H2), 3.56 (1H, app t, H4), 3.50 (3H, s, OCH₃), 2.63 (2H, m, CH₂CH₃), 1.33 (3H, d, *J*_{6,5} = 6.3 Hz, H6), 1.30 (3H, t, *J* = 7.5 Hz, CH₂CH₃).

δ_{C} (100 MHz, CDCl₃): 138.5 (Cq), 138.1 (Cq), 128.3 (Bn), 128.2 (Bn), 127.9 (Bn), 127.8 (Bn), 127.6 (Bn), 127.5 (Bn), 81.0 (C1), 80.5 (C2), 79.9 (C3), 79.7 (C4), 75.4 (CH₂Bn), 72.2 (CH₂Bn), 68.1 (C5), 58.4 (OCH₃), 25.3 (CH₂CH₃), 17.7 (C6), 14.9 (CH₂CH₃).

HRMS-ESI (*m/z*): [M + Na]⁺ calculated for C₂₃H₃₀O₄SNa 425.1763; found 425.1754.

ν_{max} (thin film): 2923 1453, 1372, 1094 cm⁻¹.

3,4-Di-benzyloxy-2-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (211)



Ethyl-3,4-benzyloxy-2-methoxy-1-thio- α -L-rhamnopyranoside **210** (470 mg, 1.17 mmol) was dissolved in anhydrous DCM (10 mL) at 0 °C under N₂. Methyl-4-hydroxy-benzoate (268 mg, 1.75 mmol) and 1.5 eq. of the activating Me₂S₂-Tf₂O solution were added. After 1 h at 0 °C the reaction was quenched with TEA (ca. 2 mL), diluted with DCM (25 mL), washed with 1M HCl solution (25 mL), sat. aq. NaHCO₃ solution (25 mL), H₂O (25 mL), dried with MgSO₄, filtered, and concentrated. Purification by column chromatography gave the product as a pale yellow oil in 47% yield (271 mg). R_f = 0.6 (hexane/EtOAc 60:40). [α]_D²⁰ -70.8° (c 0.500, CHCl₃).

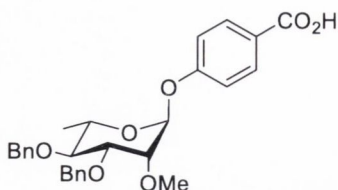
δ_H (400 MHz, CDCl₃): 8.01 (2H, m, Ar), 7.39 (10H, m, Bn), 7.08 (2H, m, Ar), 5.62 (1H, d, $J_{1,2}$ = 1.8 Hz, H1), 4.98 (1H, d, J = 10.7 Hz, CH₂Bn), 4.83 (2H, dd, J = 11.8 Hz, J = 14.2 Hz, CH₂Bn), 4.67 (1H, d, J = 10.7 Hz, CH₂Bn), 4.08 (1H, dd, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.3 Hz, H3), 3.92 (3H, s, CO₂CH₃), 3.74 (1H, m, H5), 3.72 (1H, m, H2), 3.62 (1H, app t, H4), 3.61 (3H, s, OCH₃), 1.30 (3H, d, $J_{6,5}$ = 6.2 Hz, H6).

δ_C (100 MHz, CDCl₃): 166.8 (C=O), 160.0 (Cq), 138.4 (Cq), 138.3 (Cq), 131.6 (Ar), 128.5 (Bn), 128.4 (Bn), 128.0 (Bn), 127.94 (Bn), 127.85 (Bn), 127.77 (Bn), 124.0 (Cq), 115.8 (Ar), 95.3 (C1), 80.2 (C4), 79.5 (C3), 77.9 (C2), 75.6 (CH₂Bn), 72.6 (CH₂Bn), 69.0 (C5), 59.7 (OCH₃), 52.0 (CO₂CH₃), 18.0 (C6).

HRMS-ESI (m/z): [M - H]⁻ calculated for C₂₉H₃₁O₇ 491.2070; found 491.2065.

$\nu_{\max}$ (thin film): 2925, 1718, 1454, 1278, 1099 cm⁻¹.

3,4-Di-benzyloxy-2-methoxy-1-(4-(oxycarbonyl)phenoxy)- α -L-rhamnopyranoside (212)



3,4-Di-benzyloxy-2-methoxy-1-(4-(methoxycarbonyl)phenoxy)- α -L-rhamnopyranoside **211** (224 mg, 0.455 mmol) was dissolved in dioxane/H₂O 2:1 (10 mL) along with a catalytic amount of NaOH. The mixture was refluxed at 105 °C. After 48 h the reaction was quenched with DOWEX, filtered, diluted with EtOAc (35 mL), washed with H₂O, dried with MgSO₄, filtered, and concentrated. Purification by column chromatography (EtOAc/MeOH 100-90:10) gave the product in 78% yield (166.7 mg). $[\alpha]_D^{20}$ -70.8° (*c* 0.500, CHCl₃).

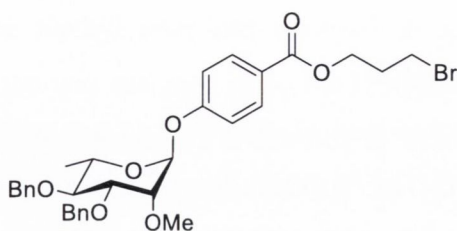
δ_H (400 MHz, CDCl₃): 8.01(2H, m, Ar), 7.34 (10H, m, Bn), 7.01 (2H, m, Ar), 5.57 (1H, app s, H1), 4.95 (1H, d, *J* = 10.7 Hz, CH₂Bn), 4.79 (2H, m, CH₂Bn), 4.65 (1H, d, *J* = 10.7 Hz, CH₂Bn), 4.05 (1H, dd, *J*_{3,2} = 2.9 Hz, *J*_{3,4} = 9.4 Hz, H3), 3.71 (2H, m, H2, H5), 3.60 (1H, m, H4), 3.57 (3H, s, OCH₃), 1.28 (3H, d, *J*_{6,5} = 6.3 Hz, H6).

δ_C (100 MHz, CDCl₃): 171.4 (C=O), 160.7 (Cq), 138.3 (Cq), 138.2 (Cq), 132.3 (Ar), 130.2 (Cq), 128.5 (Bn), 128.4 (Bn), 128.0 (Bn), 127.9 (Bn), 127.8 (Bn), 127.7 (Bn), 123.1 (Cq), 115.8 (Ar), 95.3 (C1), 80.1 (C4), 79.4 (C3), 77.8 (C5), 75.6 (CH₂Bn), 73.7 (CH₂Bn), 69.1 (C2), 59.7 (OCH₃), 17.9 (C6).

HRMS-ESI (*m/z*): [M - H]¹ calculated for C₂₈H₂₉O₇ 477.1913; found 477.1917.

$\nu_{\max}$ (thin film): 2928, 1685, 1605, 1420, 1239, 1097 cm⁻¹.

3,4-Di-benzyloxy-2-methoxy-1-(4-(3-bromopropoxycarbonyl)phenoxy)- α -L-rhamnopyranoside (213)



3,4-Di-benzyloxy-2-methoxy-1-(4-(oxycarbonyl)phenoxy)- α -L-rhamnopyranoside **212** (57 mg; 0.174 mmol) was dissolved in anhydrous DCM (7 mL). 3-bromopropan-1-ol (63 μ L; 0.696 mmol), DCC (143.6 mg; 0.696 mmol) and a catalytic amount of DMAP were added. After 18 hours the reaction mixture was filtered and concentrated *in vacuo* to give the product as a colourless oil in 66% yield (51 mg). $[\alpha]_{\text{D}}^{20}$ -117.8° (*c* 0.056, CHCl_3).

δ_{H} (400 MHz, CDCl_3): 7.97 (2H, m, Ar), 7.34 (10H, m, Bn), 7.05 (2H, m, Ar), 5.58 (1H, d, $J_{1,2} = 1.7$ Hz, H1), 4.94 (1H, d, $J = 10.8$ Hz, BnCH_2), 4.78 (2H, dd, $J = 11.3$ Hz, $J = 13.2$ Hz, BnCH_2), 4.63 (1H, d, $J = 10.8$ Hz, BnCH_2), 4.43 (2H, m, CH_2), 4.03 (1H, dd, $J_{3,2} = 3.1$ Hz, $J_{3,4} = 9.2$ Hz, H3), 3.70 (2H, m, H2, H5), 3.59 (1H, m, H4), 3.54 (3H, s, OCH_3), 3.53 (2H, m, CH_2), 2.30 (2H, m, CH_2), 1.25 (3H, d, $J_{6,5} = 6.3$ Hz, H6).

δ_{C} (100 MHz, CDCl_3): 166.1 (C=O), 159.9 (Cq), 138.4 (Cq), 138.3 (Cq), 131.6 (Ar), 128.5 (Bn), 128.4 (Bn), 128.0 (Bn), 127.9 (Bn), 127.8 (Bn), 127.7 (Bn), 123.8 (Cq), 115.8 (Ar), 95.3 (C1), 80.1 (C4), 79.5 (C3), 77.8 (C2), 75.5 (BnCH_2), 72.5 (BnCH_2), 69.0 (C5), 62.5 (CH_2), 59.7 (OCH_3), 31.9, 29.5 (CH_2), 17.9 (C6).

ν_{max} (thin film): 2925, 1684, 1421, 1240, 1100 cm^{-1} .

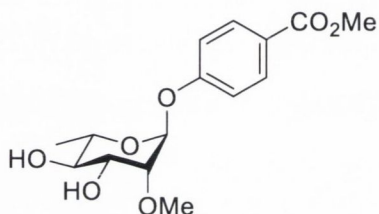
7.0 Appendix A

This appendix contains the spectra of the *p*-HBADs used for the biological evaluation in Chapter 6.

7.1 NMR spectra of compound 11

^1H NMR spectral data for compound 11 (Figure 7.1) was assigned with the aid of the 2 D techniques HSQC and COSY. The ^1H NMR spectrum for this compound is shown in Figure 7.2, with the COSY in Figure 7.3 and the HSQC displayed in Figure 7.4.

Figure 7.1 Compound 11, *p*-HBAD I



The first step of the assignment was to use the HSQC spectrum to identify the position of the anomeric proton and carbon which can be clearly distinguished from the other peaks due to the fact that they are part of an acetal group and therefore have very distinctive shifts in the HSQC spectrum. In this case the anomeric proton is at 5.67 ppm. The corresponding C-1 carbon can also be clearly seen in the ^{13}C spectrum and their relationship established using the HSQC spectrum. The orientation of the substituent at the anomeric carbon was already established in the precursor molecules but it can be seen that it remains unaltered as the H-1 proton has not changed significantly in coupling or shift remaining typical of the α compound. Using the H-1 proton and the H-H COSY (Figure 7.2) it was possible to assign the H-2 proton at 3.70 ppm using the crosspeak with H-1. In a similar manner, the H-3 proton at 3.94 ppm, the H-4 at 3.47, and the H-5 at 3.67 ppm were consecutively identified. Additional verification of the assignment can be obtained by examining the coupling constants as the H-2 proton is split by the H-1 and the H-3 meaning that their coupling values will agree. This holds true for the H-1, H-2 and H-3 protons but begins to break down for H-4 and H-5. The H-4 proton of rhamnose consistently appears as a triplet and the H-5 protons as a multiplet; this alone can make their initial identification facile (pending spectral confirmation).²⁰³ As rhamnose is a 6-deoxy sugar the H-6 protons can be found at 1.27 ppm as a doublet with an integration of 3 Hz. Using the H-6 proton and the H-H COSY the H-5 and H-4 proton locations can be confirmed. The aromatic groups are a pair of doublets between 8 and 7 ppm with a collective integration value

of 4 Hz. The remaining signals are two singlets each of which corresponds to 3 protons; one of these is the C-3 methoxy and the other the methyl ester. The aromatic methyl ester is deshielded and consequently moves further downfield than the carbohydrate methoxy. Thus the peak at 3.90 ppm corresponds to the methyl ester and the peak at 3.56 ppm to the carbohydrate methoxy. The carbons spectrum was assigned using the HSQC in conjunction with the carbon spectrum (Figure 7.5). This assignment was confirmed as being consistent with the current literature.¹⁰⁵

Figure 7.2 ¹H NMR spectrum for compound 11 (*p*-HBAD I)
δ_H (400 MHz, CDCl₃)

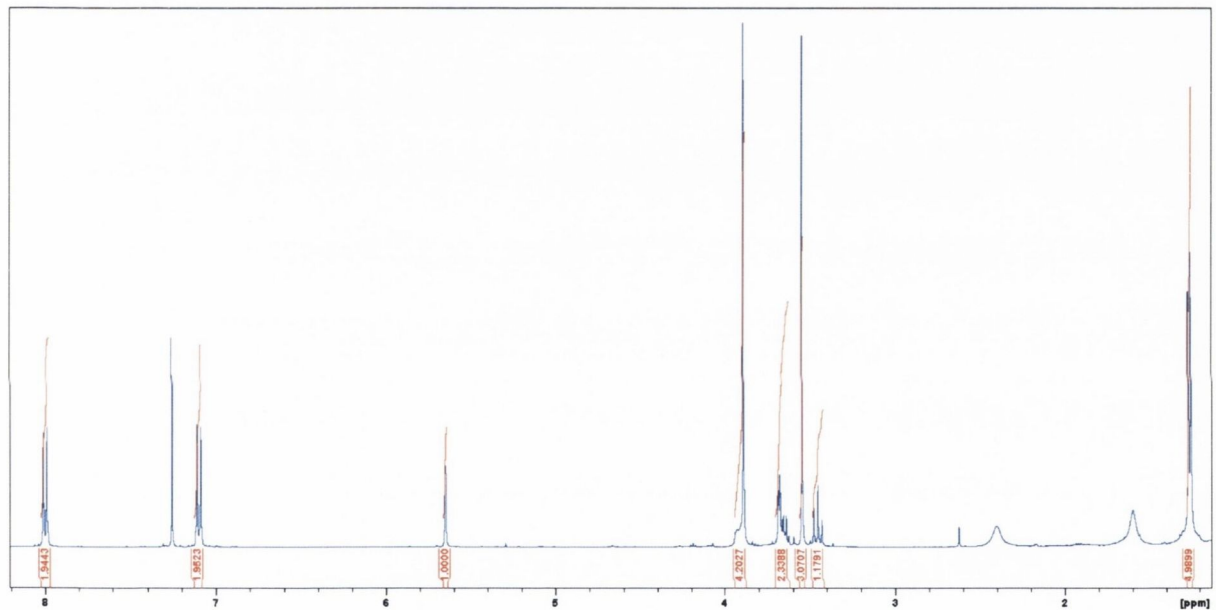


Figure 7.3 COSY of compound 11 (*p*-HBAD I)

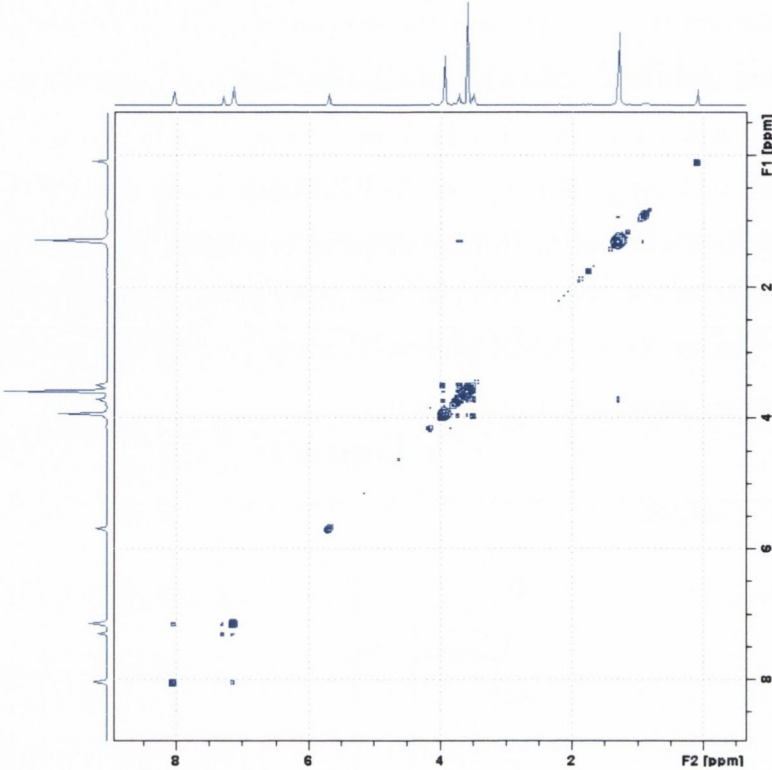


Figure 7.4 HSQC for compound 11 (*p*-HBAD I)

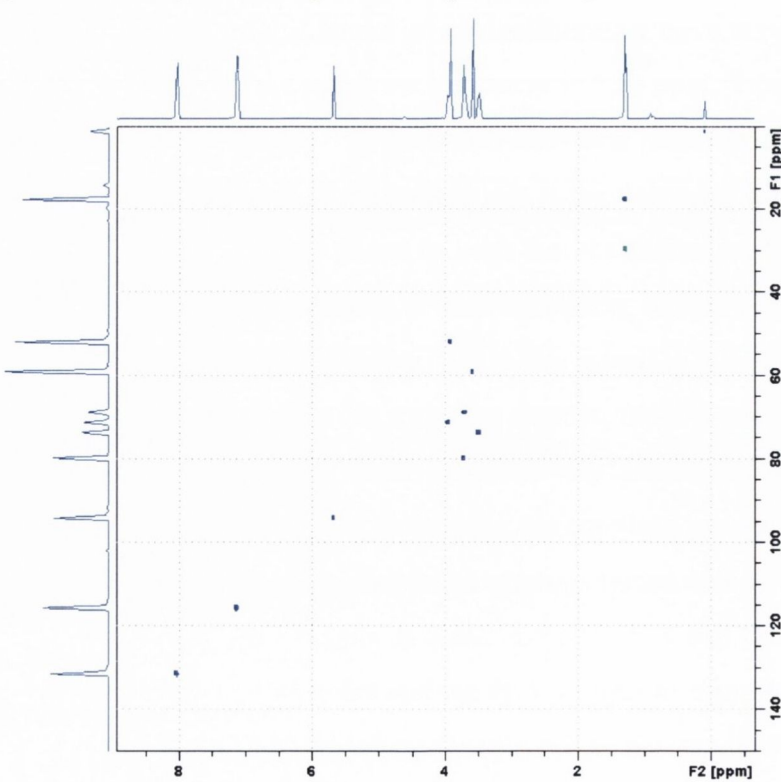
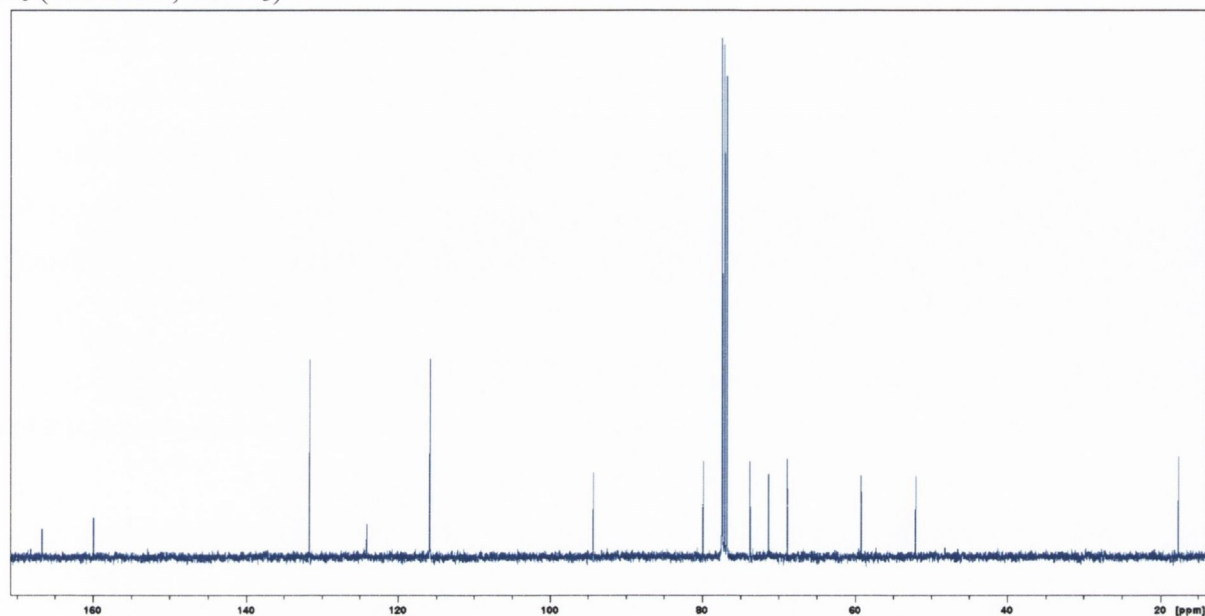


Figure 7.5 ^{13}C spectrum of compound **11** (*p*-HBAD I).
 δ_c (100 MHz, CDCl_3)



7.2 NMR spectra of compound **12**

Assigning the ^1H spectrum of trisaccharide **12**, *p*-HBAD II (Figure 7.6), is complicated by the fact that many of the proton peaks coincide. Both 2 D and 1 D spectroscopic methods were used to assign the peaks. The ^1H spectrum can be seen in Figure 7.7, the HSQC in Figure 7.8, the HMBC in Figure 7.9, three 1D TOCSYs are shown in Figures 7.10 and 7.11, and the ^{13}C spectrum is in Figure 7.12.

To begin with some key proton peaks were identified. The simplest ones to begin with were the three anomeric peaks though two of them have very similar shifts at approx. 5.16 ppm. The H-1 of the aromatic functionalised rhamnose is more deshielded than the others and hence corresponds to the signal seen at 5.65 ppm. Using the HSQC the corresponding rhamnosyl C-1 can be identified. Using this starting point and the HMBC spectrum it was possible to identify several more peaks belonging to the aromatic functionalised rhamnose in both the proton and the carbon spectrum. As with compound **11** the rhamnose proton peaks, where visible, display the typical shapes and the appropriate coupling values which aids in certainty of assignment. Overall the aromatic functionalised rhamnose peaks were identified from H-1 to H-6 (5.65, 3.80, 4.05, 3.72, 3.73 and 1.299 ppm respectively) aided by a 1 D TOCSY. The aromatic peaks can be clearly seen at 8.03 and 7.14 ppm and together with the HMBC were used to confirm which rhamnosyl ring was attached and the assignments.

Additional 1D TOCSYs helped to pick out the remaining two carbohydrate rings and as before the rhamnosyl peaks were the typically observed shaped. The TOCSY in conjunction with the HSQC was used to pick out the carbon peaks corresponding the second rhamnosyl system. The arrangement was confirmed using the HMBC as before. The fucosyl ring has already been glimpsed in a TOCSY and a similar strategy was applied using the HMBC spectrum to ascertain the order of the protons around the carbohydrate ring. A similar rational that used for compound 11 was applied to the methoxies thought again the HMBC was used to identify which methoxies were attached to which ring atoms.

In this way the ^1H and ^{13}C peaks were assigned at the same time with the assignment of each aiding the assignment of the other.

Figure 7.6 Compound 12, *p*-HBAD II

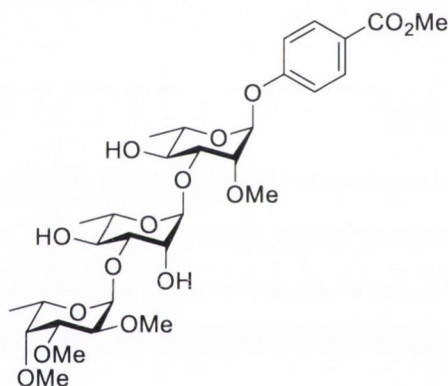


Figure 7.7 ^1H NMR spectrum for compound 12 (*p*-HBAD II)

δ_{H} (600 MHz, CDCl_3)

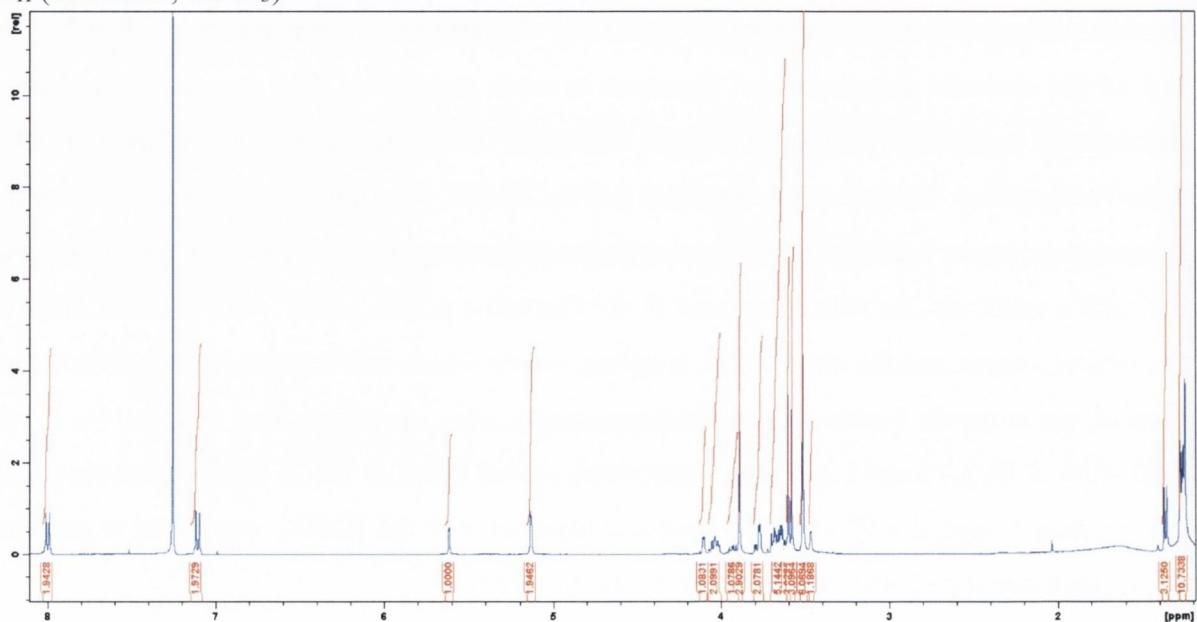


Figure 7.8 HSQC of compound 12 (*p*-HBAD II)

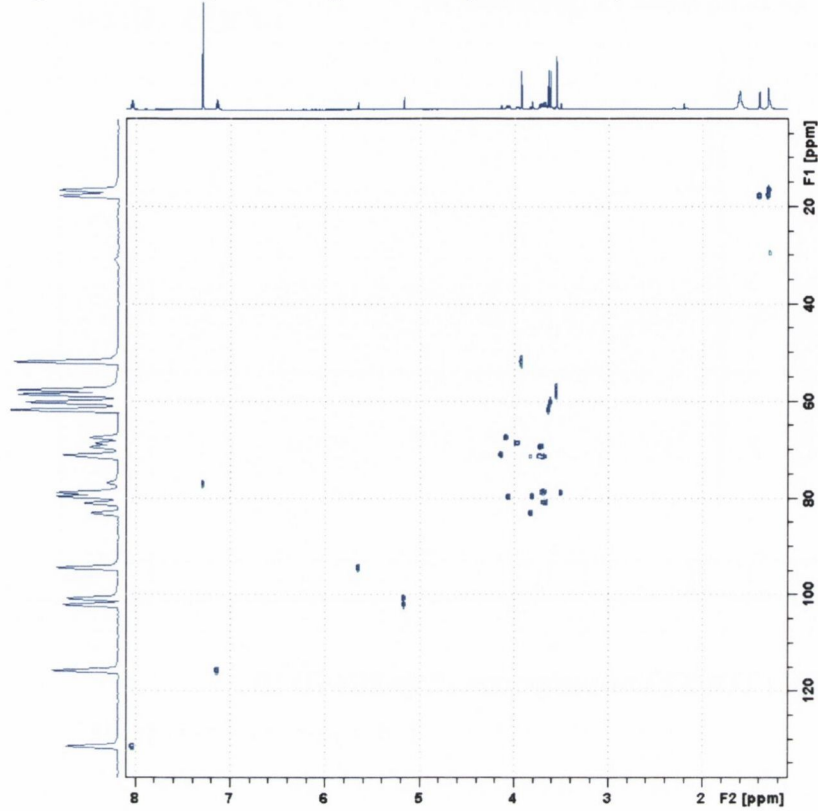


Figure 7.9 HMBC of compound 12 (*p*-HBAD II).

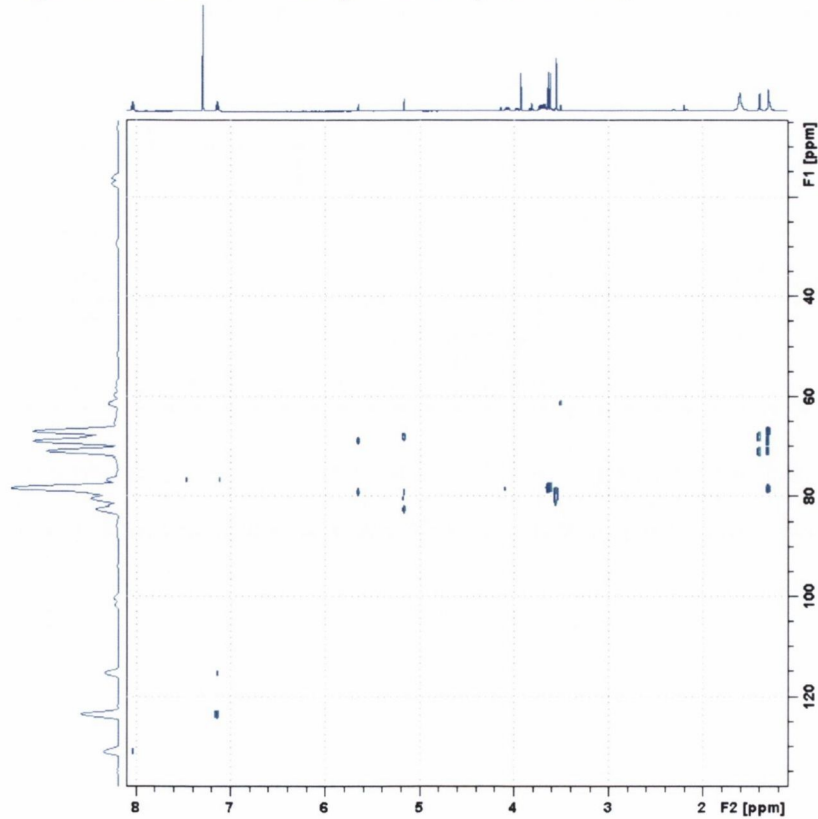


Figure 7.10 First 1D TOCSY of compound 12 (*p*-HBAD II).

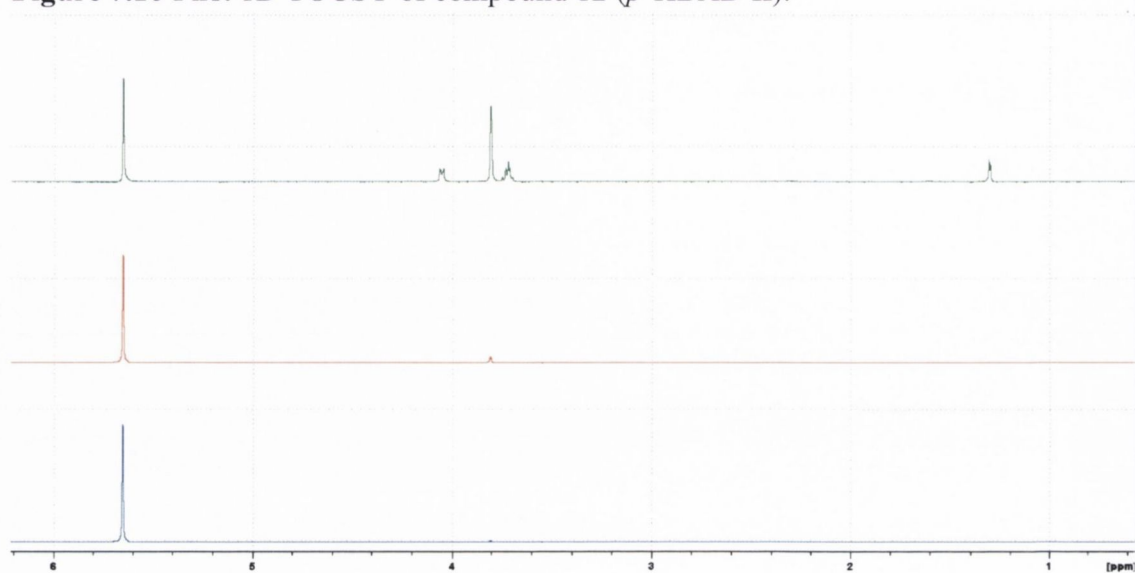


Figure 7.11 Second and third 1D TOCSYs of compound 12 (*p*-HBAD II)

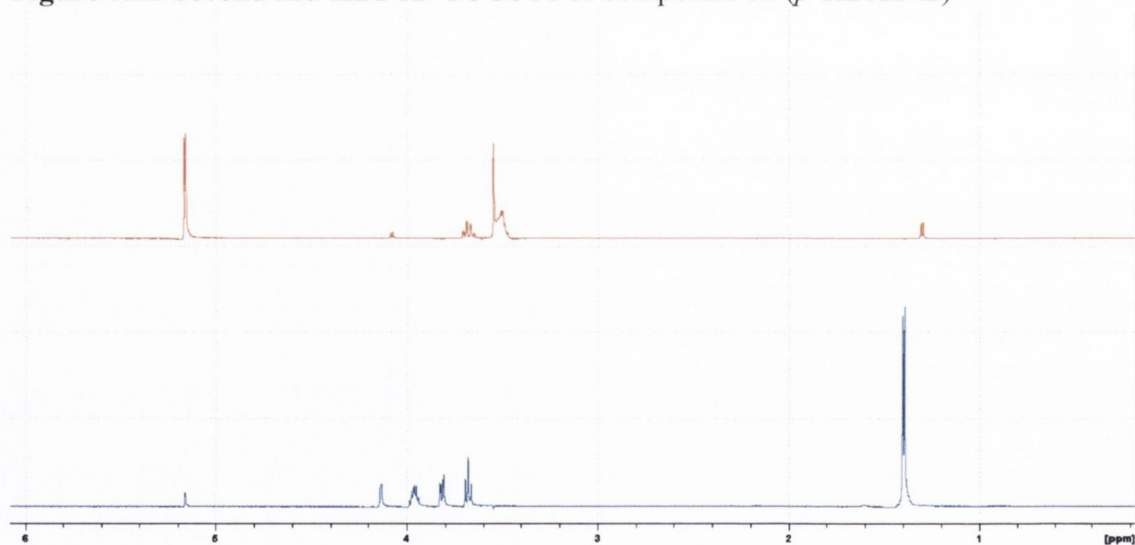
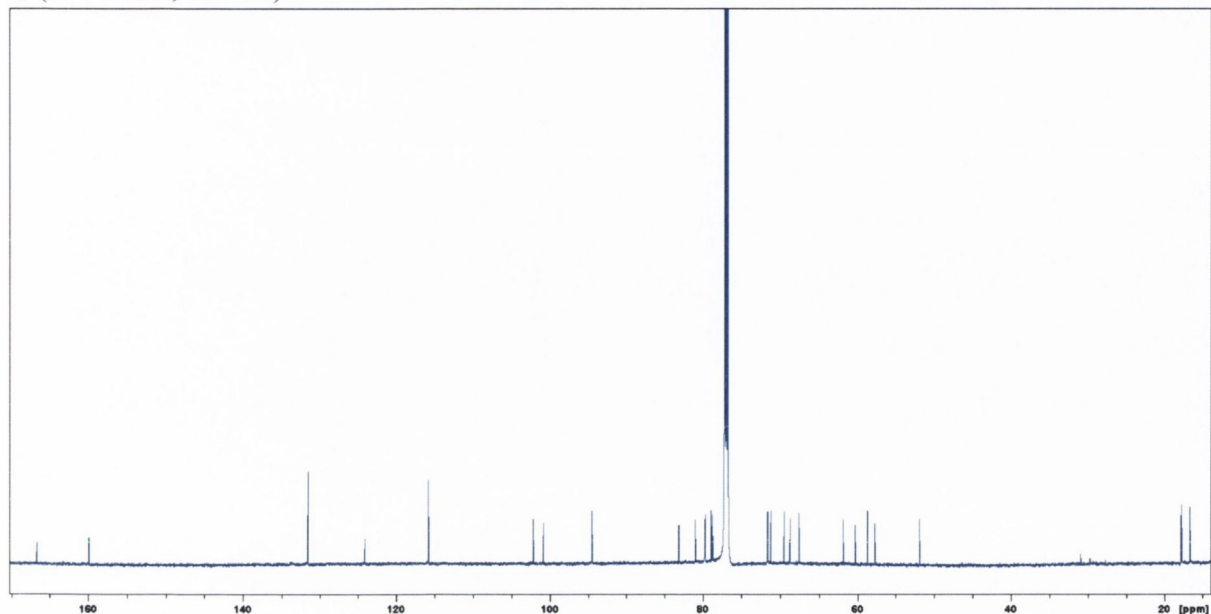


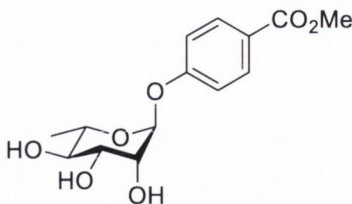
Figure 7.12 ^{13}C spectrum of compound 12, *p*-HBAD II.
 δ_{c} (150 MHz, CDCl_3)



7.3 NMR spectra of compound 13

^1H and ^{13}C NMR spectral data for compound 13 (Figure 7.13) was assigned with the aid of the 2 D techniques HSQC and COSY. The ^1H NMR spectrum for this compound is shown in Figure 7.14, with the COSY in Figure 7.15, the HSQC in Figure 7.16 and the ^{13}C spectrum in Figure 7.17.

Figure 7.13 Compound 13, UM-*p*-HBAD



As with compound 11 the anomeric proton signal and the corresponding carbon signal were clearly distinguishable and are at 5.52 and 98.7 ppm respectively. The slight shift of the H-1 signal downfield compared to compound 11 is due to the absence of a methyl at C-2. Using the COSY the relationship between the different proton peaks was established. The shapes and *J* values for the rhamnosyl proton peaks helped to confirm their assignment as did a comparison with the current literature.¹⁰⁵ The aromatic peaks were clearly distinguishable due to their shifts and the ester methoxy could clearly be seen at 3.86 ppm with its high integration value of three. The only other peak with such a high integration is the H-6 proton signal at 1.21 ppm. Using

the assignment of the proton spectrum together with the HSQC spectrum the assignment of the carbon signals was completed.

Figure 7.14 ^1H spectrum of compound 13, UM-*p*-HBAD

δ_{H} (400 MHz, CD_3OD)

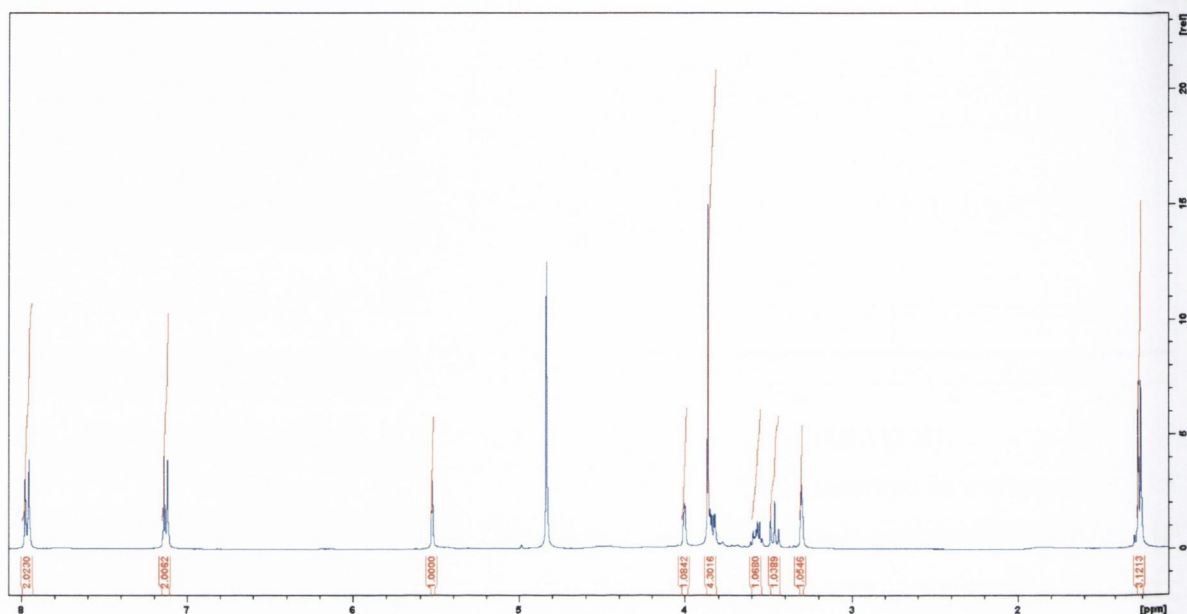


Figure 7.15 H-H COSY of compound 13, UM-*p*-HBAD

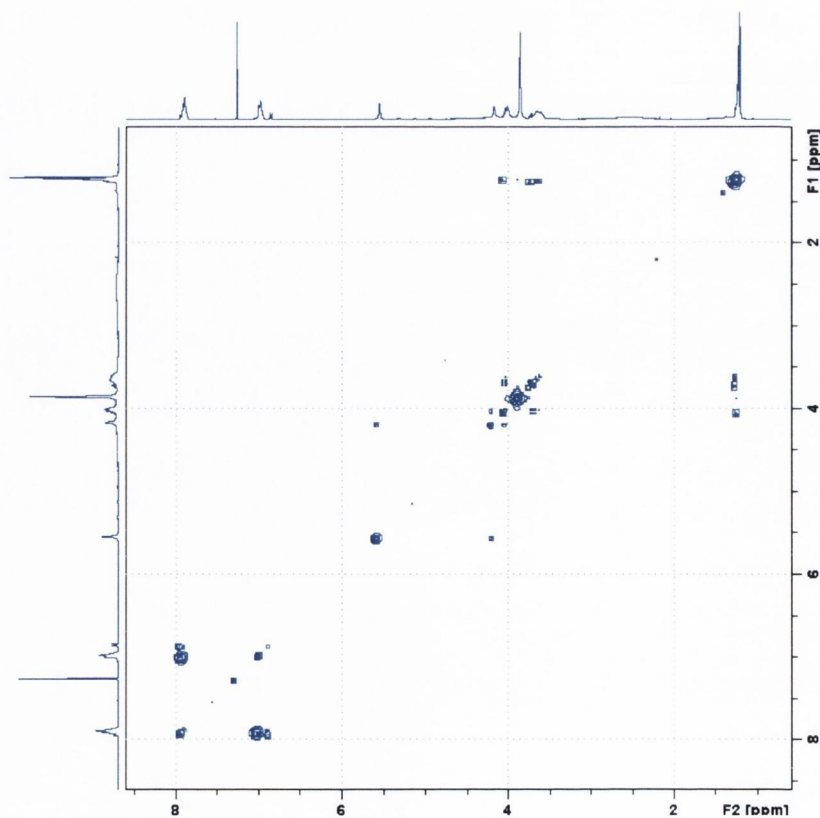


Figure 7.16 HSQC of compound 13, UM-*p*-HBAD

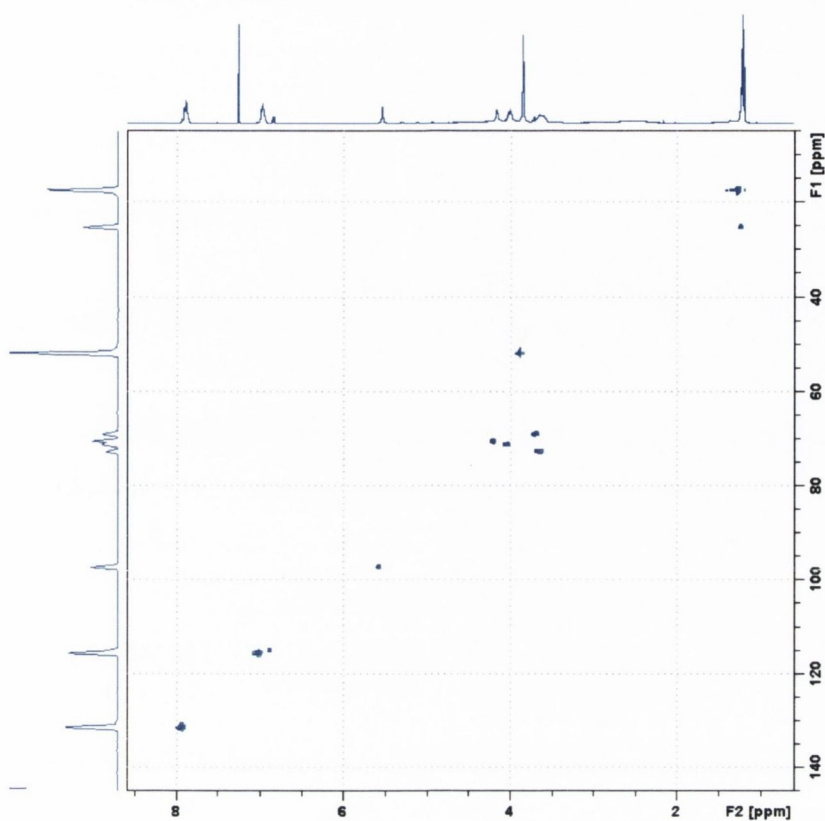
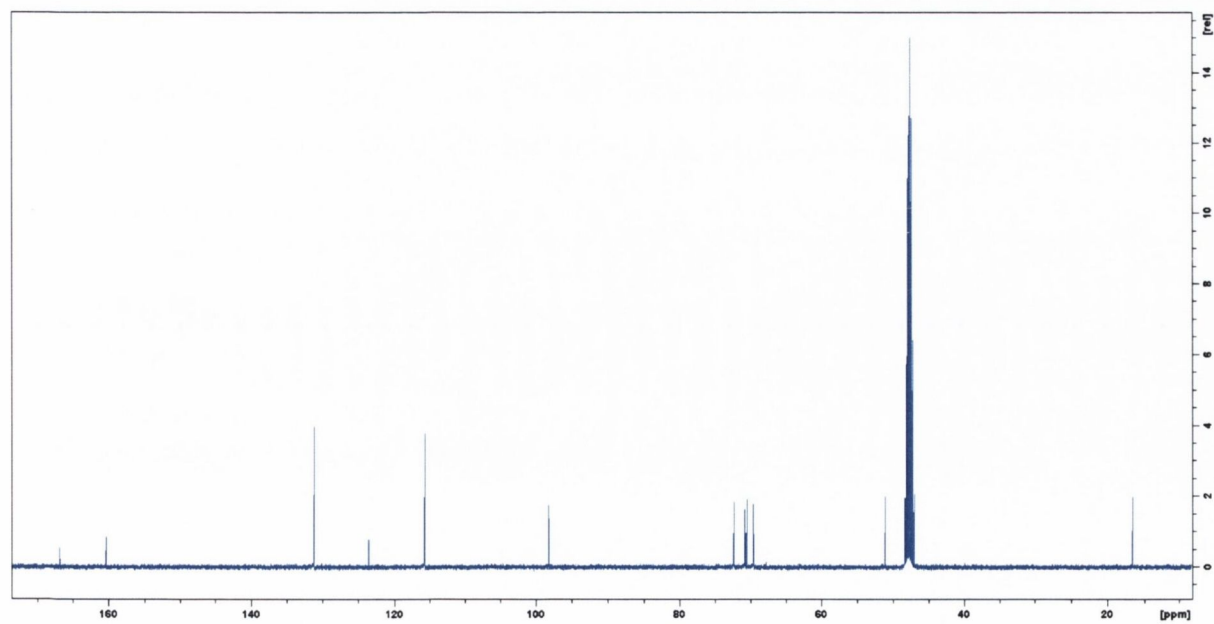


Figure 7.17 ^{13}C spectrum of compound 13, UM-*p*-HBAD
 δ_c (100 MHz, CD_3OD)



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