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Design and Synthesis of Carbohydrate Based Derivatives as Antimicrobial Compounds

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Design and Synthesis of Carbohydrate Based Derivatives as Antimicrobial Compounds

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ABSTRACT

Investigations into the design of analogues of GlcNAc-Ins, the substrate for the enzyme GlcNAc-Ins deacetylase (*msh*B), a therapeutic target on the pathway to mycothiol biosynthesis in *Mycobacterium tuberculosis* are described. Initial studies directed towards the design of a substrate analogue were based on the 3-D structure and a proposed mechanism of action of *msh*B (deduced by Dr. Andrew McCarthy, EMBL). The compounds were designed with the aim to produce an analogue which could better mimic the natural substrate for *msh*B (GlcNAc-Ins) for crystallisation and mechanistic studies to further improve the knowledge of this enzyme.

A series of fatty acid ester and ether derivatives were designed and synthesised based on carbohydrate and non-carbohydrate polyhydroxylated scaffolds with a view to testing their antimicrobial activity against microorganisms of concern to the food and healthcare industries. The synthesised compounds, along with their corresponding fatty acid monoglyceride antimicrobials, were evaluated for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Of the derivatives synthesised several of the carbohydrate based compounds have antimicrobial efficacy comparable with commercially available antimicrobials. The results suggest that the nature of the carbohydrate core plays a role in the efficacy of carbohydrate fatty acid derivatives as antimicrobials.

DECLARATION

I certify that this thesis which I now submit for examination for the award of Doctor of Philosophy, is entirely my own work and has not been taken from the work of others, save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for postgraduate study by research of the Dublin Institute of Technology and has not been submitted in whole or in part for an award in any other Institute or University.

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SYMBOLS AND ABBREVIATIONS

α	alpha
Å	angstrom
Ac	acetate
anhydr	anhydrous
apt t	apparent triplet (spectral)
aq.	Aqueous
β	beta
Bn	benzyl
br s	broad singlet
Bz	benzoyl
c	concentration
cm^{-1}	wavenumber
C=O	carbonyl bond
δ	chemical shift in ppm downfield from TMS
°C	degrees Celsius
d	doublet (spectral)
dd	doublet of doublets (spectral)
ddd	doublet of doublets of doublets (spectral)
equiv.	equivalents
Et	ethyl
FTIR	fourier transform infrared
g	gram(s)
h, min	hour(s), minute(s)

HATU	*N*,*N*,*N*',*N*'-tetramethyl-*O*-(7-azabenotriazol-1-yl)uronium hexaflurophosphate
HAOt	1-hydroxy-7-aza-benzotriazole
Hz	hertz
J	coupling constant (nmr), in Hz
L	litre
lit.	literature reference
LRMS-ES	low resolution mass spectrometry-electrospray ionization
m	multiplet
M	molar
M^+	mass of the molecular ion (mass spectrometry)
Me	methyl
mg	milligram
MHz	megahertz
mL	milliliter
mol, mmol	mole, millimole
μM, mM	micromolar, millimolar
NMR	nuclear magnetic resonance
Ph	phenyl
PMB	paramethoxybenzyl
ppm	parts per million (nmr)
q	quartet (spectral)
R_f	retention factor
rt	room temperature

$[\alpha]_D$	specific rotation
s	singlet (spectral)
t	triplet (spectral)
TIPS	triisopropylsilyl
t.l.c.	thin layer chromatography
Trityl	triphenylmethyl

SOLVENTS AND REAGENTS

AcOH	acetic acid
Ac_2O	acetic anhydride
$AlCl_3$	aluminium chloride
$BF_3{\cdot}Et_2O$	boron trifluoride·etherate
BnBr	benzyl bromide
$BrCH_2OCH_3$	bromomethyl methylether
Bu_4NI	tetrabutylammonium iodide
Bu_2SnO	dibutyltin oxide
$Bu_2Sn(MeO)_2$	dibutyltin dimethoxide
BzCl	benzoyl chloride
CAN	cerric ammonium nitrate
$CDCl_3$	deuterio chloroform
$CHCl_3$	chloroform
CH_2Cl_2	dichloromethane
CSA	camphorsulfonic acid
$CuSO_4.5H_2O$	copper sulfate pentahydrate

DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-*N*,*N* dimethylaminopyridine
DMF	*N*,*N* dimethylformamide
DMSO	dimethylsulfoxide
D_2O	deuterium oxide
EtOAc	ethyl acetate
EtOH	ethanol
Et_2O	diethylether
H_2	hydrogen gas
HCl	hydrochloric acid
H_2O	water
H_2SO_4	sulfuric acid
Im.	Imidazole
IR 120 (H^+)	ion exchange resin (IR 120+)
K_2CO_3	potassium carbonate
LaCl	lauroyl chloride
$LiAlH_4$	lithium aluminium hydride
MeCN/CH_3CN	acetonitrile
MeOH	methanol
4-MeOPhCH$(OMe)_2$	4-methoxy benzaldehyde dimethyl acetal
$MgSO_4$	magnesium sulfate
MOMCl/CH_3OCH_2Cl	chloromethyl methylether
NaCl	sodium chloride

$NaCNBH_3$	sodium cyanoborohydride
NaH	sodium hydride
$NaHCO_3$	sodium hydrogen carbonate
NaOH	sodium hydroxide
NaOMe	sodium methoxide
NAP	2-naphthylmethyl
NH_4Cl	ammonium chloride
NIS	*N*-iodosuccinimide
Pd-C	palladium on charcoal
$PhCH(OMe)_2$	benzaldehyde dimethylacetal
PhH	benzene
PhSOTf	phenylsulphenyl triflate
PMBCl	paramethoxybenzyl chloride
PPL	porcine pancreatic lipase
Py	pyridine
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPSCl	triisopropylsilyl chloride
TMSOTf	trimethylsilyl triflate
TMSCl	trimethylsilyl chloride
TrCl	triphenylmethyl chloride
TsOH/*p*TSA	*p*-toluenesulfonic acid

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1. Introduction

This thesis reports the design and synthesis of novel carbohydrate derivatives both as potential mycothiol biosynthetic enzyme substrate mimics and carbohydrate fatty acid conjugates with a view to testing their antimicrobial activity against microorganisms of concern to the food and healthcare industries.

Carbohydrates are the most abundant and the most diverse biopolymers in nature. Due to their highly specific interactions with physiological receptors, they participate in many crucial biological processes. All these processes are potential targets for therapeutic intervention and carbohydrate-based drugs are rapidly being engaged by the modern biotechnology and pharmaceutical industry.[1] Chemists and biochemists have developed new methods to rapidly synthesise oligosaccharides, enabling them to generate complex polysaccharides and analogues of natural products. Biologists have explored the physiological roles of various sugars, discovering that many play essential roles in the functioning of the major organ systems and are involved in several disease states. In addition to extending our knowledge, these findings have enabled the development of carbohydrate-based drugs and vaccines.[2]

1.1 Medicinal Chemistry and Rational Drug Design

The need for ongoing development of new drugs needs little emphasis in light of the current global situation of health and disease. Traditionally, the process of drug development has revolved around a screening approach as there can be uncertainty as to which compound or approach could serve as a drug therapy. The structure-assisted ("rational") drug design and discovery process utilizes techniques such as protein

crystallography, nuclear magnetic resonance and computational biochemistry to guide synthesis of potential drugs.[3,4,5] Structural information is used to help explain the basis of effective inhibition and to improve the potency and specificity of new lead compounds.[3] Rational drug design thus aims to realise a preconceived pharmacological activity in a directed way. This approach is based on the very reasonable assumption that if the structure of a natural ligand and its receptor is known, molecules which could mimic or antagonise the activity of the natural ligand could be synthesised.[6]

The first success story in structure-based design was the antihypertensive drug, captopril (Capoten®, Lopirin®, Squibb, now Bristol-Myers Squibb), an angiotensin-converting enzyme (ACE) inhibitor (Figure 1-1). The structure of captopril was derived in a rational manner from a binding site model, using the 3D information of the complex of benzylsuccinate with the closely related zinc proteinase carboxypeptidase A.[7]

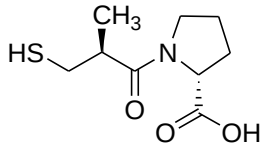


Figure 1-1 Captopril (Squibb, now Bristol-Myers Squibb).[7]

Another successful example of structure-based drug design leading to the development of an approved drug is Cimetidine, a histamine H_2-receptor antagonist that inhibits the production of acid in the stomach. This was one of the earliest drugs developed using a rational drug design approach.

1.1.1 Cimetidine – A Rational Approach to Drug Design

In 1964, Smith Kline & French (now GlaxoSmithKline) initiated an unconventional approach to seek a novel type of agent to block the action of histamine (Figure 1-2) as a stimulant of gastric acid secretion. It was hypothesized that histamine as a secretagogue acted at a hitherto uncharacterized histamine receptor (later designated as H_2).[8]

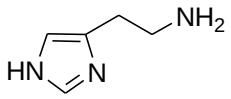


Figure 1-2 Histamine.

Histamine served as the chemical starting point for drug design and systematic structural modifications of this compound led to the early antagonists which were all imidazole derivatives. The earliest compound used as an inhibitor of this class of histamine H_2-receptors was burimamide (Figure 1-3), a thiourea derivative of a histamine homologue. Despite the activity of this compound being rather weak, it was used to verify that H_2-receptors did indeed mediate gastric acid secretion (reported in 1972).[9]

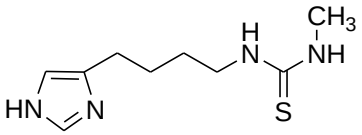


Figure 1-3 Burimamide.

The development of burimamide was followed by metiamide (Figure 1-4), which was designed to alter the electronic properties of the imidazole ring of burimamide by including an electron-releasing methyl group in the imidazole and an electron-

withdrawing isosteric sulfur replacement for CH_2 in the side chain. Metiamide was orally active, however it was suspended from clinical trials due to side effects including incidence of granulocytopenia, an abnormally low concentration of granulocytes in the blood which can reduce the body's resistance to many infections.[8]

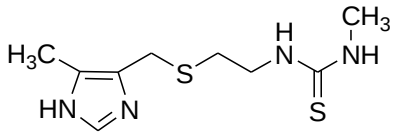


Figure 1-4 Metiamide.

The hypothesis that these side effects may have stemmed from the thioureido group led to attempts to replace the thiourea moiety. These investigations led to the cyanoguanidine analogue cimetidine (Figure 1-5), in which cyanoimino replaces thione sulfur.

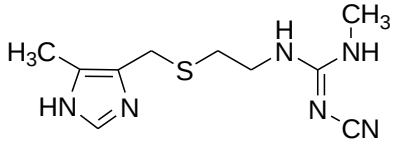


Figure 1-5 Cimetidine (Trade name Tagamet®).

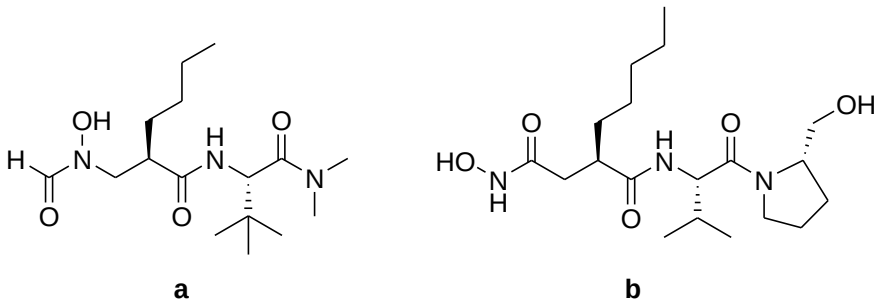
Cimetidine closely resembled metiamide but did not exhibit the granulocytopenia side effect. It was marketed as Tagamet® at the end of 1976, as the pioneer drug that revolutionised the treatment of peptic ulcer disease.[8]

1.1.2 The Role of Combinatorial Chemistry in Rational Drug Design

Combinatorial Chemistry has impacted greatly on mechanism-based drug discovery projects. Structural or functional information about the target is used to define a

generic structure that a putative inhibitor may resemble. The starting point is not a known chemical but rather theoretical pharmacophores. Combinatorial libraries are then used in order to explore this concept and to identify hits that may be used as the starting points for the next generation of libraries. This process requires an extensive knowledge of the structure and the biological properties of the target.[10]

The bacterial enzyme peptide-deformylase (PDF) is an example of how the successful combination of mechanism-based drug design with focused libraries can lead to the development of an entirely new class of antibiotics. PDF is an essential bacterial metalloenzyme which deformylates the *N*-formylmethionine of newly synthesised polypeptides. Its 3D structure has been determined.[11,12] To identify novel PDF inhibitors, Clements *et al.* screened a metalloenzymes inhibitor library and identified an *N*-formylhydroxylamine derivative, BB-3497 and a related natural hydroxamic acid antibiotic, actonin, as potent and selective inhibitors of PDF (Figure 1-6).[12]



a **b**

Figure 1-6 Structures of (a) BB-3497 and (b) Actonin, inhibitors of PDF.[12]

Actonin and BB-3497 showed minimum inhibitory values (MIC) of 32 μg/mL and 4-16 μg/mL respectively against *Staphylococcus aureus*. Combinatorial libraries played a major role in these research discovery projects.[10]

1.1.3 The Role of Carbohydrates in Rational Drug Design

As bacterial, viral and fungal drug resistance to currently administered treatments increases, the need for the development of new therapeutic strategies against infectious agents becomes more urgent. Carbohydrates are ubiquitous and important biomoloecules forming much of the structural framework of cells and tissues. As components of glycoproteins, glycolipids and other conjugates, they are key elements in a variety of processes such as signalling, cell-cell communication and molecular and cellular targeting.[13] Crucial to the survival of many pathogens are carbohydrate structures which are either themselves structurally unique or specific to non-mammalian organisms. Indeed inhibition or interference with biosynthesis of oligosaccharide materials represents an attractive and potentially highly selective strategy for the development of new classes of therapeutic agents.[14] Carbohydrates provide an excellent platform to tailor molecular diversity by appending desired substituents at selected positions around the sugar scaffold. The presence of five functionalised and stereo-controlled centres on the sugar scaffolds gives the chemist scope to custom design molecules to a pharmacophore model.[15]

1.1.3.1 Heparin

Heparin is the oldest carbohydrate-based drug and is isolated from animal sources. It has been used clinically as an antithrombotic agent since the 1940s. Heparin activates the serine protease inhibitor antithrombin III (AT), which blocks the two principle procoagulant proteases, thrombin and factor Xa, in the coagulation cascade. The rate of inactivation of these proteases by AT can increase by up to 1000-fold as a result of the binding of heparin.[16]

This drug is a highly heterogeneous mixture of polysaccharides and is associated with severe side effects including bleeding and allergic reactions. Chemically or enzymatically fragmented heparins (low-molecular-weight heparins, LMWHs) are also heterogeneous, but have greater bioavailability, longer half-life, more predictable anticoagulant activity and fewer side effects.[17]

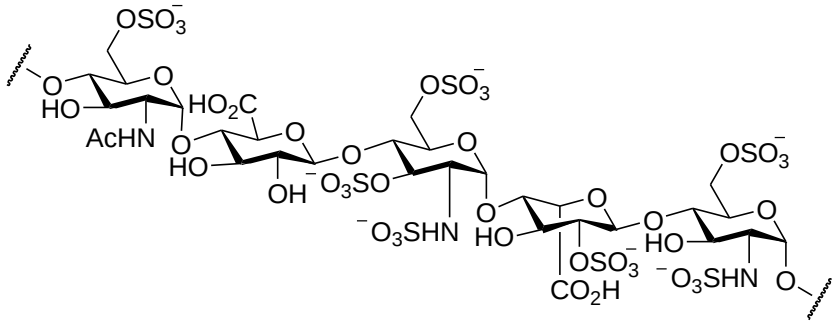


Figure 1-7 A pentasaccharide sequence of heparin. This sequence is responsible for binding to antithrombin III.[17]

After the specific pentasaccharide responsible for the anticoagulant property was identified in the early 1980s (Figure 1-7), a vast effort lasting more than 10 years began to establish a structure-function relationship using synthetic oligosaccharides. [18] As a result of this effort a synthetic pentasaccharide known as Arixtra (fondaparinux sodium; GlaxoSmithKline) has been available since 2002.[19]

1.1.3.2 Challenges of Carbohydrate Synthesis

The structural complexity that makes carbohydrates important in so many biological processes sometimes renders their chemical synthesis difficult. Though it is possible to synthesise pure oligosaccharides in the laboratory, the regioselective protection of hydroxyl groups and the stereoselective assembly of glycosidic bonds presents a

number of challenges for synthetic chemists.[20] The same regioselectivity challenges are also encountered when working with monosaccharides.

The preparation of complex carbohydrates requires the strategic placement of protective groups that mask hydroxyl groups. Whilst the ability to remove one protecting group in the presence of another is a key feature, the steric and electronic nature of the protecting groups are also important as they greatly influence both the reactivity of the building blocks and the outcome of any glycosylation reactions. The properties of the protecting group next to the anomeric centre are very important. For example, whether this group is participating or non-participating plays a significant role in the control of glycoside stereochemistry.[21] Therefore, protecting groups on sites near the anomeric centre must be chosen carefully.

The biggest challenges in carbohydrate synthesis are not only concerned with the stereoselective glycosylation of two or more sugars. The preparation of monosaccharide derivatives must also be considered. In this case protecting group manipulation is also essential to afford a regioselectively protected saccharide so that other functional groups can be introduced at the desired position. Some protection strategies are the same for a number of monosaccharides for example glucose, mannose and galactose. This is generally the case for pathways to free primary hydroxyl groups. Standard methods for the selective protection of this position include tritylation or silylation followed by acetylation or benzylation of the remaining secondary hydroxyls. The primary protecting group can then be selectively removed.[22]

Since the reactivity differences between secondary hydroxyls are not that large, one-step selective protection is challenging. Some methods developed for this purpose include Stannyl activation. By reacting carbohydrate hydroxyl groups with tin oxide reagents, stannylene ethers and acetals are formed. These stannylenes increase the nucleophilicity of the oxygens so that successive acylation or alkylation can be performed regioselectively.[23]

1.2 ENZYME INHIBITION

Development of inhibitors that are effective for specific enzymes that are involved in pathologically or physiologically important metabolic processes constitutes a viable approach for new drug discovery. In fact a majority of therapeutic agents in current use are inhibitors of enzymes.[24]

Enzyme inhibition involves the addition of a molecule that interacts with the enzyme in order to change its conformation or directly prevent substrate binding. In the latter case the inhibitor binds to the same site of the enzyme as the substrate. It binds only to the free enzyme and is usually structurally similar to the substrate. For example, succinate is the normal substrate for the enzyme succinate dehydrogenase and malonate is an effective competitive inhibitor of this enzyme (Figure 1-8).[25]

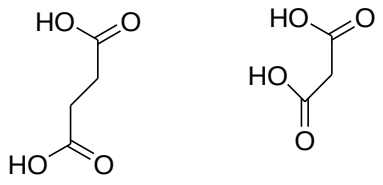


Figure 1-8 Substrate and inhibitor for succinate dehydrogenase.

Other strategies for enzyme inhibition involve the substrate being replaced with a non-cleavable isostere. For example, the following transition state mimics are commonly used to replace cleavable amide bonds (Figure 1-9).

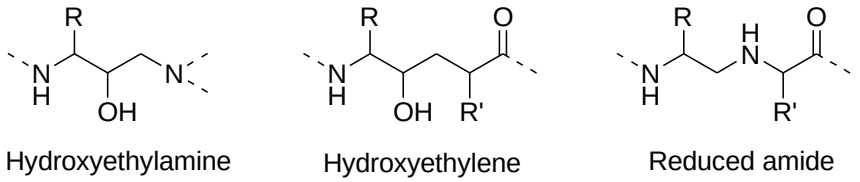


Figure 1-9 Structure of some common transition-state isosteres that have been used to replace scissile amide bonds in the development of aspartic protease inhibitors.[26]

1.2.1 Metalloenzymes

Metals play a role in approximately one-third of known enzymes. Amino acids in peptide linkages possess groups that can form coordinate-covalent bonds with the metal atom. This binding to the metal may affect the enzyme's structure resulting in its active conformation. The main function of the metal is to serve in electron transfer.[27]

Metalloenzymes are among the best studied of enzyme classes and there are excellent precedents for the mechanism-based design of their inhibitors.[26] Given the mechanistic resemblance between different metalloenzymes, the tools and knowledge developed for identifying inhibitors of one metalloenzyme can be easily applied to others of the same class which can eventually reduce the overall time and cost of identifying inhibitors of each member of this group.[28]

1.2.2 Zinc Containing Enzymes

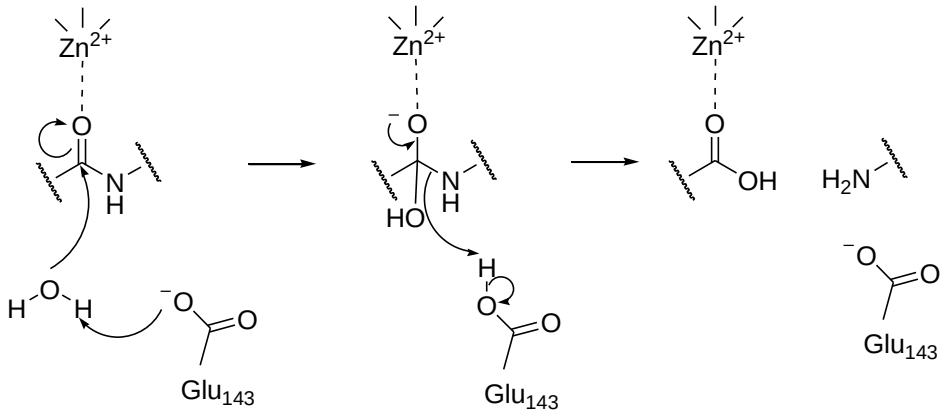
Metal ions are required for the growth of all life forms.[29,30,31,32] Zinc is one of the most abundant divalent metals in living organisms.[33] It is an essential cofactor of many metabolic enzymes and transcription factors. [31,34,35] Zinc enzymes are metalloproteins with specific enzymatic activity which may contain a single Zn (II) ion, several Zn (II) ions or different metal ions including Zn (II). In principle, the zinc ion can be functional and necessary for the catalytic action or structural and therefore not involved in the catalytic mechanism but necessary to maintain the protein in the proper conformation. Some zinc enzymes display hydrolytic activity and catalyse bond cleavage in which a reactant is water or its H^+ or OH^- components. Typical of this class of enzymes are proteases which hydrolyse peptide bonds and deacetylases which catalyse the removal of an acetyl group.[36]

1.2.2.1 Zinc Protease

All known metalloproteases use a zinc atom to effect amide bond hydrolysis. Mechanistically, the zinc-dependent metalloproteases are among the most widely studied members of the zinc hydrolase family. Zinc metalloproteases are involved in various key biological roles and as a consequence many of these enzymes are targets for the development of therapeutic agents.[37]

The design of metalloprotease inhibitors for adoption as therapeutics has become commonplace. This has also led to a deeper understanding of enzymatic action.

Thermolysin was one of the first metalloproteases to have its structure solved crystallographically. This revealed that this enzyme possessed an active-site zinc ion that activates the scissile amide bond (Scheme 1-1) toward the attack by water and stabilises the resulting tetrahedral intermediate. Collapse of the tetrahedral intermediate reforms the carbonyl group and protonation of the nitrogen from the glutamic acid generates the free amine of the cleaved substrate.[38]



Scheme 1-1 Mechanism of hydrolysis of peptides catalysed by the active-site zinc in Thermolysin.[39]

At the centre of metalloprotease inhibitor design is the selection of a functional group that will interact with the metal (Figure 1-10).[38]

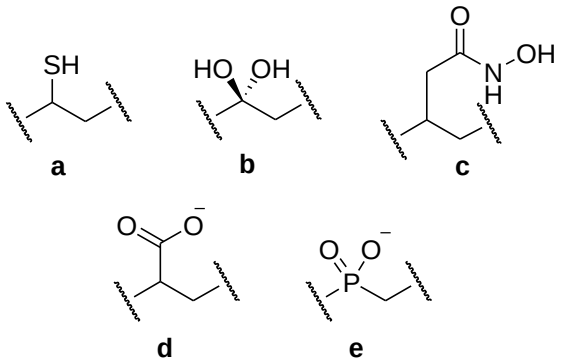
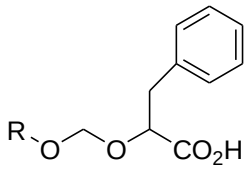


Figure 1-10 (a) Thiols, (b) Hydrated ketones, (c) Hydroxamic acids, (d) Carboxylates, (e) Phosphinates.[38]

1.2.2.2 Recent Zinc Protease Inhibitors

Carboxypeptidase A (CPA) serves as a prototypic enzyme of the zinc enzyme family.[35] This enzyme, including its active site structure and catalytic mechanism has been intensively studied. The mechanism is similar to that already outlined in Scheme 1-1 for Thermolysin.[40]

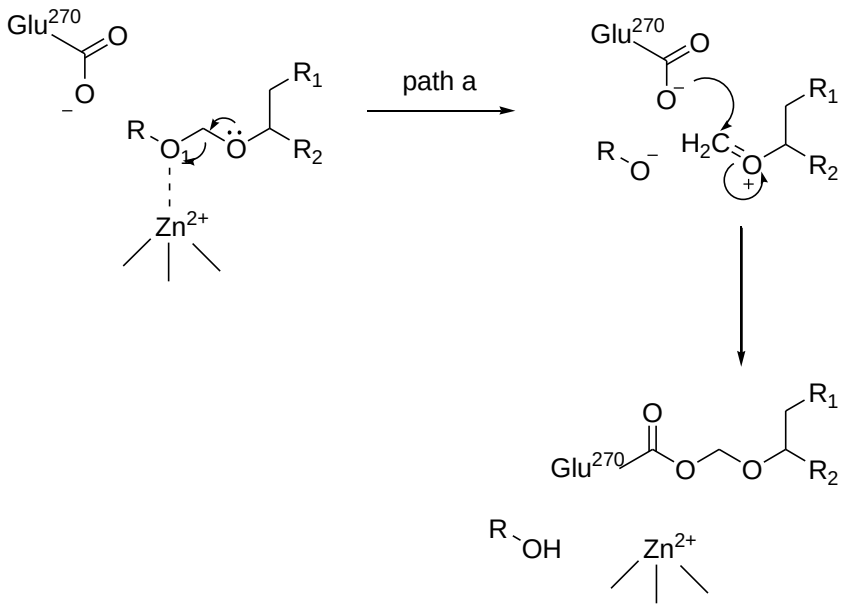
This enzyme has also been used as a model for the development of inhibitor design strategies that can be applied to other zinc-containing enzymes that are less well understood.[41,42] Min Su Han *et al.* reported a novel strategy that they developed with CPA.[24] Compounds **a-c** shown in Figure 1-11 are the potential inhibitors they designed for CPA.



a; R = CH_3
b; R = CH_2Ph
c; R = CH_2CH_2Ph

Figure 1-11 Inhibitors for CPA.[24]

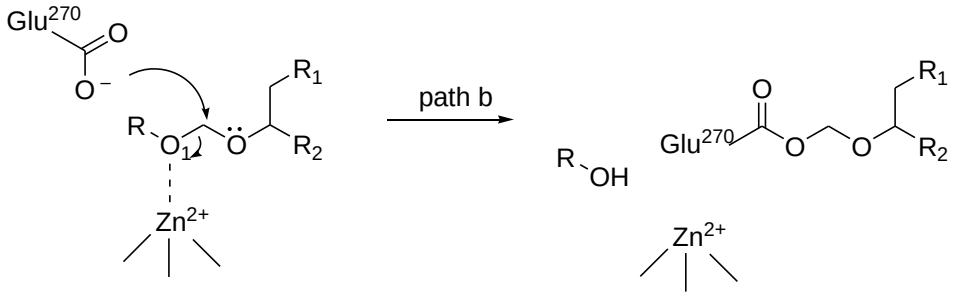
The inhibitors were synthesised readily and their activity towards CPA was evaluated. The compounds inhibited CPA in a time-dependent manner, suggesting that the inhibition occurs in an irreversible fashion.[24] These CPA inhibitors were designed on the basis of the following rationale.



Scheme 1-2 CPA enzyme inhibition path a.[24]

The O_1 of the inhibitor may be in close proximity to the zinc ion at the active site of CPA leading to the formation of a coordinate bond to the metal ion. Just as protonated acetals break down to oxacarbenium ions in acetal hydrolysis, the O_1 coordinated to the metallic Lewis acid may undergo C-O bond cleavage with the generation of an oxacarbenium ion intermediate. The oxacarbenium ion thus generated would undergo a bond formation reaction with the carboxylate of Glu-270 to result in the covalent modification of the catalytic nucleophile (Scheme 1-2). The covalently modified enzyme can no longer perform its enzymatic function.

Alternatively, the reaction path that involves nucleophilic attack of the Glu-270 carboxylate at the acetal of the inhibitor in an S_N2 fashion may also be possible (Scheme 1-3).[24]

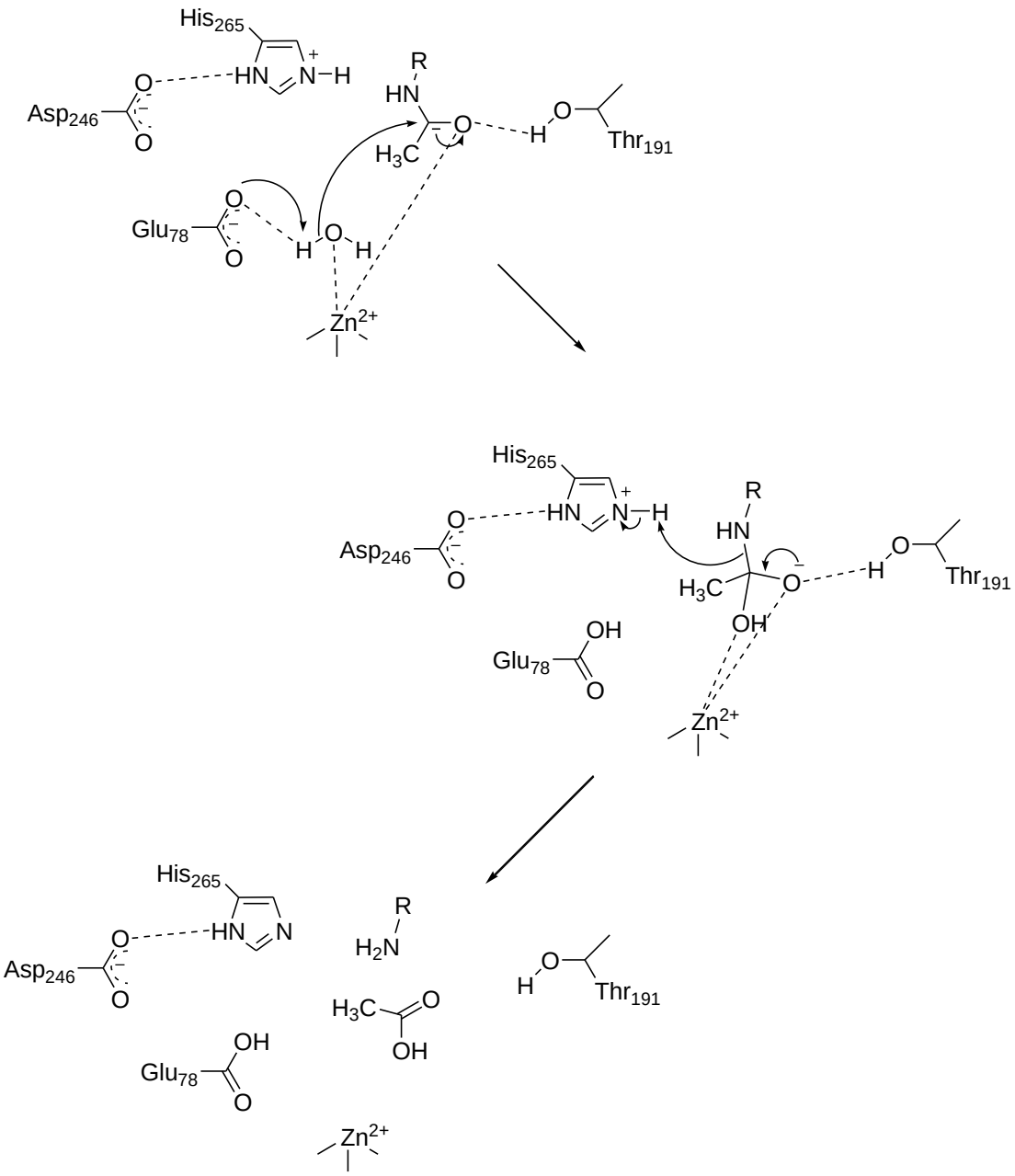


Scheme 1-3 CPA enzyme inhibition path b.[24]

1.2.2.3 Zinc Deacetylase

A typical example of a zinc deacetylase is LpxC. This enzyme catalyses the second step in the biosynthesis of Lipid A, an important outer cell-membrane component found in a number of pathogenic bacteria including *Escherichia coli* and *Salmonella typhimurium*. LpxC catalyses the removal of an acetate from nitrogen in UDP-3-*O*-acyl-*N*-acetylglucosamine and its inhibition leads to diminished production of Lipid A. Therefore it is an attractive target for antibiotic design.[43]

There are two proposed mechanisms for the reaction catalysed by LpxC. In the first, LpxC might function via a Thermolysin-like mechanism (Section 1.2.2.1). Alternatively, there is evidence consistent with the involvement of two residues, Glu78 and His265, functioning as a general acid/base pair (Scheme 1-4).[37]



Scheme 1-4 Proposed mechanism for deacetylation catalysed by LpxC.[44]

In this proposed mechanism, Glu78 accepts a proton from the Zn^{2+}-bound water, while His265-H^{+} protonates the nitrogen in the intermediate, thus leading to cleavage of the amide bond.

Recently Robinet *et al.* investigated the binding of the substrate within the active site and the deacetylase mechanism it catalyses and confirmed that LpxC uses a general acid/base pair mechanism as indeed both His265-H^+ and Glu78 are involved.[44]

1.3 TUBERCULOSIS

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB).[45] Globally, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive people.[46] TB is the major cause of death from a single infectious agent among adults in developing countries and there has been a recent revival of TB in the industrialised world.[47] The World Health Organisation (WHO) estimates that approximately one third of the world population harbours latent infection with TB and as a result it has been declared a global emergency.[46]

The mycobacterium species has been associated with AIDS/HIV and therefore, in recent years has attracted unprecedented attention.[48] The impact of tuberculosis as an opportunistic infection on developing countries has been highly significant due to increasing incidences of AIDS/HIV in these countries. For example in sub-Saharan Africa where TB notifications have on average trebled since the mid 1980's.[49] Worldwide roughly 15 million people are already co-infected with HIV and *M. tuberculosis* and 2 million new cases are added each year.[50]

Until approximately 50 years ago, there were no effective therapeutics against TB. Whilst the use of antibiotics revolutionised TB treatment, currently strains that are resistant to at least one drug have been documented in every country surveyed by the

WHO. Moreover, strains of TB resistant to all major anti-TB drugs have emerged.[45] Multidrug-resistant tuberculosis, defined as resistance to the two most important drugs isoniazid (Figure 1-12) and rifampicin (Figure 1-13), is a growing problem among HIV-infected patients.[51] The latter of these two drugs was the one most recently introduced in 1968.[52] Decades of misuse of existing antibiotics and poor compliance with a prolonged TB drug regimen that involves four different drugs administered for two months followed by two drugs for an additional four months have created an epidemic of drug resistant TB.[53]

The mechanism of action of isoniazid, as well as mechanisms conferring isoniazid resistance are complex. Evidence suggests that isoniazid inhibits mycolic acid biosynthesis. Mycolic acids are long-chain α-alkyl β-hydroxy fatty acids which are an essential component of the mycobacterium cell wall.[54] Isoniazid (Figure 1-12) is activated by the mycobacterial enzyme *Kat*G, a multifunctional catalase-peroxidase. Consequently, mutant *Kat*G deficient MTB strains are invariably isoniazid resistant.[55]

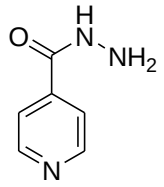


Figure 1-12 Structure of Isoniazid.[56]

Rifampicin (Figure 1-13), first introduced in 1968 as an antitubercular drug is extremely effective against *M. tuberculosis* and is a key component of anti-TB therapy.[57] However, resistance to rifampicin is increasing because of widespread application that results in the selection of mutants resistant to other components within short-course chemotherapy. Rifampicin targets the mycobacterial RNA

polymerase. RNA polymerase is a complex oligomer composed of four different subunits (α,β,β',σ, encoded by *rpo*A, *rpo*B, *rpo*C, and *rpo*D, respectively) of which rifampicin inhibits the essential *rpo*B gene.[58] It was found that in *E. coli* most mutants in *rpo*B appeared to be resistant to all rifampicins tested.[59]

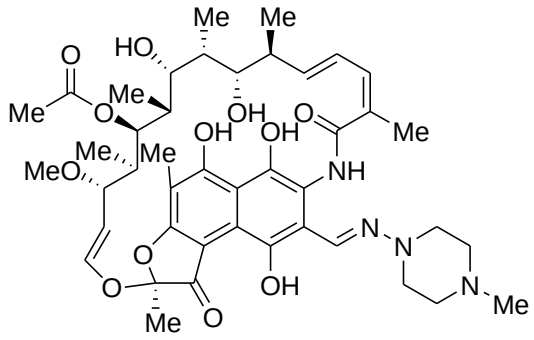


Figure 1-13 Structure of Rifampicin.[58]

It has been estimated that approximately 1 billion more people will be infected with TB in the next 20 years.[60] Clearly the lack of new anti-tuberculosis drugs is a significant problem since the frequency of antibiotic-resistant TB is growing. In order to develop new anti-tuberculosis drugs, novel metabolic pathways or metabolic intermediates used by the bacteria have been sought. One such potential target is mycothiol (MSH), the reducing agent exclusively present in the order actinomycetes to which *M. tuberculosis* belongs.[61]

1.3.1 Mycothiol and the MSH Cycle

Intracellular thiols are biomolecules involved in critical roles in living organisms. These roles include controlling intracellular redox potential and acting as cofactors for vital detoxification enzymes.[62]

The cysteine-containing tripeptide glutathione acts as a key protective agent in eukaryotes and Gram-negative bacteria against damaging peroxides and other reactive oxygen species (Figure 1-14).[63]

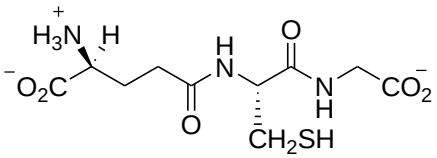


Figure 1-14 Glutathione.[62]

Bacteria of the actinomycetes order which include the pathogenic mycobacteria and antibiotic producers such as streptomycetes, do not produce glutathione but instead use mycothiol.[62] Mycothiol (1-D-myo-inositol 2-deoxy-2-(N-acetamido-L-cystein-amido-α-D-glucopyranoside, MSH (Figure 1-15), is a low molecular weight thiol produced only by actinomycetes.[64] MSH comprises one unit 1-*myo*-D-inositol (*myo*-D-Ins), glucosamine (GlcN) and *N*-acetyl-cysteine.

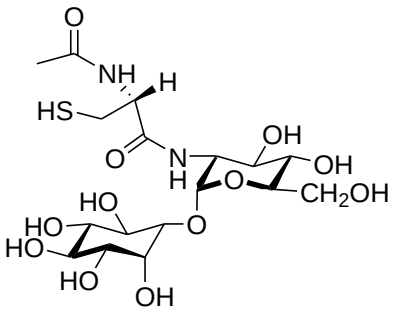
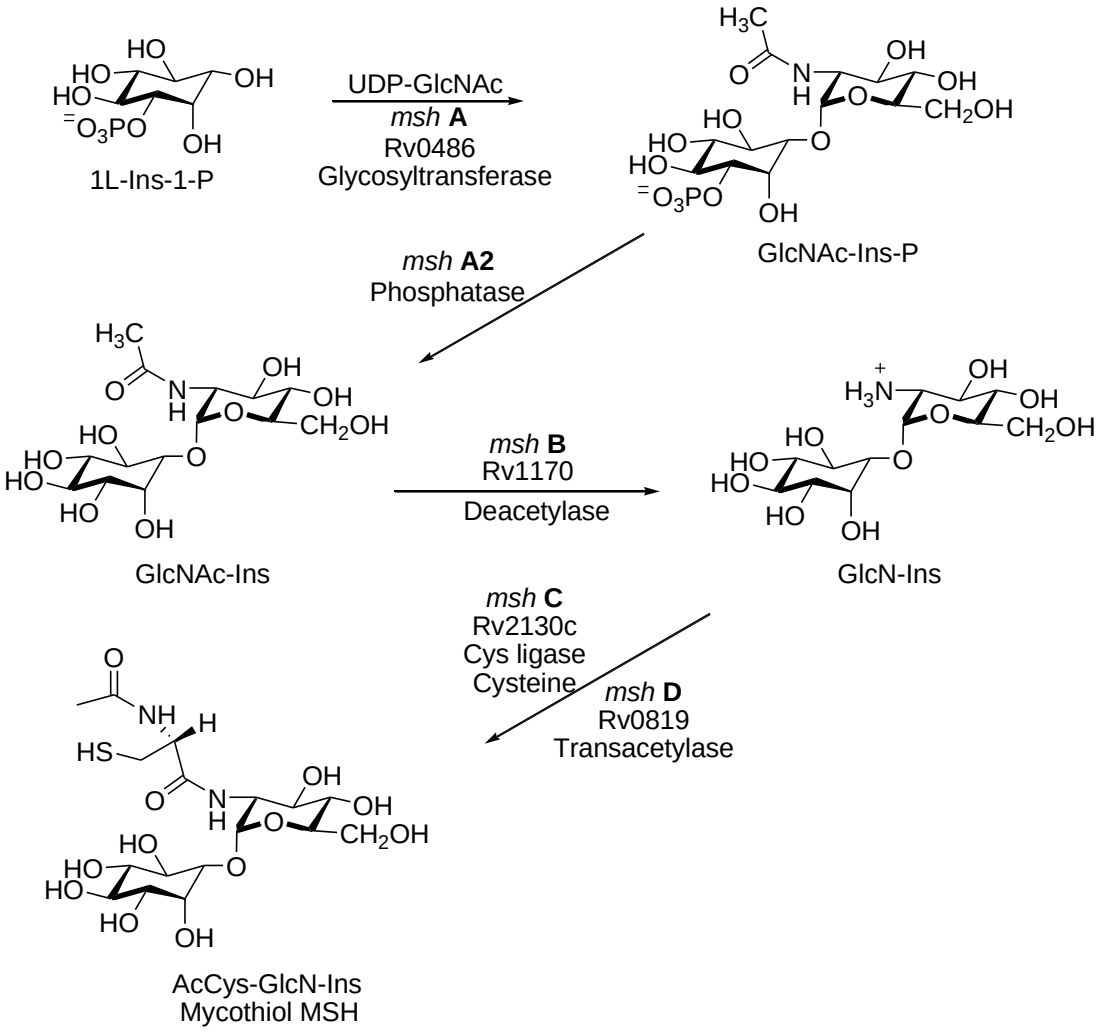


Figure 1-15 Mycothiol.[65]

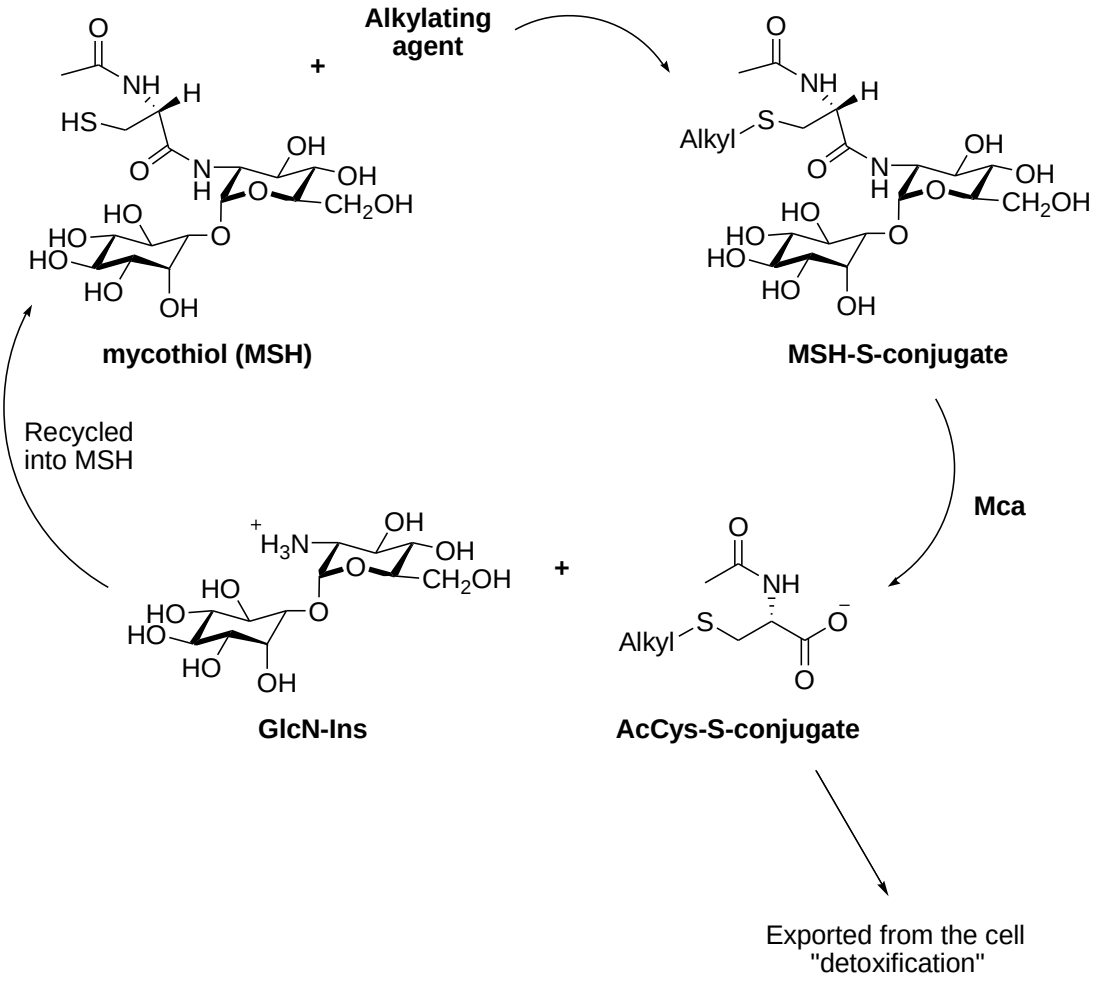
MSH is important in that it appears to play an analogous role to glutathione in maintaining a reducing intracellular environment and facilitating detoxification from alkylating agents and other toxins.[64]

Of all Gram-positive species tested, mycobacteria appear to generate the highest levels of MSH.[66] In mycobacteria biosynthesis of MSH is accomplished in four steps by the enzymes termed *msh*A-D, illustrated in Scheme 1-5.[67] Recent studies have elucidated the initial steps leading to GlcNAc-Ins.[68] The glycosyl transferase *msh*A catalyses the reaction of UDP-GlcNAc with 1L-Ins-1-P to generate GlcNAc-(α1,1)-1D-Ins-1-P which is then dephosphorylated by an as yet unidentified phosphatase *msh*A2 to generate GlcNAc-Ins. GlcN-Ins is then produced from GlcNAc-Ins by the deacetylase *msh*B. The ligase *msh*C generates Cys-GlcN-Ins from Cys and GlcN-Ins. Finally acetyl transferase *msh*D catalyses the acetylation of Cys-GlcN-Ins to complete the biosynthesis of mycothiol (MSH).[67]



Scheme 1-5 Mycothiol Biosynthetic Pathway.[67]

MSH plays a significant role in detoxification of thiol-reactive substances including formaldehyde, various electrophiles and antibiotics. Mycothiol *S*-conjugates derived from electrophiles and antibiotics are cleaved by a fifth enzyme known as mycothiol *S*-conjugate amidase (Mca) to release GlcN-Ins which is used to resynthesise MSH and also a mercapturic acid which is excreted from the cell (Scheme 1-6).[68]

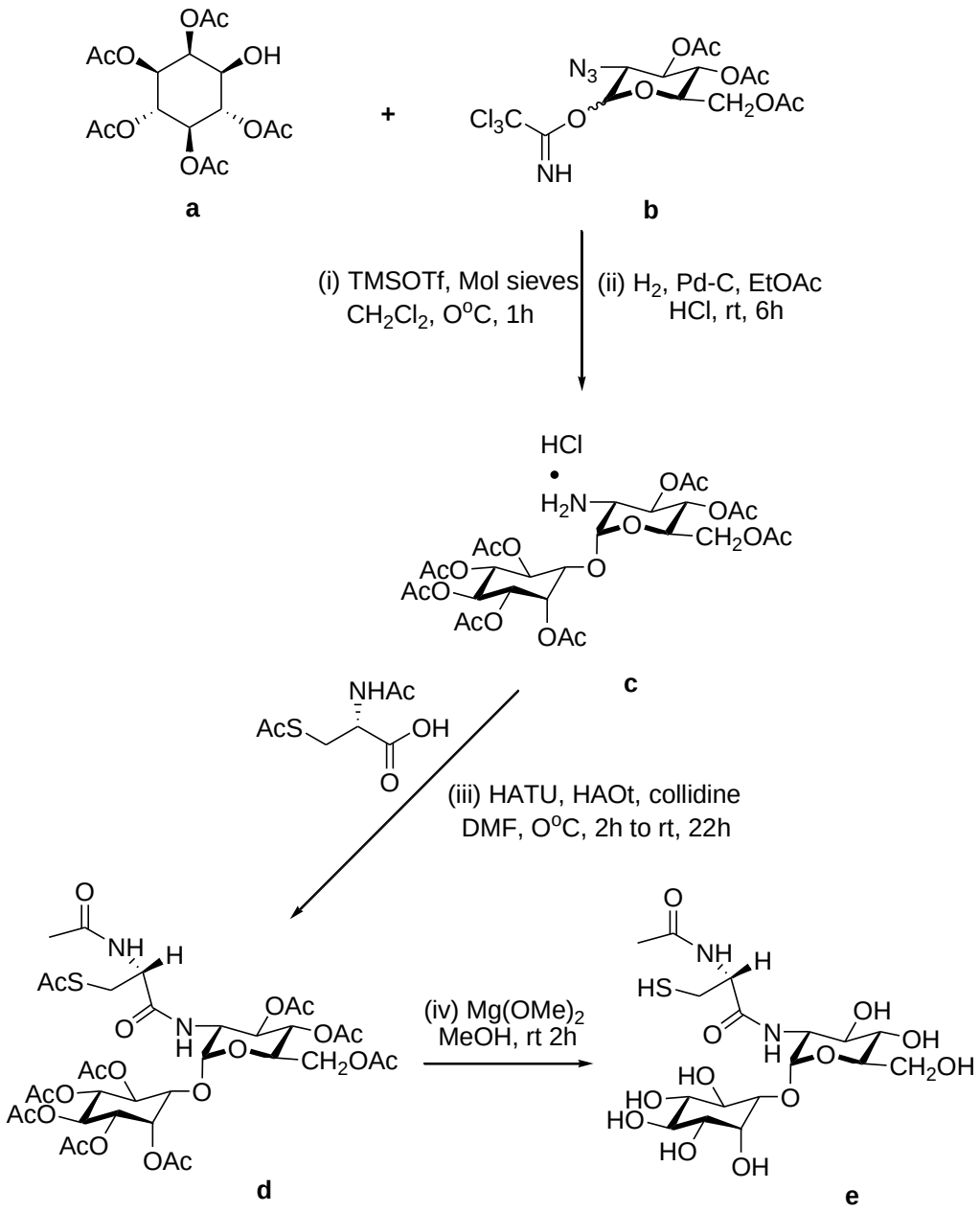


Scheme 1-6 The role of Mca in mycothiol dependent detoxification.[69]

Independent studies have demonstrated that chemically or genetically altered strains that are deficient in MSH production become hypersensitive to the most currently used antitubercular antibiotics and that MSH is indeed essential for viability of *M. tuberculosis*.[64,67,70,71,72]

Collectively, the above observations as well as the absence of mycothiol from mammalian cells suggests that molecules that can interfere with MSH associated enzymes may have therapeutic potential.[73] In one study it was observed that mycobacterial knockouts lacking *msh*B activity were more susceptible to reactive oxygen species and suggested that compounds directed to inhibit *msh*B might be able to inhibit MSH production.[74]

The total synthesis of mycothiol has been reported (Scheme 1-7).[75] The approach taken for the synthesis of the GlcN-Ins component of MSH entailed the coupling of the 1-OH of D-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol (**a** Scheme 1-7) with *O*-(3,4,6-tri-*O*-acetyl)-2-azido-2-deoxy-α,β-D-glucopyranosyl trichloroacetimidate (**b** Scheme 1-7). Synthesis of **a** (Scheme 1-7) was pursued from D/L-*myo*-inositol via literature procedures with minor modifications.[76,77,78] Details of the full synthesis are available in the literature.[75]



Scheme 1-7 Synthesis of Mycothiol.[75]

1.3.2 Crystal Structure of *Msh* B

This research project is in collaboration with Dr. Andrew McCarthy, European Molecular Biology Laboratory, Grenoble, France. The 3-D structure and a proposed mechanism of action of one of the enzymes of MSH biosynthesis, namely *msh*B, have recently been deduced by McCarthy and co-workers (Figure 1-16).[63]

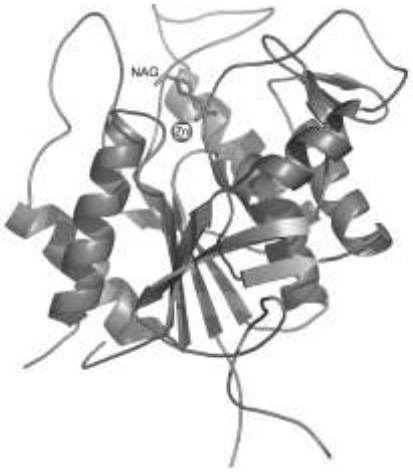


Figure 1-16 Structure of *msh*B, a modelled N-acetylglucosamine in the active site is shown in sticks, adjacent to the metal-binding site (sphere).[63]

When the structure was solved it was considered that compounds which could selectively bind to this enzyme, thus interrupting the production of MSH could leave *M. tuberculosis* vulnerable to reactive oxygen species, drugs and other stress factors. McCarthy *et al.* determined the three-dimensional structure of *msh*B to be a Zn-dependent deacetylase. The structure revealed an α/β fold in which helices pack against a seven-stranded mostly β-sheet. Large loops emanating from the C-termini of the β-strands enclose a deep cavity which is the location of the putative active site. At the bottom of the cavity is a metal-binding site associated with the sequence motif AHPDDE that is invariant in all homologues. An adventitiously bound β-octylglucoside (BOG) molecule used in crystallisation enabled them to model the binding of the true substrate and propose a metal dependent mechanistic model for deacetylation. Sequence comparisons indicate that *msh*B is representative of a wider family of enzymes that act on substituted N-acetylglucosamine residues, including a deacetylase involved in the biosynthesis of glycosylphosphalatidylinositol (GPI) anchors in eukaryotes.[63]

1.3.3 *Msh*B Proposed Active Site

The deacetylase *msh*B was the first enzyme from the mycothiol biosynthesis pathway to be characterised.[63] After crystallisation there were two forms of *msh*B, one with the octyl group of the BOG molecule directed into the hydrophobic region and another with the octyl group directed away from the active site into the hydrophilic region. The discovery of BOG molecules bound at equivalent sites on both of the *msh*B molecules in the crystal asymmetric unit clearly identifies this as the probable active site, given that the natural substrate is also a modified glucose moiety.[63]

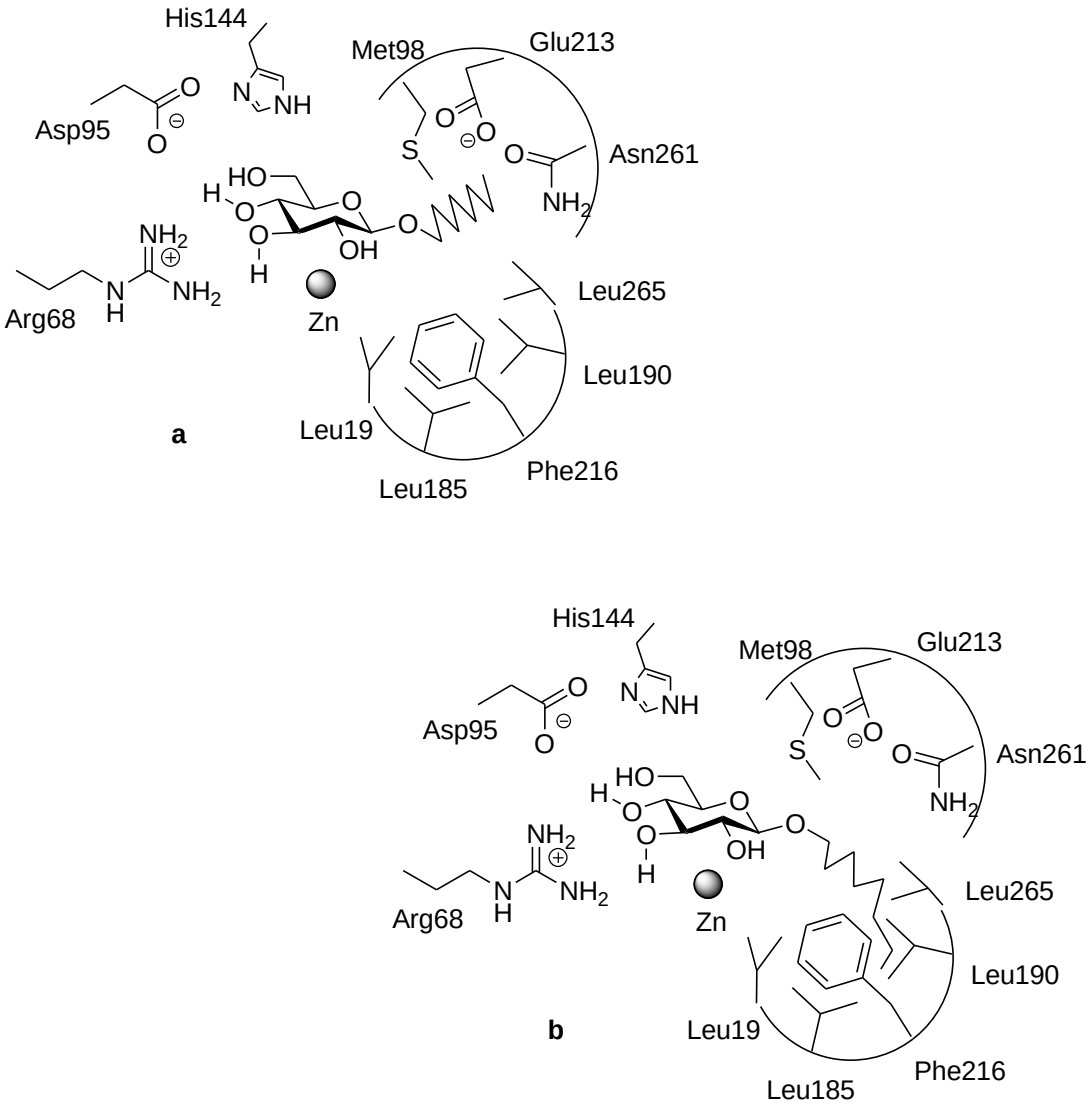


Figure 1-17 BOG molecule-binding mode in *msh*B.[63]

Whilst the active-site BOG molecules are in approximately the same locations in the two *msh*B molecules, they are in different orientations. The BOG molecule in diagram **a** (Figure 1-17) is oriented with its octyl tail projecting out of the active-site pocket in the vicinity of Met98, Glu213 and Asn261, which are contributed by three loops that form the active site entrance. The octyl tail in diagram **b** (Figure 1-17) however, is directed inwards filling a hydrophobic cavity.

Both BOG orientations were tested as a basis for modelling the natural substrate GlcNAc-Ins into the active site. Also included in the model was a metal ion bound to His13 $N^{\delta 1}$, Asp16 $O^{\delta 1}$ and His147 $N^{\epsilon 2}$ (Figure 1-18).

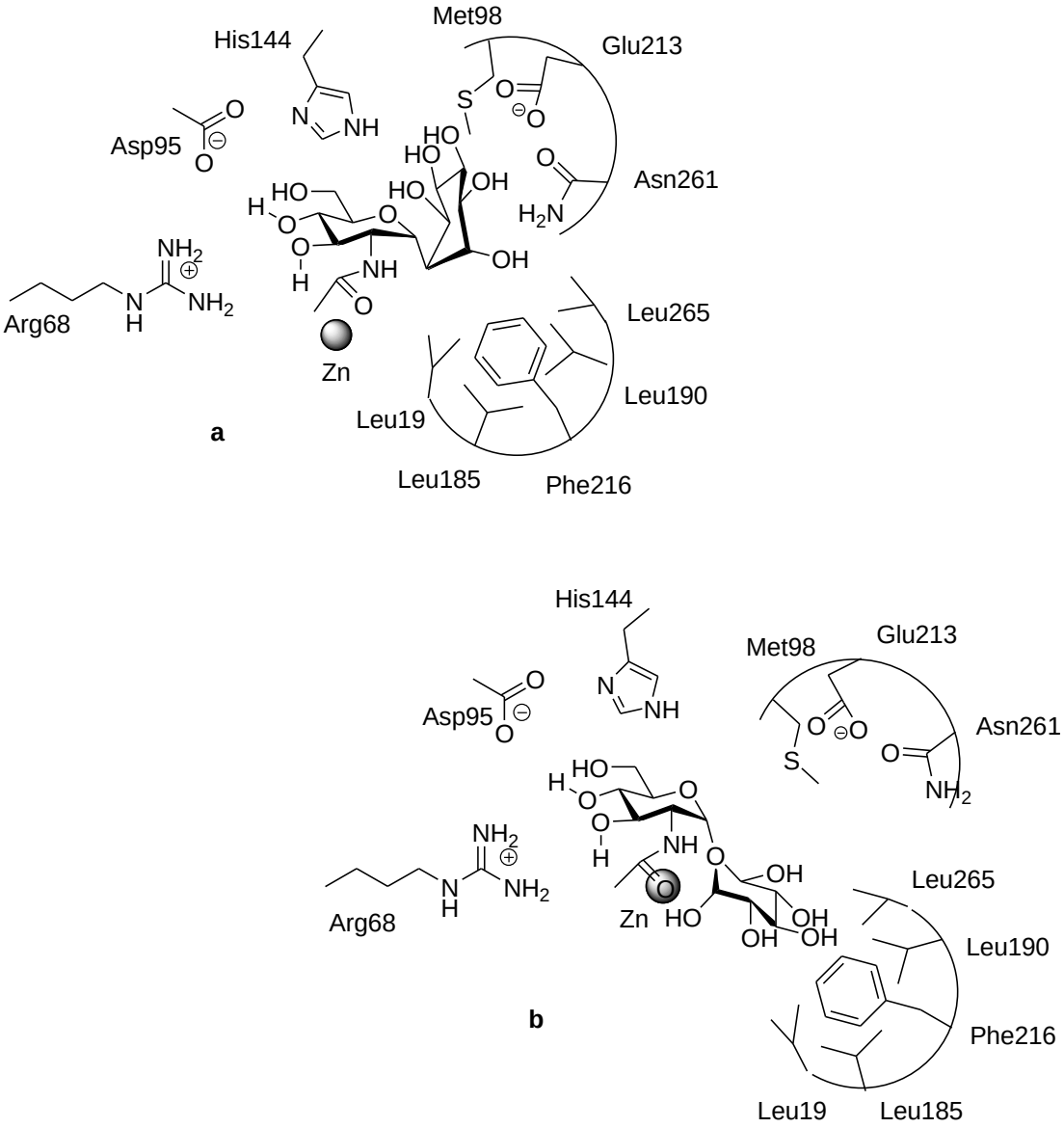


Figure 1-18 Proposed substrate-binding mode of GlcNAc-Ins in *msh*B based on molecular modelling of BOG molecule. [63]

It was immediately apparent that the conformation in diagram **b** (Figure 1-18) would require major reorientation for binding of GluNAc-Ins since the N-acetyl moiety partly overlaps the metal site and the inositol ring would project inwards into a hydrophobic region. In contrast, the conformation found in diagram **a** (Figure 1-18) provided an excellent model.[63]

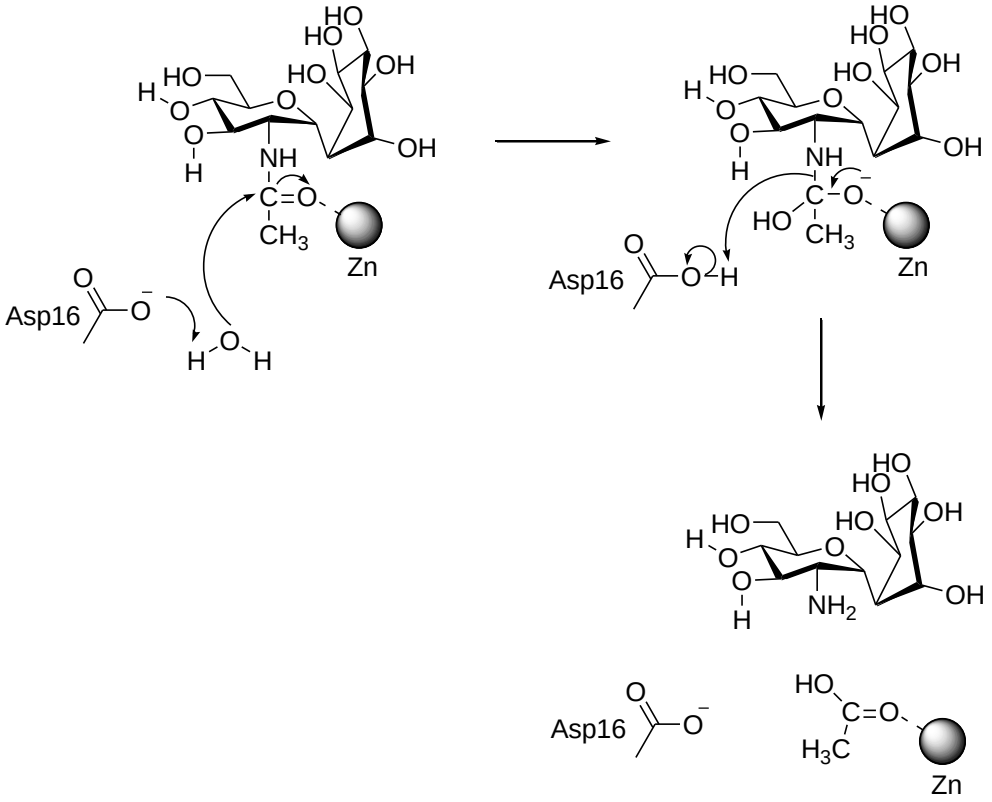


Figure 1-19 Proposed mechanism for deacetylation of GlcNAc-Ins catalysed by *msh*B.[63]

As in zinc proteases, for example carboxypeptidase,[40] the metal-binding site in *msh*B provides only three protein ligands (two His and one carboxylate), leaving vacant coordination sites for water and substrate. In the proposed mechanism (Figure 1-19), the carbonyl oxygen atom of the *N*-acetyl amide bond can bind directly to the metal ion. This is expected to polarise the C=O group as in metalloproteases, leaving a partial positive charge on the carbon atom ready for nucleophilic attack by a water molecule. The side-chain carboxylate group of Asp15 is ideally placed to serve as a catalytic base that enhances the nucleophilicity of the water molecule. The tetrahedral intermediate that is created by nucleophilic attack of water can in turn be stabilised by occupying an additional metal coordination site as in zinc proteases. This catalytic mechanism suggested for *msh*B is similar in its general features to the mechanism of

carboxypeptidase and to the proposed mechanisms of other metal-dependant deacetylases. [40,63]

1.3.3.1 Association of MshB and Mca

In order to define the protective role of mycothiol for *M. tuberculosis*, Buchmeier *et al.* constructed a *M. tuberculosis* mutant in Rv1170, the gene that encodes for the major GlcNAc-Ins deacetylase activity (*msh*B) in the MSH biosynthetic pathway of *M. tuberculosis*. During exponential growth, the Rv1170 mutant bacteria produced approximately 20% of the wild-type levels of MSH.[74]

In the mutant there was a 50-fold increase in GlcNAc-Ins, the immediate precursor molecule for the *msh*B reaction. Accumulation of GlcNAc-Ins indicated a blockage in MSH biosynthesis at the deacetylase (*msh*B) step. Levels of MSH and precursor compounds were restored to wild-type levels when the mutant was complemented with a wild-type copy of the Rv1170 gene. These results established that the Rv1170 gene is indeed responsible for the major *msh*B activity in *M. tuberculosis*. However, the presence of measurable levels of MSH in the Rv1170 mutant indicated that *M. tuberculosis* produces an additional enzyme with GlcNAc-Ins deacetylase activity that can partially substitute for Rv1170 during the synthesis of MSH.

The likely candidate for this second deacetylase activity was proposed to be Rv1082. This gene encodes for mycothiol *S*-conjugate amidase (Mca), which is normally responsible for cleaving *S*-conjugates of mycothiol to regenerate GlcN-Ins and a mercapturic acid AcCySR.[79] Rv1082 has homology throughout its sequence to Rv1170 and is 36% identical to the Rv1170 protein.[80] The minor deacetylase activity

of MCA would not be important for most mycobacteria except during times of high GlcNAc-Ins concentrations and long generation times, the conditions found in the *msh*B knockout.[74]

In a study by Steffek *et al.*, Mca of *M. tuberculosis* was cloned and expressed in *E. coli* as a native protein and purified to homogeneity by.[81] They evaluated the GlcNAc-Ins deacetylase activity of Mca and although the activity was small it was significant in that it was of a magnitude sufficient to account for the production of MSH observed in a *M. tuberculosis* mutant deficient in the normal deacetylase *msh*B as shown by Buchmeier *et al*.[74]

More recently *msh*B from *M. tuberculosis* was cloned, expressed, purified and its properties characterised by Newton *et al*. The substrate specificity of *msh*B was compared to that of *M. tuberculosis* Mca. These studies showed that *msh*B shares many properties with its homolog Mca.[82]

Results clearly indicated that the divalent metal ion was essential for the activity of *msh*B. Analogous results were found with the homologous Mca.[81] The GlcNAc-Ins deacetylase activity of both enzymes was compared and results clearly indicated that *msh*B provided the major deacetylase activity with values 4700-fold greater than those of Mca. Also as expected, the major amidase activity was attributed to Mca, showing activity 170-fold greater that *msh*B.[82]

Due to this additional deacetylase activity compounds which inhibit both *msh*B and Mca would be required to inhibit production of MSH at the deacetylase stage of the biosynthesis.

1.3.3.2 Inhibitors of MshB

Several novel and known natural and synthetic compounds that inhibit Mca with sub to low micromolar IC_{50} values have been identified. [69,83,84] Metaferia *et al.* demonstrated that a series of bromotyrosine-derived molecules, exemplified by natural products **a-c** (Figure 1-20) are competitive inhibitors of Mca.[85]

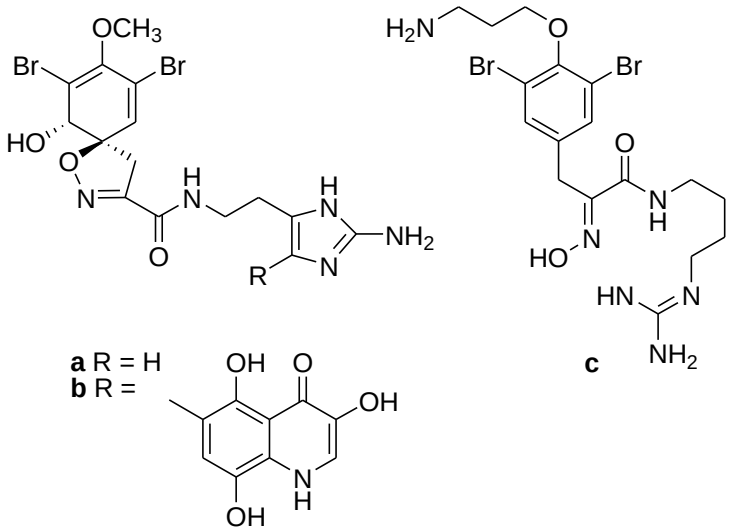


Figure 1-20 Natural product competitive inhibitors of Mca.[73]

They proposed that a small molecule incorporating features of the natural substrates, namely the pseudo disaccharide of MSH, together with those of the competitive natural product inhibitors may be able to inhibit not only Mca but also the homologous biosynthetic enzyme *msh*B.[73]

They constructed a small library of compounds built upon a GlcN or a thioglycoside scaffold and these were tested for their ability to inhibit both Mca and *msh*B (**a-k**, Figure 1-21). Of the compounds tested, sulfamides **b** and **d**, homophenylalanine trifluoroacetate **e**, homoserine analogue **g** and heterocycles **h, i, j** and **k** were the strongest inhibitors of *msh*B. For Mca, a similar pattern emerged where sulfamides **a-d**, trifluoroacetate **e** and its *N*-Boc analogue **f** and the same set of heterocycles (**h, i, j**, **k**) stood out as the best inhibitors, while analogue **g** was only weakly inhibitory.

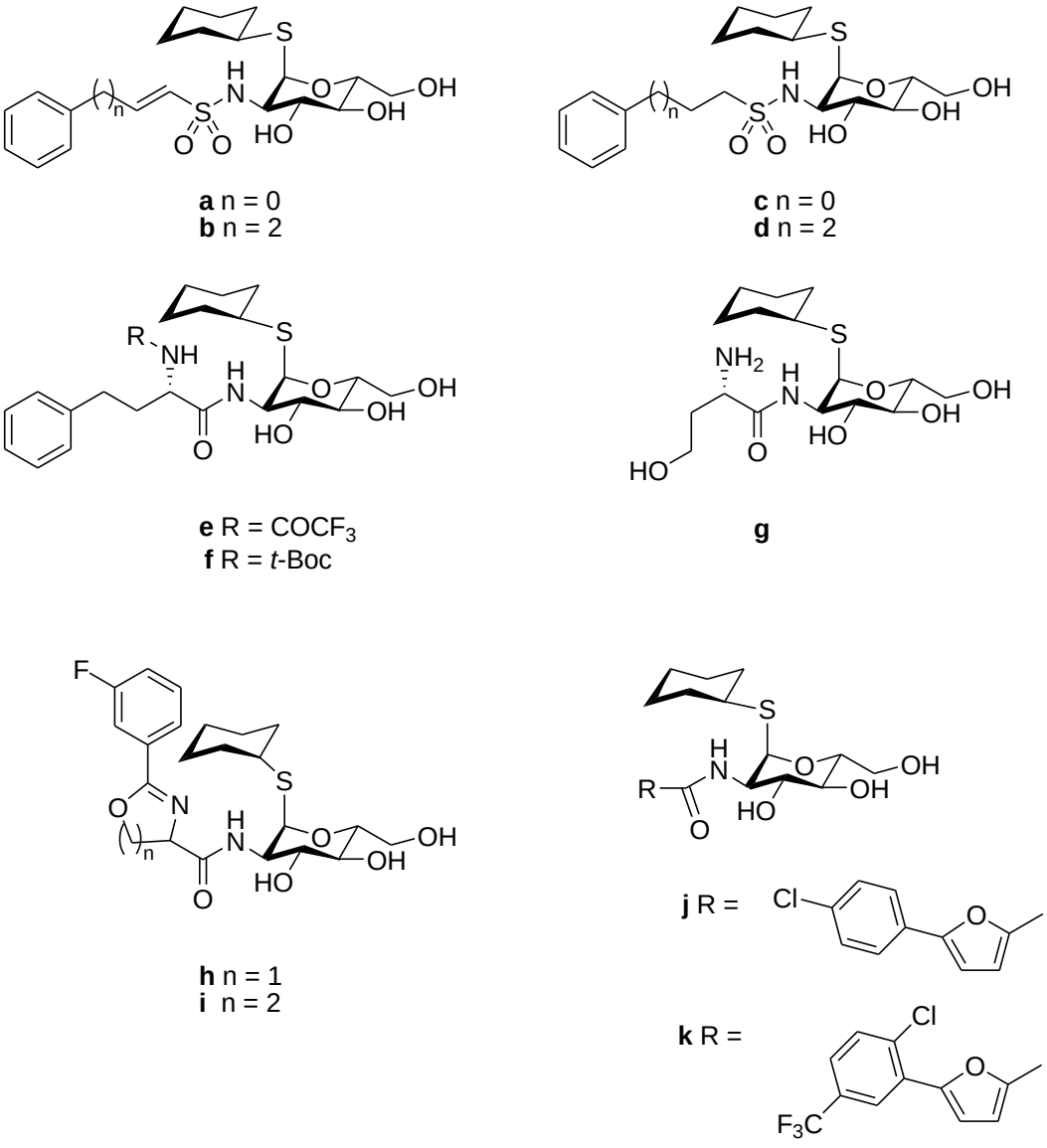


Figure 1-21 Selection of compounds tested for inhibitory effects against Mca and *msh*B.[73]

Although inhibitory concentrations for these compounds remained in the low micromolar range (data not shown), the approach was successful in that it allowed the construction of the first reported inhibitors of a MSH biosynthetic enzyme, namely *msh*B and these findings could provide the basis for further development of more potent inhibitors.[73]

1.4 FATTY ACID DERIVATIVES AS ANTIMICROBIALS

1.4.1 Food Preservation and Safety

Food Safety is the assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. Foodborne diseases present a serious challenge to public health.[86]

Virtually all food products deteriorate at some rate. The rate of deterioration is dependent on the food type, composition, formulation, packaging and storage regime. Preservation failure leads to deterioration, the consequences of which can vary greatly. Some minor effects include loss of colour, flavour or texture change within a food. At the other extreme, the most severe forms of deterioration are those associated with the presence or multiplication of micro-organisms which can lead to the transmission of life-threatening diseases, caused by the most hazardous of the food poisoning microorganisms. [87]

In the production of food it is crucial that proper measures are taken to ensure the protection and stability of the product during its shelf-life.[88] Food additives that prevent biological deterioration or antimicrobials are required to ensure that the

manufactured foods remain safe and unspoiled. Antimicrobial food additives as defined by the FDA, are additives used to control microorganisms such as bacteria, viruses, fungi, protozoa in or on food and food contact materials.[89]

1.4.1.1 Chemical Preservatives

Food antimicrobials are classified as "preservatives". Chemical preservatives are defined by the FDA as "any chemical that, when added to food tends to prevent or retard deterioration thereof, but does not include common salts, sugars, vinegars, spices or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke or chemicals applied for their insecticidal or herbicidal properties.". [90]

1.4.1.2 Natural Antimicrobials

Alternative food-preservation and processing technologies are being developed to a large extent in reaction to consumers' requirements for foods that are nutritionally healthier, more convenient in use (e.g. easier to store and prepare), fresher (e.g. chill-stored), more natural and therefore less heavily processed (e.g. mildly heated, non-thermal processes; high-pressure processing, UV processing, pulsed electric field treatment, etc), less heavily preserved (e.g. less acid, salt) and less reliant on additive preservatives (e.g. sulfite, nitrite, benzoate, sorbate) than previously.[91] To meet consumer demands, food manufacturers are searching for new as well as natural alternatives that can assure the safety of their products in the retail chain.[92]

1.4.2 Food and Health Care Industry Concerns

The greatest threat to the use of antibiotics is the emergence and spread of resistance in pathogenic bacteria that cannot be treated by previously successful regimes. Antibiotic-resistant bacteria were first identified in the 1940s, but while new antibiotics were being discovered at a steady rate, the consequences of this phenomenon were slow to be appreciated.[93] The emergence of antibiotic resistance is primarily due to excessive and often unnecessary use of antibiotics in humans and animals.[93]

Increasing numbers of hospital-acquired infections have generated much attention over the last decade. Hospitals and particularly intensive care units are an important breeding ground for the development and spread of antibiotic resistant bacteria. This is the consequence of exposing to heavy antibiotic use, a high density patient population in frequent contact with healthcare staff and the attendant risk of cross infection. Approximately 5-10 % of patients admitted to hospital will develop an infection directly related to their hospitalisation. These infections contribute to over 90,000 deaths per year in the United States.[94]

During food production and manufacturing, a range of antimicrobials (antibiotics, antifungals, sanitizers and food preservatives) are applied to improve efficiency, increase safety and to improve quality of the product. The variety of antimicrobial uses at each stage of the food system may create selective pressure that promotes antimicrobial resistance.[95] Strains of foodborne bacterial pathogens that are resistant to a variety of antibiotics have become a major health issue.[96] Some of the antibiotics and fungicides used in agriculture have identical chemical counterparts in human

medicine, for example the majority of antibiotics used in food animals belong to classes of antibiotics that are also used to treat human illness.[95]

Intense animal production in the agricultural industry involves administering livestock animals with large quantities of antibiotics to promote growth and prevent infection. This practice promotes the selection of antibiotic resistance in bacterial populations. The resistant bacteria from agricultural environments may be transmitted to humans, causing disease that cannot be treated by conventional antibiotics.[97] Major scientific and medical organizations have concluded that agricultural uses of antibiotics pose a threat to public health.[98]

Among the candidate replacements for antibiotics are fatty acids and their derivatives.[99] Some of the microorganisms of public health significance include *Staphylococcus aureus* and *Escherichia coli*.

1.4.2.1 Staphylococcus aureus

Staphylococcus aureus is a Gram-positive bacteria. Infections caused by *S. aureus* include skin infections, pneumonia, food poisoning, toxic shock syndrome and blood poisoning. *S. aureus* is one of the major infectious disease-causing pathogens in humans.[100]

Staphylococcal food poisoning is caused by staphylococcal enterotoxins produced during massive growth of *S. aureus* in food.[101] Food products that are associated with *S. aureus* include meat and meat products, poultry and egg products and dairy products amongst others. Most food intoxications caused by *S. aureus* are the result

of poor hygiene practices. In 2000, a total of 14,555 people were reported ill after drinking milk in Japan. Laboratory analysis showed that *S. aureus* enterotoxins were present in a number of packages of milk that had been linked to a dairy company. Investigations revealed poor hygiene standards within the company. It was subsequently confirmed that *S. aureus* strains isolated from pipes containing loose connections that had not been included in the automatic cleaning and disinfection system, produced the same enterotoxins that were present in the milk.[102]

Most nosocomial and community-acquired infections are caused by *S. aureus*.[103] Indeed, it is one of the most feared pathogens because of their ability to cause overwhelming sepsis and death.[104] Methicillin-resistant *S. aureus* (MRSA) infection first emerged in early 1961 and has become increasingly prevalent, with serious infections becoming more widespread during the past 20-25 years.[105]

Vancomycin (Figure 1-22) and other glycopeptide antibiotics are the current mainstay of therapy for infections caused by MRSA. However, the high prevalence of MRSA has led to increased use of vancomycin in chronic and seriously ill patients and has resulted in the emergence of MRSA with reduced susceptibility to glycopeptides. This reduced susceptibility to glycopeptide antibiotics is a serious and ongoing concern which extends beyond the hospital setting into the wider community and has heightened fears of a pan antibiotic-resistant strain.[106]

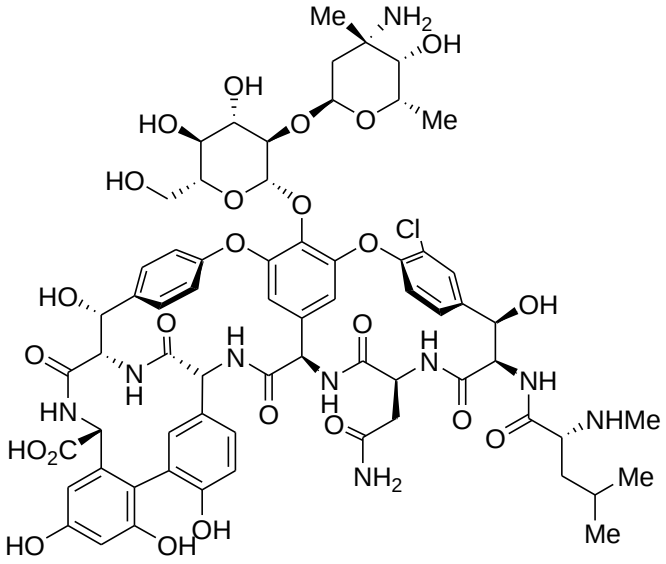


Figure 1-22 Structure of Vancomycin.[107]

1.4.2.2 Escherichia coli

Escherichia coli is a Gram-negative bacteria that is commonly found in the gut of humans and warm-blooded animals. *E. coli* is one of the most frequent causes of some of the many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), enteric infections and other clinical infections such as neonatal meningitis and pneumonia.[108]

Treatment for clinical cases of *E. coli* infection has become increasingly complicated by the emergence of resistance to most first-line antimicrobial agents, including fluoroquinolones.[109] Fluoroquinolones are a subset of the broad-spectrum antibiotics quinlones. An example of a fluoroquinolones used in treatments against *E. coli* infections is Ciprofloxacin (Figure 1-23).

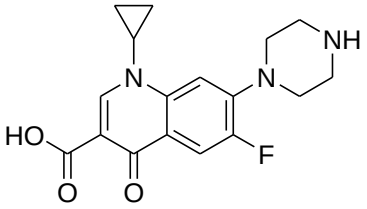


Figure 1-23 Ciprofloxacin.

E. coli is by far the most frequent uropathogen accounting for 75-95% of all positive cultures in uncomplicated cystitis which affect 40-50% of women in their lifetime. Ho *et al.* showed that resistance rates for several commonly used antibiotics against urinary *E. coli* are now highly prevalent, with resistance to one treatment using co-trimoxazole reaching 50-60% in Hong Kong.[110]

E. coli can cause severe foodborne disease. It is transmitted to humans primarily through consumption of contaminated foods such as raw or undercooked ground meat products and raw milk.[111] In 2003, two outbreaks of gastrointestinal illness due to *E.* coli O157 occurred in Dublin. One outbreak was centred around a restaurant associated with a hotel. The outbreak produced five confirmed cases and led to the closure of the restaurant upon a review of hygiene measures. The second outbreak of illness occurred at the same time in another hotel, with three confirmed cases of *E. coli* O157.[112]

1.4.3 Fatty Acids

Fatty acids and their corresponding esters are a natural class of chemicals considered to have little or no toxicity and have a long historical record for antimicrobial activity.[113] Despite this, the work carried out with fatty acids was to suffer greatly due to the boom in research into antibiotics after the discovery of Penicillin *circa* 1928.

However, interest in this class of natural antimicrobials has re-emerged in recent times.

The antimicrobial effects of fatty acids have been well documented.[114,115,116] The antimicrobial action of fatty acids is dependent on their structure and some generalisations concerning their effects can be made. For example long chain fatty acids have activity against Gram-positive bacteria, short chain fatty acids are more active against Gram-negative bacteria, while the medium chain lauric acid is regarded as the most active having reported activity against Gram-positive and Gram-negative bacteria.[113]

Lauric acid or dodecanoic acid (C 12) is a medium chain saturated fatty acid (Figure 1-24). One of the nutritional and antimicrobial factors in human milk is the presence of high levels of lauric acid fats. Lauric acid has been shown to demonstrate activity against a number of bacteria. For example, lauric acid has been shown to have activity against MRSA in combination with gentamicin.[105]

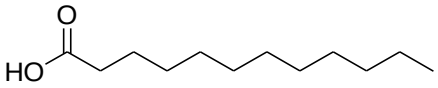


Figure 1-24 Lauric Acid.

One study which tested both saturated and unsaturated fatty acids against *Helicobacter pylori* showed that fatty acids with an equivalent carbon number (ECN) of 12 were most potent.[117] The ECN is a property which is often used during chromatographic analysis of lipids, similar ECNs imply similar retention times caused

by similar molecule size, shape, charge or polarity. According to Firestone,[118] ECN is defined as:

$$ECN = CN-2db$$

Equation 1-1Equivalent carbon number.

ECN is the equivalent carbon number, CN is the number of carbon atoms in the fatty acids and db is the number of double bonds. Therefore the ECNs, for example of lauric acid C12:0, myristoleic acid C14:1 and linolenic acid C18:3, are all equal to 12.[117]

Caprylic acid or octanoic acid (C 8) is a short chain saturated fatty acid (see Figure 1-25). Caprylic acid is also found in human milk. Nair *et al.* examined the efficacy of caprylic acid against a number of pathogens and showed that it exerts antimicrobial activity against a wide range of microorganisms including *S. aureus, Streptococcus agalactiae* and *Streptococcus dysgalactiae*.[119]

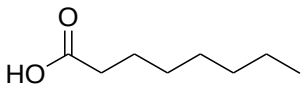


Figure 1-25 Caprylic Acid.

1.4.4 Fatty Acid Derivatives

Esterification of fatty acids with monohydric alcohols such as methanol or ethanol has been shown to reduce their antimicrobial activity. However, in contrast, this was not shown to be true when an α-hydroxy fatty acid was esterified and from these initial

results it was concluded that some hydrophilic group was necessary for biological activity. [120]

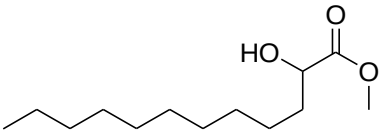


Figure 1-26 α-Hydroxymethyl laurate.[120]

Esterification of fatty acids to polyhydric alcohols such as glycerol was found to increase their antimicrobial effectiveness.[120,121] Monoacylglycerols (MAGs) are substances employed in the food and pharmaceutical industries. Due to their amphiphilic nature, they possess emulsifying properties and exhibit inhibitory effects against some types of microorganisms.[122] The most active antimicrobial fatty acid derivatives exhibit a balance between hydrophilic and lipophilic groups.[123]

1.4.4.1 Monolaurin

Monolaurin (trade name Lauricidin®), a food grade glycerol monoester of lauric acid is a food emulsifier, which in addition to its emulsification properties also possesses broad spectrum antimicrobial activity against Gram-positive bacteria, yeasts and moulds but generally not against Gram-negative bacteria (Figure 1-27). [113,124]

It has been shown that in the presence of lactic acid, monolaurin was able to suppress growth of *S. aureus* on meat products.[122]

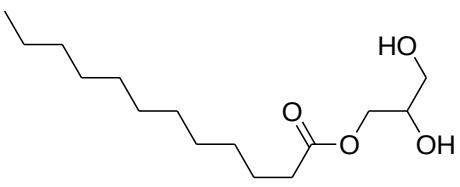


Figure 1-27 Monolaurin.

The purity of this monoglyceride is very important. Kabara *et al.* found that when tested against a range of bacteria, the 1,3-dilaurin and trilaurin derivatives were less active than the free fatty acid and that only the monoglyceride (1-mono-laurin) proved to be more active than the free acid.[120] Many monoglyceride preparations have a number of impurities particularly notable quantities of di and tri esters. It was found that monoglycerides with less than 90% monoester were not effective antimicrobials which is why the name Lauricidin® was coined to emphasise its high monoester content.[113]

1.4.4.2 Fatty Acid Ether Derivatives

As mentioned, one of the most active antimicrobial lipids identified to date is 1-dodecanoyl-glycerol (monolaurin), where the fatty acid dodecanoic acid is esterified to glycerol. However, Ved *et al.* showed that depending upon incubation conditions, the alkylglycerol ether (dodecylglycerol) can be more than 2-fold higher in antibacterial activity than monolaurin (Figure 1-28). [125]

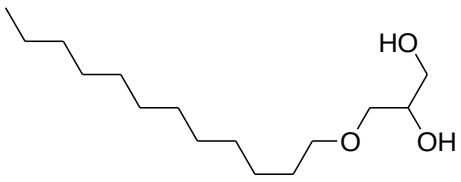


Figure 1-28 Dodecylglycerol.

As with monolaurin, there was a marked difference in the activity of dodecylglycerol against Gram-positive and Gram-negative bacteria. The growth of all Gram-positive strains tested were inhibited. However, when Gram-negative bacteria were tested there was always some growth, even with greatly increased concentrations of

dodecylglycerol and some of the Gram-negative strains were totally unaffected. However the data clearly indicated that under the conditions used, dodecylglycerol was always more potent than monolaurin (dodecanoylglycerol). As with previous trends, the antibacterial activity of the ethers peaked at a chain length of 12 carbons.[125]

1.4.5 Carbohydrate Fatty Acid Derivatives

Carbohydrate fatty acid esters are another class of fatty acid derivatives which have broad applications in the food industry.[126,127] While they are most commonly employed as surfactants, their antimicrobial properties have been reported. The use of carbohydrate esters is continually increasing as they are completely biodegradable, they are not harmful to the environment and they are non-toxic. [123] Another attractive feature of carbohydrate fatty acid derivatives is the potential to modify their properties by controlling the degree of substitution of the carbohydrate, by varying the nature of the fatty acid and also the sugar itself.

They are currently being employed in the food, cosmetic and detergent industry.[128] Sugar esters are widely used in Japan as antibacterial agents in canned drinks. The most common carbohydrate fatty acid ester utilised to date is sucrose ester, which are being produced at about 4000 ton/year.[129] One study by Kato and Shibasaki showed that the sucrose ester of lauric acid was primarily active against Gram-positive bacteria and in fact showed that in contrast to data generated for glycerides, the diester of sucrose rather than the monoester was more active. Of the diesters tested, sucrose dicaprylate possessed the highest activity.[130] It has also been shown that diesters of sucrose not only have higher antimicrobial activity than their corresponding free fatty

acids but also compare favourably in activity with commonly used antimicrobials such as parabens, sorbic acid and dehydroacetic acid.[113] However studies have shown that sucrose monoesters are also useful as antimicrobial agents in combination with EDTA against the Gram-negative *E. coli* O157:H7. Hathcox and Beuchat demonstrated that enhanced inhibition of *E. coli* O157:H7 was achieved by 500 ppm sucrose monolaurate combined with 300 ppm EDTA compared to either compound used alone.[131]

Other oligosaccharide fatty acid esters have also been synthesised. Devulapelle *et al.* synthesised pure fatty acid esters of sucrose, maltose and maltriose by an enzyme-catalysed process and tested these compounds as inhibitors of *Streptococcus sobrinus*. 6-*O*-lauroylsucrose (**a** Figure 1-29), 6'-*O*-lauroylmaltose (**b** Figure 1-29) and 6''-*O*-lauroylmaltriose (**c** Figure 1-29) at 100 μg/mL showed complete inhibition of *S. sobrinus* in agar plates. Consequently these sugar esters are potentially of significant value in the development of oral-hygiene products.[132] Although less studied than sucrose esters, fatty acid esters of maltose have potential cosmetic and medicinal applications as antitumoral agents.[133]

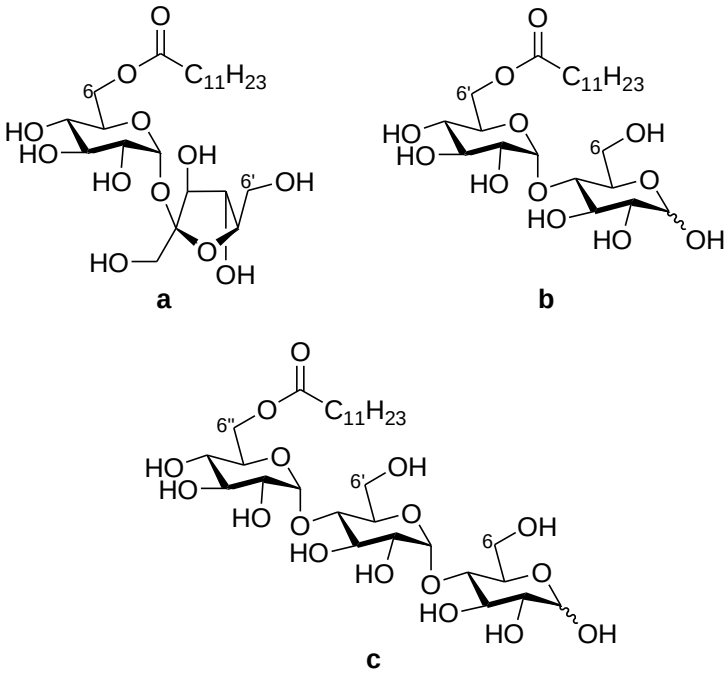


Figure 1-29 6-*O*-lauroylsucrose (a**), 6'-*O*-lauroylmaltose (**b**) and 6''-*O*-lauroylmaltriose (**c**).**[132]

One study investigating the effect of carbohydrate monoesters reported that among those synthesised, galactose laurate, fructose laurate and the reducing 6-*O*-lauroylmannose showed the highest inhibitory effect against the growth of *Streptococcus mutans*, while other analogs of hexose laurates showed no activity.[134] These findings indicate that the carbohydrate moiety itself markedly affects the activity of the fatty acid and therefore further investigation is merited.

Recent work in the area of carbohydrate fatty acid esters has focused on establishing an effective regioselective, enzyme catalysed synthesis of sugar derivatives for use as surfactants for industrial applications.[135,136] However relatively few studies have examined role of the carbohydrate in antimicrobial activity.[129,132,137]

1.4.5.1 Enzymatic Synthesis of Carbohydrate Fatty Acid Derivatives

Ester-cleaving hydrolases from different sources have been successfully used in carbohydrate chemistry for regioselective esterification. Typically lipases are employed in esterification, although proteases have also been utilized.[138] The hydrolases used in these processes share in common a similar mechanism which involves the formation of an acyl enzyme intermediate. In general, acylation takes place at the less hindered primary hydroxyls.[139]

Lipases are ubiquitous enzymes that catalyse the hydrolysis of fats and oils. In addition to their biological function in bacteria, fungi, plants and higher animals they are also used as biocatalysts in numerous industrial processes. Lipases are the most commonly used enzymes in synthetic organic chemistry.[140]

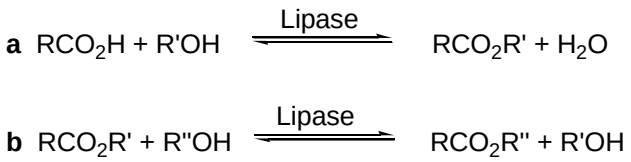


Figure 1-30 Reversible lipase-catalysed (a) esterification and (b) transesterification.

Lipases and proteases have been isolated from a variety of sources including bacteria, yeast and moulds.[135] Some examples are shown in Table 1-1.

Table 1-1 Sources of commercially available lipases/proteases used for esterification/trans-esterification of sugars.

Sugar or Related Compound	Fatty Acid/Ester	Enzyme	Enzyme Source
Glucose	Palmitic acid	Lipase P	*Pseudomonas sp*[141]
Mannose	Divinyl decanedioate	Alkaline protease	*Bacillus sublilus*[142]
Fructose	Palmitic acid	Lipase SP 435	*Candida antartica* B[136]
Glycerol	Lauric acid	Lipozyme IM	*Rhizomucor michei*[143]

Enzyme catalysed syntheses are usually specific for the carbohydrate that is being esterified and also for the degree of esterification. For example, Ferrer *et al.* found that lipase from *C. antartica* B is particularly useful for the preparation of 6,6'-di-acylsucrose, whereas *T. lanuginosus* lipase selectively catalyses the synthesis of 6-*O*-acylsucrose.[129]

Enzymatic synthesis of sugar fatty acid esters is highly regioselective,[128] although for some carbohydrates two or more minor isomers are sometimes obtained, depending on the enzyme used.[134] This can cause difficulty in specifically and unambiguously ascribing antimicrobial activity to a given species, unless purification and characterisation is carried out.

For example, as previously mentioned, Watanabe *et al.* synthesised 23 carbohydrate monoesters using lipases and proteases.[134] These compounds were then assayed for their ability to suppress the growth of *S. mutans*. Among the carbohydrate esters synthesised, fructose laurates showed one of the highest growth-inhibitory effect. The fructose laurate was synthesised by reacting lauric acid with fructose in acetone at

60°C in a sealed glass tube using immobilised *Alcaligenes* lipase and was obtained at a 51.3% yield. However, NMR spectra showed that the esters were a mixture of 1-*O* and 6-*O*acyl (α and β) esters of fructofuranose (**a** and **b** respectively, Figure 1-31) and fructopyranose (**c** Figure 1-31). This was also reported for the synthesis of fructosepalmitate by *Candida antartica* lipase in 2-methyl-2-butanol by Scheckermann *et al*.[144]

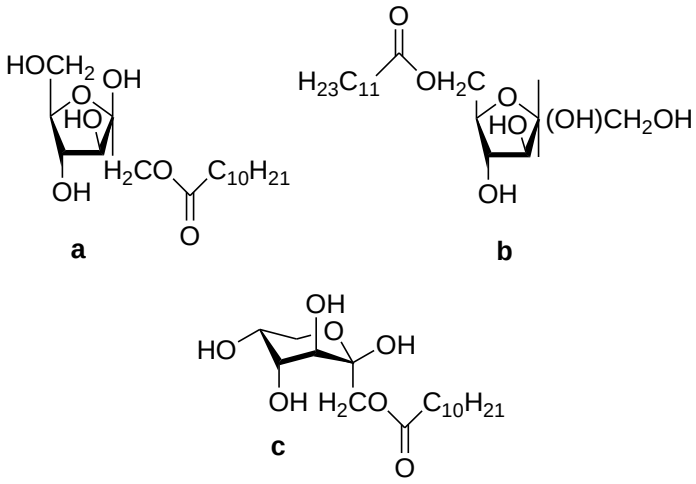


Figure 1-31 1-*O*-lauroyl fructofuranose (a), 6-*O*-lauroyl fructofuranose α/β and 6-*O*-lauroyl fructopyranose (c).[134]

1.4.6 Antimicrobial Mechanism of Action

Fatty acids and their esters may have several modes of action that are non-specific. Compounds including monolaurin and carbohydrate fatty acid derivatives are thought to affect the cell membrane, inhibit respiratory activity and membrane transport. The mode of action and inhibitory effects are reported to be concentration dependent.[113]

Bactericidal concentrations of long chain fatty acids were shown to stimulate oxygen uptake of *Bacillus megaterium* and *Micrococcus lysodeikticis* at pH 7.4. Higher concentrations of fatty acids produced complete inhibition of oxygen uptake.[145,146] In

contrast, *Pseudomonas phaseolicola* and *E. coli* showed a limited response of the oxygen uptake mechanism in these bacteria, reflecting the resistance of these bacteria to the bactericidal activity of fatty acids. However, upon partial removal of the cell wall these bacteria displayed an increase in sensitivity to inhibition of oxygen uptake. This result suggested that cell-wall lipids might be responsible for protecting the membrane from the fatty acids.[146] This finding was also supported by Hamilton on the observation that the formation of spheroplasts from Gram-negative bacteria rendered them more susceptible to metabolic inhibition by lipid-soluble antibacterial agents.[147]

Projan *et al.* showed that monolaurin at subinhibitory concentrations decreased the production of a large number of exoproteins by *S. aureus*. They observed that for TSST-1 and alpha-hemolysin this decrease was effected at the level of transcription. These results implicate signal transduction as the target for exotoxins. This led them to postulate that monolaurin may intercalate into the cytoplasmic membrane and subtly modify membrane structure.[148] They also found that the action of monolaurin wass not restricted to *S. aureus*, it also blocked the induction of vancomycin resistance in another pathogen, *Enterococcus faecalis*.[149]

Tsuchido *et al.* observed that sucrose esters cause changes in cellular morphology of *Bacillus subtilis*, which induced autolytic processes.[150,151] It was suggested that sugar esters disrupt the cell membrane, thereby altering its permeability and causing leakage of cell contents.[132]

CHAPTER 1 - REFERENCES

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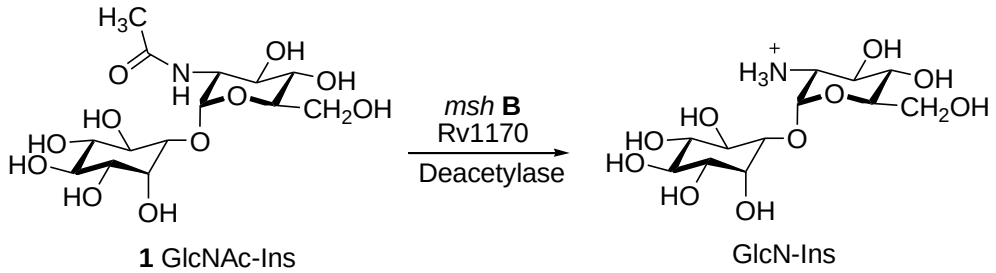
2. Design and Synthesis of Potential *Msh* B Substrate Analogs

This chapter presents the design and synthesis of compounds that could mimic GluNAc-Ins and selectively bind to *msh*B.

2.1 Design of GlcNAc-Ins Mimics

The initial aim of the project involved the design and synthesis of mycothiol (MSH) precursor analogs aimed at *msh*B. As discussed in the introduction (Section 1.3.1), *msh*B is a Zn-dependent deacetylase enzyme that catalyses the second step in the biosynthesis of mycothiol. It catalyses the removal of an acetyl group from GlcNAc-Ins **1**, producing GlcN-Ins (Scheme 2-1).



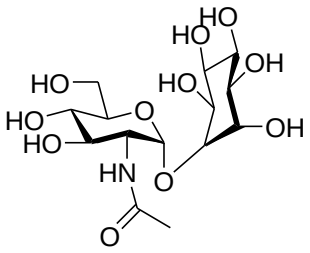
Scheme 2-1 Second step in biosynthesis of MSH, catalysed by *msh*B.[1]

The 3-D structure and a proposed mechanism of action of *msh*B have been deduced by McCarthy *et al*.[2] A β-octylglucoside (BOG) molecule was used in crystallisation and enabled them to model the binding of the true substrate and propose a metal dependent mechanistic model for deacetylation.

After crystallisation there were two forms of *msh*B, one with the octyl group BOG molecule directed into the hydrophobic region (diagram **a**, Figure 1-15) and another

with the octyl group directed into the hydrophilic region (diagram **b**, Figure 1-15). Both BOG orientations were used as a basis to represent the natural substrate GlcNAc-Ins (**1**, Figure 2-1) into the active site. The aim of this project was to design and synthesise a compound which could better mimic the natural substrate for *msh*B (GlcNAc-Ins) for crystallisation and mechanistic studies to further improve the knowledge of this enzyme.

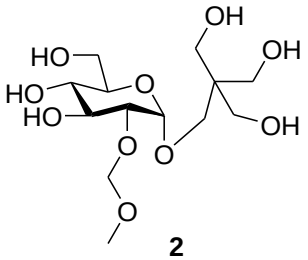
GlcNAc-Ins is composed of an α-D-glucopyranoside with an amide group at the C-2 and an inositol group at the anomeric position (Figure 2-1). The design of the mimic compound was based on its structure.



1

Figure 2-1 Natural substrate for *msh*B, GlcNAc-Ins (1).

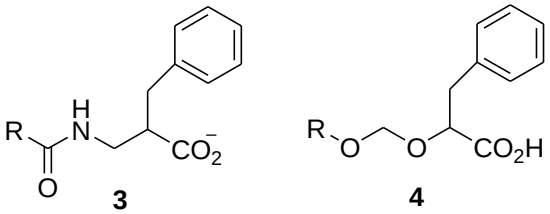
The mimic compound **2** (Figure 2-2) is also based on an α-D-glucopyranoside scaffold with the relevant pharmacophores attached. It does not contain a scissile amide bond, instead there is a methoxy methyl group (MOM) at the C-2 position and pentaerythritol replaces the inositol.



2

Figure 2-2 Proposed GlcNAc-Ins mimic compound (2).

The MOM group was chosen to replace the amide bond in the mimic compound based on work detailed earlier on a novel strategy developed for CPA (Section 1.2.2.2).[3] Figure 2-3 depicts a natural substrate of CPA (**3**)[4] and a substrate mimic successfully used by Min Su Han *et al.*, where an acetal replaces the peptide group (**4**).[3]



3 **4**

Figure 2-3 (3) Typical C-terminal portion of a peptide CPA substrate,[4] (4) Example of CPA substrate mimic.[3]

Inositol was not chosen as a component of the mimic compound due to the difficulties that would be involved in synthesis. Lee and Rosazza synthesised D-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol in a total synthesis of mycothiol (Scheme 1-7).[5] This synthesis is long and cumbersome and in general it has been avoided in the literature during synthesis of related compounds, for example Metaferia *et al.* used a cyclohexyl group to replace inositol (Section 1.3.3.2).[6] There were also problems in the literature with the assignment of orientations of the enantiomers of the *myo*-inositol component of GlcNAc-Ins.[7] Reports on the enantiomers have been in discord. The correct stereochemistry of the components of MSH was reported by Sakuda *et al.* to be L-

cysteine, D-glucosamine and 1D-*myo*-iositol.[8] However, although subsequent papers have maintained the nomenclature for the chirality, many errors in the structures drawn for MSH have occurred.[9]

Pentaerythritol was chosen due to its relative ease of synthesis. It contains four equivalent hydroxyl groups and therefore eliminates any requirements for selectivity. Also, the only compound available to McCarthy *et al.* to mimic the binding of the natural substrate was the BOG molecule which contains the hydrophobic octyl group (Figure 1-14). A pentaerythritol molecule should better mimic the inositol due to its multiple hydroxyl groups. Figure 2-4 diagram **b** depicts the mimic compound **2** which may fit into the hydrophillic region as proposed for the inositol of GlcNAc-Ins **1** in diagram **a**.

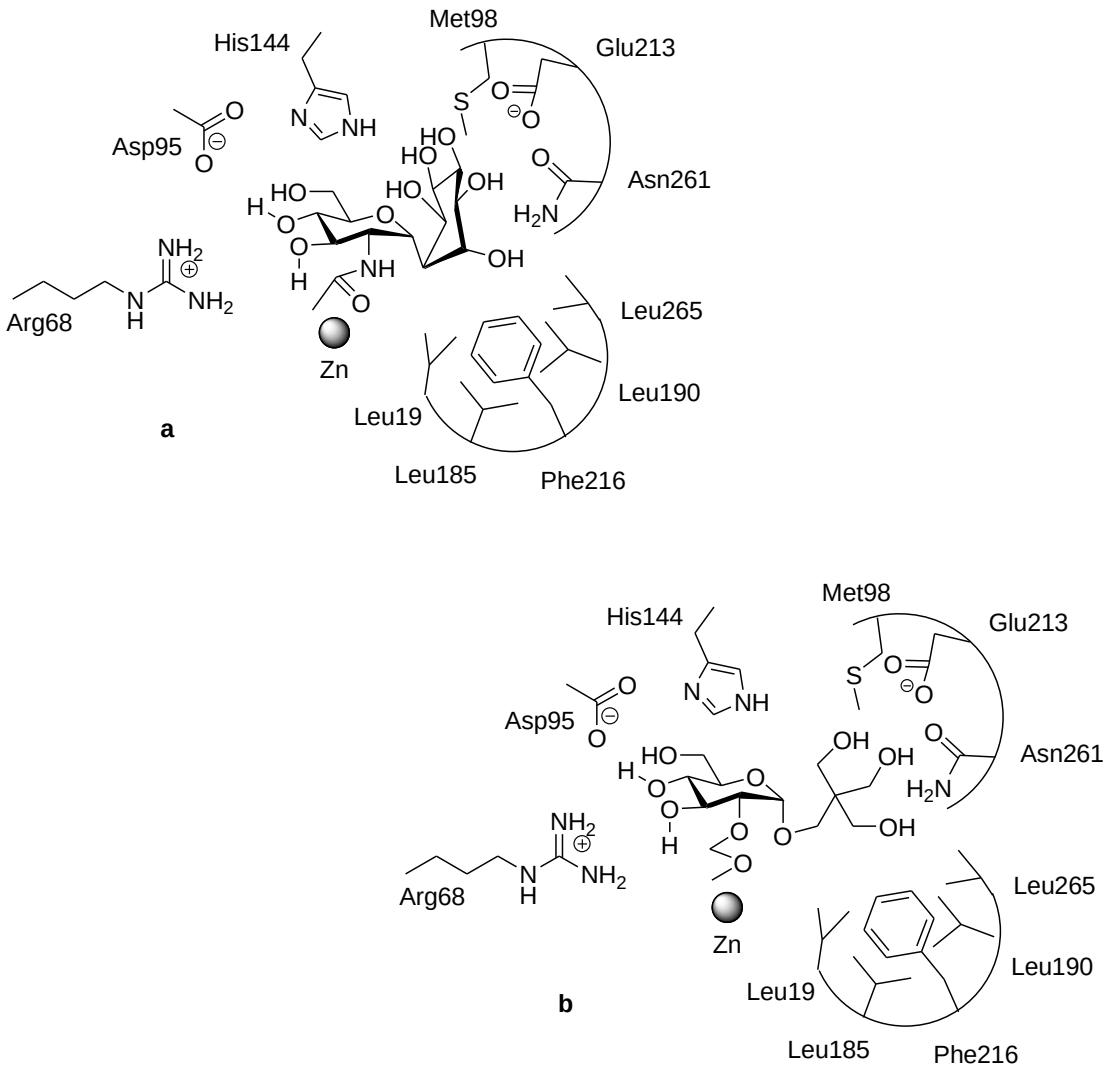
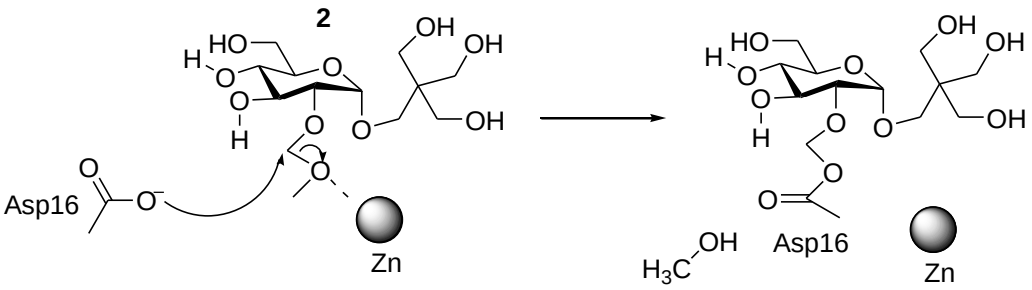


Figure 2-4 (a) Proposed substrate-binding mode of GlcNAc-Ins 2 in *msh*B based on molecular modelling of BOG molecule in Figure 1-17. (b) Possible binding of designed molecule 2.

Another advantage of the use of a MOM group at the C-2 position of the mimic compound **2** is its potential for inhibition of *msh*B. The replacement of the scissile amide bond by a non-cleavable isostere may have an inhibitory effect similar to that observed with the CPA inhibitors (section 1.2.2.2). The catalytic mechanism proposed for *msh*B is similar to the mechanism of zinc protease such as carboxypeptidase and thermolysin (section 1.2.2.1) and involves deacetylation of the C-2 amine.[2] It is proposed that the mimic compound could undergo a bond formation reaction with the carboxylate of the Asp 16 in a similar way to the proposed

mechanism for CPA inhibitors discussed in Section 1.2.2.2, resulting in a covalently modified enzyme that can no longer perform its function (Scheme 2-2).



Scheme 2-2 Possible mechanism for inhibition of *msh*B by 2.

2.2 RETROSYNTHETIC ANALYSIS

By retrosynthetic analysis it is clear a glycosyl donor and acceptor were required for the synthesis of **2**.

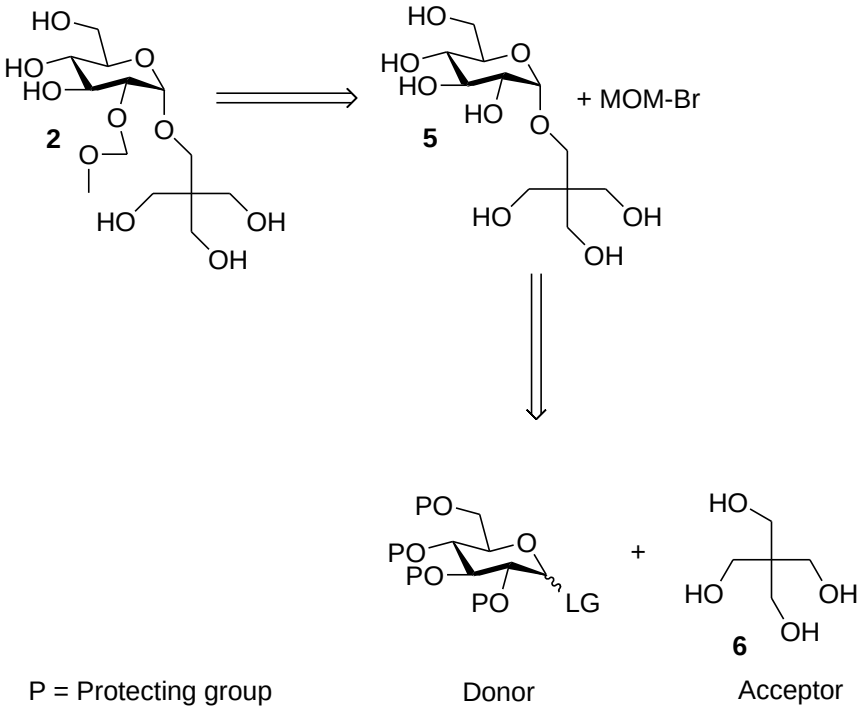


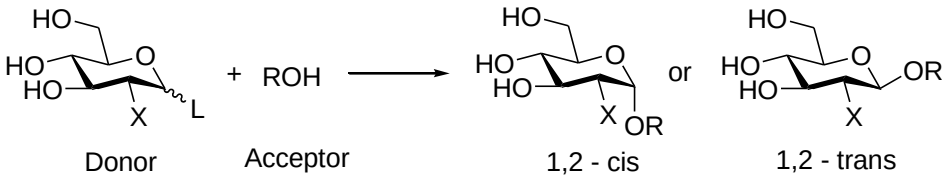
Figure 2-5 Retrosynthetic analysis of 2.

The acceptor molecule pentaerythritol **6** was chosen as an alternative to inositol (Section 2.1). The glycoside donor involved creating a good anomeric leaving group so that the pentaerythritol molecule could be attached.

2.2.1 Synthesis of Glycosides

The synthesis of *O*-glycosides is an important reaction in synthetic organic chemistry. The union of a glycosyl donor and an alcohol (glycosyl acceptor) presents major challenges.[10] Though conceptually simple, this operation has presented difficulties for chemists for over one hundred years since Köenigs and Knorr published the first chemical glycosylation reaction in 1901.[11]

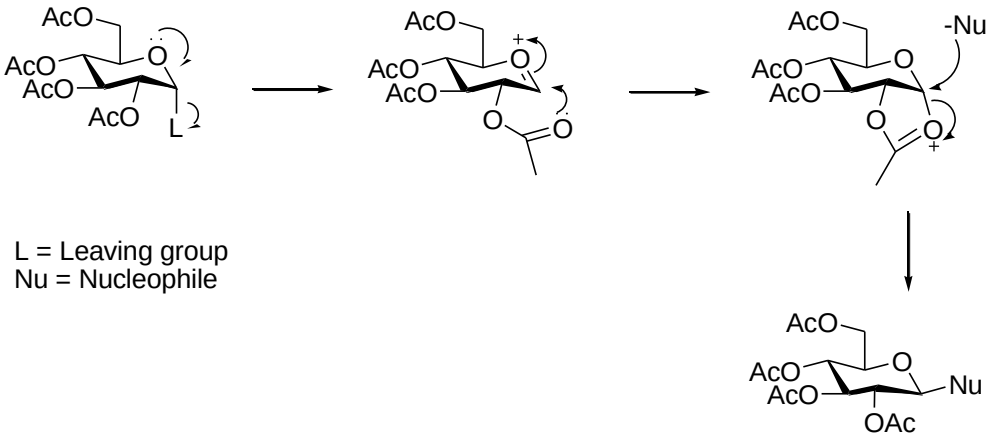
There are many problems inherent in this synthetic transformation. There are so many variables involved that each target compound requires that a particular strategy be developed following consideration of the monomers to be bonded and the types of inter-unit linkages to be made.[12] For example, controlling stereoselectivity for 1,2 – cis or 1,2 – trans-glycosidic bonds (Figure 2-6).



L = Leaving group
X = OH, NHAc, N_3, etc

Figure 2-6 Possible linkages in the formation of a glycosidic bond.[10]

One of the most useful ways to control the stereochemistry of an anomeric bond is by neighbouring group participation of an ester protecting group on the 2 hydroxyl (Scheme 2-3).[12]

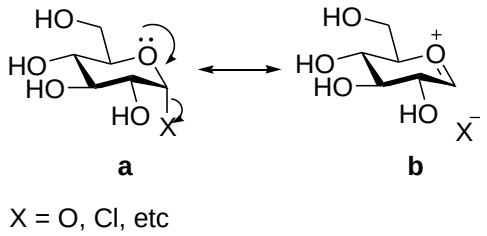


Scheme 2-3 Neighbouring group participation in glucose.

Scheme 2-3 depicts the participation of the carbonyl oxygen of the acetate protecting group at the 2 position which stabilises the intermediate glycosyl cation by forming the cyclic oxonium ion. The net result is the nucleophile is forced to attack from the β configuration and therefore the newly formed anomeric linkage is trans to the 2-hydroxyl group. If a manno starting material is used, which has an axial hydroxyl group at the 2 position, the α linkage is specifically formed.

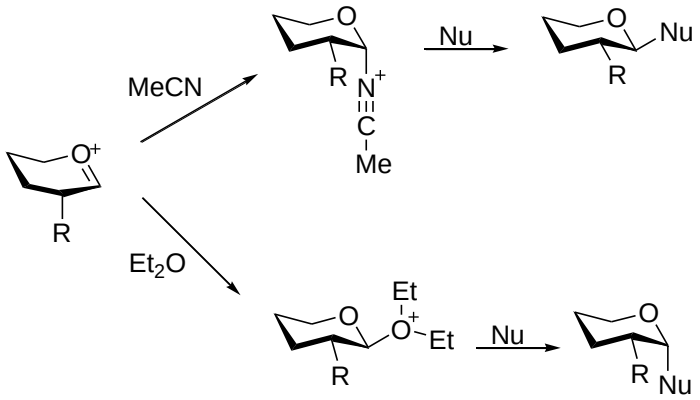
Conversely, donors displaying non-ester groups at C-2 generally favour formation of α-anomers due to the anomeric effect. This is a general phenomenon whereby the thermodynamically preferred axial position presides over the less sterically hindered equatorial position at the anomeric centre. When there is an electronegative substituent in an axial position at the anomeric centre, a lone pair of electrons on the ring oxygen is anti-planar to the C-X bond and can participate in a stabilising two

electron interaction (**a** and **b**, Scheme 2-4). This corresponds to an n → σ* interaction or partial donation of the oxygen lone pair into the anti-bonding orbital of the C-X bond. The net effect is this electronic delocalisation and therefore it is stabilising.[11]



Scheme 2-4 The anomeric effect.

The effect of solvent upon the stereoselectivity of glycosylation reactions is also an important factor (Scheme 2-5). For example even in the presence of a non-participating group at C-2, the use of acetonitrile as a solvent can allow access to the less thermodynamically favoured β-anomer. This is because acetonitrile is nucleophilic itself and can intercept the glycosyl cation initially formed allowing access to the α-nitrilium ion intermediate. The glycosyl acceptor then attacks this ion in an S_N2 reaction with inversion of configuration.[13] Ether-type solvents have a tendency to shift stereoselectivities towards the formation of *cis*-glycosides, for example diethyl ether and dioxane. This is rationalised by the preferential formation of the β-oxonium ion type intermediate from the oxocarbenium ion. This intermediate then reacts to form the axial glycoside.[14]



Scheme 2-5Effect of solvents on stereoselective outcome of glycosylation reaction.[14]

The choice of glycoside donor is in itself crucial for the outcome of the glycoside formation or the linking of a sugar unit through nucleophilic displacement of a leaving group at the anomeric centre. Therefore it is of paramount importance that a leaving group of one type or another can be introduced at the anomeric centre. Some examples follow.

Thioglycosides are extremely useful as glycosyl donors. In most of their properties these compounds are similar to the *O*-glycosides, but they show expected differences that result from the different properties of sulfur and oxygen.[15] Thioglycosides are stable in aqueous base at normal temperatures but are hydrolysed by aqueous mineral acid at a slower rate than the analogous oxygenated glycosides. They are readily prepared from the corresponding anomeric acetates by treatment with a thiol and a Lewis acid (Figure 2-7).[11]

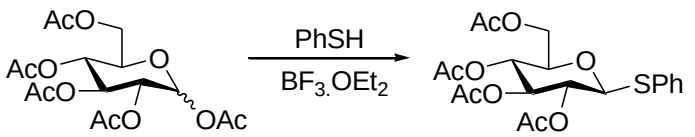


Figure 2-7 Thioglycoside formation.[11]

Note the specific formation of β-anomers due to neighbouring group participation (Scheme 2-3).

Trichloroacetimidates are a class of glycosyl donors that are accessed directly from the hemiacetal by treatment with trichloroacetonitrile and a suitable base. Depending on the type of base used, either α- or β-trichloroacetimidate may be obtained (Figure 2-8).[11]

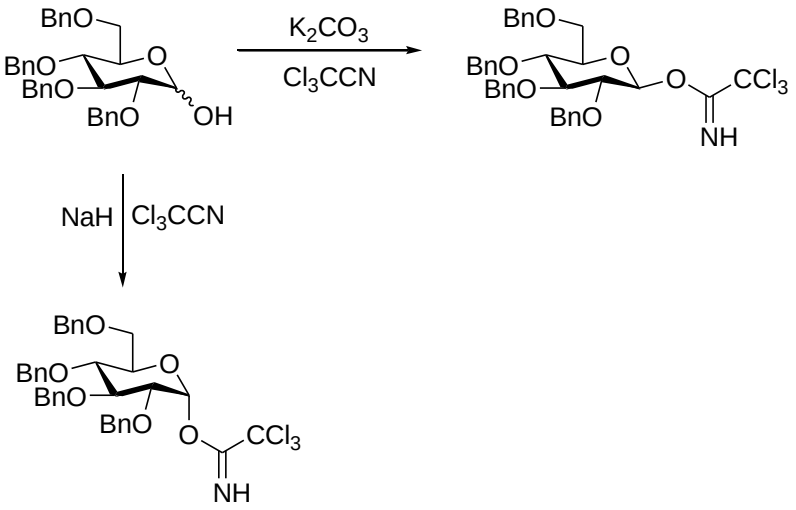
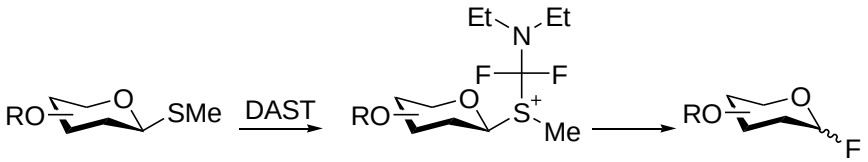


Figure 2-8 Trichloroacetimidate formation.[11]

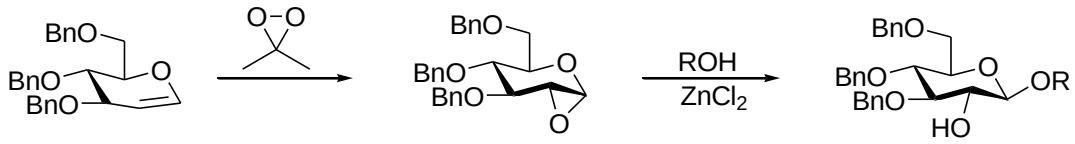
Glycosyl trichloroacetimidates undergo nucleophilic displacement reactions with predictable stereo-chemical outcome under mild conditions. Sugar trichloroacetimidates with participating groups at C-2 yield 1,2-trans-compounds as would be expected. When non-participating protective groups are selected in nonpolar solvents and supported by low temperatures, S_N2-type reactions can be quite frequently carried out, hence α-trichloroacetimidates yield β-glycosides and β-trichloroacetimidates yield α-glycosides.[12]

Glycosyl fluorides have been widely used for *O*-glycosylation reactions. A notable advantage of their use is the high thermal and chemical stability as compared with other glycosyl halides, for example glycosyl chlorides, bromides and iodides.[16] Glycosyl fluorides are commonly prepared by reaction of a protected sugar with a free anomeric hydroxyl group with diethylaminosulfur trifluoride (DAST).[14] They can also be readily synthesised from the corresponding thioglycoside using DAST (Scheme 2-6). Usually α/β-anomeric mixtures of fluorides are obtained, the ratio is often solvent dependent.[11]



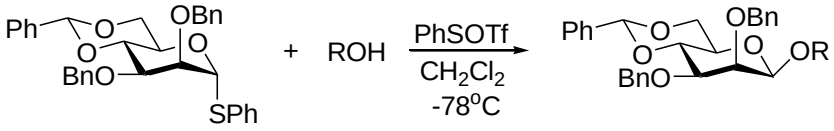
Scheme 2-6 Glycosyl fluoride formation from thioglycoside.[11]

The use of glycals for formation of glycosidic linkages can be valuable as glycals minimise protecting group manipulations and may readily be converted into different glycosylating agents. For example epoxidation of these compounds lead to the formation of 1,2-anhydrosugars which can subsequently act as glycosyl donors in $ZnCl_2$ promoted glycosylations (Scheme 2-7).[14] One of the advantages of this method is that the initial glycoside formed possesses a free hydroxyl group at C-2, available for further elaboration.



Scheme 2-7 Epoxidation of glycals and subsequent glycosylation.[11]

Glycosyl triflates are important intermediates in modern chemical glycosylation methodologies. In 1998 Crich *et al.* prepared extremely reactive α-mannosyl triflates cleanly and quantitatively at low temperatures from glycosyl sulfoxides or thioglycosides[17] (Scheme 2-8). Following their recent characterisation using low temperature NMR measurements, several methods for glycosylation on the basis of pregeneration of glycosyl triflates have been developed. They are usually generated from thioglycosides because of their stability under atmospheric conditions and a variety of reaction conditions.[18]



Scheme 2-8 β-mannoside formation from thioglycosides with PhSOTf in CH_2Cl_2.[17]

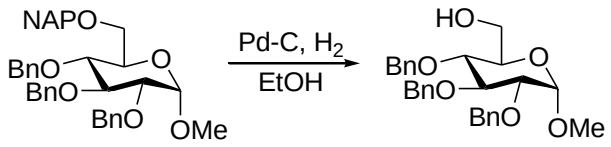
2.2.2 Regioselective Protection of Carbohydrate Hydroxyl Groups

Direct one-step protection of a single hydroxyl group of an unprotected saccharide is a prominent challenge in carbohydrate chemistry since carbohydrates contain several hydroxyl groups of similar reactivity.

Regioselective protection of the primary position is possible since it is in a sterically less encumbered environment than the other hydroxyl groups and is therefore more nucleophilic.[14] Standard methods for the selective protection of this position include tritylation or silylation with bulky silyl groups. This is usually followed by protection of the remaining secondary hydroxyl groups with an orthogonal protecting group for example by acetylation or benzylation. This is then followed by removal of the

primary hydroxyl protecting group allowing selective functionalisation of this position.

Another method involves the use of benzyl-type protecting groups at the primary hydroxyl. One of the advantages of using benzyl protecting groups is the mild conditions involved in its removal by catalytic hydrogenolysis. Studies have shown that benzyl-type protecting groups, for example a 2-naphthylmethyl group (NAP), with different reactivities can be sequentially removed via catalytic hydrogenolysis (Scheme 2-9).[19] The study identified two major factors controlling the removal sequence of different benzyl-protecting groups. These were the electronic properties of the aromatic ring and its affinity for the palladium surface.



Scheme 2-9 Selective removal of NAP group by catalytic hydrogenolysis in the presence of Bn groups (89% yield).[19]

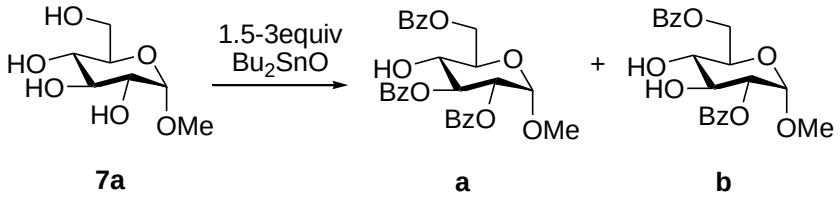
The deprotection of the benzyl group was still inhibited after removal of the NAP group had completed. This was subsequently confirmed to be the result of competition between the 2-methylnaphthalene by product and the benzyl group for the active sites on the palladium.

Unfortunately, since the reactivity difference between the secondary hydroxyl groups is not that large, direct one step protection of a single secondary hydroxyl usually results in a mixture of differently protected regioisomers. As a result, given the need for separation of these mixtures and subsequent characterisation, this method is rarely

used as a protection strategy.[14] One method used for the regioselective protection of a single secondary hydroxyl group in carbohydrates involves the use of stannylating agents.

The reaction of hydroxyl groups of saccharides and tin oxide reagents can form stannylene ethers and acetals which then enhance the nucleophilicity of the oxygen's in a regioselective way allowing for regioselective acylation or alkylation. One of the most commonly used reagents is dibutyltin oxide (Bu_2SnO).[20]

Regioselective benzoylation of carbohydrates using excess Bu_2SnO at increased reaction temperatures has been performed and shown to yield several benzoate derivatives with one or two free hydroxyl groups, for example di- and tri-benzoyl derivatives of methyl α-D-glucopyranoside **7a**[*] (Scheme 2-10).[21]



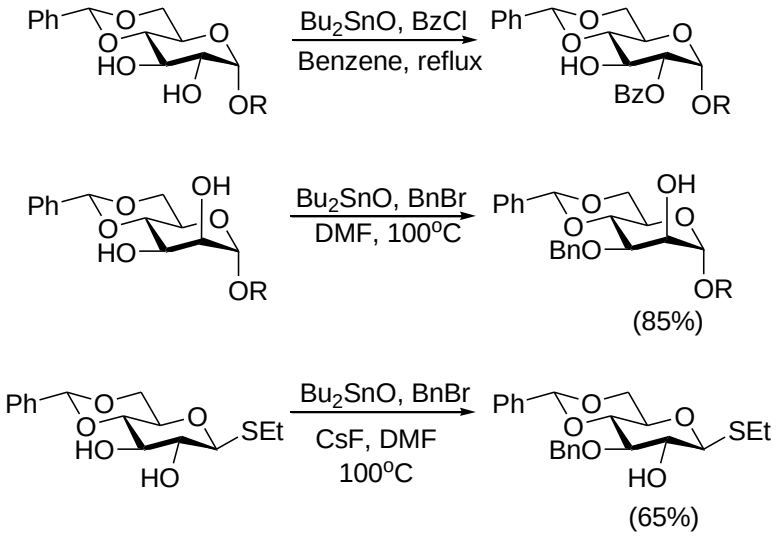
7a **a** **b**

Scheme 2-10 ***Reagents and Conditions:*** **Bu_2SnO, toluene-benzene 1:1, 100°C, BzCl (a: 91% yield, b: 6% yield).[21]**

T.l.c. analysis of the reaction using 3 equivalents of BzCl showed that 2,6-di-*O*-benzoyl derivative (**b**, Scheme 2-10) was formed first. Based on those results, the reactivities of the hydroxyl groups in the α-glucoside seemed to be 6-*O* > 2-*O* > 3-*O* > 4-*O*. It is not surprising then to see the protection of the primary hydroxyl groups

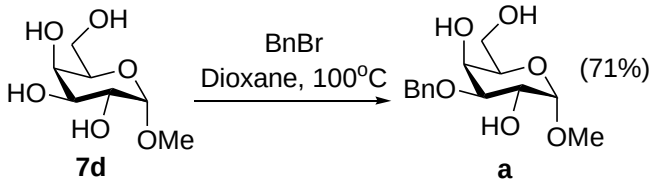
[*] Numbering system **7a** used here as this was used for publication of antimicrobial compounds discussed in Chapter 3.

prior to regioselective selection of the 2-OH or 3-OH in some instances in the literature (Scheme 2-11).



Scheme 2-11 Examples of tin-mediated regioselective benzylation of 4,6-*O*-benzylidene protected sugar derivatives.[14,22]

However regioselective protection of one hydroxyl group of free sugars has been observed, for example Scheme 2-12 depicts the regioselective protection of methyl α-D-galactopyranoside **7d**[†] at the 3-OH.



Scheme 2-12 Example of regioselective protection of single hydroxyl group in unprotected methyl α-D-galactopyranoside.[14]

[†] Numbering system **7d** used here as this was used for publication of antimicrobial compounds discussed in Chapter 3.

A PhD project recently completed in the School of Food Science and Environmental Health involved use of a tin-mediated regioselective method for functionalisation of the 2-OH of α glucopyranosides. This work involved the use of dibutyltin dimethoxide. Solvent and temperature conditions were optimised for both yield and selectivity of the 2-OH.[23]

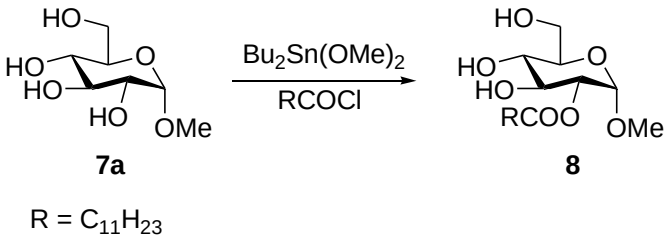


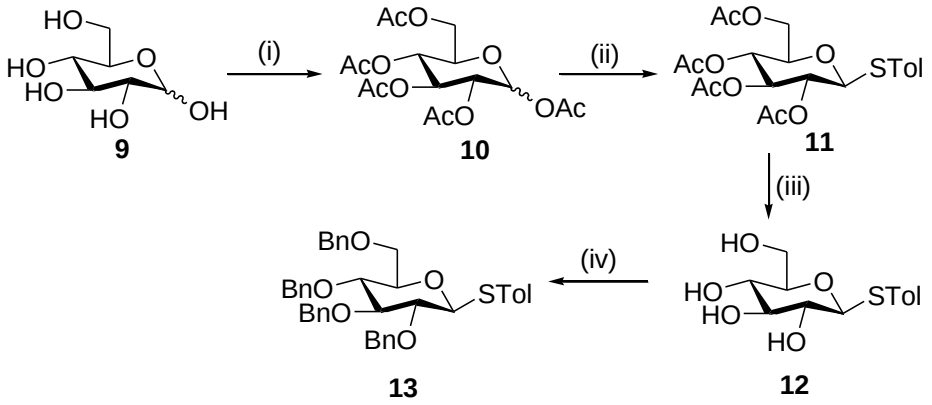
Figure 2-9 Regioselective tin-mediated synthesis.[23]

2.3 SYNTHESIS

A description of compounds synthesised in an attempt to design a synthesis for compound **2** will be given in this section. A glycosyl donor and acceptor were designed by retrosynthetic analysis (Section 2.2). This involved creating a donor with a suitable leaving group for a glycosylation reaction and also the regioselective attachment of a MOM group at the 2-OH position. Initially attempts were made at a thioglycoside donor for linkage with the pentaerythritol **6**. Following this, attempts were made for the regioselective protection of the 2-OH using a MOM group. This was performed using a model compound, methyl α-D-glucopyranoside **7a** for compound **5** for investigative synthesis**.**

2.3.1 Synthesis of Glycoside Donor

A glycosyl donor with a suitable anomeric leaving group was needed. The first attempted synthesis was for the preparation of a thioglycoside donor and is outlined below (Scheme 2-13).[24]

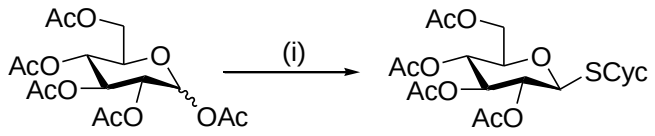


Scheme 2-13 ***Reagents and Conditions*****: (i) Ac_2O, Py, rt. (41% yield) (ii)** ***p*****-Thiocresol, CH_2Cl_2, 0°C, $BF_3{\cdot}Et_2O$. (15% yield) (iii) Na, MeOH, rt. (iv) DMF anhydr., BnBr, 0°C, NaH, rt. (53% yield over 2 steps)[24]**

The acetylated derivative **10** of D-glucose **9** was obtained from the reaction of **9** in the presence of a 1:1 solution of acetic anhydride and pyridine yielding a 3.5:1 ratio of α:β anomers (41% yield). This ratio was calculated from the ^{1}H NMR spectrum using the integration of the anomeric H signals for the α and β isomers. The H-1α peak was observed at 6.33 ppm with a coupling constant of 3.5 Hz and the H-1β peak was observed at 5.72 ppm with a coupling constant of 8.5 Hz. The introduction of a thiol leaving group provided **11**. This was achieved by activation with the Lewis acid boron trifluoride etherate and displacement with the thiol *p*-thiocresol, however this reaction did not go to completion giving a 15% yield. Only the β anomer was formed, as expected, due to neighbouring group participation of the C-2 acetate. This was confirmed by the ^{1}H NMR spectrum. The coupling constant of the anomeric proton was 10.0 Hz indicating an axial-axial coupling. Signals in the aromatic region were

integrating for 4 protons which was in accordance with the presence of only one *p*-tolyl group. Only four singlets were present between 2.00 and 2.10 ppm indicating the presence of four acetate groups and another singlet was observed at 2.35 ppm signifying the PhCH_3. Additional problems were encountered with purification as the starting material **10** and product **11** had very similar mobility by t.l.c. and separation was very difficult. Nevertheless, the deacetylation of pure compound **11** was carried out using sodium and methanol to yield **12**. Finally benzylation of crude tetrol **9** gave the fully protected thioglycoside **13** (53% yield over 2 steps).

As a result of the poor yield in step (ii) (Scheme 2-13) the synthesis of an alternative thioglycoside using cyclohexanethiol was attempted to drive the reaction to completion using cyclohexanethiol (Scheme 2-14).

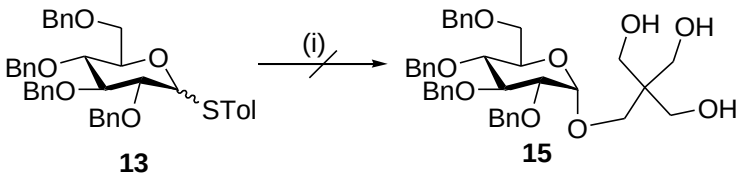


10 **14**

Scheme 2-14 ***Reagents and Conditions*****: (i) cyclohexanethiol, CH_2Cl_2 anhydr., 0°C, $BF_3{\cdot}Et_2O$.**[24]

However the reaction did not go to completion either, as revealed by t.l.c. and appeared to give a poorer yield than the previous reaction and was deemed unworthy of further pursuit.

Nevertheless using the small amount of glycoside donor available, a glycosylation reaction of **13** with pentaerythritol **6** was attempted (Scheme 2-15). However this reaction was unsuccessful and no product was seen by t.l.c. The insufficient amount of material available prohibited further investigation of this reaction.



Scheme 2-15 ***Reagents and Conditions*****: (i) Pentaerythritol, 4 Å mol. Sieve, CH_2Cl_2, NIS, TMSOTf.**

2.3.2 Regioselective Derivatisation at 2-OH using Model Compound Methyl α-D-glucopyranoside

It was decided that a model compound for **5** should be explored to ensure it would be possible to selectively derivatise an α-glycoside at the 2-OH before further effort was made on the synthesis of the glycosyl donor. Methyl α-D-glucopyranoside **7a** was used for this purpose (Figure 2-10).

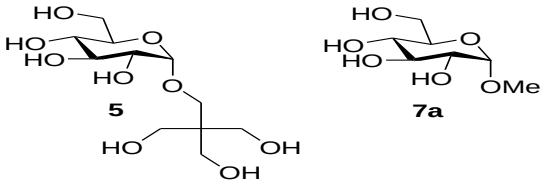
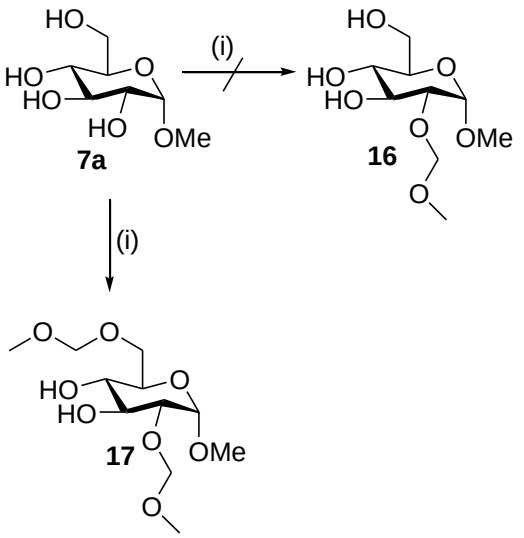


Figure 2-10 (5) α-Glycoside (7a) Methyl α-D-glucopyranoside.

From this compound, different syntheses were attempted in an effort to attach the MOM group at the 2 hydroxyl group. Several attempts were made.

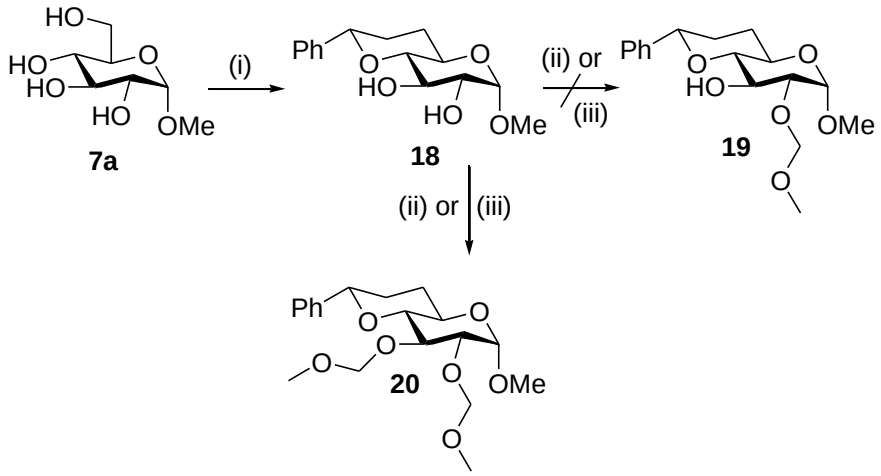
2.3.2.1 Attempted Regioselective Derivatisation of Model Glycoside Methyl α-D-glucopyranoside

Initially it was decided that based on previous work in the school (Section 2.2.2) an attempt would be made at tin mediated regioselective derivatisation of the α-glycoside **7a** (Scheme 2-16).[23]



Scheme 2-16 ***Reagents and Conditions*****: (i) $Bu_2Sn(MeO)_2$, dioxane, reflux, then $BrCH_2OCH_3$. (94% yield)**[23]

This reaction was performed using bromomethyl methylether and dibutyltin dimethoxide in dioxane and gave a disubstituted derivative presumed to be **17** (94% yield). The ^{1}H NMR spectrum indicated the presence of two MOM groups from two AB doublets at 4.83 and 4.88 ppm arising from O*CH*$_2$OMe. Also three methyl signals were observed between 3.40 and 3.45 ppm. Although the exact position of the MOM groups could not be identified, it was suspected these groups had attached at the 6- and 2-OH position (**17**, Scheme 2-16). Following this it was decided that the number of free hydroxyl groups should be reduced before attaching the MOM group.[14] This was achieved by use of 4,6-*O*-benzylidene protecting group (Scheme 2-17).

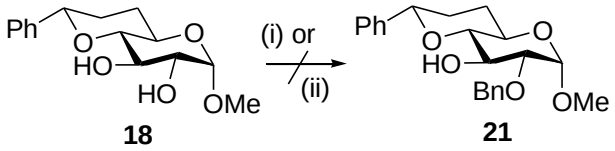


Scheme 2-17 ***Reagents and Conditions*****: (i) pTSA, $PhCH(OMe)_2$, MeCN anhydr., rt.[25] (93% yield) (ii) dioxane, DMAP, $Bu_2Sn(OMe)_2$, $BrCH_2OCH_3$, reflux. (85% yield)[23] (iii) Bu_2SnO, PhH, then Bu_4NI, $BrCH_2OCH_3$, reflux.[26] (65% yield)**

The benzylidene protected derivative **18** was obtained from the reaction of **7a** in the presence of *p*-toluenesulfonic acid and benzaldehyde dimethyl acetal in acetonitrile (93% yield). This was verified by ^{1}H NMR. Signals in the aromatic region were integrating for 5 protons, equivalent to 1 benzylidene. Also only one singlet was observed at 5.53 ppm representing C*H*Ph, this was integrating for one proton thereby verifying the presence of only 1 benzylidene. Two broad singlets were also observed at 2.47 and 3.00 ppm indicating the presence of 2 free OH groups. The following tin-mediated reaction was again performed using bromomethyl methylether and dibutyltin dimethoxide in dioxane and gave the disubstituted derivative **20**, as shown by the NMR spectra which identified two MOM groups present with two AB doublets at 4.81 and 4.80 ppm representing the two OCH_2O signals and also two singlets at 3.45 and 3.42 ppm. The two singlets were integrating for 3 protons each for each OCH_2OCH_3 group (85% yield).

After this a second method for the same reaction was employed using dibutyltin oxide.[26] NMR verified this reaction also gave the disubstituted product (65% yield)

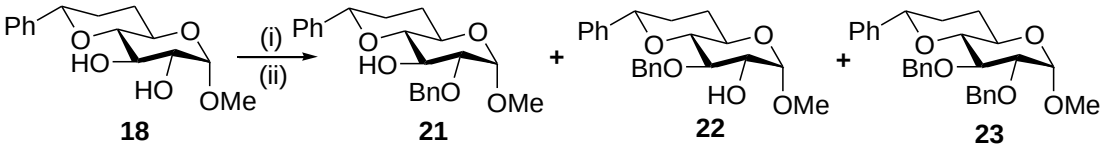
observed in the previous reaction and so an attempt was made to achieve selectivity using a benzyl protecting group instead of the MOM group since similar reactions had been seen in the literature (Scheme 2-18).[14]



18 **21**

Scheme 2-18 ***Reagents and Conditions*****: (i) Bu_2SnO, PhH, then Bu_4NI, BnBr, reflux. (ii) $Bu_2Sn(OMe)_2$, PhH, then Bu_4NI, BnBr, reflux.**[26]

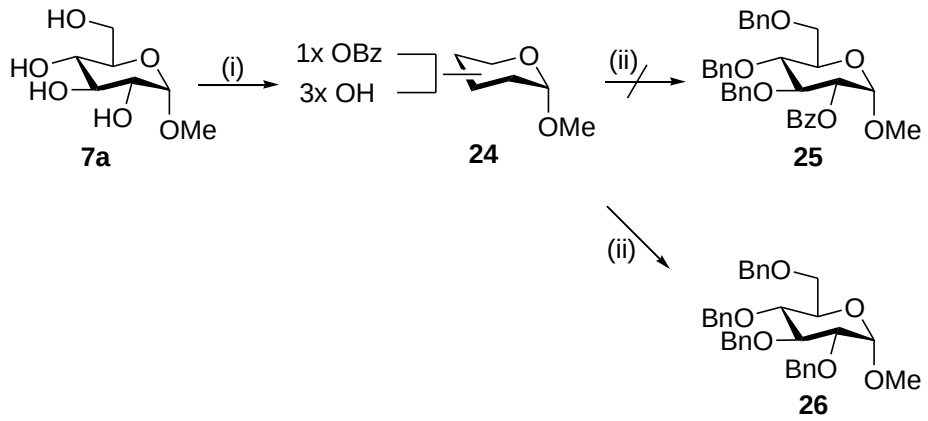
Both tin-mediated methods yielded several products observed by t.l.c. presumed to be mixtures of regioisomers **21**, **22** and **23** (Scheme 2-19).



18 **21** **22** **23**

Scheme 2-19 ***Reagents and Conditions*****: (i) Bu_2SnO, PhH, then Bu_4NI, BnBr, reflux. (ii) $Bu_2Sn(OMe)_2$, PhH, then Bu_4NI, BnBr, reflux.**

A synthesis replacing the ether group with a benzoyl ester was attempted given the previous use of esters for selectively at the 2-OH using a tin-mediated method.[23] This is outlined below (Scheme 2-20).



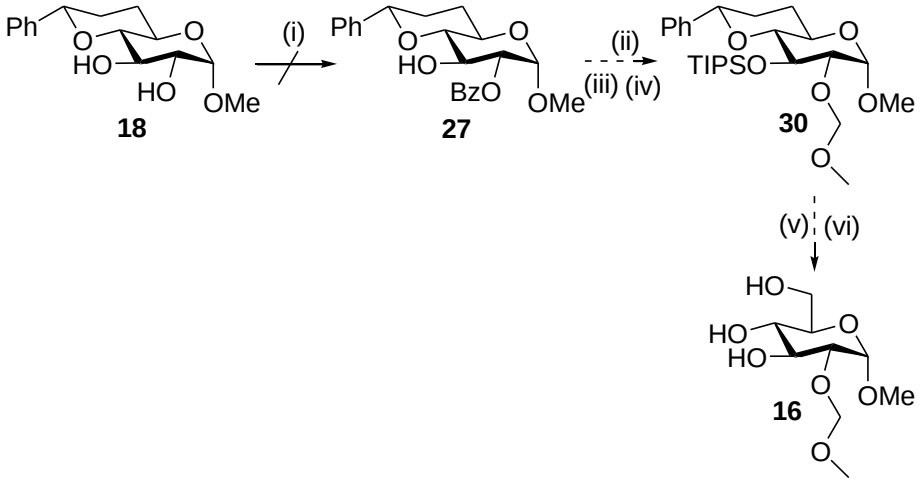
Scheme 2-20 *Reagents and Conditions*: Dioxane, $Bu_2Sn(OMe)_2$, BzCl, rt. (23% yield)[23] (ii) DMF anhdr., BnBr, 0°C, then NaH, rt. (79% yield)

The regioselective benzoylation of **7a** resulted in the formation of 2 products observed by t.l.c. presumed to be a mono and disubstituted derivative. One product appeared to be of a greater quantity by t.l.c. and was isolated by chromatography to verify which isomer it was. This was confirmed to be a monoester by mass spectrometry which was obtained in a 23% yield (Scheme 2-20). A conclusive analysis by NMR could not be obtained due to the free hydroxyl groups splitting the signals. As a result, coupling constants could not be calculated in an effort to identify the peaks and overlapping of the signals meant the COSY was of no further help. Consequently the exact position of the benzoyl group was unknown. A D_2O exchange was performed, however this did not further elucidate the position of the benzoyl group.

For NMR simplification and structural clarification it was decided to protect the remaining free hydroxyls followed by removal of the benzoyl group before attaching the MOM. The attempted benzylation of the remaining free hydroxyls resulted in the removal of the benzoyl group leaving the fully benzylated derivative **26** (79% yield). This was confirmed by the NMR spectrum. Four AB doublets were observed at 4.89, 4.82, 4.66 and 4.54 ppm indicating the presence of 4 benzyl groups. Also the H-2

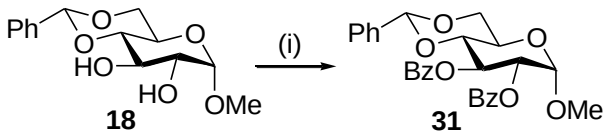
signal was not shifted further downfield than the other signal which would have been expected due to the presence of an ester group. Mass spectrometry data also established this finding.

Given the production of 2 products observed by t.l.c., problems with verification of the position of the ester and the poor yield obtained, benzylidene-protected derivative **18** was used prior to an attempt to selectively derivatise the 2-OH in an effort to reduce the number of free hydroxyl groups (Scheme 2-21).



Scheme 2-21 ***Reagents and Conditions*****: (i) Dioxane, $Bu_2Sn(OMe)_2$, BzCl, rt.[23] (ii) TIPSOTf, 2,6-lutidine, CH_2Cl_2. (iii) NaOH, MeOH, rt. (iv) $BrCH_2OCH_3$, NaH, THF (v) THF anhydr., 0°C, TBAF, rt.[27] (vi) MeOH, TsOH.[25]**

However, the regioselective benzoylation reaction of **18** gave the disubstituted derivative **31** in a 48% yield (Scheme 2-22). ^{1}H NMR verified the presence of three aromatic groups as the signals in this area were integrating for 15 protons. In addition, the H-2 and H-3 signals had both been shifted downfield by at least 1 ppm as would be expected if ester groups were attached to both these positions.



Scheme 2-22 ***Reagents and Conditions*****: (i) Dioxane, $Bu_2Sn(OMe)_2$, BzCl, rt. (48% yield)**[23]

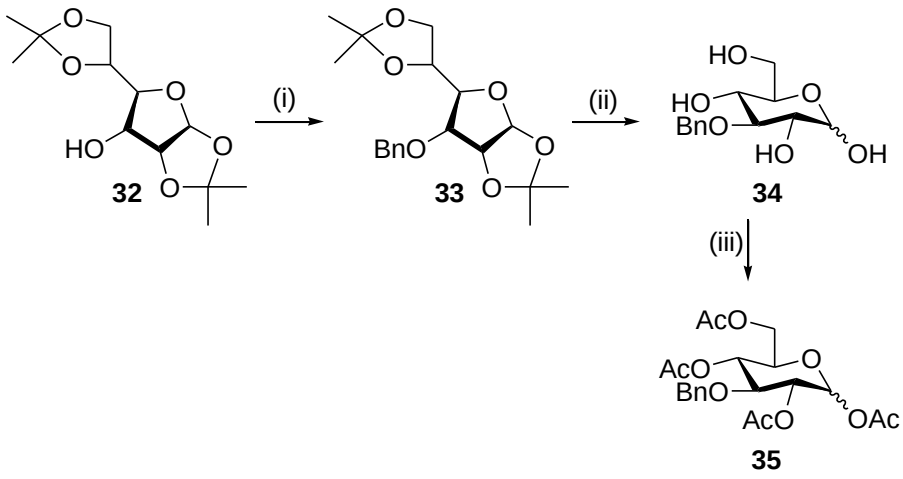
Following this, the tin mediated method was abandoned as it was considered too difficult to obtain single isomers using the α-methyl glucopyranoside **7a**.

2.3.3 Glycoside Donor Synthesis from Diacetone-D-Glucose

Studies using **7a** as a model for **5** indicated that it would be difficult to differentiate between the 2-OH and 3-OH position after the glycosylation reaction and therefore it would be wise to have these two hydroxyl groups differentiated prior to glycosylation. It was considered that diacetone-D-glucose, commercially available from Sigma, could be used due to its protection of all but the 3-OH. This free hydroxyl could therefore be orthogonally protected before removal of the acetonides thus opening the molecule into its pyranose form,[28] from which it was envisaged that a suitable glycosyl donor could be synthesised.

The first three steps of this synthesis were completed on small scale as part of an undergraduate research project (Scheme 2-23).[29] Diacetone-D-glucose **32** was benzylated at its free hydroxyl using benzyl bromide in DMF anhydrous at 0^oC, before being opened into its pyranose form by removal of the cyclic acetals by Amberlite-IR 120 (H^+) in water to give **34**. Mass spectrometry verified the presence of **34** however other compounds including D-glucose **9** and 3-*O*-Bn diacetone derivative **33** were also present indicating the benzylation of **32** in step (i) and the

deprotection of **33** in step (ii) had not completed. Nonetheless, compound **35** was synthesised after acetylation of crude **34** in a 1:1 solution of pyridine and acetic anhydride. At this step mass spectrometry also verified the presence of other compounds including pentaacetate **10** (Scheme 2-13). Co-elution due to similar mobility by t.l.c. possibly led to the presence of these compounds after purification by chromatography.



Scheme 2-23 ***Reagents and Conditions:*** **(i) BnBr, NaH, DMF anhdr. (ii) H_2O, IR 120 (H^+).[28] (iii) Py, Ac_2O.**

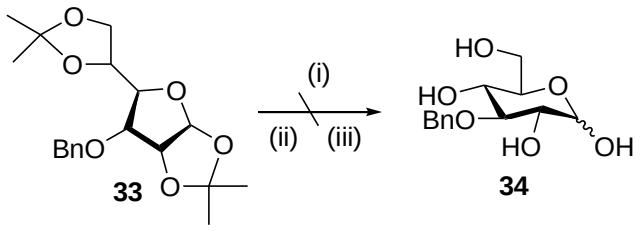
Despite the purity issues encountered in the undergraduate project, this method was believed to merit further investigation and a scale up of the reactions was performed. However upon scale up (10 g of starting material) similar problems occurred.

After several attempts compound **35** could only be obtained in a small percentage with the majority of product being an unidentified mixture. This was possibly due to the problems encountered on small scale whereby a number of compounds were observed by mass spectrometry after step (ii). It was also noted that the ratio of alpha to beta anomers had also changed on scale up with the small scale reaction yielding a

1:5 ratio of α:β anomers of **35** and the large scale yielding a 2.5:1 ratio of α:β anomers of **35**. These ratios were calculated as in Scheme 2-13 from the ^{1}H NMR spectrum using the integration of the anomeric H signals for the α and β isomers. The ^{1}H NMR spectrum of **35** from the small scale reaction showed H-1α as a doublet at 6.32 ppm with a coupling constant of 3.5 Hz. H-1β was also a doublet at 5.65 ppm with a coupling constant of 8.0 Hz. The ^{1}H NMR spectrum of **35** from the large scale reaction showed H-1α as a doublet at 6.31 ppm with a coupling constant of 3.5 Hz. H-1β was also a doublet at 5.65 ppm with a coupling constant of 8.0 Hz.

It was then decided to purify compound **34** prior to acetylation, however this compound was only soluble in water and so chromatography was not possible. Due to this, a precipitation was attempted from water and acetone, however only a slight cloudiness was observed.

Following this, different methods to cleave the acetonide groups from compound **33** were attempted (Scheme 2-24).



Scheme 2-24 ***Reagents and Conditions*****: (i) 1M HCl:THF (1:1) rt.[30] (ii) MeOH, Cat I_2, rt.[30] (iii) 60% aq. AcOH rt.[31]**

The first method involved using a 1:1 solution of 1M HCl and THF at room temperature,[30] however two products with significantly different t.l.c. mobilities were observed and believed to be a mixture of **34** and **36** (Figure 2-11).

The second method attempted used catalytic amounts of iodine in the presence of methanol.[30] Again this method yielded two products as observed by t.l.c.

The third and final method involved stirring the compound in a 60 % aqueous solution of acetic acid however two products were still observed.[31]

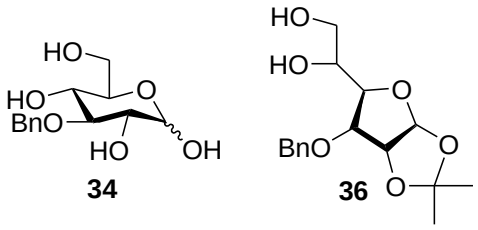


Figure 2-11 Mixture of products possibly formed after attempted deprotection of acetonides from 33.

Due to the difficulties encountered it was decided that a new approach should be adopted, however time constraints of the project at this stage did not allow for further investigation in this area. Nevertheless the following schemes (Section 2.3.4) highlight further possible attempts for future endeavours.

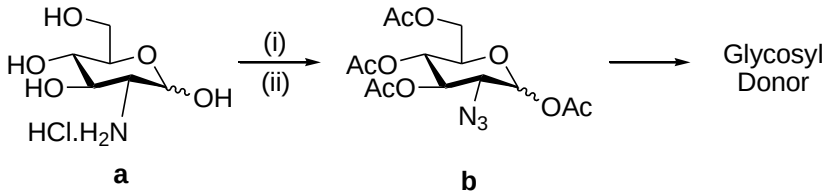
2.3.4 Future Work for the Preparation of a Possible Mimic for GlcNAc-Ins

The following examples outline possible alternative syntheses that could be investigated to obtain a mimic compound for GlcNAc-Ins **1**.

2.3.4.1 Glucosamine

Glucosamine consists of a glucose molecule functionalised at the 2-OH with an amine group. This compound may be of use as a starting material for the synthesis of a glycosyl donor due to the fact that the C-2 is already isolated and it is commercially available from Sigma.

It is envisioned that the C-2 amine could be converted to a non-participating group prior to an attempted glycoside formation. For example, the amine could be converted to an azide[32] which has been shown repeatedly in the literature to not participate in glycoside formation hence leading to preferential formation of the thermodynamically stable α-glycoside (Scheme 2-25).[33,34,35,36]



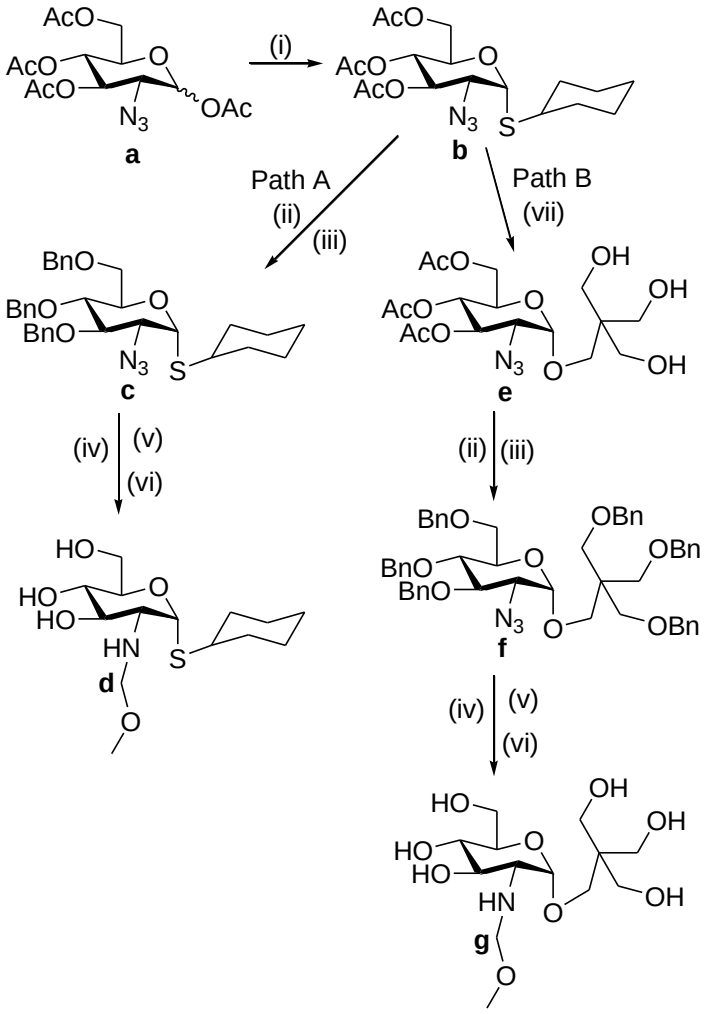
a **b**

Scheme 2-25 ***Reagents and Conditions:*** **(i) imidazole-1-sulfonyl azide hydrochloride, K_2CO_3, $CuSO_4.5H_2O$, MeOH, rt. (ii) Ac_2O, Py, rt.**[32]

The past use of thioglycosides where a cyclohexyl group replaces inositol in the synthesis of inhibitors for Mca and *msh*B[37] may also be functional in the construction of a mimic of compound **1**. The cyclohexyl group is hydrophobic and so this may bind in the hydrophobic pocket of the enzyme as was the case for the BOG molecule (diagram **b**, Figure 1-17).

Following formation of thioglycoside **b** using cyclohexyl mercaptan and $BF_3{\cdot}Et_2O$ in dichloromethane, a MOM group would need to be introduced at the C-2 amine. Prior

to this, removal of the acetate groups followed by replacement with the non-migratory benzyl groups to give **c** (Path A, Scheme 2-26), would be necessary to prevent migration of the acetates to the amine after reduction of the azide. After manipulation of the protecting groups reduction of the azide to the amine may be possible in the presence of the benzyl groups by hydrogenation using Lindlar catalyst.[38,39] Subsequent conversion to the alkoxyamine using MOMCl and NaH in THF, followed by global deprotection may yield an appropriate mimic for compound **1** (**d**, Scheme 2-26).



Scheme 2-26 ***Reagents and Conditions:*** **(i) cyclohexyl mercaptan, CH_2Cl_2, 0°C, $BF_3{\cdot}Et_2O$. (ii) Na, MeOH, rt. (iii) BnBr, NaH, DMF.[30] (iv) Lindlar catalyst, H_2, EtOH.[38,39] (v) CH_3OCH_2Cl, NaH, THF.[30] (vi) EtOH, Pd-C, H_2.[30] (vii) Pentaerythritol, 4 Å mol. Sieve, CH_2Cl_2, NIS, TMSOTf.**

Alternatively a glycosylation reaction with **b** and pentaerythritol **6** (Path B, Scheme 2-26) may give glycoside **e**. Subsequent manipulation of protecting groups, reduction of the azide to the amine, introduction of the MOM group and global deprotection could be carried out following steps (ii) to (vi) (Path A, Scheme 2-26) to yield another alternative mimic **g** for GlcNAc-Ins **1**.

CHAPTER 2 – REFERENCES

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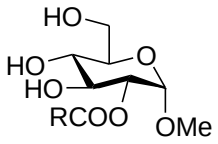
3. Design and Synthesis of Carbohydrate Fatty Acid Derivatives

This project involves the design and synthesis of novel carbohydrate and polyhydroxylated non-carbohydrate fatty acid derivatives for evaluation as antibacterial agents, with a view to examining the effect of variation of the hydrophilic moiety on antimicrobial activity.

3.1 Design of Carbohydrate Fatty Acid Derivatives

A PhD project recently completed in the School of Food Science and Environmental Health, lead to the development of a synthetic route to monosaccharides, functionalised with a range of fatty acids at the 2-OH position. A series of fatty acid esters of methyl α-D-glucopyranoside (**a-e** and **8**, Figure 3-1) and other sugars including lactose and α-D-glucuronic acid derivatives, were synthesised using dibutyltin dimethoxide as stannylating agent and were screened for antibacterial activity against a Gram-positive (*S. aureus*) and Gram-negative (*Salmonella agona*) bacteria.[1]



a; R = $C_{17}H_{35}$
b; R = $C_{15}H_{31}$
c; R = $C_{13}H_{27}$
8; R = $C_{11}H_{23}$
d; R = C_9H_{19}
e; R = C_7H_{15}

Figure 3-1 Methyl α-D-glucopyranoside 7a functionalised at the 2-OH with a range of fatty acid esters.[1]

Preliminary results indicated that only the laurate derivative of methyl α-D-glucopyranoside (**8**, Figure 3-1) had a significant inhibitory effect on the growth of *S. aureus*, while the other sugars and chain lengths (**a-e**, Figure 3-1) had little effect on the growth. None of the ester derivatives had a notable effect on the growth of *S. agona* and is in accordance with previous studies.[2,3,4]

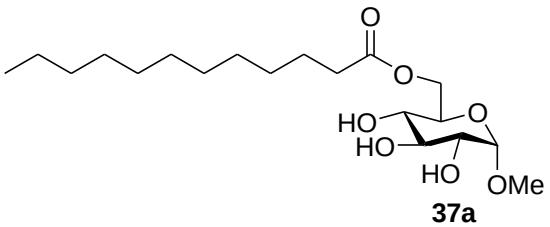
The aim of this project was to synthesise similar carbohydrate fatty acid derivatives, functionalised at the 2-OH via a tin-mediated method. These compounds could then be tested for antimicrobial activity using methods developed and implemented on site in the School of Food Science and Environmental Health. Regioisomers of these compounds would also be synthesised via a chemical route focusing on caprylic and lauric acids conjugated at the 6-hydroxyl position of various sugars. These compounds could then be tested to compare the activity of the different regioisomers and to further investigate whether the antimicrobial activity of fatty acid compounds can be enhanced with a carbohydrate delivery system. In addition, non-carbohydrate polyhydroxylated fatty acid derivatives would also be synthesised and analysed for antimicrobial activity in an effort to simplify both the structure and synthesis of these compounds.

Therefore chemical syntheses were designed to produce a range of fatty acid derivatives. This was to allow investigation of the effects of carbohydrate versus non-carbohydrate cores, the monosaccharide core itself (and the anomeric configuration with respect to glucopyranoside), the length of fatty acid chain, the glycoconjugate linkage and the number of fatty acids attached to the hydrophilic core on antimicrobial activity.

These compounds were tested as inhibitors of both a Gram-positive (*S. aureus*) and a Gram-negative (*E. coli)* microorganism of concern to the food and healthcare industries. The methodology employed and antimicrobial results from antimicrobial activity analyses will be reported and discussed in Chapter 4.

3.1.1 Carbohydrate Fatty Acid Ester Derivatives

Based on the preliminary results from the previous project in the School, whereby the lauroyl ester derivative **8** (Figure 3-1) had significantly greater efficacy against the growth of *S. aureus* than that of any other carbohydrate or fatty acid tested, it was decided to investigate whether similar results would be observed using a different regioisomer of this same compound, where the fatty acid ester would be attached at a different position on the carbohydrate. The primary hydroxyl was chosen for this approach (Figure 3-2). This position was chosen as it could be isolated chemically via a straightforward protection strategy prior to esterification. Moreover, the development of a suitable enzymatic route to the desired fatty acid ester derivative may be possible at this position, in the event of active derivatives being identified.



37a

Figure 3-2 Methyl 6-*O*-lauroyl-α-D-glucopyranoside (37a).

Whilst the antimicrobial activity of carbohydrate fatty acid ester derivatives has been well documented, few studies have investigated the potential role of the carbohydrate

moiety in antimicrobial activity.[5,6,7] This study aims to further elucidate this role through development of synthetic routes allowing the effects of structural differences on antimicrobial activity to be investigated. Therefore variations of compound **37a** using different sugar cores were synthesised. In an effort to explore the effect of structural differences in the carbohydrate on antimicrobial activity, the β-anomer **7b** (Figure 3-3) was chosen for esterification. In compound **7a** (Figure 3-3) the anomeric methoxy group is axial however in compound **7b** this group is equatorial. The use of both these sugars in the formation of fatty acid derivatives would allow for studies into the effect of anomeric configuration on the antimicrobial activity of the carbohydrate fatty acid derivatives.

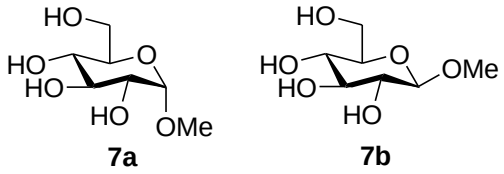


Figure 3-3 Methyl α-D-glucopyranoside (7a) and methyl β-D-glucopyranoside (7b).

Furthermore, examination of the effects of structural changes in the configuration of the hydroxyl groups of the carbohydrate might also assist in further clarification of any possible role of the sugar moiety in activity of these derivatives. Figure 3-4 illustrates the sugars chosen for this purpose.

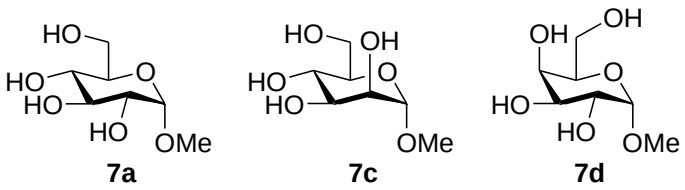


Figure 3-4 Methyl α-D-glucopyranoside (7a), methyl α-D-mannopyranoside (7c) and methyl α-D-galactopyranoside (7d).

Methyl α-D-mannopyranoside (**7c**) was chosen as the secondary hydroxyl at the C-2 position is axial, as is the hydroxyl group at the C-4 position in methyl α-D-galactopyranoside (**7d**) compared with the hydroxyl groups of methyl α-D-glucopyranoside (**7a**) which are all equatorial.

3.1.2 Carbohydrate Caprylic Ester Derivative

As discussed in the introduction (Section 1.4.3), chain length has a significant effect on the activity of both fatty acids and their derivatives.[8] Caprylic acid has been shown to exert antimicrobial activity against a wide range of microorganisms including *S. aureus*.[9] This study also showed the ability of caprylic acid and monocaprylin, the glycerol monoester of caprylic acid to inhibit Gram-negative bacteria *E. coli* in bovine milk. Monocaprylin was the more effective of the two against *E. coli*. Kato and Shibasaki showed that the sucrose diester of caprylic acid had potent antimicrobial activity against certain Gram-positive bacteria and fungi.[10] It was of particular interest to synthesise a caprylic ester carbohydrate derivative as well as a lauroyl ester carbohydrate derivative to allow comparison against both Gram-positive and Gram-negative bacteria. Compound **7a** was chosen for esterification with caprylic acid to give the sugar ester derivative **38a** (Figure 3-5) for comparison with the lauroyl derivative **37a** (Figure 3-5).

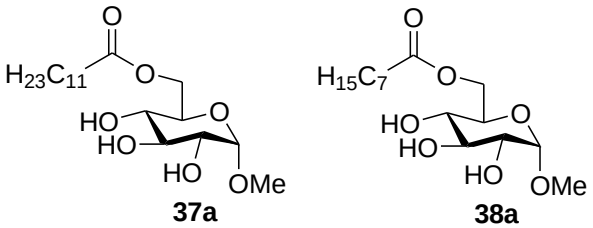
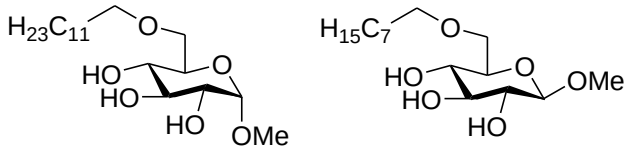


Figure 3-5 Methyl 6-*O*-lauroyl-α-D-glucopyranoside (37a) and methyl 6-*O*-octanoyl-α-D-glucopyranoside (38a).

3.1.3 Carbohydrate Alkyl Ether Derivatives

In addition to investigating the role of the carbohydrate moiety in antimicrobial activity, the glycoconjugate linkage may also play a role. Ruzin *et al.* reported a monolaurin esterase activity in association with the *S. aureus* cell membrane and cytoplasm.[11] However another study that investigated the antimicrobial effect of the ether derivative of glycerol dodecylglycerol indicated an increase in activity against Gram-positive bacteria compared with the commercially available antimicrobial monolaurin under certain conditions.[12] Ether bonds tend to have a higher chemical and metabolic stability than esters, therefore the development of a synthesis route for carbohydrate alkyl ether derivatives (**39a** and **39b**, Figure 3-6) was warranted to allow comparison of antimicrobial efficacy with the carbohydrate ester derivatives, for example **37a**.



39a **39b**

Figure 3-6 Methyl 6-*O*-dodecanyl-α-D-glucopyranoside (39a) and methyl 6-*O*-dodecanyl-β-D-glucopyranoside (39b).

3.1.4 Di-lauroyl monosaccharide Derivative

As previously mentioned in Section 1.4.5, studies have shown that the sucrose ester of lauric acid had potent antimicrobial activity against certain Gram-positive bacteria and fungi.[10] This study also found that in contrast to findings with glycerides the diester of sucrose was more active than the monoester.

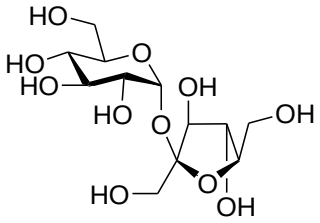
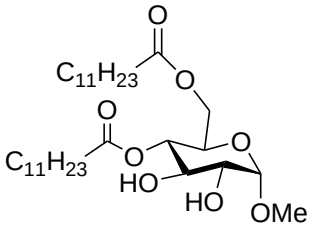


Figure 3-7 Structure of Sucrose.

Sucrose (Figure 3-7) is a disaccharide of glucose and fructose. It was of interest to investigate whether a diester of a monosaccharide might exhibit similar activity therefore the di laurate of methyl α-D-glucopyranoside was chosen (**40** Figure 3-8). The 4- and 6-OH positions were chosen for comparison with the monoesters derivatised at the 6-OH and as these positions could be easily selected regioselectively using a benzylidene group.



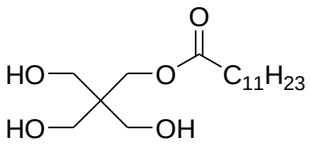
40

Figure 3-8 Methyl 4,6-di-*O*-lauroyl α-D-glucopyranoside.

3.1.5 Non-carbohydrate Polyhydroxylated Ester Derivatives

To explore whether the structure and therefore the synthesis of these ester derivatives could be simplified and still retain their antimicrobial activity, pentaerythritol **6** was chosen to synthesise a non-carbohydrate lauroyl derivative (**41** Figure 3-9). Pentaerythritol was specifically chosen due to its number of hydroxyls. After monoacylation three free hydroxyls would remain and therefore this compound would have the same number of free hydroxyl groups as the monoester carbohydrates and

would hence be useful for comparative studies. This compound also gave scope for investigation of di, tri and tetra ester derivatives for comparison with the carbohydrate derivatives such as **40** (Figure 3-8).



41

Figure 3-9 Mono lauroyl pentaerythritol.

3.1.6 Strategies for the Regioselective Synthesis of Carbohydrate Fatty Acid Derivatives

3.1.6.1 Enzymatic Synthesis of Carbohydrate Fatty Acid Esters

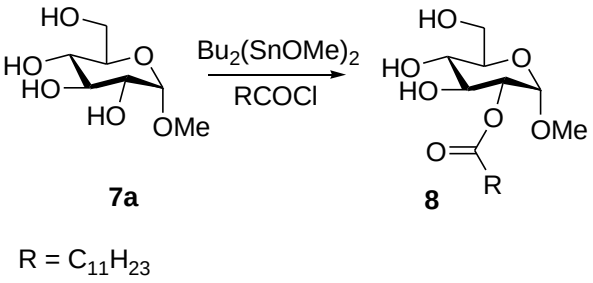
Whilst enzymatic syntheses of carbohydrate fatty acid esters has been shown to be highly regioselective[13] there is also evidence for the production of isomers for some carbohydrates depending on the enzyme used (Section 1.4.5.1).[14] The possibility of the presence of isomers makes it difficult to unequivocally attribute antimicrobial activity to any given compound without purification. However isolation of individual regioisomers by chromatography may prove difficult.

In addition, the presence of regioisomers may hinder the activity of compounds that may otherwise exhibit inhibitory affects as was the case for the presence of isomers in monoglycerides discussed in the introduction (Section 1.4.4.1), whereby monoglycerides with less than 90% monoester were not effective antimicrobials.[8]

A chemical approach was chosen to ensure pure regio-defined compounds were obtained. Thus compound purity or lack thereof could be explicitly ruled out as an influence on any antimicrobial results obtained. Also a suitable route to ether derivatives was necessary and this was not possible via an enzymatic approach.

3.1.6.2 Tin-Mediated Regioselective Synthesis of Carbohydrate Fatty Acid Esters

In the previous project mentioned above (Section 3.1), compounds were designed and synthesised that involved the fatty acid ester being attached at the 2-hydroxyl position using a tin-mediated regioselective synthesis (Scheme 3-1). Solvent and temperature conditions were optimised for both yield and selectivity of the 2-OH.



Scheme 3-1 Regioselective tin-mediated synthesis of methyl α-D-glucopyranoside functionalised at the 2-OH.[1]

In the current work, this method was attempted for the synthesis of 2-*O*-fatty acid esters for comparison with 6-*O*-derivatives against *S. aureus* and *E. coli*. However, a new approach was needed as the synthesis could not be repeated on large scale and further investigation was not merited based on the difficulties previously encountered during the attempted regioselective derivatisation of model compound **7a** for glycoside **5** (Section 2.3.2.1).

3.1.6.3 Protection Strategy for the Regioselective Synthesis of Carbohydrate Fatty Acid Derivatives

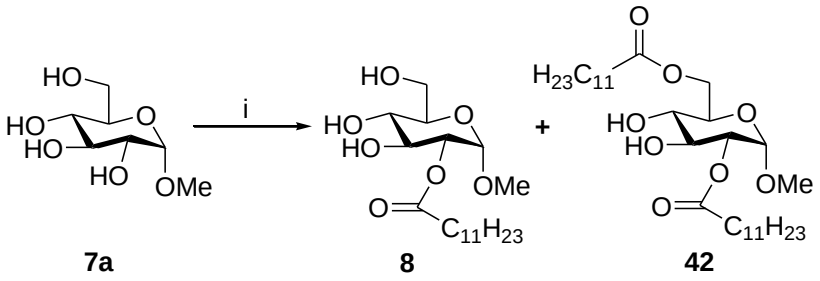
It was clear that an enzymatic synthesis was not an appropriate approach for the compounds that were needed and the level of purity required in this study. A direct esterification of compound **7a** with lauroyl chloride was attempted as part of an undergraduate research project however this method yielded regioisomers.[15] A chemical approach via a protection/deprotection strategy would allow for the synthesis of compounds functionalised at the primary hydroxyl of the monosaccharides chosen, with either an ester or ether group. These compounds would then be purified and characterised to ensure that purity was not a concern during antimicrobial evaluation. Some of the possible approaches to regioselective functionalisation of the primary hydroxyl of monosaccharides are discussed chapter 2 (Section 2.2.2).

3.2 SYNTHESIS

A description of the synthesis of compounds that were tested for antimicrobial activity against *S. aureus* and *E. coli* follows. The synthesis of these compounds was performed chemically via protection strategies with subsequent purification and characterisation to afford pure regio-defined carbohydrate and non-carbohydrate fatty acid derivatives for antimicrobial evaluation.

3.2.1 Synthesis of Monoester Carbohydrate Derivatives

The first attempt to make a lauroyl derivative was based on tin-mediated regioselective synthesis to attach the lauroyl ester at the 2-hydroxyl position previously mentioned in section 3.1.6.2.



7a **8** **42**

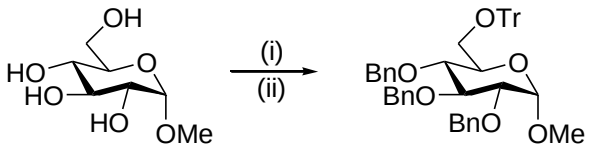
Scheme 3-2 ***Reagents and Conditions*****: (i) $Bu_2Sn(OMe)_2$, dioxane, DMAP, Lauroyl chloride, rt.**

The selectivity of the 2-hydroxyl was attempted using dibutyltin dimethoxide in dioxane with lauroyl chloride at room temperature. Two compounds were obtained, one with the fatty acid attached at the 2-OH position only (**8** Scheme 3-2) and another at both the 2- and 6-OH positions (**42** Scheme 3-2). The ^{1}H NMR spectrum of **8** showed the H-2 peak at 4.71 ppm. This was similar in the spectrum for compound **42** where H-2 was at 4.69 ppm. The difference was noted in the H-6 peak of **42** which was shifted downfield by approximately 1 ppm indicating the presence of the ester group at this position. The integration of the aliphatic peaks present in the spectrum of **42** also indicated the presence of 2 lauroyl chains.

An alternative method was used for the synthesis of regioisomers of compound **8**. This alternative synthesis involved the attachment of the fatty acid at the primary hydroxyl group at C-6 through a series of protection / deprotection steps.

Initially the synthesis commenced with the protection of the primary hydroxyl using a trityl protecting group prepared from **7a** (Scheme 3-3) using triphenylmethyl chloride in pyridine, as this group was known to show selectivity for primary hydroxyls over secondary hydroxyls.[16] The secondary hydroxyls required protection with an orthogonal protecting group allowing for the selective removal of the trityl group.

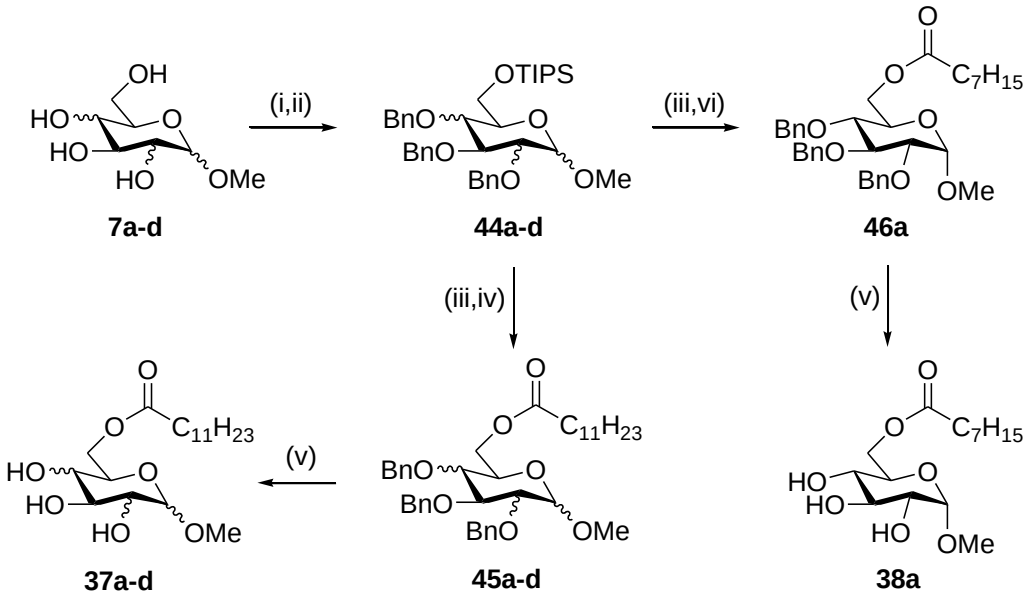
Benzyl ethers were chosen as they are non-migratory and would therefore not move to the 6-OH after deprotection of the trityl group. Benzyl ethers are also readily removed by catalytic hydrogenation after esterification of the primary hydroxyl. These mild conditions were ideal since the ester should be stable in this environment. Furthermore, purification is not necessary after hydrogenation as filtration of the catalyst and evaporation of the solvent and toluene by-product may isolate the compound. Compound **43** was obtained in a 37% yield after purification by chromatography. Proton NMR indicated the presence of the aromatic groups with multiple signals between 6.85 and 7.47 ppm. The integration of the aromatic region indicated that 6 aromatic groups were present. The ^{13}C NMR / DEPT verified the presence of the CPh_3 of the trityl group with a quaternary C at 86.3 ppm.



7a **43**

Scheme 3-3 ***Reagents and Conditions*****: (i) Py anhydr., TrCl, imidazole, 40°C. (ii) DMF anhydr., NaH, BnBr, rt. (37% yield over 2 steps).[16]**

Due to the low yield at this stage it was decided to replace the trityl group with a triisopropylsilyl (TIPS) group. A general synthesis for the production of the mono ester sugars is shown in Scheme 3-4 and is based on the following carbohydrate starting materials: **7a** methyl α-D-glucopyranoside, **7b** methyl β-D-glucopyranoside, **7c** methyl α-D-mannopyranoside and **7d** methyl α-D-galactopyranoside.



Scheme 3-4 ***Reagents and Conditions*****: (i) DMF anhydr., TIPSCl, imidazole, rt.[17] (ii) DMF anhydr., NaH, BnBr, rt.[16] (iii) THF anhydr., 0°C, TBAF, rt.[17] (iv) Py anhydr., DMAP, Lauroyl Cl, rt.[18] (v) EtOH, Pd-C, H_2.[16] (vi) Py anhydr., DMAP, Octanoyl Cl, rt.**

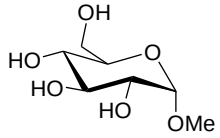
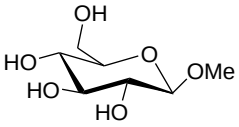
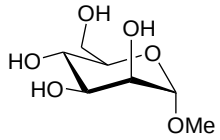
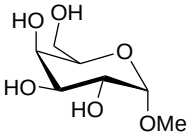
This strategy started with the selective protection of the primary hydroxyl of sugars **7a-d** with a TIPS group. The reagent used was triisopropylsilyl chloride as this method should introduce this group to primary hydroxyls selectively over secondary hydroxyls.[16] The silyl derivatives were then fully protected with benzyl groups and purified by chromatography to give **44a-d**. ^{1}H NMR for each derivative verified the presence of 15 protons in the aromatic region. The TIPS group could be seen as multiple signals between 1 and 1.3 ppm for each compound.

The removal of the TIPS group by tetrabutylammonium fluoride in THF allowed for the esterification of the free 6-OH position with either lauroyl chloride to yield **45a-d** or octanoyl chloride to yield **46a**. At this stage a careful purification by chromatography was performed so that the material after hydrogenation of the benzyl groups would not require further purification by chromatography. The replacement of the TIPS group with a lauroyl group was established by proton NMR as the H-6

signals were generally shifted downfield by approximately 0.5 ppm. This was also noted for the caprylic group in compound **46a**. Also the presence of the aliphatic chain was identified from several characteristic peaks appearing between 0.88 and 2.5 ppm. A difference was observed here in the integration of these peaks for compound **46a** which verified the variation in chain length. Further confirmation was obtained from the IR spectrum which showed peaks for each ester derivative in the region for carboxyl groups generally between 1730 and 1740 cm^{-1}.

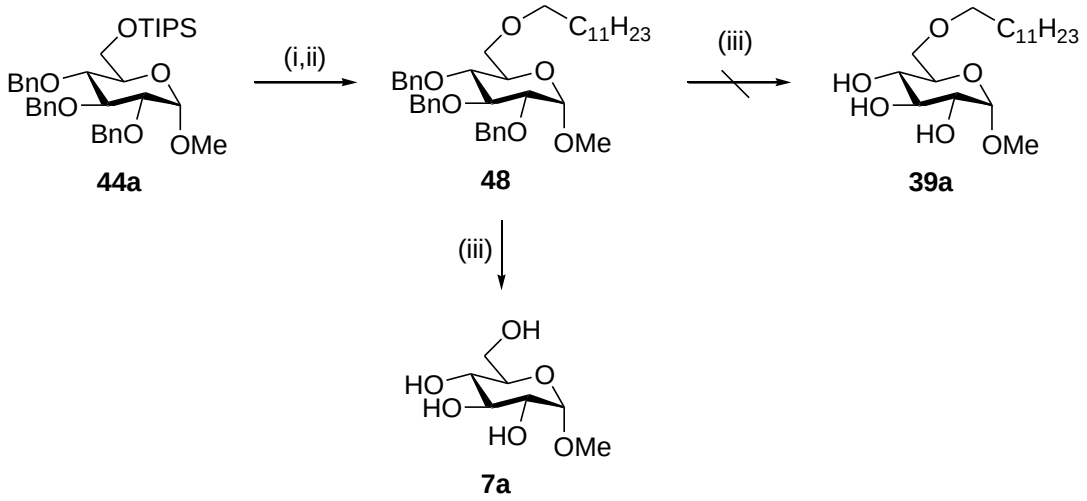
Removal of the benzyl groups form **45a-d** and **46a** by catalytic hydrogenation led to the unprotected carbohydrate esters **37a-d** and **38a**. Confirmation of the removal of the benzyl groups was obtained from the NMR spectrum as no aromatic signals were observed, however the aliphatic signals arising from the fatty acid remained. Also evidence that the ester groups had not been removed was observed in the IR spectrum where the peaks had remained in the carboxyl group region. The percentage yields of each of the characterised compounds in Scheme 3-4 are shown in Table 3-1.

Table 3-1 Percentage yields of compounds 44a-d, 45a-d, 37a-d, 46a and 38a.

Carbohydrate (**1**)	2,3,4-tri-*O*-Bn-6-*O*-TIPS (**2**)	2,3,4-tri-*O*-Bn-6-*O*-lauroyl (**3**)	6-*O*-lauroyl (**4**)	2,3,4-tri-*O*-Bn-6-*O*-octanoyl (**5**)	6-*O*-octanoyl (**6**)
 **(7a)**	**44a** 85%	**45a** 72%	**37a** 86%	**46a** 63%	**38a** 73%
 **(7b)**	**44b** 80%	**45b** 70%	**37b** 75%		
 **(7c)**	**44c** 51%	**45c** 64%	**37c** 75%		
 **(7d)**	**44d** 50%	**45d** 60%	**37d** 86%		

3.2.2 Synthesis of Ether Carbohydrate Derivatives

The first attempt at an ether derivative synthesis was carried out following the same procedure as for the ester using the TIPS and benzyl protecting groups. After removal of the TIPS the dodecanyl ether was then attached using 1-chlorododecane and NaH in DMF to give **48** (35% yield over 2 steps). The presence of the ether group was verified by aliphatic peaks between 0.88 and 1.55 ppm. Integration of these peaks indicated 25 protons. The benzyl protecting groups were then removed by catalytic hydrogenation. However the dodecanyl ether was also removed during this process (Scheme 3-5).



44a **48** **39a** **7a**

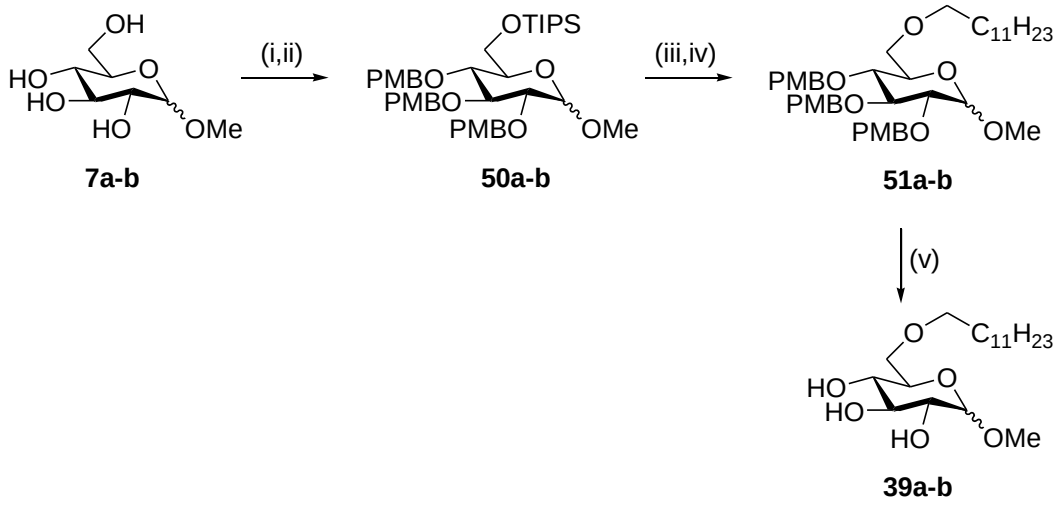
Scheme 3-5 ***Reagents and Conditions*****: (i) THF anhydr., 0°C, TBAF, rt.[17] (ii) DMF anhydr., 1-chlorododecane, 0°C, NaH, rt.[19] (35% yield over 2 steps) (iii) EtOH, Pd-C, H_2.[16]**

Time constraints did not allow for an attempted optimisation of the hydrogenation step of this synthesis for example by utilising different catalysts or varying the H_2 pressure. Instead a different protecting group was used in place of the benzyl groups for the synthesis of the ether derivatives.

The second synthesis also utilised the 6-*O*-TIPS intermediate however this diverged from the ester synthesis with an alternative secondary hydroxyl protecting group, namely the paramethoxybenzyl (PMB) group (Scheme 3-6). PMB groups were chosen to replace the benzyls as these were also non-migratory, they could be safely attached in the presence of the TIPS and their subsequent removal did not require catalytic hydrogenation.[16] The sugars were fully protected with PMBs using paramethoxy benzyl chloride, NaH and tetrabutylammonium iodide in THF and DMF to yield **50a-b** after purification by chromatography. The PMB groups were verified to be present by proton NMR showing 12 protons in the aromatic region and also the PhOCH_3 groups around 3.77 ppm.

Removal of the TIPS group gave the free primary hydroxyl. Following this the lauric ether group was attached using 1-chlorododecane and NaH in DMF and purification by chromatography gave the fully protected pure ether derivatives **51a-b**. Confirmation of the ether group attached at C-6 of both sugars was obtained by NMR spectra as for compound **48**.

Finally oxidative cleavage of the PMB groups with cerric ammonium nitrate gave the mono-dodecanyl sugars **39a-b** which were purified by chromatography. Again the NMR spectra indicated the complete removal of the PMB groups since no aromatic signals were observed.

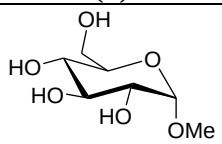
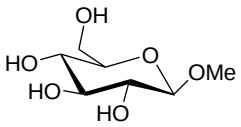


7a-b **50a-b** **51a-b** **39a-b**

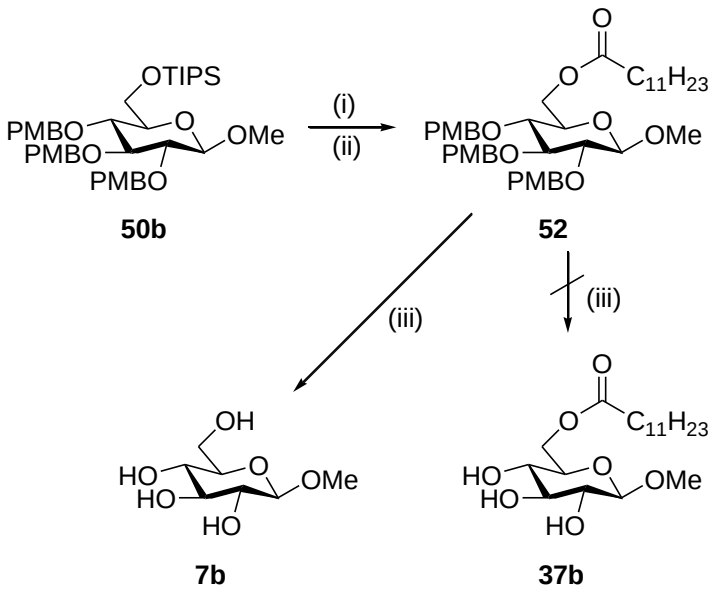
Scheme 3-6 *Reagents and Conditions*: (i) DMF anhydr., TIPSCl, imidazole, rt.[17] (ii) DMF anhydr., THF anhydr., 0°C, NaH, PMBCl, TBAI, rt.[20] (iii) THF anhydr., 0°C, TBAF, rt.[17] (iv) DMF anhydr., 1-chlorododecane, 0°C, NaH, rt.[19] (v) MeCN:H_2O 3:1, CAN, rt.[21]

The percentage yields of each of the compounds synthesised in Scheme 3-6 are shown in Table 3-2.

Table 3-2 Percentage yields of compounds 50a-b, 51a-b and 39a-b.

Carbohydrate (**1**)	2,3,4-tri-*O*-PMB-6-*O*-TIPS (**7**)	2,3,4-tri-*O*-PMB-6-*O*-dodecanyl (**8**)	6-*O*-dodecanyl (**9**)
 **(7a)**	**50a**	**51a**	**39a**
	59%	50%	73%
 **(7b)**	**50b**	**51b**	**39b**
	61%	85%	76%

In an effort to make a more convergent route with common intermediates for ester and ether derivatives, an attempt was made to synthesise the ester derivatives via the same approach used for the ethers using PMB groups. **7b** was used for this synthesis (Scheme 3-7).



Scheme 3-7 ***Reagents and Conditions*****: (i) THF anhydr., 0°C, TBAF, rt.[17] (ii) Py anhydr., DMAP, Lauroyl Cl, rt. (85% yield over 2 steps) (iii) MeCN:H_2O 3:1, CAN, rt.[21]**

This synthesis commenced in the same way as Scheme 3-6 however after removal of the TIPS group the lauroyl ester was attached instead of the ether to give **52**. The presence of the ester was verified by IR which showed a peak at 1737 cm^{-1} and ^{1}H NMR with the aliphatic peaks between 0.88 and 2.32 ppm. However on removal of the PMB groups using cerric ammonium nitrate in acetonitrile and water, the ester was also cleaved.

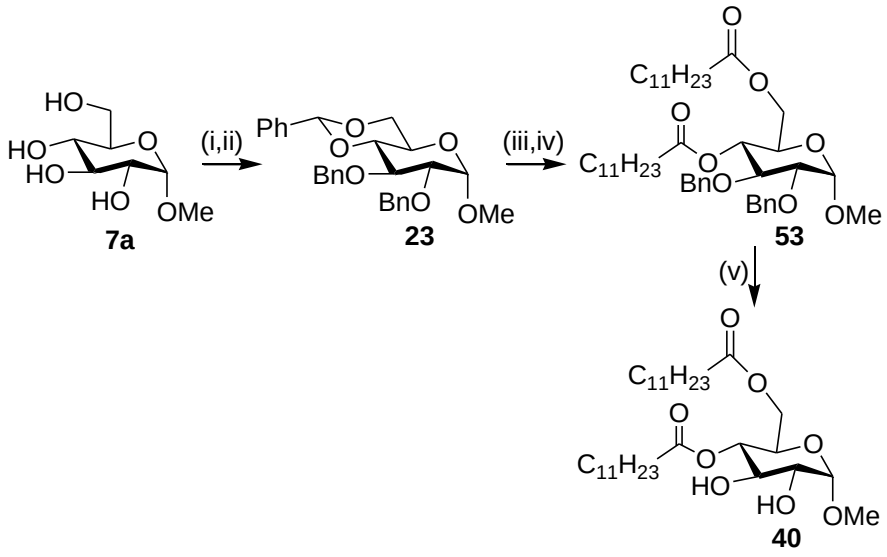
3.2.3 Synthesis of Diester Carbohydrate Derivative

A new method was needed to selectively derivatise the carbohydrate at 2 positions as the TIPS group was only useful for selective protection of primary hydroxyls. Cyclic acetals have been successfully used for this type of protection. They are favoured for their ease of preparation leading to high yields of derivatives, their inertness to a variety of reagents and also synthetic ease and high yields for deprotection.[22]

The method used to synthesise a di lauroyl derivative **40** is shown in Scheme 3-8. The 4 and 6-OH positions of methyl α-D-glucopyranoside **7a** were protected with a benzylidene group using benzaldehyde dimethyl acetal. This group was chosen as it was known to selectively protect the 4- and 6-OH positions. It was also stable to the conditions necessary for a benzylation reaction and the benzyl groups were also stable to its removal.[16] The remaining free hydroxyls were then converted to benzyl ethers to give **23** which was then purified by chromatography (95% yield over 2 steps). The ^{1}H NMR spectrum integrated correctly for aromatic peaks between 7.22 and 7.50 ppm. The presence of a singlet at 5.54 arising from C*H*Ph was verification of a benzylidene group, while two AB doublets between 4.82 and 4.85 ppm arising from OCH_2Ph verified the presence of 2 benzyl groups.

Removal of the benzylidene acetal using catalytic TsOH in MeOH enabled the esterification of the 4 and 6-OH to give **53** (38% yield over 2 steps). This product was again very carefully purified by chromatography so that the material after hydrogenation of the benzyl groups would not require further purification by chromatography. Proton NMR showed H-4 and H-6 had been shifted downfield by approximately 0.5 to 1 ppm. Aliphatic peaks were observed between 0.88 and 2.36 ppm and integrated correctly. IR showed a peak in the carboxyl region at 1743 cm^{-1}.

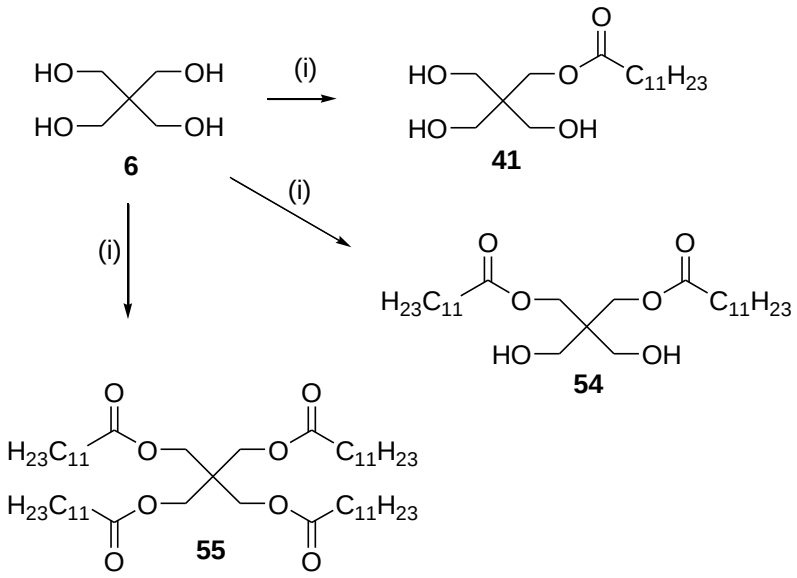
Finally removal of the benzyl groups by catalytic hydrogenation gave the diester derivative **40** in a 75% yield. Evidence for the loss of the benzyl groups was seen in the proton NMR where no aromatic signals were observed however the aliphatic signals had remained. IR verified the presence of hydroxyls with a peak at 3456 cm^{-1} and the esters with a peak at 1737 cm^{-1}.



Scheme 3-8 *Reagents and Conditions*: (i) *p*TSA, $PhCH(OMe)_2$, MeCN anhydr., rt.[20] (ii) DMF anhydr., NaH, BnBr, rt.[16] (95% yield over 2 steps) (iii) MeOH, TsOH.[20] (iv) Py anhydr., DMAP, Lauroyl Cl, rt. (38% yield over 2 steps) (v) EtOH, Pd-C, H_2.[16] (75% yield)

3.2.4 Synthesis of Pentaerythritol Ester Derivatives

Direct esterification of pentaerythritol **6** was performed using lauroyl chloride and DMAP in pyridine at room temperature. The pentaerythritol and derivatives were viewed using a potassium permanganate t.l.c. stain which indicated that 3 products had been obtained from the reaction, however a large amount of starting material had still unreacted. In order to push the reaction to completion a total of three equivalents of lauroyl chloride were added and the solution was heated to 50^{o}C. After 24 hours all starting material had reacted and three products were obtained. These were isolated by chromatography to yield the 3 non-sugar derivatives **41**, **54** and **55** shown in Scheme 3-9 (14%, 29% and 6% yields respectively).



Scheme 3-9 ***Reagents and Conditions*****: (i) Py anhydr., DMAP, Lauroyl Cl, rt. (41: 14% yield, 54: 29% yield, 55: 6 % yield)**

Mass spectrometry indicated the presence of one lauroyl group for compound **41**. The ^{1}H NMR showed a singlet integrating for two protons at 4.11 ppm arising from the CH_2OC=O$C_{11}H_{23}$ indicating there was only 1 lauroyl group attached. Further evidence for this was observed from peaks between 3.61 and 3.80 ppm which were

integrating for 9 Hs. These corresponded to three CH_2OH and three hydroxyl protons. The aliphatic signals between 0.88 and 2.34 ppm integrated for 23 protons signifying 1 lauroyl group. FTIR showed peaks at 3462 and 1737 cm^{-1} representing the OH groups and ester group respectively.

For compound **54** mass spectrometry indicated the presence of two lauroyl groups. The ^{1}H NMR singlet at 4.11 ppm integrated for 4 protons indicating 2 lauroyl groups attached. A singlet at 3.58 ppm integrated for 4 protons arising from each CH_2OH. A broad singlet at 3.22 ppm integrated for 2 protons arising from the 2 free hydroxyl groups. Finally the aliphatic signals integrated for 46 protons verifying the presence of 2 lauroyl groups. Again FTIR showed peaks at 3351 and 1739 cm^{-1} signifying the OH groups and lauroyl groups respectively.

For compound **55** mass spectrometry verified the presence of 4 lauroyl groups. The ^{1}H NMR singlet at 4.11 ppm integrated for 8 protons signifying 4 lauroyl groups attached. No signals were observed corresponding to any free hydroxyl groups or CH_2OH groups. Aliphatic signals integrated for 92 protons indicating the presence of 4 lauroyl groups. FTIR showed a peak at 1735 cm^{-1} for the ester groups however no peaks were observed in the alcohol region.

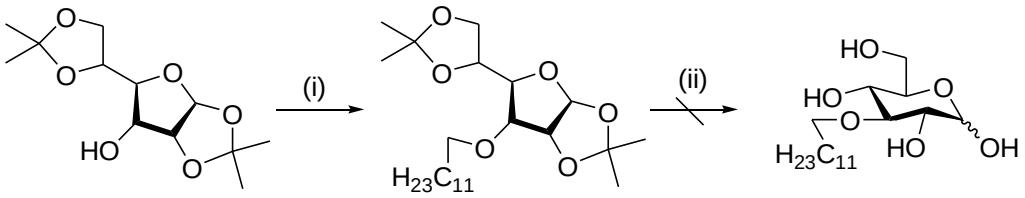
3.2.5 Synthesis of Diacetone D-Glucose Fatty Acid Derivatives

For further comparative study, synthesis of an ether derivative using a reducing sugar was attempted whereby there would be a free hydroxyl at the anomeric position instead of a methyl group. This was attempted using diacetone-D-glucose **32** which

would also have allowed for the synthesis of derivatives for evaluation in two steps. Scheme 3-10 illustrates the attempt made.

An ether was to be attached at the 3-OH of diacetone-D-glucose (**32** Scheme 3-10). This reaction was successfully performed using 1-chlorododecane in the presence of NaH in DMF to give **56** (Scheme 3-10) which was purified by chromatography to give an 89% yield. ^{1}H NMR verified the presence of the aliphatic groups as before and no signal was observed in the OH region of the IR spectrum.

An attempt to deprotect the sugar so it would open into its pyranose form was made. This compound would be different from the other ether derivatives since it was a reducing sugar and also it would be derivatised at a different position, namely the secondary hydroxyl group at C-3. This rationale was not applied for the production of an ester derivative since under the conditions necessary for the removal of the acetonide groups the ester would likely be also removed.[16]



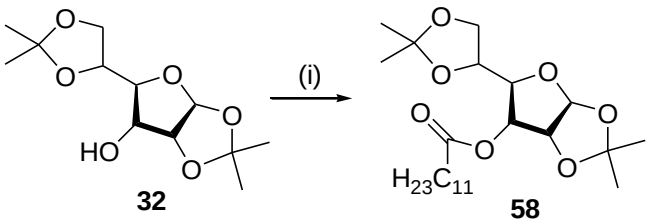
32 **56** **57**

Scheme 3-10 (i) DMF anhydr., 1-chlorododecane, 0^{o}C, NaH, rt.[19] (89% yield) . (ii) H_2O, IR 120 (H^+).[19]

Unfortunately the same problems as before in the synthesis of a glycoside donor from diacetone-D-glucose in Chapter 2 (Section 2.3.3) were encountered when an attempt was made to remove the acetonide groups since t.l.c. indicated the presence of 2

products. Due to the difficulties previously encountered with this reaction and time limitations, no further attempts were made to complete this reaction.

Nevertheless since a pure sample of compound **56** had been obtained in high yield and with relative ease, it was decided to also make the ester derivative of this protected sugar for subsequent antimicrobial analysis. The ester was attached to the 3-OH of diacetone-D-glucose **32** in the same way as with the other sugars using lauroyl chloride in the presence of DMAP in pyridine to give **58** (Scheme 3-11). This was purified by chromatography giving a 79% yield.



32 **58**

Scheme 3-11 ***Reagents and Conditions*****: (i) Py anhydr., DMAP, Lauroyl Cl, rt. (79% yield).**

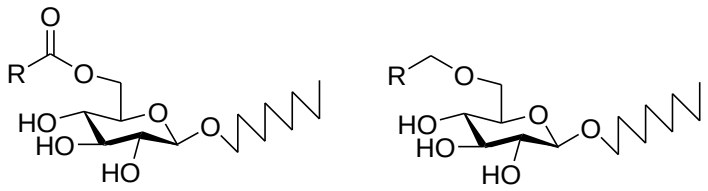
Aliphatic signals in the ^{1}H NMR again verified the presence of the lauroyl group. A peak observed at 1747 cm^{-1} in IR spectrum also verified the presence of the ester group and again as with the ether derivative **56**, no peaks were observed in the OH region.

3.3 POSSIBLE FUTURE SYNTHESIS OF CARBOHYDRATE FATTY ACID DERIVATIVES

To further optimise the syntheses with respect to a shorter route, increased yields of the fatty acid derivatives, or indeed highlighting other potentially useful compounds, the following alternative syntheses could be explored.

3.3.1 Alternative Carbohydrates and Fatty Acids for Further Investigation into the Antimicrobial Activity of Carbohydrate Fatty Acid Derivatives

The synthesis of carbohydrate fatty acid derivatives utilising alternative sugar groups may be of interest. For example the BOG molecule discussed in the introduction (Section 1.3.2) may also be used for the attachment of the ether and ester groups (Figure 3-10).



a **b**

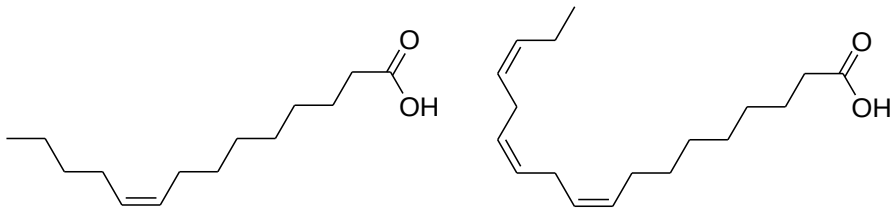
R = $C_{11}H_{23}$

Figure 3-10 Octyl 6-*O*-lauroyl-β-D-glucopyranoside (a) and octyl 6-*O*-dodecanyl-β-D-glucopyranoside (b).

These compounds would also have an alkyl ether chain at the anomeric position as well as functionalisation at the primary hydroxyl by either an ester (**a** Figure 3-10) or ether (**b** Figure 3-10). Although these compounds would have two alkyl chains, the three secondary hydroxyl groups would remain free conceivably maintaining the hydrophilic / hydrophobic balance and retaining water solubility which was shown to be crucial for antimicrobial evaluation and will be discussed in Chapter 4.

Alternative strategies could involve the attachment of different types of fatty acids. For example unsaturated fatty acids. As discussed in the introduction (Section 1.4.3) in a study by Sun *et al.* saturated and unsaturated fatty acids and monoglycerides were tested for antimicrobial activity against the *H. pylori*.[23] Of the unsaturated fatty acids

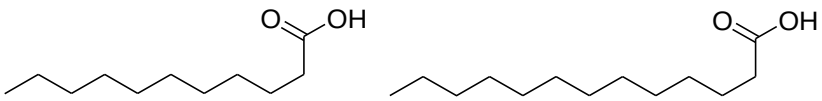
tested, the most potent possessed an equivalent carbon number of 12 discussed in section 1.4.3. These were myristoleic acid (**a** Figure 3-11) and linolenic acid (**b** Figure 3-10). The attachment of these unsaturated fatty acids to a carbohydrate moiety may also be of considerable interest in investigations into identification of active compounds.



a **b**

Figure 3-11 Myristoleic acid (a) and linolenic acid (b).

Other alternative chain lengths of saturated fatty acids might also be of interest. Although results from the previous project showed only lauric acid to exhibit significant inhibition of *S. aureus* and the literature generally considers C12 most active, fatty acids with carbon chain lengths of 11 or 13 were not tested. These chain lengths may show inhibitory effects as their chain lengths only differ from lauric acid by one carbon (Figure 3-12).



a **b**

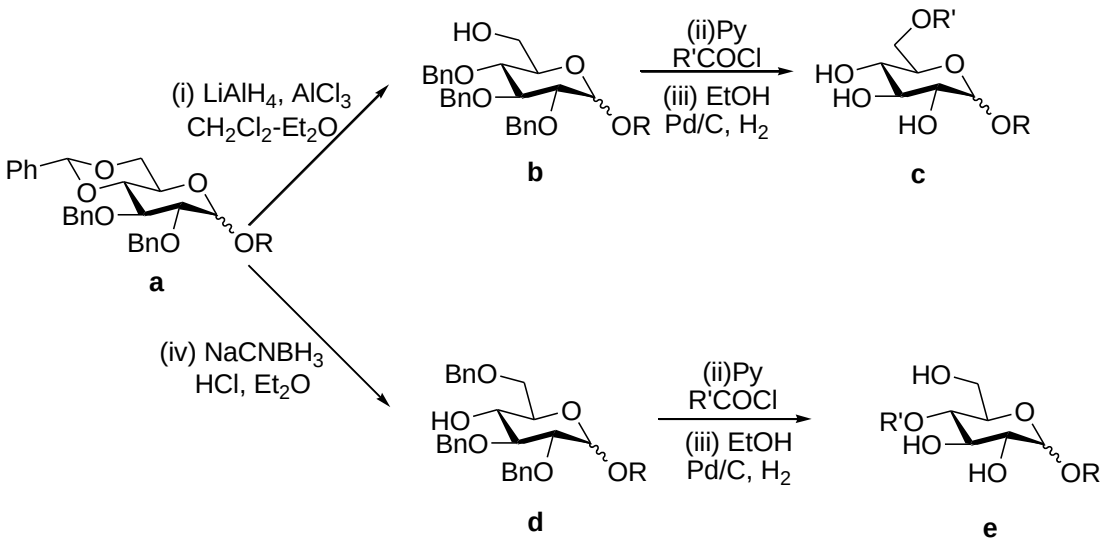
Figure 3-12 Undecanoic acid (a) and tridecanoic acid (b).

3.3.2 Alternative Strategies for the Chemical Synthesis of Carbohydrate Fatty Acid Derivatives

One alternative to the current synthesis of the monoester derivatives is the use of a benzylidene protected sugar (**a**, Scheme 3-12). The advantages of using cyclic acetals as protecting groups are discussed in Section 3.2.3. Reductive cleavage of the benzylidene yields a benzyl ether and a free hydroxyl group. Depending on the reagents used, either the 4-OH or the 6-OH is left free.

The use of $LiAlH_4$ and $AlCl_3$ in a mixture of dichloromethane and ether yield the 2,3,4-*O*-Bn derivative **b** (Scheme 3-12).[24] The free OH may then be esterified followed by hydrogenation of the benzyl groups to yield an ester derivative conjugated at the 6-OH.

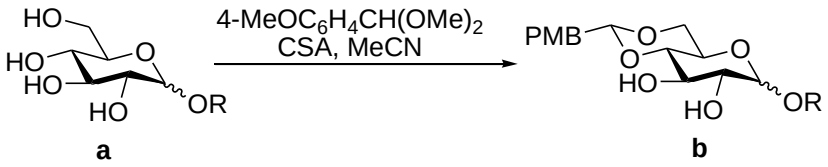
Another $NaCNBH_3$ and HCl in ether could be used to yield the 2,3,6-*O*-Bn derivative **d** (Scheme 3-12).[24] This intermediate may then be used to attach the ester at a new position followed by removal of the benzyl ethers to yield **e** (Scheme 3-12) which would allow for the comparison of two regioisomers.



R = Me or Oct
R' = Saturated fatty acid ester

Scheme 3-12 Alternative synthesis of ester derivatives by reductive cleavage of benzylidene.[24,25]

Also, a similar synthesis could be performed using a 4-methoxybenzylidene protected derivative (Scheme 3-13) for 4-*O* and 6-*O* ether derivatives. The 4,6-di-*O*-protected derivative can be synthesised using 4-methoxy benzaldehyde dimethyl acetal, in the presence of camphorsulfonic acid.[26]

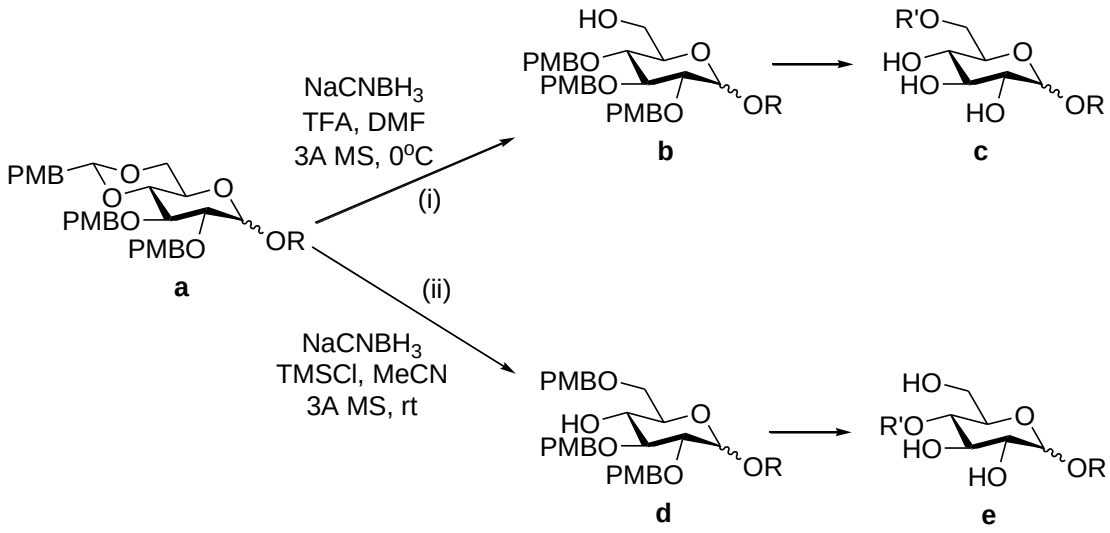


R = Me or Oct

Scheme 3-13 Formation of 4-Methoxybenzylidene protected derivative.[26]

The 4,6-di-*O*-*p*-methoxybenzylidene derivative could then be protected with PMB groups on the remaining free hydroxyls. Reductive cleavage of the *p*-methoxybenzylidene group may be performed to yield either the 4-*O*-*p*-methoxybenzyl ether or 6-*O*-*p*-methoxybenzyl ether, depending on the reagents and

solvent used (Scheme 3-14).[26] The **b** and **d** (Scheme 3-14) regioisomers respectively, could then be used to attach the desired ether at the 4- and 6-OH positions respectively. Removal of the PMB groups would give the **c** and **d** regioisomers for evaluation and comparison.



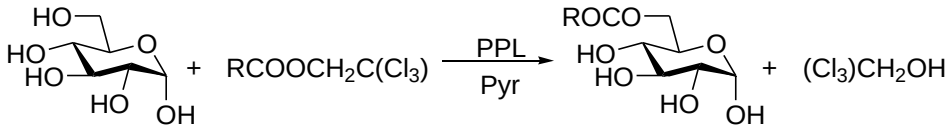
Scheme 3-14 Alternative synthesis of ether derivatives by reductive cleavage of *p*-methoxybenzylidene.[26]

3.3.3 Enzymatic Synthesis

Certain enzymes have been shown to exhibit selectivity for the same hydroxyl group on several different sugars. For example the lipase from *Thermomyces lanuginosus* displays a notable selectivity for only one hydroxyl group in the acylation of sucrose, maltose, leucrose and maltriose.[27]

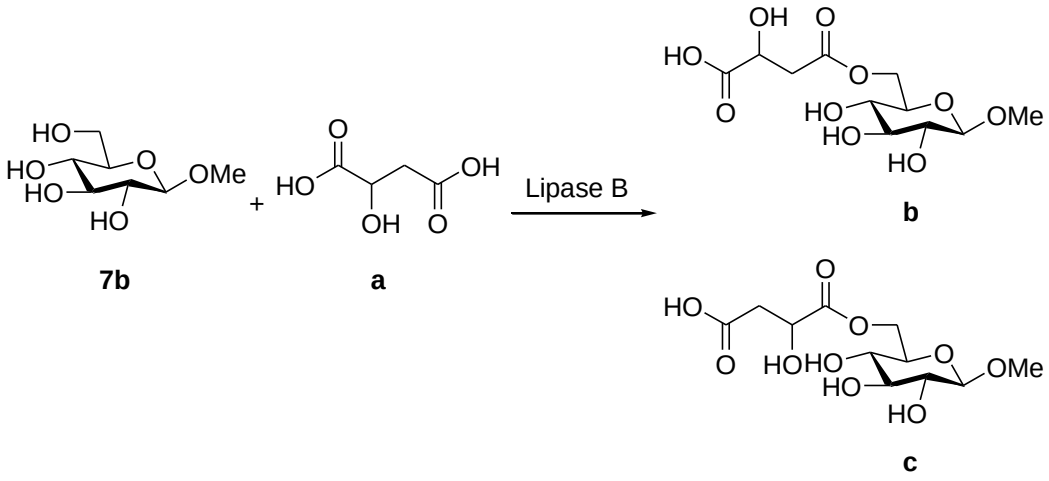
Several commercially available lipases have been used for the regioselective acylation of the primary hydroxyl group in reducing monosaccharides in different solvents. For

example porcine pancreatic lipase (PPL) catalysed acylation of sugars with activated esters of carboxylic acids in pyridine (Scheme 3-15).[28]



Scheme 3-15 PPL-catalysed regioselective esterification of glucose.[28]

The non-reducing sugar methyl β-D-glucopyranoside (**7b** Scheme 3-16) has also been successfully esterified with α-hydroxy acid, malic acid (**a** Scheme 3-16) by an enzymatic synthesis utilising alkaline protease from *Bacillus subtilis* as a catalyst for trans-esterification.[29] Malic acid has two free carboxylic groups enabling the production of isomers (**a** and **b** Scheme 3-16).



Scheme 3-16 Synthesis of β-methylglucoside malate.[29]

As described in section 3.1.6.1 an enzymatic synthesis was not appropriate for the development of several different sugar fatty acid ester derivatives as a single method has not been developed for variation in the sugar moiety. However if a library of carbohydrate fatty acid ester derivatives were obtained and analysed for antimicrobial

activity, an appropriate enzymatic synthesis may then be utilised upon identification of the most active compounds.

CHAPTER 3 - REFERENCES

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4. Antimicrobial Activity Evaluation of Fatty Acid Derivatives

In this chapter, evaluation of the antimicrobial activity of the fatty acid derivatives synthesised as described in Chapter 3 are reported and discussed.

4.1 Evaluation of Antimicrobial Activity

Minimum inhibitory concentrations (MICs) are defined here as the lowest concentration of an antimicrobial that will inhibit the growth of a microorganism after overnight incubation. MICs are used by diagnostic laboratories mainly to confirm resistance, but more often as a research tool to determine the *in vitro* activity of new antimicrobials.[1]

The traditional method of determining MIC is the broth dilution technique, where serial dilutions of antimicrobials are incorporated into the broth media in either the wells of microtiter plates or in cultural tubes. The micro broth dilution method is an adaption of the broth dilution method using small volumes. It utilises microtiter plastic plates containing 96 wells. The advantage of this system is that it utilises small volumes of reagents and allows a large number of bacteria or compounds to be screened for resistance or activity relatively quickly. [2]

4.1.1 Bacteria and Cultural Conditions

The bacteria used in this study were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. Stock cultures were maintained in tryptic soy broth (TSB, Sharlau Chemie, Spain) supplemented with 20% glycerol at -70°C. Cultures

were routinely grown by subculturing 100 µL of stock culture into 9 ml TSB and incubating at 35°C for 18 h.

Cultures were then maintained on tryptic soy agar (TSA, Sharlau Chemie, Spain) plates at 4°C. Working cultures were prepared by inoculating a loop of pure culture into TSB and incubating at 35ºC for 18 h. A bacterial suspension was prepared in saline solution (NaCl 0.85%, BioMérieux, France) equivalent to a McFarland standard of 0.5, using the Densimat photometer (BioMérieux, SA, France), to obtain a concentration of $1x10^8$ cfu/mL. This suspension was then serially diluted in TSB to obtain a working concentration of $1x10^6$ cfu/mL.

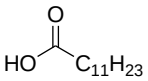
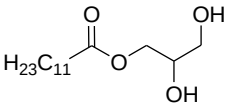
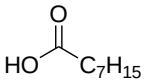
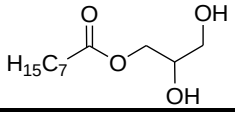
4.1.2 Preparation of Test Compounds

4.1.2.1 Fatty Acid and Monoglyceride Standards

The saturated fatty acids lauric and caprylic acid as well as their corresponding monoglycerides, monolaurin and monocaprylin (Sigma-Aldrich ~99% purity) were used as standards in this study (Table 4-1).

Standard stock solutions (100 mM) of the fatty acids and monoglycerides were prepared in sterile hydroalcoholic diluent (ethanol-distilled water, 1:1) and stored at -20^{o}C. Stock solutions were diluted in TSB to obtain working concentrations. The final concentration of ethanol (5% and 10%), corresponding to the highest concentration of compound used (10 mM for the Gram-positive and 20 mM for the Gram-negative bacteria) had a negligible effect on bacteria viability.

Table 4-1 Fatty acids and monoglycerides used as standards in antimicrobial activity assays.

Name	*Compound*
Lauric acid	
Monolaurin	
Caprylic acid	
Monocaprylin	

4.1.2.2 Fatty Acid Derivatives

A series of fatty acid derivatives were synthesised. Table 4-2 and Table 4-3 show the fatty acid derivatives assessed for antimicrobial activity. Standard stock solutions (100 mM) of the fatty acid derivatives were prepared in sterile hydroalcoholic diluent (ethanol-distilled water, 1:1) and stored at -20^{o}C. Stock solutions were diluted in TSB to obtain working concentrations.

Table 4-2 Glucopyranoside, mannopyranoside and galactopyranoside derivatives tested for antimicrobial activity.

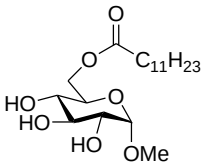
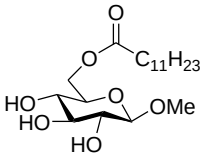
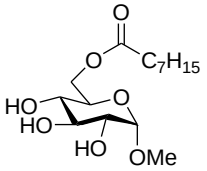
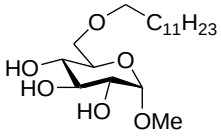
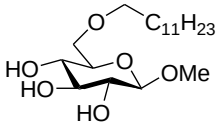
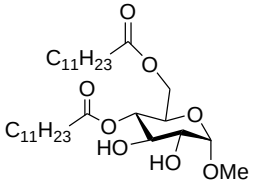
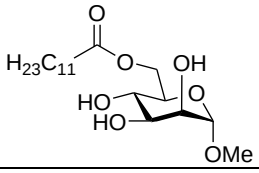
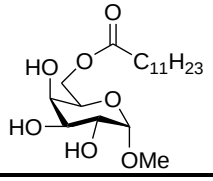
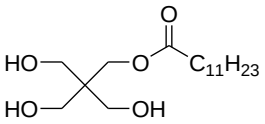
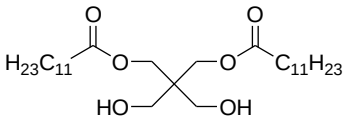
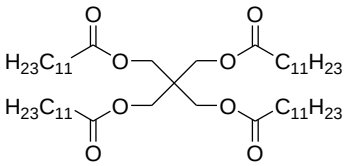
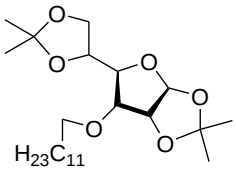
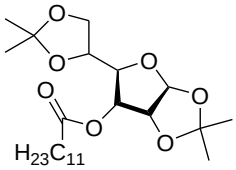
Compound Category	Name	Compound
Glucopyranoside	Methyl 6-*O*-lauroyl-α-D-glucopyranoside (**37a**)	
	Methyl 6-*O*-lauroyl-β-D-glucopyranoside (**37b**)	
	Methyl 6-*O*-octanoyl-α-D-glucopyranoside (**38a**)	
	Methyl 6-*O*-dodecanyl-α-D-glucopyranoside (**39a**)	
	Methyl 6-*O*-dodecanyl-β-D-glucopyranoside (**39b**)	
	Methyl 4,6-di-*O*-lauroyl-α-D-glucopyranoside (**40**)	
Mannopyranoside	Methyl 6-*O*-lauroyl-α-D-mannopyranoside (**37c**)	
Galactopyranoside	Methyl 6-*O*-lauroyl-α-D-galactopyranoside (**37d**)	

Table 4-3 Pentaerythritol and Diacetone-D-Glucose Derivatives Tested for Antimicrobial Activity.

Compound Category	Name	Compound
Pentaerythritol	Mono lauroyl pentaerythritol (**41**)	
	Di lauroyl pentaerythritol (**54**)	
	Tetra lauroyl pentaerythritol (**55**)	
Diacetone-D-Glucose	1,2:5,6 di-*O*-isopropylidene-3-*O*-dodecanyl-α-D-glucofuranose (**56**)	
	1,2:5,6 di-*O*-isopropylidene-3-*O*-lauroyl-α-D-glucofuranose (**58**)	

4.1.3 Antimicrobial Activity Assays

Working test compounds and standards were serially diluted in sterile TSB to a final volume of 100 µL within the 96-well plate. 100 µL of freshly prepared inoculum of the organism under study was added to each appropriate well, the final volume in each well was 200 µL. The final concentration of each microorganism in each well was approximately $5x10^5$ cfu/mL and the concentration range of chemical compounds was from 1:2 to 1:256. Each concentration was assayed in duplicate.

The following controls were used in the microplate assay for each organism and test compound;

Blank: uninoculated media without test compound to account for changes in the media during the experiment.

Negative control: uninoculated media containing only the test compound to assess background noise.

Positive control 1: inoculated media without compound to evaluate the microbial growth under optimal conditions.

Positive control 2: inoculated media without compound but including the corresponding sugar/pentaerythritol to evaluate any effect of the sugar/pentaerythritol alone.

Positive control 3: inoculated media without compound but with the equivalent concentration of ethanol used to dissolve the test compound thereby assessing any activity of the alcohol.

The 96-well plates were incubated at 35°C for 18 hours in a microtiterplate reader (PowerWave microplate Spectrophotometer, BioTek) and effects were monitored by measuring the optical density (OD) at 600 nm for each well every 20 minutes with 20 seconds agitation before each OD measurement. Each experiment was replicated three times.

4.1.4 Minimum Inhibitory Concentration Determination

The MIC was defined as the lowest concentration of compound that showed no increase in OD values for all the replicates compared to the negative control after 18 hours. Subtraction of the absorbance of the negative control eliminated interferences due to possible changes in the media.

4.2 RESULTS

Two non-carbohydrate polyhydroxylated fatty acid ester derivatives, seven carbohydrate fatty acid ester derivatives and three carbohydrate long chain alkyl ether derivatives, together with their corresponding polyhydric alcohols, fatty acids and monoglycerides as controls, were tested against a Gram-positive bacteria, *Staphylococcus aureus,* and a Gram-negative bacteria, *Escherichia coli*, to assess their antimicrobial activity.

The efficacy of the derivatives and controls were compared using Minimum Inhibitory Concentration (MIC) values. The polyhydric alcohols (carbohydrates and pentaerythritol) showed no antimicrobial activity or growth promoting effects for the microorganisms under the conditions used (results not shown).

The data in Table 4-4 show that the monoglycerides monolaurin and monocaprylin had greater activity compared to the free fatty acids lauric acid and caprylic acid against *S. aureus*. Of the monoglycerides and free fatty acids tested, monolaurin had the lowest MIC values for *S. aureus*, with a value of 0.04 mM compared to a value of 0.63 mM for lauric acid. Furthermore, monocaprylin showed MIC values of 2.5 mM against *S. aureus* compared to the value of 5.0 mM for caprylic acid. With respect to *E. coli*, monolaurin showed less inhibitory effect than lauric acid with values of 20 mM and 10 mM respectively. In contrast, monocaprylin showed activity against *E. coli* at concentrations of 6.25 mM compared with caprylic acid value of 12.5 mM. All fatty acid derivatives showed greater antimicrobial activity against *S. aureus* than *E. coli*.

Table 4-4 MIC values of fatty acid derivatives and controls.

Compound Category	***Compound Name***	**S. aureus *ATCC 25923***	**E. coli *ATCC 25922***
Control Compounds	Lauric acid	0.63 mM	10 mM
	Monolaurin	0.04 mM	20 mM
	Caprylic acid	5 mM	12.5 mM
	Monocaprylin	2.5 mM	6.25 mM
Glucose Derivatives	Methyl 6-*O*-lauroyl-α-D-glucopyranoside (**37a**)	0.31 mM	20 mM
	Methyl 6-*O*-lauroyl-β-D-glucopyranoside (**37b**)	0.04 mM	20 mM
	Methyl 6-*O*-octanoyl-α-D-glucopyranoside (**38a**)	2.5 mM	12.5 mM
	Methyl 6-*O*-dodecanyl-α-D-glucopyranoside (**39a**)	0.04 mM	20 mM
	Methyl 6-*O*-dodecanyl-β-D-glucopyranoside (**39b**)	2.5 mM	20 mM
	Methyl 4,6-di-*O*-lauroyl-α-D-glucopyranoside (**40**)	ND*	ND
Mannose Derivatives	Methyl 6-*O*-lauroyl-α-D-mannopyranoside (**37c**)	0.04 mM	20 mM
Galactose Derivatives	Methyl 6-*O*-lauroyl-α-D-galactopyranoside (**37d**)	>10 mM	>20 mM
Pentaerythritol Derivatives	Mono lauroyl pentaerythritol (**41**)	>10 mM	>20 mM
	Di lauroyl pentaerythritol (**54**)	ND	ND
	Tetra lauroyl pentaerythritol (**55**)	ND	ND
Diacetone Derivatives	1,2:5,6 di-*O*-isopropylidene-3-*O*-dodecanyl-α-D-glucofuranose (**56**)	> 10 mM	> 20 mM
	1,2:5,6 di-*O*-isopropylidene-3-*O*-lauroyl-α-D-glucofuranose (**58**)	> 10 mM	> 20 mM

* Not determined due to insolubility.

Among the sugar fatty acid esters and the sugar alkyl ethers prepared, methyl 6-*O*-dodecanyl-α-D-glucopyranoside **39a**, methyl 6-*O*-lauroyl-α-D-mannopyranoside **37c** and methyl 6-*O*-lauroyl-β-D-glucopyranoside **37b** showed the best inhibitory effects for *S. aureus*, with MIC values of 0.04 mM. The next derivative in order of efficacy was methyl 6-*O*-lauroyl-α-D-glucopyranoside **37a**, with a value of 0.31 mM.

Methyl 6-*O*-octanoyl-α-D-glucopyranoside **38a** was comparable to monocaprylin against *S. aureus* with values of 2.5 mM. This compound was also more active than any of the lauric acid derivatives against *E. coli*. Methyl 6-*O*-dodecanyl-β-D-glucopyranoside **39b** gave similar results to **38a** for *S. aureus* with values of 2.5 mM.

The galactopyranoside ester derivative **37d**, the mono-lauroyl pentaerythritol **41** and both the ether and ester derivatives of diacetone-D-glucose **56** and **58**, were the least active compounds tested, all with comparatively negligible MIC values of >10 mM for *S. aureus* and >20mM for *E. coli*.

The di-substituted methyl 4,6-*O*-lauroyl-α-D-glucopyranoside **40** did not show any activity comparable with either the monoglycerides or indeed the mono-substituted sugar derivatives against either bacteria due to poor solubility in water, as was the case for the non-sugar compounds di-lauroyl pentaerythritol **54** and tetra-lauroyl pentaerythritol **55**.

4.3 DISCUSSION

In this study the effect of polyhydroxylated fatty acid derivatives as inhibitors of a Gram-positive (*S. aureus*) and a Gram-negative (*E. coli)* microorganism of concern to the food and healthcare industries were evaluated. Several of the synthesised compounds have antimicrobial efficacy comparable with commercially available antimicrobials against *S. aureus*.

The effect of carbohydrate versus non-carbohydrate hydrophilic cores (carbohydrate and pentaerythritol laurates), the degree of substitution (monoester and diester), the monosaccharide core (glucopyranoside, mannopyranoside and galactopyranoside), the anomeric configuration (α and β glucopyranoside), the type of fatty acid carbohydrate linkage (ester and ether) and the length of fatty acid chain (lauric and caprylic) on antimicrobial activity were studied. Two protected sugar derivatives were also tested for comparative studies.

As with the monoglycerides and free fatty acids, all of the fatty acid derivatives that were found to be active, showed greater antimicrobial activity against *S. aureus* than against *E. coli*.

The non-carbohydrate pentaerythritol monoester **41**, which has the same number of free hydroxyl groups as the carbohydrate monoester derivatives, showed negligible activity against both the microorganisms tested. The result may suggest that hydrophilicity alone is not the only requirement involved in the choice of polyhydric alcohol used in the synthesis of these derivatives to achieve the desired antimicrobial

efficacy. This result also indicates the possibility that the carbohydrate moiety could play an important role in the antimicrobial activity of these compounds.

The degree of substitution of these derivatives was also shown to be crucial as the non-sugar pentaerythritol diester **54** and pentaerythritol tetraester **55** and the carbohydrate methyl α-D-glucopyranoside diester **40** were much less soluble in water than the monoesters. As a consequence, no antimicrobial activity results for these compounds could be measured. Although the diester of sucrose was shown to possess higher activity than the monoester in a study conducted by Kato and Shibasaki,[3] the results obtained in this current study clearly show the necessity of three free hydroxyl groups to ensure solubility of monosaccharides in order to measure activity of these derivatives.

With regard to investigating the effect of different sugar cores, the results showed that the lauric ester derivative of methyl α-D-mannopyranoside **37c** and methyl β-D-glucopyranoside **37b**, showed higher activity than any other ester derivatives against *S. aureus*, supporting the observation that whilst the degree of hydrophilicity may be important, the nature of the carbohydrate core itself is important in the antimicrobial efficacy of the derivatives and the orientation of these free hydroxyl groups is essential for the level of antimicrobial efficacy. These conclusions are consistent with results of an earlier study by Watanabe *et al*.[4]

Further evidence supporting this observation is seen in the results for the lauric ester anomers of methyl glucopyranoside **37a** and **37b**. A marked difference was observed between these compounds when they were tested against *S. aureus* with the beta

configuration (**37b**) showing higher activity. The lauric ether anomers of methyl glucopyranoside **39a** and **39b** also showed a marked difference in activity when tested against *S. aureus*, with the alpha configuration showing much higher activity.

In addition, the difference in activity between the ester and ether conjugates of the same carbohydrate showed that for the methyl α-D-glucopyranoside derivatives, the ether derivative **39a** was more active than the ester **37a**, however for methyl β-D-glucopyranoside, the ester **37b** was more active than the ether **39b**. These results indicate that, in combination with other factors, the nature of the bond conjugating the fatty acid to the carbohydrate could play some role in antimicrobial activity.

The importance of the chain length of the fatty acid ester was investigated using both lauric and caprylic derivatives. The lauric ester derivative **37a** showed much higher activity against *S. aureus* compared with the corresponding caprylic ester derivative **38a**. Conversely, the caprylic ester derivative **38a** showed higher activity against the Gram-negative bacteria *E. coli*, compared with the lauric derivative **37a**. This trend was also observed for the monoglyceride controls and is in accordance with general trends observed for medium and short chain fatty acids.[5]

The protected sugars, diacetone ether derivative **56** and diacetone ester derivative **58**, both gave negligible results against both organisms. This result is further evidence of the necessity of hydroxyl groups for the antimicrobial activity of these fatty acid derivatives.[6]

In conclusion, these results suggest that the nature of the carbohydrate core plays a role in the efficacy of some carbohydrate fatty acid derivatives as antimicrobials, and that therefore, further optimisation may be possible. However, to define the complex relationship between the carbohydrate moiety, the alkyl chain length and the nature of the glycoconjugate bond, further studies are warranted using a wider range of Gram-positive and Gram-negative microorganisms to eliminate species and strain effects. This work provides a framework on which further studies can be based.

CHAPTER 4 - REFERENCES

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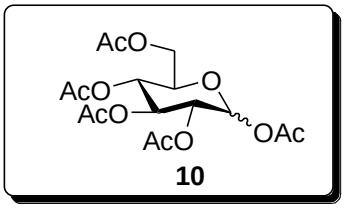
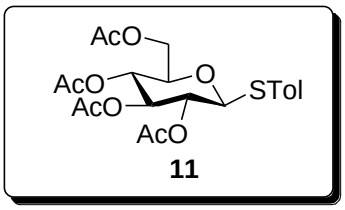
5. Experimental

All air and moisture-sensitive reactions were performed under an inert nitrogen atmosphere. All reactions performed under a hydrogen atmosphere were performed in a Parr Hydrogenator Apparatus. Toluene, benzene and dichloromethane reaction solvents were freshly distilled from calcium hydride and anhydrous DMF, THF, pyridine and acetonitrile were purchased from Sigma Aldrich. T.l.c. was performed on aluminium sheets precoated with Silica Gel 60 (HF_{254}, Fluka) and spots visualised by UV and charring with H_2SO_4-EtOH (1:20). Flash Column Chromatography was carried out with Silica Gel 60 (0.040-0.630 mm, E. Merck) and using stepwise solvent polarity gradient correlated with t.l.c. mobility. Chromatography solvents used were ethyl acetate (Riedel-deHaen), methanol (Riedel-deHaen) and petroleum ether (b.p. 40-60 ^{o}C, Fluka). Optical rotations were determined with an AA-% Series Optical Activity Ltd Polarimeter. NMR spectra were recorded with Varian Inova 300 and Varian NMRAS 400 spectrometers. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0) for ^{1}H and $CDCl_3$ (δ 77.0) for ^{13}C. Coupling constants are reported in hertz. FTIR spectra were recorded with a Nicolet FT-IR 5DXB infrared spectrometer, samples were prepared in a KBr matrix. Low-resolution electrospray-ionisation mass spectra were measured in the positive-ion mode on a Micromass Quattro tandem quadropole mass spectrometer. The D-glucose, methyl-α-D-glucopyranoside, methyl-β-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-α-D-galactopyranoside, diacetone-D-glucose, pentaerythritol, 1-chlorododecane, lauroyl chloride and octanoyl chloride were purchased from Sigma Aldrich.



10

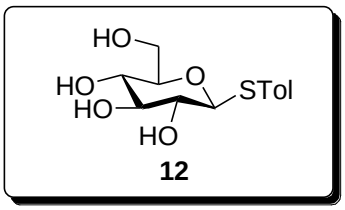
D-Glucose penta acetate (**10**) - D-Glucose (20 g, 0.11 mol) was dissolved in a solution of pyridine / acetic anhydride (1:1, 250 mL) and the mixture was stirred for 24 h. The solution was concentrated *in vacuo* and the resulting residue was dissolved in ethyl acetate (200 mL). The solution was then washed with water followed by sat. aq. $NaHCO_3$. It was then dried over $NaSO_4$ anhydrous, filtered and concentrated under diminished pressure. The crude pentaacetate was recrystallised from hexane / ethyl acetate / methanol to afford **10** as a white crystalline solid mixture of anomers. (17.52 g, 41%); $[\alpha]_D$ 63.1° (*c* 0.19, $CHCl_3$); Anal. Calcd. for $C_{16}H_{22}O_{11}$: C, 49.23; H, 5.68; Found: C, 49.48; H, 5.69; NMR data were in agreement with that previously reported.[1]



11

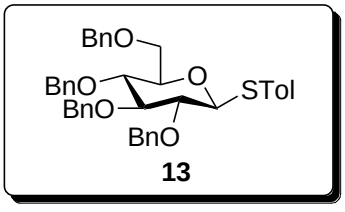
p-Tolyl 2,3,4,6-*O*-acetyl-1-thio-β-D-glucopyranoside (**11**) - Glucose pentaacetate **10** (1.1 g, 2.8 mmol) and *p*-thiocresol (1.7 g, 15.4 mmol) were dissolved in dichloromethane (12 mL). The solution was cooled to 0°C. Boron trifluoro etherate (1.9 mL, 15.4 mmol) was added and the mixture was stirred for 24 h. The solution was then diluted with dichloromethane (10 mL), washed with sat. aq. $NaHCO_3$, followed by water. The mixture was then dried over $MgSO_4$ anhydrous and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography to afford the tetra-acetate **11** as a white crystalline solid.[1] (0.185 g,

15%); $[\alpha]_D$ -3.5° (*c* 0.01, $CHCl_3$); ^1H NMR (400MHz, $CDCl_3$): δ 7.41-7.10 (ms, 4H, aromatic CH), 5.21 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.5, H-3), 5.03 (apt t, 1H, $J_{4,5}$ 10.0, H-4), 4.94 (apt t, 1H, $J_{1,2}$ 10.0, H-2), 4.64 (d, 1H, H-1), 4.20 (apt t, 2H, $J_{5,6a}$ 2.5, $J_{5,6b}$ 4.5, H-6a,6b), 3.71 (ddd, 1H, H-5), 2.35 (s, 3H, PhC*H*$_3$), 2.10, 2.09, 2.02, 2.00 (each s, 12H, each COC*H*$_3$); Anal. Calcd. for $C_{21}H_{26}O_9S$: C, 55.50; H, 5.77; Found: C, 55.25; H, 6.16; LRMS: Found, 477.0; required 477.49; $[M+Na]^+$.



12

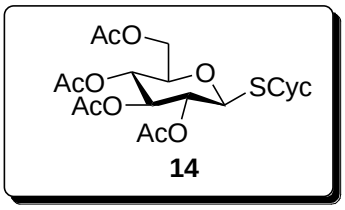
p-Tolyl 1-thio-β-D-glucopyranoside (**12**) - Tetra-acetate **11** (0.185 g, 0.41 mmol), was dissolved in methanol (2 mL) and elemental sodium (0.005 g) was added. The mixture was stirred for 24 h, at which point ion exchange resin (IR 120+) was added portion wise until the solution was neutralised. The reaction mixture was then filtered and concentrated under reduced pressure to afford the crude tetrol **12** (0.076 g, 65% crude) as a white crystalline solid.[1] $[\alpha]_D$ 36.3° (*c* 0.01, $CHCl_3$); LRMS: Found, 309.0; required 309.34; $[M+Na]^+$.



13

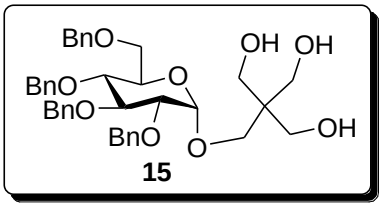
p-Tolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**13**) - Tetrol **12** (0.076 g, 0.27 mmol) was dissolved in DMF anhydrous (0.5 mL) and benzyl bromide (0.15 mL, 1.2 mmol) was added. The mixture was cooled to 0°C and sodium hydride (60% dispersion in mineral oil) (0.06 g, 1.5 mmol) was added portion wise. The solution

was stirred for 24 h and methanol (0.5 mL) was added to quench the solution, which was stirred for a further 15 min. The solution was concentrated *in vacuo* and dissolved in ethyl acetate. The mixture was then washed with brine, dried over $MgSO_4$ anhydrous, filtered and concentrated under diminished pressure. The resulting benzylated residue was purified by flash column chromatography to afford the fully protected thioglycoside **13** as a white crystalline solid. (0.14 g, 53% over 2 steps); NMR data were in agreement with that previously reported.[1] LRMS: Found, 669.2; required 669.83; $[M+Na]^+$.



14

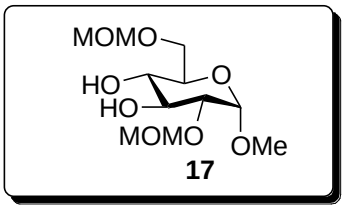
Cyclohexanethiol-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**14**) - Glucose-penta-acetate **11** (1.0 g, 2.56 mmol) and cyclohexanethiol (1.9 mL, 0.02 mol) were dissolved in dichloromethane (12 mL). The solution was cooled to 0°C and boron trifluoro etherate (1.5 mL, 15.3 mmol) was added.[1] The mixture was stirred for 24 h at which point t.l.c. indicated that this reaction did not go to completion.



15

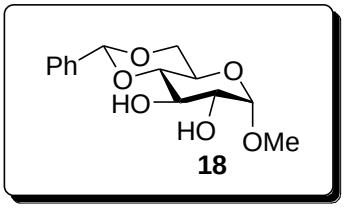
Pentaerythritol 2,3,4-tetra-*O*-benzyl-α-D-glucopyranoside (**15**) - To a stirred solution containing **13** (0.1 g, 0.2 mmol) and activated 4 Å powdered molecular sieves in dichloromethane (8 mL) at room temperature, pentaerythritol (0.06 g, 0.44 mmol), N-

iodosuccinimide (0.1 mL, 0.5 mmol) and trimethylsilyl triflate (0.031 mL, 0.17 mmol) were added. T.l.c. indicated this reaction did not complete and insufficient material prohibited further investigation.



17

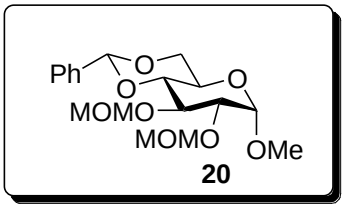
Methyl 2,6-di-*O*-lauroyl-α-D-glucopyranoside (**17**) - Methyl α-D-glucopyranoside **7a** (0.97 g, 5 mmol) was dissolved in dioxane (40 mL). A catalytic amount of 4-dimethylaminopyridine and dibutyltin dimethoxide (1.47 mL, 6.4 mmol) was added to the solution. The mixture was kept at reflux and a solution of bromomethyl methylether (0.61 mL, 7.5 mmol) in dioxane (10 mL) was added dropwise over 1 h.[2] The reaction was allowed to stir for 24 h, concentrated *in vacuo* and purified by chromatography. (1.326 g, 94%); ^{1}H NMR (400MHz, $CDCl_3$): δ 4.88 (AB d, 2H, *J* 6.0, OC*H*$_2$O), 4.83 (AB d, 2H, *J* 4.0, OC*H*$_2$O), 4.74 (d, 1H, $J_{1,2}$ 7.0, H-1), 4.16 (dd, 2H, *J* 5.0, *J* 10.0 H-6), 4.03 (apt t, 1H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0 H-3), 3.75 (m, 1H, H-5), 3.52 (m, 1H, H-2), 3.26 (apt t, 1H, $J_{4,5}$ 9.5, H-4), 3.45-3.40 (overlapping signals, 9H, each OMe), 1.26 (br s, 2H, each OH); ^{13}C NMR ($CDCl_3$): δ 104.1 (d, C-1), 99.9, 93.7 (each t, each OCH_2O), 80.7, 72.8, 71.2, 68.7 (each d), 62.5 (t, C-6), 57.4, 55.2 (each q, each OCH_3). LRMS: Found, 305.1; required 305.29; $[M+Na]^+$.



18

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**18**) - A solution of methyl α-D-

glucopyranoside **7a** (1 g, 5.2 mmol), *p*-toluenesulfonic acid (10 mg) and benzaldehyde dimethylacetal (1.5 mL, 10.3 mmol) in acetonitrile anhydrous (25 mL) was stirred for 24 h at room temperature. Trimethylamine (0.5 mL) was added to neutralise the solution which was then stirred for 1 h.[3] The product was filtered off as a white solid, washed with petroleum ether and dried. (1.37 g, 93%). $[\alpha]_D$ 8.3º (*c* 0.01, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$): δ 7.54-7.27 (ms, 5H, aromatic H), 5.53 (s, 1H, C*H*Ph), 4.78 (d, 1H, $J_{1,2}$ 4.0, H-1), 4.29 (dd, 1H, *J* 3.5, *J* 9.0 H-6), 3.92 (apt t, 1H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0 H-3), 3.84-3.62 (overlapping signals, 3H, H-2,5,6), 3.48 (apt t, 1H, $J_{4,5}$ 9.5, H-4), 3.45 (s, 3H, OMe), 2.47, 3.00 (each br s, 2H, O*H*); LRMS: Found, 305.1; required, 305.29; $[M + Na]^+$.



20

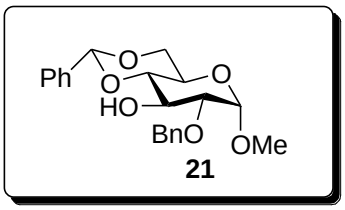
Methyl 2,3-di-*O*-methoxymethyl-4,6-*O*-benzylidene-α-D-glucopyranos*ide* (**20**)

Method using dibutyltin dimethoxide.[2] - Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **18** (0.178 g, 0.63 mmol) was dissolved in dioxane (5 mL). A catalytic amount of 4-dimethylaminopyridine and dibutyltin dimethoxide (0.2 mL, 0.82 mmol) was added to the mixture which was kept at reflux. A solution of bromomethyl methylether (0.08 mL, 0.94 mmol) in dioxane (4 mL) was then added dropwise over 1 h. The mixture was allowed to stir for 24 h and was concentrated under reduced pressure and purified by chromatography (petroleum ether-EtOAc) to yield **20**. (0.198 g, 85%); $[\alpha]_D$ 16.8º (*c* 0.4, $CHCl_3$); FTIR (KBr): 2932, 1372, 182, 1186, 1116, 1099, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49-7.27 (ms, 5H, aromatic H), 5.54 (s, 1H, C*H*Ph), 4.83 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.81, 4.80 (each AB d,

4H, *J* 6.0, *J* 6.5, OC*H*$_2$O), 4.28 (dd, 1H, $J_{5,6a}$ 4.5, $J_{6a,6b}$ 10.0, H-6a), 4.12 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.0, H-3), 3.85 (m, 1H, H-5), 3.71 (apt t, 1H, $J_{5,6b}$ 10.5, H-6b), 3.64 (dd, 1H, H-2), 3.56 (apt t, 1H, $J_{4,5}$ 9.5, H-4), 3.45, 3.42 (each s, 6H, each OCH$_2$OC*H*$_3$), 3.35 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): δ 141.8 (s, aromatic C), 133.5, 132.7, 130.6 (each d, each aromatic CH), 106.0, 104.4 (d, C-1, CHPh), 102.4, 101.8 (each t, each OCH$_2$O), 86.1, 83.0, 78.5, 66.8 (each d), 73.6 (t, C-6), 60.3, 60.0, 59.8 (each q, each OCH$_3$); LRMS: Found, 393.2; required, 393.39; $[M + Na]^+$; Anal. Calcd. for $C_{18}H_{26}O_8$: C, 58.37; H, 7.08. Found: C, 57.99; H, 7.01.

Method using dibutyltin oxide.[4] - Methyl 4,6-O-benzylidene-α-D-glucopyranoside **18** (1 g, 3.5 mmol) and dibutyltin oxide (0.97 g, 3.4 mmol) were dissolved in benzene anhydrous (120 mL) and stirred for 16 h with azeotropic removal of water. After 16 h the reaction was stopped and the solution was concentrated to 80 mL, after which bromomethyl methylether (0.6 mL, 7.35 mmol) and tetrabutylammonium iodide (1.31 g, 3.54 mmol) were added and the mixture was refluxed for a further 16 h. The solution was then concentrated *in vacuo* and purified by chromatography (petroleum ether-EtOAc) to yield **20**. (0.824 g, 65%); $[\alpha]_D$ 8.8º (*c* 0.3, $CHCl_3$); FTIR (KBr): 2927, 1372, 1281, 1184, 1115, 1100, 1051, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.33 (ms, 5H, aromatic H), 5.53 (s, 1H, C*H*Ph), 4.83 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.78, 4.77 (each AB d, 4H, *J* 6.0, *J* 6.5, OC*H*$_2$O), 4.28 (dd, 1H, $J_{5,6a}$ 4.5, $J_{6a,6b}$ 10.0, H-6a), 4.11 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.5, H-3), 3.80 (m, 1H, H-5), 3.74 (apt t, 1H, $J_{5,6b}$ 10.0, H-6b), 3.64 (dd, 1H, H-2), 3.55 (apt t, 1H, $J_{4,5}$ 9.5, H-4), 3.45, 3.42 (each s, 6H, each OCH$_2$OC*H*$_3$), 3.34 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): δ 141.7 (s, aromatic C), 133.5, 132.7, 130.6 (each d, each aromatic CH), 105.9, 104.4 (d, C-1, CHPh), 102.3, 101.8 (each t, each OCH$_2$O), 86.1, 82.9, 78.5, 66.7 (each d), 73.5 (t, C-6), 60.2, 59.9, 59.8 (each q, each OCH$_3$); LRMS: Found, 393.1; required, 393.39; $[M + Na]^+$; Anal.

Calcd. for $C_{18}H_{26}O_8$: C, 58.37; H, 7.08. Found: C, 57.98; H, 7.08.

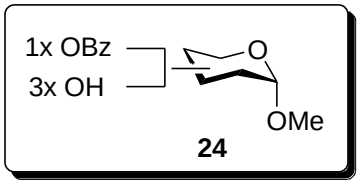


21

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**21**)

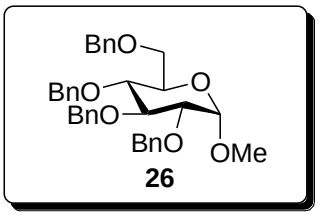
Method using dibutyltin oxide.[4] - Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **18** (0.84 g, 3.0 mmol) and dibutyltin oxide (1.5 g, 6.0 mmol) were dissolved in benzene anhydrous (100 mL) and allowed to stir for 16 h with azeotropic removal of water. After 16 h the reaction was stopped and the solution was concentrated to approximately 70 mL, after which benzyl bromide (0.36 mL, 3.0 mmol) and tetrabutylammonium iodide (1.1 g, 3.0 mmol) were added and the mixture was refluxed for a further 16 h. The solution was then concentrated *in vacuo*. T.l.c. verified the presence of several products.

Method using dibutyltin dimethoxide.[4] - Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **18** (0.8 g, 2.8 mmol) and dibutyltin dimethoxide (1.3 g, 5.6 mmol) were dissolved in benzene anhydrous (100 mL) and allowed to stir for 16 h with azeotropic removal of water. After 16 h the reaction was stopped and the solution was concentrated to approximately 70 mL, after which benzyl bromide (0.36 mL, 3.0 mmol) and tetrabutylammonium iodide (1.0 g, 3.0 mmol) were added and the mixture was refluxed for a further 16 h. The solution was then concentrated under diminished pressure. T.l.c. verified the presence of several products.



24

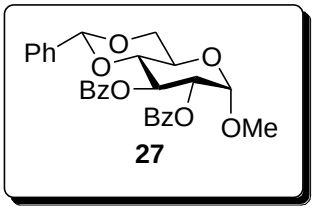
Methyl monobenzoyl-α-D-glucopyranoside (**24**) -Methyl α-D-glucopyranoside **7a** (0.97 g, 5 mmol) was dissolved in dioxane (40 mL). A catalytic amount of 4-dimethylaminopyridine and dibutyltin dimethoxide (1.47 mL, 5.5 mmol) was added. The reaction mixture was kept at room temperature and a solution of benzoyl chloride (0.64 mL, 5.5 mmol) in dioxane (10 mL) was added dropwise over 1 h.[2] The reaction was stirred for 24 h and was then concentrated *in vacuo* and purified by flash chromatography (petroleum ether-EtOAc) to yield **24**. (0.338 g, 23%); $[\alpha]_D$ 67.4º (*c* 0.02, $CHCl_3$); FTIR (KBr): 3465, 3229, 2993, 2947, 1726, 1796, 1095, 764, 710 cm^{-1} ^1H NMR (400 MHz, $CDCl_3$): δ 8.05-7.39 (ms, 5H, aromatic H), 4.93 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.85-4.79 (overlapping signals, 2H), 4.06-3.90 (overlapping signals, 2H), 3.76-3.70 (overlapping signals, 2H), 3.60-3.46 (overlapping signals, 2H), 3.32 (s, 3H, OMe); LRMS: Found, 321.0; required, 321.29; $[M + Na]^+$; Anal. Calcd. for $C_{14}H_{18}O_7$: C, 56.37; H, 6.08. Found: C, 56.67; H, 5.74.



26

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (**26**) - Compound **24** (0.338 g, 1.1 mmol) was dissolved in DMF anhydrous (8.0 mL). Benzyl bromide (0.65 mL, 5.5 mmol) was added to the solution. The mixture was cooled to 0°C and sodium hydride (0.264 g, 6.6 mmol) was added portion wise. The reaction was stirred for 24 h at room temperature after which methanol (8.0 mL) was added to quench the solution

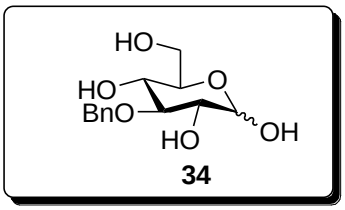
which was stirred for a further 15 min. The reaction mixture was concentrated *in vacuo* and the resulting benzylated residue was dissolved in ethyl acetate. The solution was washed with brine, dried over $MgSO_4$ anhydrous, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography (petroleum ether-EtOAc) to yield **26**. (0.48 g, 79%); ^{1}H NMR (400MHz, $CDCl_3$): δ 7.34-7.11 (ms, 20H, aromatic H), 4.89, (AB d, 2H, *J* 11.0, OC*H*$_2$Ph), 4.82, (AB d, 2H, *J* 3.5, OC*H*$_2$Ph), 4.66 (AB d, 2H, *J* 13.0, OC*H*$_2$Ph), 4.63 (d, 1H, $J_{1,2}$ 4.0, H-1), 4.54 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 3.98 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.0, H-3), 3.75-3.62 (overlapping signals, 4H, H-4,5,6), 3.56 (dd, 1H, H-2), 3.37 (s, 3H, OC*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 139.3, 139.2, 138.6, 138.5 (each s, each aromatic H), 128.45, 128.39, 128.36, 128.14, 127.97, 127.9, 127.85, 127.7, 127.6 (each d, each aromatic CH), 98.2 (d, C-1), 82.1, 79.9, 77.7, 68.5 (each d), 75.8, 75.0, 73.5, 73.4 (each t), 70.1 (t, C-6), 55.2 (q, OCH_3); LRMS: Found, 577.2 required, 577.67; $[M + Na]^+$.



27

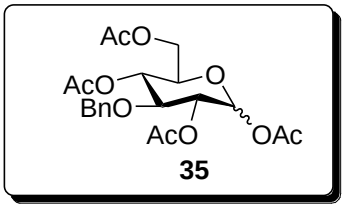
Methyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**27**) - Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **18** (0.496 g, 1.8 mmol) was dissolved in dioxane (20 mL). A catalytic amount of 4-dimethylaminopyridine and dibutyltin dimethoxide (0.5 g, 2.0 mmol) was added. The reaction mixture was kept at room temperature and a solution of benzoyl chloride (0.25 mL, 2.0 mmol) in dioxane (5.0 mL) was added drop wise over 1 h.[2] The mixture was then concentrated *in vacuo* and purified by chromatography to yield **27** (0.422g, 48%); $[\alpha]_D$ 85.4° (*c* 0.05, $CHCl_3$); FTIR (KBr):

3066, 1720, 1384, 1333, 1277, 1244, 1116, 1097, 690 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ 8.01-7.31 (overlapping signals, 15H, aromatic H), 6.06 (apt t, 1H, $J_{2,3}$ 10.0, $J_{3,4}$ 10.0, H-3), 5.57 (s, 1H, C*H*Ph), 5.26 (dd, 1H, $J_{1,2}$ 3.5, H-2), 5.19 (d, 1H, H-1), 4.39 (dd, 1H, $J_{6a,6b}$ 10.0, $J_{5,6b}$ 5.0, H-6b), 4.09 (m, 1H, H-5), 3.95-3.84 (overlapping signals, 2H, H-4,6a), 3.45 (s, 3H, OMe); LRMS: Found, 513.0 required, 513.5; $[M + Na]^+$; Anal. Calcd. for $C_{28}H_{26}O_8$: C, 68.56; H, 5.34. Found: C, 68.33; H, 5.43.



34

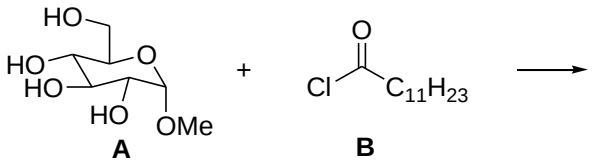
3-*O*-benzyl-(α/β)-D-glucopyranoside (**34**) - Sodium hydride (1.8 g, 46.1 mmol) was added to a solution of diacetone-D-glucose **32** (10 g, 36.4 mmol) in DMF anhydrous (100 mL) and benzyl bromide (5.5 mL, 46.1 mmol) at 0^oC. The reaction mixture was allowed to warm to room temperature and was stirred for 5 h. The solution was cooled to 0^oC and methanol (20 mL) was added dropwise. The reaction mixture was concentrated *in vacuo*. The residue was taken up in Et_2O and water was added. It was then dried over $MgSO_4$ anhydrous, filtered and concentrated under reduced pressure. Amberlite-IR 120 (H^+) (20 g) was added to a suspension of the resulting residue in H_2O (100 mL). The mixture was then stirred at reflux for 4 h, filtered and concentrated *in vacuo* to yield **34** as yellow crystals.[5] (6.36 g, 63.6% crude); FTIR (KBr): 3522, 2921, 2852, 1374, 1276, 1175, 1110, 1061 cm^{-1}; LRMS: Found, 293.1 required, 293.28; $[M + Na]^+$.



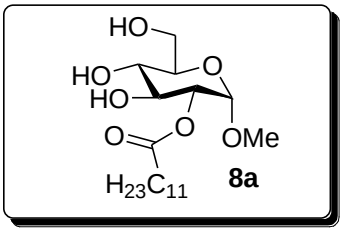
35

1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-(α/β)-D-glucopyranoside (**35**) - Crude compound **34** (1 g, 3.7 mmol) was added to a pyridine / acetic anhydride solution (1:1, 7 mL). The mixture was stirred overnight concentrated *in vacuo* and the residue was dissolved in ethyl acetate. The resulting solution was then washed with water followed by sat. aq. $NaHCO_3$. The mixture was dried over $NaSO_4$ anhydrous, filtered and finally concentrated under reduced pressure before being purified by chromatography to yield compound **35**. (0.54 g, 33.3%); $[\alpha]_D$ 2.8º (*c* 0.11, $CHCl_3$); FTIR (KBr): 2921, 2852, 1743, 1713, 1374, 1276, 1110, 1061 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ 7.35-7.24 (ms, 5H, aromatic CH), 6.31 (d, 1H, $J_{1\alpha,2\alpha}$ 3.5, H-1α), 5.65 (d, 1H, $J_{1\beta,2\beta}$ 8.0, H-1β), 5.19-5.12 (overlapping signals, 3H, H-4α,6αa,2β), 5.06 (dd, 1H, $J_{2\alpha,3\alpha}$ 10.0, H-2α), 4.68 (AB d, 2H, *J* 11.5, OCH_2Ph), 4.24-4.19 (overlapping signals, 3H, H-4β,6β), 4.14-3.95 (overlapping signals, 3H, H-3α,5α,6αb), 3.80-3.73 (overlapping signals, 2H, H-3β,5β), 2.16-1.97 (ms, 12H, each OCOCH_3); LRMS: Found, 461.1 required, 461.43; $[M + Na]^+$; Anal. Calcd. for $C_{21}H_{26}O_{10}$: C, 57.53; H, 5.98. Found: C, 57.91; H, 6.12.

Lauroylation of methyl α-D-glucopyranoside **7a**:

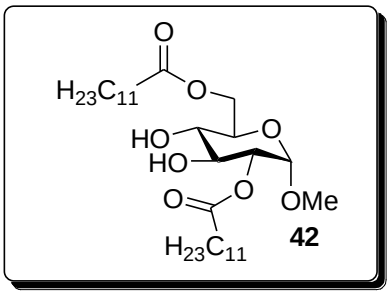


Methyl α-D-glucopyranoside **7a** (0.97 g, 5 mmol) was dissolved in dioxane (40 mL). Dibutyltin dimethoxide (2.4 mL, 10.5 mmol) was added to the solution. The mixture was kept at room temperature and a solution of lauroyl chloride (1.2 mL, 5 mmol) in dioxane (10 mL) was added dropwise over 1 h. The mixture was stirred overnight.[2] The solution was then concentrated *in vacuo* and the following products were isolated by chromatography (petroleum ether-EtOAc):



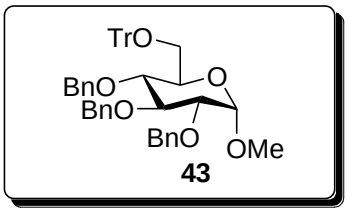
Methyl 2-*O*-lauroyl-α-D-glucopyranoside (**8a**) - (0.273 g, 15%); ^{1}H NMR (400MHz, $CDCl_3$): δ 4.89 (d, 1H, $J_{1,2}$ 3.0, H-1), 4.71 (m, 1H, H-2), 4.62 (br s, 1H, OH), 3.91-3.80 (overlapping signals, 3H, H-3,5,6b), 3.71-3.56 (overlapping signals, 2H, H-4,6a), 3.36 (s, 3H, OMe), 2.39 (t, 2H, *J* 7.0, *J* 7.5, aliphatic $OCOC\mathit{H}_2C_{10}H_{21}$), 2.05, 1.80 (each br s, 2H, each OH), 1.67-1.43 (m, 2H, aliphatic $OCOCH_2C\mathit{H}_2C_9H_{19}$), 1.41-1.09 (overlapping signals, 16H, aliphatic $OCOC_2H_4C_8\mathit{H}_{16}CH_3$), 0.88 (t, 3H, *J* 5.5, *J* 7.0, aliphatic $OCOC_{10}H_{20}C\mathit{H}_3$); ^{13}C NMR ($CDCl_3$): δ 174.1 (s, C=O), 97.2 (d, C-1), 73.1, 71.6, 71.1, 69.9 (each d), 61.3 (d, C-6), 55.2 (q, OMe), 34.2, 31.9, 29.63, 29.52, 29.33, 29.07, 24.9, 22.7 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS:

Found, 399.3 required, 399.48; $[M + Na]^+$; Anal. Calcd. for $C_{19}H_{36}O_7$: C, 60.61; H, 9.64. Found: C, 60.97; H, 9.63.



42

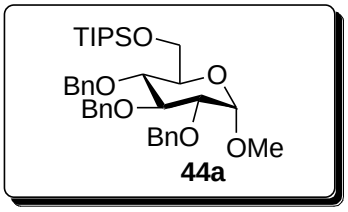
Methyl 2,6-*O*-lauroyl-α-D-glucopyranoside (**42**) - (0.274 g, 9.8%); $[\alpha]_D$ 43º (*c* 0.01, $CHCl_3$); FTIR (KBr): 3522, 2921, 2852, 1743, 1715, 1415, 1374, 1276, 1110, 1061 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ 4.92 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.69 (dd, 1H, $J_{2,3}$ 10.0, H-2), 4.55 (dd, 1H, $J_{6a,6b}$ 12.0, $J_{5,6b}$ 4.0, H-6b), 4.33-4.22 (m, 1H, H-6a), 3.97 (apt t, 1H, $J_{3.4}$ 9.5, H-3), 3.75 m, 1H, H-5), 3.43-3.37 (overlapping signals, 4H, H-4, OMe), 3.20, 2.63 (each br s, 2H, each OH), 2.41-2.36 (m, 4H, each aliphatic $OCOC\mathit{H}_2C_{10}H_{21}$), 1.80-1.53 (m, 4H, each aliphatic $OCOCH_2C\mathit{H}_2C_9H_{19}$), 1.46-1.09 (overlapping signals, 32H, each aliphatic $OCOC_2H_4C_8\mathit{H}_{16}CH_3$), 0.98-0.86 (m, 6H, each aliphatic $OCOC_{10}H_{20}C\mathit{H}_3$); LRMS: Found, 581.5 required, 581.79; $[M + Na]^+$; Anal. Calcd. for $C_{31}H_{58}O_8$: C, 66.63; H, 10.46. Found: C, 66.70; H, 10.20.



43

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (**43**) - A solution of methyl α-D-glucopyranoside **7a** (5 g, 25 mmol) in pyridine anhydrous (50 mL) was treated with triphenylmethyl chloride (10.7 g, 38.55 mmol) and imidazole (5

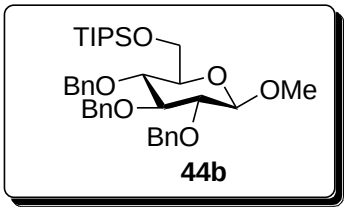
g, 75 mmol) and was heated to approximately 40^{o}C. The suspension was allowed to stir for 24 h and was then concentrated *in vacuo*.[6] The crude trityl protected product was dissolved in DMF anhydrous (50 mL) and cooled to 0^{o}C. NaH (4 g, 100 mmol) was added portion wise. BnBr (9 mL, 75 mmol) was added to the solution which was then allowed to warm to room temperature and stir for 24 h. Methanol (20 mL) was added to quench the mixture which was stirred for 1 h. The solution was concentrated under diminished pressure, dissolved in ethyl acetate and washed with water. It was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The fully protected sugar was then purified by chromatography (petroleum ether-EtOAc) to give **43** (6.59 g, 37%); $[\alpha]_D$ 22.1º (*c* 0.07, $CHCl_3$); FTIR (KBr): 1597, 1491, 1460, 1449, 899, 745, 696 cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.47-6.85 (ms, 30H, aromatic H), 4.89 (AB d, 2H, *J* 10.5, OC*H*$_2$Ph), 4.79 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.76 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.50 (AB d, 2H, *J* 10.5, OC*H*$_2$Ph), 3.97 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.5, H-3), 3.81 (m, 1H, H-5), 3.64-3.59 (overlapping signals, 2H, H-2,4), 3.50 (dd, 1H, $J_{5,6a}$ 2.0, $J_{6a,6b}$ 10.0, H-6a), 3.44 (s, 3H, OC*H*$_3$), 3.19 (dd, 1H, $J_{5,6b}$ 5.0, H-6b); 13C NMR ($CDCl_3$): δ 144.0, 138.7, 138.3, 137.9 (each s, each aromatic C), 128.8, 128.6, 128.5, 128.43, 128.38, 128.3, 128.2, 128.1, 128.0, 127.97, 127.90, 127.85, 127.8, 127.7, 127.5, 126.9 (each d, each aromatic CH), 97.9 (d, C-1), 86.3 (s, CPh_3), 82.3, 80.2, 78.2, 70.3 (each d), 75.9, 74.9, 73.4 (each t, each CH_2Ph), 62.6 (d, C-6), 54.9 (q, OMe); LRMS: Found, 729.4; required, 729.86; $[M + Na]^+$.



44a

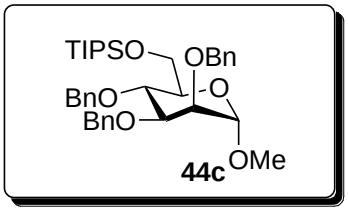
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-glucopyranoside (**44a**) - A solution of **7a** (5 g, 25 mmol) in DMF anhydrous (120 mL) was treated with triisopropylsilyl chloride (15 mL, 75 mmol) and imidazole (5 g, 75 mmol) and allowed to stir at room temperature for 24 h. The crude TIPS protected intermediate was then concentrated *in vacuo* and dissolved in ethyl acetate. It was washed with 10% HCl, water, followed by sat. aq. $NaHCO_3$ and finally sat. aq. NaCl. It was then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure.[7] The crude product was dissolved in DMF anhydrous (50 mL) and cooled to 0^oC. NaH (5 g, 125 mmol) was added portion wise. BnBr (9 mL, 75 mmol) was added and the mixture was allowed to warm to room temperature and stir for 24 h. Methanol (20 mL) was added to quench the mixture which was stirred for 1 h. The fully protected sugar was then concentrated *in vacuo* and dissolved in ethyl acetate. The solution was washed with water, dried over anhydrous $MgSO_4$ and concentrated under diminished pressure. The resulting residue was purified by chromatography (petroleum ether-EtOAc) to give **44a**. (13.2 g, 85%); $[\alpha]_D$ 10.7º (*c* 0.07, $CHCl_3$); FTIR (KBr): 2923, 1733, 1498, 1455, 909, 884, 791, 695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.36-7.27 (ms, 15H, aromatic H), 4.91, (AB d, 2H, *J* 11.0, OCH_2Ph), 4.78, (AB d, 2H, *J* 11.0, OCH_2Ph), 4.74 (AB d, 2H, *J* 12.0, OCH_2Ph), 4.61 (d, 1H, $J_{1,2}$ 3.5, H-1), 3.99 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.5, H-3), 3.84 (d, 2H, $J_{5,6}$ 4.5, H-6a,6b), 3.64 (m, 1H, H-5), 3.55-3.49 (overlapping signals, 2H, H-2,4), 3.37 (s, 3H, OCH_3), 1.10-1.02 (ms, 18H, each TIPS CH_3), 0.88 (m, 3H, each TIPS C*H*); ^{13}C NMR ($CDCl_3$): δ 139.1, 138.7, 138.5 (each s, each aromatic C), 128.65, 128.63, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8 (each d,

each aromatic CH), 98.0 (d, C-1), 82.5, 80.5, 78.1, 76.1 (each d), 76.1, 75.3, 73.6 (each t, each CH_2Ph), 62.9 (t, C-6), 55.0 (q, OCH_3), 18.3, 18.2 (each q, each TIPS CH_3), 12.2 (each d, each TIPS CH); LRMS: Found, 643.3; required, 643.9; $[M + Na]^+$.

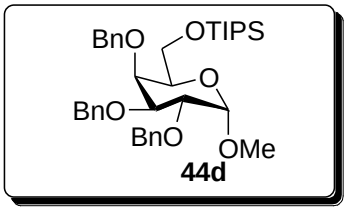


44b

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triisopropylsilyl-β-D-glucopyranoside (**44b**) - Treatment of **7b** (4.5 g, 23.17 mmol) as described for **7a** gave **44b**. (8.7 g, 80%); $[\alpha]_D$ 23º (*c* 0.01, $CHCl_3$); FTIR (KBr): 2863, 1730, 1497, 1454, 1399, 1277, 882, 802, 751, 697. cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.28 (ms, 15H, aromatic H), 4.90, 4.88, 4.83 (each AB d, 6H, *J* 11.0, OC*H*$_2$Ph), 4.30 (d, 1H, $J_{1,2}$ 7.5, H-1), 4.00-3.90 (overlapping signals, 3H, H-5,6), 3.66 (m, 1H, H-3), 3.53 (s, 3H, OC*H*$_3$), 3.41 (m, 1H, H-2), 3.34 (m, 1H, H-4), 1.26-1.05 (ms, 21H, TIPS); ^{13}C NMR ($CDCl_3$): δ 138.98, 138.92, 138.7 (each s, each aromatic C), 128.69, 128.65, 128.62, 128.5, 128.3, 128.2, 128.0, 127.9, 127.8 (each d, each aromatic CH), 104.7 (d, C-1), 84.9, 82.9, 77.8, 76.2 (each d), 76.0, 75.3, 75.0 (each t, each CH_2Ph), 62.7 (t, C-6), 56.9 (q, OCH_3), 18.3, 18.2 (each q, each TIPS CH_3), 12.3 (d, TIPS CH); LRMS: Found, 643.3 required, 643.9 $[M + Na]^+$.

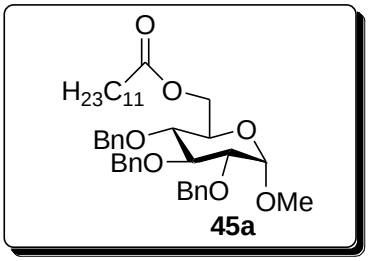


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-mannopyranoside (**44c**) - Treatment of **7c** (4 g, 20 mmol) as described for **7a** gave **44c**. (6.5 g, 51%); $[\alpha]_D$ 25.5º (*c* 0.05, $CHCl_3$); FTIR (KBr): 3056, 2864, 1496, 1363, 1324, 970, 882, 790, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.24 (multiple signals, 15H, each aromatic H), 4.79 (AB d, 2H, *J* 11.0, OC*H*$_2$Ph), 4.72 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.71-4.64 (overlapping signals, 3H, OC*H*$_2$Ph, H-1), 3.95 (dd, 1H, $J_{2,3}$ 2.0, $J_{3,4}$ 11.0, H-3), 3.93-3.87 (overlapping signals, 3H, H-4,6a,6b), 3.76 (dd, 1H, $J_{1,2}$ 2.5, H-2), 3.59 (dd, 1H, *J* 5.5, *J* 7.0, H-5), 3.31 (s, 3H, OMe), 1.12-1.04 (multiple signals, 21H, TIPS); ^{13}C NMR ($CDCl_3$): δ 138.68, 138.61, 138.4 (each s, each aromatic C), 128.3, 128.2, 127.9, 127.67, 128.63, 127.5, 127.4 (each d, each aromatic CH), 98.5 (d, C-1), 80.3, 76.7, 74.9, 73.3 (each d), 75.1, 72.5, 72.1 (each t, each CH_2Ph), 63.2 (t, C-6), 54.4 (q, OMe), 18.0, 17.9 (each q, each TIPS CH_3), 12.3 (each d, each TIPS CH_2); LRMS: Found, 638.5 required, 638.9; $[M + H_2O]^+$.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-galactopyranoside (**44d**) - Treatment of **7d** (4.0 g, 20.0 mmol) as described for **7a** gave **44d**. (6.4 g, 50%); $[\alpha]_D$ 20.6º (*c* 0.07, $CHCl_3$); FTIR (KBr): 3030, 2865, 1496, 1454, 1350, 1194, 1054, 882, 793, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ; 7.41-7.22 (multiple signals, 15H,

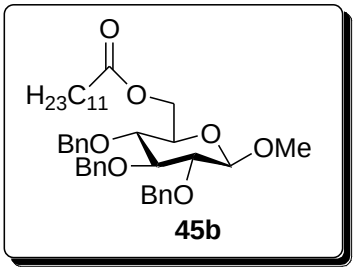
each aromatic H), 4.82 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.71 (AB d, 2H, *J* 11.5, OC*H*$_2$Ph), 4.77 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.68 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.04 (dd, 1H, $J_{2,3}$ 10.0, H-2), 3.95-3.92 (overlapping signals, 2H, H-3,5), 3.74-3.64 (overlapping signals, 3H, H-4,6), 3.36 (s, 3H, OMe), 1.12-0.86 (multiple signals, 21H, TIPS); ^{13}C NMR ($CDCl_3$): δ 137.9, 137.7, 137.5 (each s, each aromatic C), 127.33, 127.28, 127.22, 127.15, 127.06, 126.62, 126.48, 126.45 (each d, each aromatic CH), 97.6 (d, C-1), 78.1, 75.4, 74.0, 70.1 (each d), 73.7, 72.5, 72.2 (each t, each CH_2Ph), 61.4 (t, C-6), 54.1 (q, OMe), 16.94, 16.93 (each q, each TIPS CH_3), 10.8 (each d, each TIPS CH_2); LRMS: Found, 638.5 required, 638.9; $[M + H_2O]^+$.



45a

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-lauroyl-α-D-glucopyranoside (**45a**) - Compound **44a** (3.0 g, 4.8 mmol) was dissolved in THF anhydrous (80 mL) and was cooled to 0^{o}C. Tetrabutylammonium fluoride (1 g, 4 mmol) was added and the solution was allowed to warm to room temperature and stir for 1 h.[7] It was then concentrated *in vacuo* and approximately 1 mmol of the resulting 6-OH residue was dissolved in pyridine anhydrous (25 mL). A catalytic amount of 4-dimethylaminopyridine and lauroyl chloride (0.29 mL, 1.22 mmol) were added and the solution was allowed to stir at room temperature for 24 h.[8] It was then concentrated under reduced pressure and the resulting benzylated ester derivative was purified by chromatography (petroleum ether-EtOAc) to give **45a**. (0.47 g, 72%); $[\alpha]_D$ 7.5^o (*c* 0.02, $CHCl_3$); FTIR (KBr): 2924, 2853, 1738, 1603, 1502, 1454, 1249, 1072 cm^1; ^{1}H NMR (400 MHz, $CDCl_3$): δ

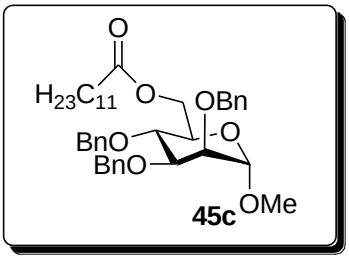
7.35-7.26 (ms, 15H, aromatic H), 4.92, (AB d, 2H, *J* 10.5, OC*H*$_2$Ph), 4.72, (AB d, 2H, *J* 10.5, OC*H*$_2$Ph), 4.64 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.59 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.27 (d, 2H, $J_{5,6}$ 3.5, H-6a,6b), 4.01 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.0, H-3), 3.82 (d apt t, 1H, $J_{4,5}$ 10.0, H-5), 3.53 (dd, 1H, H-2), 3.48 (apt t, 1H, H-4) 3.37 (s, 3H, OC*H*$_3$), 2.35 (m, 2H, aliphatic OCOC*H*$_2$C$_{10}$H$_{21}$), 1.61 (m, 2H, aliphatic OCOCH$_2$C*H*$_2$C$_9$H$_{19}$), 1.28-1.24 (ms, 16H, aliphatic OCOC$_2$H$_4$C$_8$*H*$_{16}$CH$_3$), 0.87 (m, 3H, aliphatic OCOC$_{10}$H$_{20}$C*H*$_3$); ^{13}C NMR (CDCl$_3$): δ 171.1 (s, C=O), 138.6, 138.1, 137.9 (each s, each aromatic C), 128.5, 128.48, 128.46, 128.1, 128.03, 127.98, 127.90, 127.7 (each d, each aromatic CH), 98.0 (d, C-1), 88.0, 79.9, 77.6, 68.6 (each d), 75.8, 75.1, 73.4 (each t, each CH$_2$Ph), 60.4 (t, C-6), 55.2 (q, OCH$_3$), 34.2, 31.9, 29.8, 29.6, 29.5, 29.3, 29.2, 24.9, 22.7, 21.1 (each t, each aliphatic CH$_2$), 14.2 (q, aliphatic CH$_3$); LRMS: Found, 669.39; required, 669.85; [M + Na]$^+$; Anal. Calcd. for C$_{40}$H$_{54}$O$_7$: C, 74.27; H, 8.41. Found: C, 73.98; H, 8.30.



45b

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-lauroyl-β-D-glucopyranoside (**45b**) - Treatment of **44b** (3.0 g, 4.8 mmol) as described for **44a** gave **45b**. (2.2 g, 70%); [α]$_D$ 8.3º (*c* 0.03, CHCl$_3$); FTIR (KBr): 2924, 2853, 1739, 1497, 1454, 1356, 1151, 1070, 735 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.36-7.24 (ms, 15H, aromatic H), 4.87, 4.84, 4.72 (each AB d, 6H, *J* 10.5, OC*H*$_2$Ph), 4.37 (d, 2H, $J_{5,6}$ 11.5, H-6a,6b), 4.31 (d, 1H, $J_{1,2}$ 8.0, H-1), 4.25 (m, 1H, H-5), 3.67 (apt t, 1H, $J_{2,3}$ 8.5, $J_{3,4}$ 8.5, H-3), 3.56 (s, 3H, OC*H*$_3$), 3.54 (m, 1H, H-4), 3.43 (dd, 1H, H-2), 2.32 (m, 2H, aliphatic OCOC*H*$_2$C$_{10}$H$_{21}$), 1.62 (m,

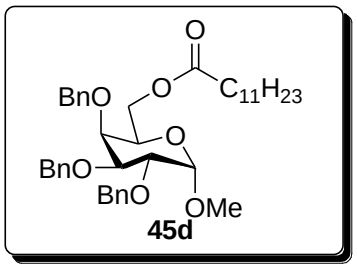
2H, aliphatic $OCOCH_2CH_2C_9H_{19}$), 1.26-1.24 (ms, 16H, each aliphatic $OCOC_2H_4C_8H_{16}CH_3$), 0.88 (t, 3H, *J* 6.0, *J* 7.0, aliphatic $OCOC_{11}H_{23}CH_3$); ^{13}C NMR ($CDCl_3$): δ 173.6 (s, C=O), 138.43, 138.42, 137.8 (each s, each aromatic C), 128.8, 128.5, 128.4, 128.38, 128.34, 128.26, 128.11, 128.07, 127.97, 127.92, 127.8, 127.7, 127.69, 127.64, 127.5 (each d, each aromatic CH), 104.7 (d, C-1), 84.6, 82.3, 77.6, 72.9 (each d), 75.7, 75.1, 74.8 (each t, each OCH_2Ph), 62.9 (t, C-6), 57.1 (q, OCH_3), 34.2, 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 24.9, 24.7, 22.6 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 669.2 required, 669.9 $[M + Na]^+$; Anal. Calcd. for $C_{40}H_{54}O_7$: C, 74.27; H, 8.41. Found: C, 73.91; H, 8.79.



45c

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-lauroyl-α-D-mannopyranoside (**45c**) - Treatment of **44c** (6.2 g, 10.0 mmol) as described for **44a** gave **45c**. (4.1 g, 64%); $[\alpha]_D$ 23.3º (*c* 0.04, $CHCl_3$); FTIR (KBr): 3031, 2924, 2853, 1737, 1496, 1454, 1362, 1066, 1027, 970, 909, 735, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.25 (multiple signals, 15H, each aromatic H), 4.77 (AB d, 2H, *J* 10.5, OCH_2Ph), 4.74 (d, 1H, $J_{1,2}$ 2.0, H-1), 4.72 (AB d, 2H, *J* 12.5, OCH_2Ph), 4.61 (s, 2H, OCH_2Ph), 4.38 (dd, 1H, $J_{5,6a}$ 2.5, $J_{6a,6b}$ 12.0, H-6a), 4.33 (dd, 1H, $J_{5,6b}$ 5.0, H-6b), 3.94-3.88 (overlapping signals, 2H, H-3,4), 3.78 (dd, 1H, $J_{2,3}$ 2.5, H-2), 3.76 (m, 1H, H-5), 3.31 (s, 3H, OMe), 2.32 (t, 2H, *J* 7.5, *J* 7.5, aliphatic $OCOCH_2C_{10}H_{21}$), 1.61 (m, 2H, aliphatic $OCOCH_2CH_2C_9H_{19}$), 1.31-1.54 (ms, 16H, aliphatic $OCOC_2H_4C_8H_{16}CH_3$), 0.91-0.86 (m, 3H, aliphatic $OCOC_{10}H_{20}CH_3$); ^{13}C NMR ($CDCl_3$): δ 173.7, (s, C=O), 138.32, 138.21, 138.17

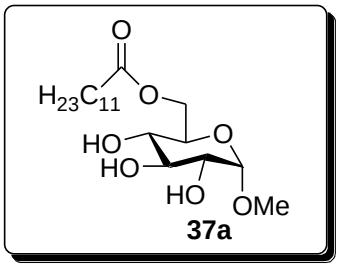
(each s, each aromatic C), 128.4., 128.38, 128.33, 128.05. 127.90, 127.76, 127.63, 127.23 (each d, each aromatic CH), 98.9 (d, C-1), 75.2, 74.6, 74.4, 69.9 (each d), 80.1, 72.6, 72.1 (each t, each CH_2Ph), 63.3 (t, C-6), 54.8 (q, OCH_3), 34.2, 33.9, 31.9, 29.61, 29.48, 29.44, 29.33, 29.27, 29.17, 29.07, 24.9, 24.7, 23.8, 22.7, 21.1 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 664.6 required, 664.9; $[M + H_2O]^+$; Anal. Calcd. for $C_{40}H_{54}O_7$: C, 74.27; H, 8.41. Found: C, 74.35; H, 8.25.



45d

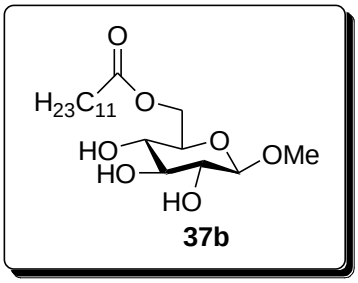
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-lauroyl-α-D-galactopyranoside (**45d**) - Treatment of **44d** (5.7 g, 9.2 mmol) as described for **44a** gave **45d**. (3.6 g, 60%); $[\alpha]_D$ 27.8º (*c* 0.09, $CHCl_3$); FTIR (KBr): 3030, 2924, 2853, 1738, 1496, 1454, 1350, 1099, 1049, 735, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.41-7.23 (multiple signals, 15H, each aromatic H), 4.83 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.81 (AB d, 2H, *J* 11.5, OC*H*$_2$Ph), 4.77 (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.68 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.16 (dd, 1H, *J* 7.5, *J* 11.5, H-4), 4.07-4.03 (overlapping signals, 2H, H-2,5), 3.94 (dd, 1H, *J* 3.0, *J* 10.0 H-6a), 3.86-3.84 (overlapping signals, 2H, H-3,6b), 3.35 (s, 3H, OMe), 2.23 (m, 2H, aliphatic OCOC*H*$_2$C$_{10}$H$_{21}$), 1.57 (m, 2H, aliphatic OCOCH$_2$C*H*$_2$C$_9$H$_{19}$), 1.31-1.18 (ms, 16H, aliphatic OCOC$_2$H$_4$C$_8$*H*$_{16}$CH$_3$), 0.88 (t, 3H, *J* 6.5, *J* 7.0, aliphatic OCOC$_{10}$H$_{20}$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 173.4 (s, C=O), 138.7, 138.4, 138.2 (each s, each aromatic C), 128.42, 128.36, 128.32, 128.11, 127.90, 127.75, 127.59, 127.51, 127.21 (each d, each aromatic CH), 98.7 (d, C-1), 78.9, 76.3, 74.9, 68.4 (each d), 74.6, 73.63, 73.54 (each t, each CH_2Ph), 63.3 (t, C-6), 55.3 (q, OCH_3), 34.1, 33.8, 31.9,

29.359, 29.45, 29.32, 29.26, 29.12, 24.9, 24.8, 22.7 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 664.6 required, 664.9; $[M + H_2O]^+$; Anal. Calcd. for $C_{40}H_{54}O_7$: C, 74.27; H, 8.41. Found: C, 74.67; H, 8.68.



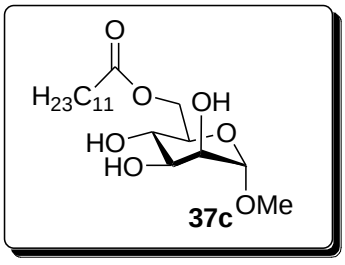
37a

Methyl 6-*O*-lauroyl-α-D-glucopyranoside (**37a**) - Compound **45a** (0.34 g, 0.2 mmol) was dissolved in ethanol (1 mL) and Pd-C (0.1 g) was added. The mixture was allowed to shake under hydrogen atmosphere of 2 psi until all protecting groups had been removed as verified by t.l.c. to yield **37a**. The suspension was filtered and concentrated *in vacuo*.[6] (0.17 g, 86%); $[\alpha]_D$ 19° (*c* 0.02, $CHCl_3$); FTIR (KBr): 3734, 3445, 2955, 2924, 2850, 2359, 2341, 1728. cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.75 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.33 (m, 2H, H-6), 3.75-3.73 (overlapping signals, 2H, H-3,5), 3.35 (apt t, 1H, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5, H-4), 3.54 (dd, 1H, $J_{2,3}$ 9.5, H-2), 3.41 (s, 3H, OMe), 2.35 (t, 2H, *J* 7.5, aliphatic $OCOC\mathit{H}_2C_{10}H_{21}$), 1.63 (m, 2H, aliphatic $OCOCH_2C\mathit{H}_2C_9H_{19}$), 1.38-1.23 (ms, 16H, aliphatic $OCOC_2H_4C_8\mathit{H}_{16}CH_3$), 0.88 (t, 3H, *J* 7.0, aliphatic $OCOC_{10}H_{20}C\mathit{H}_3$); ^{13}C NMR ($CDCl_3$): δ 174.2 (s, C=O), 99.4 (d, C-1), 74.1, 71.9, 70.4, 69.8 (each d), 63.5 (t, C-6), 55.2 (q, OCH_3), 34.2, 31.9, 29.66, 29.64, 29.5, 29.4, 29.3, 29.2, 24.9, 22.7 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 399.3 required, 399.5; $[M + Na]^+$; Anal. Calcd. for $C_{19}H_{36}O_7$: C, 60.61; H, 9.64. Found: C, 60.69; H, 9.83.



37b

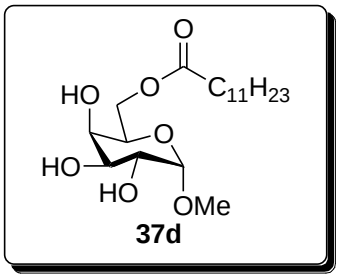
Methyl 6-*O*-lauroyl-β-D-glucopyranoside (**37b**) - Treatment of **45b** (2.0 g, 3.0 mmol) as described for **45a** gave **37b**. (0.86 g, 75%); $[\alpha]_D$ –25.5º (*c* 0.05, $CHCl_3$); FTIR (KBr): 3421, 2921, 1744, 1703, 1016 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.40 (d, 1H, $J_{1,2}$ 11.5, H-1), 4.28 (dd, 1H, $J_{2,3}$ 6.0, H-2), 4.21 (d, 2H, $J_{5,6}$ 7.5, H-6), 3.54 (s, 3H, OC*H*$_3$), 3.49 (m, 1H, H-3), 3.39-3.31 (overlapping signals, 2H, H-4,5), 2.34 (m, 2H, aliphatic OCOC*H*$_2$$C_{10}H_{21}$), 2.02 (s, 3H, OH), 1.62 (m, 2H, aliphatic $OCOCH_2CH_2C_9H_{19}$), 1.28-1.26 (ms, 16H, aliphatic $OCOC_2H_4C_8H_{16}CH_3$), 0.88 (t, 3H, *J* 6.5, aliphatic $OCOC_{10}H_{20}CH_3$); ^{13}C NMR ($CDCl_3$): δ 174.2 (s, C=O), 103.6 (d, C-1), 76.5, 73.9, 73.4, 70.3 (each d), 63.6 (t, C-6), 57.0 (q, OCH_3), 34.2, 31.9, 29.61, 29.60, 29.5, 29.3, 29.2, 29.1, 24.9, 22.7 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 399.1 required, 399.5 $[M + Na]^+$; Anal. Calcd. for $C_{19}H_{36}O_7$: C, 60.61; H, 9.64. Found: C, 60.25; H, 9.91.



37c

Methyl 6-*O*-lauroyl-α-D-mannopyranoside (**37c**) - Treatment of **45c** (3.3 g, 5.0 mmol) as described for **45a** gave **37c**. (1.4 g, 75%); $[\alpha]_D$ 33.3º (*c* 0.01, $CHCl_3$); FTIR (KBr): 3421, 2923, 1736, 1466, 1197, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.70 (s, 1H, H-1), 4.45 (br s, 1H, OH), 4.36 (d, 2H, *J* 4.0, H-6), 3.96-3.92

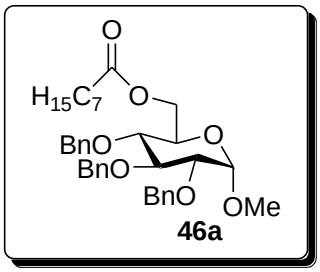
(overlapping signals, 2H, OH, H-2), 3.78 (dd, 1H, $J_{2,3}$ 2.5, $J_{3,4}$ 9.0, H-3), 3.71 (m, 1H, H-5), 3.62 (apt t, 1H, $J_{4,5}$ 9.5, H-4) 3.36 (s, 3H, OMe), 2.35 (t, 2H, *J* 7.5, *J* 7.5, aliphatic $OCOC\mathit{H_2}C_{10}H_{21}$), 1.61 (m, 2H, aliphatic $OCOCH_2C\mathit{H_2}C_9H_{19}$), 1.29-1.25 (ms, 16H, aliphatic $OCOC_2H_4C_8\mathit{H_{16}}CH_3$), 0.88 (t, 3H, *J* 6.5, *J* 7.0, aliphatic $OCOC_{10}H_{20}C\mathit{H_3}$);); ^{13}C NMR ($CDCl_3$): δ 174.7 (s, C=O), 100.9 (d, C-1), 71.5, 70.5, 70.4, 67.7 (each d), 63.9 (t, C-6), 54.9 (q, OCH_3), 34.2, 31.9, 29.7, 29.6, 29.5, 29.4, 29.36, 29.34, 29.19, 24.9, 22.7 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 377.3 required, 377.5; $[M + H]^+$; Anal. Calcd. for $C_{19}H_{36}O_7$: C, 60.61; H, 9.64. Found: C, 60.71; H, 9.53.



37d

Methyl 6-*O*-lauroyl-α-D-galactopyranoside (**37d**) - Treatment of **45d** (2.8 g, 4.4 mmol) as described for **45a** gave **37d**. (1.43 g, 86%); $[\alpha]_D$ 56.25º (*c* 0.01, $CHCl_3$); FTIR (KBr): 3250, 2918, 1741, 1467, 1194, 1025cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.63 (apt t, 1H, *J* 6.5, *J* 5.0, OH-3), 4.57 (d, 1H, *J* 6.5, OH-2), 4.55 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.13 (dd, 1H, $J_{5,6a}$ 8.0, $J_{6a,6b}$ 11.5, H-6a), 4.07 (dd, 1H, $J_{5,6b}$ 4.0, H-6b), 3.75 (dd, 1H, H-5), 3.68 (apt t, 1H, $J_{3,4}$ 3.5, $J_{4,5}$ 3.0, H-4), 3.58 (ddd, 1H, $J_{2,3}$ 10.0, $J_{2,OH}$ 16.5, H-2), 3.52 (m, 1H, H-3), 3.24 (s, 3H, OMe), 2.28 (t, 2H, *J* 7.5, aliphatic $OCOC\mathit{H_2}C_{10}H_{21}$), 1.63 (t, 2H, *J* 7.0, aliphatic $OCOCH_2C\mathit{H_2}C_9H_{19}$), 1.28-1.23 (ms, 16H, aliphatic $OCOC_2H_4C_8\mathit{H_{16}}CH_3$), 0.85 (t, 3H, *J* 7.0, aliphatic $OCOC_{10}H_{20}C\mathit{H_3}$); ^{13}C NMR ($CDCl_3$): δ 178.2 (s, C=O), 104.8 (d, C-1), 74.9, 74.1, 73.7, 73.1 (each d), 68.8 (t, C-6), 59.8 (q, OCH_3), 38.9, 36.5, 34.24, 34.10, 33.97, 33.93, 33.75, 29.5, 27.3,

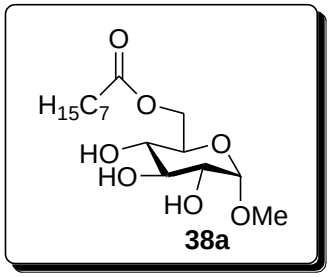
(each t, each aliphatic CH_2), 18.9 (q, aliphatic CH_3); LRMS: Found, 399.3 required, 399.5; $[M + Na]^+$; Anal. Calcd. for $C_{19}H_{36}O_7$: C, 60.61; H, 9.64. Found: C, 60.60; H, 9.88.



46a

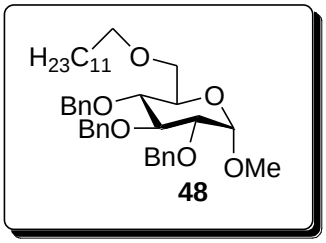
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-octanoyl-α-D-glucopyranoside (**46a**) - Compound **44a** (5.0 g, 8.5 mmol) was dissolved in THF anhydrous (150 mL) and was cooled to 0°C. Tetrabutylammonium fluoride (2.2 g, 8.5 mmol) was added and the solution was warmed to room temperature and stirred for 1 h.[7] The mixture was then concentrated *in vacuo* and the resulting 6-OH residue was dissolved in pyridine anhydrous (100 mL). A catalytic amount of 4-dimethylaminopyridine and octanoyl chloride (2.9 mL, 17 mmol) was added and the mixture was stirred at room temperature for 24 h.[8] The solution was then concentrated under reduced pressure and purified by chromatography (petroleum ether-EtOAc) to give **46a**. (3.9 g, 63%); $[\alpha]_D$ 20.8° (*c* 0.07, $CHCl_3$); FTIR (KBr): 2927, 1738, 1497, 1454, 1360, 1163, 1093, 738, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.26 (ms, 15H, aromatic H), 4.93, (AB d, 2H, *J* 10.5, OC*H*$_2$Ph), 4.74, (AB d, 2H, *J* 12.0, OC*H*$_2$Ph), 4.73 (AB d, 2H, *J* 10.5, OC*H*$_2$Ph), 4.60 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.28 (d, 2H, $J_{5,6}$ 3.0, H-6), 4.01 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.5, H-3), 3.81 (m, 1H, H-5), 3.54 (dd, 1H, H-2), 3.48 (dd, 1H, $J_{4,5}$ 10.5, H-4), 3.37 (s, 3H, OC*H*$_3$), 2.31 (m, 2H, aliphatic OCOC*H*$_2$C$_6$H$_{13}$), 1.62 (m, 2H, aliphatic OCH$_2$C*H*$_2$C$_5$H$_{11}$), 1.30-1.05 (ms, 8H, aliphatic OC$_2$H$_4$C$_4$*H*$_8$CH$_3$), 0.87 (m, 3H, aliphatic OC$_6$H$_{12}$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 173.8 (s, C=O), 138.8, 138.3, 138.1

(each s, each aromatic C), 128.7, 128.6, 128.3, 128.29, 128.27, 128.3, 128.25, 128.20, 128.1 127.9 (each d, each aromatic CH), 98.3 (d, C-1), 82.2, 80.2, 77.8, 68.9 (each d), 76.1, 75.3, 73.6 (each t, each OCH_2Ph), 63.1 (t, C-6), 55.4 (q, OCH_3), 34.4, 31.9, 29.2, 25.0, 22.8, 17.9 (each t, each aliphatic CH_2), 14.3 (q, aliphatic CH_3); LRMS: Found, 613.4 required, 613.7; $[M + Na]^+$; Anal. Calcd. for $C_{36}H_{46}O_7$: C, 73.19; H, 7.85. Found: C, 73.25; H, 7.61



38a

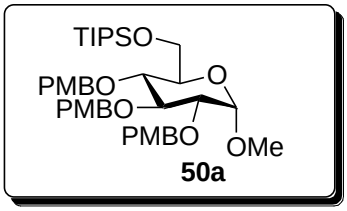
Methyl 6-*O*-octanoyl-α-D-glucopyranoside (**38a**) - Treatment of **46a** (3.6 g, 6.2 mmol) as described for **45a** gave **38**[a]. (1.44 g, 73%); $[\alpha]_D$ 27.9º (*c* 0.4, $CHCl_3$); FTIR (KBr): 3388, 2922, 1712, 1465, 1193, 1106, 724 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.82 (s, 3H, each O*H*), 4.76 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.35 (d, 2H, $J_{5,6}$ 4.0, H-6), 3.78-3.72 (overlapping signals, 2H, H-3,5), 3.54 (dd, 1H, $J_{2,3}$ 9.5, H-2), 3.41 (s, 3H, OCH_3), 3.36 (dd, 1H, $J_{3,4}$ 9.5, $J_{4.5}$ 10.0, H-4), 2.35 (m, 2H, aliphatic $COCH_2C_6H_{13}$) 1.64 (t, 2H, *J* 7.0, aliphatic $COCH_2CH_2C_5H_{11}$), 1.31-1.05 (ms, 8H, aliphatic $COC_2H_4C_4H_8CH_3$), 0.88 (t, 3H, *J* 5.5, *J* 7.0, aliphatic $COC_6H_{12}CH_3$); ^{13}C NMR ($CDCl_3$): δ 179.5 (s, C=O), 99.4 (d, C-1), 74.1, 72.0, 69.7, 70.3 (each d), 63.4 (t, C-6), 55.3 (q, OCH_3), 34.1, 31.7, 31.6, 29.9, 28.9, 24.8 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 343.1 required, 343.4; $[M + Na]^+$; Anal. Calcd. for $C_{15}H_{28}O_7$: C, 56.23; H, 8.81. Found: C, 56.47; H, 8.73.



48

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-dodecanyl-α-D-glucopyranoside (**48**) - Compound **44a** (3 g, 4 mmol) was dissolved in THF anhydrous (80 mL) and was cooled to 0°C. Tetrabutylammonium fluoride (1 g, 4 mmol) was added and the solution was allowed to warm to room temperature and stir for 1 h.[7] It was then concentrated *in vacuo* and approximately 3 mmol of the resulting 6-OH sugar was dissolved in DMF anhydrous (10 mL). 1-Chlorododecane (2.2 mL, 9.5 mmol) was added and the suspension was cooled to 0°C before NaH (0.38 g, 9.5 mmol) was added portion wise. The solution was then allowed to warm to room temperature and stirred for 24 h. Methanol (50 mL) was added to quench the mixture which was stirred for 1 h.[5] It was then concentrated *in vacuo* and purified by chromatography (petroleum ether-EtOAc) to give the benzylated ether **48**. (0.70 g, 35 %); $[\alpha]_D$ 3.3° (*c* 0.03, $CHCl_3$); FTIR (KBr): 2924, 2853, 1496, 1454, 1360, 1160, 1072, 736 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ 7.37-7.25 (ms, 15H, aromatic H), 4.91, (AB d, 2H, *J* 11.0, OC*H*$_2$Ph), 4.75, (AB d, 2H, *J* 11.0, OC*H*$_2$Ph), 4.71 (AB d, 2H, *J* 13.0, OC*H*$_2$Ph), 4.62 (d, 1H, $J_{1,2}$ 3.5, H-1), 3.98 (apt t, 1H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0, H-3), 3.71 (m, 1H, H-5), 3.68-3.59 (overlapping signals, 3H, H-4,6a,6b), 3.55 (dd, 1H, H-2), 3.37 (s, 3H, OC*H*$_3$), 1.55 (m, 2H, aliphatic OC*H*$_2$$C_{11}H_{23}$), 1.31-1.23 (ms, 20H, aliphatic OCH_2C_{10}*H*$_{20}$$CH_3$), 0.88 (t, 3H, *J* 7.0, aliphatic C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 139.1, 138.7, 138.4 (each s, each aromatic H), 128.7, 128.63, 128.60, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8 (each d, each aromatic CH), 98.4 (d, C-1), 82.4, 80.1, 78.0, 70.3 (each d), 76.0, 75.3, 73.6, 72.0 (each t), 69.4 (t, C-6), 55.4 (q, OCH_3), 32.1, 29.9, 29.86, 29.82, 29.7, 29.6, 26.4, 22.9

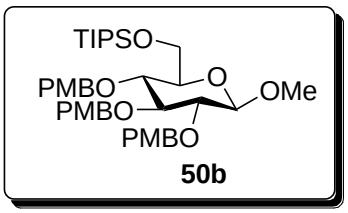
(each t, each aliphatic CH_2), 14.4 (q, aliphatic CH_3); LRMS: Found, 655.4; required, 655.87; $[M + Na]^+$. After this step an attempt was made to remove the benzyl groups from compound **48** using the same method as for compound **45a**, however during the reaction, the dodecanyl ether group was also removed leaving methyl-α-D-glucopyranoside **7a** confirmed by t.l.c.



50a

Methyl 2,3,4-tri-*O*-paramethoxybenzyl-6-*O*-triisopropylsilyl-α-D-glucopyranoside (**50a**) - A solution of **7a** (5.0 g, 25.0 mmol) in DMF anhydrous (120 mL) was treated with triisopropylsilyl chloride (15 mL, 75 mmol) and imidazole (5 g, 75 mmol) and allowed to stir at room temperature for 24 h. The crude TIPS protected intermediate was then concentrated *in vacuo* and the resulting residue dissolved in ethyl actetate. It was then washed with 10% HCl, water, followed by sat. aq. $NaHCO_3$, and finally sat. aq. NaCl before being dried over anhydrous $MgSO_4$ and concentrated under reduced pressure.[7] The TIPS protected crude residue was then split in two and half was dissolved in DMF anhydrous (30 mL) and THF anhydrous (20 mL). This solution was then added dropwise at 0^oC to a suspension of NaH (2.5 g, 62.5 mmol) in DMF anhydrous (10 mL) and THF anhydrous (7 mL), paramethoxybenzyl chloride (17 mL, 125 mmol) and tetrabutylammonium iodide (18.5 g, 50 mmol). This was stirred at approximately 10^oC for 30 min and then allowed to warm to room temperature and stir for 24 h. Methanol (50 mL) was added to quench the mixture which was stirred for 1 h. The solution was then concentrated under diminished pressure and dissolved in ethyl acetate. It was washed with water, dried over anhydrous $MgSO_4$ and

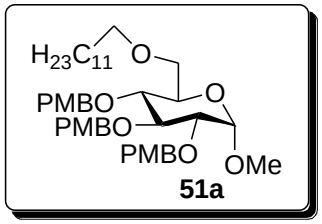
concentrated *in vacuo*.[3] The resulting residue was purified by chromatography (petroleum ether-EtOAc) to give **50a**. (5.15 g, 59%); $[\alpha]_D$ 11.6º (*c* 0.05, $CHCl_3$); FTIR (KBr): 3479, 2936, 2864, 1464, 1421, 1360, 1302, 883, 820. cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.34-6.73 (ms, 12H, aromatic H), 4.88 (AB d, 2H, *J* 10.5 OCH_2Ph), 4.78 (d, 1H, $J_{1,2}$ 5.0, H-1), 4.75, 4.71 (each AB d, 2H, *J* 12.0 OCH_2Ph), 4.63 (m, 1H, H-2), 3.99 (apt t, 1H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.0, H-4), 3.89 (m, 2H, H6), 3.77 (m, 9H, each PhOCH_3), 3.57-3.49 (overlapping signals, 2H, H-3,5), 3.39 (s, 3H, OCH_3), 1.28 (m, 3H, each TIPS C*H*), 1.16-1.06 (ms, 18H, each TIPS CH_3); ^{13}C NMR ($CDCl_3$): δ 159.6, 159.5, 159.4, 131.6, 131.4, 131.0 (each s, each aromatic C), 129.99, 129.93, 129.8, 114.13, 114.08, 113.6 (each d, each aromatic CH), 98.1 (d, C-1), 82.2, 80.2, 77.8, 72.1 (each d), 75.8, 74.9, 73.2 (each t, each OCH_2Ph), 63.1 (t, C-6), 55.47, 55.40, 55.36 (each q, each $PhOCH_3$), 55.0 (q, OCH_3), 18.27, 18.25 (each q, each TIPS CH_3), 12.3 (d, each TIPS CH); LRMS: Found, 733.3 required, 733.9 $[M + Na]^+$.



50b

Methyl 2,3,4-tri-*O*-paramethoxybenzyl-6-*O*-triisopropylsilyl-β-D-glucopyranoside (**50b**) - Treatment of **7b** (4.5 g, 23.17 mmol) as described for **7a** gave **50b**. (10.1 g, 61%); $[\alpha]_D$ 4.8º (*c* 0.05, $CHCl_3$); FTIR (KBr): 2939, 1586, 1464, 883, 821, 760, 683. cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.30-6.84 (ms, 12H, aromatic H), 4,85, 4.80, 4.73 (each AB d, 2H, *J* 10.5, OCH_2Ph), 4.27 (d, 1H, $J_{1,2}$ 7.5, H-1), 3.95 (m, 1H, H-6a), 3.87 (dd, 1H, $J_{4,5}$ 11.0, $J_{5,6}$ 4.5, H-5), 3.78 (m, 9H, PhOCH_3), 3.59 (m, 1H, H-3), 3.53 (s, 3H, OCH_3) 3.36 (apt t, 1H, $J_{2,3}$ 9.0, H-2), 3.29-3.24 (overlapping signals, 2H, H-4,6b), 1.10-1.04 (ms, 21H, TIPS); ^{13}C NMR ($CDCl_3$): δ 159.5, 159.4, 131.2, 131.1,

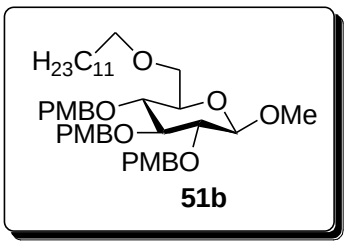
130.9, (each s, each aromatic C), 129.9, 129.8, 128.7, 114.1, 114.04, 114.01 (each d, each aromatic CH), 104.7 (d, C-1), 84.7, 82.6, 77.5, 76.2 (each d), 75.7, 74.9, 74.7 (each t, each OCH_2PH), 62.7 (t, C-6), 56.8 (q, OCH_3), 55.5 (each q, each $PhOCH_3$), 18.3, 18.2 (each q, each TIPS CH_3), 12.2 (d, each TIPS CH); LRMS: Found, 733.3; required, 733.9 $[M + Na]^+$.



51a

Methyl 2,3,4-tri-*O*-paramethoxybenzyl-6-*O*-dodecanyl-α-D-glucopyranoside (**51a**) - Compound **50a** (4.0 g, 5.5 mmol) was dissolved in THF anhydrous (100 mL) and was cooled to 0°C. Tetrabutylammonium fluoride (1.4 g, 5.5 mmol) was added and the solution was allowed to warm to room temperature and stir for 1 h.[7] The mixture was then concentrated *in vacuo* and the resulting 6-OH residue was dissolved in DMF anhydrous (100 mL). 1-Chlorododecane (1.8 mL, 11 mmol) was added and the solution was cooled to 0°C before NaH (0.11 g, 2.75 mmol) was added portion wise. The mixture was then allowed to warm to room temperature and was stirred for 24 h. Methanol (50 mL) was added to quench the solution which was stirred for 1 h.[5] The crude PMB protected ether was then concentrated under diminished pressure and purified by chromatography (petroleum ether-EtOAc) to give **51a**. (1.89 g, 50%); $[\alpha]_D$ –8.6° (*c* 0.06, $CHCl_3$); FTIR (KBr): 2924, 2854, 1613, 1586, 1464, 1359, 1301, 1248, 1172, 1037, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.85-7.30 (ms, 12H, aromatic H), 4.92 (d, 1H, $J_{1,2}$ 10.5, H-1), 4.85 (AB d, 2H, *J* 10.5, OCH_2PhOCH_3), 4.74 (dd, 1H, $J_{2,3}$ 9.5, H-2), 4.69, (AB d, 2H, *J* 10.5, OCH_2PhOCH_3), 4.60 (AB d, 2H, *J* 11.5 OCH_2PhOCH_3), 4.55 (apt t, 1H, $J_{3,4}$ 9.5, H-3), 3.95 (m, 1H, H-5), 3.80 (s, 9H,

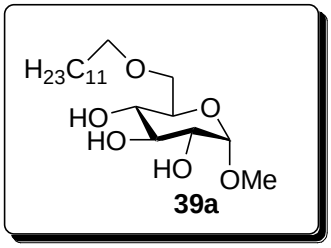
each PhOCH_3), 3.53-3.37 (overlapping signals, 3H, H-4,6a,6b), 3.36 (s, 3H, OCH_3), 1.60 (m, 2H, aliphatic CH_2C$_{11}$H$_{23}$), 1.30-1.25 (ms, 20H, aliphatic CH$_2$C$_{10}$$H_{20}CH_3$), 0.89 (t, 3H, *J* 7.0, aliphatic C$_{11}$H$_{20}$CH_3); ^{13}C NMR ($CDCl_3$): δ 159.6, 159.5, 159.4, 131.3, 131.0, 130.6 (each s, each aromatic C), 130.0, 129.8, 129.6, 114.07, 114.05, 114.03 (each d, each aromatic CH), 98.5 (d, C-1), 82.1, 79.8, 77.7, 70.2 (each d), 75.7, 74.9, 73.3 (each t, each OCH$_2$Ph), 72.0 (t, aliphatic OCH$_2$C$_{11}$H$_{23}$), 69.5 (t, C-6), 55.5 (q, PhOCH$_3$), 55.3 (s, OCH$_3$), 32.2, 29.94, 29.91, 29.89, 29.87, 29.84, 29.7, 29.5, 28.4 (each t, each aliphatic CH$_2$), 14.4 (q, aliphatic CH$_3$); LRMS: Found, 745.5; required, 745.9; $[M + Na]^+$; Anal. Calcd. for $C_{43}H_{62}O_9$: C, 71.44; H, 8.64. Found: C, 71.09; H, 8.73.



51b

Methyl 2,3,4-tri-*O*-paramethoxybenzyl-6-*O*-dodecanyl-β-D-glucopyranoside (**51b**) - Treatment of **50b** (3.2 g, 4.5 mmol) as described for **50a** gave **51b**. (0.55 g, 85%); [α]$_D$ 2^o (*c* 0.01, $CHCl_3$); FTIR (KBr): 2923, 2851, 1614, 1464.40, 1421, 1359, 1302, 1254, 1173, 1072, 813 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-6.84 (ms, 12H, aromatic H), 4.79, 4.75, 4.67 (each AB d, 2H, *J* 10.5, OCH_2Ph), 4.26 (d, 1H, $J_{1,2}$ 7.5, H-1), 3.79-3.58 (overlapping signals, 2H, H-3,5), 3.79 (m, 9H, PhOCH_3), 3.68 (m, 2H, H-6a,6b), 3.56 (s, 3H, OCH_3), 3.43-3.39 (overlapping signals, 2H, H-2,4), 1.63 (m, 2H, aliphatic OCH_2C$_{11}$H$_{23}$), 1.29-1.24 (ms, 20H, aliphatic OCH$_2$C$_{10}$$H_{20}CH_3$), 0.88 (t, 3H, *J* 7.0, aliphatic OC$_{11}$H$_{22}$CH_3); ^{13}C NMR ($CDCl_3$): δ 159.3, 159.2, 159.1, 130.9, 130.8, 130.5 (each s, each aromatic C), 129.8, 129.6, 129.5, 113.8, 113.7 (each

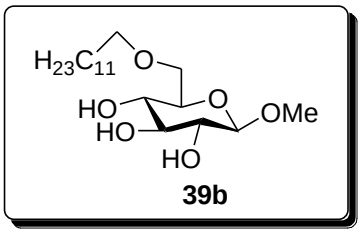
d, each aromatic CH), 104.8 (d, C-1), 84.4, 82.1, 77.7, 75.3 (each d), 74.9, 74.6, 74.4 (each t, each OCH_2Ph), 71.9 (t, aliphatic CH_2), 69.7 (t, C-6), 57.1 (q, OCH_3), 55.3, 55.2 (each q, each $PhOCH_3$), 31.9, 29.7, 29.68, 29.65, 29.63, 29.5, 29.4, 26.2, 22.7 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 745.3; required, 745.9; $[M + Na]^+$; Anal. Calcd. for $C_{43}H_{62}O_9$: C, 71.44; H, 8.64. Found: C, 71.19; H, 8.70.



39a

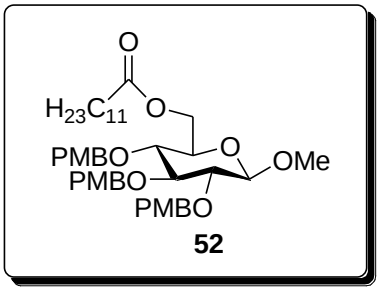
Methyl 6-*O*-dodecanyl-α-D-glucopyranoside (**39a**) - Compound **51a** (1.45 g, 2.0 mmol) was dissolved in a mixture of MeCN:H_2O (3:1, 21 mL) and cerric ammonium nitrate (8.85 g, 16.16 mmol) was added. The solution was allowed to stir at room temperature for 24 h.[9] It was then concentrated *in vacuo* and purified by chromatography (petroleum ether-EtOAc) to give **39a**. (0.53 g, 73%); $[\alpha]_D$ 78.8° (*c* 0.04, $CHCl_3$); FTIR (KBr): 3416, 2919, 2851, 1467, 1372, 1128, 1043, 1019 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.98 (br s, 1H, OH), 4.75 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.34 (br s, 1H, OH), 4.01 (br s, 1H, OH), 3.75 (apt t, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.5, H-3), 3.66 (m, 2H, H-6), 3.54-3.44 (overlapping signals, 3H, H-2,4,5), 3.37 (s, 3H, OCH_3), 1.58 (m, 2H, aliphatic $CH_2C_{11}H_{23}$), 1.28-1.25 (ms, 20H, each aliphatic $CH_2C_{10}H_{20}CH_3$), 0.88 (t, 3H, *J* 6.5, *J* 7.0, aliphatic $C_{11}H_{20}CH_3$); ^{13}C NMR ($CDCl_3$): δ 99.7 (d, C-1), 74.5, 72.3, 72.2, 71.2 (each d) 70.6 (t, aliphatic CH_2), 69.5 (t, C-6), 55.4 (q, OCH_3), 32.1, 29.9, 29.88, 29.86, 29.83, 29.7, 29.6, 26.3, 22.9 (each t, each aliphatic CH_2), 14.3 (q,

aliphatic CH_3); LRMS: Found, 385.2; required, 385.5; $[M + Na]^+$; Anal. Calcd. for $C_{19}H_{38}O_6$: C, 62.95; H, 10.57. Found: C, 62.60; H, 10.67.



39b

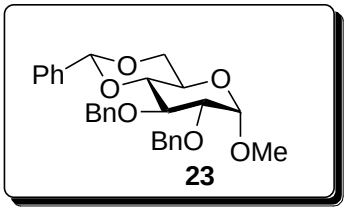
Methyl 6-*O*-dodecanyl-β-D-glucopyranoside (**39b**) - Treatment of **51b** (0.44 g, 0.6 mmol) as described for **51a** gave **39b**. (0.17 g, 76%); $[\alpha]_D$ –1° (*c* 0.03, $CHCl_3$); FTIR (KBr): 3405, 2922, 2850, 1470, 1391, 1128, 1109, 1048 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.20 (d, 1H, $J_{1,2}$ 7.5, H-1), 3.89 (s, 1H, OH), 3.74 (m, 2H, H-6a,6b), 3.66 (m, 1H, H-5), 3.54 (s, 3H, OC*H*$_3$), 3.52-3.44 (overlapping signals, 2H, H-3,4), 3.35 (apt t, 1H, $J_{2,3}$ 8.0, H-2), 1.58 (m, 2H, aliphatic OC*H*$_2$C$_{11}$H$_{23}$), 1.28-1.11 (ms, 20H, aliphatic OCH$_2$C$_{10}$*H*$_{20}$CH$_3$), 0.88 (t, 3H, *J* 6.5, *J* 7.0, aliphatic OC$_{11}$H$_{22}$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 103.5 (d, C-1), 76.5, 74.4, 73.4, 72.1, (each d), 71.6 (t, aliphatic CH_2), 70.9 (t, C-6), 57.1 (q, OCH_3), 31.9, 29.7, 29.66, 29.65, 29.58, 29.53, 29.4, 26.0, 22.7 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 385.2; required, 385.5; $[M + Na]^+$; Anal. Calcd. for $C_{19}H_{38}O_6$: C, 62.95; H, 10.57. Found: C, 62.83; H, 10.36.



52

Methyl 2,3,4-tri-*O*-paramethoxybenzyl-6-*O*-lauroyl-β-D-glucopyranoside (**52**) - Compound **50b** (3.2 g, 4.5 mmol) was dissolved in THF anhydrous (150 mL) and was cooled to 0°C. Tetrabutylammonium fluoride (1.2 g, 4.5 mmol) was added and the solution was allowed to warm to room temperature and stir for 1 h.[7] The mixture was then concentrated *in vacuo* and the resulting PMB protected residue was divided up and approximately 1 mmol was dissolved in pyridine anhydrous (25 mL). Lauroyl chloride (0.42 mL, 1.8 mmol) and a catalytic amount of 4-dimethylaminopyridine was added to the solution which was stirred at room temperature for 24 h.[8] It was then concentrated under reduced pressure and purified by chromatography (petroleum ether-EtOAc) to give **52**. (0.56 g, 85 %); $[\alpha]_D$ 7.8° (*c* 0.05, $CHCl_3$); FTIR (KBr): 2924, 1737, 1600, 1501, 1464, 1302, 1249, 1173, 821 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ; 7.29-6.84 (ms, 12H, aromatic H), 4.77, 4.70, 4.63 (each AB d, 6H, *J* 10.5, OC*H*$_2$Ph), 4.28 (d, 1H, $J_{1,2}$ 7.5, H-1), 4.25 (dd, 1H, $J_{2,3}$ 12.0, H-2), 3.61 (m, 1H, H-5), 3.56 (s, 3H, OC*H*$_3$), 3.52-3.47 (overlapping signals, 3H, H-4,6a,6b), 3.39 (apt t, 1H, $J_{3,4}$ 8.0, H-3), 2.31 (m, 2H, aliphatic OCOC*H*$_2$$C_{10}H_{21}$), 1.61 (t, 2H, *J* 7.0, aliphatic $OCOCH_2CH_2C_9H_3$), 1.29-1.24 (ms, 16H, aliphatic $OCOC_2H_4C_8H_{16}CH_3$), 0.88 (t, 3H, *J* 8.0 aliphatic $OCOC_{10}H_{20}CH_3$); ^{13}C NMR ($CDCl_3$): δ 173.6 (s, C=O), 159.4, 159.3, 159.2, 130.7, 130.6, 129.9 (each s, each aromatic C), 129.8, 129.7, 129.5, 113.9, 113.82, 113.80 (each d, each aromatic CH), 104.7 (d, C-1), 84.3, 81.9, 77.3, 72.9 (each d), 75.4, 74.7, 74.4 (each d, each OCH_2Ph), 62.9 (t, C-6), 57.1 (q, OCH_3), 55.3 (q, each $PhOCH_3$), 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 24.9, 22.7 (each d, each aliphatic

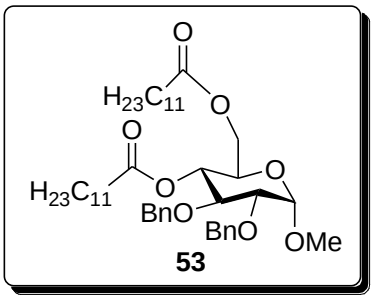
CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 759.3; required, 759.9; $[M + Na]^+$. After this step an attempt was made to remove the paramethoxybenzyl groups from compound **52** using the same method as for compound **51b**, however during the reaction, the lauroyl ester group was also removed leaving methyl-β-D-glucopyranoside **7b** confirmed by t.l.c.



23

Methyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-glucopyranoside (**23**) - A solution of **7a** (1.0 g, 5.2 mmol), *p*-toluenesulfonic acid (10 mg) and benzaldehyde dimethylacetal (1.5 mL, 10.3 mmol) in acetonitrile anhydrous (25 mL) was stirred for 24 h at room temperature. Trimethylamine (0.5 mL) was added to neutralise the solution which was then stirred for 1 h. The product was filtered off as a white solid, washed with petroleum ether and dried.[3] The benzylidene protected intermediate was then dissolved in DMF anhydrous (15 mL) and the solution was cooled to 0^oC. NaH (0.74 g, 18.4 mmol) was added slowly followed by benzyl bromide (2.5 mL, 20 mmol). The mixture was then warmed to room temperature and stirred over night. Methanol (10 mL) was added to quench the solution which was stirred for a further 1 hr. The mixture was then concentrated under diminished pressure and purified by chromatography (petroleum ether-EtOAc) to give **23**. (2.0 g, 95%); $[\alpha]_D$ 0.7º (*c* 0.05, $CHCl_3$); FTIR (KBr): 3063, 3031, 1109, 1088, 735, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.50-7.22 (ms, 15H, each aromatic H), 5.54 (s, 1H, C*H*Ph), 4.85 (AB d, 2H, *J* 4.0, OCH_2Ph), 4.82 (AB d, 2H, *J* 12.0, OCH_2Ph), 4.59 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.26 (dd, 1H, $J_{5,6a}$ 10.0, $J_{6a,6b}$ 4.5, H-6a), 4.05 (apt t, 1H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0, H-3), 3.83 (m,

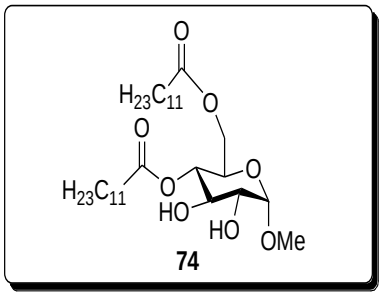
1H, H-5), 3.70 (apt t, 1H, $J_{5,6b}$ 10.5, H-6b), 3.62-3.54 (overlapping signals, 2H, H-2,4), 3.39 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 138.7, 138.1, 137.4 (each s, each aromatic C), 128.89, 128.43, 128.29, 128.20, 128.10, 128.01, 127.90, 127.57, 126.0 (each d, each aromatic CH), 101.2 (d, C-1), 99.2 (d, C*H*Ph), 82.1, 79.2, 78.6, 62.3 (each d), 75.3, 73.8 (each t), 69.1 (t, C-6), 55.3 (q, OCH_3); LRMS: Found, 463.3 required, 463.5; $[M + H]^+$; Anal. Calcd. for $C_{28}H_{30}O_6$: C, 72.71; H, 6.54. Found: C, 72.31; H, 6.56.



53

Methyl 2,3-di-*O*-benzyl-4,6-di-*O*-lauroyl-α-D-glucopyranoside (**53**) - Compound **23** (1.7 g, 3.6 mmol) was dissolved in Methanol (50 mL) and a catalytic amount of TsOH was added. The solution was stirred at room temperature overnight after which Et_3N (1 mL) was added to quench the reaction.[3] The mixture was concentrated under diminished pressure and the crude diol residue was dissolved in pyridine anhydrous (70 mL). A catalytic amount of 4-dimethylaminopyridine and lauroyl chloride (3.3 mL, 14.4 mmol) was added and the reaction was stirred at room temperature for 3 h.[8] The solution was then concentrated under diminished pressure and purified by chromatography (petroleum ether-EtOAc) to give **53**. (1.0 g, 38%); FTIR (KBr): 2925, 2853, 1743, 1455, 1360, 1167, 1105, 1045, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.26 (multiple signal, 10H, each aromatic H), 5.01 (dd, 1H, $J_{3,4}$ 9.5, $J_{4,5}$ 10.0, H-4), 4.78 (AB d, 2H, *J* 11.5, OCH_2Ph), 4.73 (AB d, 2H, *J* 12.0, OCH_2Ph),4.59 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.15 (dd, 1H, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 12.5, H-6a), 4.04

(dd, 1H, $J_{5,6b}$ 2.0, H-6b), 3.92 (apt t, 1H, $J_{2,3}$ 9.5, H-3), 3.87-3.82 (m, 1H, H-5), 3.59 (dd, 1H, H-2), 2.36-2.27 (m, 4H, each aliphatic OCOC*H*$_2$C$_{10}$H$_{21}$), 1.67-1.56 (m, 4H, each aliphatic OCOCH$_2$C*H*$_2$C$_9$H$_{19}$), 1.26-1.16 (ms, 32H, each aliphatic OCOC$_2$H$_4$C$_8$*H*$_{16}$CH$_3$), 0.88 (t, 6H, *J* 6.5, *J* 7.0, each aliphatic OCOC$_{10}$H$_{20}$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 173.6, 172.4 (each s, each C=O), 138.4, 137.9 (each s, each aromatic C), 128.51, 128.32, 128.18, 128.05. 127.69, 127.57 (each d, each aromatic CH), 98.2 (d, C-1), 79.51, 79.18, 69.5, 67.7 (each d), 75.4, 73.6 (each t, each CH_2Ph), 62.2 (t, C-6), 55.4 (q, OCH_3), 34.15, 34.03, 33.99, 31.9, 29.62, 29.60, 29.49, 29.44, 29.35, 29.34, 29.28, 29.26, 29.15, 29.13, 29.07, 24.76, 24.70, 22.69 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3).

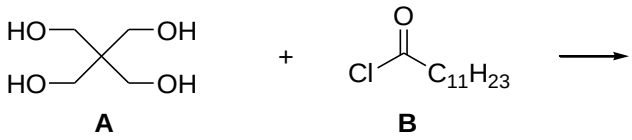


74

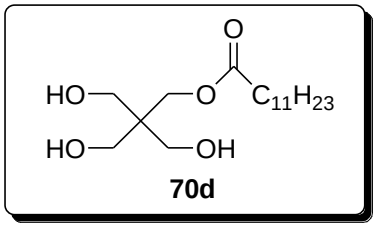
Methyl 4,6-di-*O*-lauroyl-α-D-glucopyranoside (**40**) - Compound **53** (0.84 g, 1.14 mmol) was dissolved in Ethanol (2.5 mL) and Pd-C (0.3 g) was added. The mixture was allowed to shake under hydrogen atmosphere of 2 psi until all protecting groups had been removed as shown by t.l.c. to yield **40**. The suspension was filtered and concentrated *in vacuo*.[6] (0.47 g, 75%); $[\alpha]_D$ 4.33° (*c* 0.03, $CHCl_3$); FTIR (KBr): 3456, 2918, 2849, 1737, 1701, 1468, 1301, 1240, 1187, 1046 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.87 (dd, 1H, $J_{3,4}$ 9.5, $J_{4,5}$ 10, H-4), 4.82 (d, 1H, $J_{1,2}$ 4.0, H-1), 4.23 (dd, 1H, $J_{5,6b}$ 2.0, $J_{6a,6b}$ 12.0, H-6b), 4.12 (dd, 1H, $J_{5,6a}$ 2.0, H-6a), 3.91 (ddd, 1H, H-5), 3.84 (apt t, 1H, $J_{2,3}$ 9.5, H-3), 3.64 (m, 1H, H-2), 3.44 (s, 3H, OMe), 2.37-2.32 (m, 4H, each aliphatic OCOC*H*$_2$C$_{10}$H$_{21}$), 1.68-1.55 (m, 4H, each aliphatic

$OCOCH_2CH_2C_9H_{19}$), 1.30-1.26 (multiple signals, 32 H, each aliphatic $OCOC_2H_4C_8H_{16}CH_3$), 0.88 (t, 6H, *J* 6.5, *J* 7.0, each aliphatic $OCOC_{10}H_{20}CH_3$); ^{13}C NMR ($CDCl_3$): δ 173.63, 173.58 (each s, each C=O), 99.0 (d, C-1), 72.9, 72.7, 70.3, 67.7 (each d), 62.2 (t, C-6), 55.5 (q, OMe), 34.2, 34.1, 34.0, 31.9, 29.63, 29.61, 29.50, 29.47, 29.45, 29.36, 29.30, 29.27, 29.14, 29.08, 24.84, 24.82, 24.70, 22.70 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found, 559.5 required, 559.8; $[M + H]^+$; Anal. Calcd. for $C_{31}H_{58}O_8$: C, 66.63; H, 10.46. Found: C, 66.66; H, 10.79.

Lauroylation of Pentaerythritol:

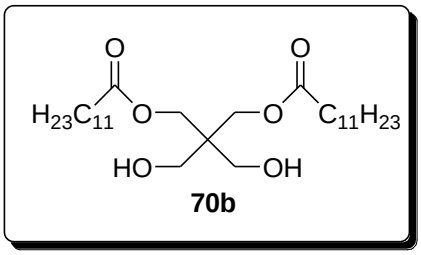


Pentaerythritol **6** (1.0 g, 7.3 mmol), lauroyl chloride (4.8 mL, 21 mmol) and a catalytic amount of 4-dimethylaminopyridine were dissolved in pyridine anhydrous (50 mL) and stirred at 50°C for 24 h. The solution was then concentrated *in vacuo* and the following mono lauroyl **41**, di lauroyl **54** and tetra lauroyl **55** products were isolated by chromatography (petroleum ether-EtOAc):



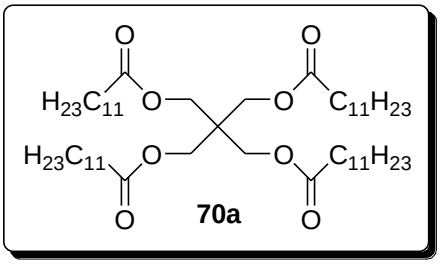
Mono lauroyl pentaerythritol (**41**) - (0.33 g, 14%); FTIR (KBr): 3462, 2914, 2848, 1737, 1712, 1476, 1187, 1038, 1005 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.10 (s,

2H, C*H*$_2$OC=O), 3.80-3.61 (overlapping signals, 9H, 3 x C*H*$_2$OH, 3 x O*H*), 2.34 (t, 2H, *J* 6.0, *J* 7.0, aliphatic OCOC*H*$_2$C$_{10}$H$_{21}$), 1.61 (m, 2H, aliphatic OCOCH$_2$C*H*$_2$C$_9$H$_{19}$), 1.26 (ms, 16H, aliphatic OCOC$_2$H$_4$C$_8$*H*$_{16}$CH$_3$), 0.88 (m, 3H, aliphatic OCOC$_{10}$H$_{20}$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 175.0 (s, C=O), 62.7, 62.4 (each t, each CH_2O), 45.3 (s, $C(CH_2)_4$), 34.2, 31.9, 29.59, 29.57, 29.44, 29.30, 29.23, 29.15, 24.9, 22.6 (each t, each aliphatic CH_2), 14.1 (q, aliphatic CH_3); LRMS: Found 341.2, required 341.45 $[M+Na]^+$; Anal. Calcd. for $C_{17}H_{34}O_5$: C, 64.12; H, 10.76. Found: C, 64.08; H, 10.79.



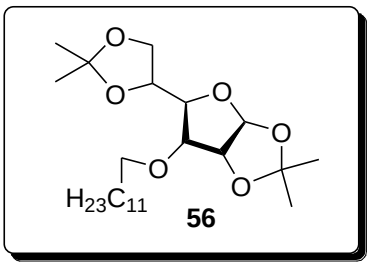
70b

Di lauroyl pentaerythritol (**54**) - (1.074 g, 29%); FTIR (KBr): 3351, 2915, 2850, 1739, 1701, 1471, 1163, 978, 719 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.12 (s, 4H, each C*H*$_2$OC=O), 3.58 (s, 4H, each C*H*$_2$OH), 3.22 (br s, 2H, each O*H*) 2.34 (t, 4H, *J* 7.5, *J* 7.5, each aliphatic OCOC*H*$_2$C$_{10}$H$_{21}$), 1.62 (t, 4H, *J* 6.5, *J* 6.5, each aliphatic OCOCH$_2$C*H*$_2$C$_9$H$_{19}$), 1.29-1.26 (ms, 32H, each aliphatic OCOC$_2$H$_4$C$_8$*H*$_{16}$CH$_3$), 0.88 (t, 6H, *J* 6.5, *J* 6.5, each aliphatic OCOC$_{10}$H$_{20}$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 174.4 (s, each C=O), 62.4 (t, each CH_2O), 44.7 (s, $C(CH_2)_4$), 34.2, 31.9, 29.56, 29.29, 29.21, 29.11, 24.9, 22.6 (each t, each aliphatic CH_2), 14.1 (q, each aliphatic CH_3); LRMS: Found 501.5, required 501.75 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{56}O_6$: C, 69.56; H, 11.27. Found: C, 69.64; H, 11.31.



70a

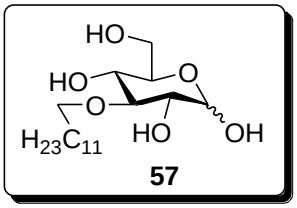
Tetra lauroyl pentaerythritol (**55**) - (0.39 g, 6%); FTIR (KBr): 2917, 2849, 1735, 1336, 1299, 1250, 1154, 1111, 1002 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.11 (s, 8H, each CH_2OC=O), 2.30 (t, 8H, *J* 7.5, *J* 8.0, each aliphatic $OCOCH_2C_{10}H_{21}$), 1.60 (t, 8H, *J* 6.5, *J* 7.0, each aliphatic $OCOCH_2CH_2C_9H_{19}$), 1.41-1.26 (ms, 64H, each aliphatic $OCOC_2H_4C_8H_{16}CH_3$), 0.88 (t, 12H, *J* 6.5, *J* 7.0, each aliphatic $OCOC_{10}H_{20}CH_3$); ^{13}C NMR ($CDCl_3$): δ 173.2 (s, each C=O), 62.1 (t, each CH_2O), 41.8 (s, $C(CH_2)_4$), 34.1, 31.9, 29.59, 29.45, 29.31, 29.23, 29.11, 24.8, 22.7 (each t, each aliphatic CH_2), 14.1 (each q, each aliphatic CH_3); LRMS: Found 888.7, required 888.36 $[M+Na]^+$; Anal. Calcd. for $C_{53}H_{100}O_8$: C, 73.56; H, 11.65. Found: C, 73.60; H, 11.58.



56

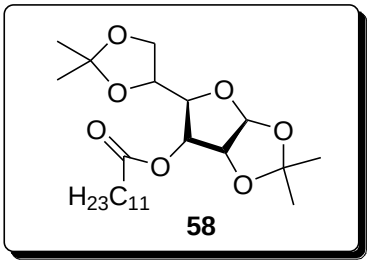
3-*O*-dodecanyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**56**) - Diacetone-D-glucose **32** (1 g, 3.84 mmol) was dissolved in DMF anhydrous (10 mL) and 1-chlorododecane (3.63 ml, 15.35 mmol). The mixture was cooled to 0°C and NaH (0.92 g, 23.04 mmol) was added portion wise. The solution was then warmed to room temperature and stirred for 24 h. The mixture was cooled to 0°C and methanol (10 mL) was added to quench the mixture which was stirred for 1 h.[5] The solution was

concentrated *in vacuo* and purified by chromatography (petroleum ether-EtOAc) to give **56**. (1.43 g, 89 %); $[\alpha]_D$ –21.3º (*c* 0.2, $CHCl_3$); FTIR (KBr): 2927, 2855, 1457, 1371, 1216, 1166, 1074, 1022, 721 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ 5.87 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.42 (dd, 1-H, $J_{2,3}$ 4.0, H-2), 4.13 (dd, $J_{4,5}$ 3.0, 1H, $J_{5,6a}$ 8.0, H-5), 4.08 (dd, 1H, $J_{5,6b}$ 8.5, 1H, $J_{6a,6b}$ 6.0, H-6b), 3.98 (dd, 1H, $J_{3,4}$ 6.0, H-4), 3.85 (d, 1H, H-6a), 3.61 (apt t, 1H, H-3), 3.51 (m, 2H, aliphatic $OCH_2C_{11}H_{23}$), 1.55 (m, 2H, aliphatic $OCH_2CH_2C_{10}H_{21}$), 1.49, 1.42, 1.35, 1.31 (each s, each CH_3), 1.30-1.26 (ms, 18H, aliphatic $OC_2H_4C_9H_{18}CH_3$), 0.88 (t, 3H, *J* 6.5, *J* 7.0, aliphatic $OC_{11}H_{22}CH_3$); ^{13}C NMR ($CDCl_3$): δ 111.8, 106.9 (each s), 105.4 (d, C-1), 82.7, 82.2, 81.3, 72.7 (each d), 70.8 (t, aliphatic CH_2), 67.3 (t, C-6), 32.0, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 26.2, 22.8 (each t, each aliphatic CH_2) 26.9, 26.8, 26.4, 25.5 (each q), 14.2 (q, aliphatic CH_3); LRMS: Found, 451.5 required, 451.6; $[M + Na]^+$; Anal. Calcd. for $C_{39}H_{54}O_9$: C, 67.26; H, 10.35. Found: C, 67.17; H, 10.35.



57

3-*O*-dodecanyl-(α/β)-D-glucopyranoside (**57**) - Amberlite-IR 120 (H^+) (3 g) was added to a suspension of **56** (1.43 g, 3.34 mmol) in H_2O (15 mL). The mixture was then stirred at reflux for 4 h[5] however at this stage t.l.c indicated the presence of several products.



58

3-*O*-lauroyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**58**) - Diacetone-D-glucose **32** (0.5 g, 2 mmol) was dissolved in pyridine anhydrous (25 mL). A catalytic amount of 4-dimethylaminopyridine and lauroyl chloride (0.56 ml, 2.4 mmol) was added and the solution was allowed to stir at room temperature for 24 h.[8] It was then concentrated *in vacuo* and the crude ester derivative was purified by chromatography (petroleum ether-EtOAc) to give **58**. (0.71 g, 79 %); $[\alpha]_D$ –24.2° (*c* 0.03, $CHCl_3$); FTIR (KBr): 2924, 1747, 1457, 1373, 1160, 1022, 943, 722 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ 5.87 (d, 1H, $J_{1,2}$ 4.0, H-1), 5.27 (d, 1H, $J_{3,4}$ 1.0 H-3), 4.47 (d, 1H, $J_{2,3}$ 3.5, H-2), 4.21 (dd, 1H, $J_{4.5}$ 4.0 H-4), 4.11 (m, 2H, H-6a,6b), 4.01 (m, 1H, H-5), 2.36-2.32 (ms, 2H, aliphatic $COCH_2C_{10}H_{21}$), 1.69-1.60 (ms, 2H, aliphatic $COCH_2CH_2C_9H_{19}$), 1.52-1.26 (overlapping signals, 28H, aliphatic $COC_2H_4C_8H_{16}CH_3$, each acetonide CH_3), 0.88 (t, 3H, *J* 7.0, aliphatic $COC_{10}H_{20}CH_3$); ^{13}C NMR ($CDCl_3$): δ 172.4 (s, C=O), 112.3, 109.3 (each s), 105.0 (d, C-1), 83.4, 79.9, 75.8, 72.5 (each d), 67.3 (t, C-6), 34.3, 31.9, 29.7, 29.6 (each t, each aliphatic CH_2), 29.5, 29.3, 29.2, 29.0 (each q), 26.8, 26.7, 26.2, 25.3, 24.9, 22.7 (each t, each aliphatic CH_2); LRMS: Found, 465.2 required, 465.6; $[M + Na]^+$; Anal. Calcd. for $C_{36}H_{46}O_7$: C, 65.13; H, 9.57. Found: C, 65.01; H, 9.63.

CHAPTER 5 - REFERENCES

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LIST OF PUBLICATIONS

Papers:

Synthesis and antimicrobial evaluation of carbohydrate and polyhydroxylated non-carbohydrate fatty acid ester and ether derivatives.

Aoife Smith, Patricia Nobmann, Gary Henehan, Paula Bourke and Julie Dunne. *Carbohydr. Res.* (2008), doi: 10.1016/j.carres.2008.07.012

The antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against Listeria spp. and food spoilage microorganisms.

Patricia Nobmann, Aoife Smith, Julie Dunne, Gary Henehan, Paula Bourke. Submitted to *Int. J. Food Microbiol.* Under review.

Conference Poster Presentations:

Synthesis and Antimicrobial Evaluation of Novel Carbohydrate Fatty Acid Derivatives. Aoife Smith, Patricia Nobmann, Julie Dunne, Paula Bourke and Gary Henehan. A poster presentation at the 60th University Chemistry Research Colloquium, University College Cork, June 2008.

Synthesis and Antimicrobial Evaluation of Novel Carbohydrate Fatty Acid Derivatives. Aoife Smith, Patricia Nobmann, Julie Dunne, Paula Bourke and Gary Henehan. A poster presentation at the 3rd ERA-Chemistry "Flash" Conference, Carbohydrates at the Interfaces of Biology, Medicine and Materials Science, Gleneagle Hotel Killarney, March 2008.

Synthesis and Antimicrobial Evaluation of Novel Carbohydrate Fatty Acid Derivatives. Aoife Smith, Patricia Nobmann, Julie Dunne, Paula Bourke and Gary Henehan. A poster presentation at the 59th University Chemistry Research Colloquium, Dublin City University, June 2007.