DOCTOR OF PHILOSOPHY

Synthesis of Complex Sugar Mycolates of Mycobacterium Tuberculosis

Ali, Omar

Award date:
2017

Awarding institution:
Bangor University

Synthesis of Complex Sugar Mycolates of *Mycobacterium Tuberculosis*

A thesis submitted to Bangor University

for the degree of Doctor of Philosophy

By

Omar Thanoon Ali

2017

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Acknowledgments

I would like to thank my supervisors Professor Mark S. Baird and Dr. Juma'a R. Al-Dulayymi for giving me the opportunity to carry out a PhD under their supervision and for all their excellent advice and support throughout my study. I am extremely grateful to them for their guidance, teaching, valuable discussions and assistance.

I am also grateful to all the staff at the school of chemistry especially my research committee, Dr. Martina Lahmann and Dr. Paddy J. Murphy, for their editing and corrections that continue to benefit me. Also, special thanks to all the technicians David, Gwynfor, Denis, Glyn and Nick and the secretaries Caroline, Tracey, Siobhan and Bryony for always being very helpful.

I would like to thank Prof. Martin Vordermeier, Animal Health and Veterinary Laboratories Agency (AHVLA), Surrey, UK, for making the samples that were known to be infected with bovine TB available.

I am also thankful to Mr. Paul Mason (school of chemistry, Bangor University, UK) for running ELISA assays.

I would like to thank Dr. Andy Chancellor (University of Southampton, UK) for running THP-1 cell assays.

I am also thankful to Dr. Alison Jones for her assistance with the mass spectrometry, and all her help throughout the project.

Many thanks to all my friends on the 10^{th} floor who have helped me throughout this project, especially Dr. Mohsin O. Mohammed.

I would like to thank my country (IRAQ) and the Iraqi Ministry of Higher Education and scientific research (MOHESR)-University of Mosul for sponsoring me.

Finally, I would also like to thank every member of my family in Iraq and Bangor. Special thanks go to my wife Intisar, my daughter Maryam and my son Mustafa, for their support, encouragement and patience.

Abbreviations and acronyms

$[\alpha]_D$	Specific rotation
Ac	Acetyl
Å	Angstrom
AIDS	Acquired immunodeficiency syndrome
AG	Arabinogalactan
Aq	Aqueous
All	Allyl
br	Broad
Bn	Benzyl
Bu	Butyl
Bz	Benzoyl
CAN	Ceric ammonium nitrate
ºC	Degrees Celsius
cm^{-1}	Wavenumbers(s)
COSY	Correlation spectroscopy
δ	Chemical shift
d	Doublet
dt	Doublet of triplet
dd	Double doublet
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DEPT	Distortionless enhancement by polarization transfer
DCs	Dendritic cells
DMAP	4-(*N*,*N*-Dimethylamino)pyridine

DMAG	Di-mycolyl-di-ara*f*-glycerol
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DMTAG	Di-mycolyl-tri-ara*f*-glycerol
ELISA	Enzyme-linked immunosorbent assay
Ether	Diethyl ether
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	Ethyl
equiv	Equivalents
GAM	Glycerol-arabino-mycolates
GMM	Glucose mono-mycolate
GroMM	Glycerol mono-mycolate
h	Hours
HIV	Human immunodeficiency virus
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
IMS	Industrial methylated spirit
I.R.	Infra-red
i-Pr	Isopropyl
ISO	Isopropyl alcohol
J	Coupling constant
LAM	Lipoarabinomannan
$LiAlH_4$	Lithium aluminium hydride
lit.	literature value

LM	Lipomannan
LPS	Lipopolysaccharide
m	Multiplet
M	Molar (moles per liter)
M^+	Parent molecular ion (in MS)
MA	Mycolic acid
MAC	*Mycobacterium avium* complex
mAG	Mycolyl arabino galactan
MALDI	Matrix-assisted laser desorption/ionization
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
MAM	Methyl arabino-mycolates
MDR-TB	Multiple drug resistant tuberculosis
Me	Methyl
MHz	Megahertz
min	Minute(s)
mL	Milliters
mmol	Millimol
m/z	Mass to charge ratio
Mincle	Macrophage-inducible C-type lectin
mol eq.	Molar equivalents
m.p.	Melting Point
MS	Mass spectrometry
MTADM	Methyl tri-ara*f*-di-mycolates

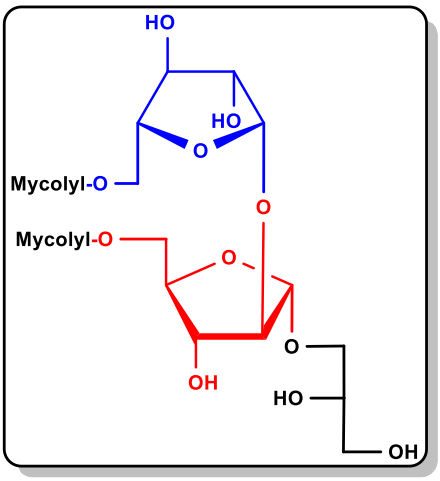
M.tb	*Mycobacterium tuberculosis*
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
NSI	Nano-electrospray Ionization
Petrol	Petroleum spirit (boiling point 40 to 60 ^{o}C)
PG	Peptidoglycan
Ph	Phenyl
PIMs	Phosphatidylinositol mannosides
PMB	*p*-Methoxybenzyl
ppm	Parts per million
Pyr	Pyridine
q	Quartet
R_f	Retention factor
r.t	Room temperature
sat.	Saturated
s	Singlet
S_N2	Nucleophilic substitution, bimolecular
Sensitivity	Probability (%) of being test positive when disease present
Specificity	Probability (%) of being test negative when disease absent
STol	4-Methylbenzenethiol
t	Triplet
T-cells	T Lymphocytes
TB	Tuberculosis
TBAF	Tetrabutylammonium fluoride

TBAI	Tetrabutylammonium iodide
TBDMSCl	Tetrabutyldimethylsilyl chloride
TBDPSCl	Tetrabutyldiphenylsilyl chloridc
TDM	Trehalose dimycolate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TIPDS	1,3-(1,1,3,3)-Tetraisopropyldisiloxanylidene
TLC	Thin layer chromatography
TMM	Trehalose monomycolate
TMS	Tetramethylsilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNF-α	Tumor necrosis factor-α
Ts	*p*-Toluene sulfonyl
Trityl	Triphenylmethyl
p-TsCl	*p*-Toluene sulfonyl chloride
p-TsOH	*p*-Toluene sulfonic acid
VLA	Veterinary laboratories agency
WHO	World health organisation
XDR-TB	Extensively drug resistant tuberculosis

Abstract

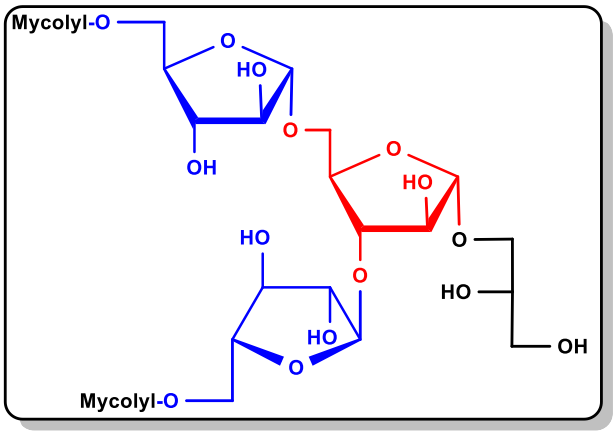
Mycobacteria are present in many environments and complex mixtures of sugar esters and mycolic acids are present in their cell wall structure. This complicated mixture is thought to be responsible for their high resistance to known antibiotics and chemotherapeutic treatments. Mycolic acids are high molecular weight α-alkyl-branched β-hydroxy long-chain fatty acids, have 60-90 carbon atoms, and various classes of mycolic acids are made by different species of mycobacteria. Sugar esters of mycolic acids associated with the cell wall of mycobacteria have very interesting toxic and immunological properties, and thus could be useful for the control and treatment of mycobacterial infections. The main objectives of this thesis will be discussed in three parts.

The main target of **the first part** involved the first synthesis of a single enantiomer of the glycolipid di-mycolyl di-arabino glycerol **(DMAG) (I)**, which has interesting toxicological and immunological properties. This was achieved by a successful synthesis of the glycan moiety of DMAG with the L-stereochemistry of the glycerol component, followed by the successful esterification of the glycan di-arabino glycerol with three normal fatty acids, a model mycolic acid, and five different mycolic acids. The NMR spectra of the synthetic isomer of the DMAG penta-acetate analogue, in the sugar region, matched very well those reported for the peracetate formed from the natural mixtures, confirming the stereochemistry of the arabinose units and establishing the absolute stereochemistry of the glycerol unit. An efficient route to prepare the DMAG glycan with excellent β-selectivity and in excellent yield was achieved.



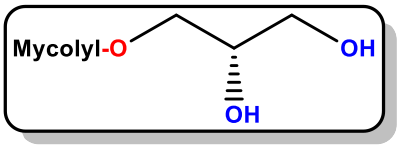
(I)

The second part entailed the first preparation of di-mycolyl tri-arabino glycerol **(DMTAG) (II)**, which involved the synthesis of the donor moiety part according to literature methods with slight modifications, and the new arabino glycerol acceptor. The coupling of the donor and the acceptor to prepare the desired glycan was carried out using known coupling conditions. After the success in synthesising this tetra-saccharide, a model glycolipid was prepared through esterification with a normal fatty acid. Furthermore, a series of three DMTAG compounds were prepared, based on two common classes of mycolic acids.



(II)

The final part of this project was the synthesis of glycerol mycolates **(GroMM) (III)**, which have interesting adjuvant properties in vaccines, by coupling five common classes of synthetic mycolic acids with the *S*-glycerol stereoisomer. One model GroMM was prepared from a simple fatty acid. These compounds were prepared to study whether the stereochemistry of the glycerol component (*R* & *S*) has any effect on their biological activities.



(III)

Initial studies of the biological activity of the synthetic DMAGs showed that some could be used to distinguish serum from cattle infected with bovine TB from uninfected cattle, and that they selectively activate THP-1 cells. In contrast, initial ELISA results with the synthetic GroMM showed little response to serum from patients with active TB.

Chapter 1

Introduction

Chapter 1

Introduction

Mycolyl-arabinogalactan complex (mAG) is the major component in the cell wall of mycobacteria, and acts as a permeability barrier that prevents the passage of known antibiotics. Therefore, blocking the biosynthesis of this component is an important strategy for developing new anti-tuberculosis (anti-TB) medications. This thesis reports the synthesis of three different complex sugar mycolates of the *Mycobacterium tuberculosis (M.tb)* cell wall, which allows their biological activity to be investigated. The following introduction will provide some background information on tuberculosis and some related topics.

1.1 Tuberculosis

Tuberculosis (TB) is a contagious bacterial disease caused by an infection with a type of bacterium and several closely related mycobacterial species belonging to the so-called *M.tb* complex. The origin of this infection is not clearly known but it is believed to have originated in cattle and then transferred to human beings. Overcoming the immune system defences by the TB bacilli and the start of reproduction changes TB from infection to disease.[1,2] The lung is the most common place of infection in the body, however, any other organ can be infected, such as the lymph nodes, kidneys and central nervous system. The bacteria of TB are spread easily in the air by talking, coughing, breathing or exchange of blood fluids, and the symptoms, which may not appear immediately, are fever, loss of appetite, weight loss, chest pain and prolonged coughing, night sweats, swollen glands and no response to antibiotics.[3,4,5]

TB has been found in humans from the early dawn of history, and is believed to date back more than one hundred and fifty million years.[6,7] It is known by several names, for instance: 'The king of diseases' in India, 'The captain of all these men of death',[8] and 'phthisis' by the Greek physician Hippocrates.[9] The earliest specific discoveries of TB were in the remains of a bison from 18000 years ago.[10] Studies have demonstrated the presence of TB in Egyptian mummies over 5000 years old, and have also shown signs of death as a result of TB.[11] There is evidence which shows the presence of TB in China 2300 years ago and in India 3300 years ago.[12,13] It is believed that TB in the North and South America was present before the arrival of European explorers, with a similarity to that found in Egypt.[14,15] TB was well documented in ancient Greece and its treatment was devised by the physician Clarissimus Galen as sea voyages, fresh air and milk.[16]

In 1720, Marten first suggested that TB was caused by a microscopic air-bound organism.[17] Regrettably, his findings were ignored until 1865 when surgeon Jean-Antoine Villemin, demonstrated the nature of TB transmission from humans to rabbits by injecting a rabbit with liquid from the lung of a patient who had died from infection with TB, and so confirmed the theory proposed by Marten that TB was a contagious disease.[18] In 1882 Robert Koch, who was awarded the Nobel Prize in Medicine and Physiology in 1905 for his work on tuberculosis, first described the causative agent of TB when he discovered a stain which enabled the bacilli of *M.tb* to be seen.[19,20,21] Since Koch's discovery, and due to the complexity of TB disease, a reliable vaccine was not developed until 1921, when Albert Calmette and Camelle Guérin developed a vaccine from *Mycobacterium Bovis (M.bovis)* called Bacillus Calmette-Guérin (BCG).[22] The BCG vaccine became more widespread in the 1940's, being used in Scandinavia, France, Spain, Russia, Germany, Latin America and some Eastern European countries.[23,24] Currently, more than 115 million units of BCG as a freeze-dried form are dispersed annually in 172 countries on average.[25]

In the 18th and 19th centuries, TB spread over Asia, Africa, South America and Europe, and approximately one fourth of all deaths in the world was caused by TB.[26] In Europe it was responsible for 25% of adult deaths in major cities.[27] Population intensity, poor living standards and a shortage in health services led to an increase in TB infection and led to it being considered a public health problem.[28] In the 20th century, the disease was slowly controlled as living conditions and health care improved, and several medicines were discovered, such as streptomycin in 1944, and isoniazid in 1952, which are now used to treat TB.[27]

In 1990, a significant rise in the number of deaths of TB patients was reported, in contrast to the number of deaths due to other illnesses. In 1993, the World Health Organization (WHO) announced TB as a major health problem and declared a global health emergency.[29] According to WHO, TB is among the top ten causes of death and disability globally. Between 2013-2015, new incidents of TB increased, with around 10.4 million new cases of TB being recorded worldwide in 2015 leading to 1.8 million deaths, with most being concentrated in six countries, South Africa, China, India, Nigeria, Indonesia and Pakistan.[30,31] **Figure 1** shows the estimated number of new cases of TB per 100,000 population in 2016.[31]

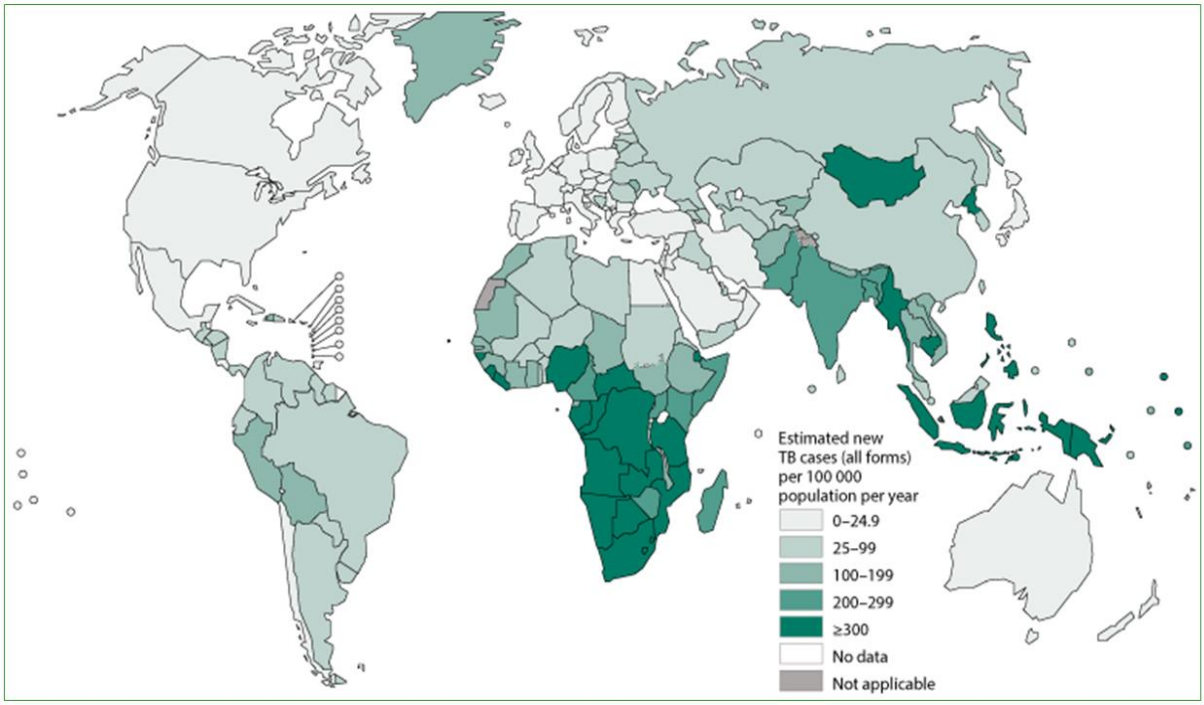


Figure 1: Map showing the estimated number of new TB cases in 2016.[31]

Infection with TB can be active or latent, depending on the immune system response of the host. In the case of a strong immune response, the bacilli are killed after being inhaled into the lung, and no symptoms of TB infection will appear. However, when the immune system becomes weak for any reason, the bacteria will start to spread through the blood, and then the infection will become serious. An individual infected with active TB will produce small droplets containing *M.tb***,** which are spread as aerosolised drops when an infected patient sneezes.[32,33]

The continuing occurrence of TB remains globally uncontrolled, due to the evolution of strains highly resistant to drug treatment and a higher incidence of human immunodeficiency virus (HIV) co-infection. TB/HIV together produce a fatal combination, each increasing the progression rate of the other disease, with HIV infection being one of the major conditions that make people more vulnerable to developing active TB. HIV has contributed to a significant rise in the global rate of TB by weakening the individual's immune system. The risk of developing active TB for individuals infected with HIV is up to 30 times greater than for uninfected persons.[31] Although HIV and TB are both preventable and treatable, they continue to increase in developing countries in which TB infection and HIV are prevalent and resources are limited.[31,34,35] Similarly, TB may also destructively affect the natural progress of HIV infection.[36,37] The effect of TB on HIV illness development is assumed to be assignable to increased immune system activation.[38] The WHO estimates that nearly one third of the world's population is currently infected with *M.tb* and about 22% of these are believed to be co-infected with HIV **Figure 2**.[31,39]

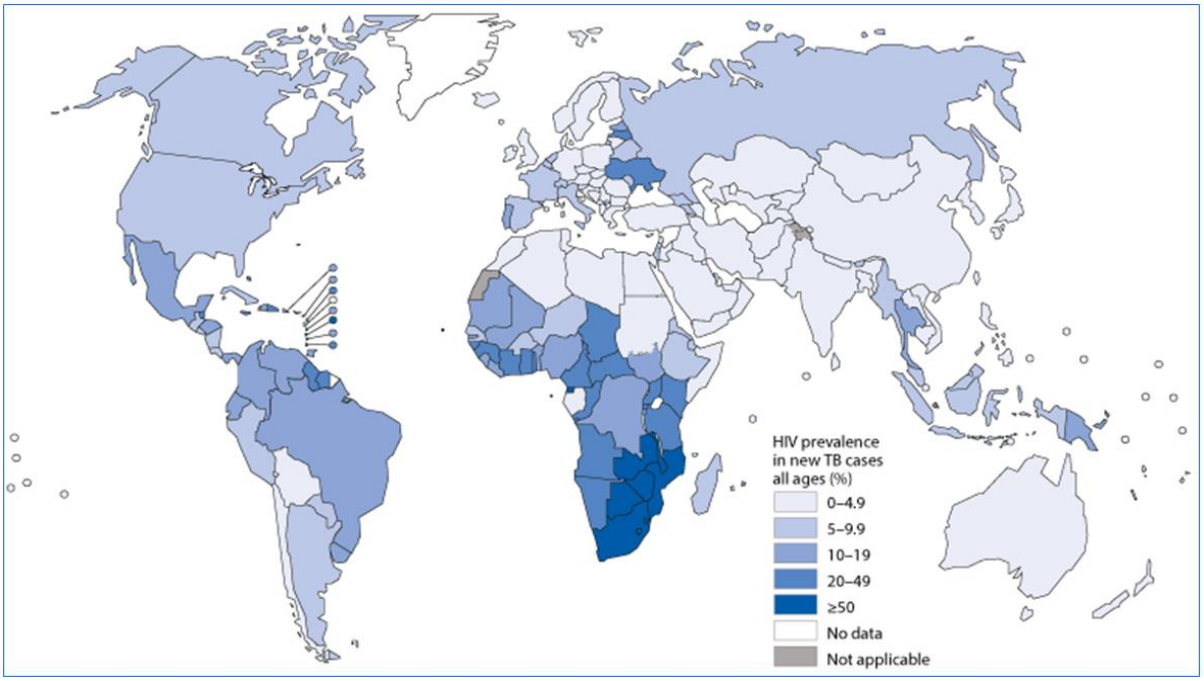


Figure 2: Map showing the estimated number of TB patients co-infected with HIV in 2016.[31]

The treatment of TB is difficult and involves long courses of multiple antibiotics.[40,41] In addition to an increase in the number of people infected with TB since 1980, there has also been a large increase in the number of drug-resistant tuberculosis cases. The emergence of multi drug resistant tuberculosis (MDR-TB) is another factor which contributes to the failure of controlling TB. Strains of *M.tb* showing resistance to the anti-TB drug streptomycin developed during early attempts to treat TB.[42] Nowadays, strains of *M.tb* showing resistance to other anti-TB drugs have developed. When a strain of TB is found to be resistant to two or more front line drugs like Isoniazid, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin, it is considered to be multiply drug resistant MDR.[43] The WHO estimated that there were about 480,000 new cases of MDR-TB in 2015 and their last report estimated that 3.5% of new cases and 20% of previously treated cases are multidrug-resistant.[31,44,45] More recently, extensively drug-resistant tuberculosis (XDR-TB) cases have emerged where the *M.tb* is resistant to Rifampicin, Isoniazid, a Fluoroquinolone and a seconD-line injectable drug (Capreomycin, Amikacin). This type of resistance was reported for the first time in KwaZulu-Natal in South Africa.[46] In 2008, 963 infections of XDR-TB were reported,[45] while in 2015 around 45600 people with MDR-TB had XDR-TB globally.[30] Resistance of *M.tb* to the treatment makes this disease more problematic and an estimated budget of approximately $2 billion was promised in 2016 in order to address and control, by diagnosis and treatment, the serious problem of TB.[30]

1.2 Mycobacteria

Mycobacteria are a genus of bacteria which can cause fatal diseases in both humans and animals. There are over 150 known species, which can be divided into two classes, tuberculosis mycobacteria and non-tuberculosis mycobacteria.[47,48] They include both pathogenic and non-pathogenic species.[49,50] Species of pathogenic mycobacteria causing TB in mammals are *M.tb*, *Mycobacterium bovis* (which is responsible for causing bovine TB), *Mycobacterium africanum* (a heterogenous group of strains isolated from equatorial African inhabitants) and *Mycobacterium microti* (a rodent pathogen).[51] Other mycobacterial species causing disease in man are *Mycobacterium leprae* (which causes leprosy), *Mycobacterium ulcerans* (which is responsible for the dangerous and potentially fatal Buruli ulcer, a skin and sometimes bone infection).[52,53] Other pathogens include *Mycobacterium marinum* (which causes disease in fish and skin infections in humans) and *Mycobacterium avium* (an illness of poultry first discovered in 1890, also known as Battery bacillus).[54,55] *M. bovis* has the most diverse range of hosts, as it is found not just in bovine animals but also in man, dogs, cats, pigs, goats and wild animals such as deer.[51] Nevertheless, unlike *M. tb*, *M. bovis*, *M. microti*, *Mycobacterium kansasii* and *Mycobacterium smegmatis*, a large number of other mycobacterial strains only affect individuals whose immune system is suppressed, for example HIV/AIDS sufferers or transplant patients.[54,55]

1.2.1 Non-Tuberculosis Mycobacteria

Non-tuberculosis mycobacteria, also known as environmental mycobacteria, are small, rod shaped bacilli which enter the human body through environmental sources such as natural water, soils, foods, and water pipes. The reason for the survival of these bacteria in water pipes is due to their resistance to chlorine in water.[56] These species do not cause tuberculosis, but have the ability to cause other diseases in both humans and animals such as skin diseases, disseminated disease (a diffuse disease-process, either infectious or neoplastic, throughout the body over a considerable area), and pulmonary disease in HIV negative patients.[48] Unlike tuberculosis, non-tuberculosis mycobacteria are not transmitted from one person to another, the organism being acquired exclusively from environmental sources.[57]

1.2.2 Tuberculosis Mycobacteria

The tuberculosis mycobacteria constitute a collection that contains more than 70 different species, together known as *Mycobacterium tuberculosis (M.tb)* complex, which can all cause tuberculosis disease in both humans and animals.[58] Some species cause TB in humans only, such as *M.tb*, while some, such as *M. bovis,* can cause TB in both humans and animals. [51,59,60] The most common in the family of tuberculosis mycobacteria is *M.tb*, which was isolated and identified for the first time by the German physician, Robert Koch, in 1882. It is highly aerobic (grows most successfully in tissues with oxygen content, such as the lungs), appears under the microscope as straight or slightly curved rods approximately 1-4 x 0.3-0.6 μm in size **(Figure 3)**, non-encapsulated, non-spore forming, aciD-fast (do not retain the methyl violet stain well), weak Gram-positive bacilli.[61] However, it was reported recently that they have features of both Gram-positive and Gram-negative bacteria (Gram-positive bacteria have a greater amount of peptidoglycan, lower lipid content and they retain the gram's stain, while, Gram-negative bacteria have a thin peptidoglycan cell wall and they do not retain the gram's stain), divides aerobically every 16 to 20 hours,[8,62,63,64] an extremely slow rate compared with other bacteria, such as *Escherichia coli* that can divide roughly every 20 minutes.[65] *M. tuberculosis* is very resistant to environmental conditions, and can survive in dry conditions for a long time; this is due to its unique cell wall structure compared with the cell walls of other bacteria. The cell wall is rich in mycolic acids (MAs) and glycolipids, which are crucial for the survival and growth of *M. tuberculosis* inside the infected organism.[66]

Figure 3: Scanning electron micrograph of *M. tuberculosis*.[67]

1.3 The Mycobacterial Cell Wall

It is important to have an understanding about the nature, the organisation and the structure of the cell envelope in the fight against TB and other related diseases. The cell wall of *M.tb* has an unusual structure. The thick, multi-layered and extremely hydrophobic structure of the cell wall, is important for the organism, because it prevents the passage of antibiotics into the cell and protects it from the immune system of the host, by allowing it to survive in macrophages, acting as a permeability barrier. A high resistance to the majority of antibiotics, therapeutic agents and disinfectants is a big problem in the eradication of mycobacterial organisms, and this resistance is thought to be related to the unique structure of the mycobacterial cell wall. The general structure of the mycobacterial cell wall is now well understood, and it was basically proposed by Minnikin with its complex architecture of lipids, glycolipids, polysaccharides and proteins.[68,69,70] Generally, the *M.tb* cell wall consists of the significant components shown in **Figure 4**, namely, the plasma membrane (PM) (inner membrane), peptidoglycan (PG), mycolyl-arabinogalactan (mAG), and an outer capsule-like layer.[71,72,73]

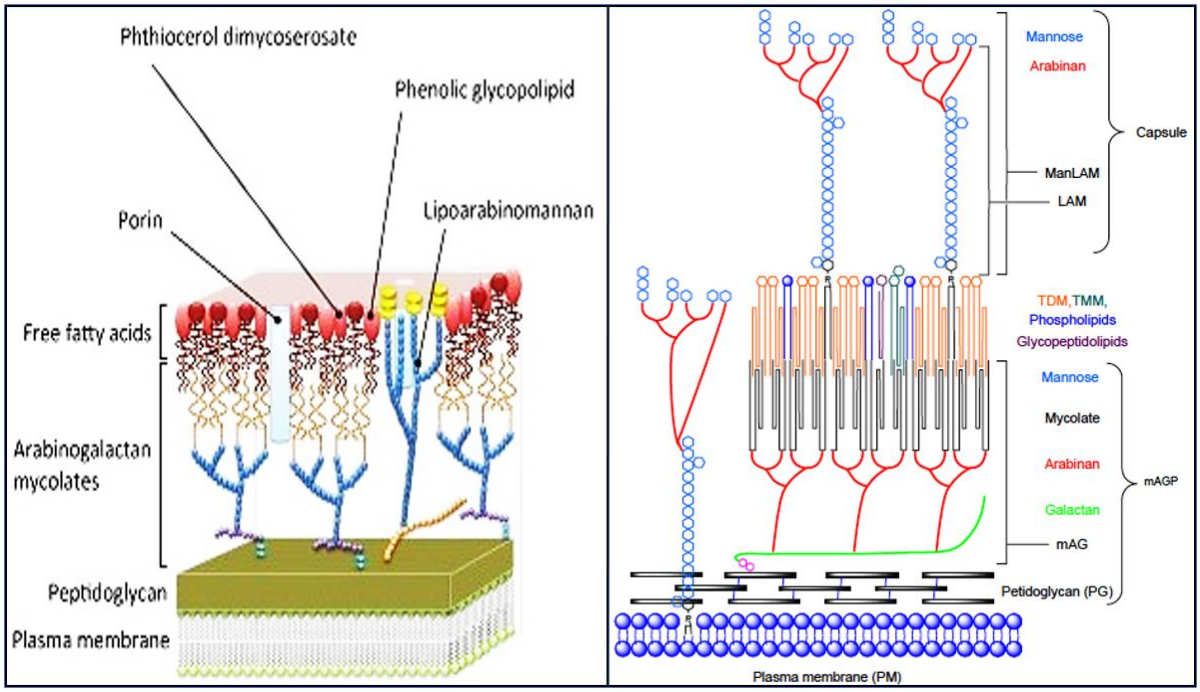


Figure 4: General structure of *M. tuberculosis* cell wall.[74,75]

The main components of the capsule outer layer are polysaccharides and proteins with low amounts of lipids.[76] The plasma membrane is about 5 nm thick, and its composition is similar to that in other organisms. The PG is a polymer that forms the backbone of the cell wall skeleton, consisting of *N*-acetylglucosamine (NAG), and *N*-acetylated muramic acid (NAM)

saccharides.[77,78] The mAG, is a heteropolysaccharide consisting of D-arabinose in the furanose form joined to linear D-galactose units.[79,80] Mycolic acids (MAs), are α-alkyl branched β-hydroxy long chain fatty acids (60-90 carbon atoms, see section 1.4). Together, mycolic acids, arabinogalactan and peptidoglycan produce the tightly packed bilayer of the *M. tb* cell wall and are known as the mycoyl-arabino-galactan-peptido-glycan complex (mAPG).[79] Moreover, though the main component of the cell wall is mycolic acids, there are also large amounts of very complicated lipids amounting to around 40% dry weight of the cell wall. These lipids are highly complex and are thought to be responsible for the high resistance and low permeability of the cell wall to hydrophilic compounds. In addition, there are many glycolipids present in the cell wall of mycobacteria, for example di-mycolyl di-arabino-glycerol ester (DMAG ester), which is di-arabino-glycerol esterified with mycolic acids, has been reported to have interesting toxicological and immunological properties.[81,82]

1.3.1 Mycolyl-arabinogalactan complex

The mycolyl-arabino-galactan complex (mAG) **(Figure 5)**, is the largest component in the cell wall of mycobacteria and is located directly outside the PG layer. It is believed that the mAG complex acts as a permeability barrier that prevents the passage of antibiotics. It forms from cross bonding between both α-D-arabinofuranose (α-D-Ara*f*) and β-D-galactofuranose (β-D-Gal*f*) esterified with mycolic acid (long chain α-alkyl branched β-hydroxylated fatty acid). Carbohydrates (Ara*f*) and (Gal*f*), creating about 35% of the cell wall mass, are bound to NAG residues of PG through a covalent bond at the non-reducing end of the wall by a unique linker disaccharide, α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosylphosphate. The galactan part is a linear chain of around 30 - 40 units of (β-D-Gal*f*) with alternating β-(1→5) and β-(1→6) galactofuranose residues. The arabinan unit is composed of 60 - 70 units of linear (1→5) (α-D-Ara*f*) residues and branches to form a (3,5-α-D-Ara*f*) linked fork.[83,84,85,86] Galactan and arabinan are bonded from the C-5 position in the galactan core.[79,74,87,88,89] The arabinose motif of the cell wall contains 1,3-branched Ara*f*-based mycolated hexasaccharides, via ester linkages at each of the four primary hydroxyl groups to form the mycolyl-arabinan moiety.[72,90]

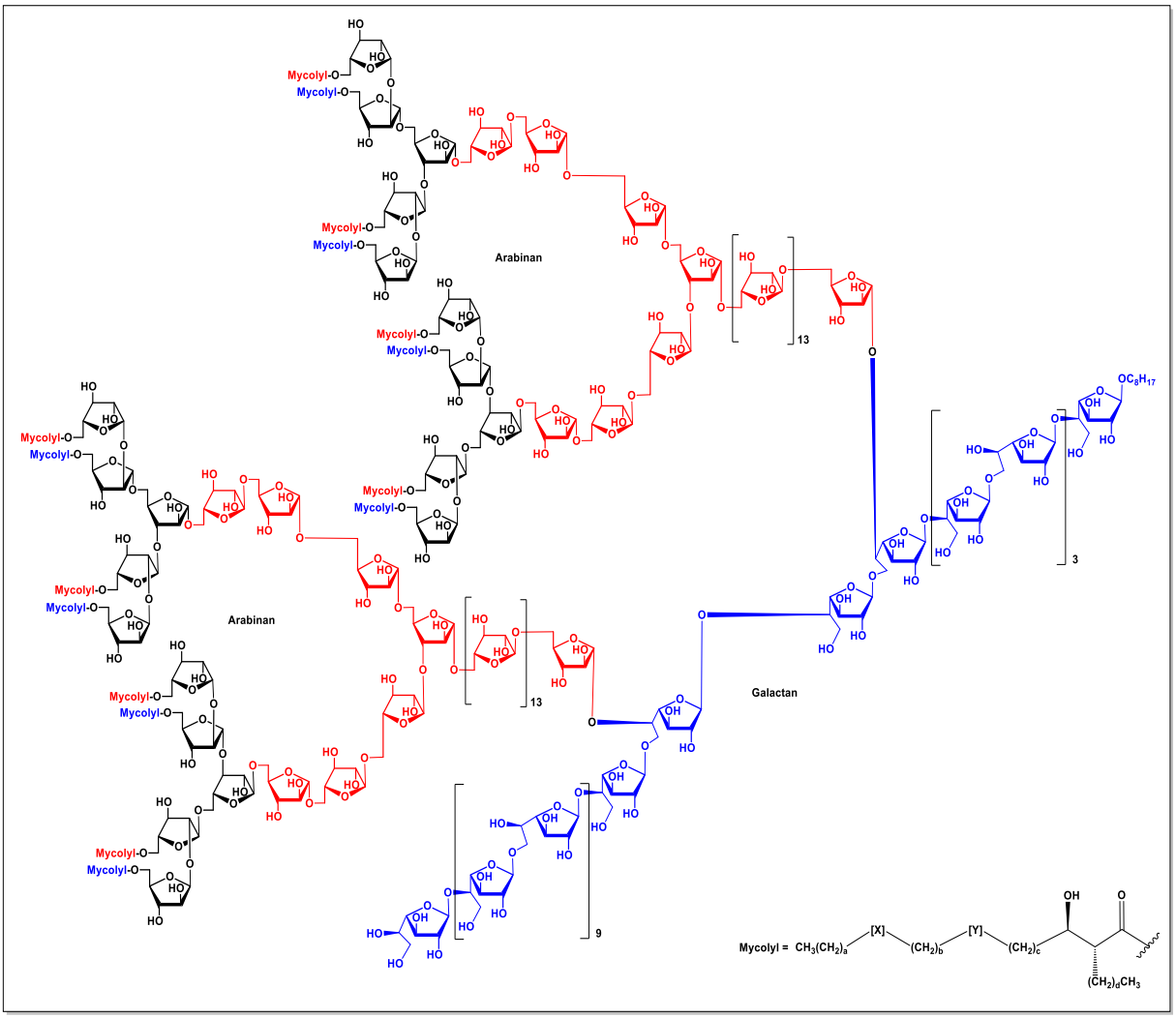


Figure 5: Structure of the mAG complex.

Both galactosyl and arabinosyl units in the mAG complex are in the furanose form which is less thermodynamically stable than the pyranose form (the six-membered pyranose ring is thermodynamically more stable than the five-membered furanose ring, because the six membered ring can be in the chair form in which there is no angle or eclipse strain).[91] It is believed that this plays an important role in raising the flexibility of the polysaccharide and making the MAs pack strongly by van der Waals interactions. Thus, the structure of the cell wall has extremely low permeability, which provides the organism with high protection from drugs and from its environment. On the other hand, given its importance to the life cycle of the organisms, mycobacteria must produce a complete mAG complex.[92] As mentioned previously, targeting mAG biosynthesis is therefore an important strategy for developing new anti-TB drugs. Indeed, isoniazid and ethambutol, two of the standard antibiotics, target the mAG complex biosynthesis; ethambutol inhibits arabinosyltransferases which contribute to the biosynthesis of the arabinan part of the polysaccharides, while isoniazid inhibits MA biosynthesis.[93]

1.4 Mycolic Acids

Mycolic acids (MAs) are high molecular weight (60-90 carbon atoms) hydrophobic fatty acids alkylated at the α-position and hydroxylated at the β-position and can be isolated from the waxy extract of *M.tb*.[94] Mycolic acids are unique to mycobacteria, and are considered as one of the main and characteristic components of the cell wall of all the mycobacterial species. In 1938, Anderson *et al.* first reported and isolated mycolic acids as unsaponifable ether-soluble hydroxy acids from the human tubercle bacillus. Mycolic acid is very difficult to purify and not possible to crystallize.[95,96] The first mycolic acid structures were published by Minnikin *et al* in 1967.[97,98,99,100] Mycolic acids exist in the mycobacterial cell wall in the free form or esterified with other lipids, such as trehalose mono-mycolates (TMM) and trehalose di-mycolates (TDM), glucose mono-mycolate (GMM), glycerol mono-mycolate (GroMM), and di-mycolyl di-arabino glycerol (DMAG).

The structure of mycolic acids can be divided in to two parts. The main part is called the meromycolate moiety and the second part is called the mycolic motif. The mycolic motif part contains the α-alkyl β-hydroxy fatty acid, which is similar in all mycolic acids except for a slight variation in the chain length at the α-alkyl position. The main part is the meromycolate moiety, which normally has two intra-chain functional groups at the distal and proximal positions labelled as [X] and [Y] **(Figure 6)**. The proximal position can be a *cis* or *trans* cyclopropane, or double bonds, while the distal position can be a *cis* or *trans* cyclopropane, *cis* or *trans* double bond, epoxy group, methoxy group, carbonyl group, hydroxyl group or ester group. Due to the possible variations in the functional groups that could be present in a certain mycolic acid, Watanabe *et al.* proposed a broad classification method to divide the mycolic acids into three types: Types 1, 2 and 3 as shown in **(Figure 6)**. Type 1 MAs have a cyclopropane ring at the proximal position (which can be either *cis* or *trans*), type 2 MAs have a *trans* double bond at the proximal position, while type 3 MAs contain a *cis* double bond at the proximal position.[101,102]

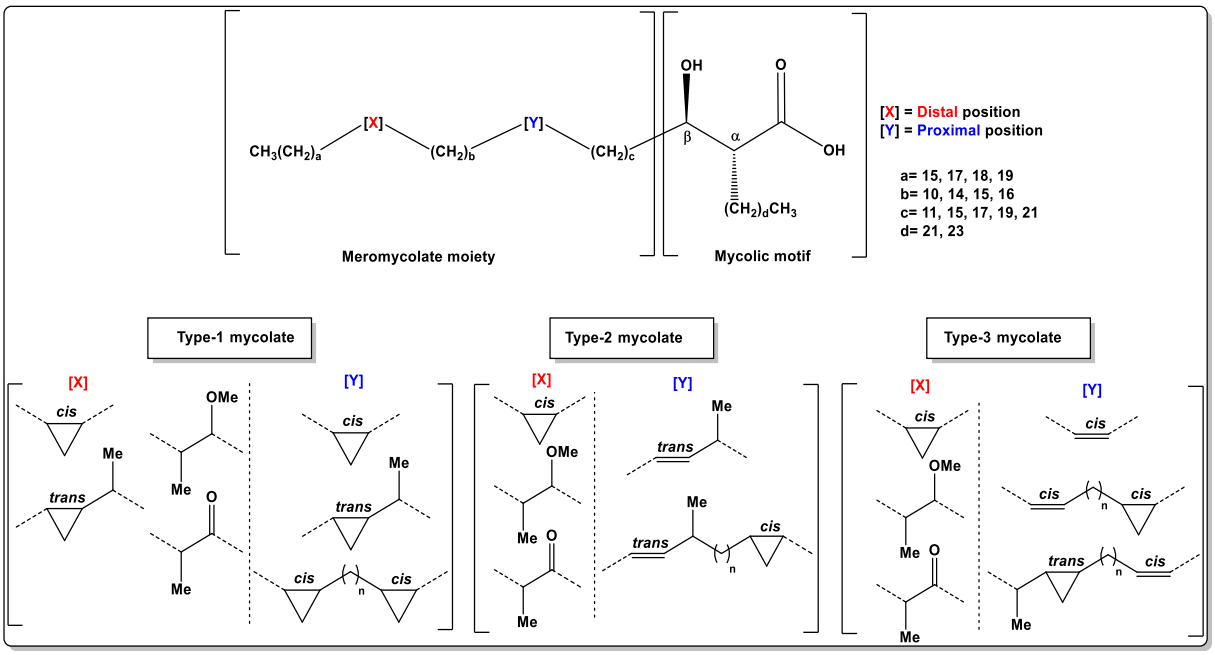
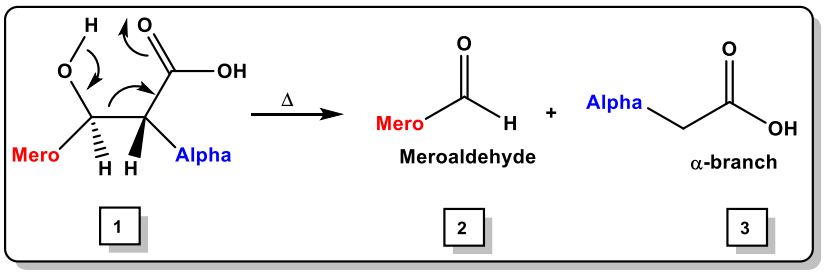


Figure 6: General structure of mycolic acids and various functional groups.[101,102]

Asselineau *et al.* reported the pyrolysis of mycolic acid (MA) **(1)**, and confirmed the positions of the hydroxyl group (β) and a long alkyl chain (α) in relation to the carboxylic acid **(Scheme1)**.[103]



Scheme 1: Thermal cleavage of the β-hydroxy group of MA.[94]

Mycolic acids (MAs) have a role in the permeability of the outer cell envelope of the bacteria, but the stacking and arrangement of the long hydrocarbon chains of the acids within the cell wall is complicated.[94,101] The cell wall of *M.tb* is believed to contain a mixture of over 500 different mycolic acids with a varying combination of functional group type and chain length. These most important components in the cell wall with this large number of different structures have significant biological properties.[94] The isolation of mycolic acids and separation from the mixture of various similar structures in the cell wall was the main problem in identifying their individual structures. For this reason, highly developed analytical techniques were required in order to determine the correct structures of these series of mycolic acids. Over the last fifty years, by using new analytical techniques such as TLC,

HPLC, GC, MS, and NMR, it has been less difficult to separate and identify several mycolic acids.[104,105,106,107,108]

Based on the nature of the functional groups present in the meromycolate chains, mycolic acids from *M.tb* can be divided into three main categories:

1. Alpha-mycolic acids with no oxygen-containing intra-chain groups.
2. Methoxy-mycolic acids in which the distal group has a methoxy group.
3. Keto-mycolic acids in which the distal group has a carbonyl group (with a *cis* or *trans* cyclopropane).

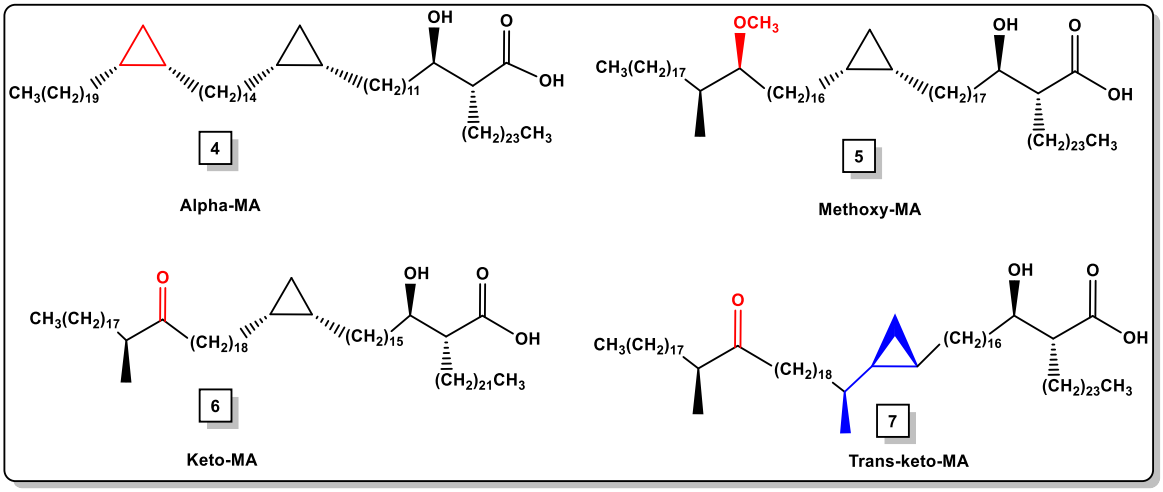


Figure 7: Major types of mycolic acids from *M. tuberculosis*.

In 2003, Al-Dulayymi *et al*. reported the synthesis of a single enantiomer of an alpha mycolic acid from *M. tuberculosis*.[109] Various types of single enantiomers of mycolic acids have also been prepared by the same group such as methoxy,[110] keto[111,112] and alpha-mycolic acids[113] which are major mycolic acids present in *M. tuberculosis*.[114]

As discussed before MAs are often present in the cell wall esterified to sugars. The different types of sugar esters with different glycolipid linkages are described in the following sections.

1.5 Synthesis and control the stereochemistry of different sugars

1.5.1 Carbohydrates

Carbohydrates are the most abundant and diverse bio-molecules in nature. They were named as glycans or saccharides (Greek, meaning sugar). Virtually all important biomolecules have a glycan in their structures, *e.g.* secondary metabolites, t-RNA, lipids and proteins.[115] Initially, the function of carbohydrates was known as a source of energy; however, it has since been proven that they play an essential role in many biological progressions, for instance growth, development and the survival of living organisms.[116] Although carbohydrates have significant characteristics in biological systems, due to their complex forms, their functions and structures are still less understood in comparison with other biological molecules like proteins and nucleotides.[117] Carbohydrates often contain a glycosidic bond in their structures, which is in either an α- or β-configuration, and this bond is created when two glycan units are bound to form a disaccharide. In nature, this stereochemistry plays a significant role in biological activity. Furthermore, each glycan unit contains many hydroxyl groups which can also react with another molecule to produce oligosaccharides, which can be linear or branched macromolecules. In addition to that, the hydroxyl groups in glycans can be modified through different reactions such as esterification, oxidation and methylation.[118] Monosaccharides can adopt various forms due to free rotation around the glycosidic bond, therefore monosaccharides have a heterogeneous conformation. In addition, in oligosaccharides there is an internal rotation around exocyclic bonds, such as the primary hydroxyl groups in most glycan forms. The saccharide molecule itself can adopt different ring forms such as furanose rings. In conclusion carbohydrates can possess complex branched and modified forms, and they are more complicated compared with the two other main classes of molecules, nucleotides and proteins.[119,120]

1.5.2 Carbohydrate Conformations

The overall structure of an oligosaccharide is determined by many factors such as, the stereochemistry of the glycosidic bond (α or β-anomer); pseudo-rotation of the furanose rings; stereoelectronic effects and the conformation of the attached groups in the glycan ring. The stereochemistry of the glycosidic bond is important for the biological functionality of the oligosaccharide.[118] The main conformations of the furanose ring are: **envelope (E) (8)**, in this form, four adjacent atoms are all in one plane and only one atom is out of the plane (above or below) and

twist (T) (9), in this form, two atoms are outside the plane and the other three atoms are in the plane **(Figure 8)**.

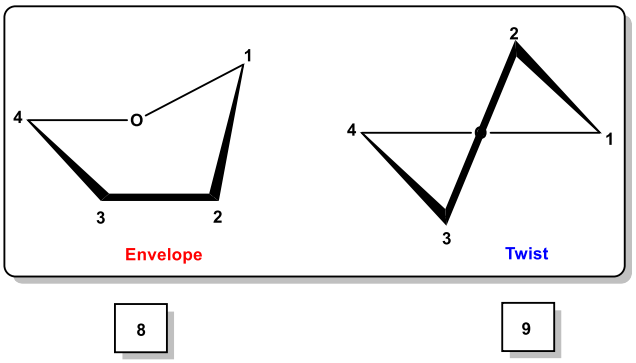


Figure 8: Envelope and twist conformations in furanose rings.

The energy difference between the E and T conformations in the case of the mono-saccharide is small, therefore the furanose is present in a dynamic equilibrium through a pseudorotation. Consequently, due to the flexibility of the furanose ring, the exocyclic methyl hydroxy link and the flexibility of the glycosidic bond, there are twenty different conformations, ten different twist conformations and ten different envelope conformations. These conformations are represented on the pseudorotational wheel for the D-aldofuranose ring **(Figure 9)**.[121]

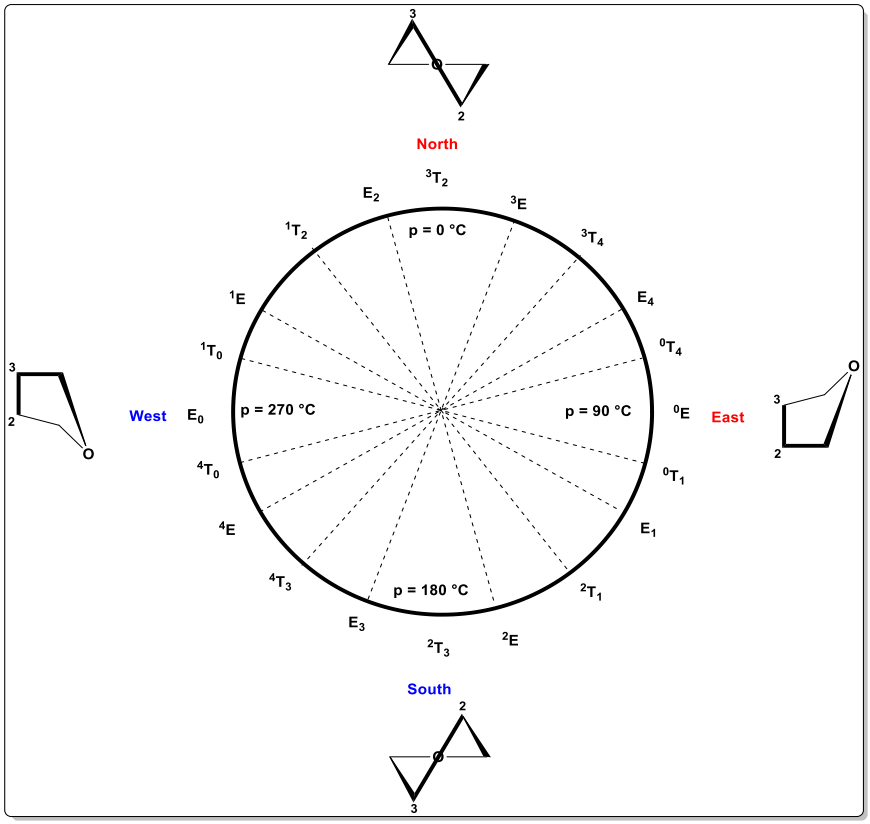


Figure 9: Pseudorotational wheel for a-D-aldofuranose ring.[121]

In solution, furanose rings are present as a mixture of conformers, therefore, analysis of NMR data such as measuring the $^{3}J_{H\text{-}H}$ coupling constant or the chemical shifts is more complicated because all the data is an average from more than one conformation.[122]

Stereoelectronic effects (defined as the kinetic and chemical consequences of molecular orbital overlapping in space) are other factors which have an effect on the conformation of the oligosaccharide. According to molecular orbital theory, the total energy of any molecule is equal to the summation of the occupied molecular orbitals. Different reactivities and conformations are obtained due to the overlap between occupied and unoccupied orbitals because this overlap causes a change in energy (lower energy). In carbohydrate chemistry, this effect is known as the anomeric effect, which was first proved by Jungins in 1905 and revived by Edward in 1955 and by Lemieux and Chiu in 1958. They showed the predominance of alkyl α-D-glucopyranosides **(11)** compared to the corresponding β-anomers of this compound **(Figure 10)**.[123,124]

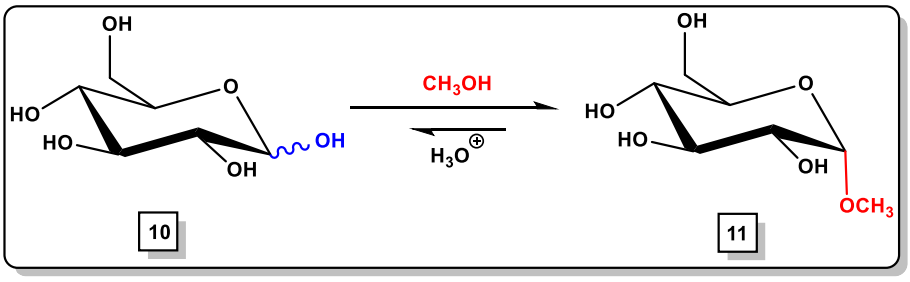


Figure 10: Glycosylation of D-glucose.

Further studies on the anomeric effect showed that highly electronegative substituents, for example aryl derivatives, S- or *O*-alkyl and halides, at the anomeric carbon (C-1) on the ring typically favour the axial α-anomer, which lead to stabilization of the axial substituent compared to the equatorial substituent.[125,126]

The Gauche effect is another example of a stereoelectronic effect which shows that the two vicinal heteroatom groups prefer the synclinal orientation which allows a good interaction between the anti-bonding orbital of C-X and the bonding orbital of C-H leading to minimizing the energy of the molecule **(Figure 11)**.[127]

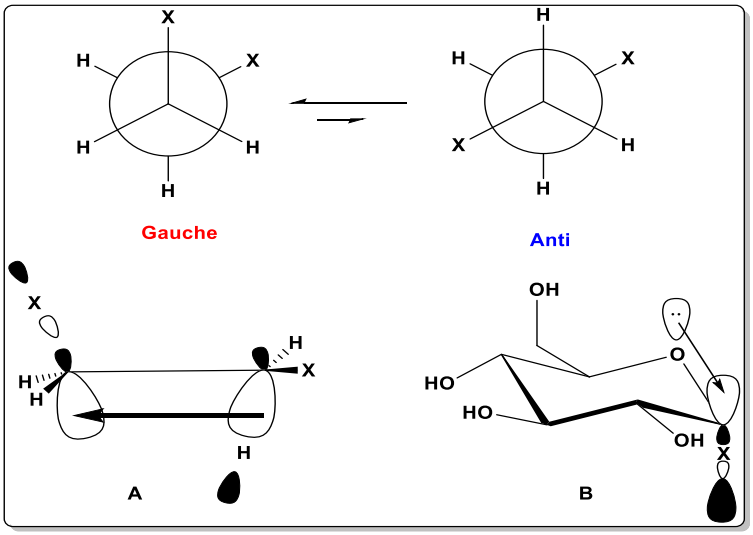


Figure 11: Stereoelectronic effects: **A.** Gauche effect; **B.** Anomeric effect.

1.6 Preparation of Glycosidic Linkages in furanose system

Disaccharides or higher molecules can be formed through glycosidic bonds between two mono-saccharides. Chemical or enzymatic methods are the main approaches for their synthesis. In nature, the glycosidic linkages originate through a reaction involving an enzyme (glycosyltransferase). Enzymatic preparation is a highly stereospecific and regiospecific method to synthesise significant carbohydrates.[128] However, it is high in cost and requires a specific enzyme (sometimes not available) which limits its application, therefore, chemical methods are usually applied. Glycosylation is the reaction between the glycosyl acceptor, which has a free hydroxyl group (nucleophile), with a glycosyl donor, which has a leaving group (electrophile). The coupling between these two glycans is done in the presence of an activator to give a disaccharide with a new anomeric centre. The product could be either the α- or the β- anomer, depending on many factors. Controlling this is a challenge which has been broadly studied **(Figure 12)**.[129,130,131,132,133,134,135,136,137]

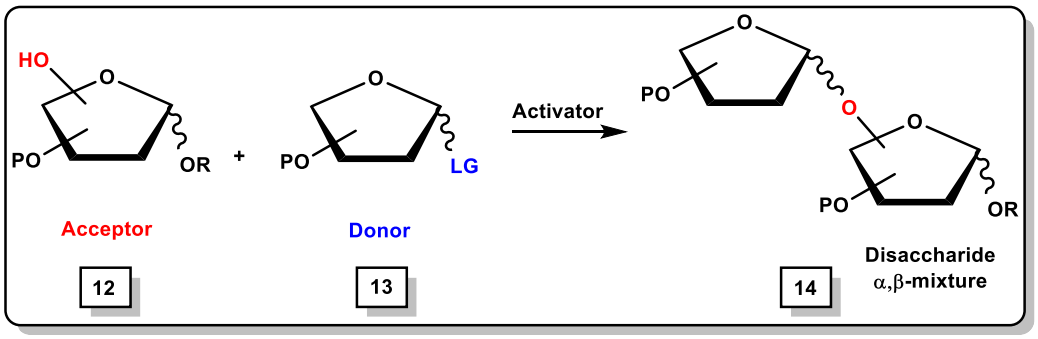


Figure 12: Glycosidic linkage formation.

Mostly, glycosidic linkages exist as two types, 1,2-*cis* and 1,2-*trans* glycosides **(Figure 13)**. *Cis* and *trans* refers to the stereochemistry of the substituents at the C-1 and C-2 positions.

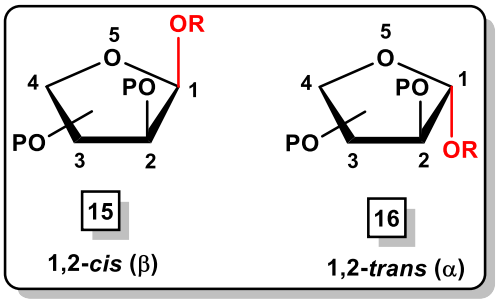


Figure 13: Types of the glycosidic linkages **(15&16)**.

1.6.1 Type 1,2-*trans*-Glycosidic Linkages

Type 1,2-*trans* glycosidic bonds, can be straightforwardly achieved by using a donor protected at the C-2 position with an *O*-acyl which allows for neighbouring group participation. As illustrated **(Figure 14)**, losing the leaving group from the anomeric centre through Lewis acid activation, leads to the formation of an oxocarbenium ion which is attacked directly by the *O*-acyl protecting group to produce a dioxolenium ion intermediate. The desired 1,2-*trans* glycoside is thus the major product because the dioxolenium ion blocks one face of the molecule and hence the acceptor is forced to attack the anomeric centre from the less hindered face, through a process that is kinetically favoured. The dioxolenium ion intermediate could, however, form several by-products through a series of rearrangements.

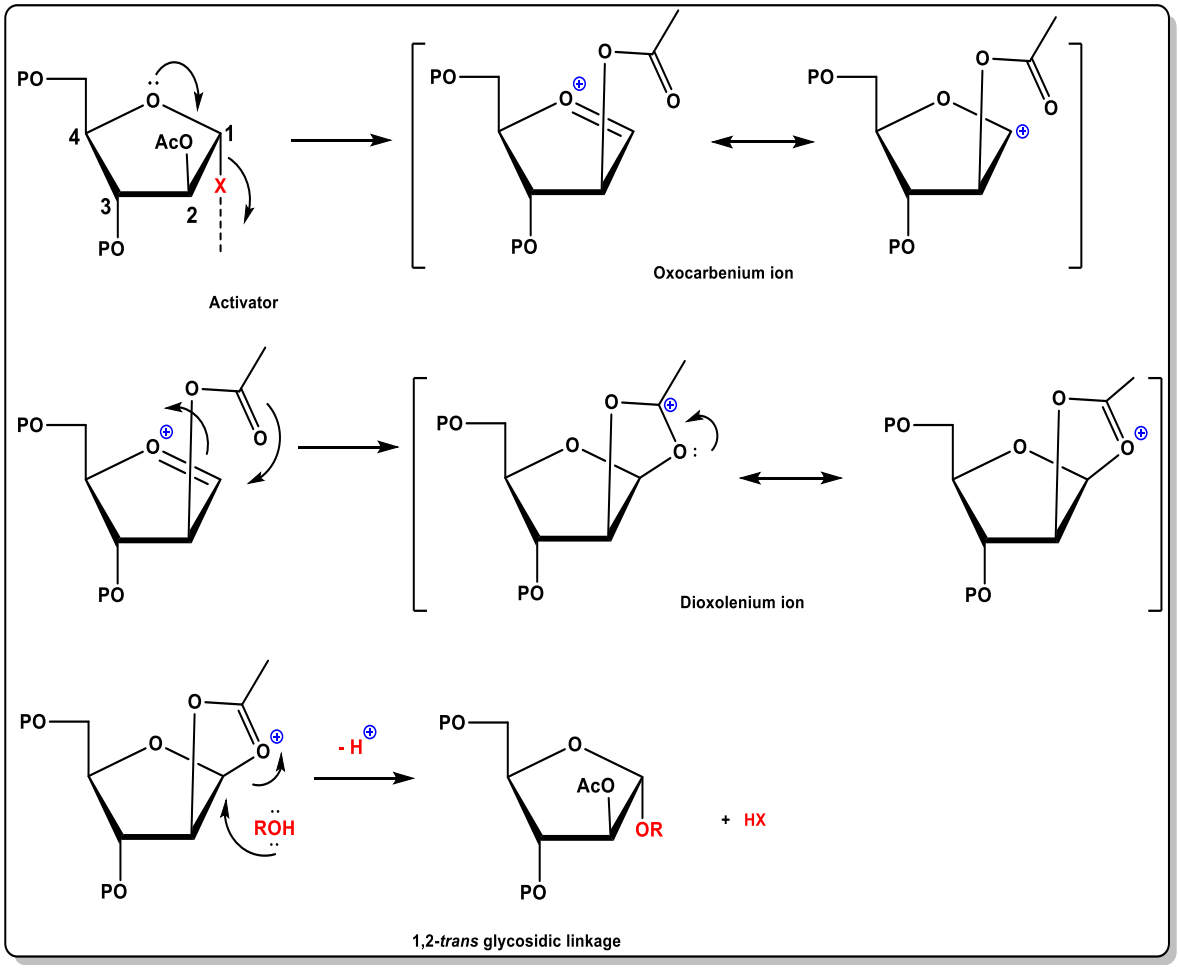


Figure 14: Proposed formation of stabilized cation.[138]

When the *O*-acyl group bears an electron-withdrawing substituent, the stereoselectivity of the glycosylation is reduced because of the reduction of the electron density on the carbonyl oxygen atom, and thus its nucleophilicity; therefore, formation of the dioxolenium ion cannot proceed effectively. In this case, this compound shows more oxocarbenium ion character, and a mixture of both α and β anomers is produced.[139]

In some cases, the participating group can be an electronegative atom, for example nitrogen, or a chiral auxiliary **(Figure 15)**. However, the application of chiral auxiliaries is limited, due to the difficulty of fixing and removal of these groups. In addition there is the possibility of forming both glycosidic anomers because the intermediate species can be formed in diverse orientations.[140,141,142]

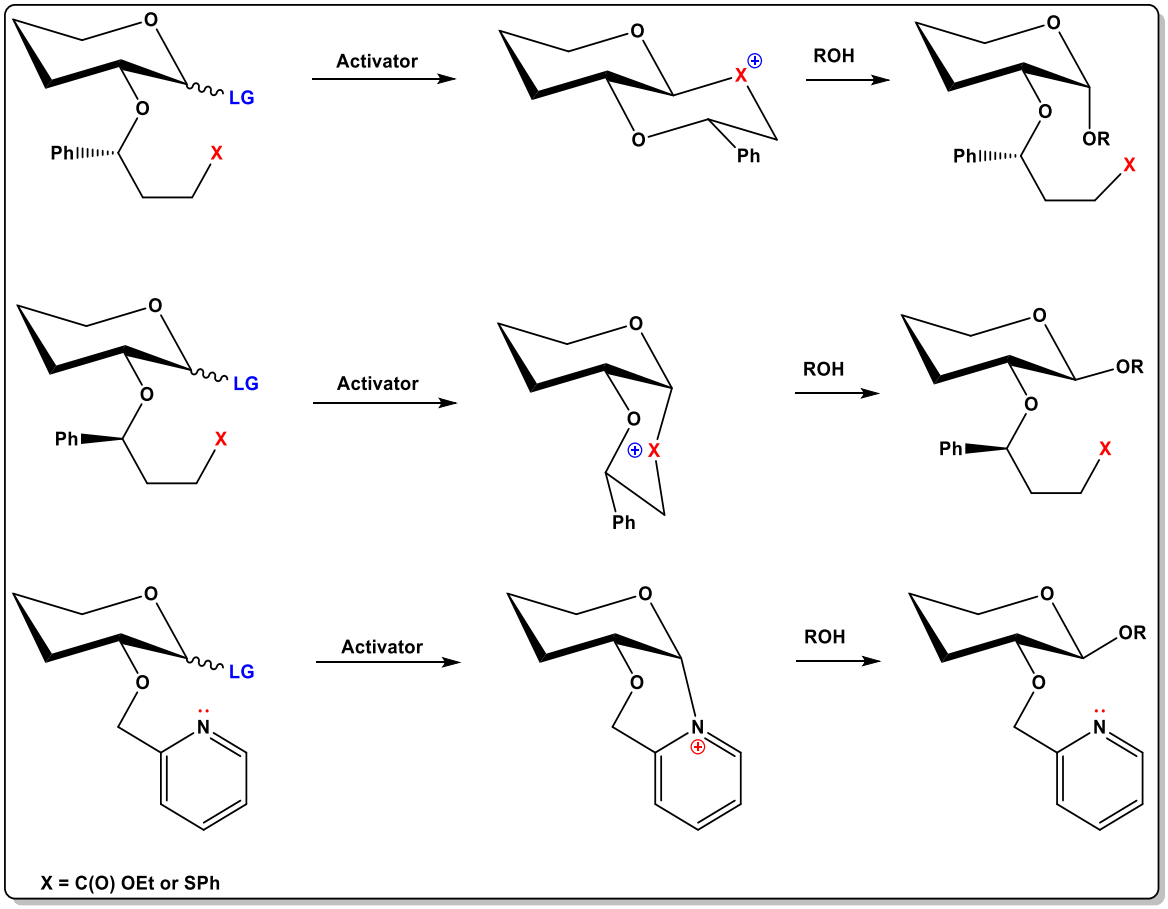
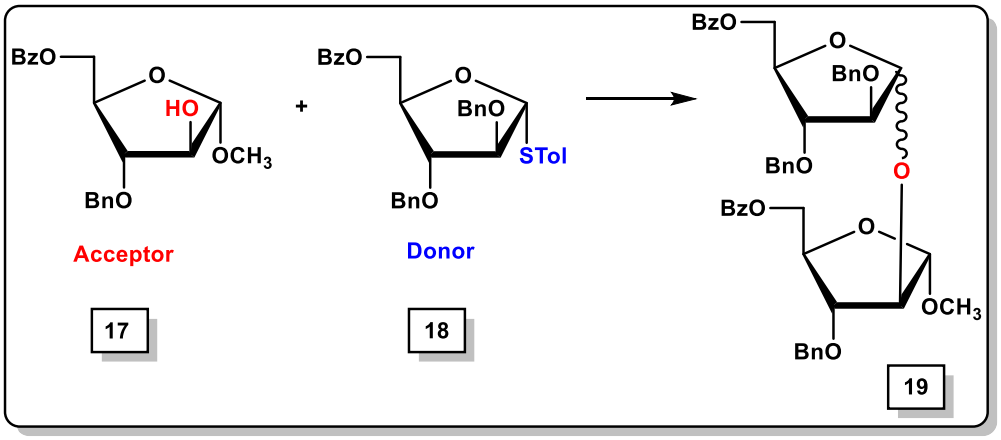


Figure 15: Neighbouring group participating approaches.

1.6.2 Type 1,2-*cis*-Glycosidic Linkages

A general route for the synthesis of β-arabinofuranoside (1,2-*cis* glycosidic linkage) has not been established, thus an understanding of the factors that control this reaction is still limited; however, most of these reactions are believed to occur through an S_N1-type reaction via an oxocarbenium ion intermediate, therefore, the acceptor can attack the donor from both faces and the selectivity is difficult to predict.[143] Numerous strategies for the preparation of β-glycosidic linkages have been reported, however most of these focus on pyranose glycan classes. Lowary and co-workers,[144] reported a study to find the best conditions to use in the glycosylation between the donor and the acceptor to improve β-selectivity. They reacted the acceptor **17** and the donor **18** in CH_2Cl_2 **(Scheme 2)**, using the promoter silver trifluoromethanesulfonate and *N*-iodosuccinimide (AgOTf-NIS) as a coupling reagent. Firstly, they studied the effect of the temperature of the reaction on the ratio of α/β and the yield of the product (entries 1–5) **(Table 1)**. They developed an approach where the reaction was initiated at a temperature of -60 ºC and then gradually warmed to -40 ºC over 2 h. The reaction gave improved yield and stereoselectivity.[144] Studying the effect of the reactant

concentration (entries 6-8) was also carried out, and it was established that by using a low concentration, a slight increase in the β-selectivity was observed; variation of the concentration did not affect the yield. Finally, the effect of the activator (entries 9 and 10) was tested, and they proved that the utilisation of different promoters, such as NIS-trimethylsilyl trifluoromethane sulfonate (TMSOTf) and diphenylsulfoxide, 2,4,6-tri-*tert*-butylpyrimidine, and trifluoromethane sulfonic anhydride (Ph_2SO-TTBP-Tf_2O) **(Table 1)**, gave a low yield and β-selectivity.



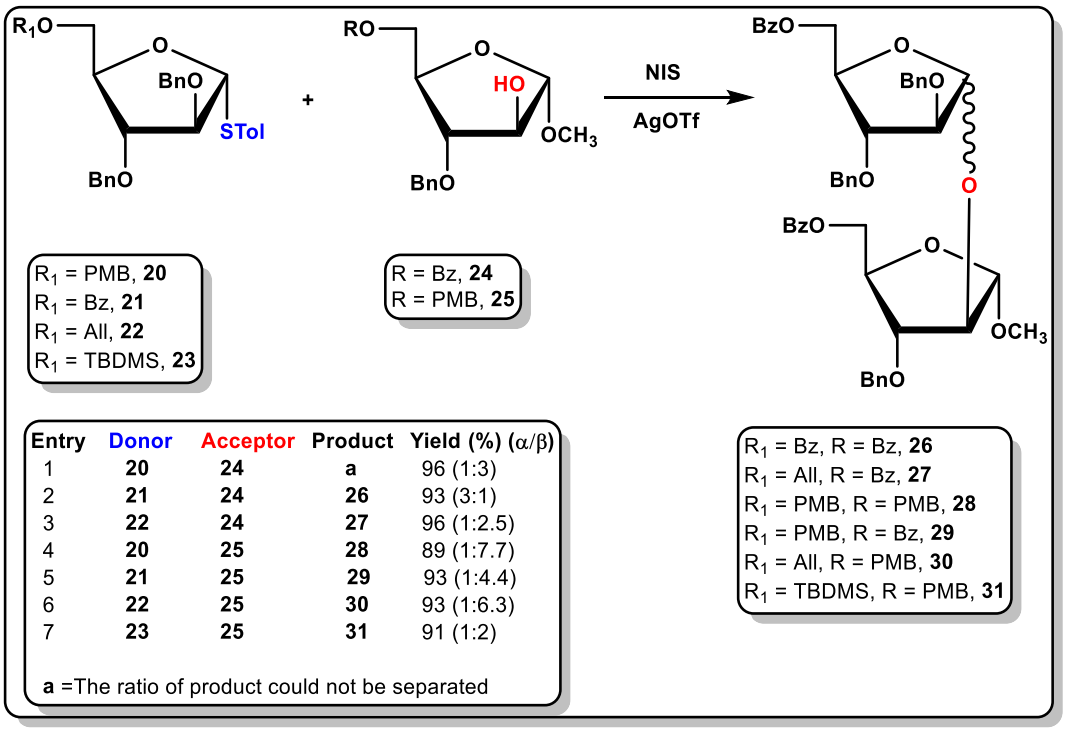
Scheme 2: Synthesis of di-saccharides.[144]

Table 1: Optimization of β-Arabinofuranosylation.[144]

Entry	Temperature °C	Time (h)	Acceptor c (M)	Activator	Yield % (α/β)
1	- 78 → R.T.	4	0.08	NIS-AgOTf [a]	81% (3.1:1)
2	- 78	6	0.08	NIS-AgOTf [a]	85% (3:1)
3	- 60	4	0.08	NIS-AgOTf [a]	91% (3.4:1)
4	- 40	0.5	0.08	NIS-AgOTf [a]	74% (4.6:1)
5	- 60 → - 40	1	0.08	NIS-AgOTf [a]	89% (4.2:1)
6	- 60 → - 40	1	1.00	NIS-AgOTf [a]	84% (4.3:1)
7	- 60 → - 40	1.5	0.05	NIS-AgOTf [a]	85% (4:1)
8	- 60 → - 40	2	0.01	NIS-AgOTf [a]	93% (3:1)
9	- 60 → - 40	0.5	0.01	NIS-AgOTf [b]	78% (5:1)
10	- 60 → - 40	6	0.01	Ph_2SO-TTBP-Tf_2O [c]	63% (4:1)

All reactions were carried out in CH_2Cl_2: [a]**Acceptor 17** (1 equiv), donor 20 (1.2 equiv), NIS (1.2 equiv), AgOTf (0.1 equiv). [b]**Acceptor 17** (1 equiv), donor 20 (1.2 equiv), NIS (1.2 equiv), TMSOTf (0.1 equiv). [c]**Acceptor 17** (1 equiv), donor 20 (1.2 equiv), Ph_2SO (3 equiv), TTBP (6 equiv), Tf_2O (1.1 equiv).

In the same study, an investigation of the effect of the protecting groups on both the donor and the acceptor was undertaken, as illustrated in **Scheme 3**. The best α/β ratio achieved was 1:7.7 (entry 4), and the two anomers were inseparable. This ratio was obtained by utilising PMB as a protecting group at the C-1 position on both the donor and the acceptor.

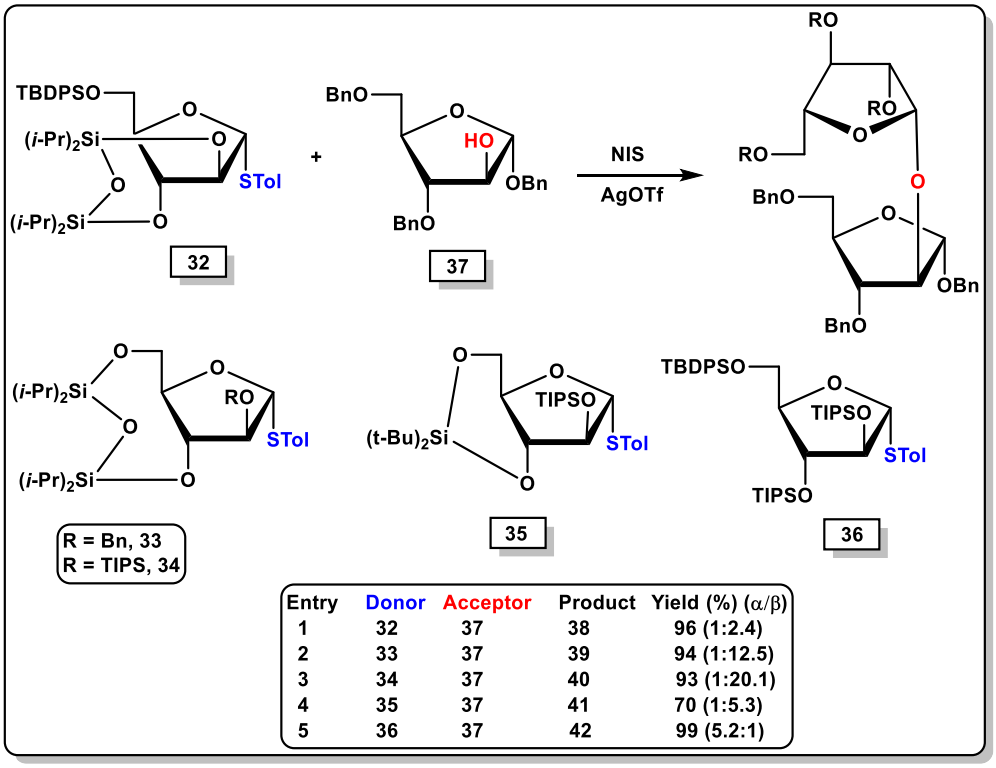


Entry	Donor	Acceptor	Product	Yield (%) (α/β)
1	**20**	**24**	**a**	96 (1:3)
2	**21**	**24**	**26**	93 (3:1)
3	**22**	**24**	**27**	96 (1:2.5)
4	**20**	**25**	**28**	89 (1:7.7)
5	**21**	**25**	**29**	93 (1:4.4)
6	**22**	**25**	**30**	93 (1:6.3)
7	**23**	**25**	**31**	91 (1:2)

a =The ratio of product could not be separated

Scheme 3: Reaction of different donors and acceptors.[187]

Ishiwata and co-workers,[145] reported a new strategy for conducting β-selective glycosylation using donors protected with 3,5-TIPDS. An enhancement of β-selectivity was achieved by utilising a donor with an eight-membered ring **(33, 34)**, which gave the best α/β ratio of 1:20 **(**entry 3, **Scheme 4)**.



Scheme 4: Effect of protection of the glycosyl donor in Arabinofuranosylation.[145]

Using a donor containing an eight-membered ring 3,5-*O*-protection **(34)**, in comparison to a six-membered ring 3,5-*O*-protection **(35)**, with the same acceptor **(37)** showed a marked difference in β-selectivity (entries 3 and 4). In the case of α-attack of the anomeric carbon **(Figure 16)**, there seems to be a large steric repulsion from the α-hydrogen atom at C-2.[145]

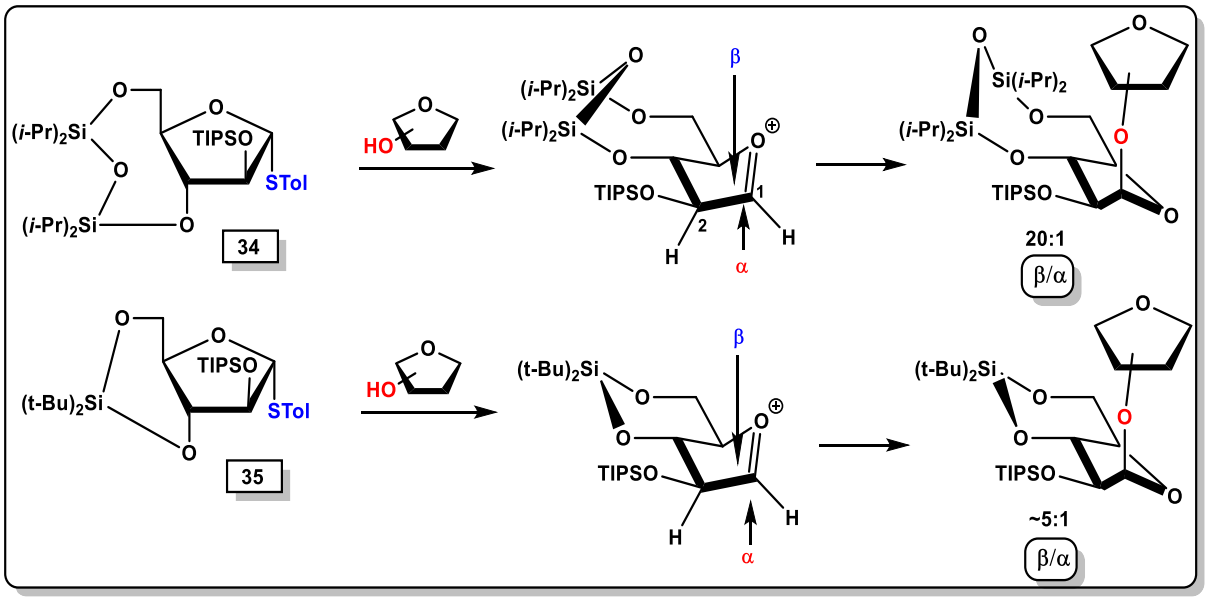


Figure 16: A reasonable explanation for the β-selective addition to the activated donor.[145]

Since this work involved the preparation of different sugar esters, the following section describes various sugar mycolates in the mycobacterial cell wall.

1.7 Trehalose Mycolates (Cord Factor):

Cord Factor is a non-reducing disaccharide, in which two glucose units (linked by an α,α-1,1-glycosidic linkage) has been esterified at both primary alcohol positions with mycolic acids (MAs) creating trehalose di-mycolate (TDM) or esterified at one primary alcohol position to form trehalose mono-mycolate (TMM).[146] It is one of the most interesting and potentially valuable glycolipids found in the cell wall of *M.tb*. The mycobacterial cell wall contains high levels of the free trehalose and esterified trehalose TDM **(43)** and TMM **(44) (Figure 17)**.

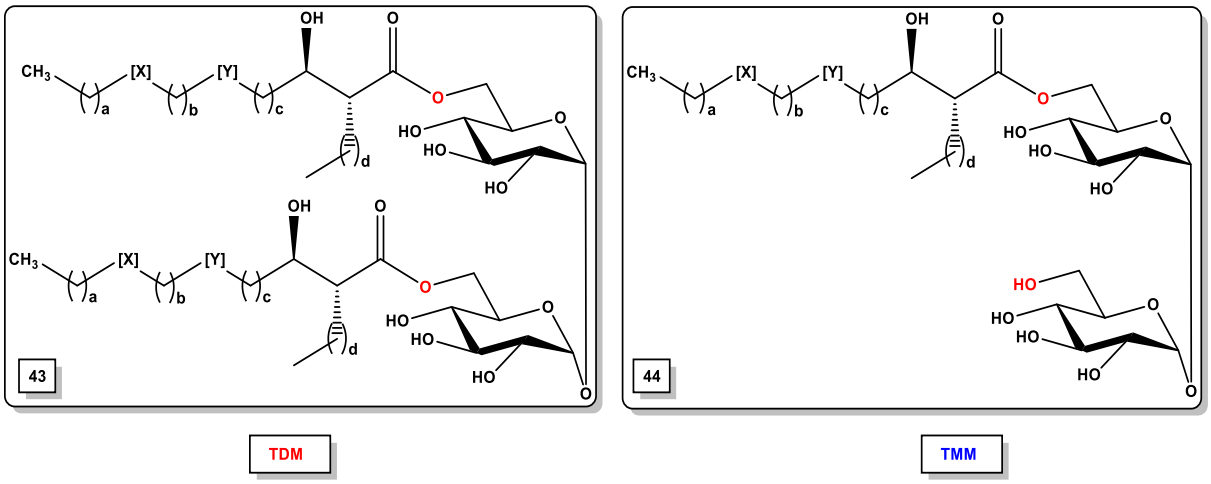


Figure 17: Structures of TDM and TMM.

The name cord factor has been used in the early papers, as far back as 1947.[147] The toxicity of cord factors in mycobacteria was reported in 1953 by Bloch *et al*. when they extracted four different strains of cord factors and they tested them on mice. It was proven that cord factors caused inhibition of respiratory disease and inflammation.[148,149] It has also been confirmed that cord factors have anti-bacterial, anti-tumour and anti-parasitic characteristics.[150] In 1956, the structure of *M.tb* cord factor was identified by Noll *et al.*[151] Mycobacterial cord factors are known as very interesting compounds due to their biological activity, which has been the subject of many investigations. Studies showed that these components of mycobacteria have immunogenic activity. TDM is necessary for the survival of the mycobacteria inside macrophages.[152] It can also induce a wide range of cytokine and chemokine production in the host's immune system such as (IL-1β, IL-6, and TNF).[153] Early studies showed that cord factors can be used as an adjuvant (an agent that can enhance the immune response to an antigen) against immunological problems. Meyer and Azuma in 1975 discovered that the cell wall components of mycobacteria show adjuvant activity.[154] A study by Saito confirmed that mycobacterial cord factor was a good adjuvant and could enhance the immune system in mice and rats through antibody production. Also, it can cause delayed hypersensivity.[155]

Finally, TMM, TDM and mAG complex are responsible for an extremely hydrophobic surface in the cell wall of mycobacteria, which plays a significant role in providing the bacterium with high protection from antibodies and from their environment. The enantioselective synthesis of these compounds is important to understand their biological properties such as their effects on the immune system, in diagnosis and controlling several diseases.

1.8 Glucose Mono-Mycolate (GMM)

Glucose mono-mycolate (GMM) is a glycolipid consisting of a mycolic acid attached to a glucose molecule at the C-6 position. GMM is present in several bacterial species including *Mycobacterium Nocardia, Mycobacterium Rhodococcus* and *M. tuberculosis* (**Figure 18**).[156,157]

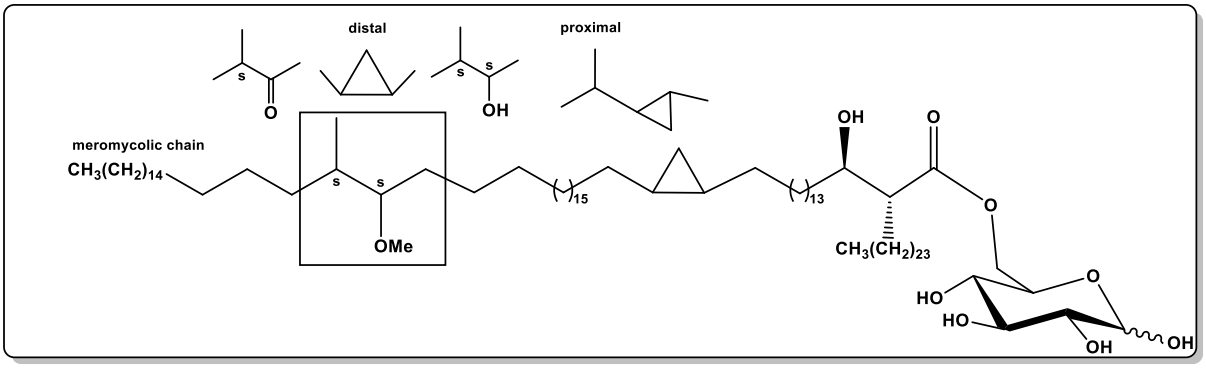


Figure 18: Glucose mono-mycolate (GMM, 6-O-mycoloyl-D-glucose) from *M. tb*.[158]

GMM can induce a memory T cell response by acting as a protein antigen.[157] GMM and other antigenic mycobacterial glycolipids are presented to T cells by the CD1 family of proteins and the antigen–protein complexes mediate the T cells response in the human host.[159] However, depending on the species, the meromycolate chain carries variable functionalities in the proximal and distal positions, which are characteristic of the species.[158] The structure of GMM from *M. tuberculosis* is shown in **Figure 18**.

The structure of human CD1b and CD1a in a complex with specific GMM glycolipids illustrates the binding of a natural bacterial lipid to CD1b and shows how its novel structural features fit this molecule for its role in the immune response to intracellular bacteria.[160] Many health problems globally are caused by mycobacterial infections and it has been suggested that anti lipid antibodies may contribute to protection against mycobacterial infection.[161] GMMs have the ability to produce T cell reproductive responses in a number of species including cattle,[162] humans,[163] mice [164] and guinea pigs.[165] Nguyen *et al.* have described cell-mediated and humoral immune responses in cattle upon vaccination with GMM as the only antigen; as a result a T cell response was produced but no antibody responses, while the

vaccine comprising a pure protein as the only antigen generated both T cell and antibody responses. However, in humans and cattle,[166,167] Nguyen *et al* assumed that a conjugate of GMM with a protein may provide T cell help for B cells to produce antibodies against surface exposed glycolipids on mycobacteria.[162]

The synthesis of GMM from *Mycobacterium phlei* has been described by Branch *et al*,[168,169] and the synthesis of 6-*O*-mycolylglucoses (GMMs) from single synthetic mycolic acids matching the overall structure of some of the major natural glucose monomycolates of Mycobacterium tuberculosis and other mycobacteria, has been reported by Sahb *et al*.[170]

1.9 Di-Mycolyl di-Arabino Glycerol (DMAG)

The mAG complex is the largest component structure in the mycobacterial cell wall and acts as a permeability barrier that prevents the passage of antibiotics. It's formed from both galactan and arabinan in the furanose form.[88,89,171] It is believed that this plays an important role in raising the flexibility of the polysaccharide, causing the cell wall to have extremely low permeability, which provides the organism with high protection from drugs and from its environment.[172] Mycolylated glycolipids like GMM, TDM, or GroMM **(see Section 1.11)**, play a significant role in the variation of the immune system of the host and among them the mAGP serves as an anchoring matrix. Therefore, mAGP is seen as a target for several anti-tuberculosis drugs.[82,173] Components such as triacylglycerols (TAGs), C70–90 mono-mycolyl glycerol (C70–90 GroMM) and phenolic glycolipids (PGLs), separated from the subcutaneous immunisation of mice, induced extremely high levels of all three cytokines IL-12, TNF-α and IL-6.[174] An antigenic glycolipid, 5-mycoloyl-β-arabinofuranosyl-(1→2)-5-mycoloyl-α-arabinofuranosyl-(1→1`)-glycerol (DMAG) **(Figure 19),** was isolated for the first time by Watanabe and co-workers in all 12 strains of the *M. avium-M. intracellulare* complex (MAI) and reacted immunologically with antisera from rabbits.[81,82,173]

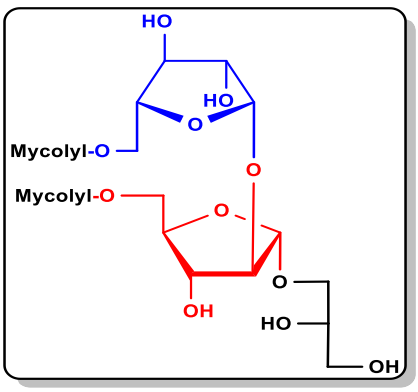


Figure 19: General structure of DMAG.[81]

DMAG also showed applicability for serodiagnosis of MAI infection by ELISA.[175,176,177] In 1997 DMAG was obtained from *M. kansasii* among glycolipid fractions of the cell wall.[178] Recently, Rombouts and co-workers identified DMAG in large quantities, in slow growing pathogenic species, including *M.tb, M. bovis*, BCG and *M. Scrofulaceum,* making this glycolipid more biologically potent and possibly important in mycobacterial pathogenesis. Studies showed that DMAG isolated from *M. marinum* and *M. bovis BCG* are very similar to each other except for the terminal lipid moiety MA, which consists of a mixture of alpha, keto and methoxy-mycolates in *M. marinum* while only alpha and keto-mycolates are found in *M. bovis* BCG. Furthermore, the cyclopropane ring in *M. marinum* seems more likely to be in the *trans* stereochemistry.[82] Construction of DMAG in the growing mycobacterium requires the presence of glycerol. In addition, drugs used for the inhibition of mAG also inhibit DMAG, which again indicates the similarity between these two components and raises a possibility of metabolic interconnectivity between them. DMAG is formed during infection with *M.tb* and is not synthesized along with other lipids/glycolipids. It is considered a surface-exposed immunogenic molecule, suggesting that it is synthesized through TB infection. In addition, the existence of the anti-DMAG antibodies in the sera of patients infected with *M. avium* further suggested that DMAG is an immunogenic compound produced during infection.[173,175] TNF-α has been proven as a significant inflammatory mediator, that can affect different kinds of cells.[179,180,181] TNF-α, IL-1β, and IL-8 secretions have been widely used to investigate the biological activity of mycobacterial glycolipids; for instance DMAG isolated from *M. marinum* induced TNF-α, IL-1β, and IL-8 on separated cells.[82,182,183] Depending on the high similarity between TDM and DMAG in their location in the mycobacterial cell wall and their analogous structures, it is expected that both glycolipids will show the same characteristics which are relevant to mycobacterial pathogenesis, for example, formation of granuloma and tissue-destructive lesions and proinflammatory cytokine production.[184]

1.10 Di-Mycolyl tri-Arabino Glycerol (DMTAG)

A new glycolipid, di-mycolyl tri-arabinofuranosyl glycerol (DMTAG) **(Figure 20)**, was obtained by McNeil and co-workers after studying the effects of Smith degradation on the terminal tetra-mycolyl-penta-arabinofuranosyl unit of mAGP of *M. tuberculosis*.[80]

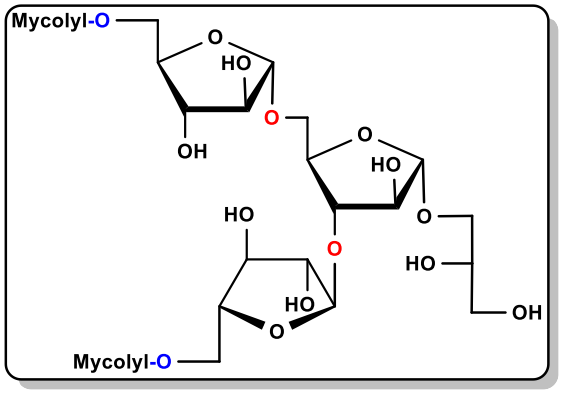


Figure 20: General structure of DMTAG.[80]

Methyl tri-arabinofuranosyl di-mycolate (MTADM) compounds derived from a natural mixture of MAs have been reported in the literature and showed a very high response in the stimulation of TNF-α cytokines.[80,185] The synthesis, biological activity, particularly the antigenicity, of DMTAG glycolipids was not investigated. Furthermore, the stereochemistry of the glycerol, *i.e.* whether it is in the D- or L- configuration, has not been proven.

1.11 Glycerol Mono-Mycolate (GroMM)

Complex mixtures of mycolic acids are characteristic components of mycobacterial cells, either bound to the wall as arabinose esters or not bound to the wall, as free acids or esterified to sugars such as glucose (GMM), or trehalose (dimycolate, TDM; monomycolate, TMM).[185] As early as 1956, it was shown that *M. tuberculosis* contains C-90 mycolylglycerols.[146,186,187,188] Later, shorter chain glycerols esters were isolated from *Nocardia rhodochrous*, which contains 40 – 44 carbon atoms (nocardomycoloylglycerols),[189] from Corynebacterium pseudoytuberculosis,[190] from Nocardia asteroids,[191] and from *Rhodococcus lentifragmentus.*[192] Glycerol monomycolate (GroMM, also known as MMG) **(Figure 21)**, is also present in the wax C fraction of BCG.[193] GroMM has been extracted from *M. bovis* by Layre et *al.* and the identification of this novel antigen is supported by ^{1}H-NMR analysis for the structure of *M. bovis* BCG GroMM and by the mass spectrum.[194]

Andersen *et al.* identified GroMM *in vitro* as the most immunopotentiating compound among several different lipids isolated from the mycobacterial cell wall.[195] Hattori *et al.* identified

GroMM as a specific immune target in human individuals with latent, but not active tuberculosis. The *in vivo* response to GroMM and the relevance of it to latent infection remain poorly understood.

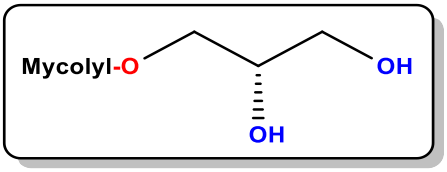
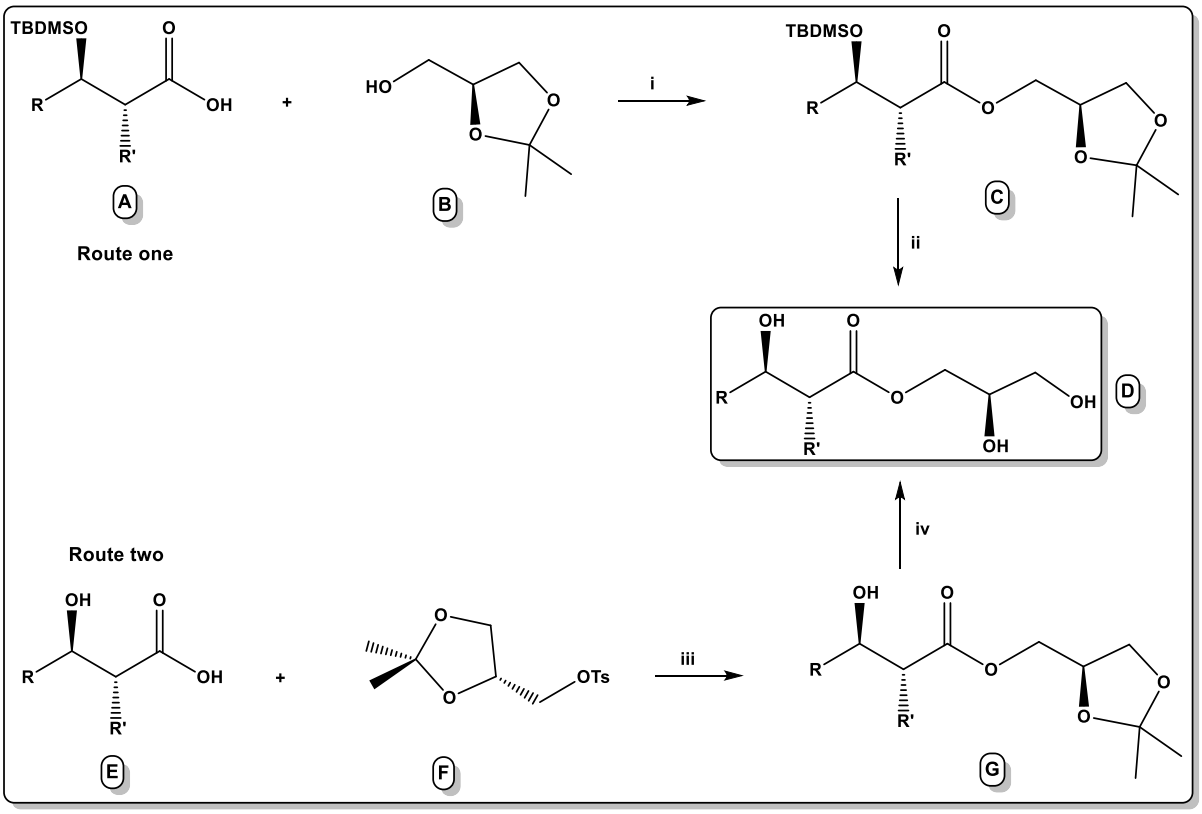


Figure 21: General structure of GroMM.

Hattori *et al.* immunized guinea pigs with bacillus Calmette–Guerin (BCG) expressing high levels of GroMM and then monitored skin reactions at the site of inoculation with GroMM-containing liposome. The host responses to GroMM produced by inactive mycobacteria contribute to their long-term survival in the host.[196] Glycerol esters of complex mixtures of natural mycolic acids have strong effects in the immune system.[197] The identification of GroMM lipid species formed by mycobacteria infected hosts, as well as the analysis of the host response directed toward them, will provide important new insights into host-microbe interactions in tuberculosis. These latent mycobacteria are unlikely to produce cell wall lipids at a level comparable with that for actively replicating microbes, as in the case of the former the bacteria can be eradicated by the host's immune system.[198] but a recent study has identified GroMM as a mycobacterial lipid species potentially associated with latent infection.[199] Mycobacteria have been known as modulators of the immune system and as a source of adjuvant preparations. The role of the adjuvants is to potentiate or prolong the specific action of a vaccine.[174] GroMM has been observed to have adjuvant activity in murine models,[200,201] with the fine structure of the mycolate components being of importance for its proinflammatory activity.[202] The GroMM lipid, isolated from *M. bovis* bacillus Calmette- Guérin (BCG), has potent immunostimulatory activity on human dendritic cells, which play an essential role in directing the immune response upon infection with pathogens, such as *M. tuberculosis*. This activity was shown by a synthetic analogue of GroMM with shorter fatty acids.[197] Bhowruth *et al.* separated a lipid extract of *M. bovis* BCG showing GroMM as one of four lipid fractions, which showed an ability to induce high levels of some pro-inflammatory cytokines (IL-12 and TNF-α).[174]

The synthesis of GroMM using single synthetic mycolic acids will result in a rich knowledge of the nature of the cell wall of *M. tuberculosis,* and hence give a better understanding of their effects in the immune system.[197]

The development of such a novel class of synthetic lipids would be suitable for presence in vaccines to investigate their use in humans. Such synthetic GroMMs with a *R*-glycerol component (**Scheme 5**) have been reported by M. Sahb.[203,204] They were prepared by two methods (Scheme 5). In the first, the TBDMS protected MA was coupled with acetonide **(B)** promoted by DCC and DMAP. In the second, the unprotected acid was coupled with tosylate **(F)** in the presence of cesium hydrogen carbonate.



Scheme 5: Reagents & conditions: (i) DMAP, DCC; (ii) TFA, CH_2Cl_2; (iii) $CsHCO_3$; (iv) HCl, THF, H_2O.[203]

In order to determine whether the biological activitiy of the GroMMs is dominated by the *S*-glycerol unit or the mycolic acid unit, a series of GroMMs containing single enantiomers of synthetic mycolic acids were prepared. This will allow the biological activity of these compounds to be compared to those of the previously synthesised *R*-glycerol esters.

1.12 TB Detection

TB infection can be detected through several existing methods; these all meet the WHO's standards, but they still have disadvantages such as high cost, being time consuming, inaccurate results, interference by BCG vaccination and their inability to distinguish between latent and active TB. A brief description of the tests is given below:

- **Bacterial Culture**: This method is considered as a gold standard for detecting active TB,[205] and it has one of the highest sensitivities (sensitivity is defined as the proportion of actual positive samples correctly detected by the antigen to the total TB-positive sera) among TB diagnostics. It can also distinguish TB patients with drug resistant strains from those with non-drug resistant strains,[206] providing valuable information for the treatment of the disease. This method does however take around 3 to 8 weeks to obtain a result.[207,208]

- **Tuberculin Skin Test**: The tuberculin skin test (TST) was one of the earliest diagnostic tools developed against the disease, and it requires 2 to 3 days to obtain a result.[206] One drawback is that it does not distinguish between latent and active TB; moreover, it cannot distinguish an active TB patient from one that was previously infected by the disease.[209] False negative samples include patients co-infected with HIV, while false positive samples include patients vaccinated with BCG.[207,210,211,212,213,214]

- **Sputum Smear Microscopy**: This is a simple, cheap and fast method of diagnosing TB, and relies on direct observation of mycobacteria under a light microscope. It cannot however distinguish TB from other mycobacterial diseases.[206] An advantage of this method over bacterial culture is that the time required for the result is much shorter, the staining process being performed in less than 1 hour,[215] making this valuable as a quick screening method for mycobacterial disease.[216,217]

- **Interferon γ-Release Assay**: The principle of the IFN-γ assay is the stimulation of T-cells to produce IFN-γ when they re-encounter the antigen of *M.tb*.[218] This method detects both latent and active TB and again cannot distinguish between them.[209,219] It is also reasonably fast, with results available in 24 h; no false positive samples are seen caused by BCG vaccination. One disadvantage however is there is only a limited amount of clinical and laboratory experience with this assay. [220,221,222]

- **Nucleic Acid Amplification Test**: Detects the presence of the *M.tb* complex, but cannot distinguish the individual mycobacteria. This method does however have low limits of detection, and the results are obtained in 2.5 to 3.5 h.[223,224]

There are some newly available tests which are simple, faster, less expensive and give better results such as:

- **Biosensor Detection of TB:** The principle of this method depends on the binding of the host antibody to specific antigens such as mycolic acids, cord factors or their corresponding thiolated compounds. The main advantages of this method include high levels of detection, it is simple to use and very fast.[225,226,227]

- **Serodiagnostic assays**: ELISA assays for the detection of TB are used to detect antibodies produced against *M.tb*. However, the WHO has strongly recommended that the current assays are not to be used for the detection of TB. It does however indicate that an assay of this kind which does meet its criteria would be valuable. [225,228]

1.13 ELISA in the serodiagnosis of TB

A number of natural mixtures of cord factors and mycolic acids have been shown to have strong effects on the immune system and have been used in ELISA (Enzyme-linked immunosorbent assay) to investigate both their biological activity and diagnosis applications for the serodiagnosis of TB.[229,230,231] ELISA is widely used in clinical medicine to detect many infectious diseases such as TB. The principle of the method is based on the detection of the antibodies in an infected sample through antibody–antigen reaction which produces a detectable signal. The test is a rapid and simple screening method.[232] ELISA tests have been used for TB detection because *M.tb* has many different antigens which were widely used for detection; however, it suffers from low sensitivity and specificity (specificity is the proportion of actual negative samples correctly detected by the antigen to the total TB-negative sera).[233] Schleicher *et al.,* used ELISA to try and detect anti-mycolic acid antibodies from *M.tb* in serum samples from patients infected with TB and HIV, and from patients infected with TB alone. They used natural mycolic acids, isolated from *M.tb*, as antigens and they showed that the antibody levels were pointedly higher for TB positive sera than for TB negative sera and that antibody levels remained largely unchanged between HIV-positive and HIV-negative samples, signifying that antibody responses to mycolic acids are also preserved in patients who have tested HIV-positive.[234] Beukes *et al.* used synthetic mycolic acids

prepared by Al Dulayymi *et al.* and natural mycolic acids extracted from *M.tb* to compare the antibody responses, in addition to examining their corresponding methyl esters.[235] They proved that in the case of free mycolic acids the antibody recognition is much higher than their corresponding methyl esters, signifying that the carboxylic acid unit of the mycolic acids either has a large contribution to the binding of the mycolic acids to the antibodies, or that they stabilise a particular antigen conformation. They also established that oxygenateD-mycolic acids are more antigenic than alpha-mycolic acids. However, none of the synthetically produced mycolic acids could reproducibly distinguish TB positive sera from TB negative sera.[235]

1.15 Overall Aim of this Research:

The aim of this study was to synthesise two different glycolipids related to the mAG complex, as well as, glycerol mycolates, which are present in the cell wall of mycobacteria. In particular, the synthesis of:

1- Di-Mycolyl-Di-Arabino-L-Glycerol (DMAG).
2- Di-Mycolyl Tri-Arabino-L-Glycerol (DMTAG).
3- Glycerol Mono-Mycolates (GroMM) with *S*-stereochemistry of the glycerol component, to compare the biological activity of *R*-GroMM.

The object of this work was to ascertain if these fragments **(Figure 22**) have any capacity for the stimulation of the production of co-stimulatory molecules and certain pro-inflammatory cytokines (*e.g.* TNF-α, IL-1β, IL-6), in addition to investigating their antigenicity for the detection of TB and other mycobacterial infections.

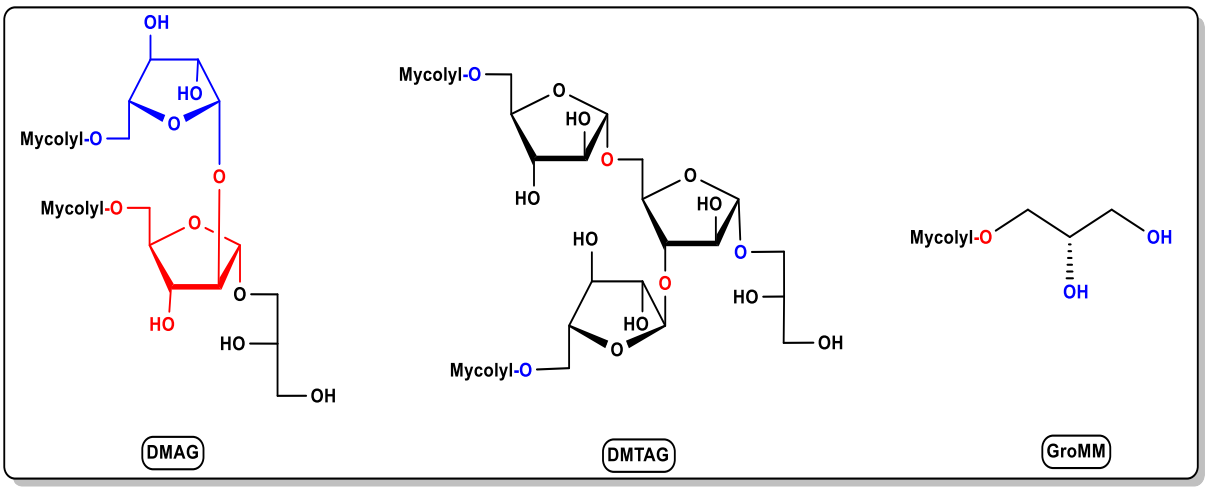


Figure 22: Structures of target molecules.

Chapter 2

Results & discussion

Chapter 2

Results and Discussion

Section 1

2.1 Synthesis of a single enantiomer of di-mycolyl di-arabinofuranosyl glycerol (DMAG)

2.1.1 The aims of this part

- Synthesis of di-arabino-glycerol (DAG), 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-α-D-arabinofuranoside.

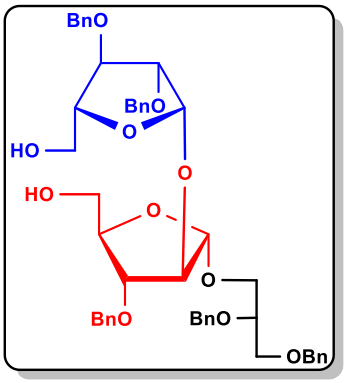


Figure 23: Structure of the glycan (DAG).

- Esterification of the above glycan (DAG) with simple fatty acids as model glycolipids.
- Preparation of a number of di-mycolyl di-arabino glycerol (DMAG) esters through esterifying the glycan moiety with common classes of synthetic mycolic acids (MAs).
- Investigation of the biological activity of the synthetic compounds.

2.1.2 Synthesis of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-α-D-arabinofuranoside (DAG)

In the glycosidation reaction, a new stereogenic centre is created at the C-1 position on the ring and as a result two different diasteromeric products can be obtained **(Figure 24)**. Thus, control of stereochemistry at the anomeric position has been a challenge to organic chemists. In nature, glycosidic linkages are formed through reactions catalysed by enzymes (glycosyltransferases). The biosynthesis is a highly stereoselective processes due to the specificity of the enzymes.[236]

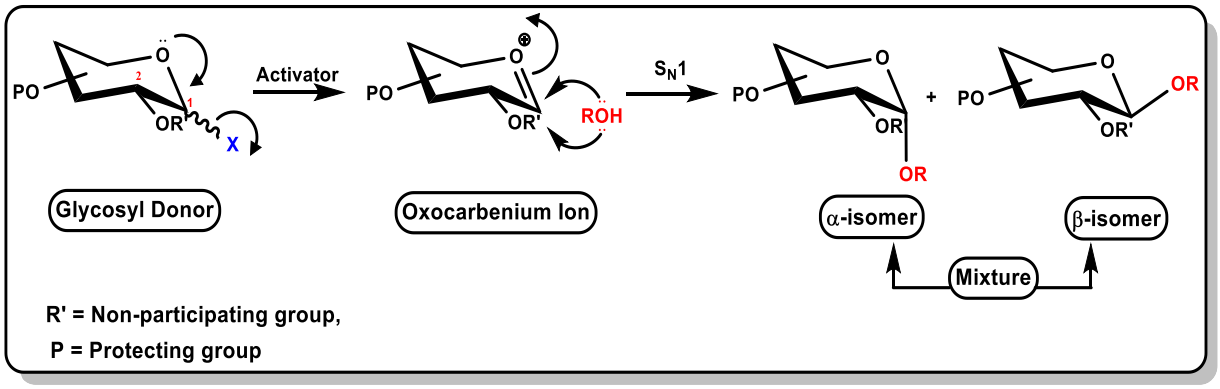


Figure 24: Glycosylation using a donor with a nonparticipating group at C-2.

In this part of the study, the preparation of the target glycan molecules was undertaken, which contains both α- and β- arabinofuranosyl linkages **(Figure 25)**. Formation of the α-glycosidic bond can be directly achieved by using neighbouring group participation of the 2-*O*-acyl functionalities in the donor species. The leaving group at the anomeric centre of the donor departs by the activation of the promoter to form an oxocarbenium ion, which is immediately attacked by the protecting group 2-*O*-acyl to generate a dioxolenium ion intermediate. The dioxolenium ion blocks one face of the molecule and hence, the acceptor has to attack the anomeric centre from the less sterically hindered face, through a process that is kinetically favoured. Discrimination between α- and β- isomers was achieved using NMR data, particularly the $\delta_{C\text{-}1}$ and $^{3}J_{H\text{-}1,H\text{-}2}$ values. For the β-isomer, $\delta_{C\text{-}1}$ = 97 – 103 ppm and $^{3}J_{H\text{-}1,H\text{-}2}$ = 3 – 5 Hz, whereas for the α-isomer, $\delta_{C\text{-}1}$= 104 –111 ppm and $^{3}J_{H\text{-}1,H\text{-}2}$ = 0 – 2 Hz.[237]

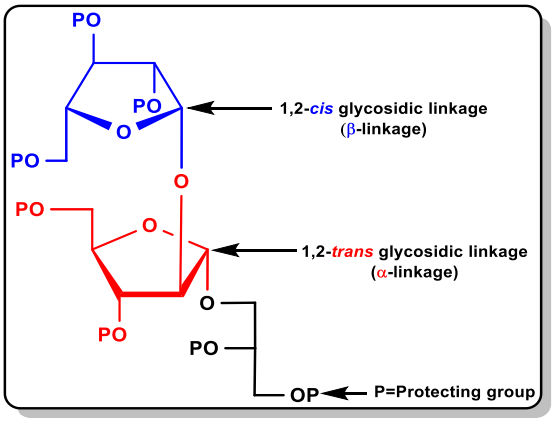
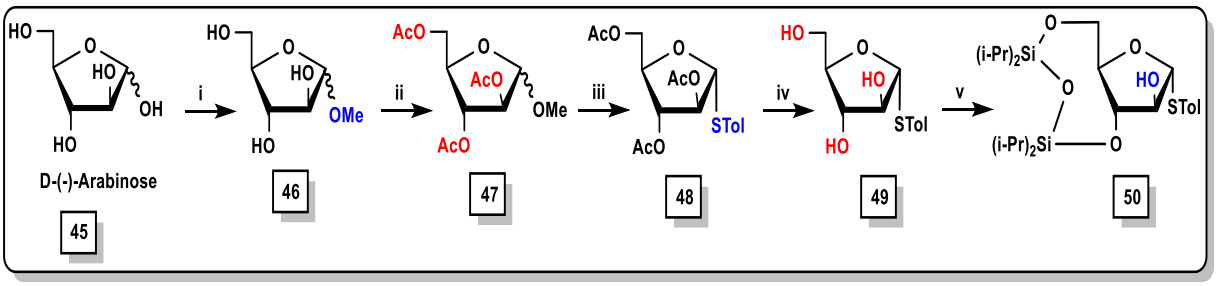


Figure 25: Glycosidic linkages in the target molecule.

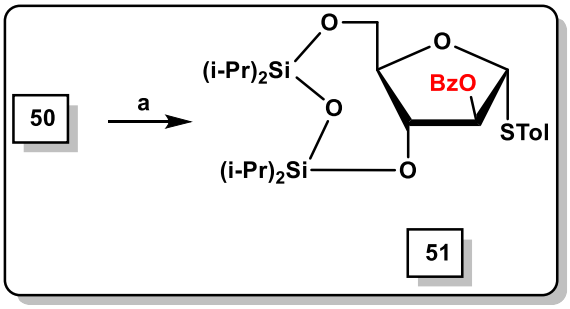
The synthesis of the DMAG's acceptor **(60)** began with commercially available D-arabinose **(45)** as starting material **(Scheme 5A)**. Fischer glycosylation of **(45)** in methanol under kinetic control provided a mixture of methyl-α,β-arabinofuranoside **(46).** The crude product was suspended in dry pyridine without further purification, before acetic anhydride was added, to give triacetate (**47)** in 80% yield. Methyl glycoside **(47)** was stereoselectively

transformed to the α-thioglycoside **(48)** as the principal product upon reaction with *p*-thiotoluene in the presence of boron trifluoride dietherate in CH_2Cl_2 in 65% yield. Subsequent treatment of **(48)** with $NaOCH_3$ in methanol afforded thioglycoside triol **(49)** in 89% yield, which was converted to silyl-protected arabino-thioglycoside **(50)** in one step. All data was identical to those of the authentic samples reported in the literature.[238]



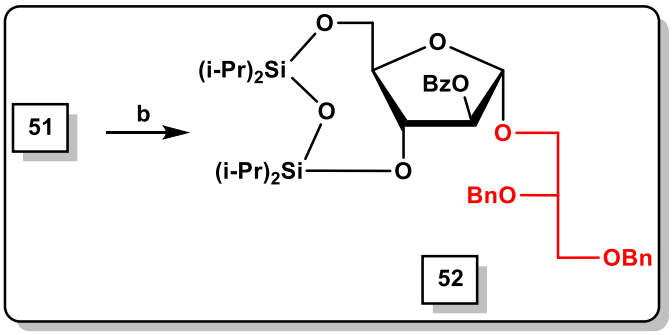
Scheme 5A: Synthesis of compound **(50):** Reagents & conditions: (i) MeOH, HCl, 78%; (ii) Ac_2O, pyridine. 0 ^{0}C, 80%; (iii) *p*-$CH_3C_6H_4SH$, $BF_3.Et_2O$, CH_2Cl_2, 0 ºC/R.T., 8 h, 65%; (iv) $NaOCH_3$, MeOH:CH_2Cl_2 (1:1), R.T., 3 h, 89%; (v) $TIPDSCl_2$, pyridine, 6 h, 0 ºC/R.T., 76%.

Subsequently, benzoyl chloride was used under standard conditions to protect the hydroxyl group of **(50)** at the C-2 position on the sugar ring affording silyl, benzoyl-protected arabino-thioglycoside **(51)** in 89% yield **(Scheme 6)**.[239]



Scheme 6: Reagents & conditions: (a) BzCl, pyridine, 0 ºC/R.T., 89%.

The initial step in the synthetic route was to construct the previously prepared L-glycerol moiety in DMAG's acceptor and in order to achieve that the silyl benzoyl-protected arabino-thioglycoside **(51)** was subjected to a glycosidation reaction with 2,3-di-*O*-benzyl-L-glycerol **(51G)** [247] using *N*-iodosuccinimide (NIS) and silver triflate (AgOTf) in dichloromethane (CH_2Cl_2) at -36 °C **(Scheme 7)**.



Scheme 7: Reagents & conditions: (b) HO, BnO, OBn , NIS/AgOTf, CH_2Cl_2, -36 ºC, 91%.

This type of glycosidation is highly efficient and highly stereoselective due to the neighbouring group participation (benzoyl group at the C-2 position on the glycan ring), thus, only the α-isomer (1,2-*trans* isomer) was obtained in an excellent yield of 91% for the fully-protected arabiofuranosyl-glycerol **(52).** The ^{1}H-NMR spectrum showed the α-anomer glycosidic linkage, which appeared as the only product, one downfield signal occurring as a broad doublet at δ 4.98 ppm (*J* 1.0 Hz) for the proton at the anomeric centre, while the ^{13}C-NMR showed a peak at δ 105.6 ppm due to the carbon at the C-1 position on the glycan ring **(Figure 26)**, both indicating that only the α-anomer was present.

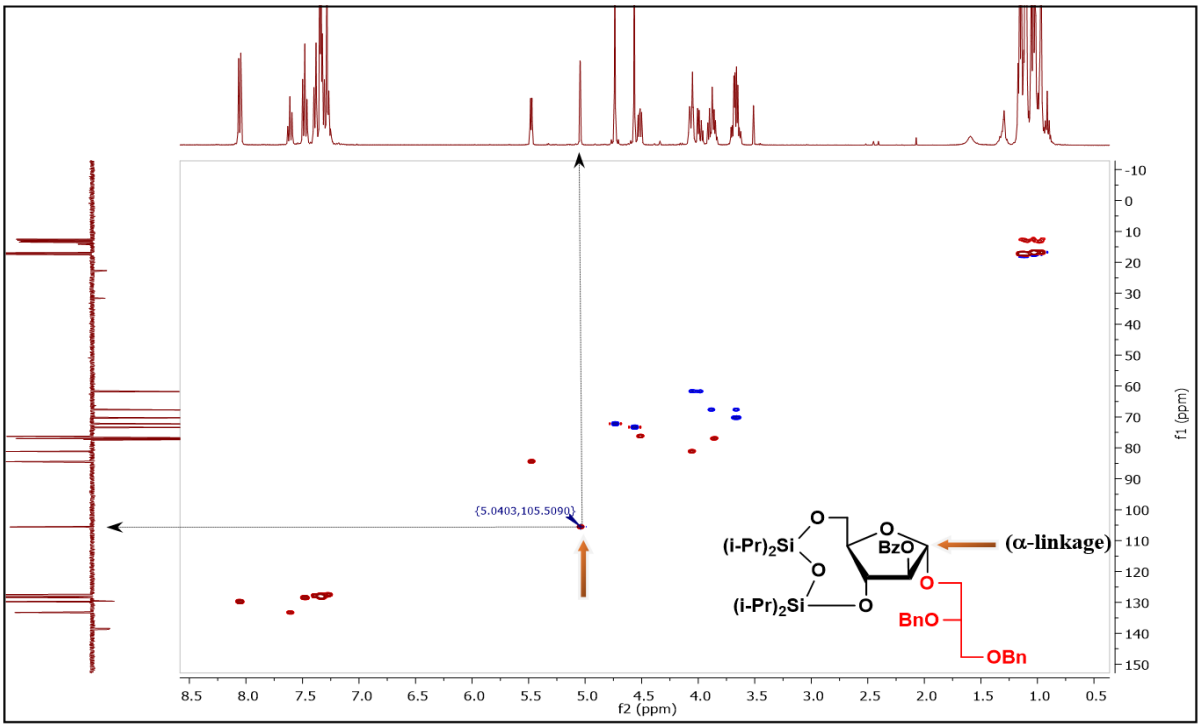
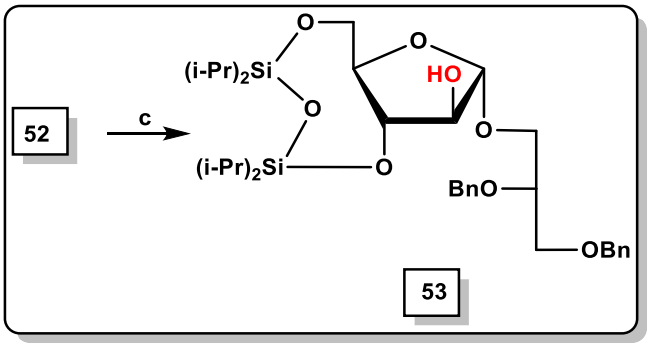


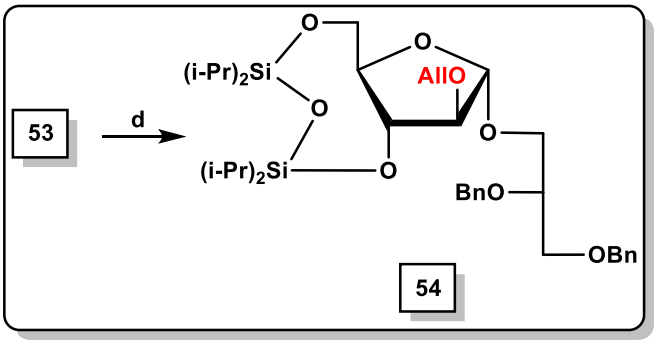
Figure 26: HSQC-NMR of compound **(52)**.

Deprotection of the benzoyl ester-protecting group of the glycan **(52)** under Zemplén conditions using methanol and a catalytic amount of sodium methoxide afforded **(53)** in 89% yield **(Scheme 8)**.



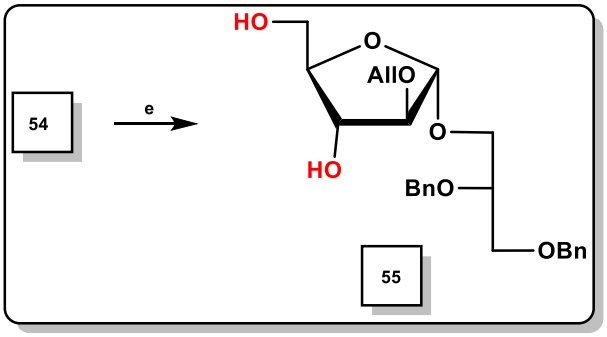
Scheme 8: Reagents & conditions: (c) $NaOCH_3$, CH_3OH: CH_2Cl_2 (1:1), R.T.,1 h, 89%.

The next step was the protection of the free hydroxyl group at the C-2 position using an allyl (All) group. This was achieved through reaction with allyl bromide (AllBr) and NaH in dry DMF at 0 ºC to give **(54)** in 75% yield **(Scheme 9)**.



Scheme 9: Reagents & conditions: (d) NaH, AllBr, DMF, 0 ºC/R.T., 1 h, 75%.

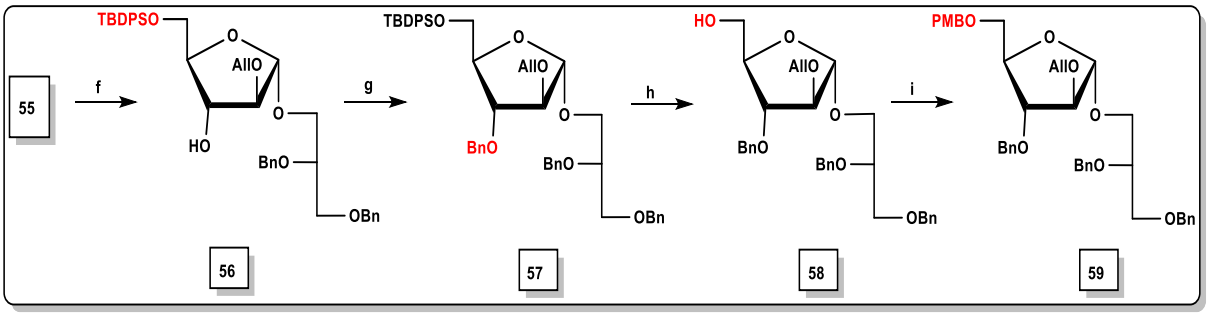
Removal of the silyl group *(tetra*-isopropyldisiloxane) was achieved using fluoride ion [tetrabutylammonium fluoride solution 1.0 M (TBAF)] in dry THF. This gave two free hydroxyl groups at the C-3 and C-5 positions **(55)** in 95% yield **(Scheme 10)**.



Scheme 10: Reagents & conditions: (e) TBAF, THF, 0 ºC/R.T., 16 h, 95%.

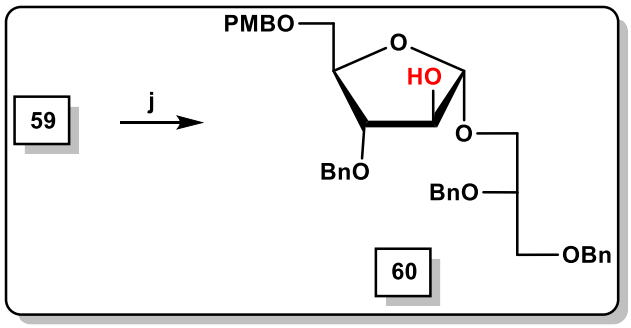
Two different protecting groups were required at the C-3 and C-5 positions of the DMAG's acceptor. The presence of a *p*-methoxybenzyl (PMB) protecting group at the C-5 position on the acceptor is found to give good β-selectivity when coupling with the donor to form a disaccharide.[144] In order to obtain this, the hydroxyl group at the C-5 position in **(55)** was

firstly protected using *tert*-butyldiphenylsilyl ether (TBDPS) to afford **(56)** in 65% yield, followed by protection of the hydroxyl group at the C-3 position with a benzyl (Bn) group using benzyl bromide (BnBr) and sodium hydride in DMF at 0 ºC to give **(57)** in 72% yield. De-silylation using TBAF to give a free hydroxyl group at the C-5 position was then carried out affording **(58)** in 91% yield, which was then treated with sodium hydride and freshly prepared *p*-methoxybenzyl bromide (PMBBr) in dry DMF to give **(59)** in 76% yield **(Scheme 11)**. The formation of all these compounds **(50-59)** was proved by NMR and I.R spectroscopy and mass spectrometry.



Scheme 11: Reagents & conditions: (f) *t*-$BuPh_2SiCl$, imidazole, DMF, 0 ºC/R.T., 1/2 h, 65%; (g) NaH, BnBr, DMF, 0 ºC/R.T., 2 h, 72%; (h) TBAF, THF, 0 ºC/R.T.,16 h, 91%; (i) NaH, PMBBr, DMF, 0 ºC/R.T., 2 h, 76%.

The final step to prepare the target acceptor was the removal of the allyl protecting group at the C-2 position, which was successfully achieved by stirring **59** in a mixture of CH_3OH:CH_2Cl_2 and adding 0.2 equivalents of $PdCl_2$ to give the target molecule, the DMAG's acceptor **(60)** in 84% yield **(Scheme 12)**.



Scheme 12: Reagents & conditions: (j) $PdCl_2$, CH_3OH:CH_2Cl_2 (1:1), R.T., 16 h, 84%.

Formation of the target acceptor **(60)** was confirmed by ^{1}H-NMR, which showed one downfield signal as a broad singlet at δ 5.03 corresponding to the proton attached to the C-1 position on the sugar, while the ^{13}C-NMR spectrum showed a signal at δ 109.3 corresponding to the C-1 position on the glycan ring. **Figure (27 A)** shows the correlation of these signals by HSQC-NMR.

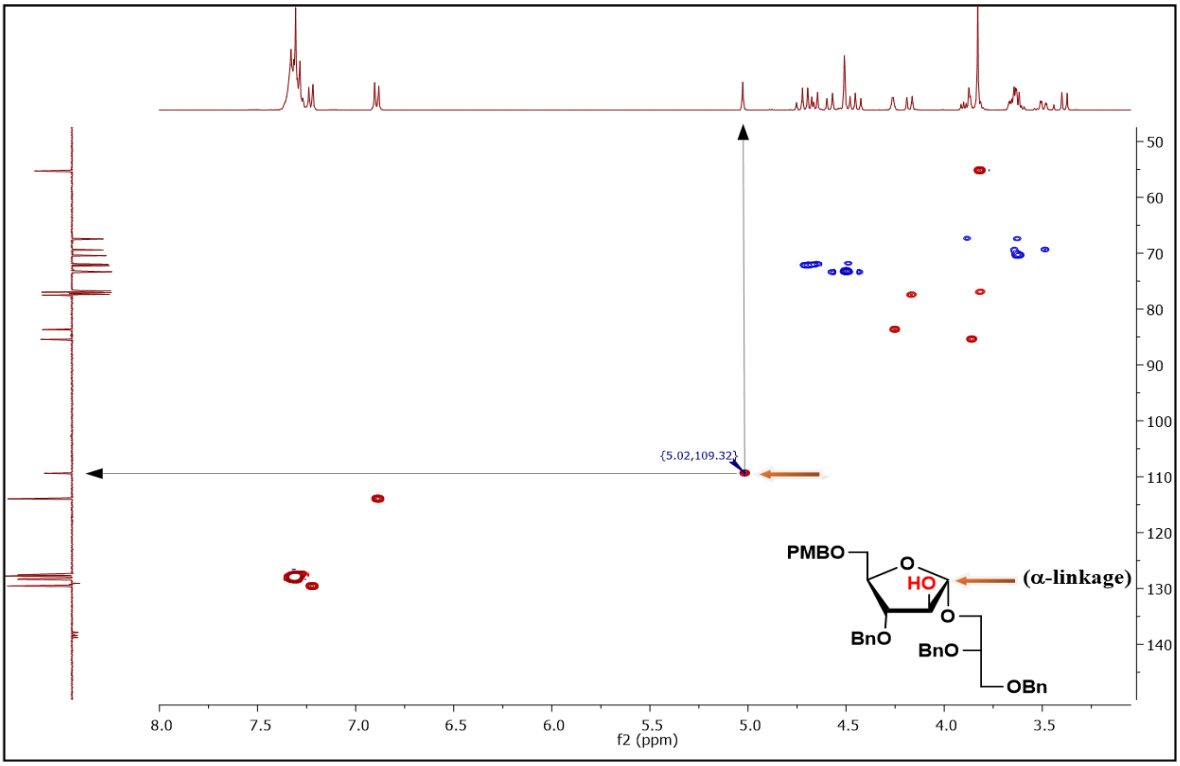


Figure 27 A: HSQC-NMR of the DMAG's acceptor compound **(60)**.

The structure of **(60)** was also confirmed by mass spectrometry. Nano Electrospray Ionization (NSI) mass technique, was used to reduce fragmentation and enhance the abundance of the intact molecular ion. The [NSI–Found $(M+NH_4)^+$: 632.3209; $C_{37}H_{46}O_8N$, requires: 632.3218] **(Figure 27 B)**, and the specific rotation which was $[\alpha]_D^{22}$ +41.7 (*c* 1.6, $CHCl_3$) for **(59)** changing to $[\alpha]_D^{22}$ +60.0 (*c* 4.6, $CHCl_3$) for the target molecule **(60)**.

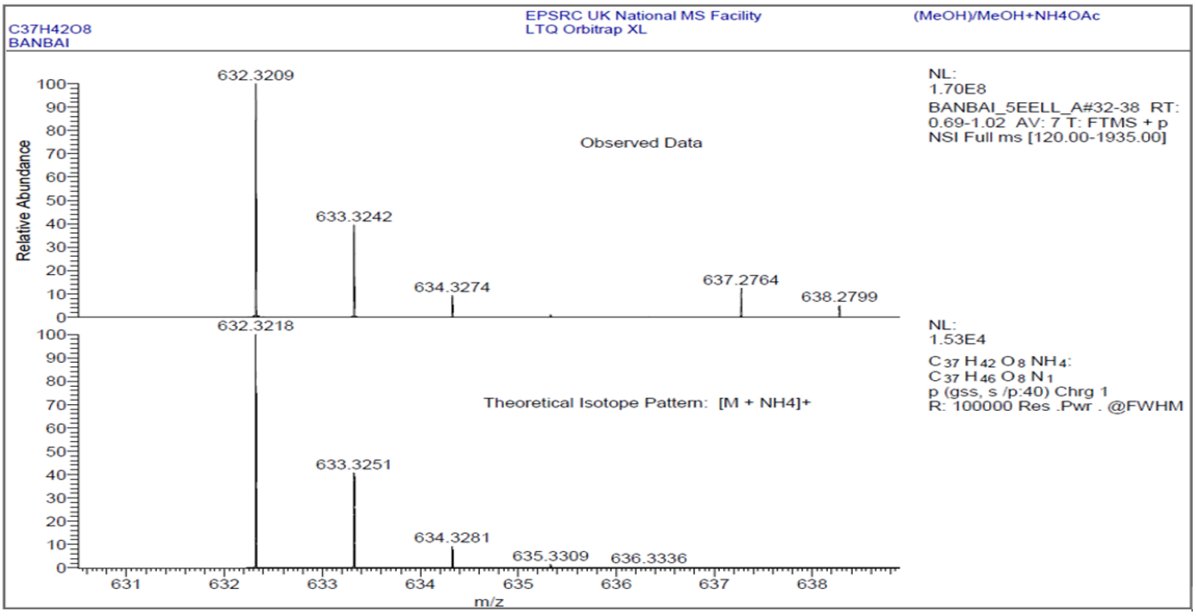
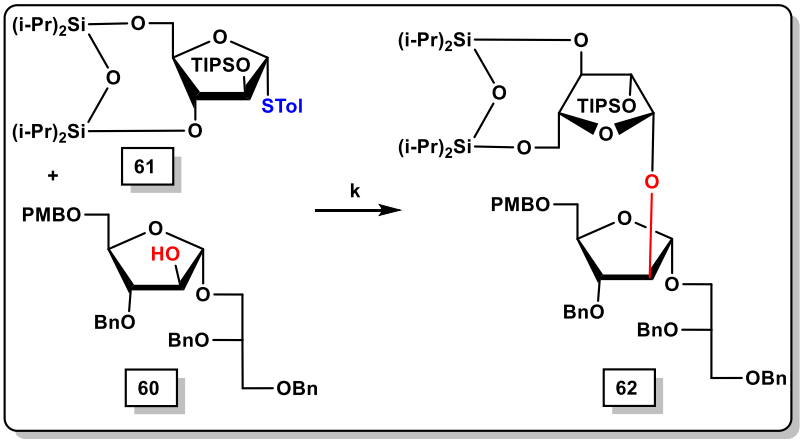


Figure 27 B: Mass spectrum of the DMAG's acceptor compound **(60)**.

2.1.3 Coupling the donor and the acceptor

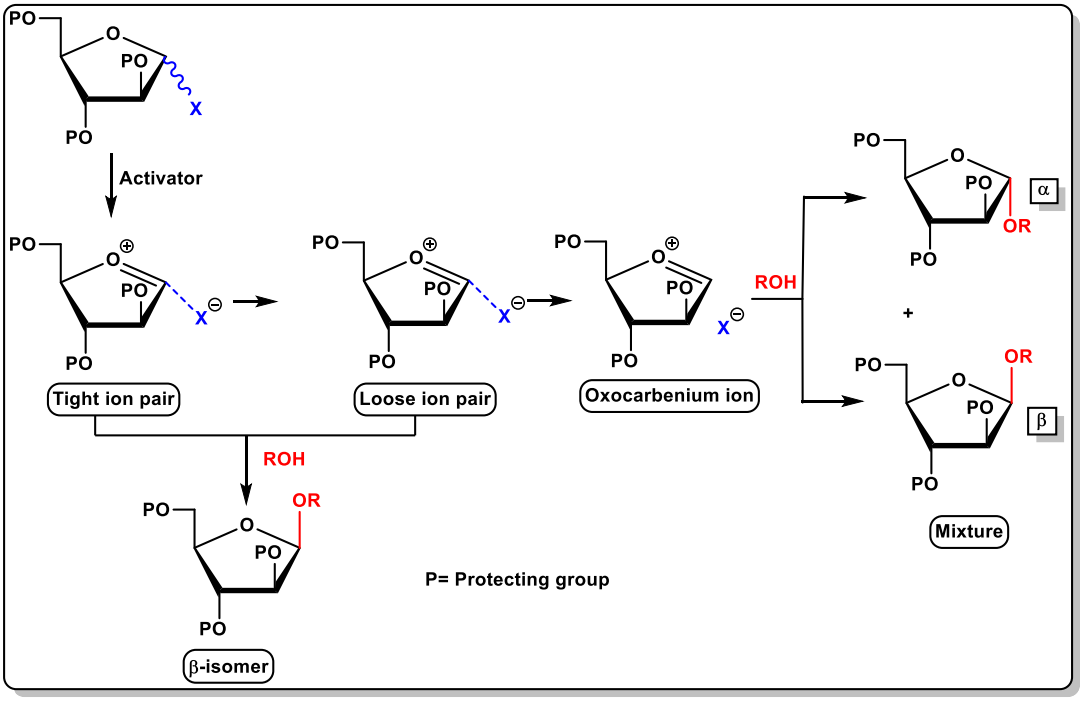
As mentioned earlier, good β-selectivity can be obtained in the case of glycosylation of active acceptors and armed donors.[145] Active acceptors are species that are not sterically hindered and possess electron-donating protecting groups and thus are more nucleophilic, while armed donors are referred to as those protected with electron-rich ethers. The next step was the coupling of the newly synthesized active acceptor **(60)**, and the armed donor **(61)**, which was prepared as reported in the literature,[145] to afford the di-saccharide **(62) (Scheme 13)**.



Scheme 13: Reagents & conditions:(k) NIS/AgOTf, CH_2Cl_2, -78 ºC, 86%.

The reaction of the 5-*O*-*p*-methoxybenzylated glycosyl acceptor **(60)** and thioglycoside donor **(61)** was carried out in dry CH_2Cl_2 by cooling the reaction mixture to -78 ºC. Then NIS and AgOTf were added and the reaction was warmed to -60 ºC over 60 – 90 min. The reaction was quenched with trimethylamine and after work-up afforded the desired disaccharide **(62)** in 86% yield with excellent β-selectivity, as confirmed by the ^1H, ^{13}C and 2D-NMR spectra.

It was desirable to carry out the coupling reaction between the acceptor and the donor at low temperature (-78 ºC) in order to obtain an oxocarbenium ion pair, instead of the free oxocarbenium ion intermediate. It has been suggested that at higher temperatures, it is possible that this ion pair would dissociate quickly to give the free oxocarbenium ion, which would give mixtures of products. This suggested pathway is consistent with earlier studies on the glycosylation of fully protected arabinofuranosyl chlorides **(Scheme 14)**.[240]



Scheme 14: Possible mechanism of the glycosylation from the low temperature activation of thioglycosides.

The ^{1}H-NMR spectrum **(Figure 28A)** of the new di-saccharide **(62)** indicated the presence of two different anomeric hydrogens due to the two signals downfield at δ 4.96 (br. s) and at δ 4.79 (br.d, *J* 4.3 Hz) corresponding to the protons at the α- and β-anomeric centres respectively. The ^{13}C-NMR spectrum **(Figure 28B)** confirmed the presence of glycosidic linkages in the glycan due to the signals at δ 106.2 and δ 100.6 ppm belonging to the two carbons at the α- and β-anomeric centres respectively.

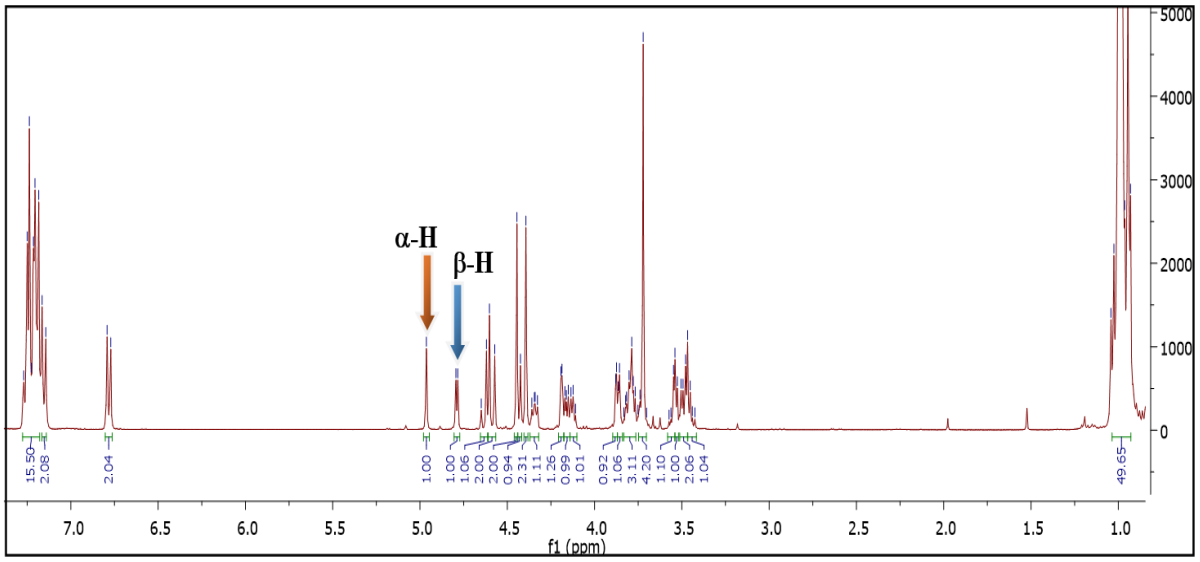


Figure 28 A: ^{1}H-NMR of the di-saccharide **(62)**.

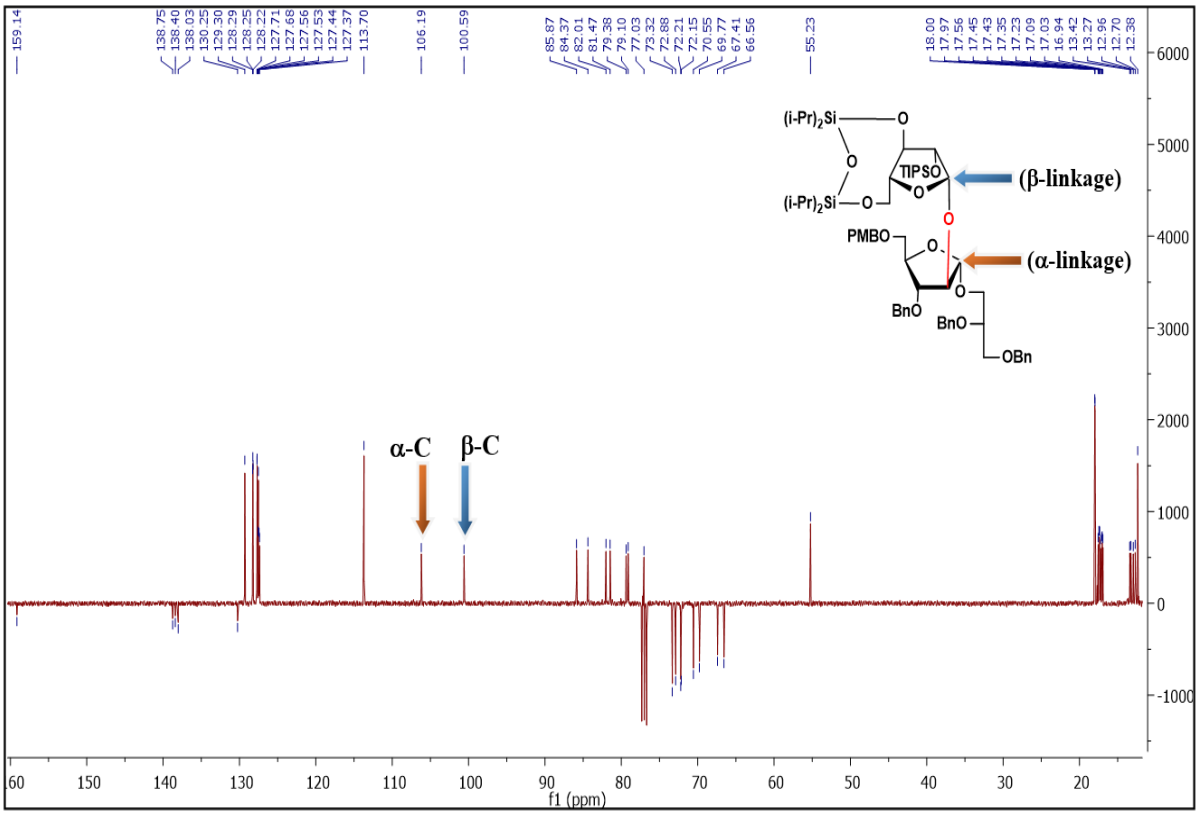


Figure 28 B: ^{13}C-NMR of the di-saccharide compound **(62)**.

The HSQC-NMR spectrum **(Figure 29 A)** confirmed the structure of **(62)**, where the acetal proton signals were correlated to their carbons.

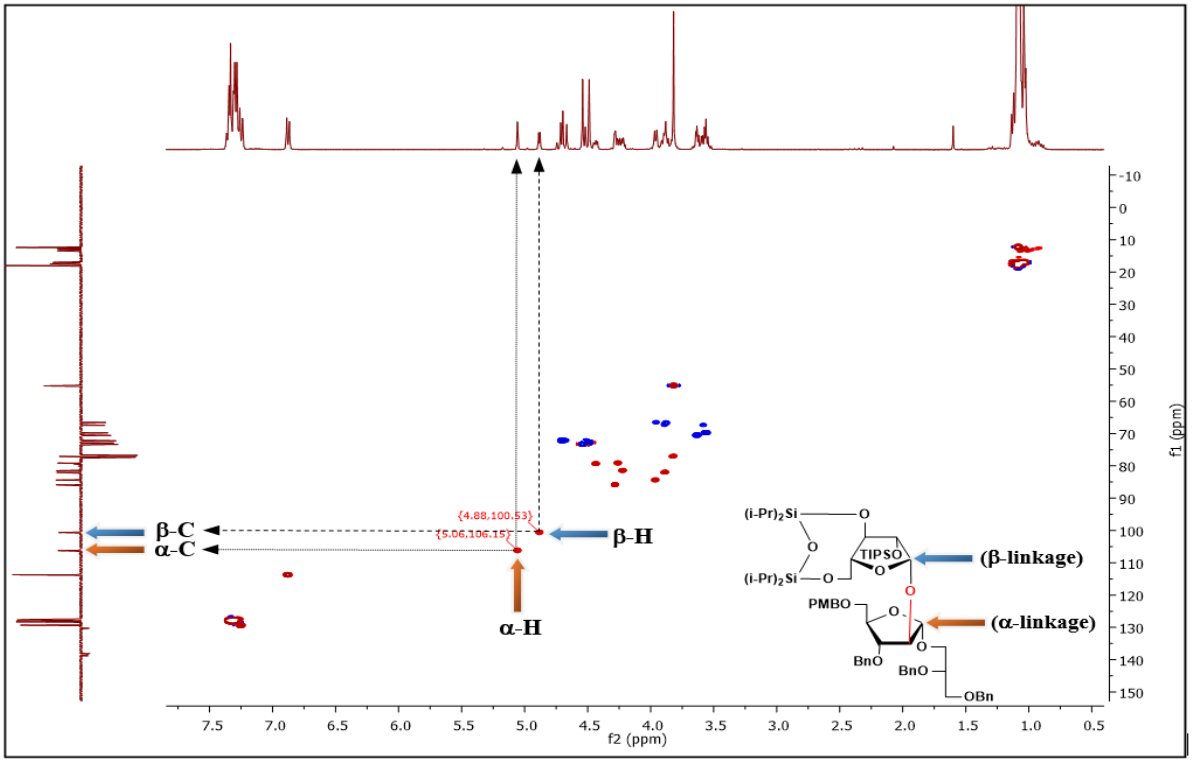


Figure 29 A: HSQC-NMR of the di-saccharide compound **(62)**.

The structure of **(62)** was also confirmed by mass spectrometry [NSI–Found $(M+NH_4)^+$: 1162.6495; $C_{63}H_{100}O_{13}Si_3N$, requires: 1162.6497] **(Figure 29 B)**.

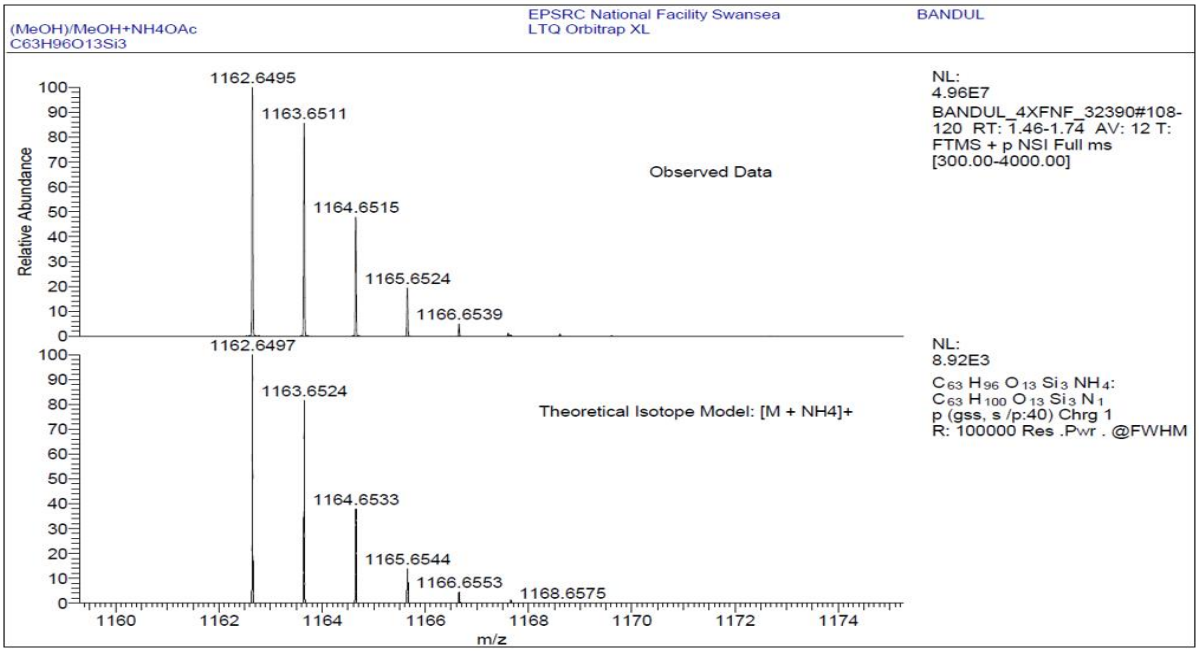
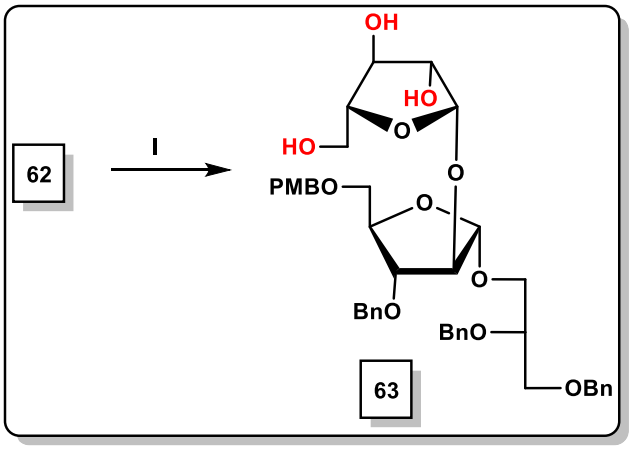


Figure 29 B: Mass spectrum of the di-saccharide compound **(62)**.

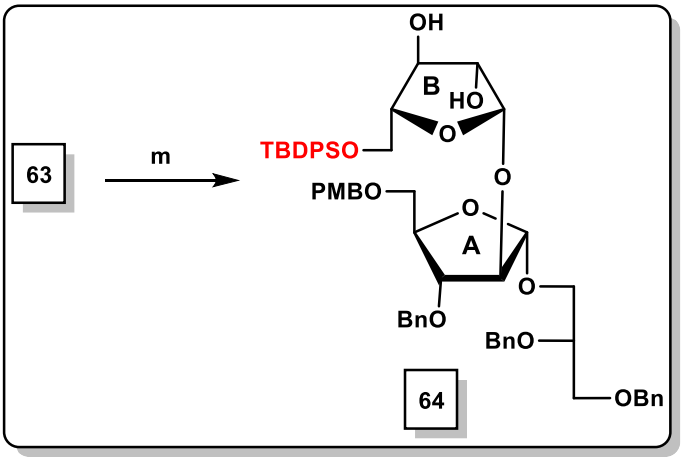
Since the di-arabino glycerol (DAG) derivative was planned to be used as precursor for a range of mono- and di-substituted glycolipids, two orthogonal protecting groups were required for the primary hydroxyl groups in the C-5 positions of the arabinosyl residue. This would allow one protecting group to be removed in the presence of the other, later in the synthesis, so that symmetrical or unsymmetrical glycolipids could be prepared. This will be discussed in more detail later **(see page 73)**.

Subsequently, the cyclic siloxane-protecting group in the pure β-anomer **(62)** was removed by reacting with TBAF for 6 h in dry THF to give the tri-ol **(63)** in 95% yield **(Scheme 15)**.



Scheme 15: Reagents & conditions: (l) TBAF, THF, 0 ºC/R.T., 6 h, 95%.

The first primary hydroxyl group (in the bottom arabinose molecule) has been successfully protected with a PMB group, through the synthesis of the DMAG's acceptor. The new primary hydroxyl group (in the top arabinose molecule) needed to be protected with a different group, and in order to achieve that, compound **(63)** was protected with a TBDPS group, in the presence of imidazole in dry pyridine at 0 ºC, to afford **(64)** in 77% yield **(Scheme 16)**.



Scheme 16: Reagents & conditions: (m) *t*-$BuPh_2SiCl$, imidazole, DMF, 0 ºC/R.T., 2 h, 77%.

The structure of this compound was confirmed by NMR spectroscopy and mass spectrometry. **Figures 30 and 31** show the proton and HSQC-NMR where the key peaks corresponding to the two different protecting groups (*i.e.* TBDPS and PMB) at the primary carbons (C-5 positions) of each arabinose molecule are shown.

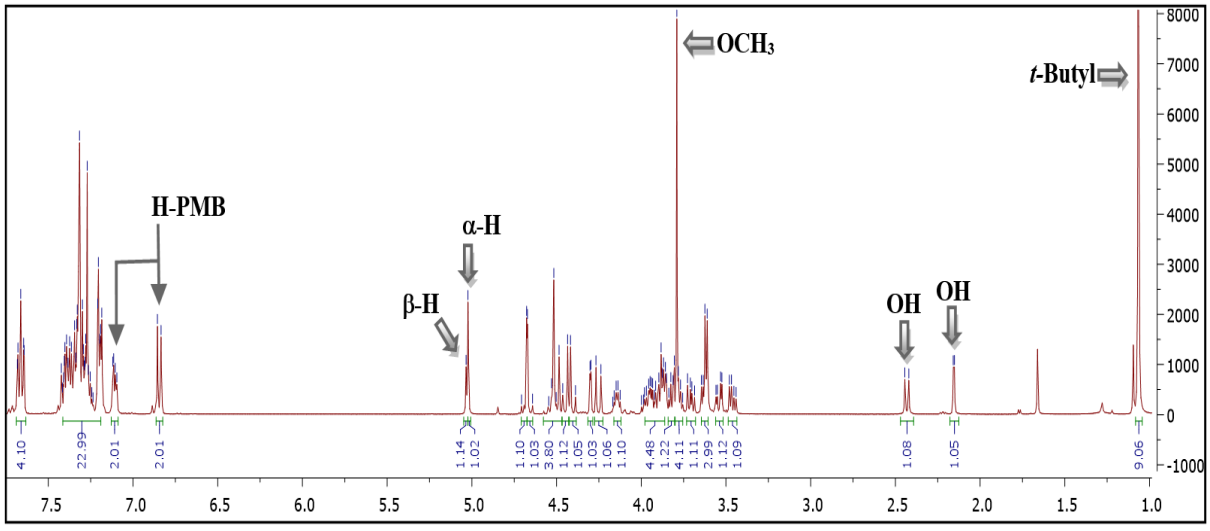


Figure 30: ^{1}H-NMR of the di-saccharide compound **(64)**.

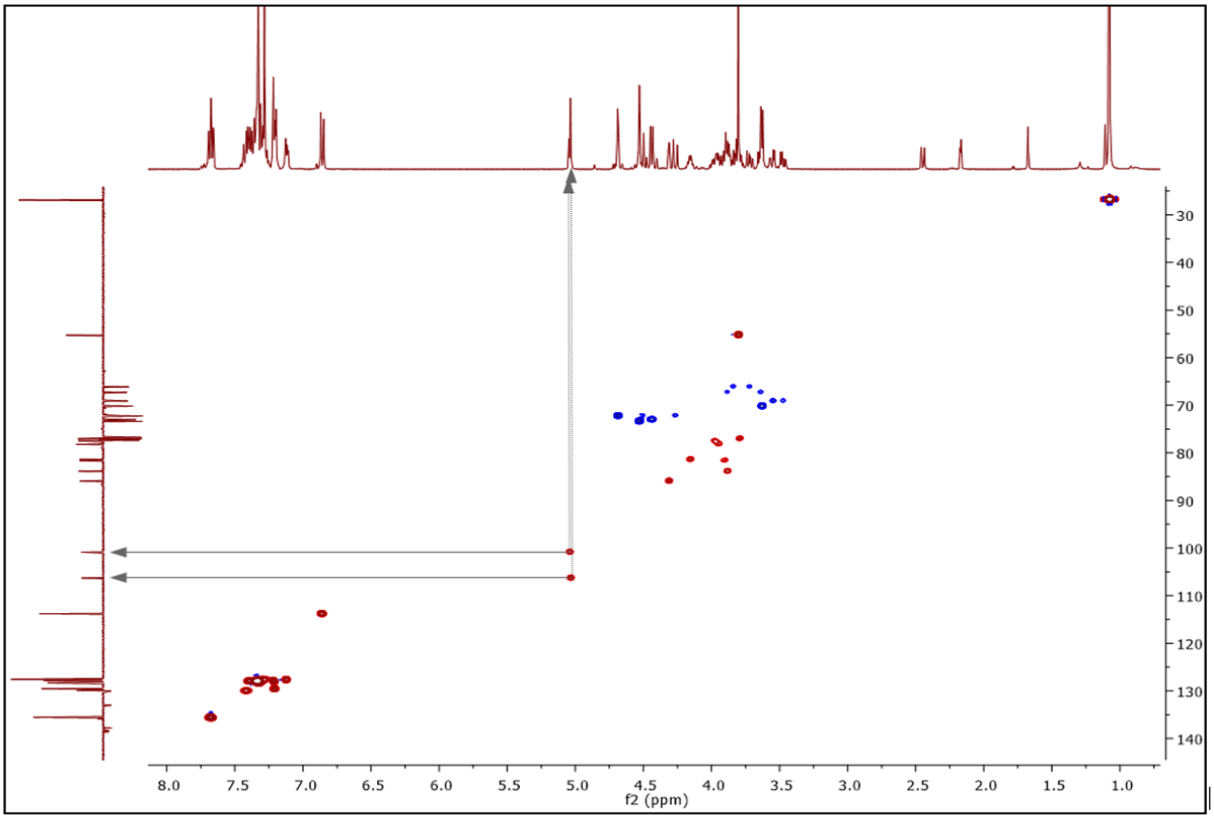
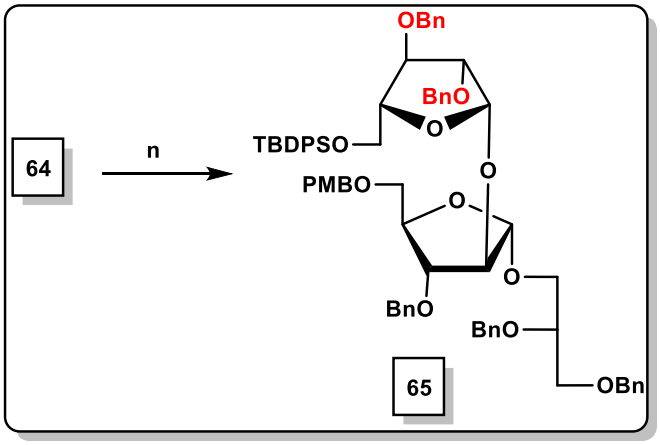


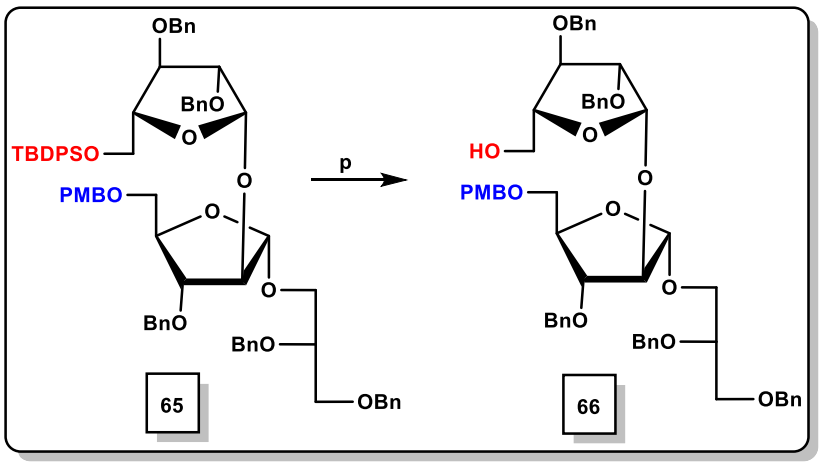
Figure 31: HSQC-NMR of the di-saccharide compound **(64)**.

The next step was the protection of the free hydroxyl groups at the C-2 and C-3 positions (in the top arabinose molecule) of compound **(64)** by reaction with benzyl bromide (BnBr) and NaH in dry DMF at 0 ºC – R.T for 2 h to afford **(65)** in 90% yield **(Scheme 17)**. This compound is a key intermediate for the preparation of the glycolipids selectively as will be illustrated later **(see page 73)**.



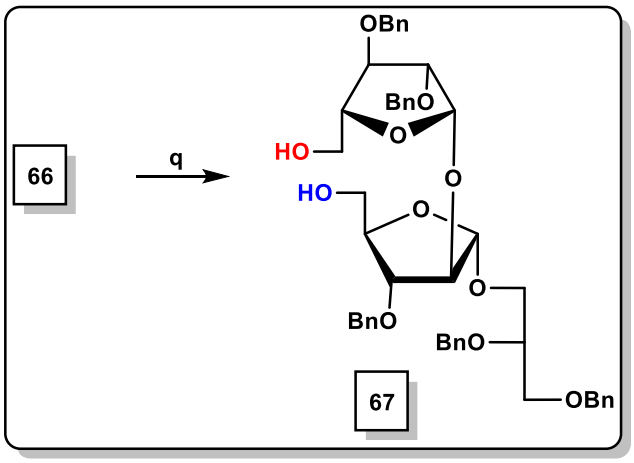
Scheme 17: Reagents & conditions: (n) NaH, BnBr, DMF, 0 ºC/R.T., 2 h, 90%.

Having successfully synthesised **(65)**, the next step was to investigate the coupling of the disaccharide with commercially available simple fatty acids. In order to achieve this, de-protection of the two hydroxyl groups at the C-5 positions was required. Firstly, the TBDPS group on the top arabinose molecule was removed by subjecting compound **(65)** to TBAF in dry THF at 0 ºC, affording **(66)** in 93% yield **(Scheme 18)**.



Scheme 18: Reagents & conditions: (p) TBAF, THF, 0 ºC/R.T., 6 h, 93%.

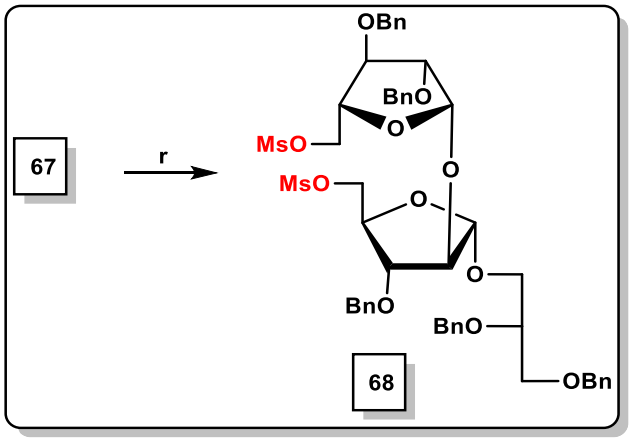
After the first de-protection, the alcohol **(66)** was treated with cerium ammonium nitrate (CAN) in a mixture of CH_3CN:H_2O (9:1) at 0 ºC to R.T for 1h to remove the PMB group, when TLC indicated the conversion had finished. The mixture was diluted with chloroform, washed with aq. $NaHCO_3$, dried ($MgSO_4$) and the solvent was evaporated under reduced pressure, the product was purified to afford the di-ol **(67)** in 89% yield **(Scheme 19).**



Scheme 19: Reagents & conditions: (q) Cerium ammonium nitrate (CAN)/ CH_3CN:H_2O (9:1), 0 ºC/R.T., 1h, 89%.

Finally, the benzyl-protected diol **(67)** was treated with methanesulfonyl chloride (MsCl) in dry pyridine and catalytic 4-dimethylaminopyridine **(**DMAP**)** at room temperature to activate

the free primary hydroxyl groups, affording the mesylate benzyl-protected di-saccharide **(68)** in 85% yield **(Scheme 20)**.



Scheme 20: Reagents & conditions: (r) CH_3SO_2Cl, DMAP, Pyr. 16 h, 85%.

Once again, the structure of **(68)** was confirmed by NMR spectroscopy, which gave the signals illustrated in **Table 2**. These assignments were made based on literature values for the arabinoglycerol fragment,[241] and a detailed analysis of the HSQC-NMR spectrum for the top arabinose **(Figure 33)**.

Table 2: ^{1}H and ^{13}C-NMR data analysis of the mesylate compound **(68)**.

Proton	Shift	H's	Multiplicity	*J/Hz*	Carbon	*δ/ppm*
$H_{\text{-aromatic}}$	7.27	25	m	-	$C_{1\text{-alpha}}$	106.4
H_b	5.01	1	br.d	4.4	C_2	81.1
H_a	4.93	1	br. s	-	C_3	83.5
$H_{\text{-Bn}}$	4.70	1	d	11.7	C_4	85.9
$H_{\text{-Bn}}$	4.67	1	d	11.7	C_5	69.9
$H_{\text{-Bn}}$	4.63	2	br.s	-	C_6	37.5
$H_{\text{-Bn}}$	4.58	1	d	11.7	$C_{1\text{''-beta}}$	101.2
$H_{\text{-Bn}}$	4.51	1	d	11.7	$C_{2\text{''}}$	78.4
$H_{\text{-Bn}}$	4.46	4	br.s	-	$C_{3\text{''}}$	80.9
H_h	4.32	1	br. q	4.6	$C_{4\text{''}}$	83.5
$H_{i,\ l',\ n,\ n',f}$	4.20	5	m	-	$C_{5\text{''}}$	69.8
$H_{g,j,k}$	4.10	3	m	-	$C_{6\text{''}}$	37.4
H_m	4.00	1	br.dd	4.4, 6.9	$C_{\text{-Bn}}$	72.7
H_c	3.80	1	dd	5.2, 10.3	$C_{\text{-Bn}}$	72.6
H_d	3.74	1	br. p	5.1	$C_{\text{-Bn}}$	72.4
$H_{c',\ e,\ e'}$	3.56	3	br. dd	4.6, 7.2	$C_{\text{-Bn}}$	72.3
$(CH_3)_p$	2.85	3	s	-	$C_{\text{-Bn}}$	73.4
$(CH_3)_{p'}$	2.84	3	s	-	$C_{1'}$	69.0
-	-	-	-	-	$C_{2'}$	76.9
-	-	-	-	-	$C_{3'}$	67.3

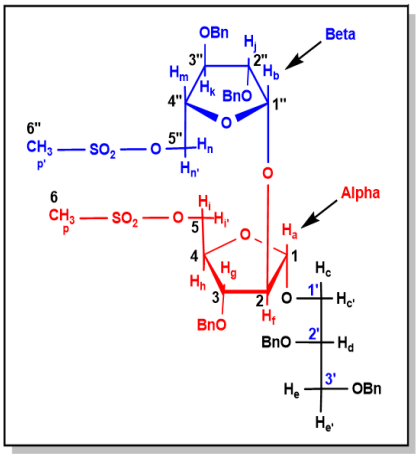


Figure 32: Structure of the mesylate **(68)**.

The specific rotation of **(67)** was $[\alpha]_D^{21}$ - 4.3 and changed to $[\alpha]_D^{22}$ +2.8 for the target molecule **(68)**.

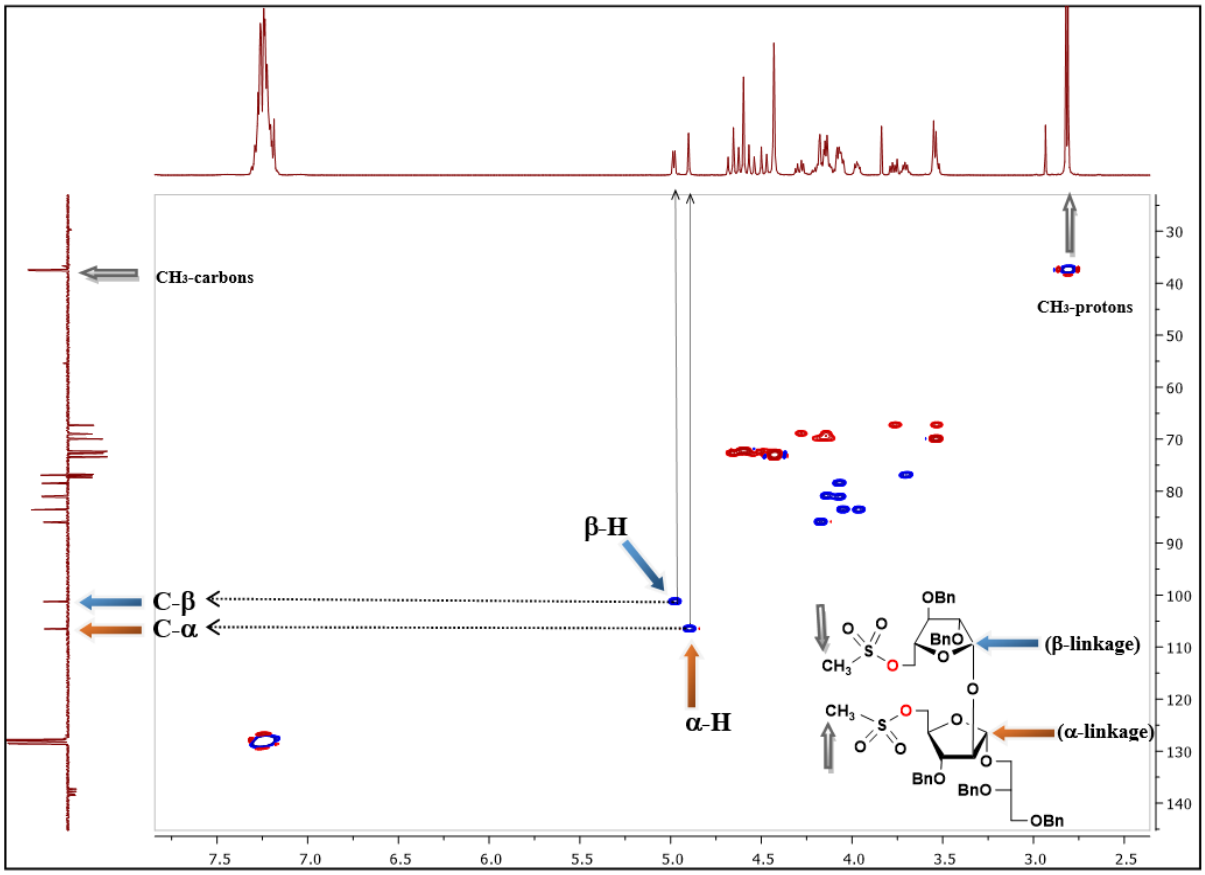


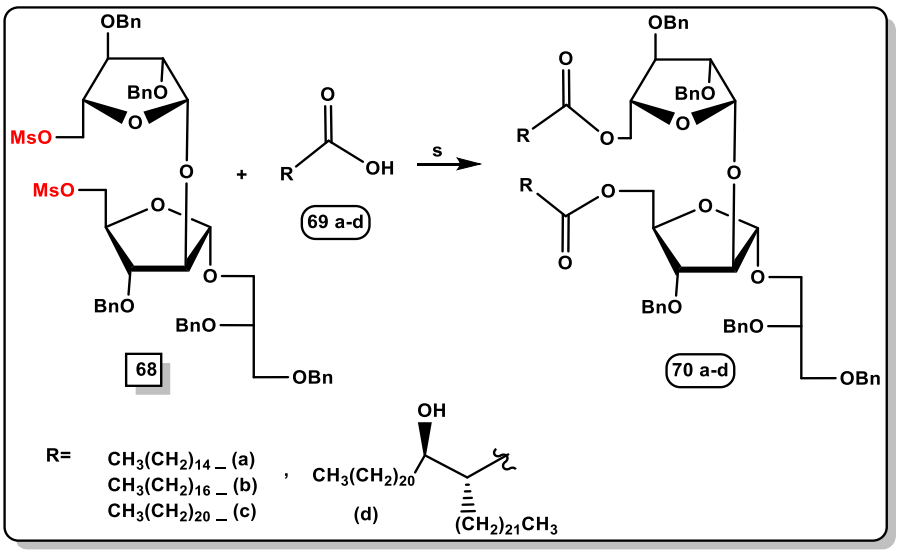
Figure 33: HSQC-NMR of the mesylate compound **(68)**.

For the initial series of analogues, fatty acids that are commercially available were chosen, as it was expected that they would be easy to connect to the glycan (hydroxylated or mesylated) by esterification. Two different methods were used to achieve the esterification:

1. An alkylative coupling using cesium hydrogen carbonate ($CsHCO_3$) after mesylation of the primary hydroxyl groups in the glycan; this method was employed when using free simple fatty acids or unprotected mycolic acids (*i.e.* those with a free hydroxyl group at the β- position).
2. Direct coupling of the glycan alcohol with the fatty acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI); this coupling method was used when the mycolic acids were protected (*i.e.* did not have a free hydroxyl group at the β- position).

2.1.4 Esterification of the glycan (68) with simple fatty acids using the $CsHCO_3$ method

The di-mesylate **(68)** was reacted with three different simple fatty acids **(69a-c)**, and a model β-hydroxy acid **(69d)** *via* the alkylative esterification strategy, using caesium hydrogen carbonate as a coupling reagent in dry THF:DMF (5:1) at 70 ºC for 4 days, to give the expected fully protected di-esters **(70a-d)** in 92, 89, 87 and 55% yield respectively **(Scheme 21)**. Mass spectrometry of the products confirmed the structures (as expected) as did I.R. and NMR spectroscopy.



Scheme 21: Synthesis of fully protected DMAG glycolipid analogues **(70 a-d)**: Reagents & conditions: (s) $CsHCO_3$, THF: DMF (5:1), 70 ºC 3 days, 92, 89, 87 and 55% respectively.

As an example, the ^{1}H-NMR spectrum for the fully protected di-stearyl di-arabino-glycerol **(70b)**, showed two downfield signals at δ 5.01 (br.d *J* 4.2 Hz) and δ 4.95 (br.s) corresponding to the protons of the glycan at the β- and α-anomeric centers respectively. Protons corresponding to the CH_2 of the five-benzyl groups appeared between δ 4.69 – 4.38. The remaining 15 protons on the sugar moiety and the glycerol appeared in the range from δ 4.30 – 3.55. Four protons corresponding to the CH_2 adjacent to the carbonyl groups in the acid came around δ 2.32 as a triplet (*J* 7.6 Hz) and δ 2.18 as a double triplet (*J* 1.9, 7.4 Hz) respectively. The CH_2 chain ranged from δ 1.69 – 0.95 and the protons of the terminal CH_3 of the acid part came up-field around δ 0.85 as a triplet (*J* 6.8 Hz).

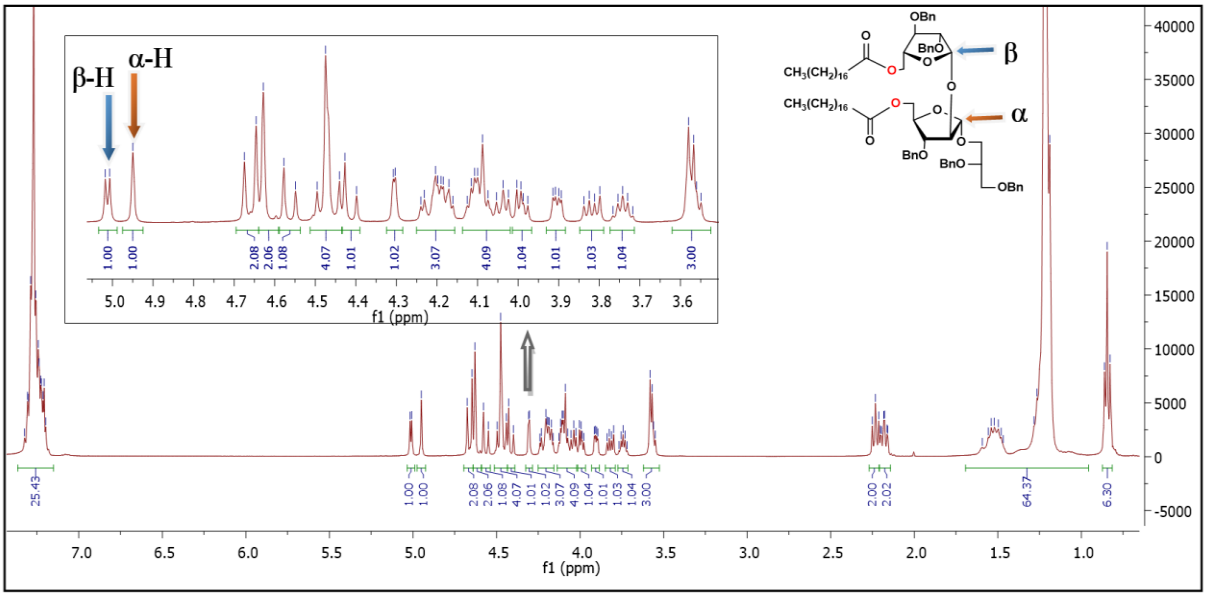


Figure 34: ^{1}H-NMR of fully protected DMAG analogue compound **(70b)**.

The ^{13}C-NMR spectrum showed two carbonyl signals at δ 173.5 and δ 173.3. Signals for the carbon at the α-anomeric centre was at δ 106.1 while that at the β-anomeric centre was at δ 100.4. The remaining sugar and glycerol carbons were in the region of δ 85.5 – 63.7. The carbons for the CH_2 chain ranged from δ 34 - 22 and the carbons of the CH_3 came up-field around δ 14.1. The specific rotation of **(68)** was $[\alpha]_D^{22}$ +2.8 changing for the protected di-ester **(70b)** to $[\alpha]_D^{22}$ -14.6. The I.R. spectrum of **(70b)** gave bands for the two carbonyl groups at 1740 and 1731 cm^{-1}.

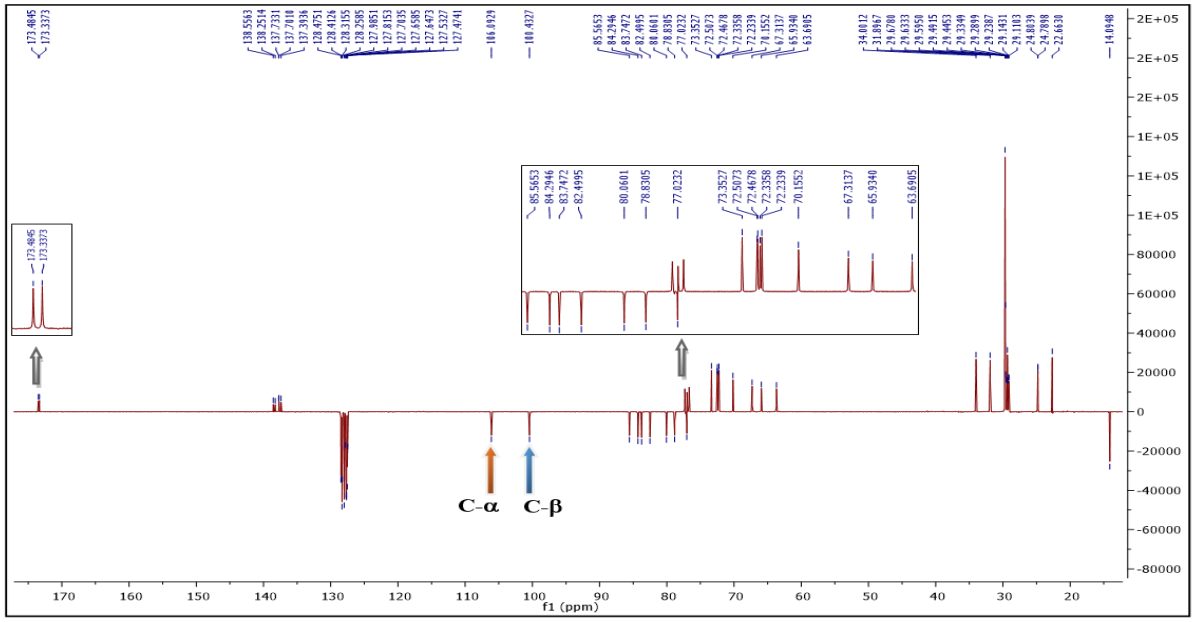
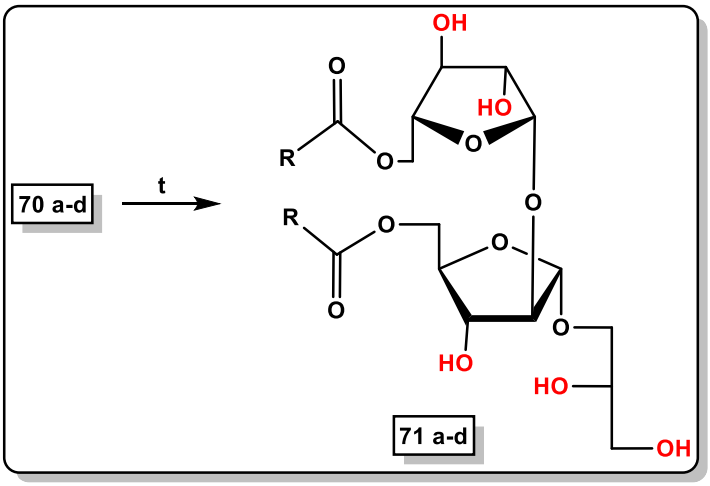


Figure 35: ^{13}C-NMR of fully protected DMAG analogue compound **(70b)**.

The protected esters **(70a-d)** were then subjected to hydrogenolysis to afford the target DMAG glycolipid analogues **(71a-d) (Scheme 22).**



Scheme 22: Synthesis of DMAG glycolipid analogues **(71 a-d)**: Reagents & conditions: (t) $(Pd(OH)_2$-C/20%), H_2 atmosphere, CH_3OH:CH_2Cl_2 (1:1), R.T., 36 h, 82, 81, 87 and 74% respectively.

The debenzylation was achieved by stirring **(70a-d)** in dry CH_2Cl_2:MeOH (1:1) in the presence of $Pd(OH)_2$ (0.75 eq. fold by weight) under a hydrogen atmosphere (using a hydrogen filled balloon)**.** Once again, mass spectrometry, I.R. and NMR spectroscopy proved the formation and the structure of the products **(71a-d)**. The ^{1}H-NMR spectrum of **(71b)** for example, showed clearly the disappearance of those signals corresponding to the methylene protons of the benzyl groups between δ 4.69 – 4.38, and the aromatic protons between δ 7.32 – 7.15. Two downfield signals appearing as a broad doublet at δ 5.01 (*J* 4.3 Hz) and a broad singlet at δ 5.00, corresponded to the protons at the β- and α-anomeric centers respectively. The remaining protons on the glycan moiety and the glycerol appeared in the range from δ 4.35 – 3.51. The CH_2 adjacent to the carbonyl groups in the acid came around δ 2.33 as a triplet (*J* 7.5 Hz). The CH_2 chain ranged from δ 1.7 – 0.90 and the protons of the terminal CH_3 of the acid part came up-field around δ 0.86 as a triplet (*J* 6.3 Hz). The ^{13}C-NMR spectrum showed two carbonyl signals at δ 174.3 and δ 174.0, while the α-anomeric centre appeared at δ 105.8 and that of the β-anomeric centre at δ 101.9. The remaining sugar and glycerol carbons were in the region of δ 88.5 – 63.3. The carbons of the CH_2 chain ranged from δ 34.0 – 22.0 and the carbons for the CH_3 groups came up-field around δ 14.0.

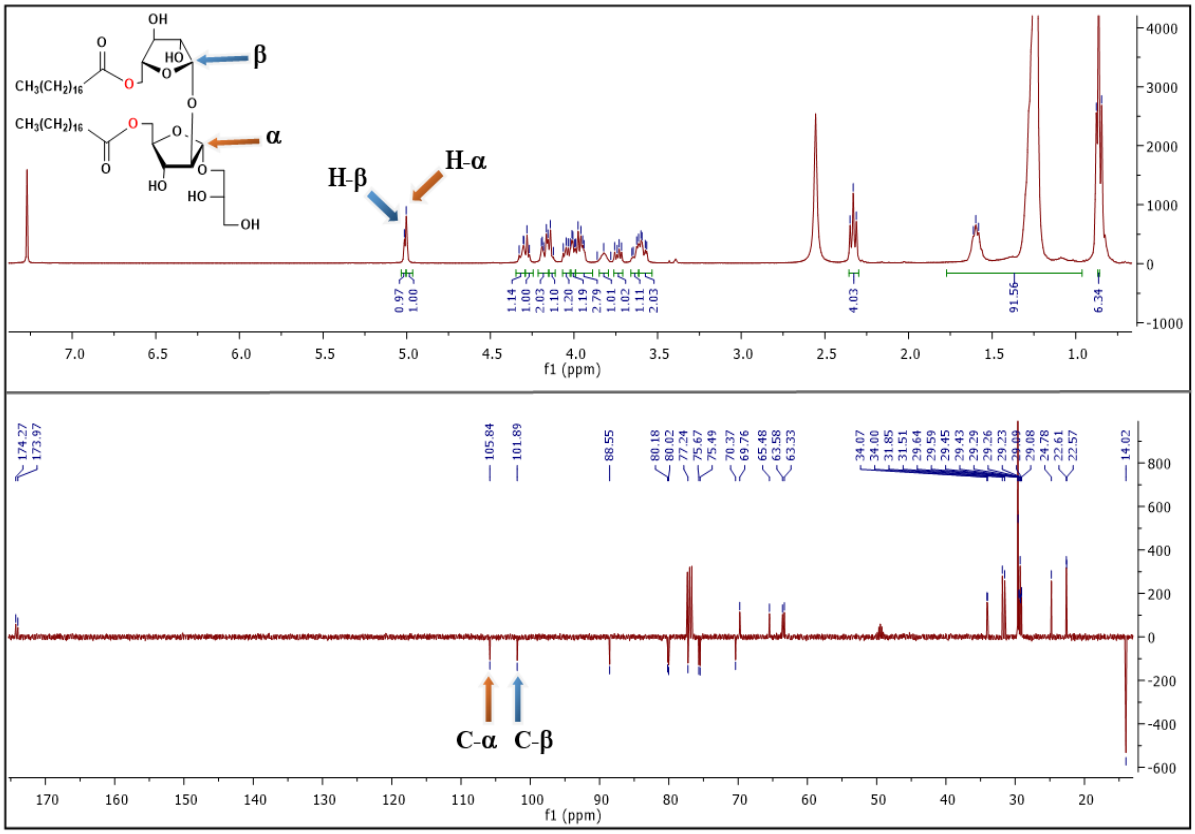


Figure 36: ^{1}H and ^{13}C-NMR of DMAG analogue compound **(71b)**.

The 2D-NMR spectrum **(Figure 37)** for **(71b)** showed the acetal proton signals were correlated to their carbons.

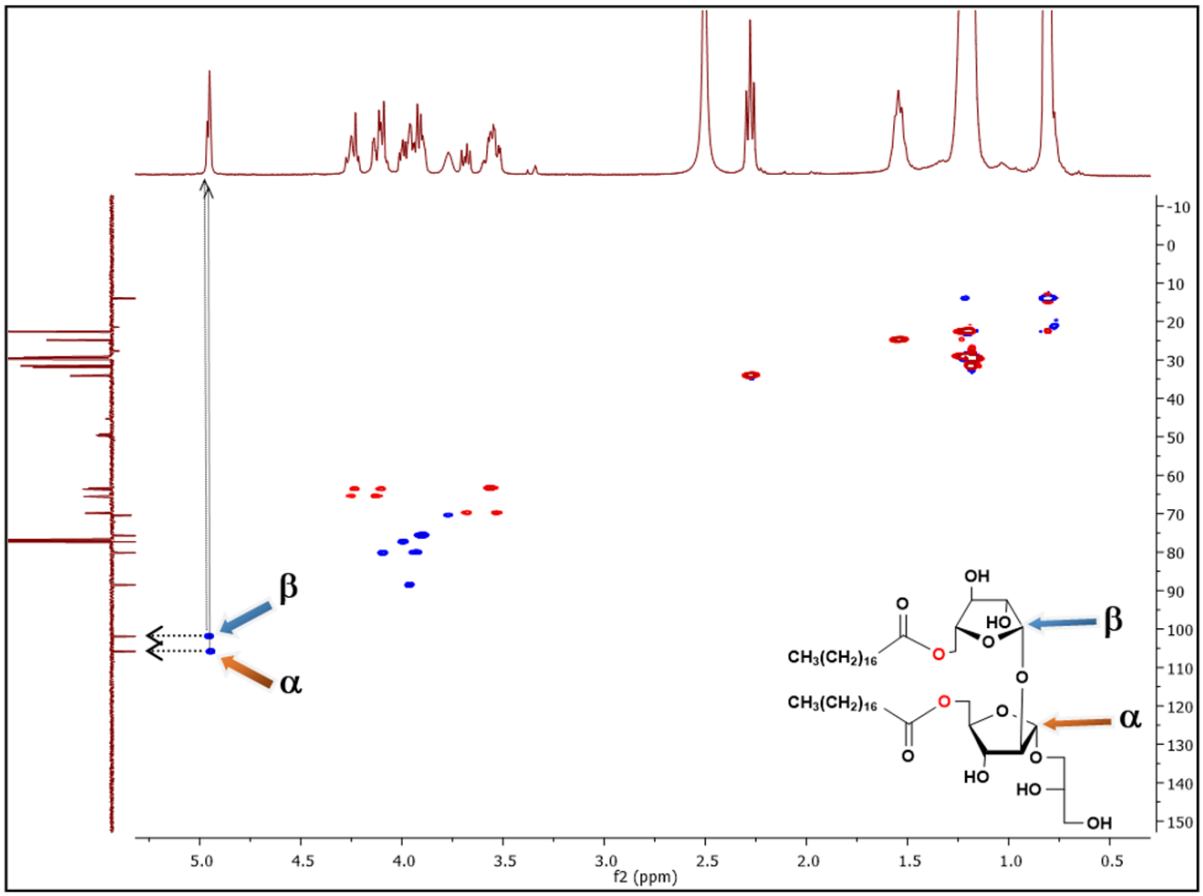


Figure 37: HSQC-NMR of DMAG analogue compound **(71b)**.

The specific rotation of **(70b)** was $[\alpha]_D^{22}$ -14.6 changing for the target DMAG glycolipid analogue **(71b)** to $[\alpha]_D^{25}$ -3.4. The MS gave the correct mass ion, and the I.R. spectrum of **(71b)** gave a broad absorbance at 3430 cm^{-1} for the five hydroxyl groups and a band for the carbonyl groups at 1737 cm^{-1}.

2.1.5 Comparison of the NMR data for the glycan part of the synthetic and natural DMAG

After successfully synthesising the DMAG analogues, a comparison of the synthesised DMAG glycolipid analogue (particularly the sugar part) with that reported for the natural mixture (as DMAG per-acetate) was needed to confirm the structure of the synthetic DMAG. Therefore, the five free hydroxyl groups in **(71c)** were acetylated with acetic anhydride in anhydrous pyridine at 0 ºC to give the penta-acetate **(72)** in 83% yield **(Figure 38)**.

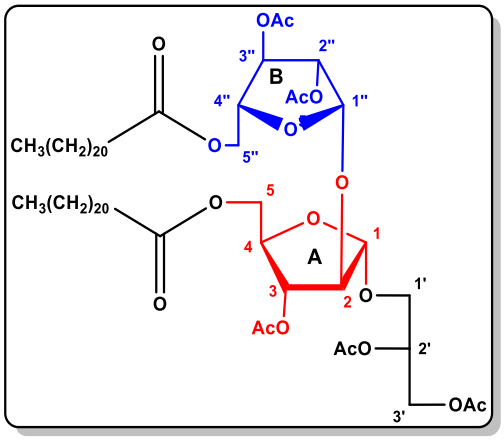


Figure 38: Structure of synthetic DMAG penta-acetate analogue compound **(72)**.

The NMR data for **(72)** gave the signals illustrated in **Table 3**.

Table 3: A comparison of di-arabino-glycerol fragment of carbon and proton NMR spectra of synthetic and natural DMAG penta-acetates.

	Natural DMAG peracetate[81]			**Synthetic DMAG penta-acetate analogue (72)**	
		^{13}C δ/ppm	***^{1}H Shift, Class, J/Hz***	***^{1}H Shift, Class, J/Hz***	***^{13}C δ/ppm***
Glycerol	**C1'**	65.3	3.60 (dd, *J* 4.5, 11.0), 3.80 (dd, *J* 5.2, 11.0)	3.60 (dd, *J* 4.5, 11.0), 3.80 (dd, *J* 5.2, 11.0)	65.3
	C2'	69.8	5.20	5.21(m)	69.8
	C3'	62.8	4.25 (dd, *J* 4.0, 11.7), 4.17 (dd, *J* 5.2, 11.7)	4.37 (dd, *J* 4.6, 11.6), 4.20 (m)	62.6
Arabinose B	**C1**	99.5	5.39 (d, *J* 4.7)	5.40 (br.d, *J* 4.7)	99.4
	C2	77.2	4.98 (dd, *J* 4.7, 6.6)	4.95 (br.dd, *J* 4.7, 6.6)	77.5
	C3	75.4	5.34 (dd, *J* 5.1, 6.6)	5.34 (dd, *J* 5.3, 6.3)	75.6
	C4	79.0	4.12 (dt, *J* 4.6, 5.1, 7.8)	4.12 (m)	79.1
	C5	65.2	4.38 (dd, *J* 4.6, 11.6), 4.22 (dd, *J* 7.8, 11.6)	4.37 (dd, 4.6, 11.6), 4.20 (m)	65.2
Arabinose A	**C1''**	105	4.91 (s)	4.91 (br.s)	105
	C2''	84.0	4.22 (m)	4.21 (m)	84.0
	C3''	77.5	4.98	4.95 (br.dd, *J* 4.7, 6.6)	77.5
	C4''	80.8	4.17	4.17 (m)	80.6
	C5''	63.8	4.18, 4.30 (dd, *J* 2.7,10.3)	4.18 (m)	63.6

The 2D-NMR spectrum **(Figure 39)** for **(72)** also showed the acetal proton signals were correlated to their carbons.

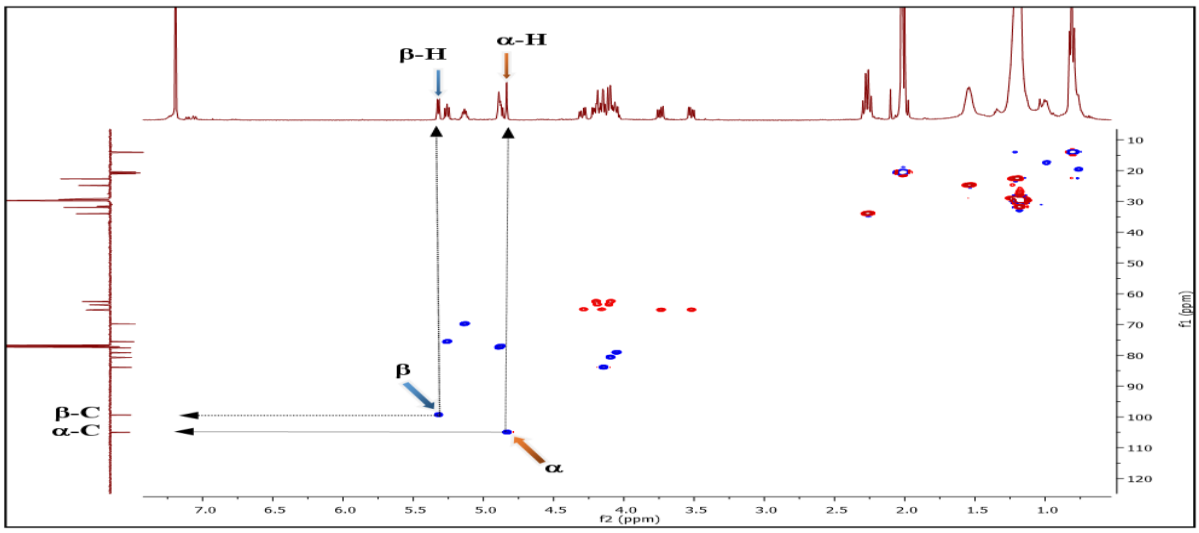


Figure 39: HSQC-NMR of DMAG penta-acetate analogue compound **(72)**.

The comparison of the reported data for the natural mixture (containing a mixture of mycolic acids) with that of the synthetic compound, confirmed the structure of the DMAG core and showed a very good agreement between the signals for the di-arabino-glycerol fragments of the natural and synthetic molecules **(Figure 40)**.

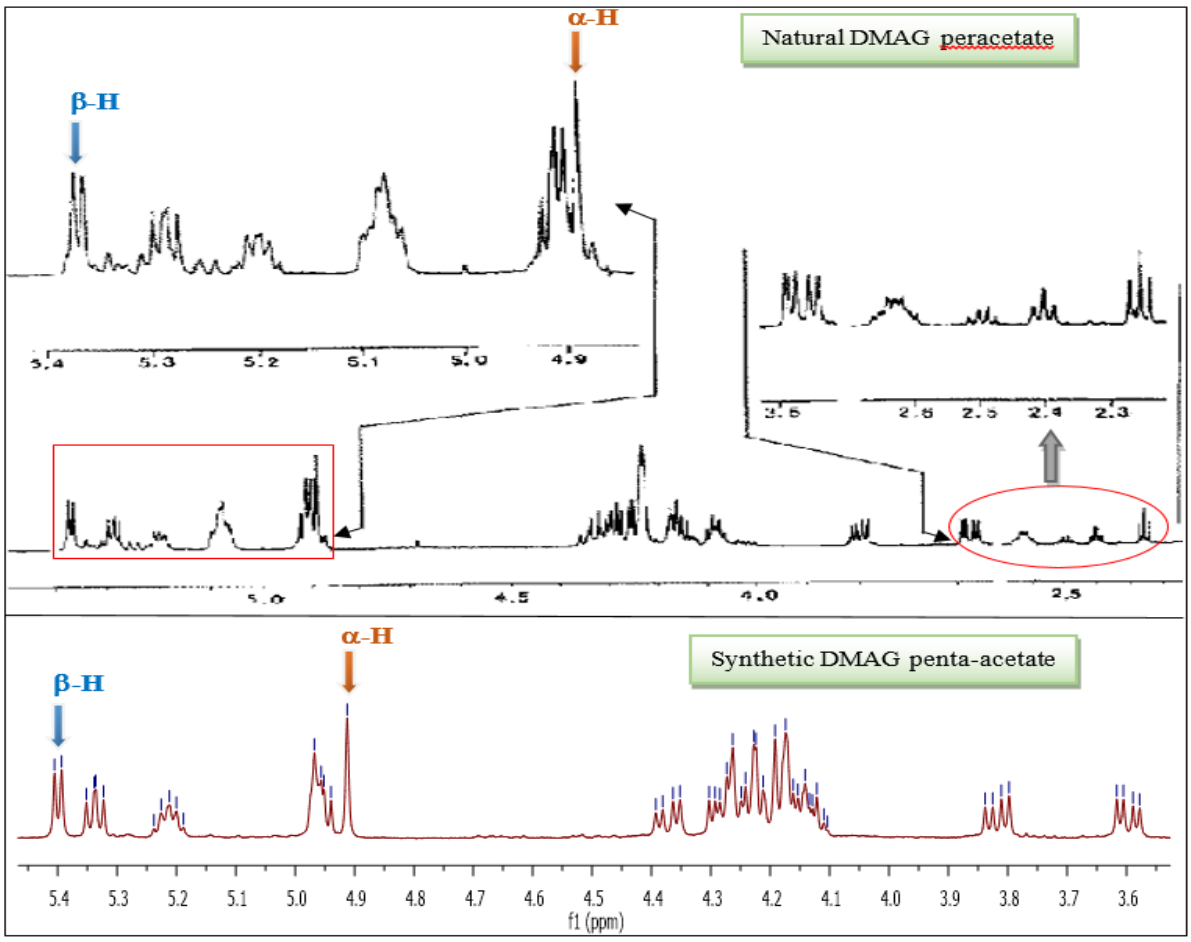
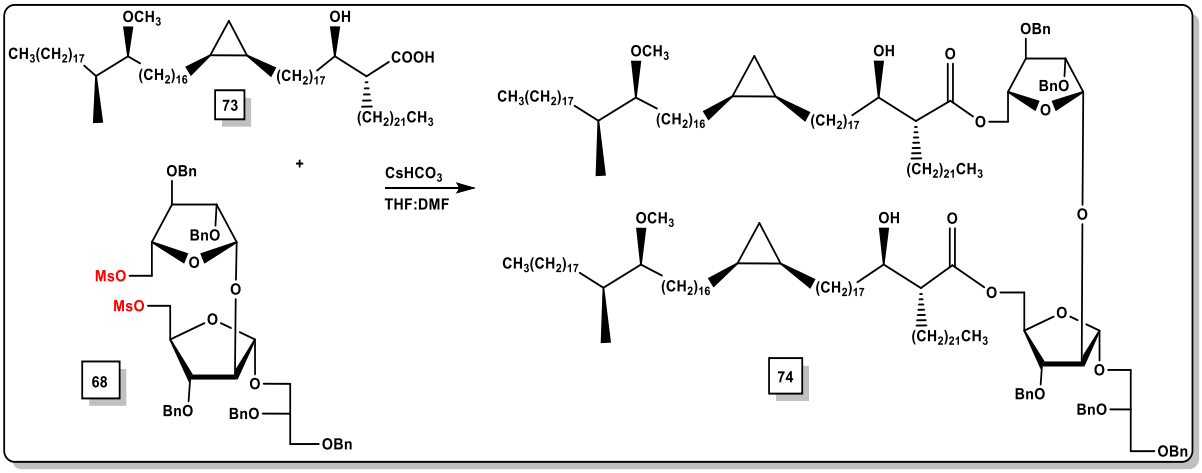


Figure 40: A comparison of ^{1}H-NMR spectra of synthetic DMAG penta-acetate analogue **(72)** with that reported for the natural mixture.[81]

2.1.6 Esterification of the glycan (68) with synthetic methoxy-MA (73) using the $CsHCO_3$ method

Having secured a successful method for synthesizing model analogues of DMAG containing simple fatty acids, an attempt to prepare the desired DMAG containing a mycolic acid by the same method as above was undertaken. Firstly, the structurally defined synthetic MA **(73)** provided by Dr. Al Dulayymi,[110] a methoxy-*cis*-cyclopropane with a 22 carbon α-alkyl chain, which is present in nature in *M. kansasii*, was reacted with the mesylate **(68)**, using cesium hydrogen carbonate (10 eq.) in dry DMF:THF (1:5) at 70 ºC for 4 days to afford **(74)** in 54% yield **(Scheme 23)**. This yield was lower than those observed when preparing DMAG glycolipids with simple fatty acids (**70a-c**); a possible reason for this may be due to the presence of more functional groups in the methoxy-MA chain and also due to there being more steric hindrance.



Scheme 23: Synthesis of fully protected DMAG glycolipid **(74)**.

The success of the esterification was demonstrated by mass spectrometry, I.R and ^{1}H-NMR spectroscopy, where the characteristic signals corresponding to the protons at the α- and β-anomeric centres appeared as a broad singlet at δ 4.93 and broad doublet at δ 4.97 (*J* 4.3 Hz) respectively. The region of interest is between δ 0.61 and - 0.36 (cyclopropane reigon), which corresponds to the eight protons of the two *cis*-cyclopropane rings. The proton H_a (**Figures 41 & 42)** gave a doublet of triplets, the broadness of this signal shown at δ 0.56 to 0.48 is possibly because H_c and $H_{c`}$ are not magnetically equivalent and the signal observed is actually a double doublet of doublets. However, due to the signals being at a nearly identical chemical shift it appears as a doublet of triplets. The proton H_b should show a doublet of triplets. However, due to the difference of H_c and $H_{c`}$ the signal at δ - 0.3 to - 0.4 is distorted and it appears as a broad quartet. Protons $H_c$ and $H_{c`}$ again showed a distorted multiplet at δ 0.67 to 0.57 for the same reason. A singlet at δ 3.31 corresponded to the methoxy group in

the MA. The α-proton H_d exhibits a multiplet at δ 2.42 – 2.32, which is not consistent with the expected splitting pattern (should be a doublet of triplets), due to the complexity of the ring the signal was distorted. H_f is seen as a doublet of triplets at δ 2.95 – 2.90 due to it being adjacent to the methoxy group, and the splitting pattern observed is consistent with the expected splitting pattern. Signals corresponding to the benzylic protons were seen as three doublets at δ 4.41, 4.58, 4.67 (the coupling constant for each signal being 11.6 Hz) and two multiplets at δ 4.47 and 4.63. All the remaining protons on the glycan moiety and the glycerol part appeared in the range from δ 4.30 – 3.50.

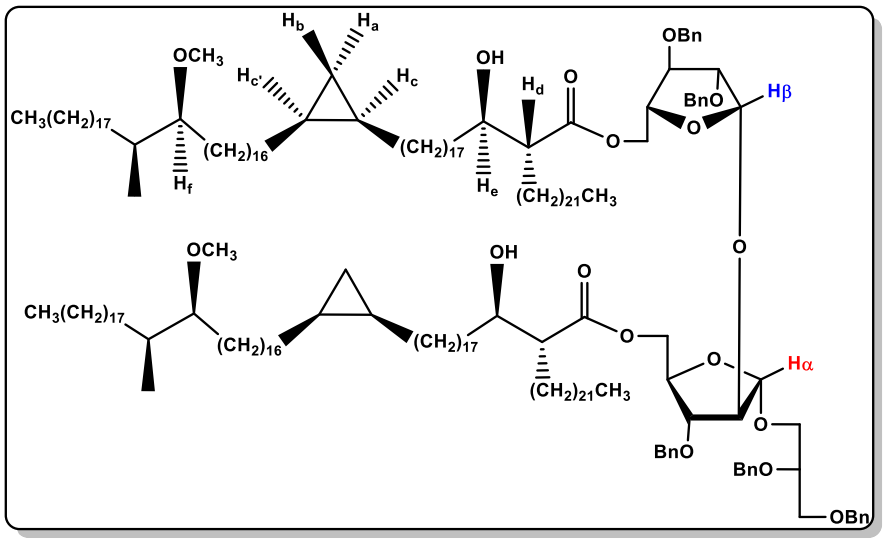


Figure 41: Structure of fully protected DMAG compound **(74)**.

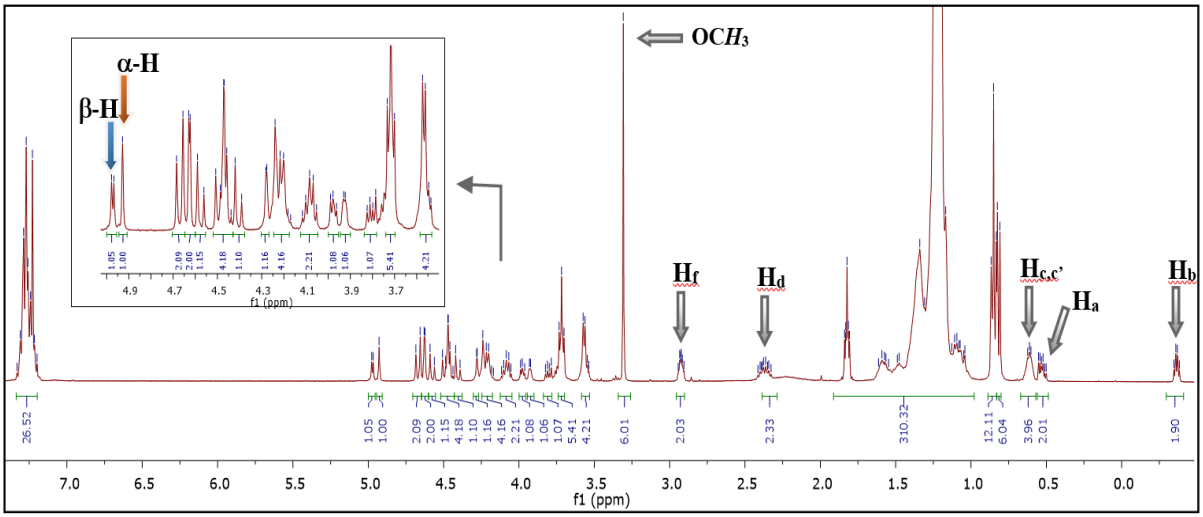


Figure 42: ^{1}H -NMR of fully protected DMAG compound **(74)**.

The ^{13}C-NMR spectrum showed two carbonyls at δ 175.04 and δ 175.03 with a slight downfield shift, the carbon of the α-anomeric centre appeared at δ 105.9 and that of the β-anomeric centre at δ 100.4. The remaining sugar and glycerol carbons were in the region of δ 85.5 – 63.7, and the methoxy group at δ 57.7, The carbons of the CH_2 chain ranged from δ 35.0 – 22.0 and the carbons of the CH_3 came up-field at around δ 14.0 (**Figure 43)**.

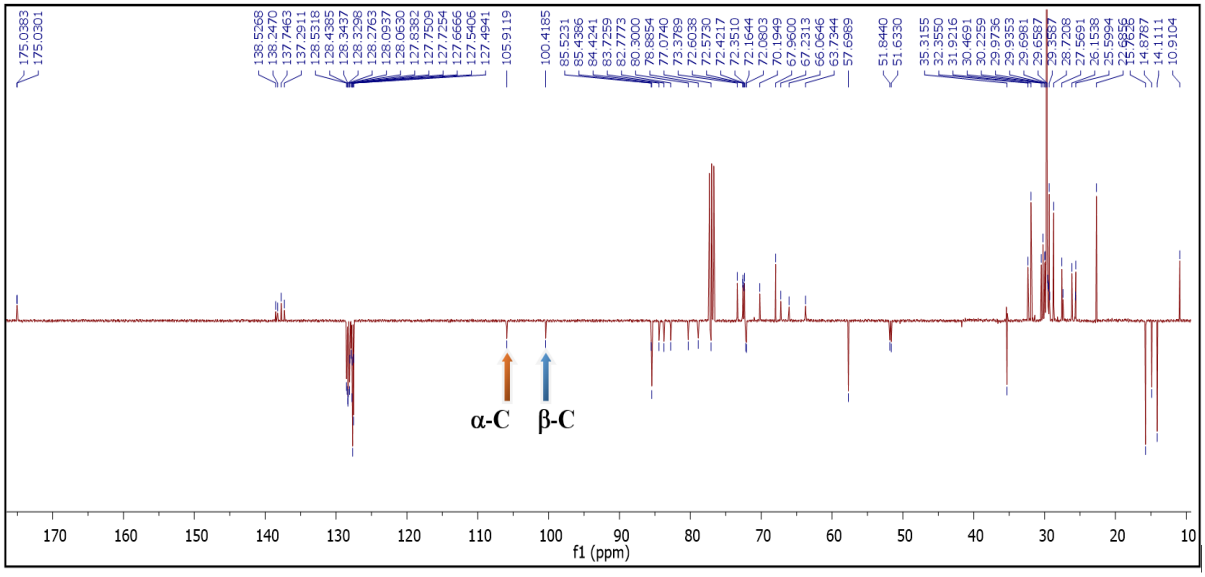
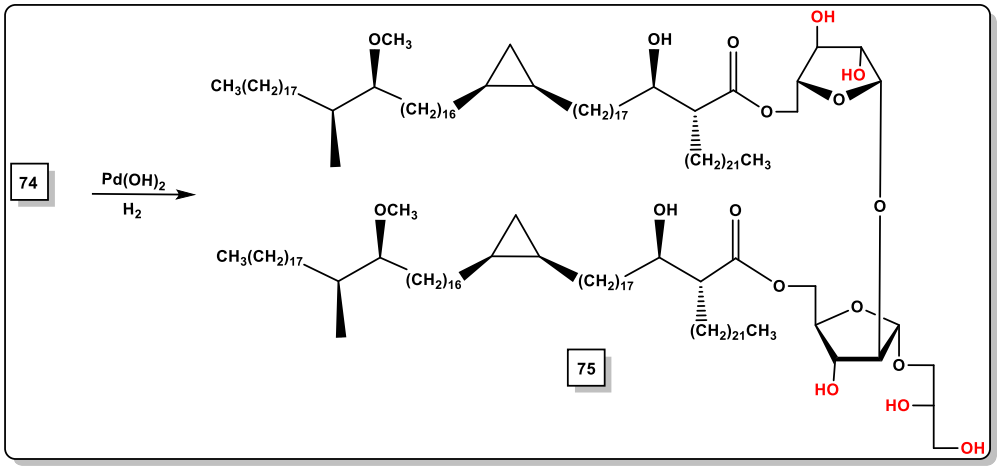


Figure 43: ^{13}C-NMR of fully-protected DMAG compound **(74)**.

Debenzylation of **(74)** was achieved by the method described previously to give the target DMAG **(75)** in 73% yield **(Scheme 24)**.



Scheme 24: DMAG glycolipid of methoxy-MA **(75)**.

Once again, the ^{1}H and ^{13}C-NMR spectra for **(75)** showed the disappearance of those signals corresponding to the methylene group of the benzyl groups, as well as, the aromatic signals. All the remaining signals corresponding to the glycan core, the glycerol and the methoxy-MA are similar to those of **(74)** as discussed before.

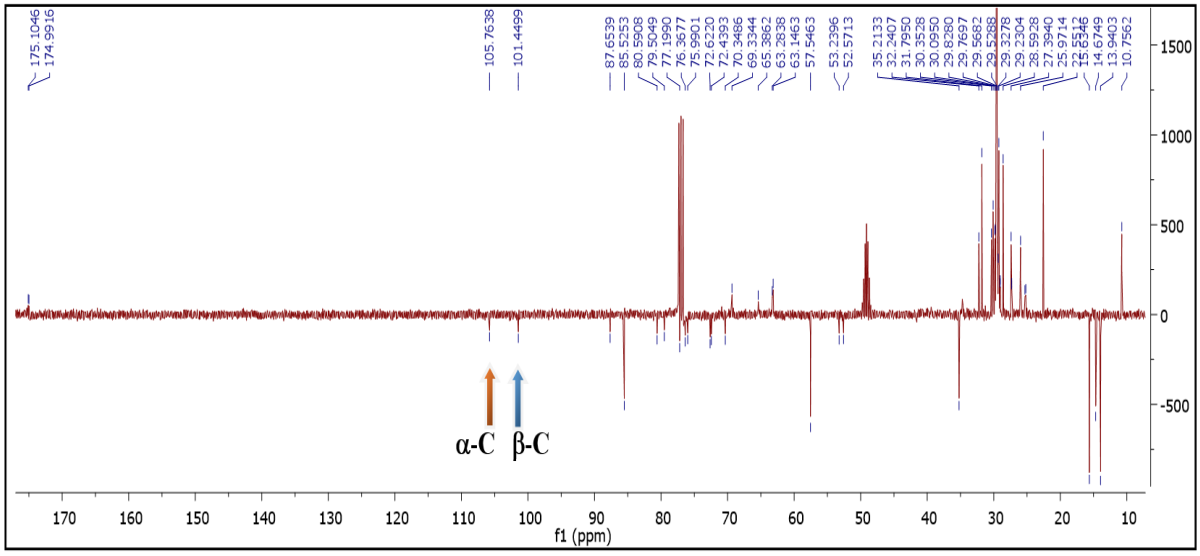
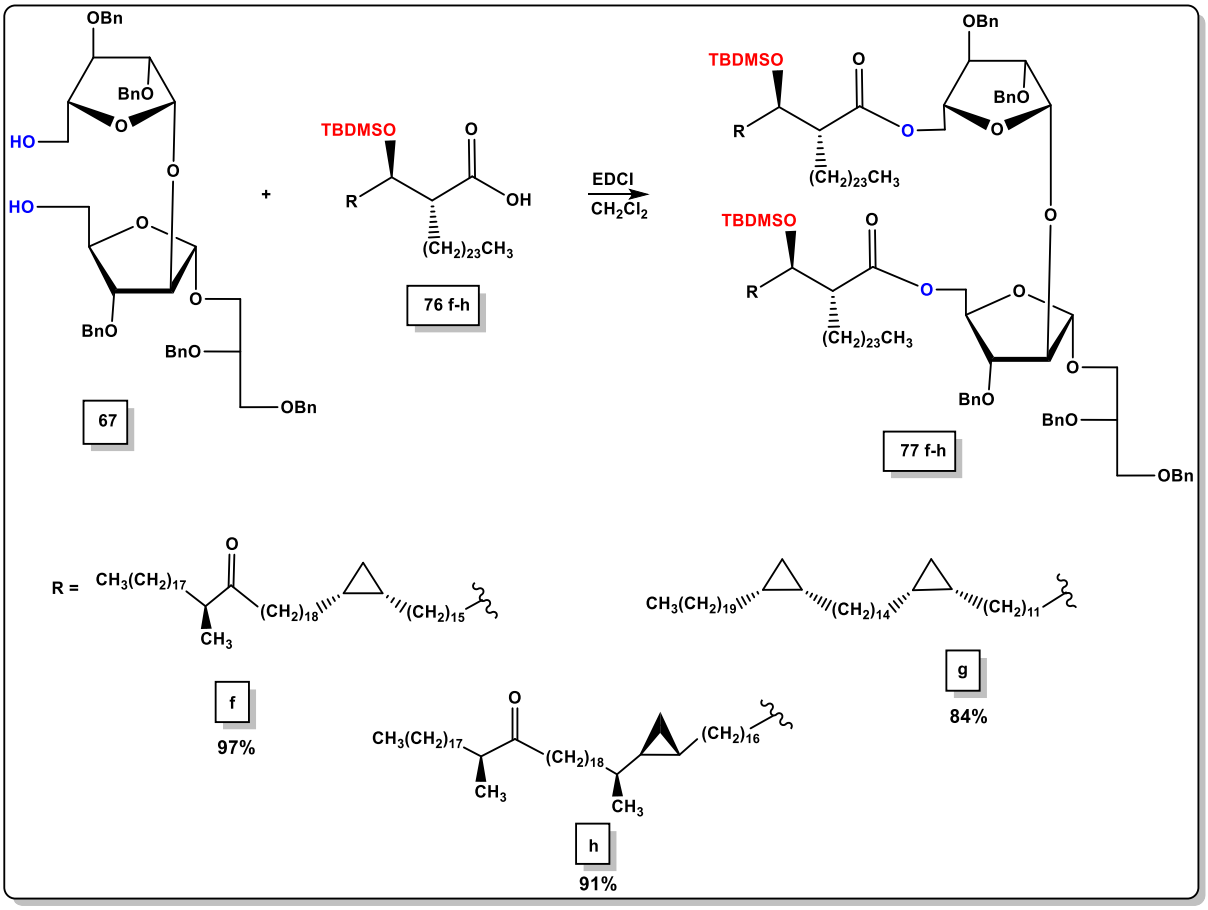


Figure 44: ^{13}C-NMR of DMAG compound **(75)**.

2.1.7 Esterification of the glycan (67) with different synthetic MAs using the EDCI method

In order to explore another route for esterification and to produce a series of DMAG glycolipids from the most common classes of synthetic mycolic acids, a direct esterification method was undertaken using the EDCI method. The advantage of this is that it avoids protecting the primary hydroxyl groups of the sugar and gives a good yield. According to the literature, direct esterification between the primary hydroxyl group of α-D-Ara*f* and a carboxyl group in natural MA was achieved in a low yield (30%) due to the tendency of the hydroxy acid to undergo self-condensation; however, by using a MA protected at the β-position (the secondary hydroxyl group) with *tert*-butyldimethylsilyl (TBDMS), the yield of the coupling was raised to 97%. Therefore, the direct condensation of the sugar-diol **(67)** with three different protected synthetic MAs **(76f-h)** was investigated, using EDCI as an activating agent and DMAP as catalyst in dry CH_2Cl_2 and stirring for five days at room temperature, to give the fully protected DMAG's **(77f-h) (Scheme 25)**.



Scheme 25: Synthesis of fully protected DMAG glycolipids **(77f-h)**.

Confirmation of the formation of **(77f-h)** was achieved by mass spectrometry, I.R and NMR spectroscopy. The NMR spectra for **(77f) (Figure 45)** showed characteristic signals for both the glycan and keto-MA illustrated in **Table 4**.

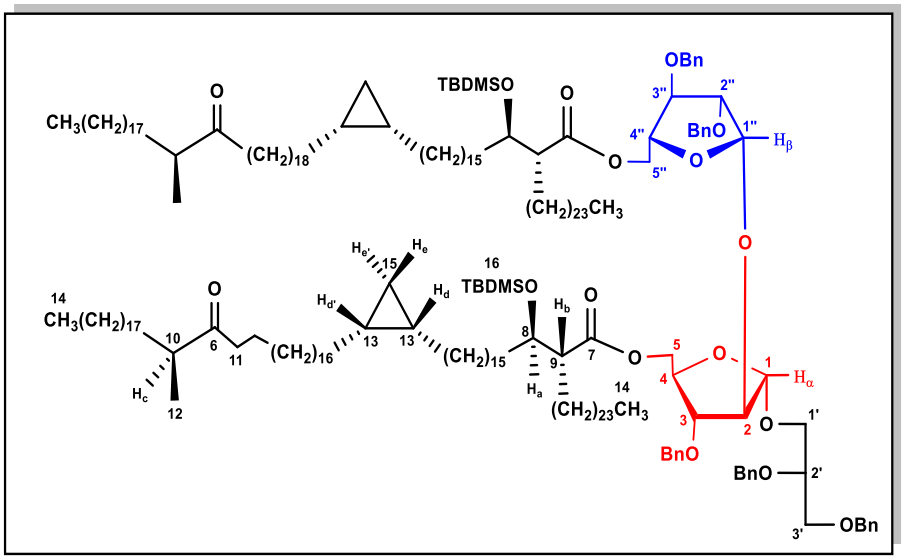


Figure 45: Structure of compound **(77f)**.

Table 4: The ^{1}H and ^{13}C-NMR data analysis of compound **(77f)**.

Proton	Shift	H's	Class	*J/Hz*	Carbon	*δ/ppm*
$H_{\text{-aromatic}}$	7.30	25	m	-	C_6	215.2
$H_{(\beta)}$	5.03	1	br.d	4.2	C_7	174.3
$H_{(\alpha)}$	4.97	1	br. s	-	$C_{7''}$	174.1
$H_{\text{-Bn}}$	4.72	1	d	11.6	$C_{1\text{-alpha}}$	105.9
$H_{\text{-Bn}}$	4.68	3	d	11.6	$C_{1''\text{-beta}}$	100.2
$H_{\text{-Bn}}$	4.62	1	d	11.7	C_4	84.9
$H_{\text{-Bn}}$	4.52	4	m	-	$C_{4''}$	84.6
$H_{\text{-Bn}}$	4.43	1	d	11.7	C_3	83.6
H_4	4.37	1	br. q	4.6	$C_{3''}$	83.3
$H_{(3'', 4'', 5'', 5)}$	4.20	6	m	-	C_2	80.1
H_2	4.06	1	t	6.0	$C_{2''}$	79.1
$H_{2''}$	4.00	1	br.dd	4.3, 6.5	$C_{2'}$	77.1
$H_{Ha, 3', 1'}$	3.88	4	m	-	$C_{\text{-Bn}}$	73.4
$H_{2'}$	3.78	1	br.p	4.7, 9.7	C_8	73.1
$H_{3, 1', 3'}$	3.60	3	br.dd	4.4, 10.4	$(C_{\text{-Bn}})_{x2}$	72.5
H_{11}	2.53	4	sextet	6.8	$C_{\text{-Bn}}$	72.4
$H_{b, c}$	2.42	4	dt	1.0, 7.2	$C_{\text{-Bn}}$	72.2
$(CH_{2\text{-Chain}})$	1.35	288	m	-	$C_{1'}$	70.3
$(CH_3)_{\text{-12}}$	1.06	6	d	6.9	$C_{3'}$	67.2
$(CH_3)_{\text{-14}}$	0.89	12	t	6.8	C_5	66.3
$H_{\text{(tert-butyl)-16}}$	0.86	9	s	-	$C_{5''}$	64.3
$H_{\text{(tert-butyl)-16'}}$	0.84	9	s	-	C_9	51.5
$H_{d,d'}$	0.67	4	m	-	C_{10}	51.4
$H_{e'}$	0.57	2	dt	4.1, 8.4	C_{11}	46.3
$(CH_3)_{\text{-16}}$	0.04	3	s	-	$C_{11''}$	41.1
$(CH_3)_{\text{-16}}$	0.03	3	s	-	C_{12}	16.4
$(CH_3)_{\text{-16'}}$	0.02	3	s	-	C_{13}	15.8
$(CH_3)_{\text{-16'}}$	0.01	3	s	-	C_{14}	14.1
H_e	- 032	2	br.q	5.1	C_{15}	10.9

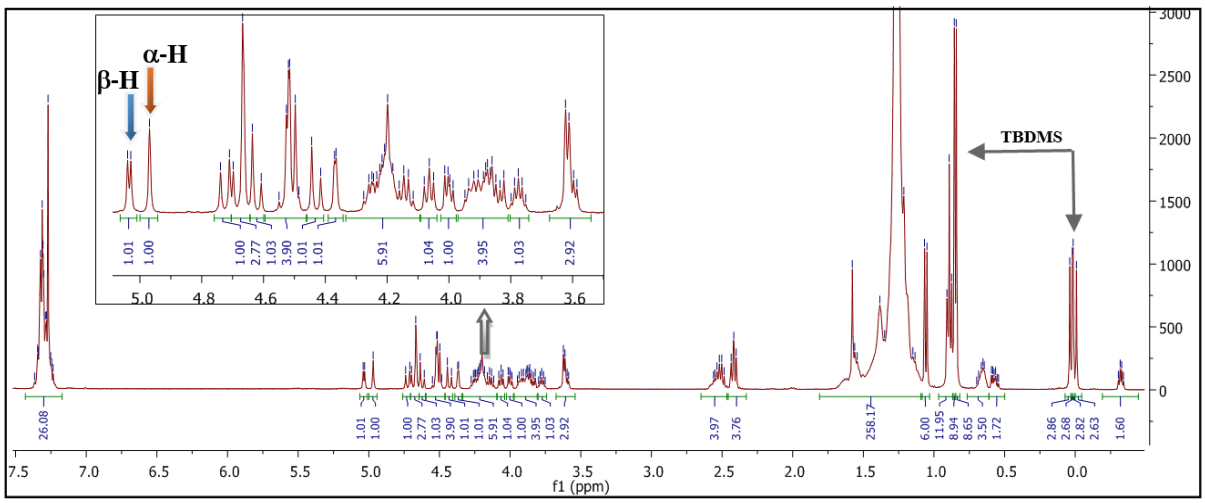


Figure 46: ^{1}H-NMR of compound **(77f)**.

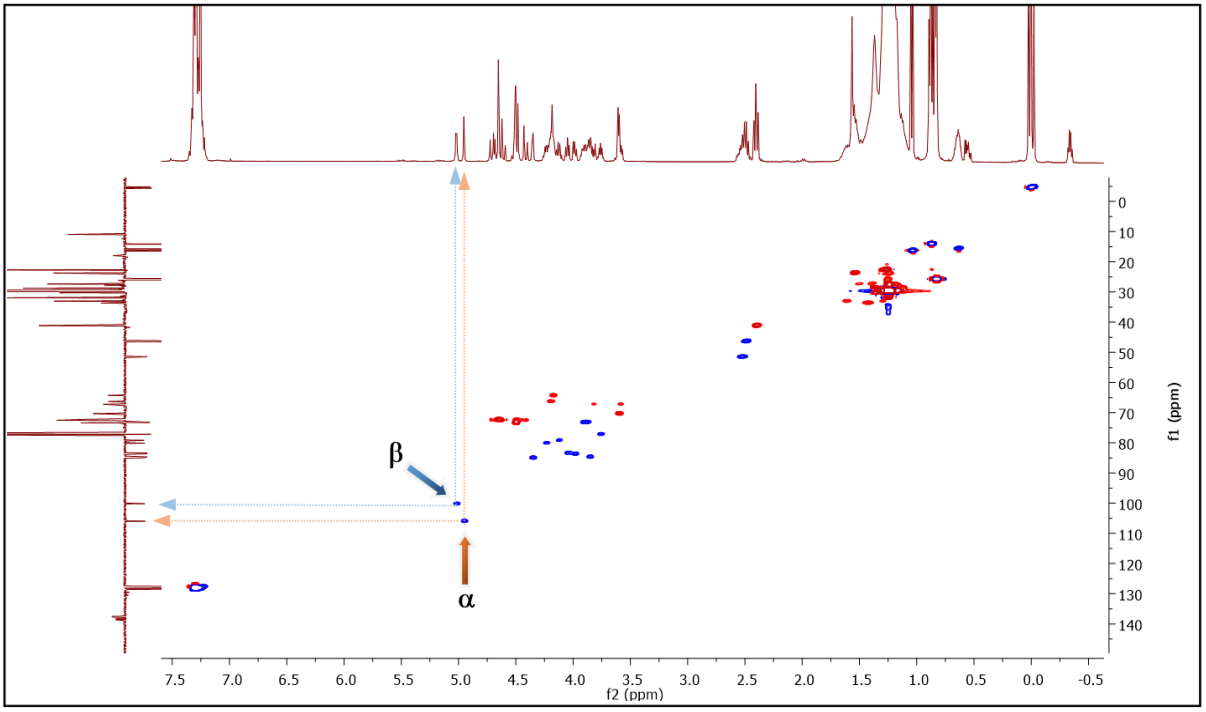
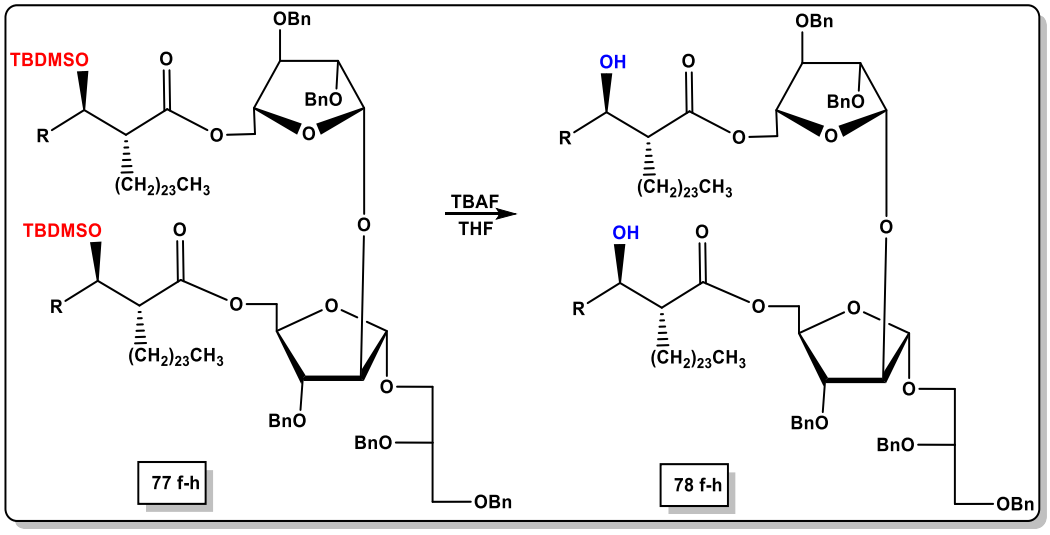


Figure 47: HSQC of compound **(77f)**.

2.1.7.1 Deprotection of TBDMS groups from the MAs core:

Compounds **(77f-h)** were then de-protected in two steps. Firstly, removal of the TBDMS group from the β-position of the MA part by using TBAF in dry THF at 0 °C – R.T under nitrogen atmosphere for 16 hr gave **(78f-h)** in 38, 64 and 31% yield respectively **(Scheme 26)**.



Scheme 26: Removing of TBDMS group from compounds **(78f-h)**.

For example, the ^{1}H-NMR spectrum for **(78f)** confirmed the disappearance of the signals for the TBDMS groups at δ 0.86 and δ 0.84 ppm corresponding to two *tert*-butyl groups, and

between δ 0.04 – 0.01 ppm corresponding to four methyl groups, which confirmed that the deprotection was successful.

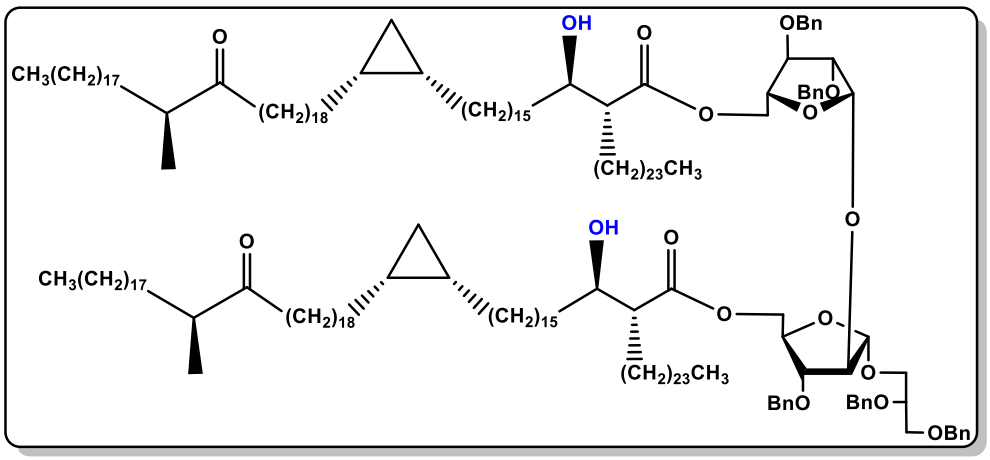


Figure 48: Structure of compound **(78f)**.

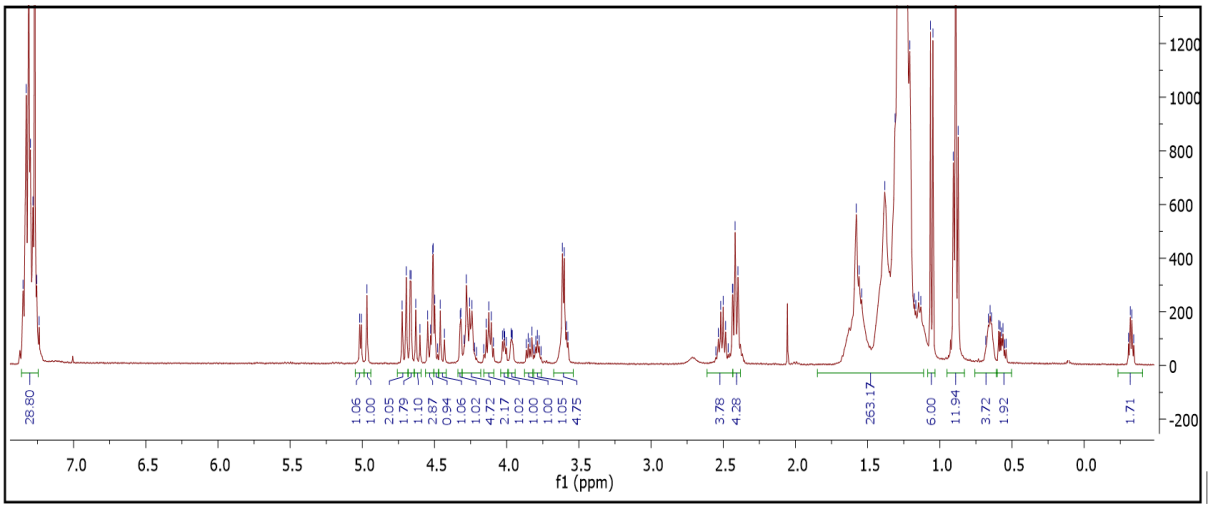
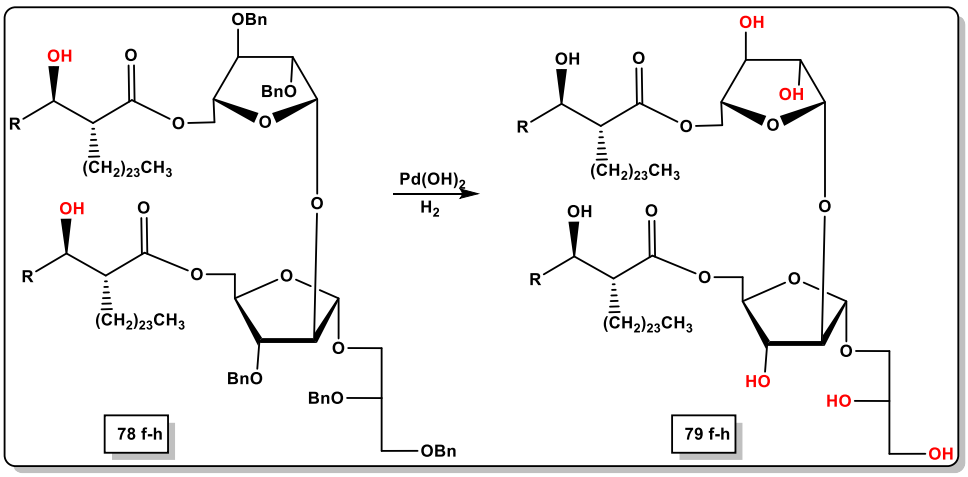


Figure 49: ^{1}H-NMR of compound **(78f)**.

Secondly, the removal of the benzyl groups (hydrogenolysis) from **(78f-h)** was accomplished by stirring in dry CH_2Cl_2:MeOH (1:1) in the presence of $Pd(OH)_2$ under a hydrogen atmosphere leading to the target DMAG glycolipids **(79f-h)** in 71, 70 and 72% yield respectively **(Scheme 27)**.



Scheme 27: Hydrogenolysis of compounds **(79f-h)**.

The ^{1}H-NMR spectrum (**Figure 51)** for **(79f)** showed the disappearance of the signals for the aromatic protons at δ 7.20 ppm, as well as, the complete loss of the signals for the CH_2 of the benzyl groups between δ 4.71 – 4.45 ppm, which confirmed the success of the hydrogenolysis.

The ^{13}C-NMR spectrum showed the anomeric carbon signals at δ105.8 and 101.5 ppm for the α and β anomers respectively. The remaining NMR data for the sugar and MAs appeared approximately similar to that of **(78f)**.

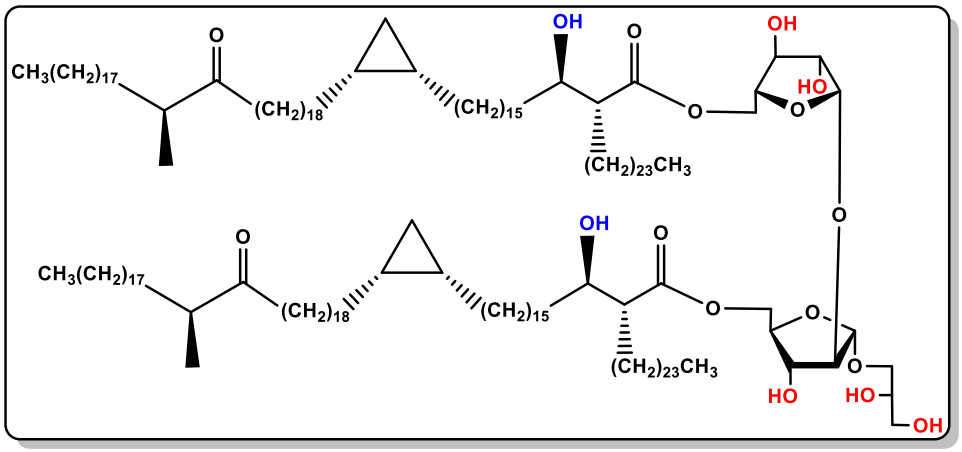


Figure 50: Structure of compound **(79f)**.

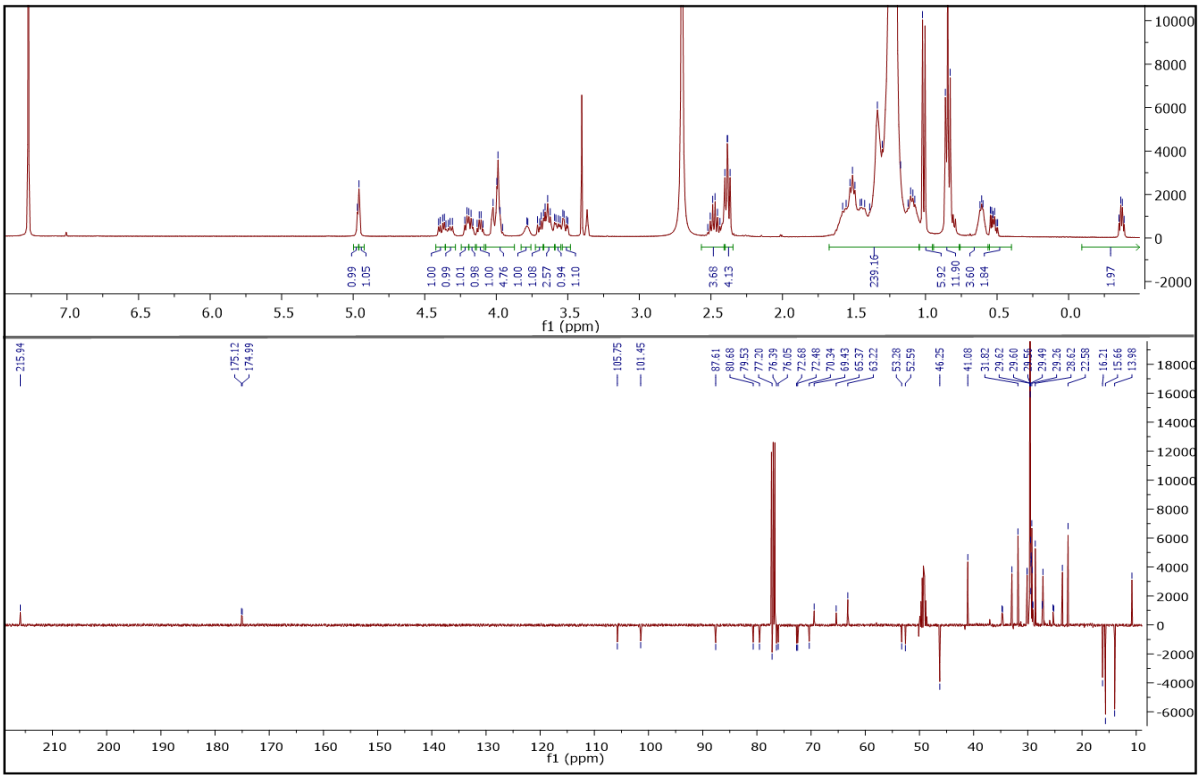


Figure 51: ^{1}H and ^{13}C-NMR of compound **(79f)**.

The HSQC-NMR spectrum **(Figure 52)** for **(79f)** showed the acetal proton signals were correlated to their carbons.

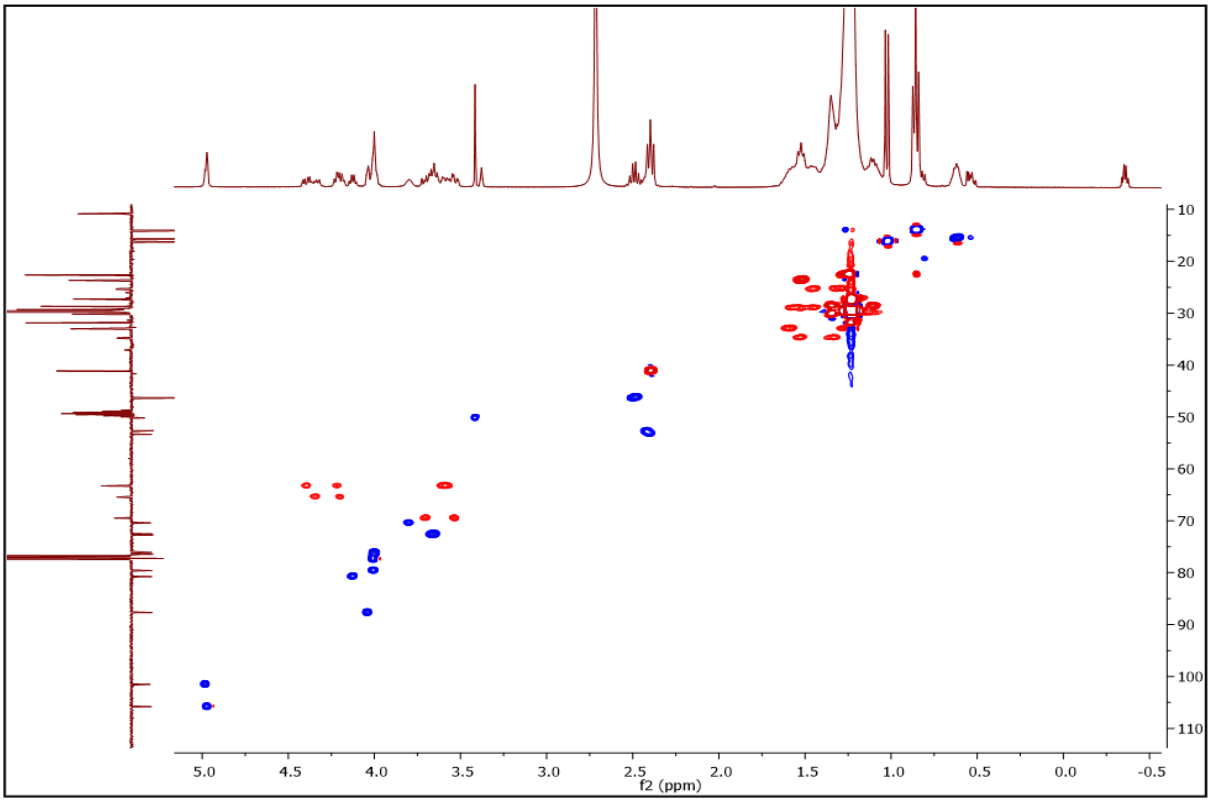


Figure 52: HSQC of compound **(79f)**.

2.1.7.2 Esterification of the glycan (67) with α-MA (76g) using the EDCI method

The Synthetic α-MA **(76g)**[109] was esterified with the glycan **(67)** using EDCI to give **(77g)** **(Scheme 25)**.

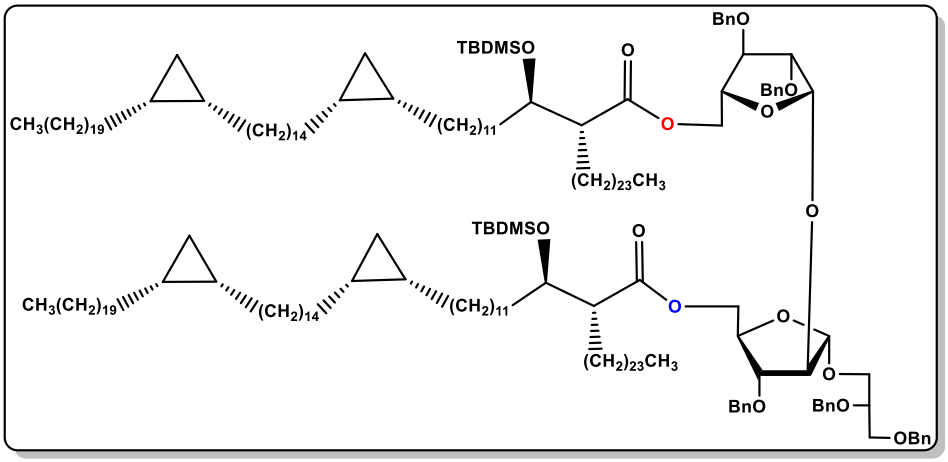


Figure 53: Structure of compound **(77g)**.

The ^{1}H-NMR spectrum of this compound showed 4 singlets at δ 0.04, 0.02, 0.01 and -0.01 for 12 protons corresponding to the TBDMS group, and the two protons at the β-position to the carboxylic group in the two MAs as a multiplet at δ 3.97 – 3.80. The signal for the proton at the α-position in the MA appeared as a multiplet at δ 2.63 – 2.46 for two protons. The signals of the cyclopropane protons were seen as a broad quartet (4H) at δ - 0.32 (*J* 5.1 Hz), a doublet of triplets (4H) at δ 0.57 (*J* 4.1, 8.5 Hz) and a multiplet (8H) at δ 0.80 – 0.59. Signals corresponding to the glycan moieties were similar to those of the previously prepared DMAG's. Formation of **(77g)** was proved by the ^{13}C-NMR spectrum, which showed two signals for the carbonyls at δ 174.3 and δ 174.1, and the anomeric carbons at the α- and β-positions which resonated at δ 105.9 and δ 100.2 respectively.

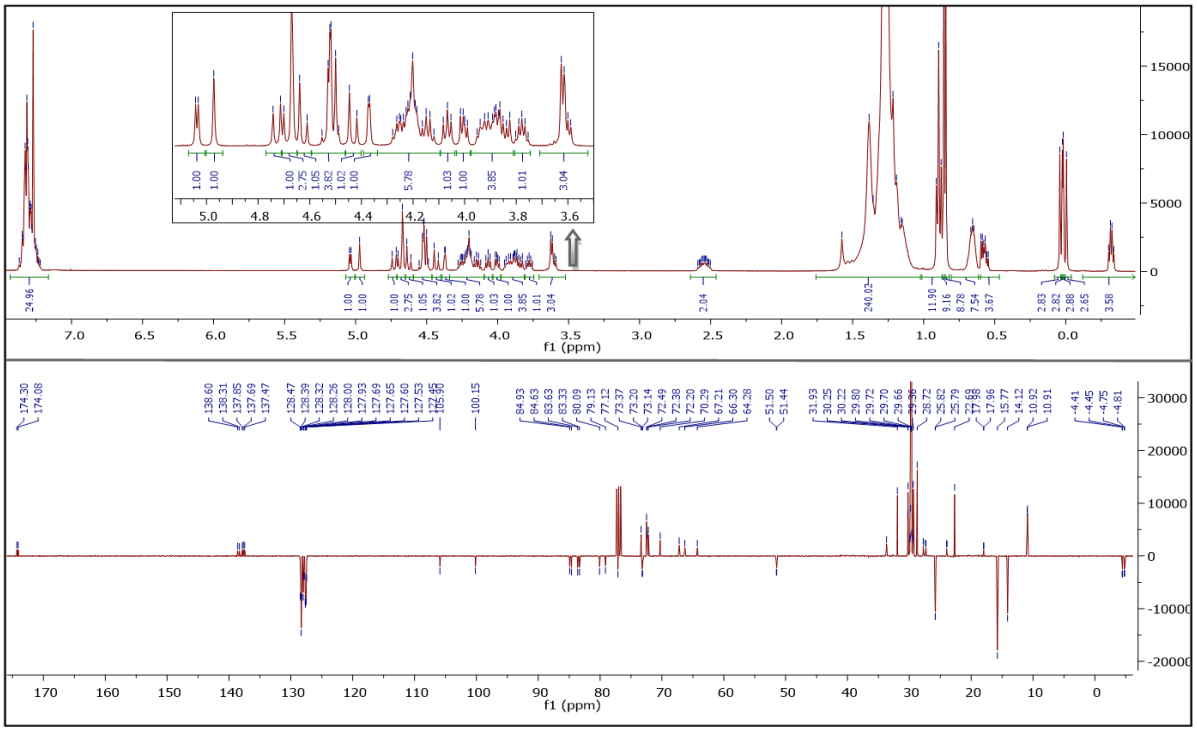


Figure 54: ^{1}H and ^{13}C-NMR of compound **(77g)**.

This compound was then deprotected to remove the TBDMS groups using the same conditions as before, to give **(78g)** in 64% yield **(Figure 55)**.

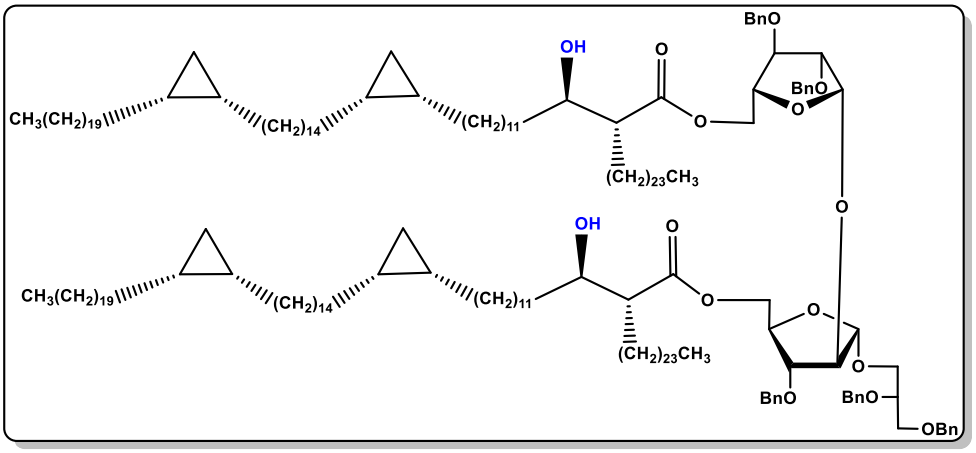


Figure 55: Structure of compound **(78g)**.

The ^{1}H-NMR spectrum for **(78g)** confirmed the complete disappearance of the signals for the TBDMS group, which confirmed that the de-silylation had been successful.

The hydrogenolysis of this compound by the method described before afforded **(79g)**.

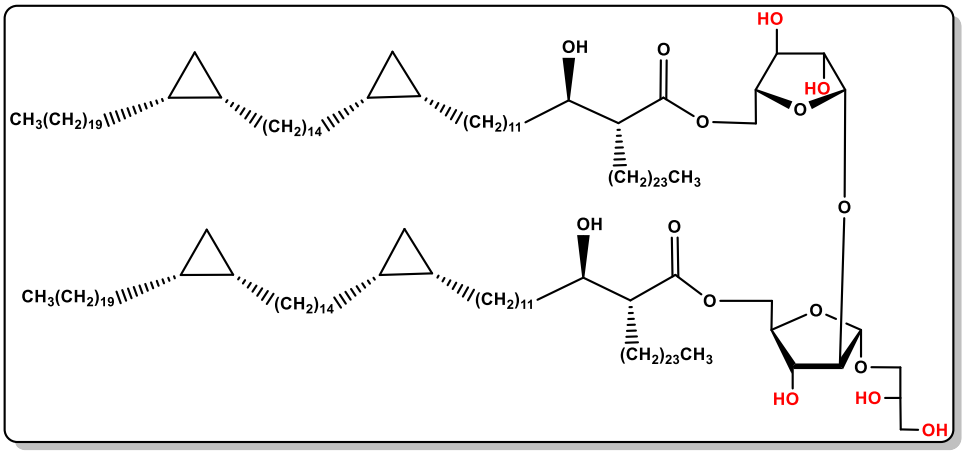


Figure 56: Structure of compound **(79g)**.

Confirmation of the formation of **(79g)** was achieved by ^{1}H-NMR spectroscopy. Signals for the cyclopropane appeared at approximately the same chemical shift as those in the spectrum of **(78g)**. The ^{13}C-NMR spectrum showed two ester carbonyls at δ 175.1 and 175.0. Two signals at δ 105.9, 100.4 corresponded to the carbons at the positions α- and β- in the glycan respectively. The remaining sugar carbons were in the region of δ 87 – 52. The CH_2 chain ranged from δ 35 – 22 and the CH_3 came up-field to around δ 14.0. 2D-NMR was used to provide further proof of the structure. **Figure 57** shows the HSQC spectrum of **(79g)** where the acetal proton signals were correlated to their carbons.

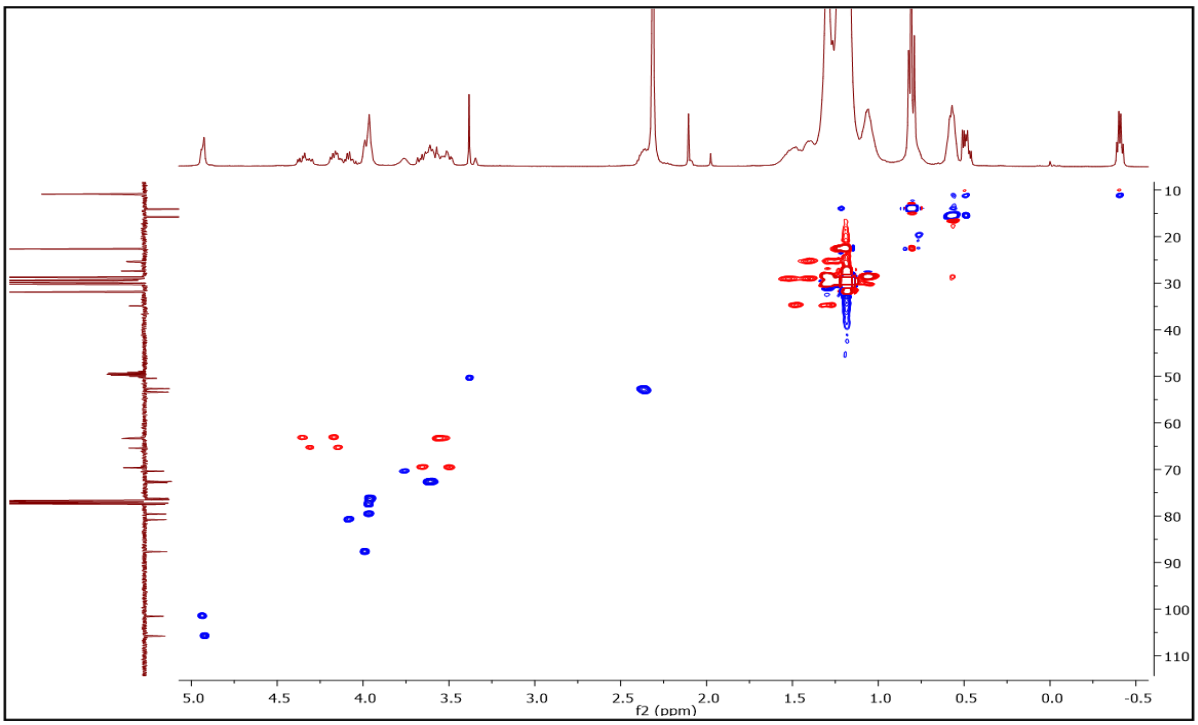


Figure 57: HSQC of compound **(79g)**.

2.1.7.3 Esterification of the glycan (67) with keto-MA (76h) using the EDCI method

The keto-MA with a *trans*-cyclopropane **(76h)**[111] has been found in the cell wall of *M. tuberculosis.* The sugar-diol **(67)** was coupled with this synthetic keto-MA by the method described above to prepare **(77h) (Figure 58)** in 91% yield.

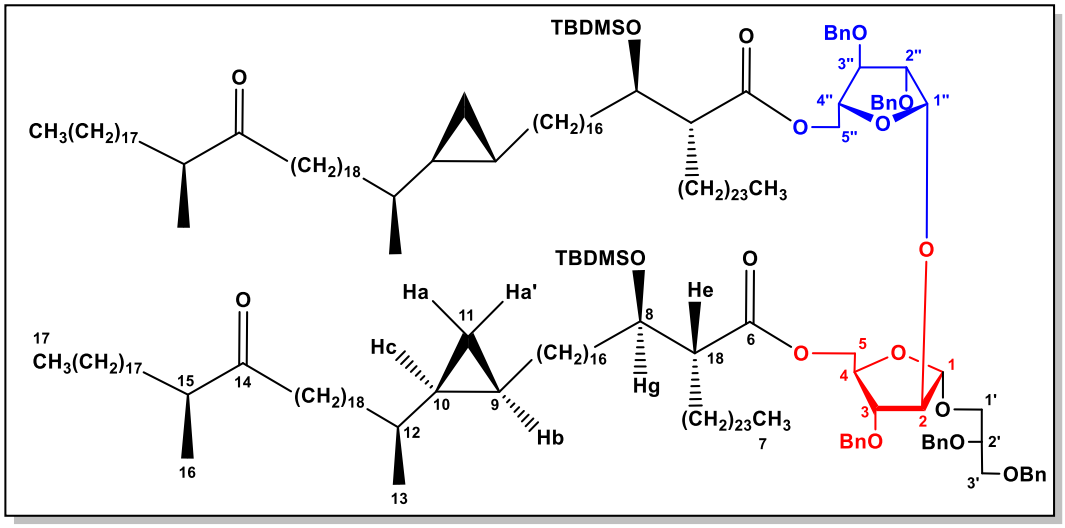


Figure 58: Structure of compound **(77h)**.

The most interesting signals in the ^{1}H-NMR spectrum **(Figure 59)** for each MA in this compound were in the area between δ 0.50 and δ 0.06 and corresponded to the four protons directly bound to the *trans*-cyclopropane. The region between δ 0.35 – 0.05 contained signals for the three different hydrogens. This is because H_a and $H_{a`}$ are non-equivalent and each has three couplings, each signal splitting to give a double doublet of doublets (8 lines), which leads to 16 lines. H_b should give a double double doublet of doublets of doublets (32 lines) as it is coupled to five non-equivalent protons; however, due to overlap with the signals for H_a and $H_{a`}$, H_b cannot be resolved fully at δ 0.24 – 0.18. H_c, represented by the broad multiplet at δ 0.50 – 0.38, should give a doublet of doublets of doublets of doublets (16 lines). However, a complex broad multiplet is observed due to the presence of four similar coupling constants leading to the overlapping of peaks. The β-chiral centre proton H_g gave a doublet of triplets at δ 2.42 ppm (*J* 1.0, 7.5 Hz). The α-proton H_e and the CH_2 group adjacent to the distal position appeared as a multiplet at δ 2.53. The region between δ 0.91 – 0.87 (9H, including a triplet at 0.89 with *J* 7.5 Hz) corresponding to the three terminal methyl groups and the doublet at δ 1.06 (*J* 6.9 Hz) corresponds to the other α-methyl groups.

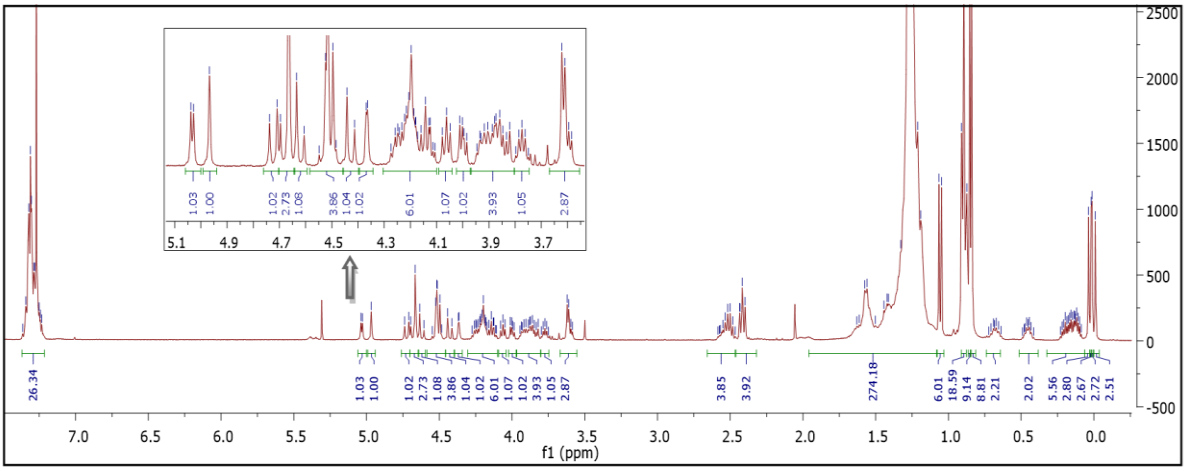


Figure 59: ^{1}H-NMR of compound **(77h)**.

The ^{13}C-NMR of compound **(77h) (Figure 60)** showed characteristic signals illustrated in **Table 5**.

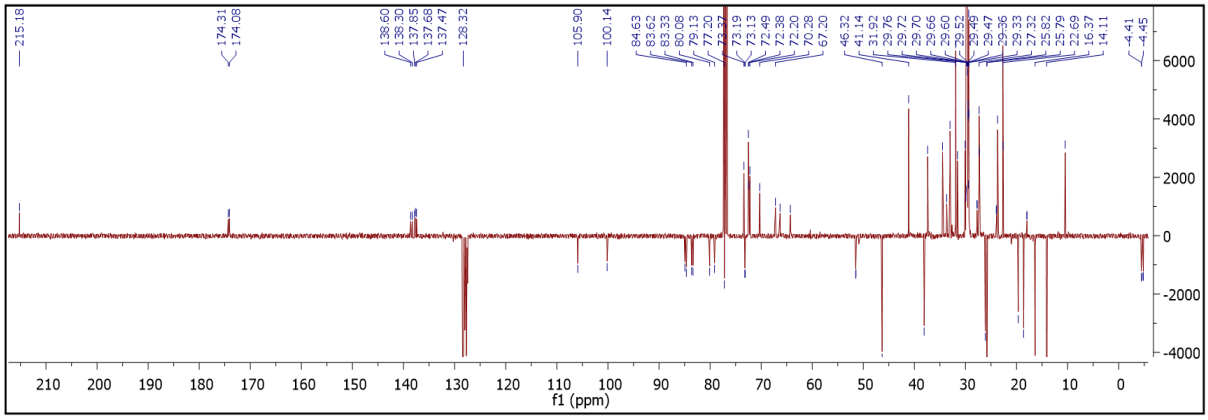


Figure 60: ^{13}C-NMR of compound **(77h)**.

Table 5: The ^{13}C-NMR data analysis of **(77h)**.

C_n	C_1 / $C_{1''}$	C_2 / $C_{2''}$	C_3 / $C_{3''}$	C_4 / $C_{4''}$	C_5 / $C_{5''}$	C_6 / $C_{6''}$	C_7	C_8	C_9	C_{10}	C_{11}
***δ*/ ppm**	105.9/ 100.1	80.1/ 79.1	83.6/ 83.3	84.9/ 84.6	67.2/ 66.3	174.3/ 174.1	14.1	72.4	18.6	26.1	10.5
C_n	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	$C_{1'}$	$C_{2'}$	$C_{3'}$	
***δ*/ ppm**	38.1	19.7	215.2	46.3	16.4	14.1	51.5	70.3	77.2	67.2	

This compound then was de-silylated by using the method described previously to give **(78h) (Figure 61)**.

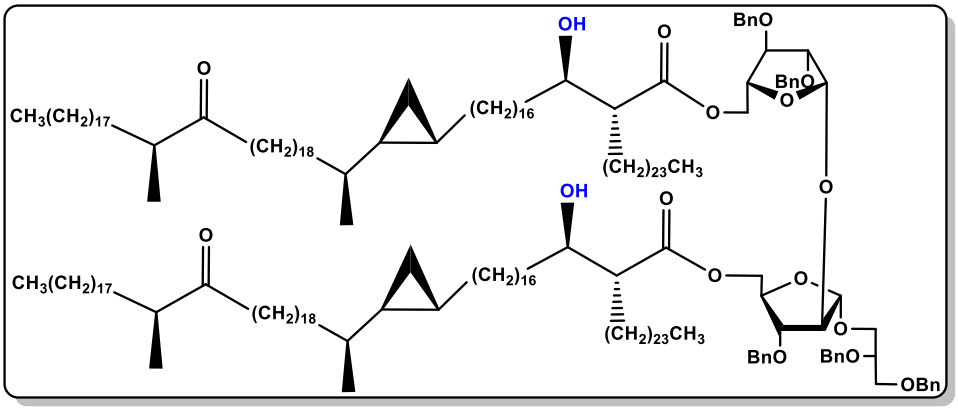


Figure 61: Structure of compound **(78h)**.

The ^{1}H and 2D-NMR spectrum for this compound showed the success of the de-protection through the disappearance of the signals belong to TBDMS group **(Figure 62 and 63)**.

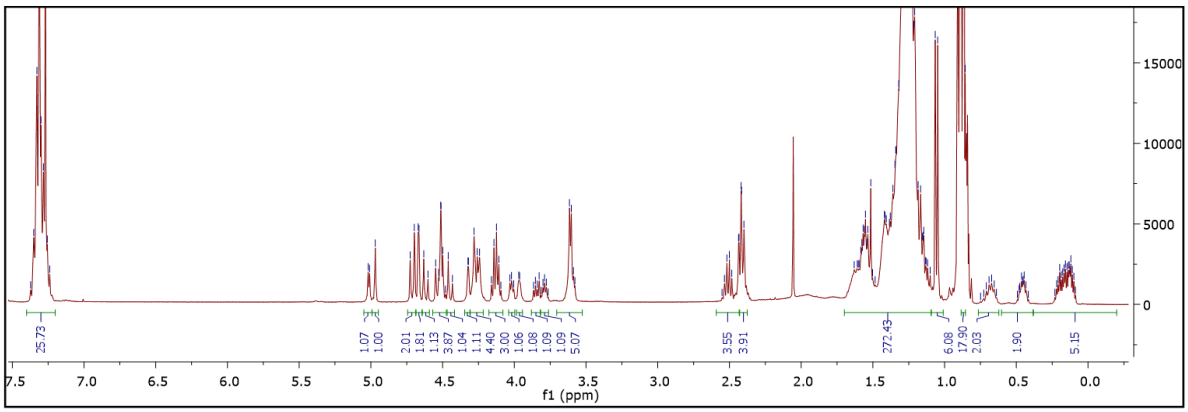


Figure 62: ^{1}H-NMR of compound **(78h)**.

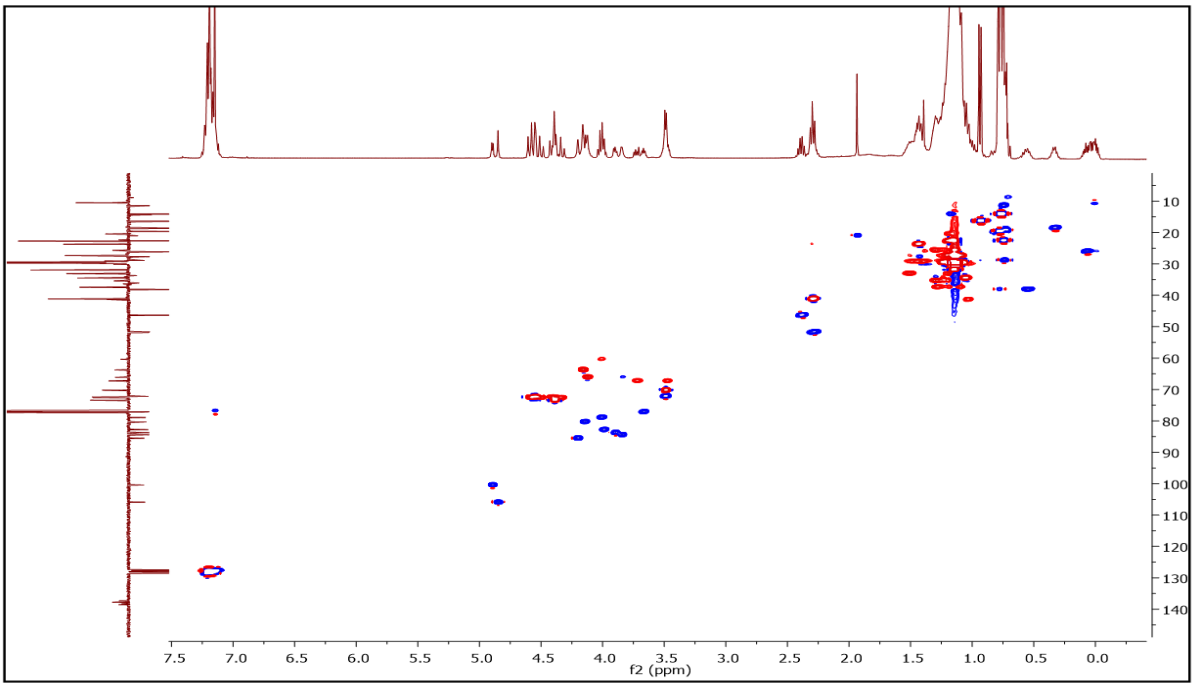


Figure 63: 2D-NMR of compound **(78h)**.

Hydrogenolysis of **(78h)** was achieved as above to afford **(79h) (Figure 64)**.

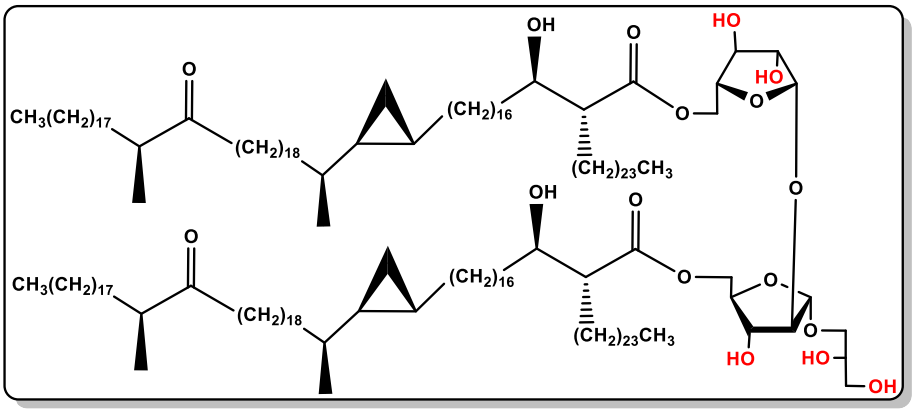


Figure 64: Structure of compound **(79h)**.

Once again, compound **(79h)** showed characteristic NMR signals **(Figure 65)** between δ 0.51 to 0.05 for the protons of the *trans*-cyclopropane. The signals for protons on the α- and β-anomeric centres appeared as a broad singlet at δ 5.03 and a broad doublet at δ 5.04 respectively. The remaining 17 protons on the glycerol and the glycan moieties, as well as the proton at the β-hydroxy position of the MAs, appeared between δ 4.54 - 3.58.

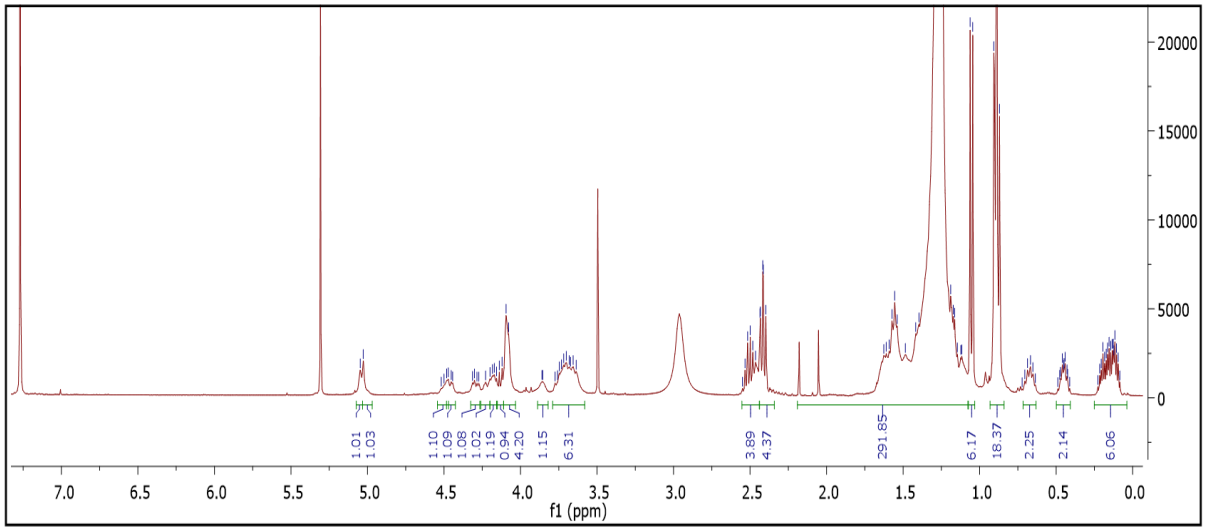


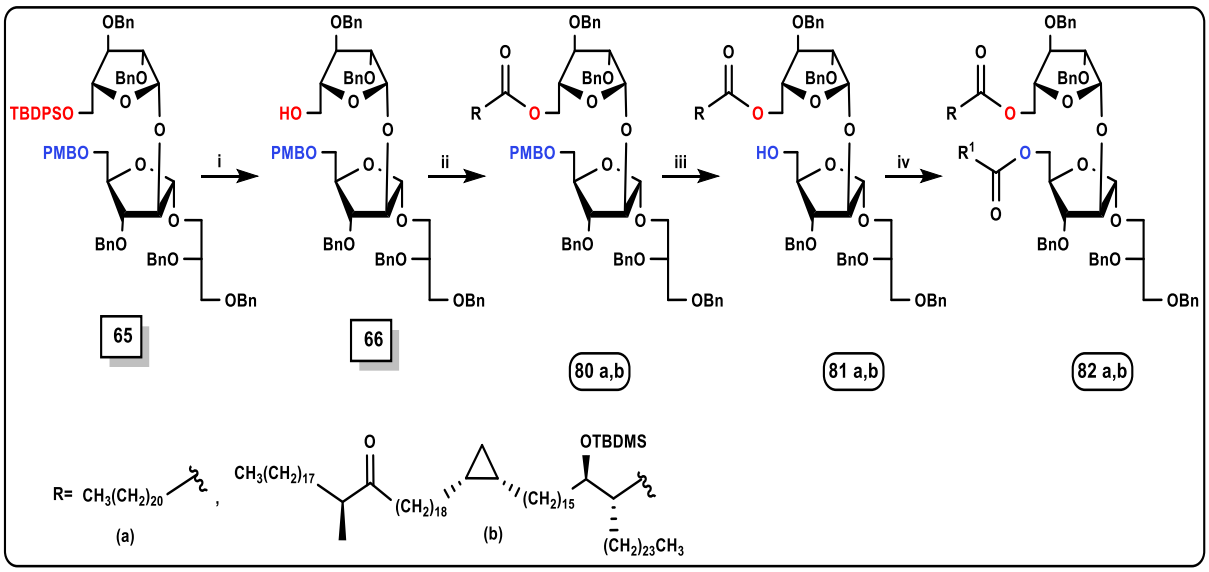
Figure 65: ^{1}H-NMR of compound **(79h)**.

Section 2

Orthogonal protection

2.2 Selective esterification at each primary alcohol position

As mentioned earlier, the strategy in the synthesis of the glycan di-arabino glycerol was to put two different protecting groups at the primary hydroxyl group (C-5) of each arabinose molecule. This was achieved successfully by having a PMB group on the lower arabinose molecule through the synthesis of the DMAG's acceptor, and after coupling the donor and acceptor, a TBDPS group was also added to the top arabinose molecule **(65)**. The reason for having two different protecting groups, was to allow selective esterification with different acids at each primary alcohol position, and control the synthesis of either mono- or di- (symmetrical or unsymmetrical) glycolipids. Thus, if the esterification process is carried out before removal of the PMB group, a mono-mycolyl di-arabino-glycerol can be produced, esterifing only on the top arabinose, which could then be selectively acylated on the lower arabinose as illustrated in **(scheme 28)**.



Scheme 28: Selective esterification to make mono and symmetrical di-glycolipid.

Reagents and conditions: **(i)** TBAF, THF, 0 ºC/R.T., 6 h, 93%; **(ii)** EDCI, RCOOH, CH_2Cl_2, DMAP, R.T., 48 h, 85%.; **(iii)** Cerium ammonium nitrate (CAN), CH_3CN:H_2O:THF (9:1:0.2), 0 ºC-R.T., 16 h, 83%; **(iv)** EDCI, R^1COOH, CH_2Cl_2, DMAP, R.T., 72 h, 50%.

2.2.1 Selective esterification of the glycan (66) with behenic acid

After removing the TBDPS group from **(65)** using TBAF to give **(66)**, direct condensation with behenic acid was carried out using EDCI as a coupling reagent and DMAP as catalyst in dry CH_2Cl_2 for 2 days to give the expected mono-ester **(80a)** in 85% yield **(Figure 66)**.

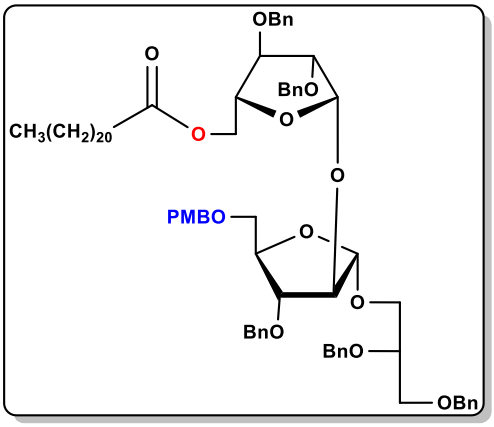


Figure 66: Structure of compound **(80a)**.

The NMR data confirmed the formation of the mono-ester **(80a)** and appeared similar to that of the DMAG analogue **(70c)** but with only half of the behenate integration as expected. The ^{1}H-NMR spectrum included two downfield signals at δ 7.15 (d *J* 8.6 Hz) and δ 6.77 (d *J* 8.5 Hz) corresponding to the protons of the PMB ring, and two downfield signals at δ 5.00 (br.d *J* 4.1 Hz) and δ 4.95 (br.s) corresponding to the glycan protons at the β- and α-anomeric centres respectively. The protons of the methoxy group of the PMB in the glycan gave a singlet at δ 3.72; the presence of this singlet and the two-doublets mentioned above for the PMB ring, is good evidence that the PMB group remains within the glycan structure. Twelve protons corresponding to the CH_2 of five benzyl groups, and one methylene of the PMB appeared between δ 4.61 – 4.25. The remaining 15 protons of the sugar moiety and the glycerol part appeared in the range from δ 4.25 – 3.47. Two protons corresponding to the CH_2 adjacent to the carbonyl group in the acid came around δ 2.13 as a double triplet (*J* 3.6, 7.7 Hz). The CH_2 chain ranged from δ 1.53 – 1.02 and the protons of the terminal CH_3 group of the acid part came up-field around δ 0.81 as a triplet (*J* 6.7 Hz).

The ^{13}C-NMR spectrum showed a characteristic signal at δ 173.4 due to one carbonyl group, and the four CH groups of the PMB ring were seen in the region δ 130.2 and δ 113.7 ppm. Carbons corresponding to the α- and β-anomeric centres appeared at δ 106.0 and δ 100.5 respectively, while the remaining carbons of the sugar and glycerol part were seen in the region of δ 85.9 – 66.0. The carbons of the CH_2 chain ranged from δ 34.0 – 22.7 and the carbons of the

CH_3 came up-field around δ 14.1. All the signals described above are shown in the HSQC-NMR spectrum **(Figure 67)**, which confirmed the structure of the compound.

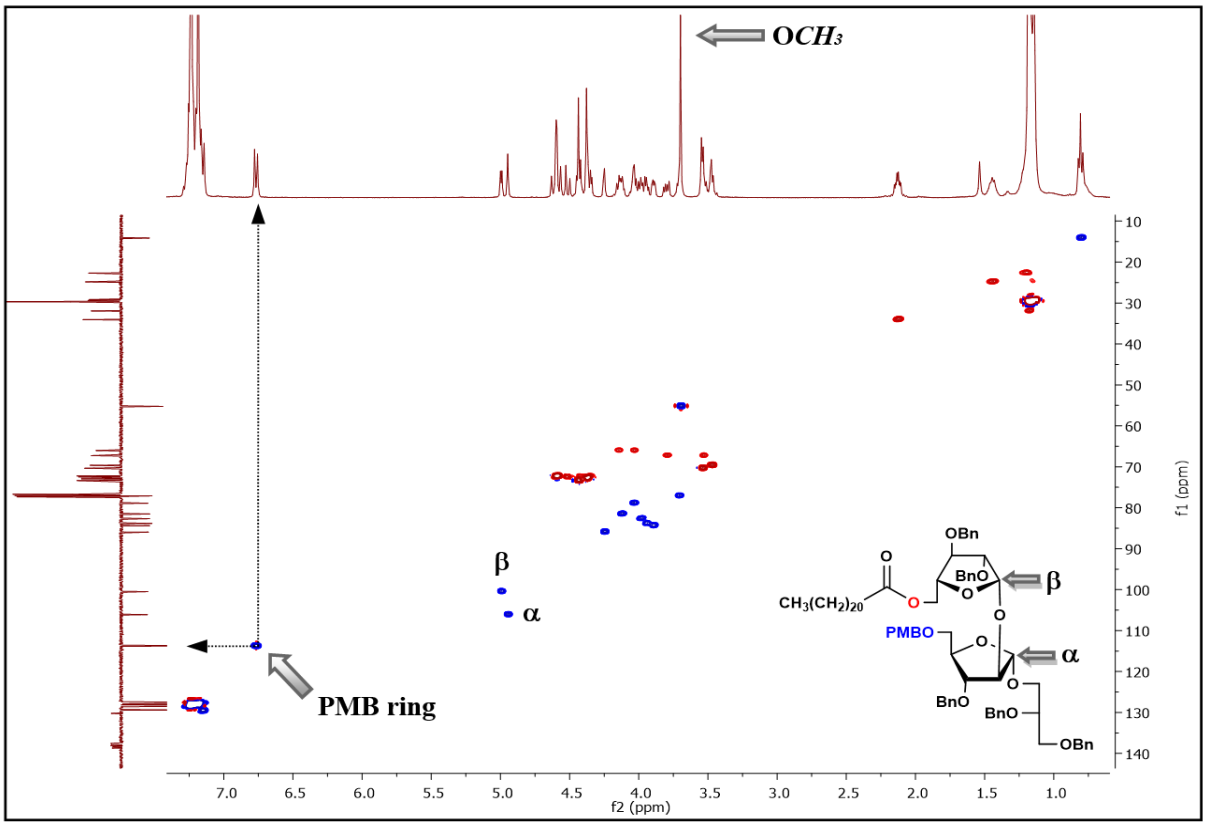


Figure 67: ^{1}H, ^{13}C and HSQC-NMR of compound **(80a)**.

De-protection of **(80a)**, removing the PMB group, was carried out by using ceric ammonium nitrate (CAN) in a mixture of CH_3CN: H_2O (9:1) and stirring at 0 ºC – R.T for 1 h. to afford **(81a)** in 62% yield **(Figure 68)**.

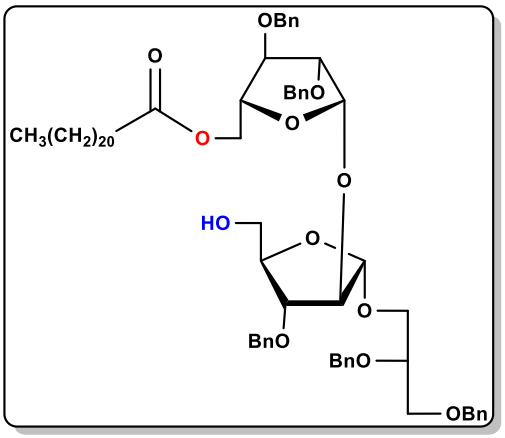


Figure 68: Structure of compound **(81a)**.

Once again, the formation of this compound was proved by mass spectrometry and NMR spectroscopy (^{1}H and ^{13}C), which showed approximately similar signals for the glycan and the acid to those of **(80a)**, except those signals corresponding to the PMB group **(Figure 69)**. The I.R. spectrum of **(81a)** gave characteristic bands for the hydroxyl group at 3414 and the carbonyl group at 1737 cm^{-1}.

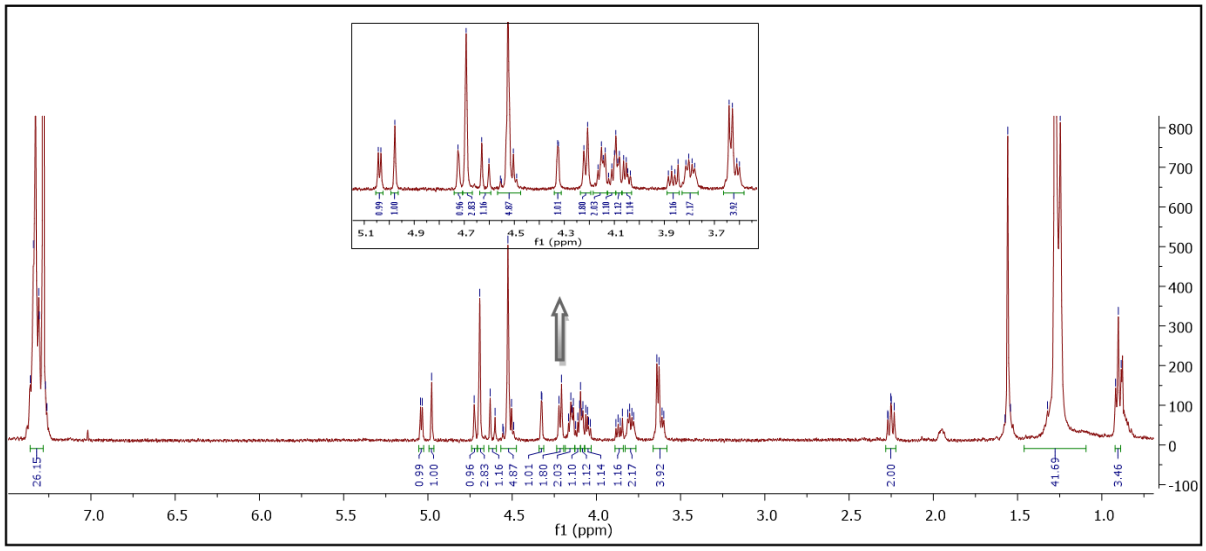


Figure 69: ^{1}H-NMR of mono-ester alcohol compound **(81a)**.

Now, by utilising the same coupling conditions as before, compound **(81a)** and a second molecule of behenic acid were coupled again to make a model glycolipid. This time the reaction mixture was stirred for 72 h, and afforded a symmetrical fully protected DMAG analogue compound **(82a)** in 50% yield **(Figure 70)**.

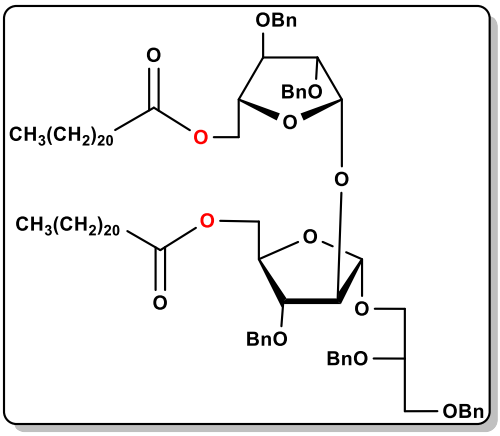


Figure 70: Structure of compound **(82a)**.

The ^{1}H-NMR spectrum (**Figure 71)** of this compound showed signals and integration corresponding to the glycan and the acid part moieties that were identical to those of the previously prepared and discussed compound **(70c) (Scheme 21)**.

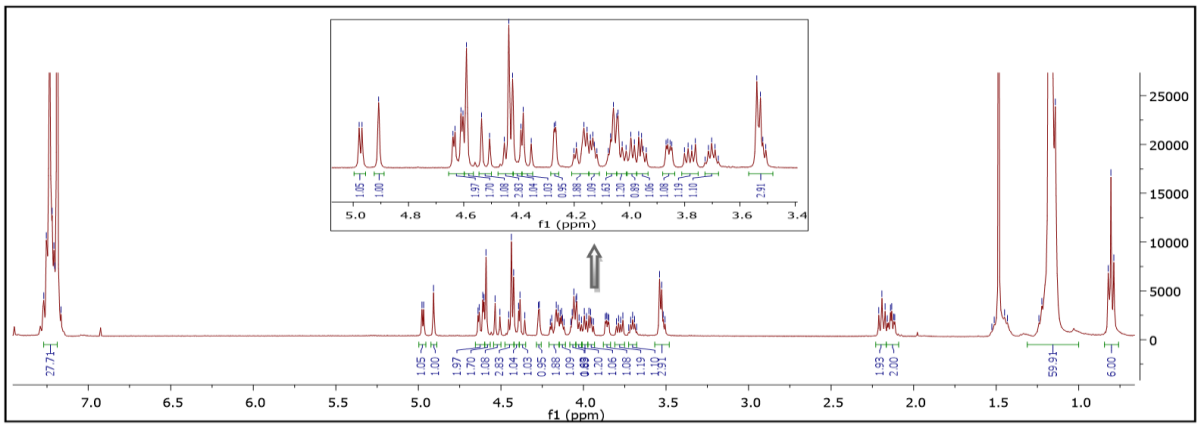


Figure 71: ^{1}H-NMR of a symmetrical fully protected DMAG analogue compound **(82a)**.

2.2.2 Selective esterification of the glycan (66) with a protected keto-MA (76f)

The glycan **(66)** was coupled with a TBDMS-protected synthetic keto-MA **(76f)**[111] by the same method described above to prepare **(80b)** in 86% yield **(Figure 72)**.

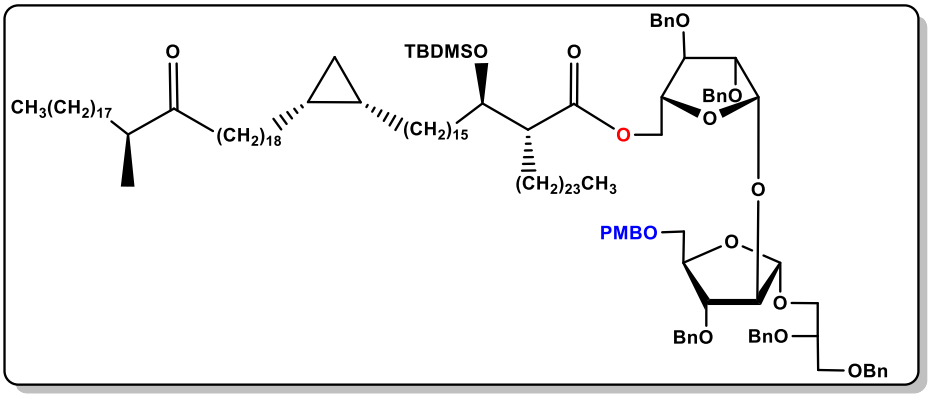


Figure 72: Structure of compound **(80b)**.

This showed characteristic NMR signals **(Figure 73 and 74)**; all the signals corresponding to the glycerol glycan part were approximately similar to those of **(80a)** and the signals belonging to the mycolic acid were identical to those discussed before for the keto-MA.

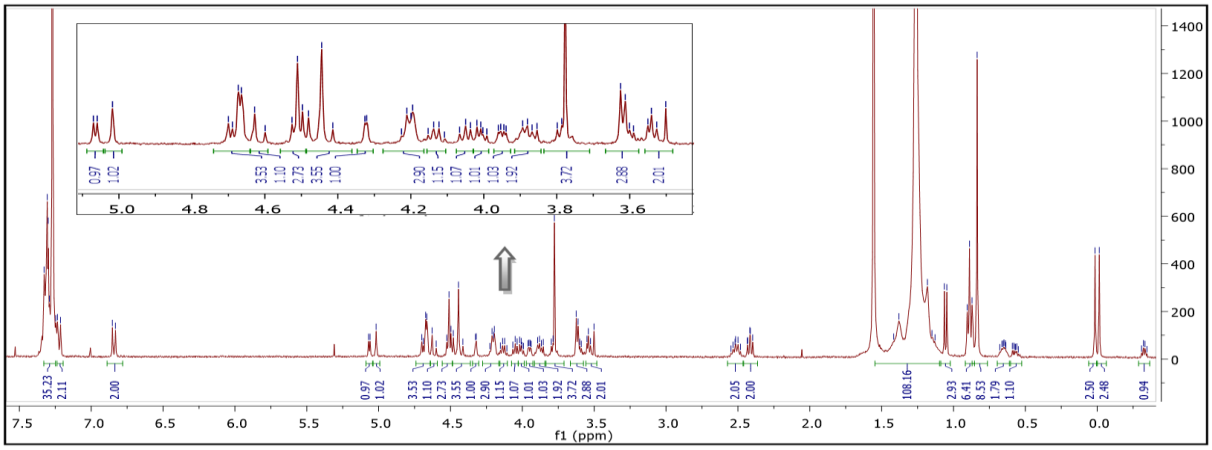


Figure 73: ^{1}H-NMR of compound **(80b)**.

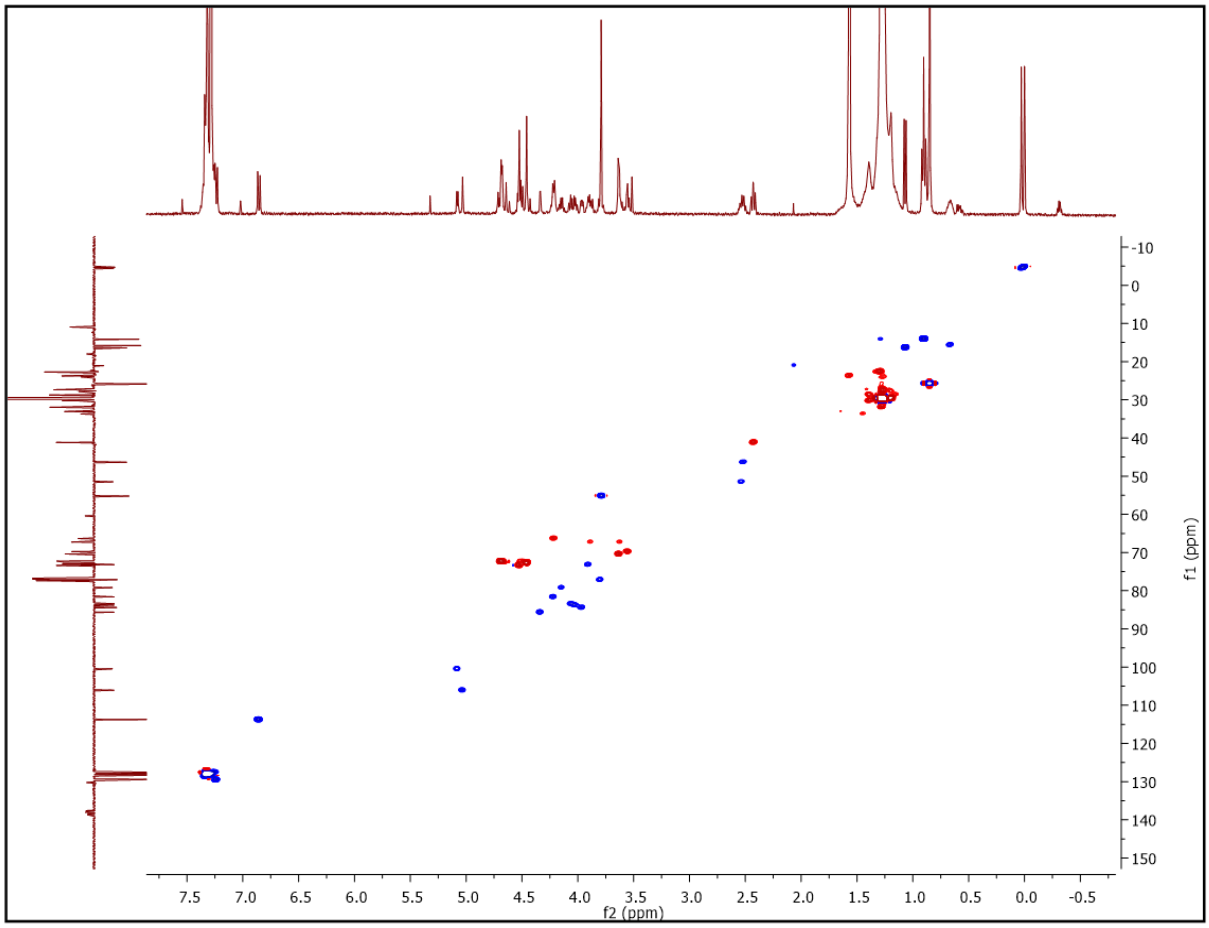


Figure 74: HSQS-NMR of compound **(80b)**.

Next, ceric ammonium nitrate (4 eq.) was used, this time in a mixture of CH_3CN: H_2O: THF (9:1:0.2) and stirring at room temperature for 16 h, to remove the PMB group. Surprisingly, this resulted in the removal of both the PMB group from the lower arabinose molecule, as expected, and also the TBDMS group from the β-position of the mycolic acid core, to afford **(81b)** in 83% yield **(Figure 75)**.

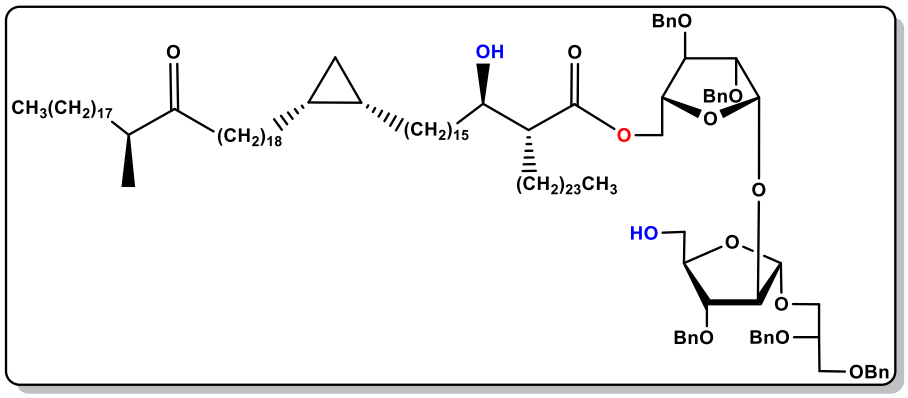


Figure 75: Structure of compound **(81b)**.

The ^{1}H-NMR spectrum showed characteristic signals for the glycan and the acid part similar to those of **(80b),** apart from those signals corresponding to the PMB and TBDMS groups **(Figure 76)**.

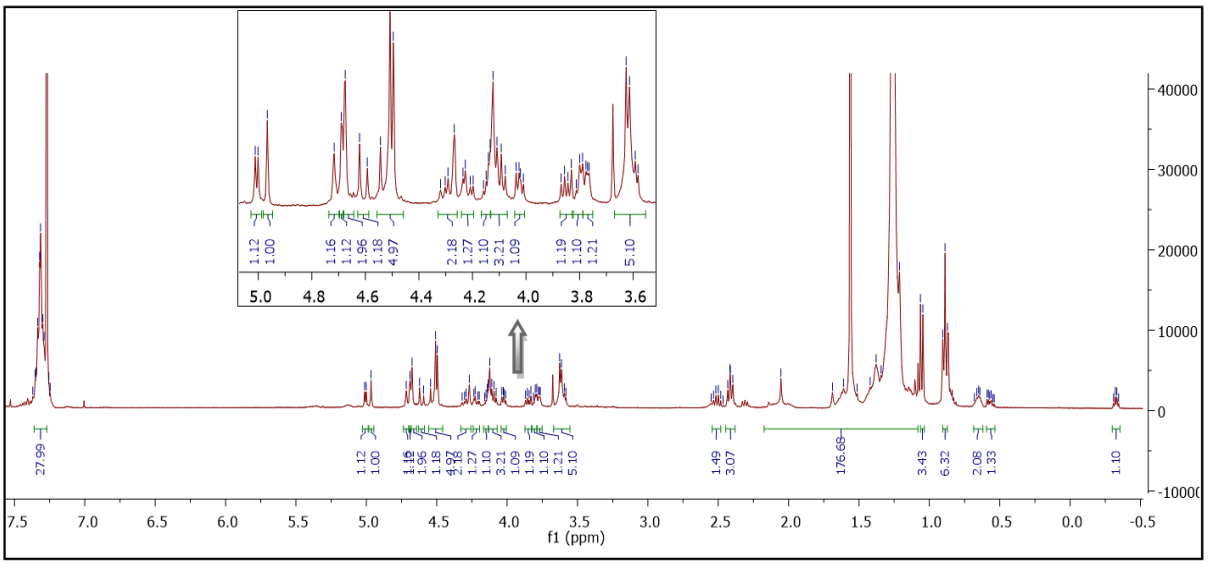


Figure 76: 1H-NMR of compound **(81b)**.

To examine the coupling at the free hydroxyl group on the bottom arabinose molecule, and to avoid the complexity of the ^{1}H-NMR for the resulting product, a second esterification of **(81b)** with a second protected keto-MA (same MA) was achieved by the method given previously, and afforded **(82b)** in 50% yield **(Figure 77)**. The low yield in comparison to the previous method, may be related to the sterically hindred structure of **(82b)**.

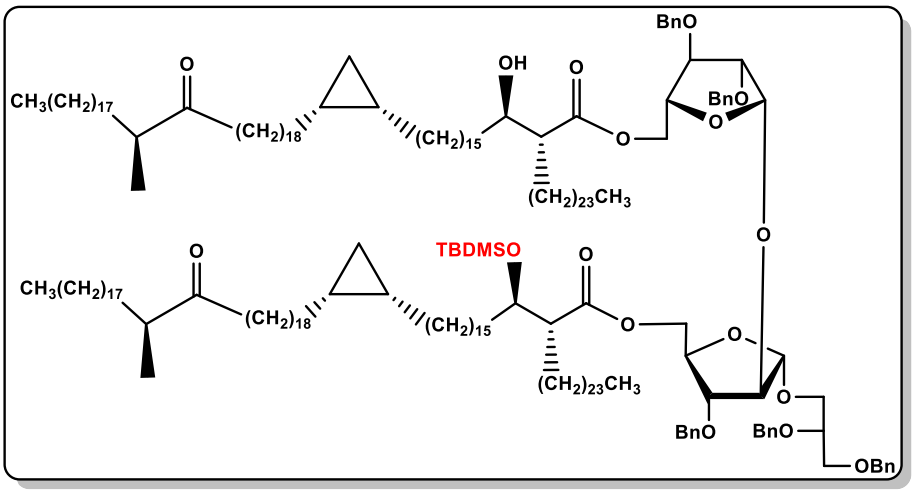


Figure 77: Structure of compound **(82b)**.

Compound **(82b)** showed characteristic NMR signals for the glycan and the acid part, again similar to those for **(81b)** with double integration, and new signals for the protected mycolic acid. **Figure 78** shows the ^{1}H-NMR spectrum of **(82b)** which confirmed the structure of the compound.

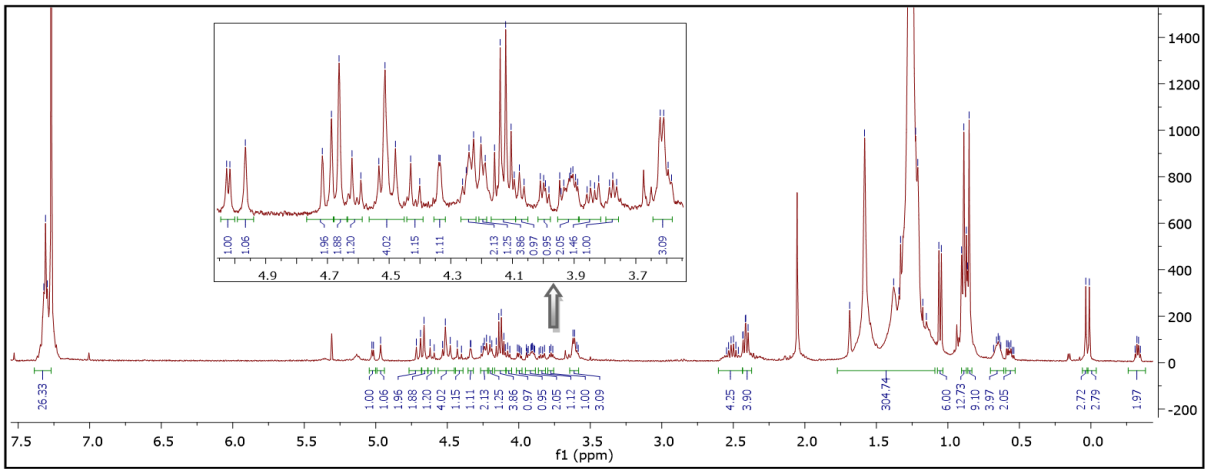


Figure 78:1H-NMR of compound **(82b)**.

The ^{13}C-NMR spectrum of **(82b)** showed characteristic signals at δ 215.2 for the keto-MAs and at δ 175.0 and 174.4 for the ester carbonyls.

It is worth mentioning that by esterifying the DMAG in two separate steps, this will allow unsymmetrical DMAG's, with two different MAs, to be prepared. This may be useful for the biological assays, and may lead to compounds with improved biological activity.

2.2.3 Summary

In this part of the thesis, the following targets have been achieved successfully:

1. The glycan moiety di-arabino glycerol (DAG) incorporating L-glycerol was synthesised with the correct stereochemistry.
2. Esterification of the above moiety with three different simple fatty acids to make the DMAG glycolipid analogues has been carried out.
3. A model DMAG using a β-hydroxy acid coupled to DAG, was prepared.
4. Esterification with four common classes of synthetic mycolic acids, to make the DMAG glycolipids has been achieved. The synthesised glycolipids are shown below **(Figure 79)**.

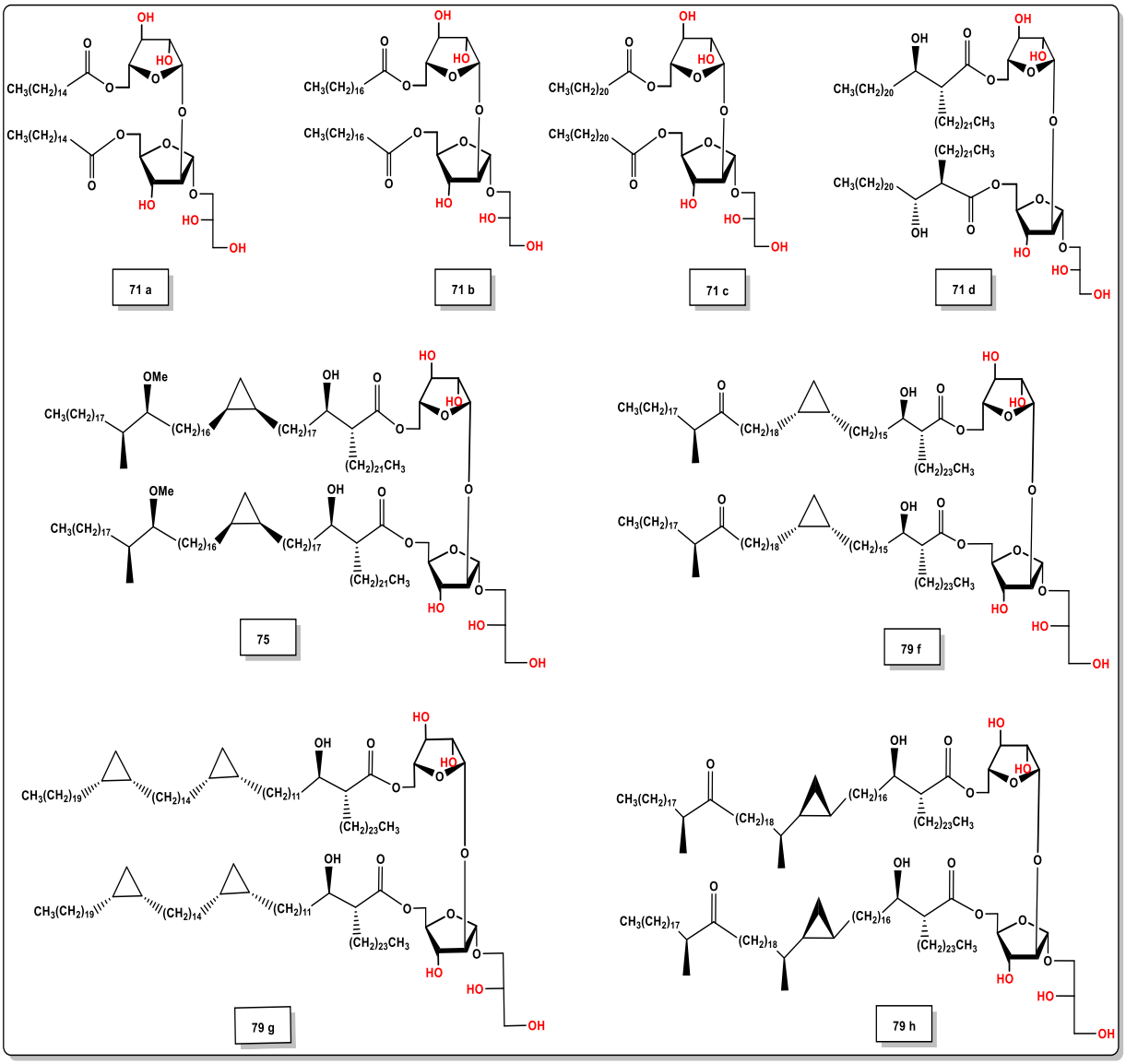


Figure 79: Structures of synthesised DMAG glycolipids.

5. The penta-acetate analogue of the DMAG of behenic acid was prepared to compare it with the natural mixture. This proved and confirmed the structure and stereochemistry of this novel sugar of the DMAG glycolipid to be α-β-di-arabino-furanosyl glycerol.

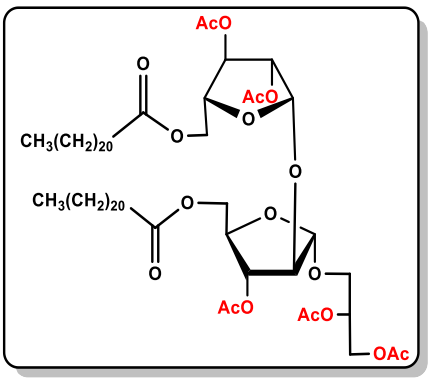


Figure 80: Structure of synthetic DMAG penta-acetate analogue **(72)**.

6. Selective esterification with different acids at each primary alcohol position was carried out, to produce prepared mono or symmetrical di-glycolipids. The prepared mono- and di-glycolipids are shown below **(Figure 81)**.

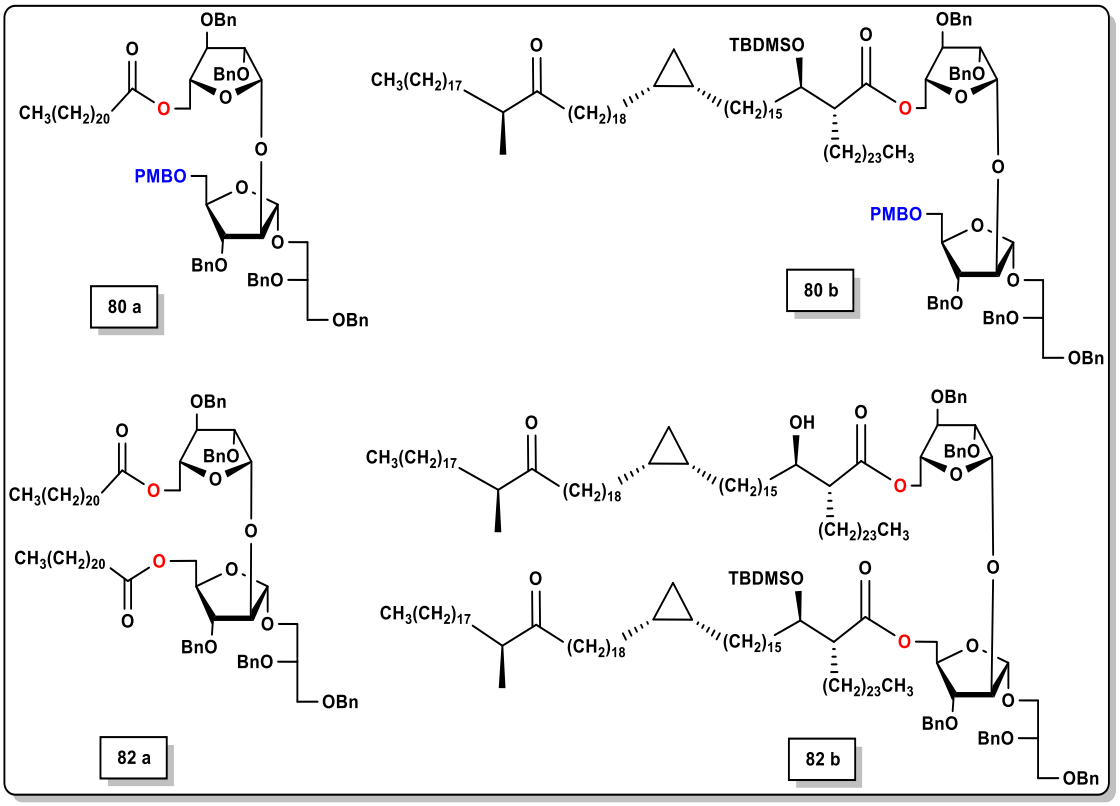


Figure 81: Structures of selective mono- and di-symmetrical protected glycolipids.

Section 3

2.3.1 Synthesis of di-mycolyl tri-arabinofuranosyl glycerol (DMTAG)

2.3.2 The aims of this part

- To prepare the glycan tri-arabinofuranosyl glycerol (TAG) **(Figure 82)**, which is 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl -α-D-arabinofuranoside.

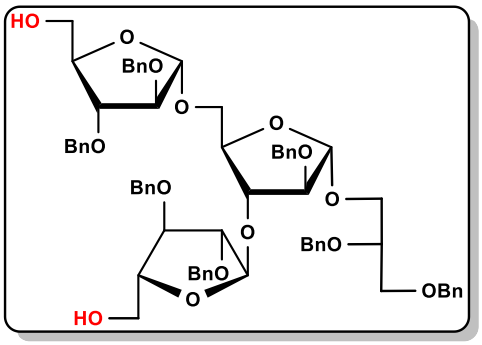
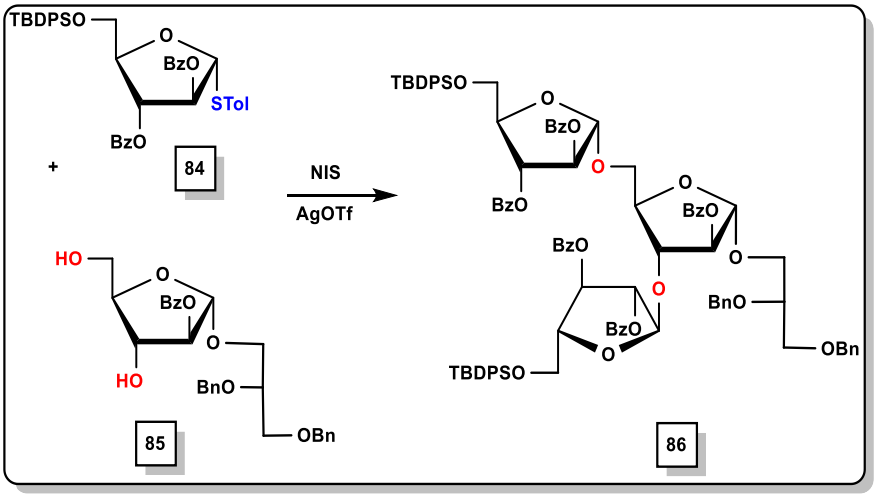


Figure 82: Structure of the glycan **(TAG)**.

- To prepare a model glycolipid formed by the esterification of TAG with a simple fatty acid.
- To prepare a series of DMTAG glycolipids, through esterification of the above glycan with structurally defined synthetic MAs.
- To investigate the biological activity of the synthesised compounds, as they are important components of the cell wall of mycobacteria. In particular, their antigenicity will be studied.

2.3.3 Synthesis of fully protected tri-arabino-furanosyl glycerol (TAG)

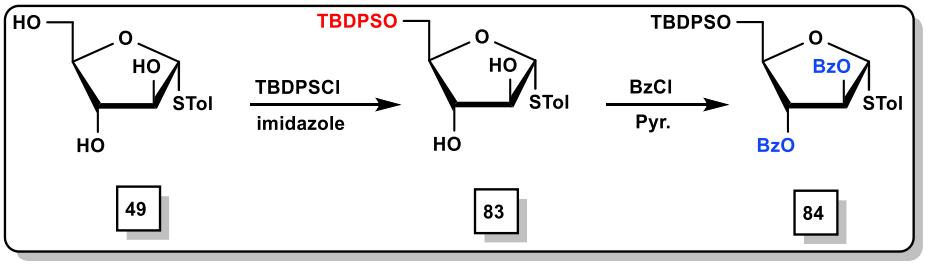
Although the methyl tri-saccharide of D-arabinofuranoside had been prepared and esterified with different fatty acids, such as behenic, palmatic and butyric acids earlier,[144] there is no report of the synthesis of tri-arabinofuranosyl-L-glycerol. The target tri-saccharide structure **(86)** has three α-glycosidic linkages, and can be assembled readily from the following building blocks, the donor **(84)**[242] and the diol acceptor **(85) (Scheme 29)**.



Scheme 29: Synthesis of fully protected glycan tri-arabino glycerol (TAG) **(86)**.

2.3.3.1 Synthesis of the donor (84)

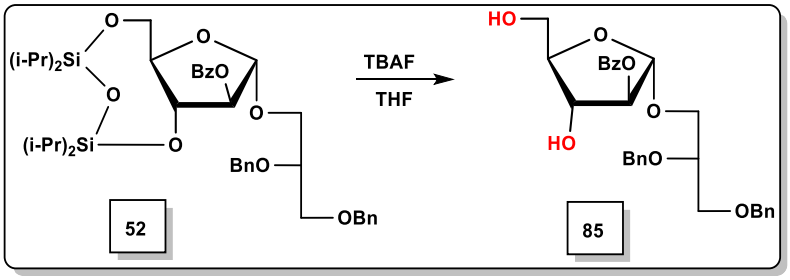
The synthetic route to the 2,3-*O*-benzoyl protected thioglycosyl donor **(84)** started from thioglycoside triol **(49)**. The primary hydroxyl group at the C-5 position was protected by treating **(49)** with TBDPS in dry DMF in the presence of a catalytic amount of imidazole to afford **(83)** in 82% yield. In order to protect the two secondary hydroxyl groups at the C-2 and C-3 positions, compound **(83)** was suspended in pyridine before benzoyl chloride was added, to give **(84)** in 85% yield. The NMR data of **(84)** were identical to the literature **(Scheme 30)**.[242]



Scheme 30: Synthesis of the TAG's donor **(84)**.

2.3.3.2 Synthesis of the acceptor (85)

Having the donor target in hand, the exploration of the synthesis of the acceptor **(85)** was carried out. The initial step in the synthetic route, was preparing the fully protected arabinofuranosyl glycerol **(52)** from D-arabinose as described earlier **(Scheme 7)**, then, removal of the silyl group using TBAF in dry THF gave two free hydroxyl groups at the C-3 and C-5 positions respectively, affording the target acceptor **(85)** in 95% yield **(Scheme 31)**.



Scheme 31: Synthesis of TAG's acceptor **(85)**.

The structure of the acceptor **(85)** was confirmed by mass spectrometry [NSI–Found $(M+Na)^+$: 531.2; $C_{29}H_{32}NaO_8$, requires: 531.2]. The 2D-NMR spectrum (**Figure 83)** showed the α-anomeric proton was correlated to its carbon.

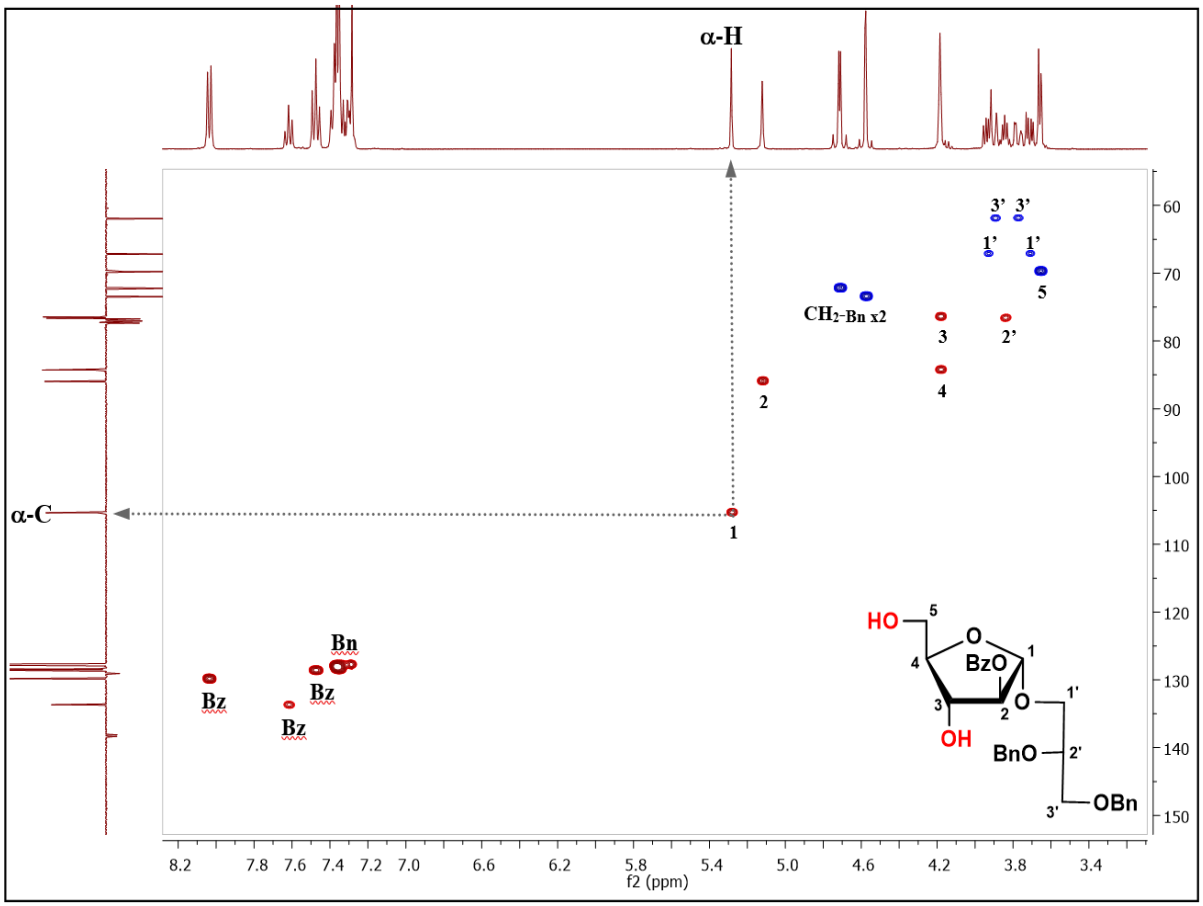


Figure 83: HSQC-NMR spectrum for TAG's acceptor **(85)**.

2.3.3.3 Coupling the donor and the acceptor

Lowary *et al*. have used NIS/AgOTf in dry CH_2Cl_2 to couple the thioglycoside donor **(84)** with a 2-*O*-benzylated diol acceptor but not with the acceptor **(85)** used here. In order to investigate the effect of the protecting group at the C-2 position, the 5-silylated thioglycoside donor **(84)** and the new 2-*O*-benzoylated acceptor **(85)** were reacted under the reported conditions to give the desired glycan **(86)** as mentioned earlier **(Scheme 29)**. The tri-arabino-glycerol **(86)** was obtained in 91% yield, which was similar to that reported by Lowry, when using the 2-*O*-benzylated acceptor with a methoxy group at the anomeric centre rather than a glycerol as described here. The ^{1}H-NMR spectrum of **(86)** showed three signals downfield corresponding to the three protons at the anomeric centre of each ring, as broad singlets at δ 5.61, 5.31 and 5.22. The ^{13}C-NMR spectrum **(Figure 84)** established the presence of the glycosidic linkages in the tri-arabinofuranosyl glycerol **(86)**, with the signals at δ 106.1 and 105.2 ppm belonging to the three carbons at the anomeric centers. Those signals together confirmed the three α-glycosidic linkages in the glycan.

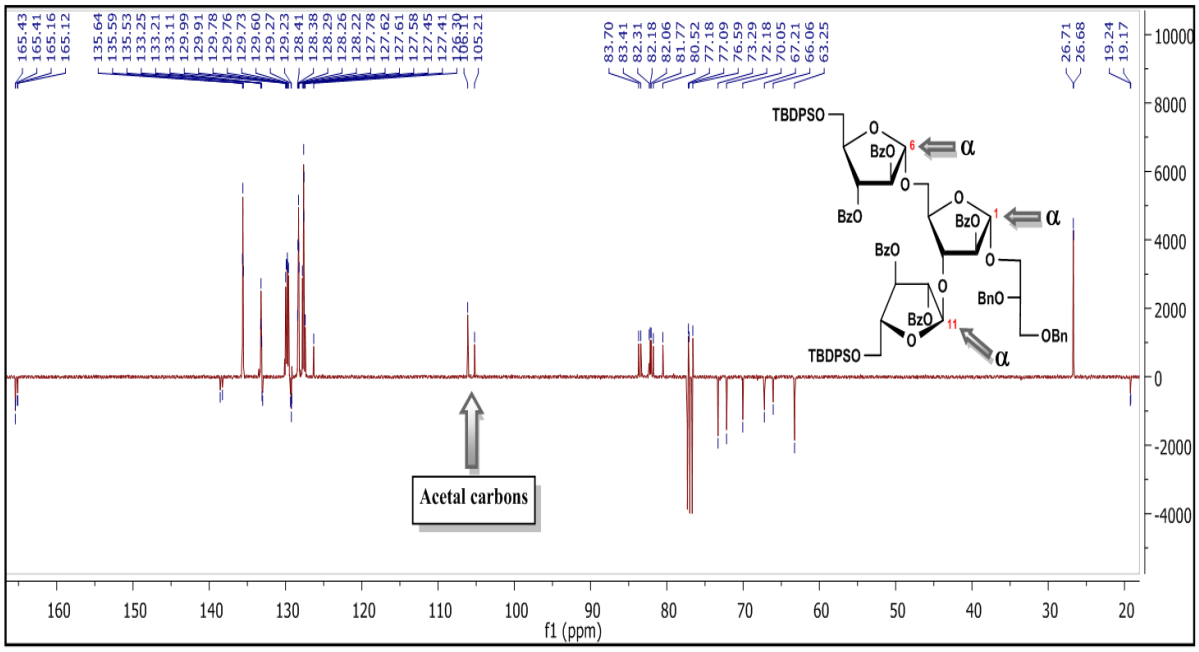


Figure 84: ^{13}C-NMR spectrum for fully protected tri-arabino glycerol **(86)**.

The HSQC-NMR of **(86)** confirmed the structure of the compound **(Figure 85)**, the spectrum showed three significant peaks corresponding to the acetal protons, at δ 5.61, 5.31 and 5.22, correlated to their carbons, which confirmed that the coupling between the donor **(84)** and the acceptor **(85)** was successful**.**

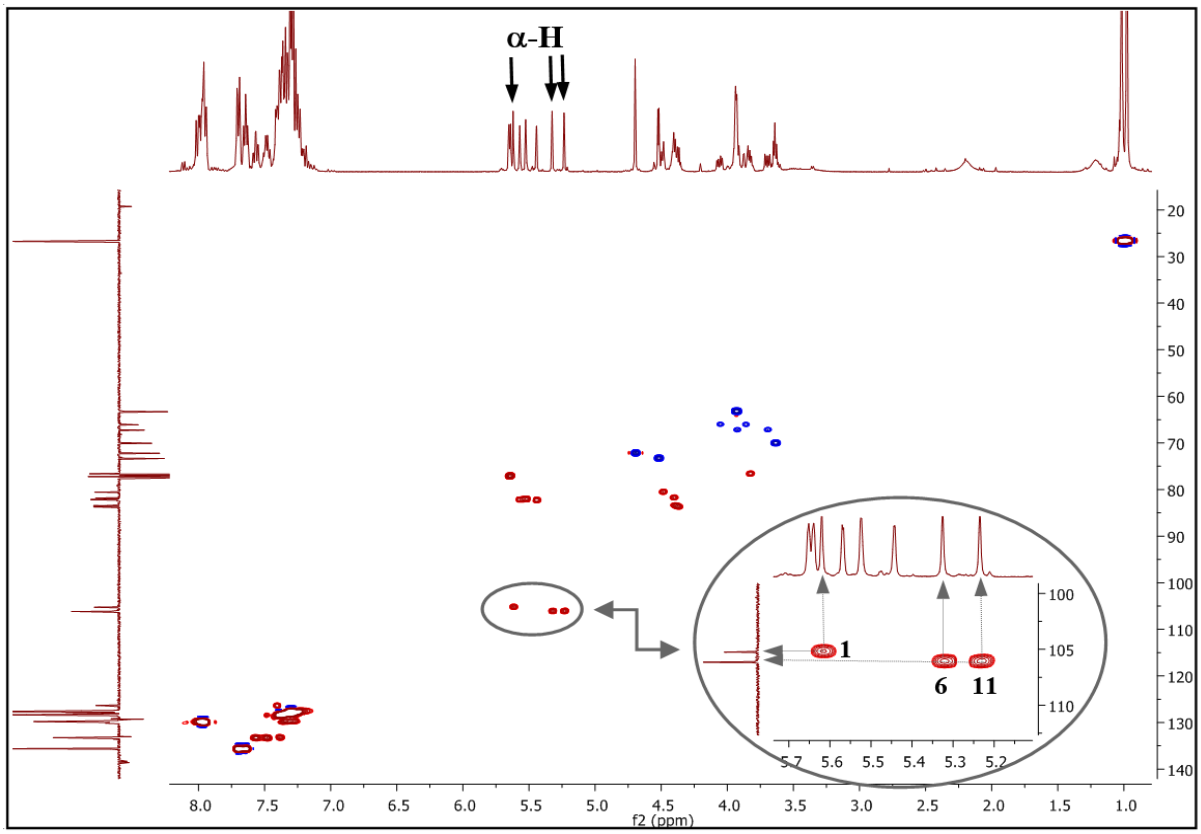


Figure 85: HSQC-NMR spectrum for tri-arabino glycerol **(86)**.

The structure of **(86)** was also confirmed by mass spectrometry **(Figure 86)**.

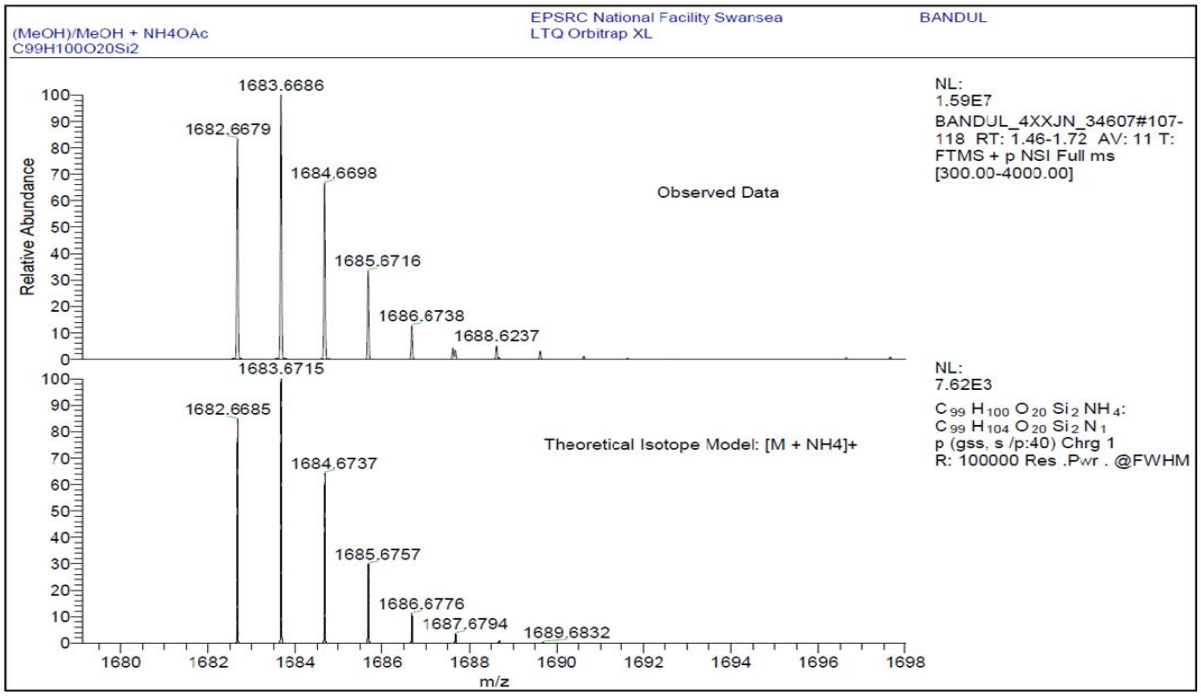
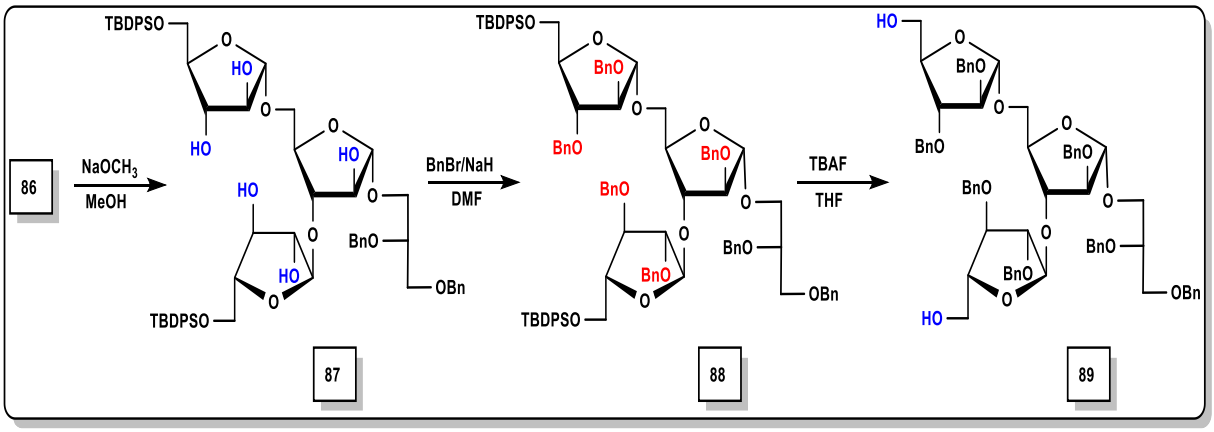


Figure 86: Mass spectrum of the tri-saccharide compound **(86)**.

The tri-saccharide **(86)** was deprotected with sodium methoxide to give **(87)** as a thick oil in 83% yield. Formation of the penta-hydroxy saccharide **(87)** was confirmed by ^{1}H-NMR, where all the signals corresponding to the protons on the carbon adjacent to the benzoyl ester were shifted up-field. The ^{13}C-NMR spectrum showed the disappearance of the carbonyl signals which indicated the success of the hydrolysis. Compound **(87)** was benzylated to protect the five secondary hydroxyl groups using benzyl bromide and sodium hydride in dry DMF to give **(88)** in 65% yield, followed by de-protection of the two primary hydroxyl groups using TBAF to afford **(89)** in 87% yield **(Scheme 32)**.



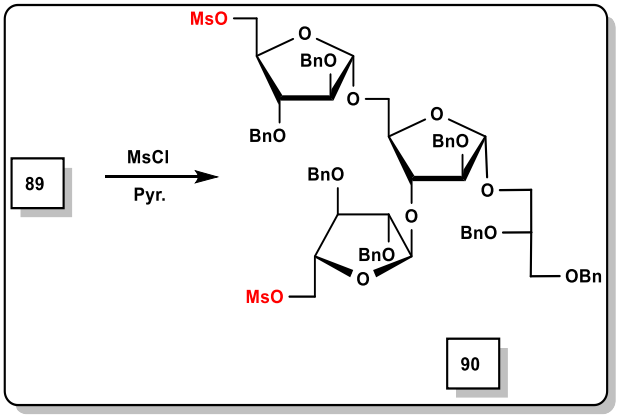
Scheme 32: Synthesis of compounds **(87-89)**.

The material obtained, **(89)**, was split into two portions, each portion being used in different esterification methods as follows (*cf*. DMAG section):

1. An alkylative coupling using cesium hydrogen carbonate after mesylation of the primary hydroxyl groups in the glycan.

2. Direct coupling of the sugar alcohol with the fatty acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI).

In order to use the $CsHCO_3$ method, the two primary hydroxyl groups in the first portion of **(89)** were activated, by treating with MsCl in dry pyridine in the presence of catalytic DMAP in dry CH_2Cl_2 at 0 ºC to afford the corresponding mesylate **(90)** in 87% yield **(Scheme 33)**.



Scheme 33: Structure of the tri-arabino-glycerol di-mesylate **(90)**.

The structure of **(90)** was confirmed by mass spectrometry and NMR spectroscopy. The ^{1}H-NMR spectrum of **(90)** (**Figure 87)** showed the expected signals, including two singlets at δ 2.94 and 2.89 for the CH_3 of the mesylate groups.

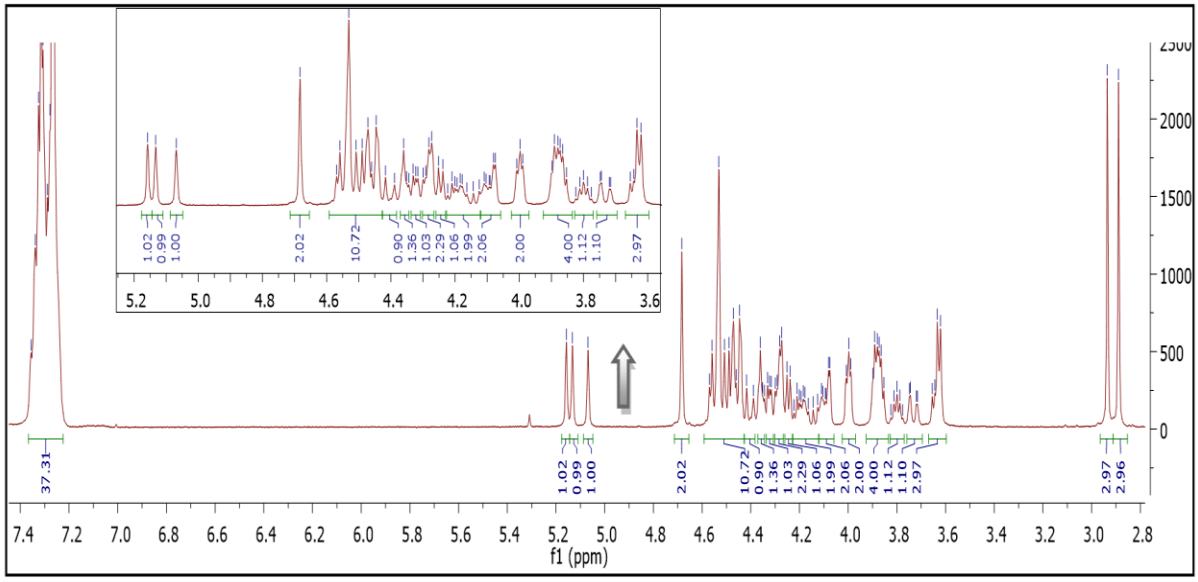


Figure 87: ^{1}H-NMR spectrum for compound **(90)**.

The ^{13}C-NMR spectrum **Figure 88** showed two signals at δ 37.6 and 37.5 for the carbons of the mesylate groups. The assignments of the signals were made by comparison with literature values reported for the methoxy tri-arabinose compound, which is identical to (**90**) except for the absence of the glycerol moiety.[241]

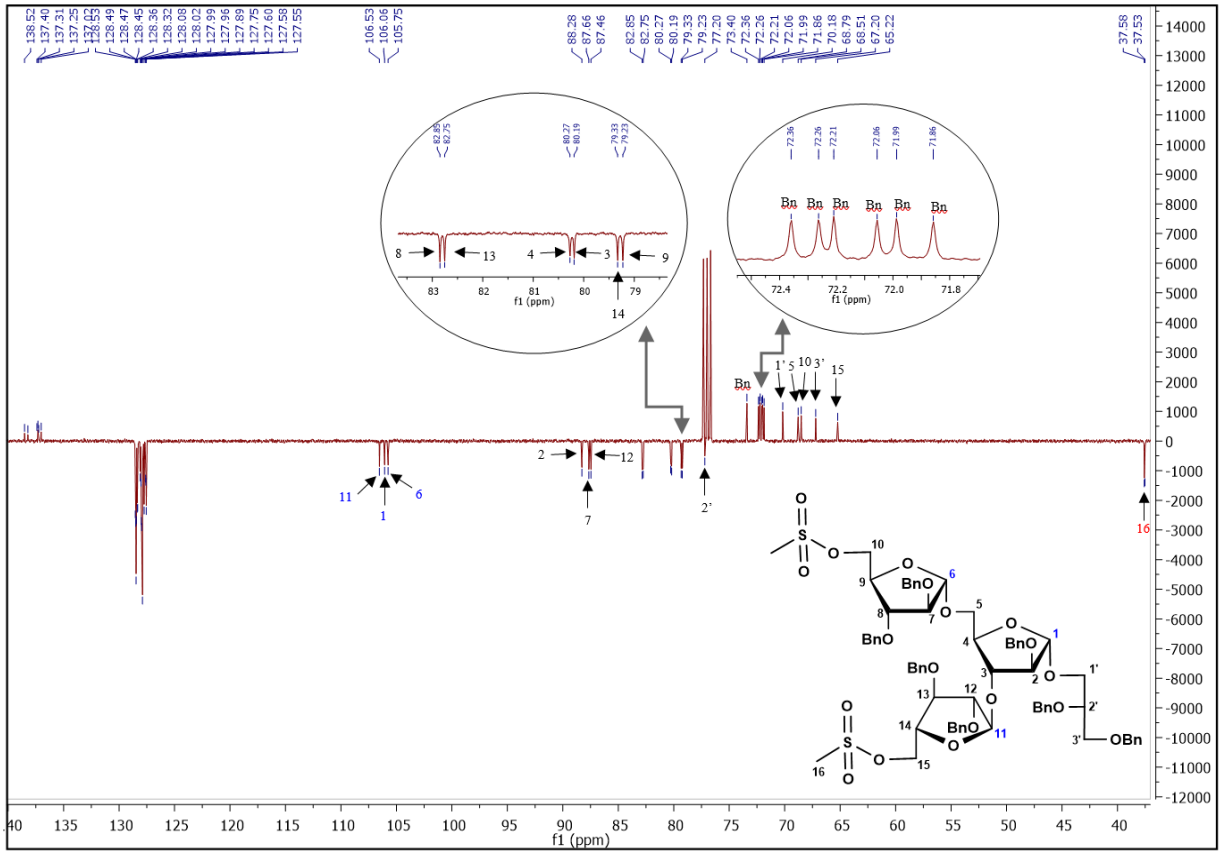


Figure 88: ^{13}C-NMR spectrum for compound **(90)**.

The 2D-NMR **Figure 89** confirmed the structure of **(90)** and showed the signals of the CH_3 groups and the acetal protons correlated to their carbons.

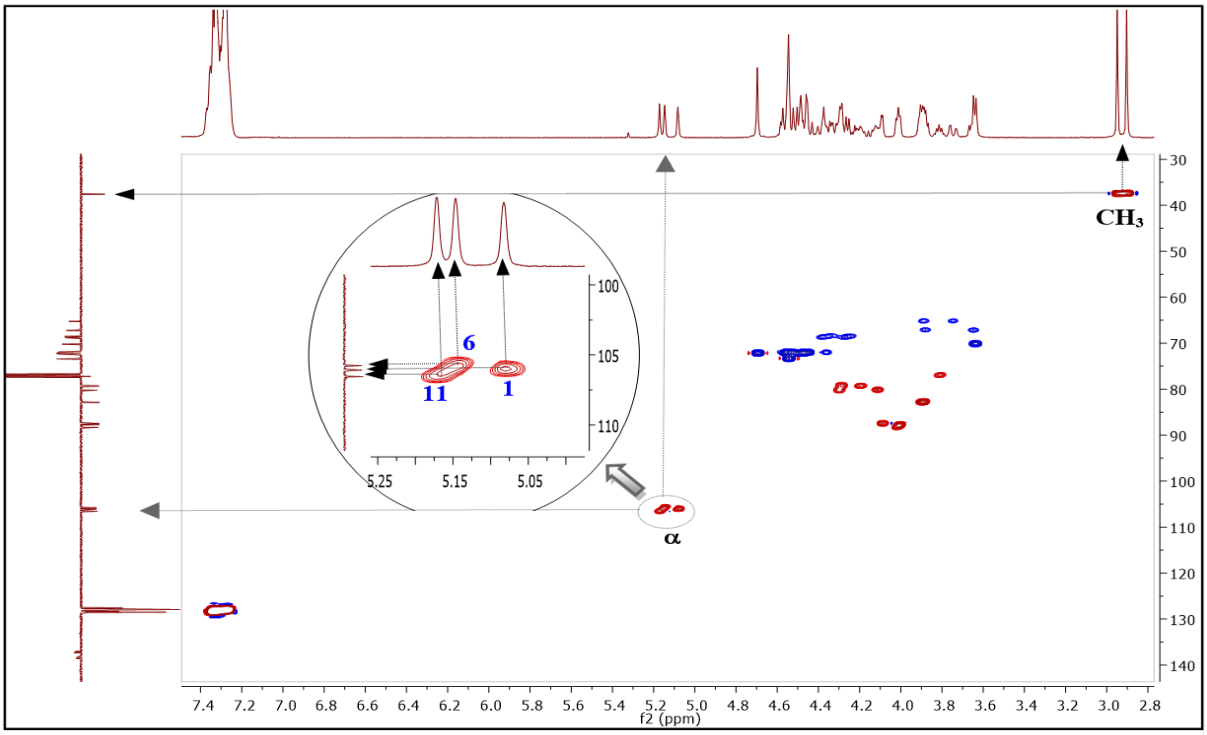
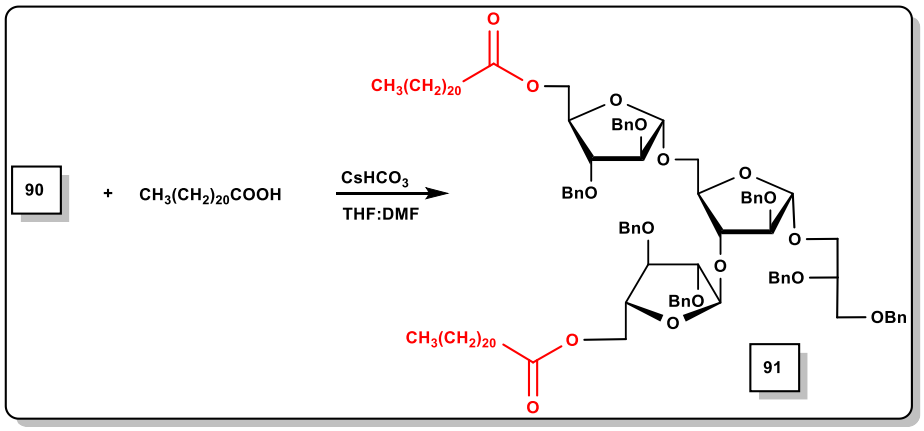


Figure 89: 2D-NMR spectrum for compound **(90)**.

2.3.4 Esterification of lipids with the glycan tri-arabino glycerol (90)

2.3.4.1 Esterification with a simple fatty acid

Having prepared the mesylate **(90)**, the exploration of coupling with behenic acid to make a model analogue, was now undertaken. The fully protected di-behenoyl-tri-arabino-glycerol **(91)** was prepared by coupling the mesylate (**90)** with behenic acid through the alkylative esterification strategy using cesium hydrogen carbonate in dry THF: DMF at 70 ºC for 3 days and afforded **(91)** in 80% yield **(Scheme 34)**.



Scheme 34: Synthesis of a model analogue of fully protected di-behenoyl tri-arabino glycerol **(91)**.

The formation of **(91)** was confirmed by NMR spectroscopy. The region of the ^{1}H-NMR spectrum which was of most interest was between δ 5.15 – 4.90, which corresponds to the three protons at the anomeric centres on the glycan rings. Signals at δ 5.09, 5.06 and 4.97, integrating to one proton each, occurred as broad singlets. The CH_2 groups adjacent to the carbonyls gave a triplet at δ 2.17 (*J* 7.6 Hz) integrating to 4 protons. The terminal methyl group showed a triplet signal at δ 0.81 (*J* 6.8 Hz) integrating to 6 protons.

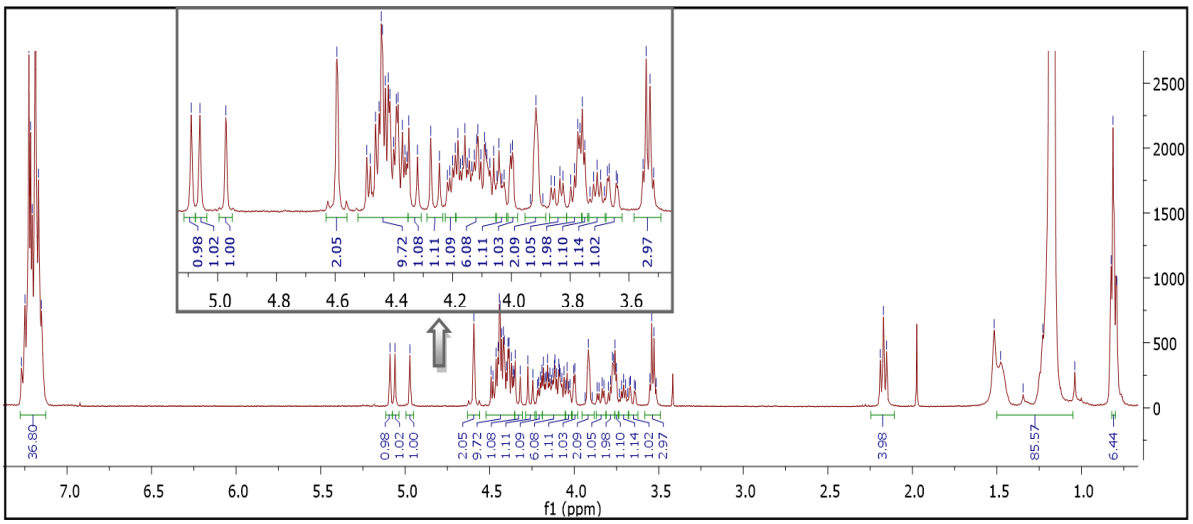


Figure 90: ^{1}H-NMR spectrum for compound **(91)**.

The ^{13}C-NMR spectrum showed two signals at δ 173.6 and 173.5 for the carbonyl groups. Signals corresponding to the carbon at the anomeric centre for the three rings appeared at δ 106.5, 106.2 and 105.5. The carbon of the CH_2 group adjacent to the carbonyl in the acid appeared at δ 34.1. The CH_2 chain ranged from δ 32 - 22 and the CH_3 came up-field at δ 14.1.

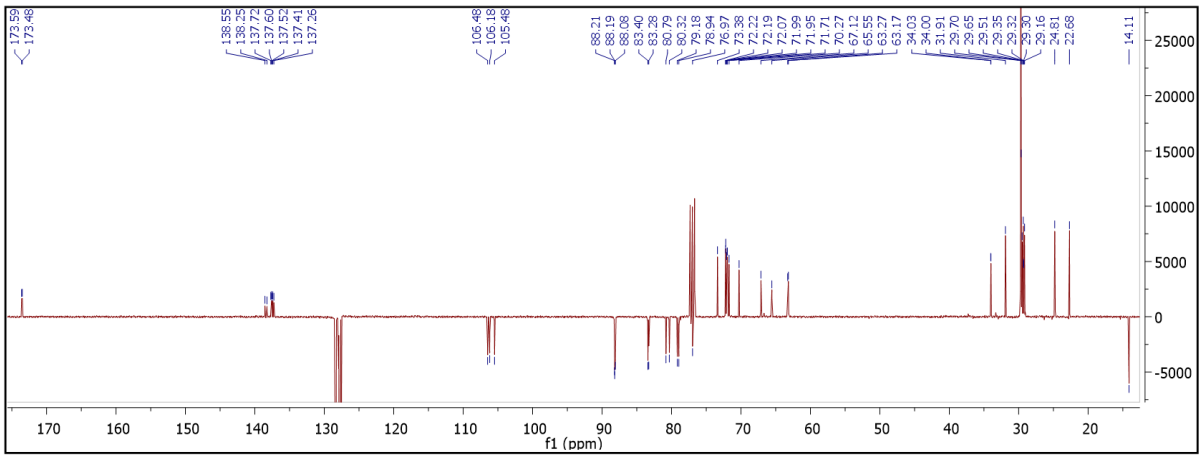


Figure 91: 13C-NMR spectrum for compound **(91)**.

All the signals described above are shown in the HSQC-NMR spectrum, where the anomeric protons and the behenic acid (the CH_2 adjacent to carbonyl and the terminal CH_3) signals were correlated to their carbons **(Figure 92)**.

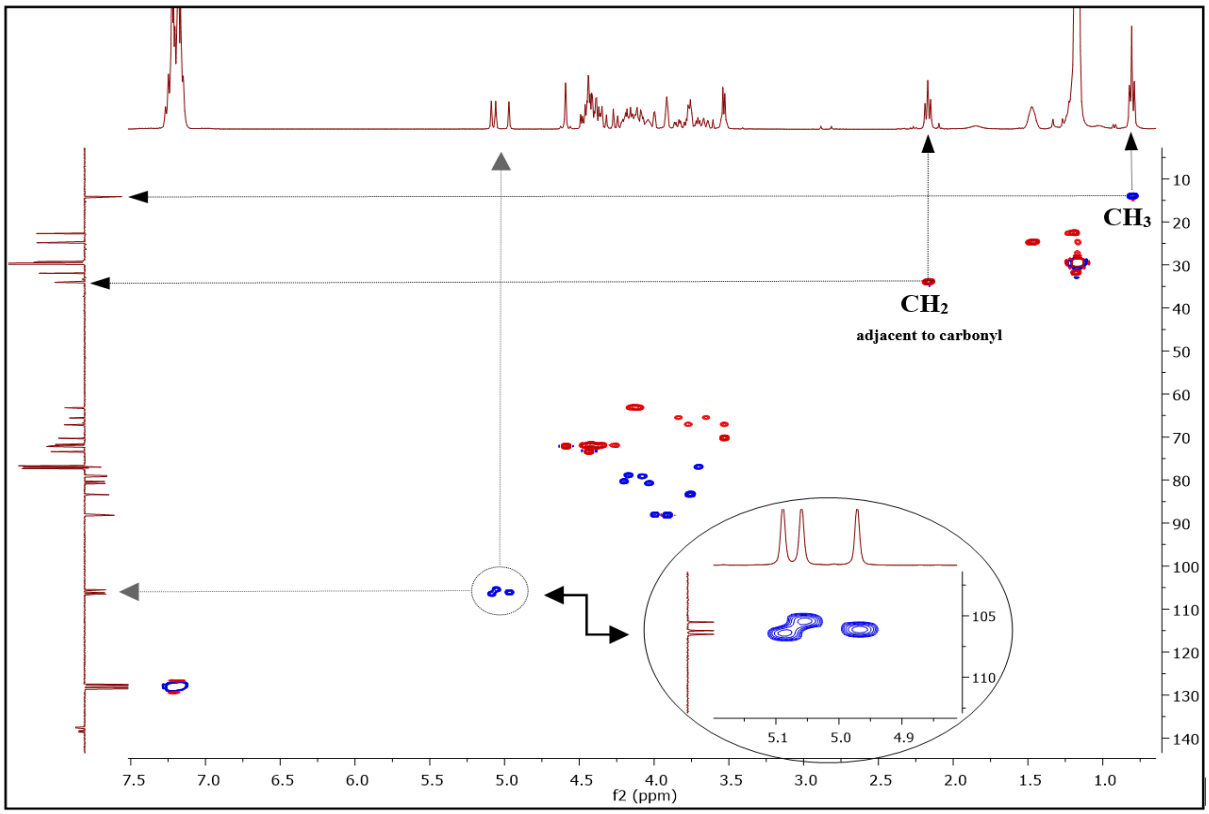
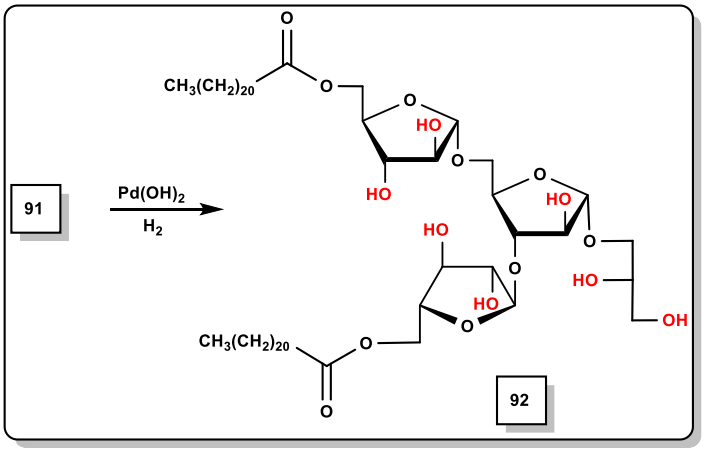


Figure 92: 2D-NMR spectrum for compound **(91)**.

Compound **(91)** was debenzylated by stirring vigorously in a suspension of $Pd(OH)_2$ in dry CH_2Cl_2:MeOH (1:1) under an atmosphere of hydrogen for 36 h to give the target DMTAG analogue **(92)** in 82% yield **(Scheme 35)**.



Scheme 35: Synthesis of a model analogue of di-behenoyl tri-arabino glycerol **(92)**.

The ^{1}H-NMR spectrum **(Figure 93)** of compound (**92**) confirmed the success of the hydrogenolysis, and showed three broad singlets at δ 5.01, 4.97 and 4.90 ppm for the three α-position protons respectively, the remaining 20 protons of the sugar and glycerol moieties appeared between δ 4.30 – 3.50 ppm. The four protons next to the two carbonyls gave a triplet at δ 2.30 ppm (*J* 7.6 Hz), while the terminal CH_3 appeared at δ 0.83 ppm as a triplet (*J* 6.5 Hz).

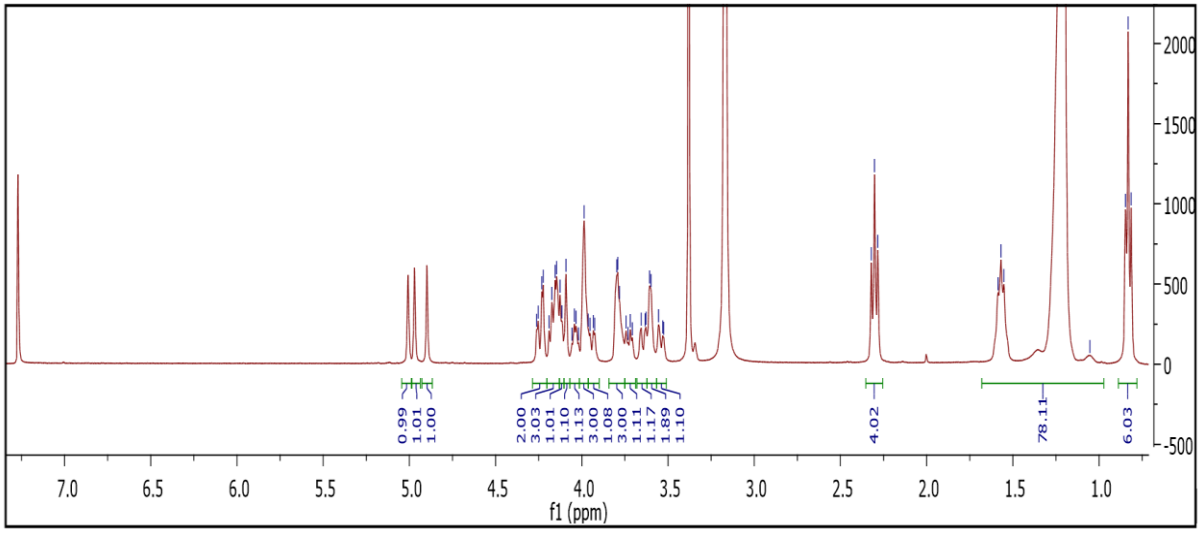


Figure 93: ^{1}H-NMR spectrum for compound **(92)**.

The ^{13}C-NMR spectrum obtained for the glycolipid analogue **(92)** gave signals illustrated in **(Figure 94)**, which were essentially identical to those for an analogue in the literature,[241] bearing a methoxy substituent at C-1 rather than the glycerol substituent in compound **(92)**, and are assigned on that basis **(See Table 6)**.

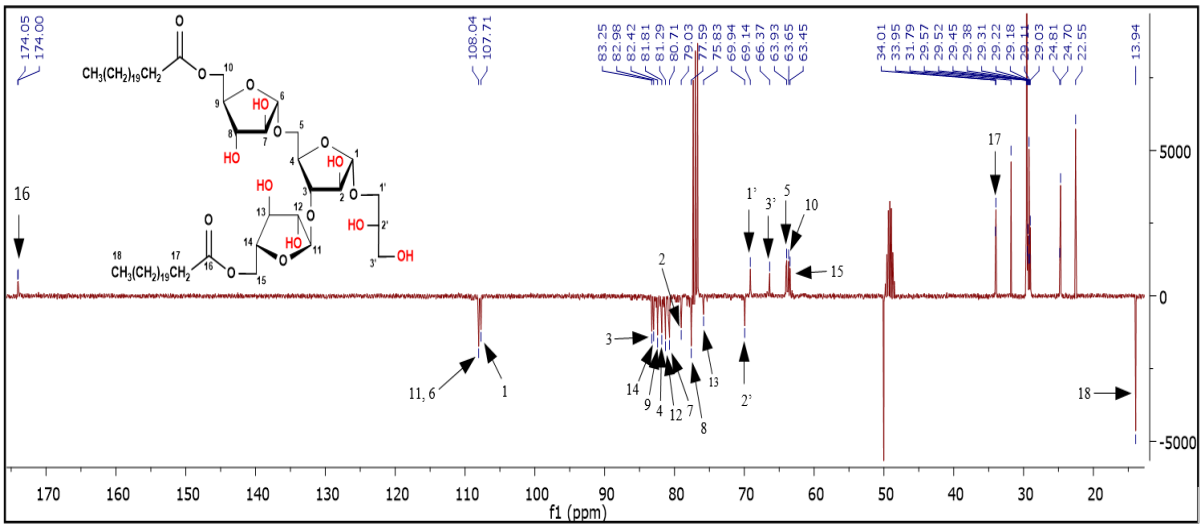


Figure 94: ^{13}C-NMR spectrum for compound **(92)**.

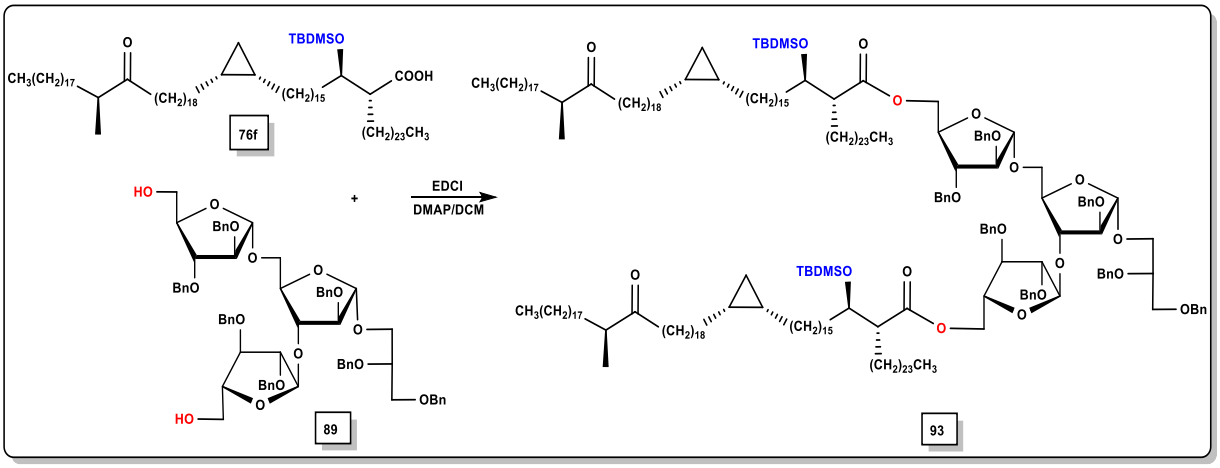
Table 6: The ^{1}H and ^{13}C-NMR data analysis of the glycolipid compound **(92)**

Proton	Shift	H's	Class	*J*/Hz	Carbon	*δ/ppm*
H_{11}	5.01	1	br. s	-	C_{16}	174.05
H_6	4.97	1	br. s	-	$C_{6,\ 11}$	108
H_1	4.90	1	br. s	-	C_1	107.7
$H_{10,\ 3}$	4.24	2	br.dd	3.2, 11.7	C_3	83.3
$H_{15,\ 1'',\ 13}$	4.17	3	br.dd	5.0, 11.8	C_{14}	83.0
$H_{15`}$	4.12	1	br.d	4.3	C_9	82.4
$H_{10`}$	4.09	1	m	-	C_4	81.8
H_4	4.04	1	br.q	5.5	C_{12}	81.3
$H_{2,\ 7,\ 9}$	3.95	3	br.m	-	C_7	80.7
$H_{5`}$	3.94	1	dd	3.6, 11.5	C_2	79.0
$H_{5,\ 2',\ 8}$	3.79	3	m	-	C_8	77.6
$H_{1'}$	3.73	1	br.dd	4.8, 10.1	C_{13}	75.8
$H_{3''}$	3.64	1	m	-	$C_{2'}$	69.9
$H_{3',\ 12}$	3.60	2	br.d	3.1	$C_{1'}$	69.1
H_{14}	3.54	1	m	-	$C_{3'}$	66.4
CH_2-Next to carbonyl	2.30	4	t	7.6	C_5	63.9
CH_2-Chain	1.39	83	m	-	C_{10}	63.7
CH_3-Terminal	0.83	6	t	6.5	C_{15}	63.5
-	-	-	-	-	C_{17}	34.0
-	-	-	-	-	C_{18}	13.9

2.3.5 Esterification of the glycan (89) with mycolic acids (76f-h) using the EDCI method

2.3.5.1 Esterification of the glycan (89) with keto-MA (76f)

Having secured a successful method for preparing a model analogue, the tri-arabinosyl glycerol di-behenate **(92)**, now, the glycan **(89)** was used to explore the coupling with three common classes of structurally defined synthetic MAs **(76f-h)**.[111] First, the glycan **(89)** was coupled with the synthetic keto-MA **(76f)** using the EDCI method as before (see **Scheme 24**, Section 1), and afforded **(93)** in 51% yield **(Scheme 36)**.



Scheme 36: Synthesis of fully protected DMTAG of keto-MA **(93)**.

This compound showed NMR signals for the keto-MA and the glycan similar to those discussed before **(Figure 95)**.

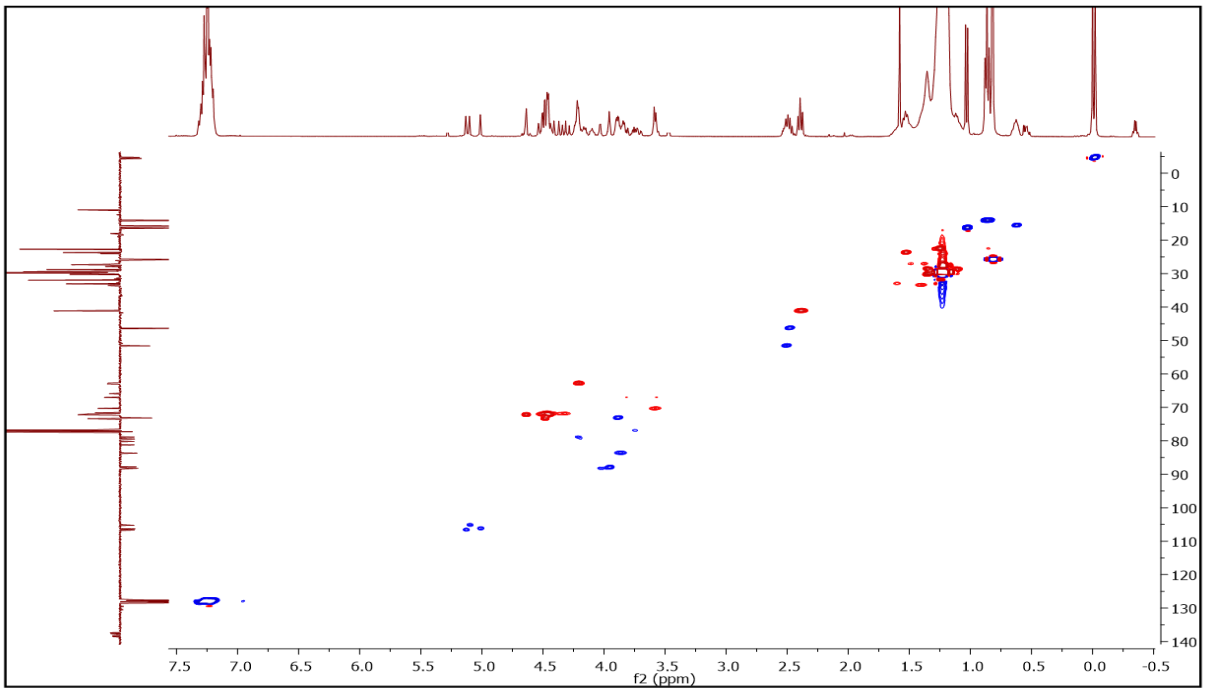
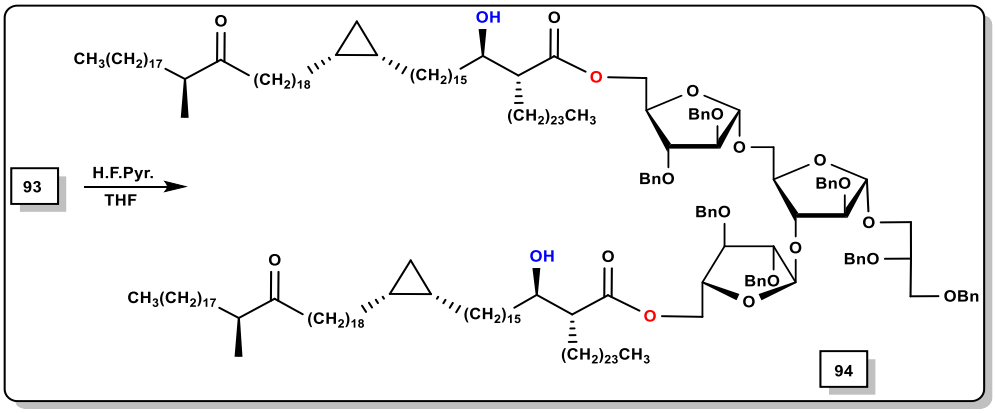


Figure 95: HSQC-NMR spectrum for compound **(93)**.

To improve the percentage yield of the de-silylation of **(93)**, an alternative method was used, compared to that used with the DMAG glycolipids (see **Scheme 25**, Section 1). This time, the TBDMS group at the β-position in the keto-mycolic acid was removed using hydrogen fluoride-pyridine complex (~70% HF) in dry THF, stirring the reaction mixture at 43 ºC for 24 h to afford **(94)** in 76 % yield **(Scheme 37)**, **(Figure 96)**.



Scheme 37: Synthesis of fully protected DMTAG **(94)**.

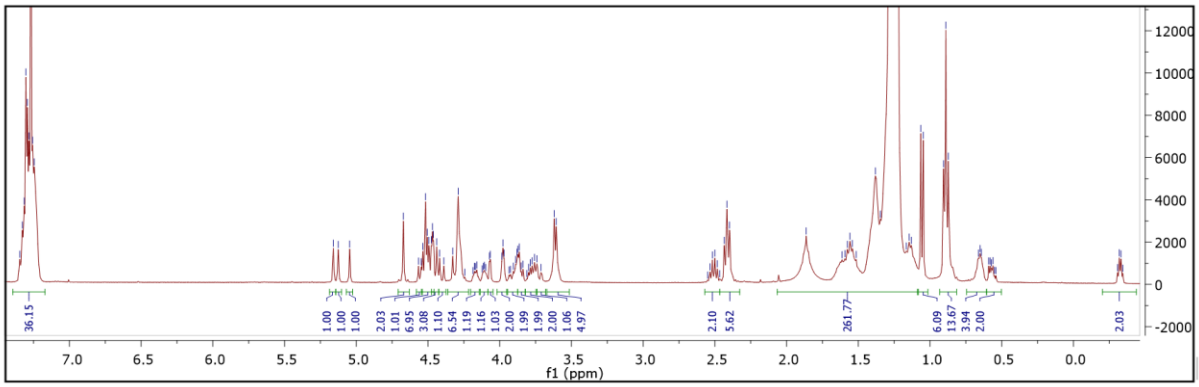
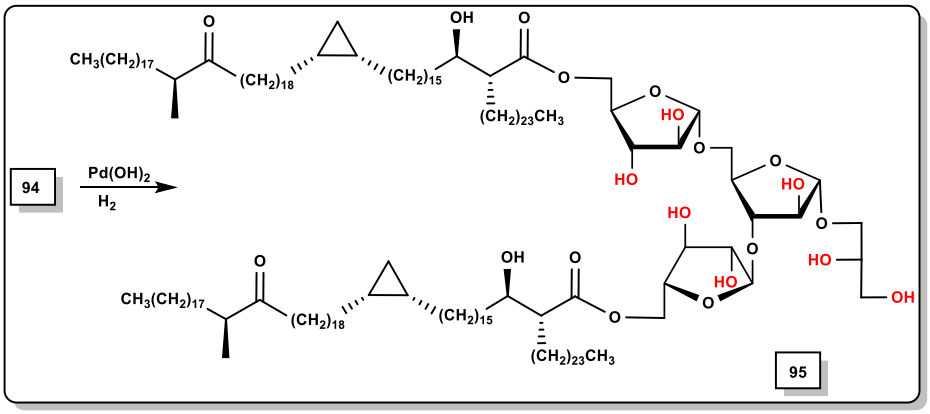


Figure 96: ^{1}H-NMR spectrum for compound **(94)**.

Hydrogenolysis of **(94)** by stirring it in dry CH_2Cl_2:MeOH (1:1) in the presence of $Pd(OH)_2$ and under a hydrogen atmosphere gave **(95)** in 86% yield **(Scheme 38)**.



Scheme 38: Synthesis of DMTAG of keto-MA **(95)**.

Compound **(95)** gave NMR signals **(Figure 97)** corresponding to the keto-MA and the glycan moiety similar to those discussed before.

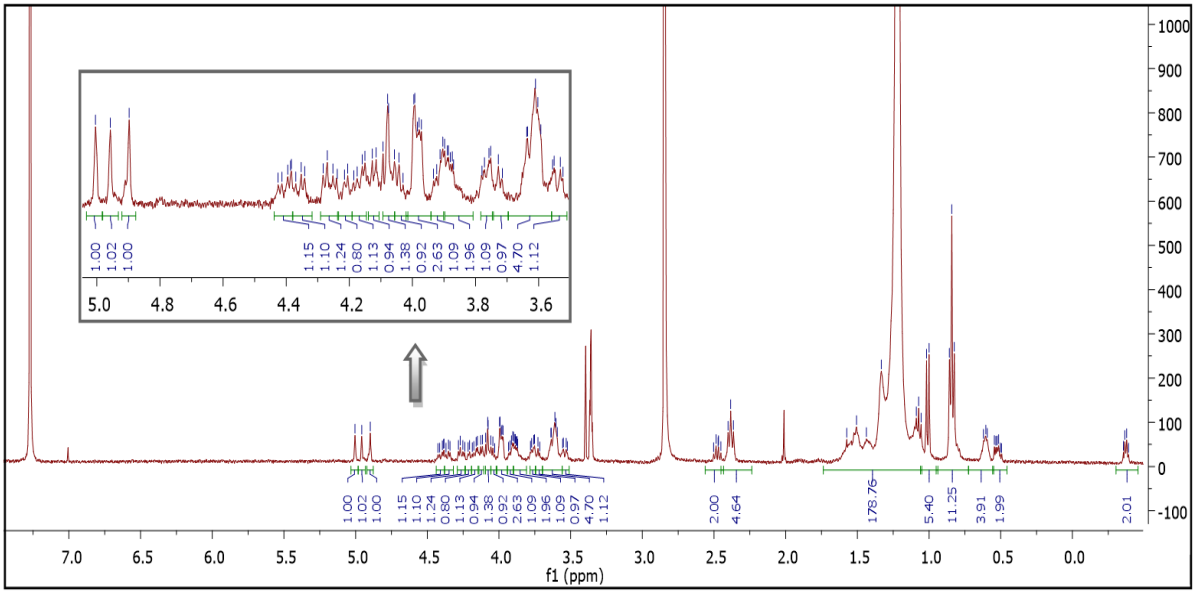
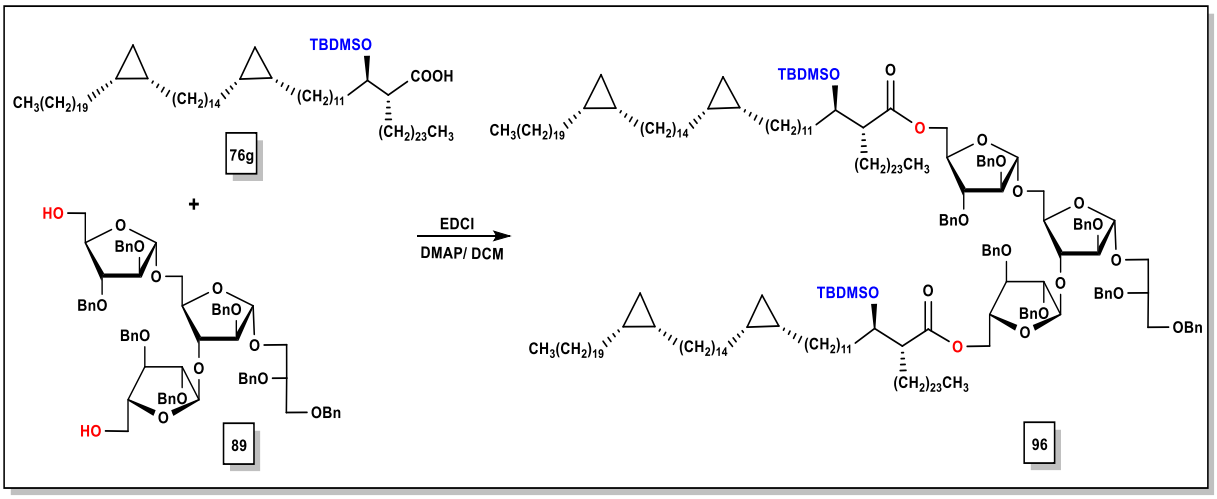


Figure 97: ^{1}H-NMR spectrum for compound **(95)**.

2.3.5.2 Esterification of the glycan (89) with α-mycolic acid (76g)

The TBDMS-protected α-MA with a 24 carbon α-alkyl chain **(76g)**,[109] was reacted with the sugar-diol **(89)** by the EDCI method, and afforded **(96)** in 84% yield **(Scheme 39)**.



Scheme 39: Synthesis of fully protected DMTAG of α-MA **(96)**.

The ^{1}H-NMR spectrum showed the success of the coupling between the tri-arabino-glycerol and the α-MA **(Figure 98)**.

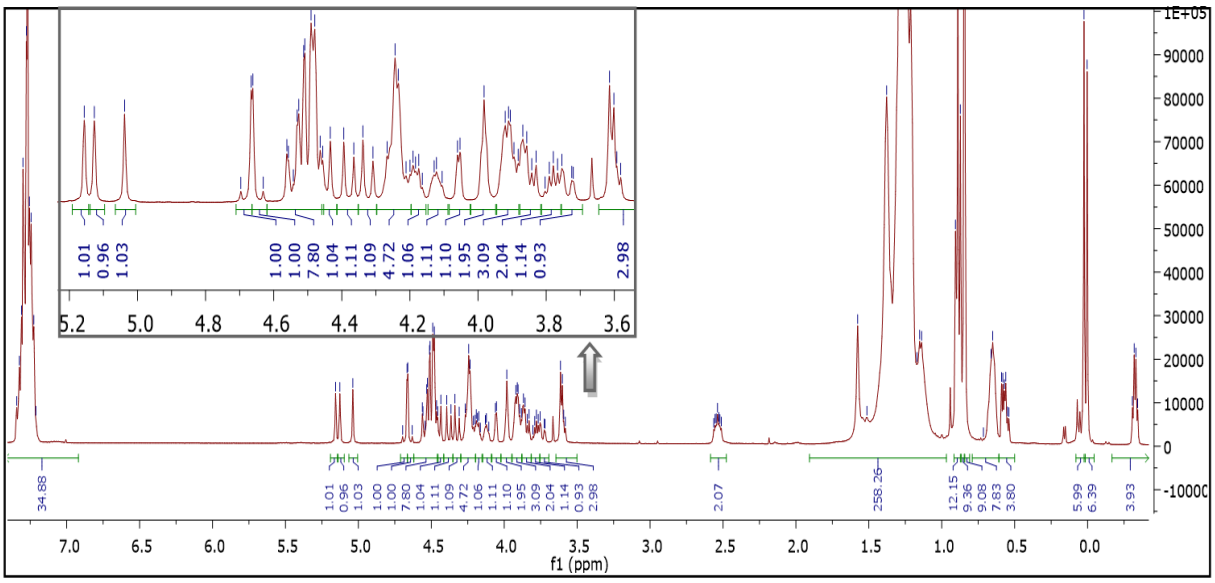
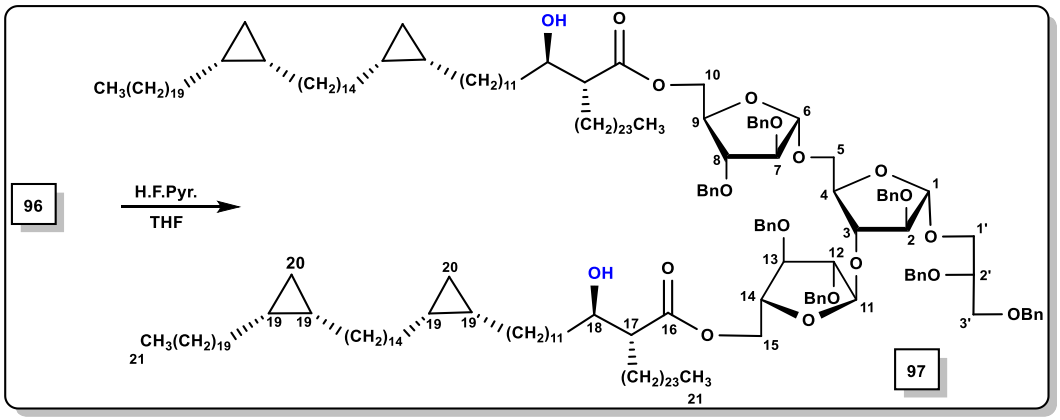


Figure 98: ^{1}H-NMR spectrum for compound **(96)**.

Removal of the silyl group from the β-position in the α-mycolic acid was achieved by the same method described previously (using hydrogen fluoride-pyridine complex), and afforded **(97)** in 51% yield **(Scheme 40)**.



Scheme 40: Synthesis of compound **(97)**.

The ^{1}H-NMR spectrum **(Figure 99)** of this compound showed the disappearance of signals corresponding to the TBDMS groups and gave characteristic NMR signals for the α-MA and the glycan moiety **(Table 7)**. These were assigned by comparison to values reported for the methoxy tri-arabino-dimycolate,[241] which is essentially identical to the DMTAG shown here.

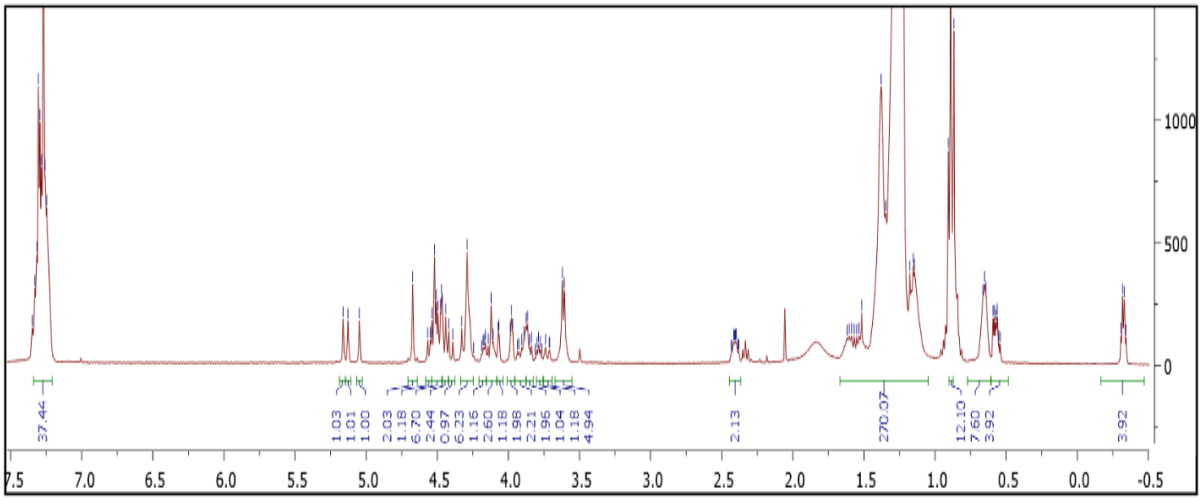
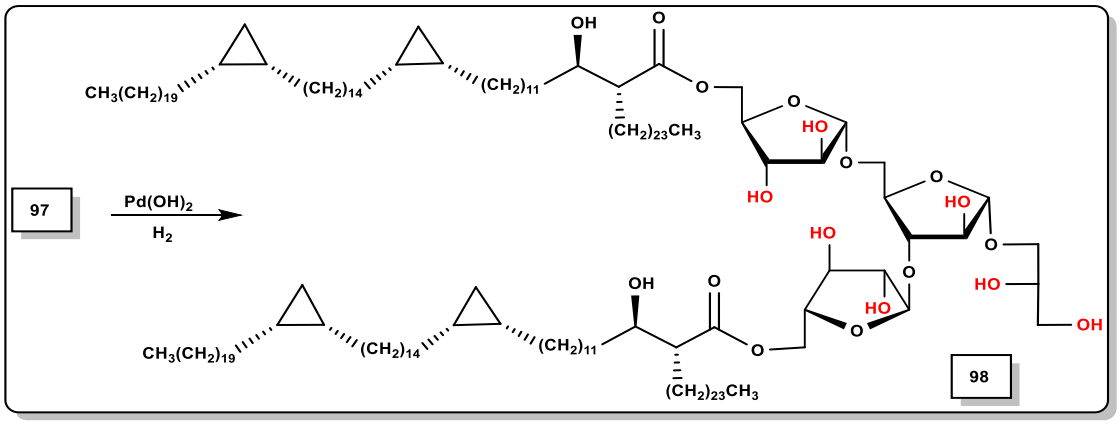


Figure 99: ^{1}H-NMR spectrum for compound **(97)**.

Table 7: The ^{1}H and ^{13}C-NMR data analysis of compound **(97)**.

Proton	***Shift***	***H's***	***Class***	***J/Hz***	***Carbon***	***δ/ppm***
H_1	5.16	1	br. s	-	C_{16}	175.1
H_6	5.13	1	br. s	-	$C_{16'}$	175.0
H_{11}	5.05	1	br. s	-	C_1	106.3
$H_{3, 10, 10`, 15, 15`, 9}$	4.29	6	m	-	C_6	106.2
H_{14}	4.17	1	br.dd	3.7, 6.9	C_{11}	105.5
H_4	4.11	1	dd	2.7, 4.4	C_2	88.2
H_7	4.07	1	br. d	2.1	C_7	88.0
H_{12}	3.98	1	m	-	C_{12}	87.9
$H_{5`,2}$	3.91	2	m	-	$C_{8, 13}$	83.6
$H_{13, 8}$	3.86	2	br.dd	4.6, 10.3	C_4	80.7
$H_{2'}$	3.79	1	br.p	4.8	C_3	80.3
H_5	3.72	1	m	-	C_{14}	79.3
$H_{1',3',18}$	3.61	5	br.d	5.3	C_9	79.2
H_{17}	2.41	2	m	-	$C_{2'}$	77.2
H_{21}	0.89	12	t	8.1	C_{18}	72.2
H_{19}	0.65	8	m	-	$C_{1'}$	67.1
$H_{20'}$	0.57	4	dt	4.2, 8.5	C_5	65.4
H_{20}	-0.32	4	br.q	4.9	$C_{15, 10}$	63.1
PhCH_2O	4.67	2	br.s	-	$C_{3'}$	63.0
PhCH_2O	4.55	1	d	11.9	C_{17}	51.9
PhCH_2O	4.50	8	m	-	$C_{17'}$	51.8
PhCH_2O	4.46	2	d	11.8	C_{19}	15.8
PhCH_2O	4.41	1	d	11.9	C_{21}	14.1
$Ph_{\text{-aromatic}}$ × 7	7.29	35	m	-	C_{20}	10.9
-	-	-	-	-	PhCH_2	73.4
-	-	-	-	-	PhCH_2	72.4
-	-	-	-	-	PhCH_2 × 2	72.0
-	-	-	-	-	PhCH_2	71.9
-	-	-	-	-	PhCH_2	71.7
-	-	-	-	-	PhCH_2	70.3

Hydrogenolysis of **(97)** was achieved by the method given previously, and afforded **(98)** in 88% yield **(Scheme 41)**.



Scheme 41: Synthesis of DMTAG of α-MA **(98)**.

Confirmation of the formation of **(98)** was achieved by ^{1}H-NMR (**Figure 100)**, which showed the disappearance of the signals corresponding to the benzyl protons. Signals of the glycan moiety and the α-MA appeared similar to those discussed before.

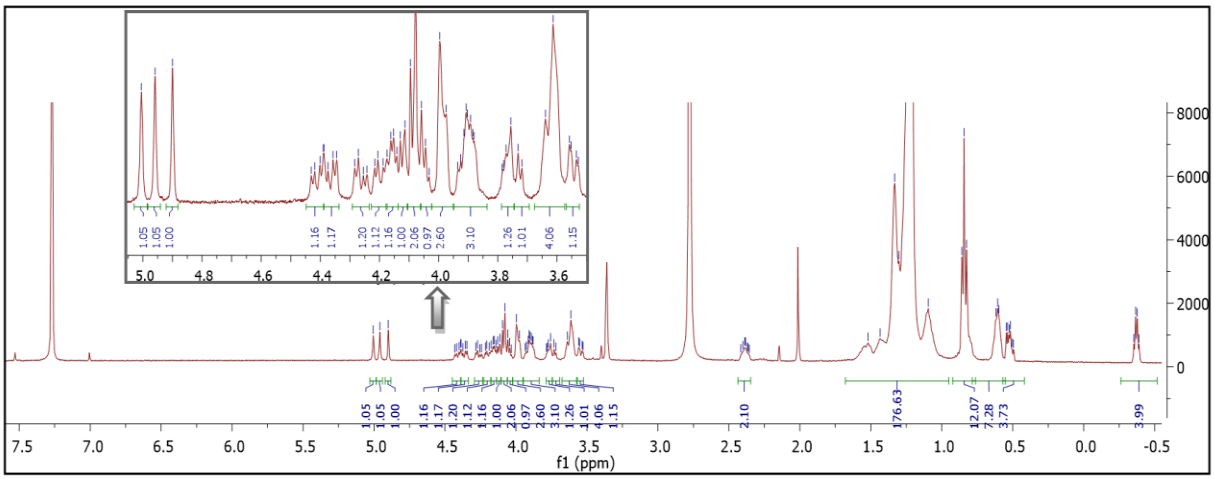
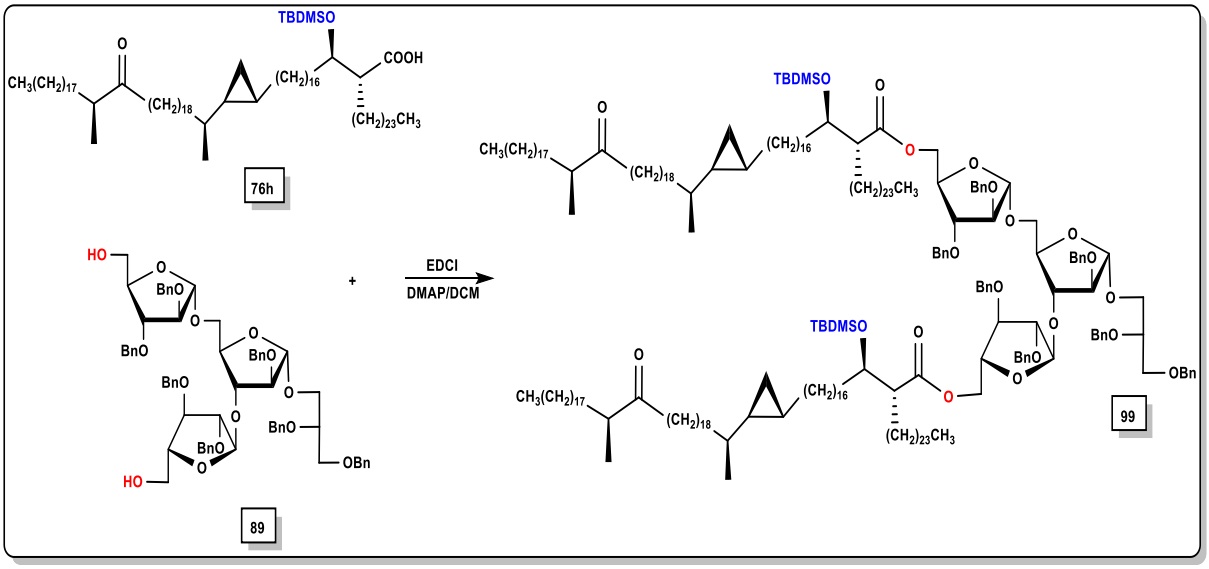


Figure 100: ^{1}H-NMR spectrum for compound **(98)**.

2.3.5.3 Esterification of (89) with *trans*-cyclopropane keto-MA (76h)

The sugar-diol **(89)** was esterified with the keto-MA with a *trans*-cyclopropane **(76h)**[111] by the same method as before, and afforded **(99)** in 46% yield **(Scheme 42)**.



Scheme 42: Synthesis of fully protected DMTAG of *trans*-keto-MA **(99)**.

All the ^{1}H-NMR signals belonging to the MAs appeared in the same area as discussed before, as did the signals for the tri-arabino glycerol. The ^{1}H and ^{13}C-NMR spectra for **(99)** are shown in **Figure 101**.

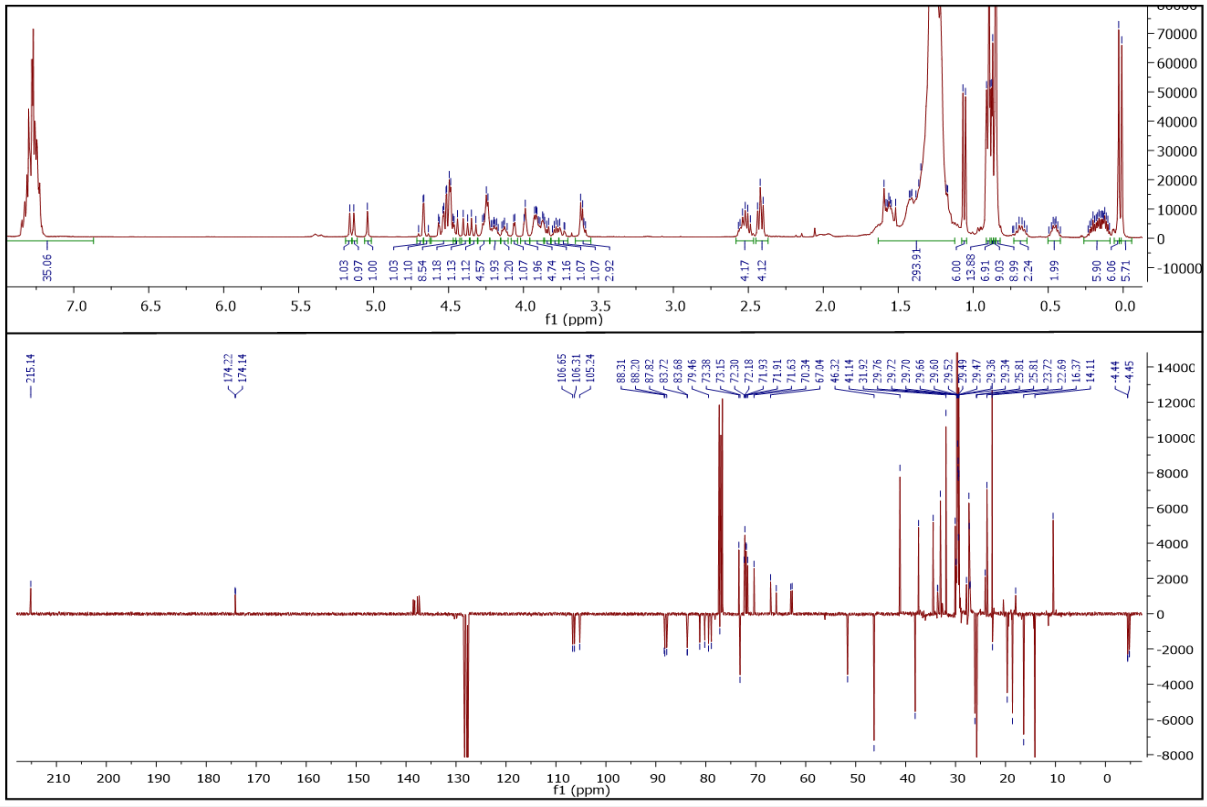
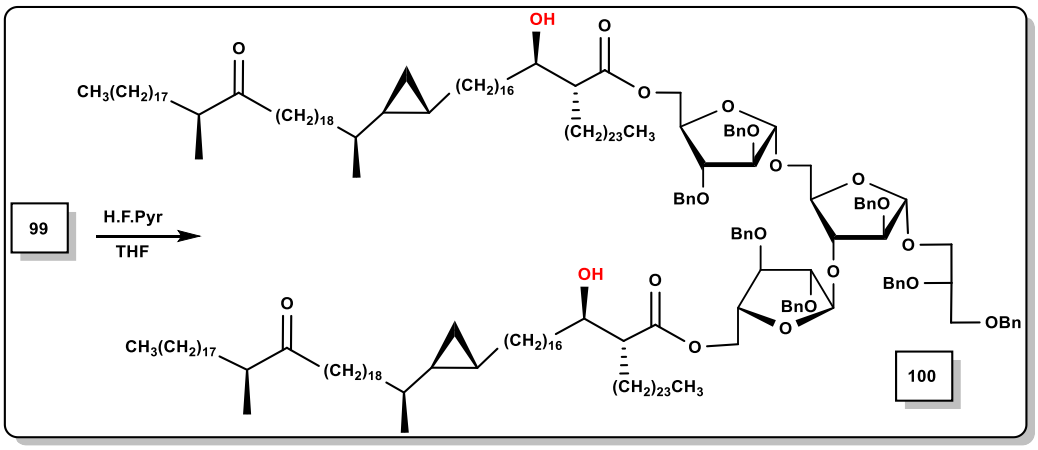


Figure 101: ^{1}H and ^{13}C-NMR spectra for compound **(99)**.

De-silylation of **(99)** was carried out next to remove the TBDMS group from β-position in the MAs by the same method mentioned above, and afforded **(100)** in 66% yield **(Scheme 43**, **Figure 102)**.



Scheme 43: Synthesis of DMTAG of *trans*-keto-MA **(100)**.

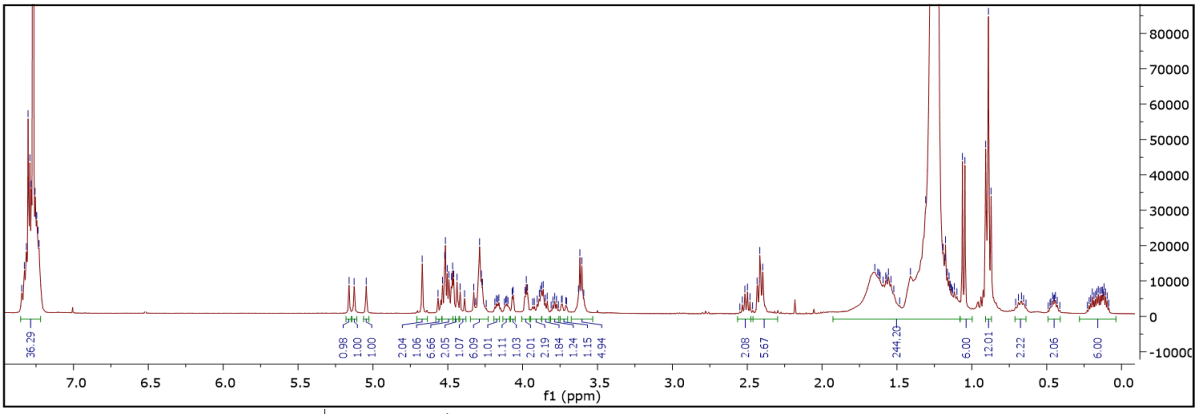
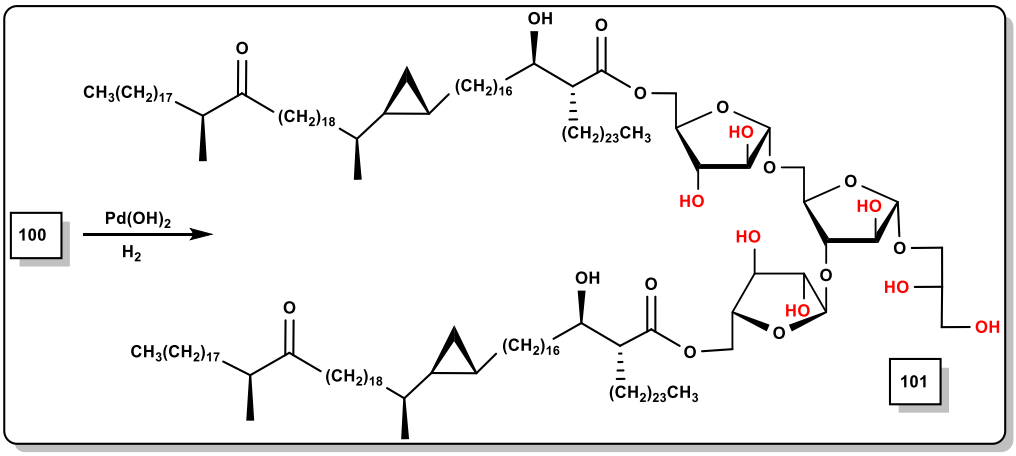


Figure 102: ^{1}H-NMR spectrum for compound **(100)**.

Compound **(100)** was hydrogenolysed by a similar method to that discussed before, to give **(101)** in 82% yield **(Scheme 44)**.



Scheme 44: Synthesis of DMTAG of *trans*-keto-MA **(101)**.

Compound **(101)** showed ^{1}H-NMR signals for the glycan and the keto-MA, similar to those obtained before. The disappearance of the signals corresponding to the benzylic protons and the protons in the aromatic area, confirmed the successful of hydrogenolysis (debenzylation).

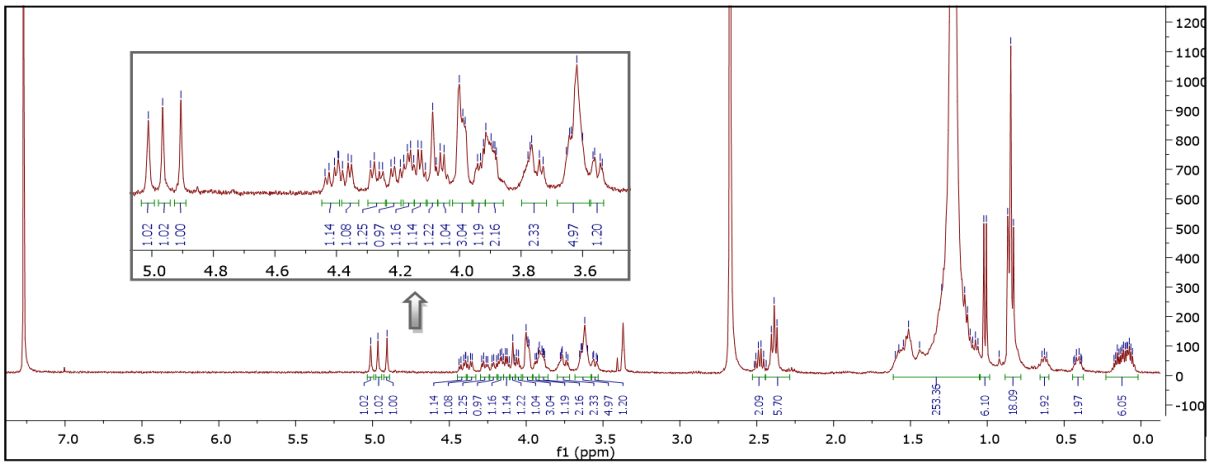


Figure 103: ^{1}H-NMR spectrum for compound **(101)**.

2.3.6 Summary:

In this part of the thesis, the preparation of DMTAG glycolipids was described, and the following targets have been achieved successfully:

1. The first synthesis of the glycan moiety tri-arabino glycerol (TAG) incorporating L-glycerol has been achieved.

2. Esterification of the above moiety with a simple fatty acid to prepare the DMTAG glycolipid analogue has been done.

3. Esterification with three common classes of synthetic mycolic acids, to make the DMTAG glycolipids has been achieved. No literature data are available for DMTAGs, so it will be interesting in future work to have authentic samples for comparison.

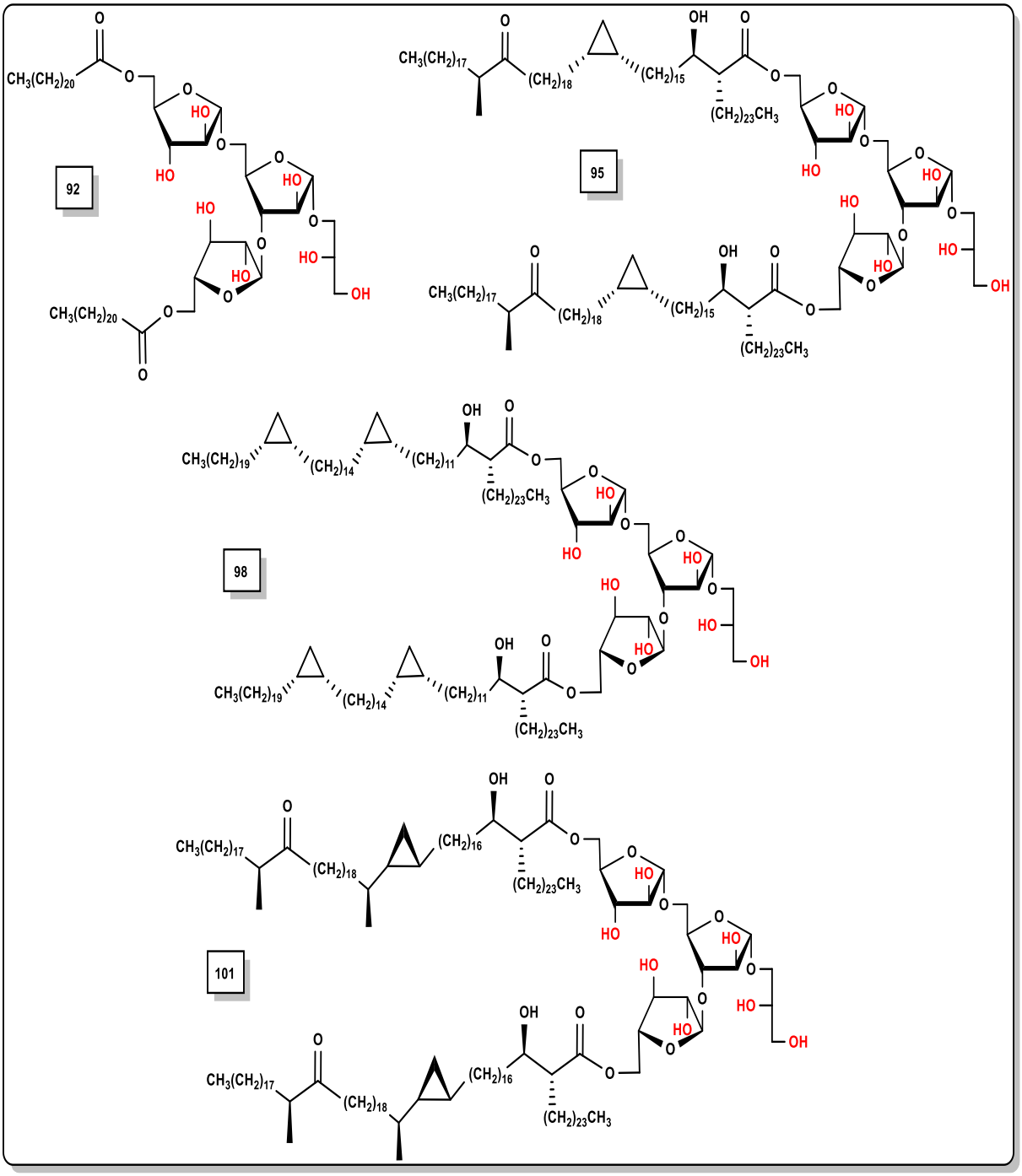


Figure 104: Structures of the target DMTAG glycolipids.

Section 4

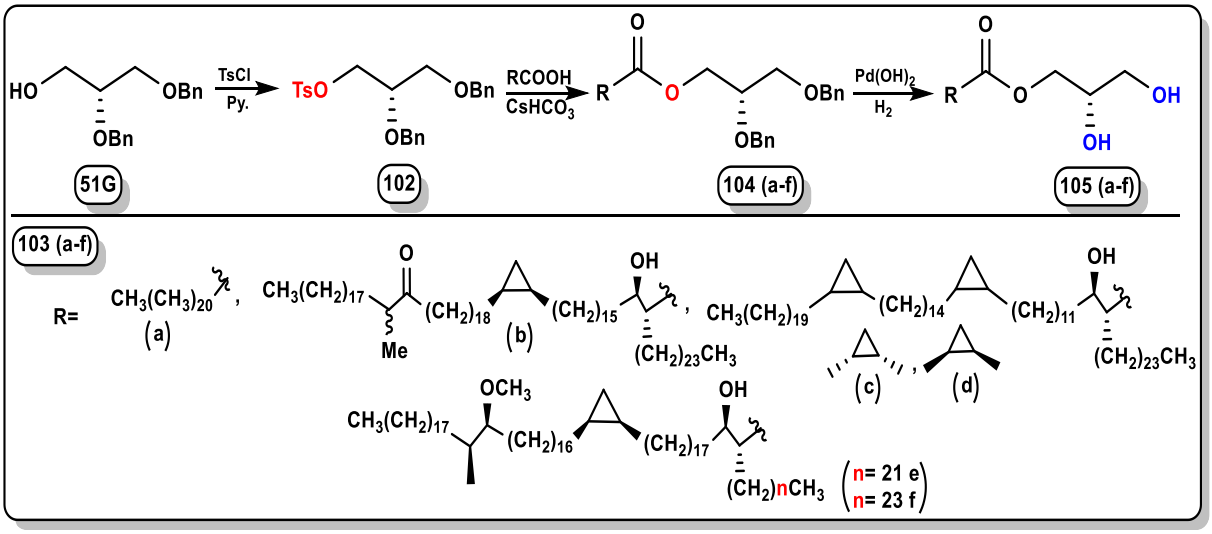
2.4 Synthesis of Glycerol Mono-mycolate (GroMM)

2.4.1 The aims of this part

- Synthesis of benzyl protected glycerol [(4-methylbenzenesulfonyl)-2,3-di-*O*-benzyl-*S*-glycerol].
- Synthesis of a model glycolipid through esterification with a simple fatty acid.
- Synthesis of a series of GroMM esters through esterification of the benzyl protected *S*-glycerol with different synthetic MAs.
- Investigate the biological activity of the synthetic *S*-glycerol esters and compare it to those of the *R*-glycerol esters to determine whether the stereochemistry matters.

2.4.2 Synthesis of 1-*O*-*p*-toluenesulfonyl-2,3-di-*O*-benzyl-*S*-glycerol (102)

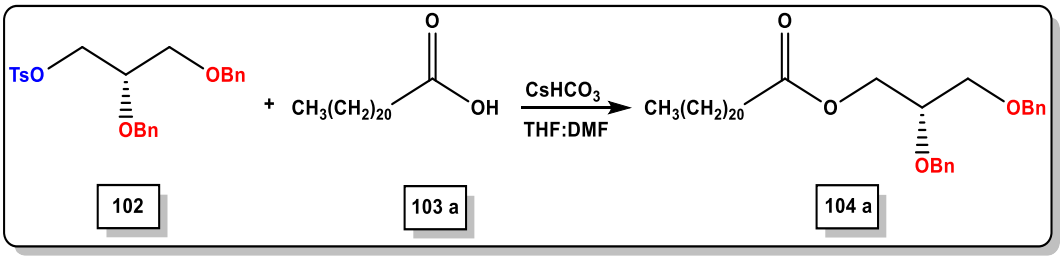
The overall aim of this part of the work was the preparation of 2,3-di-*O*-benzyl-*S*-glycerol **(51G)**, which could first be esterified with a simple fatty acid as a model, then with a number of different synthetic MAs from common classes. The protected *S*-glycerol **(51G)** was synthesized from D-mannitol as reported in the literature.[243,244,245,246,247]. For the synthesis of glycerol mono-mycolate (GroMM) **(Scheme 45)** from benzyl protected *S*-glycerol **(51G)** and fatty or mycolic acids **(103 a-f)**, it was necessary to convert the free hydroxyl group at the C-1 position in the benzyl protected *S*-glycerol into a good leaving group. The free hydroxyl group was therefore converted to a tosylate by reaction with *p*-toluenesulfonyl chloride (TsCl) in dry pyridine and catalytic DMAP in dry CH_2Cl_2 at 0 ºC to afford the tosylate **(102)** in 62% yield. The synthesis of this compound was confirmed and all the data obtained were consistent with the literature.[248]



Scheme 45: Synthesis of GroMM incorporating *S*-glycerol.

2.4.3 Esterification of the tosylate (102) with a simple fatty acid

Compound **(102)** was reacted with behenic acid **(103a)** *via* the alkylative esterification strategy, using cesium hydrogen carbonate (10 eq.) in dry DMF:THF (1:5) at 70 ºC for 2 days to give protected ester **(104a)** in 83% yield **(Scheme 46).**



Scheme 46: Synthesis of protecteD-glycerol behenate **(104a)**.

The ^{1}H-NMR spectrum (**Figure 105)** confirmed the formation of **(104a)**, which showed six signals in region δ 4.61-3.53 belonging to the glycerol. The protons corresponding to the CH_2 group of the two benzyl groups appeared as two broad singlets at δ 4.61 and 4.49 respectively, and the two doublets of doublets at δ 4.25 (*J* 4.2, 11.7 Hz) and 4.12 (*J* 5.8, 11.7 Hz) respectively corresponded to the CH_2 protons adjacent to the carboxylic group. The multiplet at δ 4.75 belonged to the CH group on the glycerol, while the CH_2 group on the glycerol appeared as a muliplet at δ 3.53. The protons corresponding to the CH_2 adjacent to the carbonyl group in the acid came around δ 2.2 as a triplet (*J* 7.6 Hz) and the three protons in the terminal position of the acid chain appeared as an up-field triplet at δ 0.83 (*J* 6.8 Hz).

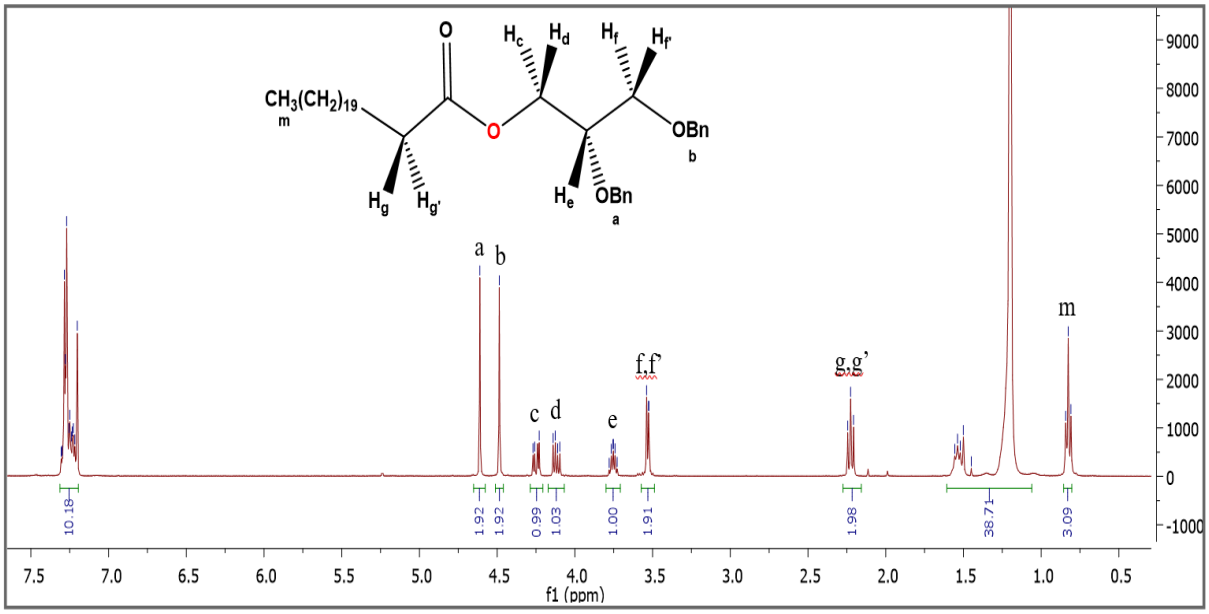


Figure 105: ^{1}H-NMR spectrum for compound **(104a)**.

The ^{13}C-NMR spectrum (**Figure 106)** of **(104a)** showed a carbonyl signal at δ 173.6, the aromatic carbons at δ 138 – 127, the remaining glycerol ester carbons at δ 75 – 63, the CH_2 chain ranged from δ 34 – 22 and the terminal CH_3 came up-field at around δ 14.0.

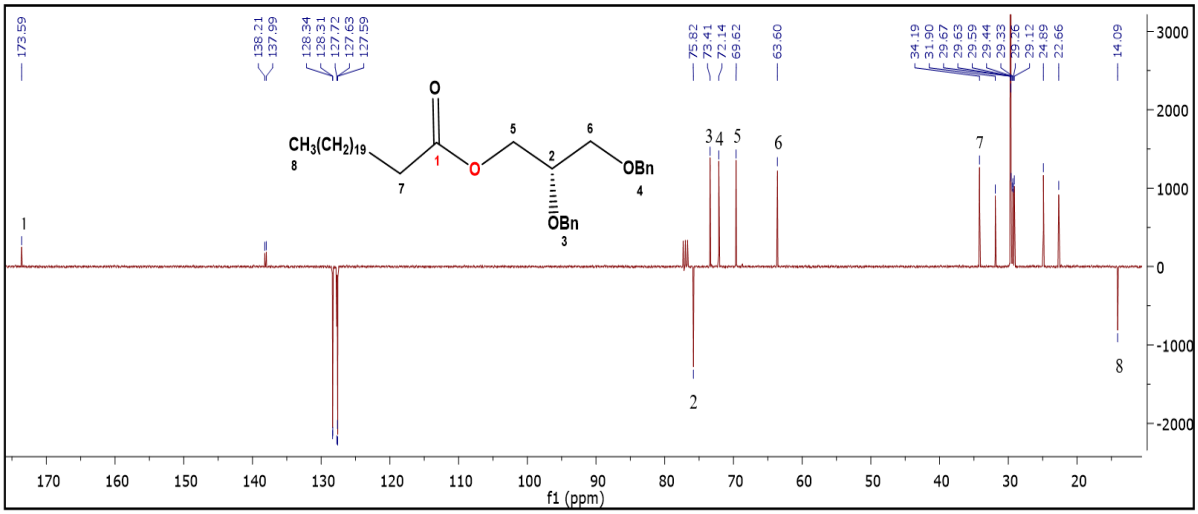
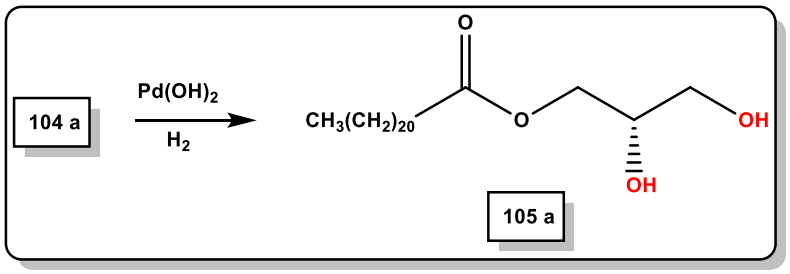


Figure 106: ^{13}C-NMR spectrum for compound **(104a)**.

Debenzylation of **(104a)** was achieved by stirring in dry CH_2Cl_2:MeOH (1:1) in the presence of $Pd(OH)_2$ (0.15 eq. fold by weight) under a hydrogen atmosphere to give **(105a)** in 70% yield **(Scheme 47).**



Scheme 47: Synthesis of glycerol behenate **(105a)**.

Once again, the formation of this compound was proven by ^{1}H-NMR spectroscopy, which clearly showed the disappearance of those signals corresponding to the benzyl groups between δ 4.6 – 4.5. Two doublets of doublets at δ 4.07, (*J* 3.6, 9.9 Hz) and 4.03 (*J* 4.4, 9.9 Hz) corresponded to the CH_2 adjacent to the carboxylic group, a multiplet at δ 3.80 belonged to the CH group on the glycerol core, while the CH_2 group on the glycerol appeared as two doublets of doublets at δ 3.57 (*J* 4.1, 11.5 Hz) and 3.48 (*J* 6.1, 11.5 Hz).

The ^{13}C-NMR spectrum (**Figure 107)** showed a carbonyl at δ 174.4, the remaining glycerol ester carbons appeared at δ 70 – 63, the CH_2 chain ranged from δ 34 – 22 and the terminal CH_3 came up field around δ 14.0.

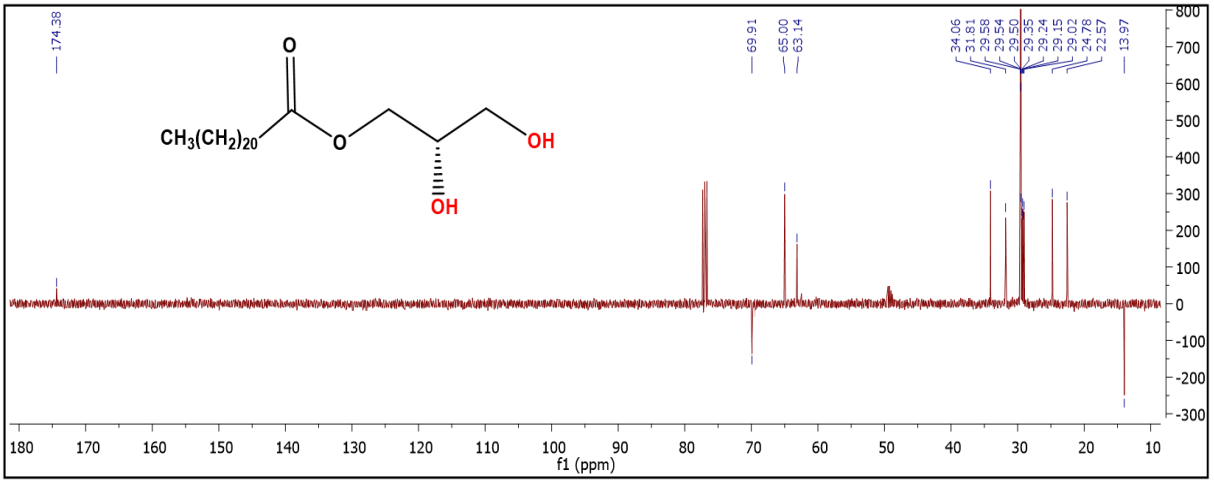
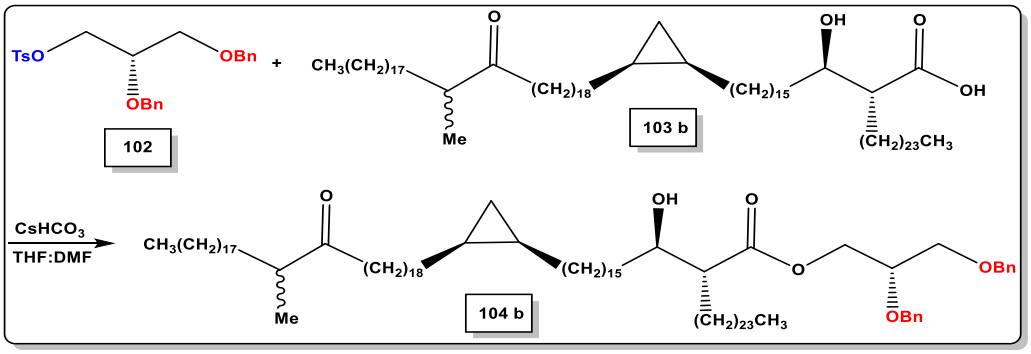


Figure 107: ^{13}C-NMR spectrum for compound **(105a)**.

2.4.4 Esterification of the tosylate (102) with synthetic mycolic acids (103b-f)

2.4.4.1 Esterification of the tosylate (102) with the keto-mycolic acid (103b)

Keto-MAs are the major oxygenated MAs in the cell wall of mycobacteria. Synthetic keto-MA **(103b)** is present in nature in *M. kansasii*. Keto-MAs encourage the growth of the bacterial cell. Their formation in the cell increases during growth in macrophages and at low oxygen concentrations.[249] The α-chain of the keto-MA in *M. tuberculosis* has 24 carbons. Having secured a successful method for synthesising a model of the *S*-glycerol behenate **(105a)**, the coupling of the tosylate **(102)** with a synthetic mycolic acid was now undertaken. The synthetic material of keto-mycolic acid **(103b)**[111], was provided by Dr. Al Dulayymi. Firstly, this compound was esterified with the tosylate **(102)** to give the corresponding protected glycerol mycolate **(104b)** in 59% yield using the same procedure as above **(Scheme 48)**.



Scheme 48: Synthesis of protecteD-glycerol-keto-mycolates **(104b)**.

The success of the esterification was demonstrated by the ^{1}H-NMR spectrum (**Figure 108)**. The region of most interest is between δ 0.56 and - 0.40, which corresponds to the four protons of the *cis*-cyclopropane ring. The proton H_p gave a doublet of triplets; the broadness of this signal shown at δ 0.52 to 0.45 is possibly because H_n and $H_{n'}$ are not magnetically

equivalent and the signal observed is actually a double doublet of doublets, but due to the signals being at a nearly identical chemical shift it appears as a doublet of triplets. The proton H_q should show a doublet of triplets; however, due to the magnetic inequivalence of H_n and $H_{n'}$ the signal at δ - 0.35 to - 0.45 is distorted and appears as a broad quartet. Protons H_n and $H_{n'}$ again showed a distorted multiplet at δ 0.65 to 0.54 for the same reason. The α-proton H_h exhibits a multiplet seen at δ 2.45 – 2.38. More proton and ^{13}C-NMR data **(Figure 109)** of protected ester (**104b**) was analysed as shown in **Table 8**.

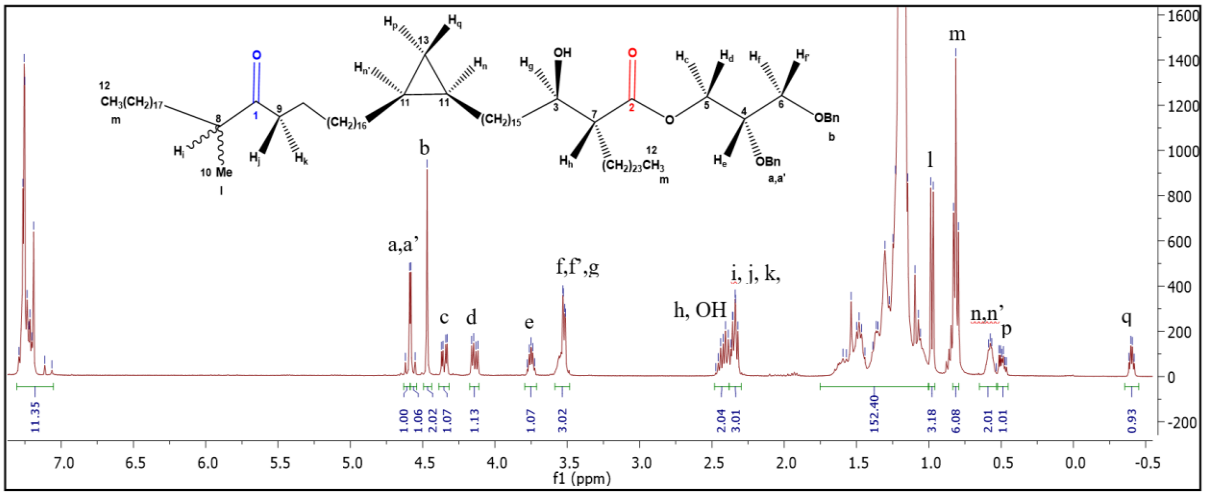


Figure 108: ^{1}H-NMR spectrum for compound **(104b)**.

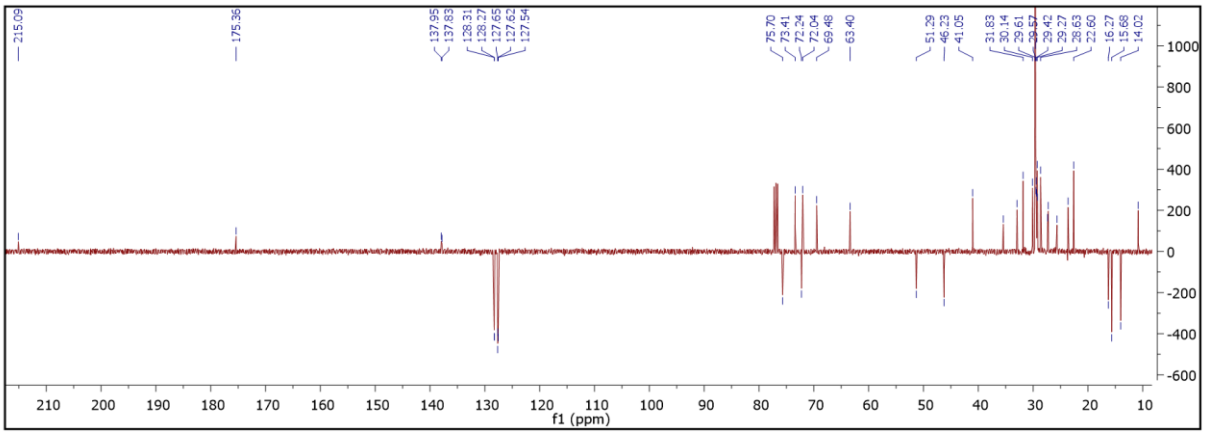
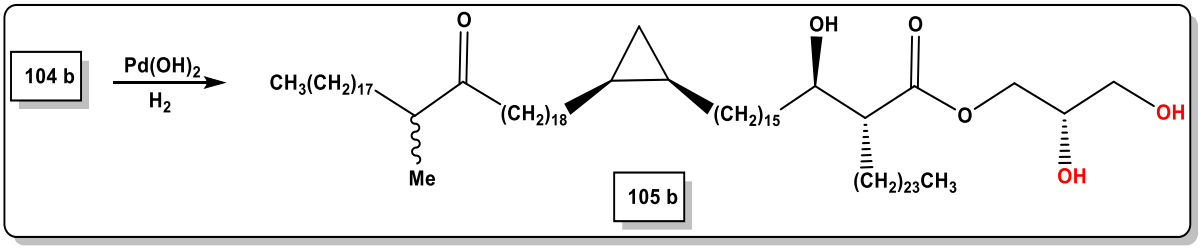


Figure 109: ^{13}C-NMR spectrum for compound **(104b)**.

Table 8: The ^{1}H and ^{13}C-NMR data analysis of compound **(104b)**.

Proton	**H_a**	**$H_{a'}$**	**H_b**	**H_c**	**H_d**	**H_e**	**$H_{f,f',g}$**	**$H_{h,OH}$**	**$H_{i,j,k}$**	**H_l**	**H_m**	**$H_{n,n'}$**	**H_p**	**H_q**
Shift	4.60	4.57	4.47	4.35	4.14	3.75	3.52	2.43	2.35	0.98	0.81	0.56	0.49	-0.40
H's	1	1	2	1	1	1	3	2	3	3	6	2	1	1
Class	d	d	br.s	dd	dd	m	br.dd	m	dt	d	t	m	dt	br.q
***J*/Hz**	11.8	11.8	-	4.0, 11.7	5.5, 11.7	-	1.4, 5.4	-	5.4, 7.9	6.9	6.7	-	4.0, 8.5	5.1
Carbon	**C_1**	**C_2**	**C_3**	**$C_{a,a'}$ / C_b**	**C_4**	**C_5**	**C_6**	**C_7**	**C_8**	**C_9**	**C_{10}**	**C_{11}**	**C_{12}**	**C_{13}**
δ/ppm	215.1	175.4	75.7	73.4/ 72.2	72.0	69.5	63.4	51.3	46.2	41.0	16.3	15.7	14.0	10.8

Hydrogenolysis of **(104b)** was achieved by the method described previously to give **(105b)** in 87% yield (**Scheme 49)**.



Scheme 49: Synthesis of glycerol-keto-mycolates **(105b)**.

Compound **(105b)** showed characteristic NMR signals (**Figure 110)** corresponding to the cyclopropane protons and glycerol moiety approximately similar to those signals of **(104b)** without the benzylic protons, which confirm the success of the debenzylation**.**

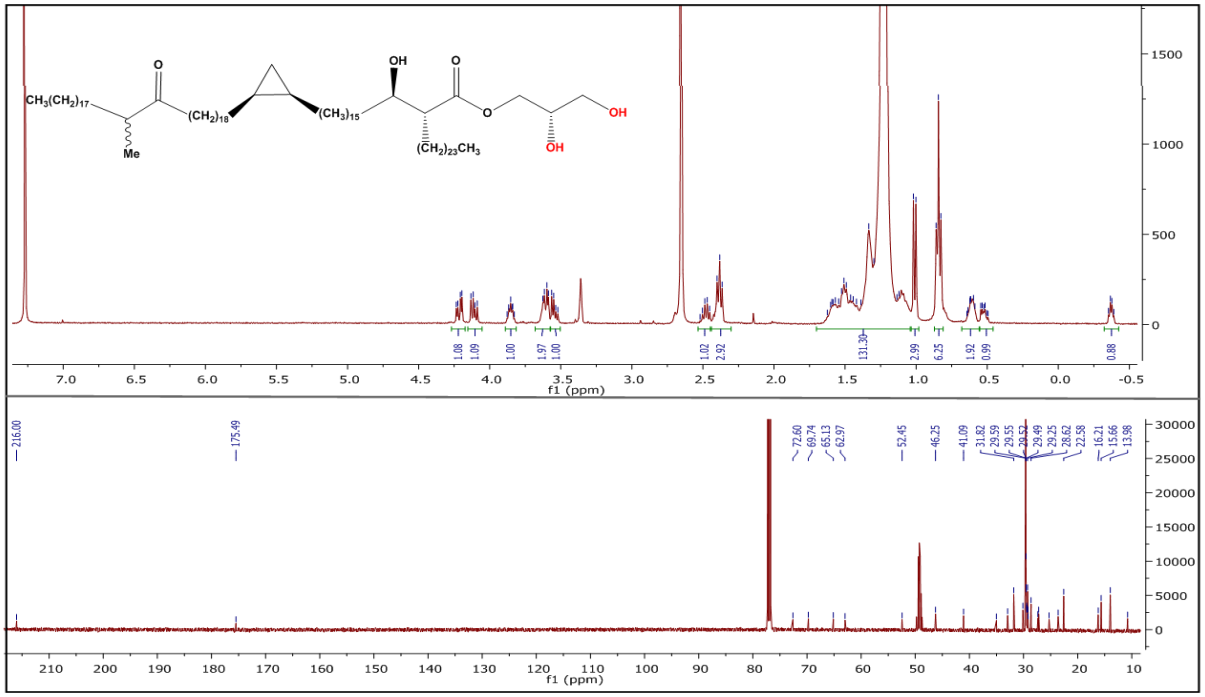
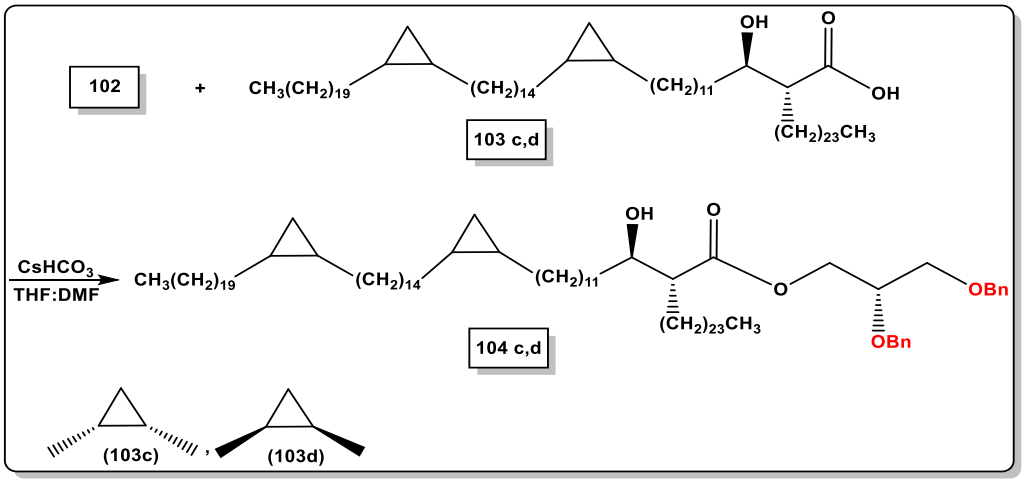


Figure 110: ^{1}H and ^{13}C-NMR spectra for compound **(105b)**.

2.4.4.2 Esterification of the tosylate (102) with α-MAs (103c,d)

The α-MA type is the most abundant among MAs in *M. tuberculosis*, and characterized by containing two *cis*-cyclopropanes. This acid was reported by Minnikin and Polgar to be the major mycolic acid of *M. tuberculosis* var *hominis*.[99] The absolute stereochemistry of the *cis*-cyclopropane in the MA has not been proven, *i.e.* the (*S*, *R*) or (*R*, *R*) or (*S*, *S*) or (*R*, *S*) configurations respectively, therefore the two of the four possible structures were used in this study.

Synthesising MA esters may provide valuable information for determining the stereochemistry of naturally occurring MAs, which subsequently may provide further understanding of the biosynthetic pathway. The structurally defined synthetic MAs **(103c,d)** reported by Al Dulayymi *et al.*[109], were reacted with tosylate **(102)**, using the conditions mentioned earlier to give **(104c)** in 58% yield, and compound **(104d)** in 71% yield **(Scheme 50)**.



Scheme 50: Synthesis of protecteD-glycerol-α-mycolates **(104c,d).**

The ^{1}H-NMR spectrum (**Figure 111)** of **(104c)** showed a broad doublet of doublets at δ 3.60 (*J* 1.6, 5.4 Hz) for the proton at the β-position. The signal corresponding to the proton at the α-position in the MA appeared as a broad doublet of doublets at δ 2.43 (*J* 3.8, 10.5 Hz). Protons corresponding to the cyclopropane appeared as a 2H broad quartet at δ - 0.32 (*J* 5.2 Hz), a 2H doublet of triplets at δ 0.57 (*J* 3.9, 8.4 Hz), and a 4H multiplet at δ 0.73 – 0.60. Signals corresponding to the glycerol moiety were approximately similar to those of the previously prepared glycerol-mycolates. Formation of **(104c)** was proven by the ^{13}C-NMR spectrum which showed a signals for the carbonyl ester at δ 175.4, and the carbon on the β-hydroxy acid position at δ 72.3.

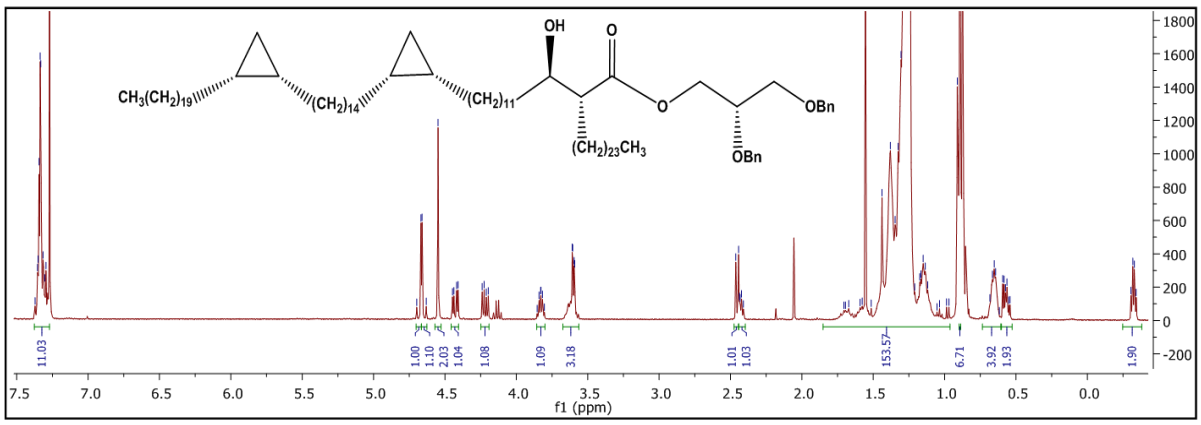
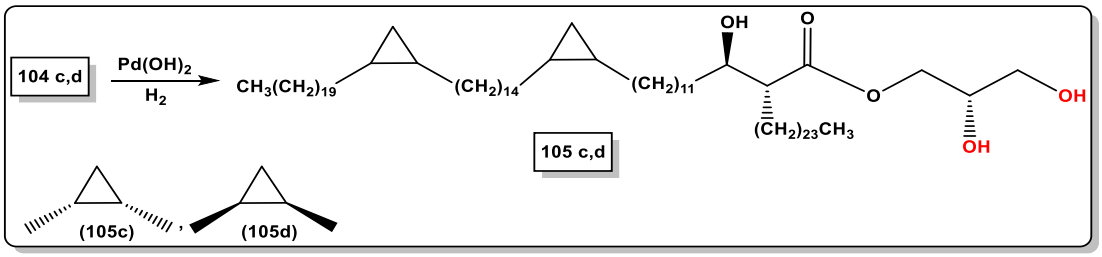


Figure 111: ^{1}H-NMR spectrum for compound **(104c)**.

Hydrogenolysis of **(104c,d)** using the same method mentioned earlier afforded **(105c,d)** in 74% and 92% yield respectively **(Scheme 51)**.



Scheme 51: Synthesis of glycerol-α-mycolates **(105c,d)**.

Confirmation of the formation of **(105c)** was achieved by ^{1}H-NMR. Two doublets of doublets at δ 4.21 and δ 4.10 (*J* 4.3, 11.4 and 6.4, 11.5 Hz respectively) correspond to the 2 protons attached to the C-4 of the glycerol core. The remaining protons of the glycerol moiety were approximately similar to those of the previously prepared glycerol-mycolates. The α-proton in the MA appeared as a doublet of doublet of doublets at δ 2.39. Signals for the cyclopropane appeared at approximately the same chemical shift as those in the spectrum of compound **(104c)**. The ^{13}C-NMR spectrum showed a carbonyl at δ 175.5, and the remaining glycerol carbons in the region of δ 83 – 54. The β-hydroxy carbon appeared at δ 72.6. The CH_2 chain ranged from δ 37 – 22.5 and the CH_3 came up-field to around δ 14.0. The MA α-carbon resonated at δ 52.6. 2D-NMR **(Figure 112)** was used to provide further proof of the structure of the glycerol mycolate **(105c)**.

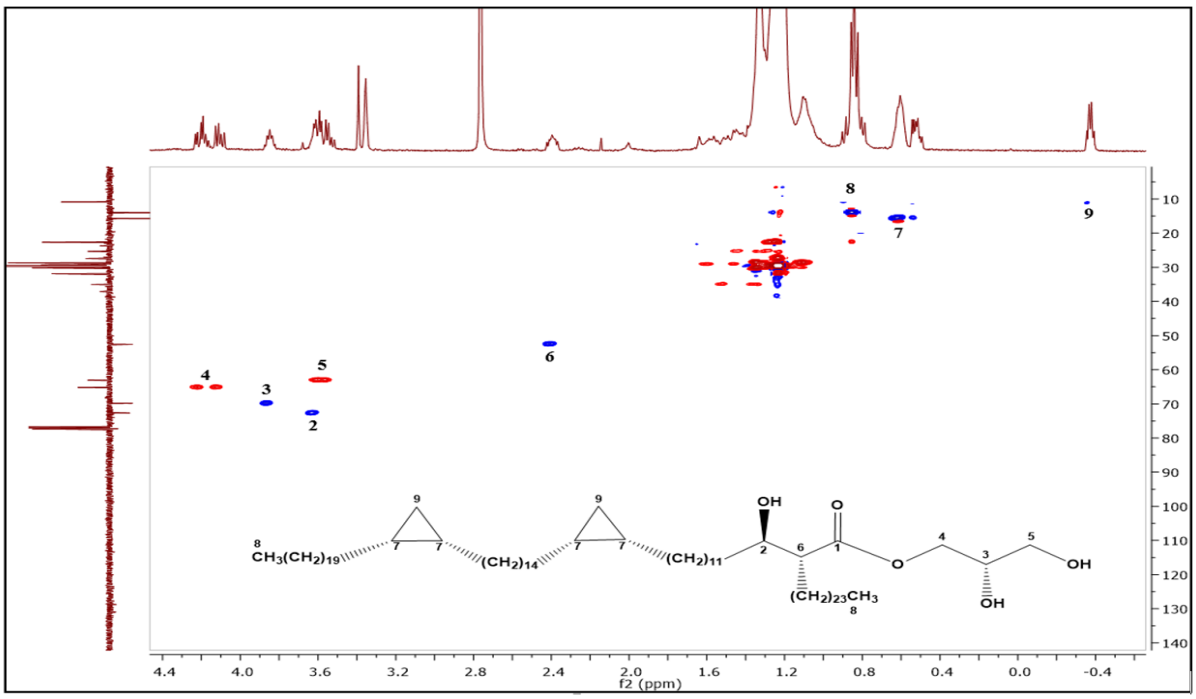
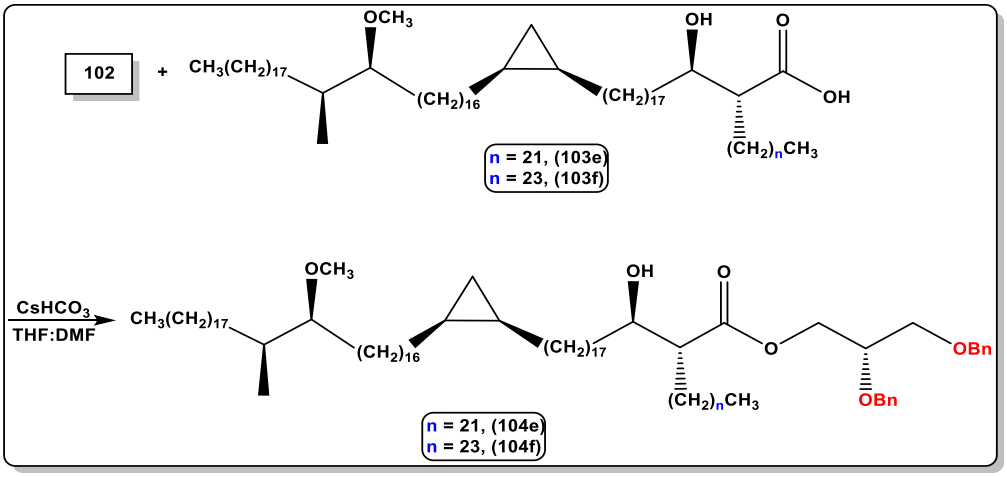


Figure 112: HSQC-NMR spectrum for compound **(105c)**.

2.4.4.3 Esterification of the tosylate (102) with the methoxy-MAs (103e,f)

Methoxy-MAs in *M. tuberculosis* increase in stationary phase cells and in addition, the oxygenated mycolates in *M. tuberculosis* affect the growth rate of intramacrophages.[249] In order to probe the biological effects of varying the α-alkyl chain length, methoxy-MAs with 22 and 24 carbon α-chains, were used to obtain glycerol mycolates **(104e,f)**. Firstly, the structurally defined synthetic MA reported by Al Dulayymi *et al.*[110], a methoxy-*cis*-cyclopropane with 22 carbon α-alkyl chain **(103e)**, which is present in nature in *M. kansasii*, was reacted with the tosylate **(102)**, using the same procedure as above, giving compound **(104e)** in 62% yield. The tosylate **(102)** was coupled with **(103f)** by the same method as above to prepare compound **(104f) (Figure 113)** in 72% yield **(Scheme 52)**; this showed NMR signals as shown in **Table 9**. These assignments were made by comparison to the data reported for the *R*-GroMM compounds.[203]



Scheme 52: Synthesis of protecteD-glycerol-methoxy-mycolates **(104e,f)**.

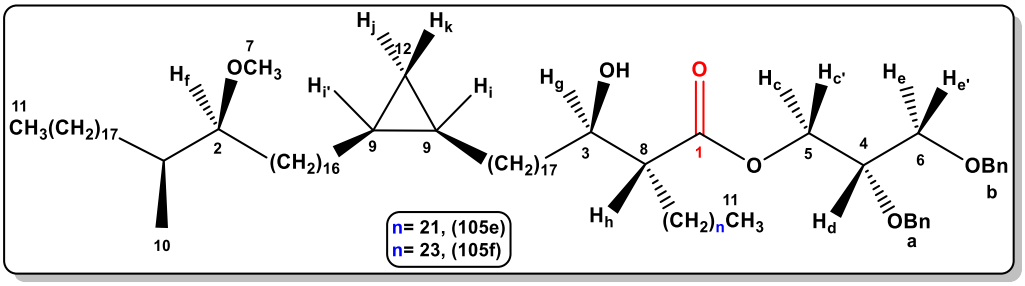


Figure 113: Structure of protecteD-glycerol-methoxy mycolates **(104e,f)**

Table 9: The ^{1}H and ^{13}C-NMR data analysis of compounds **(104e)**.

Proton	*Shift*	*H's*	*Class*	*J/Hz*	*Carbon*	*δ/ppm*
$H_{a\text{-}Bn}$	4.62	1	d	11.8	C_1	175.4
$H_{a'\text{-}Bn}$	4.58	1	d	11.8	C_2	85.3
$H_{b\text{-}Bn}$	4.48	2	br.s	-	C_3	75.7
H_c	4.36	1	dd	4.1, 11.7	$C_{a\text{-}Bn}$	73.4
$H_{c'}$	4.15	1	dd	5.5, 11.7	C_4	72.2
H_d	3.76	1	m	-	$C_{b\text{-}Bn}$	72.0
$H_{e,e'}$	3.53	3	br.dd	1.6, 5.4	C_5	69.5
$(OCH_3)_7$	3.28	3	s	-	C_6	63.4
H_f	2.90	1	m	-	$(OCH_3)_7$	57.6
$H_{g,h}$	2.37	2	m	-	C_8	51.3
$(CH_3)_{11}$	0.83	6	t	6.8	C_9	15.7
$(CH_3)_{10}$	0.79	3	d	6.9	$(CH_3)_{10}$	14.8
$H_{i,i'}$	0.60	2	m	-	$(CH_3)_{11}$	14.0
H_j	0.50	1	dt	4.0, 8.4	C_{12}	10.8
H_k	-0.39	1	br.q	5.2	-	-

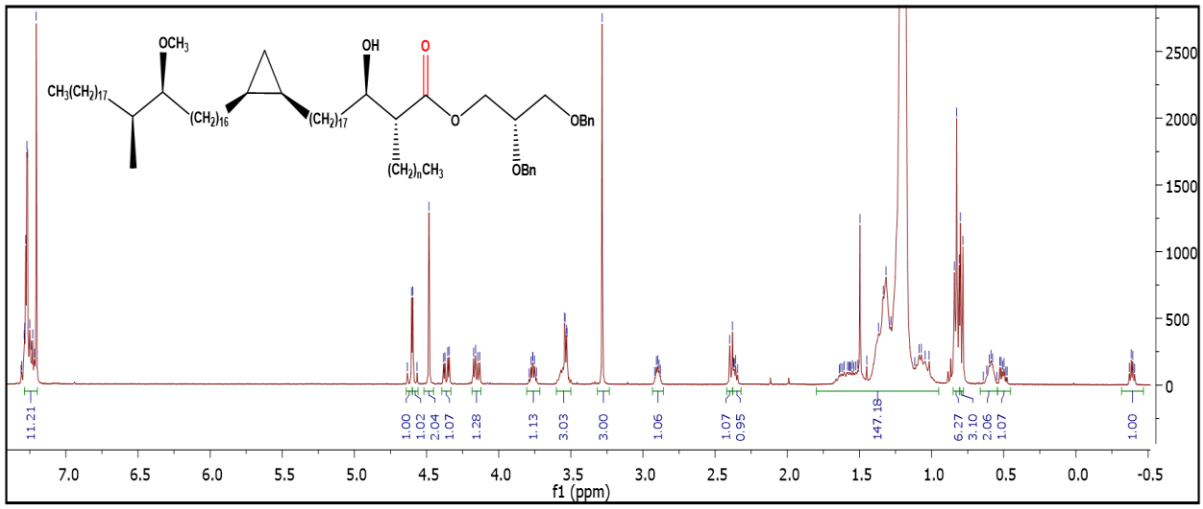
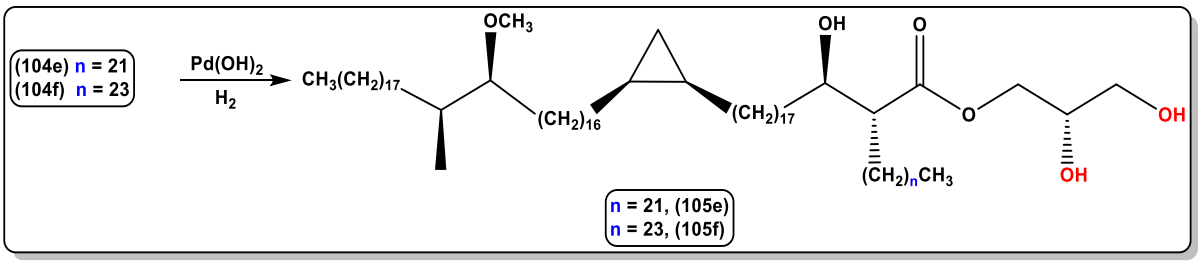


Figure 114: ^{1}H-NMR spectrum for compounds **(104e)**.

Hydrogenolysis of **(104e,f)** by the same method as above afforded **(105e,f)** in 85 and 92% yield respectively **(Scheme 53)**.



Scheme 53: Synthesis of glycerol-methoxy-mycolates **(105e,f)**.

Once again, compounds **(105e,f)** showed characteristic NMR signals **(Figure 115)** corresponding to the cyclopropane protons and the remaining protons in the methoxy-MA and glycerol moiety were approximately the same to those previously discussed.

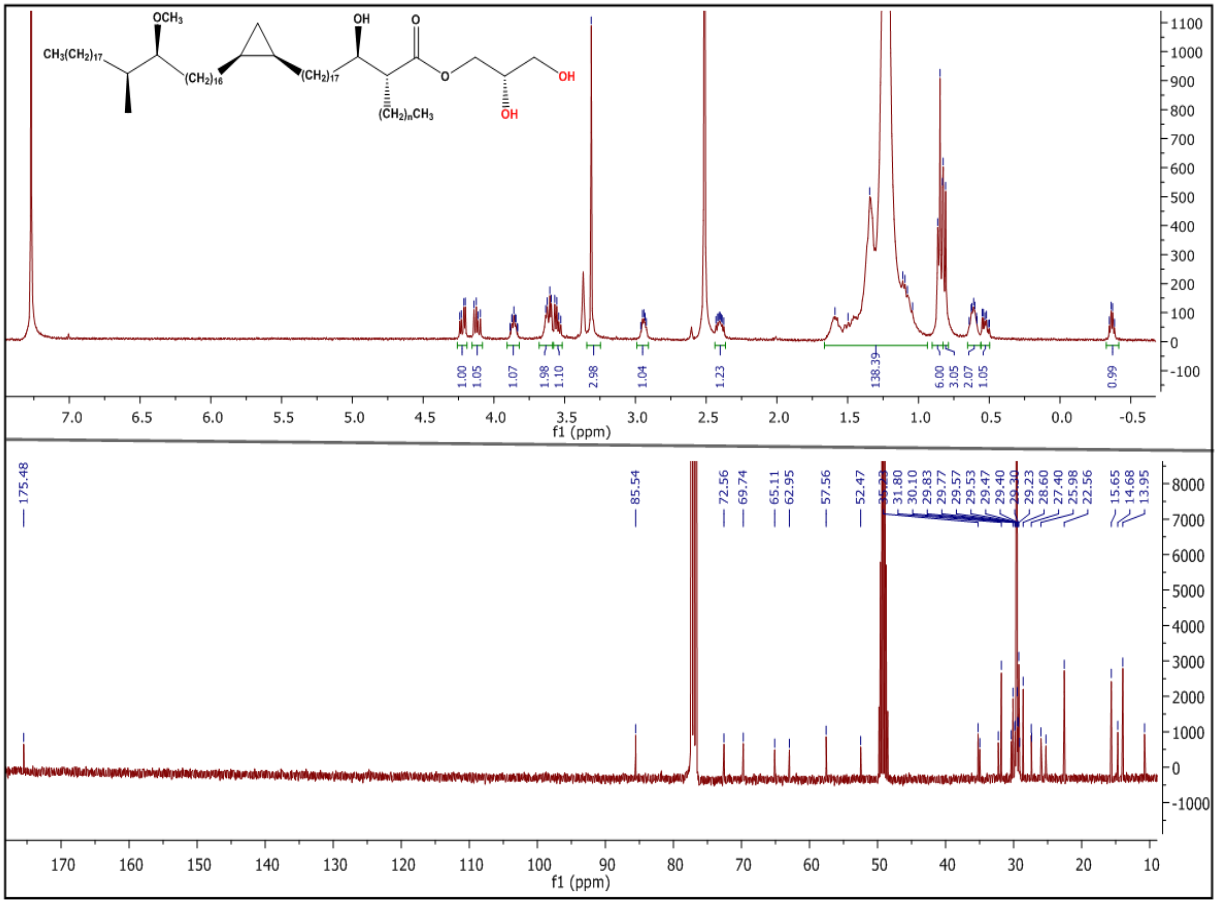


Figure 115: ^{1}H and ^{13}C-NMR spectra for compound **(105e)**.

2.4.5 Summary

In this part of the thesis, five examples of GroMM esters **(105b-f)** were prepared based on the three common classes of synthetic MAs, and one linear alkyl acid as a model **(105a)**. The prepared GroMMs are shown below **(Figure 116)**.

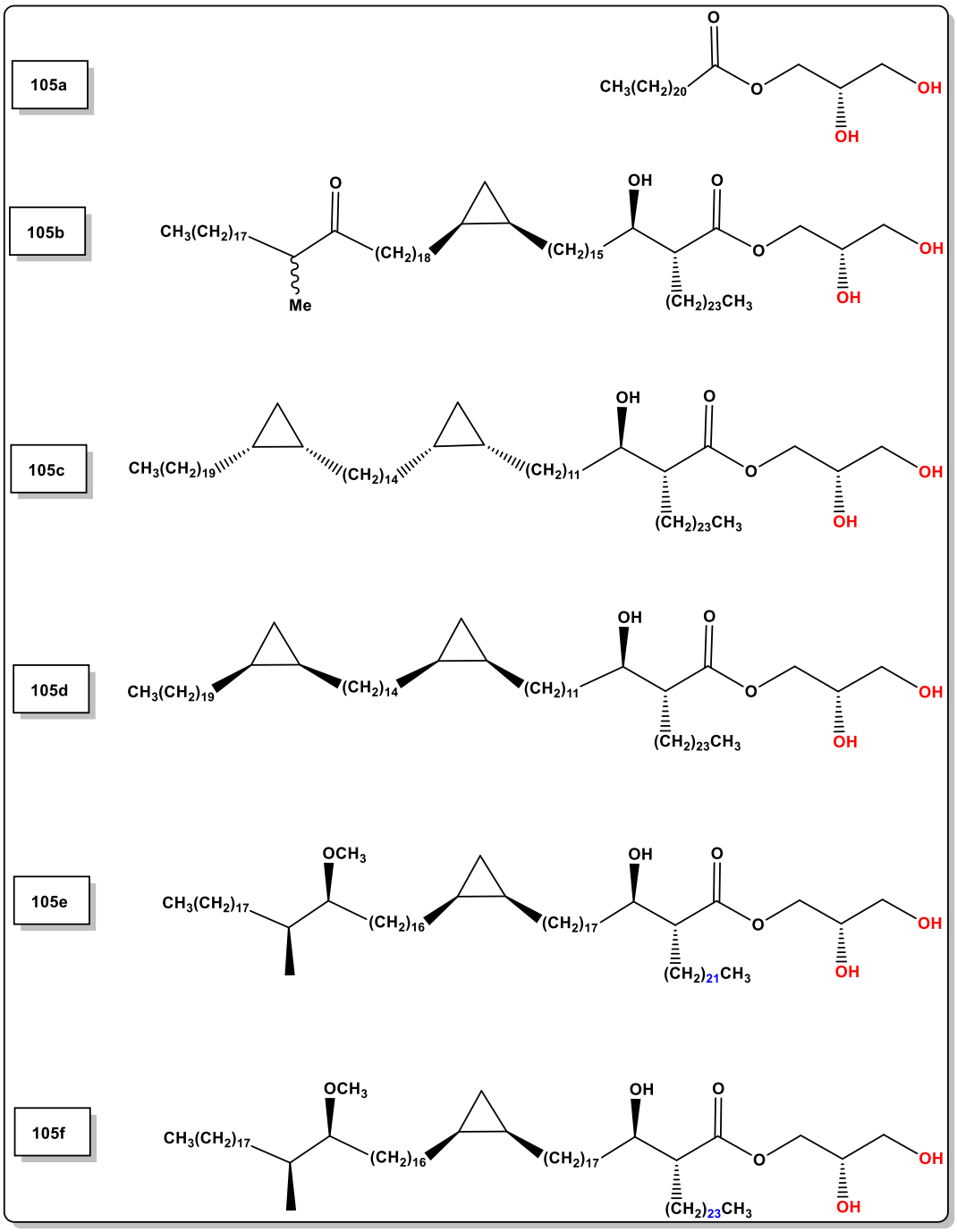


Figure 116: Structures of the target glycerol mono-mycolates **(GroMMs 105a-f)**.

2.5 DMAGs as antigens in the serodignosis of bovine tuberculosis

2.5.1 ELISA assay

The use of ELISA as a tool for diagnosing TB is attractive for a number of reasons, in particular it is a relatively simple, cheap and quick technique. However, the results obtained from ELISA predominately depend on how well the antibodies in the TB patient's blood sample are detected by the antigens used. MAs and their derivatives are the dominant lipids in the mycobacterial cell wall, and are considered to be among the best antigens. Being able to use this method of detection for TB would mean patients would not need to spend time in quarantine and would be able to receive anti-TB therapy more quickly.[229,230,231,232]

According to the literature, DMAG showed applicability for serodiagnosis of *Mycobacterium avium-intracellulare* complex (MAI) infection by ELISA.[175,176,177] Recently, Rombouts and co-workers identified DMAG in large quantities in slow growing pathogenic species, *e.g. M.tb, M. bovis,* BCG and *M. Scrofulaceum,* identifying this glycolipid as more biologically potent and possibly important in mycobacterial pathogenesis.

One purpose of synthesizing the DMAG compounds described in this thesis is that their antigenic activity can be studied by ELISA. Investigating the antigenic activity of specific synthetic DMAG antigens could lead to the development of improved methods for the detection and diagnosis of mycobacterial diseases such as bovine TB. It is possible that single synthetic antigens could give a better distinction between TB+ and TB- serum samples, compared to a natural mixture, which contains a number of different homologues.

Preliminary ELISA assays, using the synthetic DMAG glycolipids **(71a)**, **(71b)**, **(71c)**, **(71d)**, **(75)**, **(79f)**, **(79g)** and **(79h)** as antigens, have been carried out by Mr. Paul Mason at the School of Chemistry, Bangor University. A synthetic keto TDM **(AD132)** known to distinguish active TB was also used as a control antigen. For these initial assays bovine serum samples were used at a 1 in 40 dilution in Casein / PBS buffer and an anti-bovine IgG Fc specific HRP secondary antibody was used. This was visualised by adding a colour reagent (OPD/H_2O_2 solution), and the absorbance was measured at 492 nm. A detailed method for the assay can be seen in the Appendix.

These initial assays were carried out to determine whether any response was observed to samples obtained from the Veterinary Laboratories Agency (VLA) that were known to be infected with bovine TB. Two samples that were believed to be free from bovine TB were also run as negative controls. **(Figure 117)**.

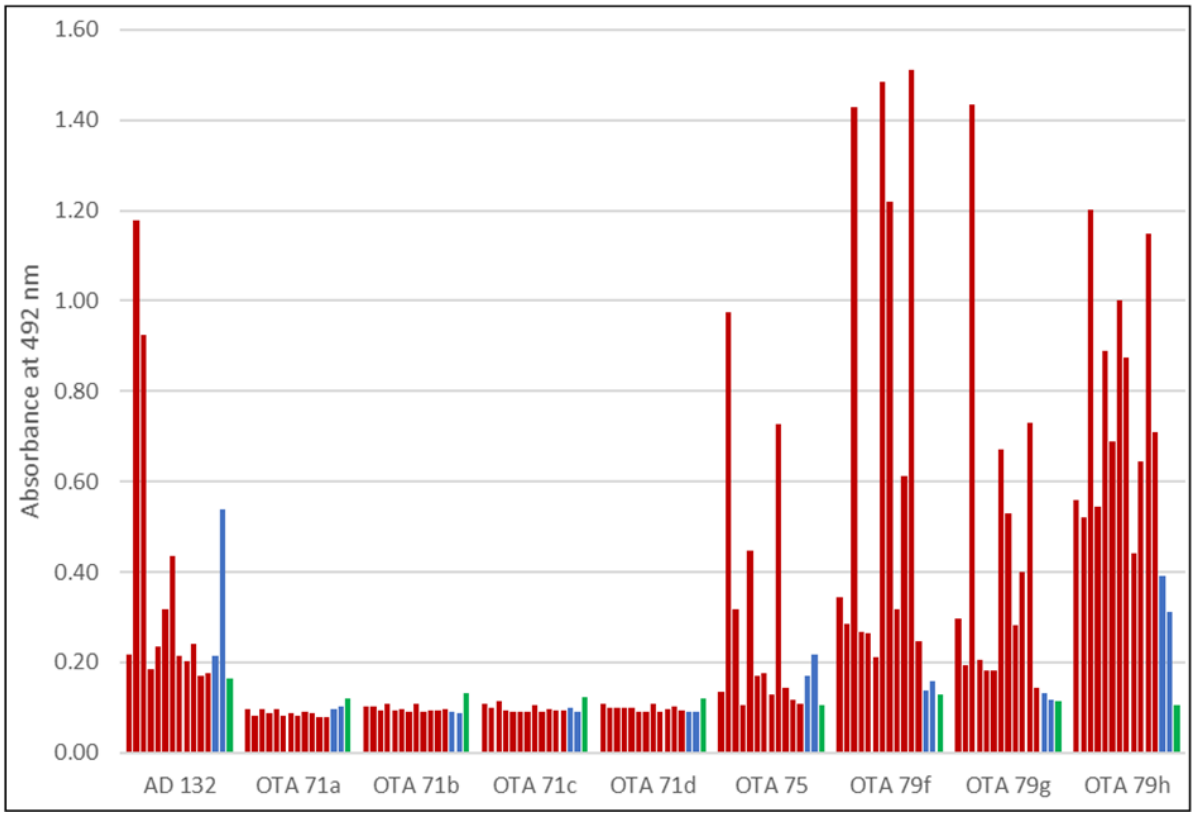


Figure 117: Response of 14 samples - 12 infected with bovine TB (red) and 2 samples free from bovine TB (blue) to a range of DMAG antigens. Green – blank / control.

As can be seen from Figure 1 the model DMAG antigens (simple esters) **71a**, **71b**, **71c** and **71d** have a very low response to all serum samples, both positive and negative, with the values being comparable to those observed for the blank / control wells. The DMAG antigens that have a mycolic acid attached to the diarabinoglycerol did however show a response to the serum samples, with 3 of the antigens, **79f**, **79g** and **79h** showing a distinction between the positive and negative samples. The other antigen **75** also showed a response to some of the serum samples, however a number of the positive samples gave responses that were lower than that observed for the negative controls. These results therefore suggest that the mycolic acid moiety of the DMAG plays a role in the antigenicity of these compounds and that the presence of different types of mycolic acid also influences the response observed. In addition a set of *Mycobacterium Avium* subspecies *Paratuberculosis* (MAP) infected and uninfected samples, obtained from the Veterinary and Agrochemical Research Centere, Brussels, were also tested **(Figure 118)**.

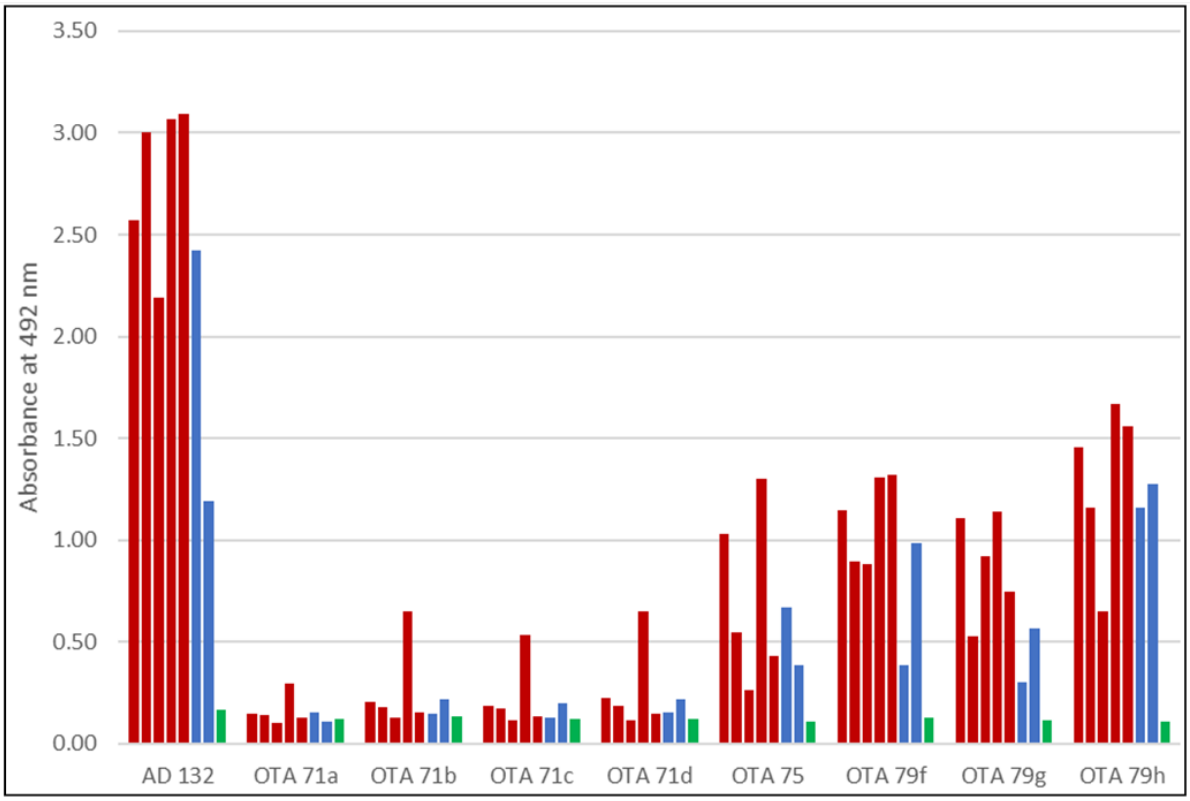


Figure 118: Response of 7 samples - 5 infected with MAP (red) and 2 uninfected (blue) to a range of DMAG antigens. Green – blank / control.

As was the case with the previous assay, apart from 1 sample, a poor response was again observed with the model DMAG antigens **71a**, **71b**, **71c** and **71d**. The other antigens all gave a response, however in this case there was not such a good distinction between the positive and negative samples.

These initial results show that the DMAG antigens **75**, **79f**, **79g** and **79h** do give a response to bovine serum samples infected with bovine TB and MAP, while the model DMAG antigens **71a**, **71b**, **71c** and **71d** give no response, this suggesting that the mycolic acid moiety plays a role in the recognition. For the samples obtained from the VLA some of the antigens **79f**, **79g** and **79h** show some distinction between the positive and negative samples. Although these results are encouraging they are only for a very small set of serum samples, therefore a much larger sample set needs to be tested in order to see whether these observations are real. It will also be interesting to test these antigens using TB+ and TB- human serum samples to determine whether they show a distinction between the two sets and could thus be used for the detection of TB. Based on these intial results, the use of DMAGs in serodiagnosis of such infections shows promise and is being further studied.

2.5.2 TNF-α cytokine stimulation

Many mycobacterial components have been demonstrated to be strong adjuvants. For example, natural TDM is able to stimulate the immune system so it will produce a range of chemokines (*e.g.* IL-8) and cytokines (*e.g.* TNF-α).[250] Another component which induced high levels of TNF-α is the lipid extract from *Mycobacterium avium-intracellulare* complex (MAI).[175]

TNF-α is stimulated from the cells of macrophages or monocytes in response to organisms such as *M. tuberculosis*, *M. bovis* BCG, and *Listeria monocytogenes*, in addition, some glycolipids can also release TNF-α.

TNF-α has been proven to be a significant inflammatory mediator, that can affect different kinds of cells. Studies in mice infected with *M. bovis* BCG and *L. monocytogenes*, and injected with anti-TNF-α antibody, showed inhibition of granuloma production in the host organs, and widespread growth of organisms *in vivo*.[251] It has been widely shown that activation of dendritic cells (DCs) is required to initiate immune responses. Presently, one of the suggested mechanisms for the route of DCs after activation, is that these cells are programmed to respond to certain activators. This process stimulates the production of co-stimulatory molecules and certain pro-inflammatory cytokines (*e.g.*, TNF-α, IL-12, IL-6).[252] As described in the Introduction **(Section 1.9)**, natural mixtures of DMAG show very strong effects on a number of immune system responses.

Biological assay experiments using the synthetic DMAG glycolipids **(71a)**, **(71b)**, **(71c)**, **(71d)**, **(75)**, **(79f)**, **(79g)** and **(79h)** against the production of certain pro-inflammatory cytokines (*e.g* TNF-α), were carried out by Dr. Andy Chancellor, University of Southampton, UK.

To study the stimulation of the TNF-α different concentrations were used (10, 50 and 100 μg/mL). The TNF-α signal was measured for these synthetic DMAG glycolipids and compared to that of a control sample. **(Figure 119)**. As can be seen, some of the compounds show a good stimulation of TNF-α compared to the control sample, with compound **(OTA 79g)** showing a very high level of TNF-α production at all concentrations. These initial results clearly indicate that synthetic single glycolipids show a selectivity in the inducing the TNF-α cytokine, and show an improved stimulation compared to the control.

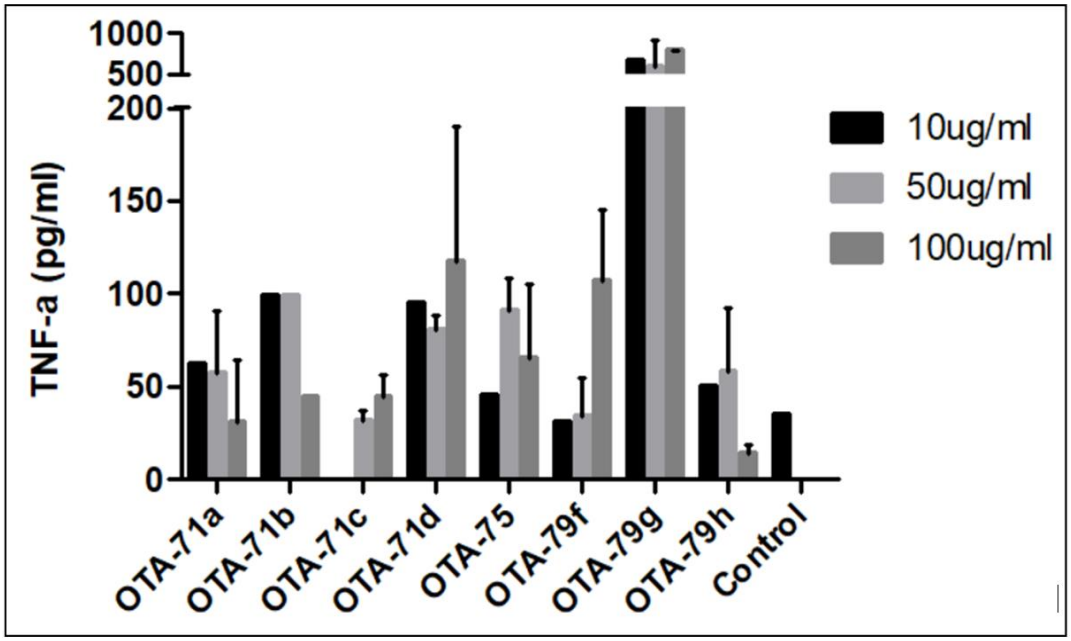


Figure 119: TNF-α stimulation.

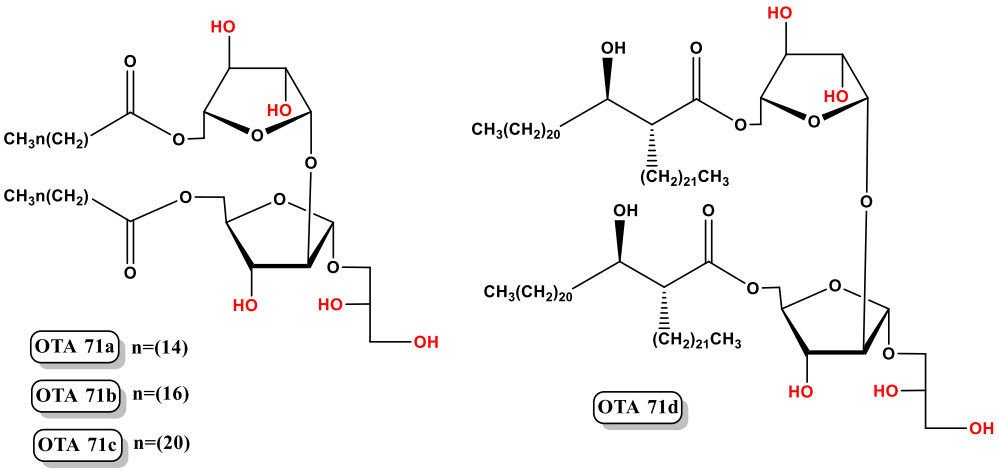


Figure 120: Structure of synthetic DMAG models used for TNF-α stimulation.

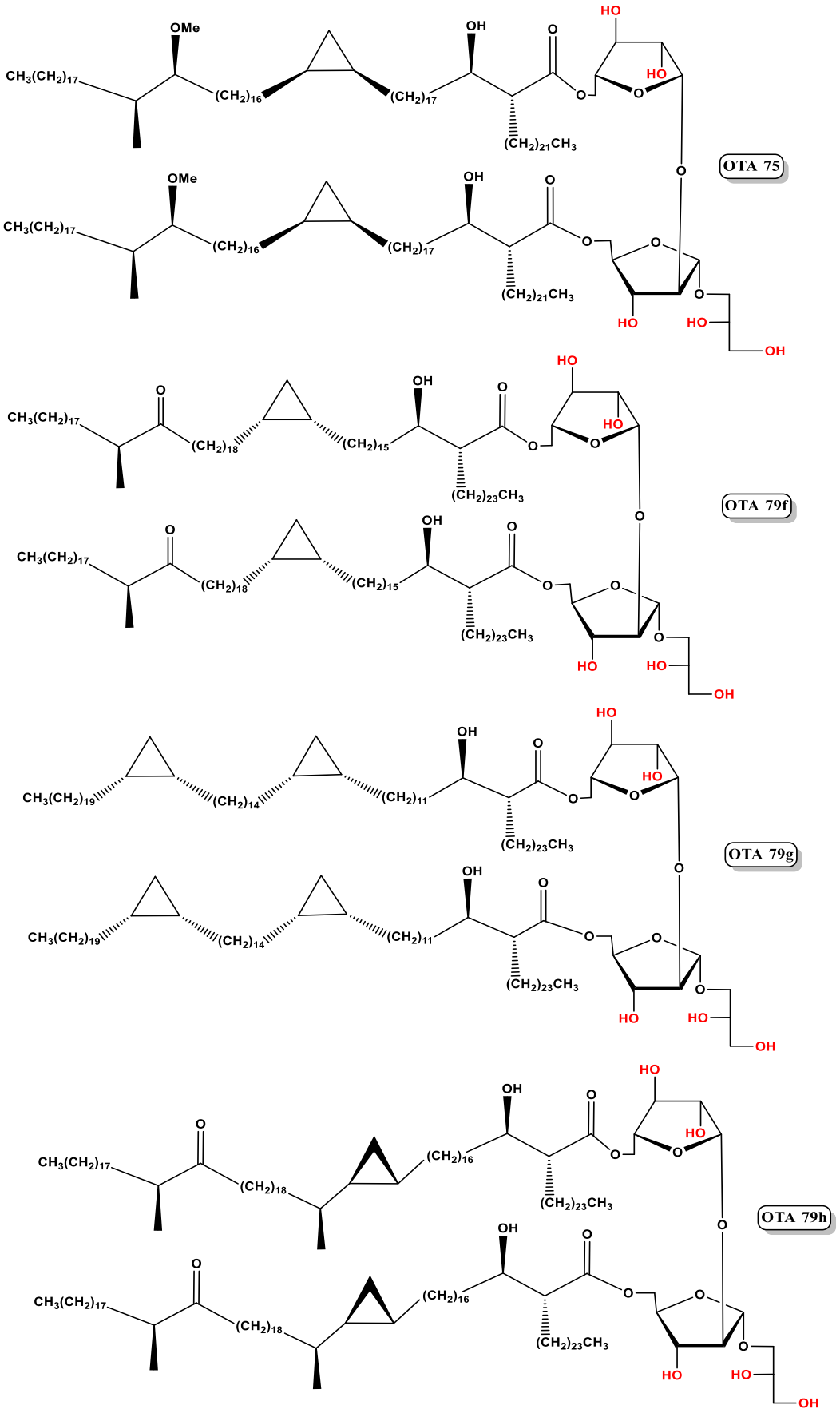


Figure 121: Structure of synthetic DMAGs used for TNF-α stimulation.

Chapter 3

Conclusion and Further Work

Chapter 3

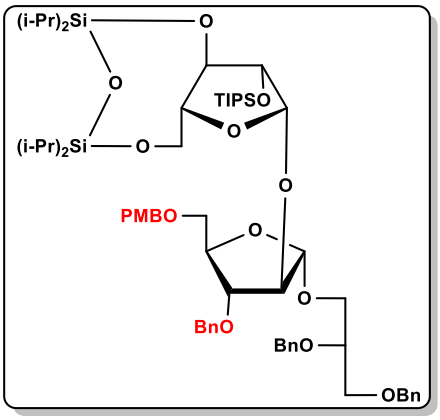
Conclusion and further work

3.1 Conclusions:

This work involved the successful synthesis of: eight Di-Mycolyl-Di-Ara*f*-Glycerol **(DMAG)** compounds, which had the L-stereochemistry of the glycerol component; four Di-Mycolyl-tri-Ara*f*-Glycerol **(DMTAG)** compounds; and finally, a range of Glycerol-Mono-Mycolates **(GroMM)**, with *S*- stereochemistry of the glycerol component. All the synthetic MAs used in this project were provided by researchers within the Prof. M. S. Baird group.

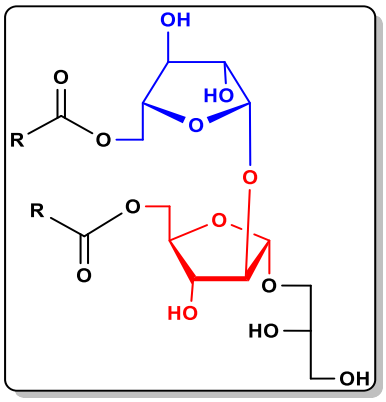
The first aim of this project was the synthesis for the first time of single enantiomers of DMAG from structurally defined synthetic MAs. These compounds will be assayed for their capability to stimulate a variety of cytokines in the immune system as well as being tested for their antigenicity in the detection of TB disease through ELISA assays.

The synthesis of DMAG was carried out by firstly preparing the glycan moiety of DMAG with L-stereochemistry of the glycerol component.



An efficient route to prepare the DMAG glycan moiety as a single anomer in excellent β-selectivity and a very good yield was successfully developed. This was achieved by having a benzyl protecting group and a PMB protecting group on the C-3 and C-5 positions, respectively, in the acceptor. The armed donor was prepared according to the literature.[145] Such coupling reactions have been reported to produce a mixture of α- and β-anomer, however, the synthesis of this di-saccharide has not previously been reported. After synthesising the di-saccharide unit in a large quantity, it was followed by the preparation of three new model analogues of DMAG, and one model using a β-hydroxy acid coupled to the glycan.

Finally, a series of four compounds of DMAG glycolipids was prepared based on the three common classes of synthetic MAs. The following compounds were successfully synthesised:



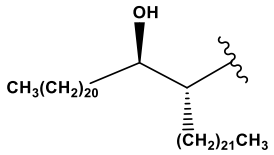
R=

$CH_3(CH_2)_{14}$ **Di-palmitoyl-di-Ara*f*-Glycerol. (71a)**

$CH_3(CH_2)_{16}$ **Di-stearoyl-di-Ara*f*-Glycerol. (71b)**

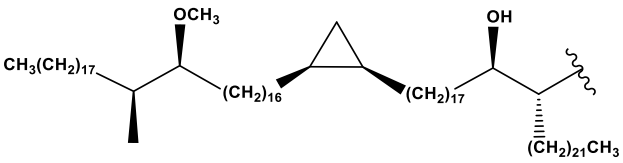
$CH_3(CH_2)_{20}$ **Di-behenoyl-di-Ara*f*-Glycerol. (71c)**

R=



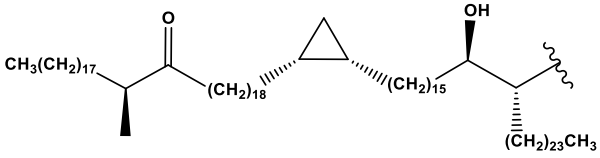
Di-tetracosanoyl-di-Ara*f*-Glycerol. (71d)

R=

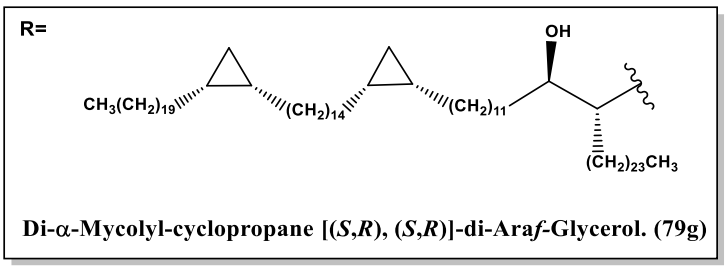


Di-methoxy-Mycolyl-cyclopropane [(*R*,*S*)]-di-Ara*f*-Glycerol. (75)

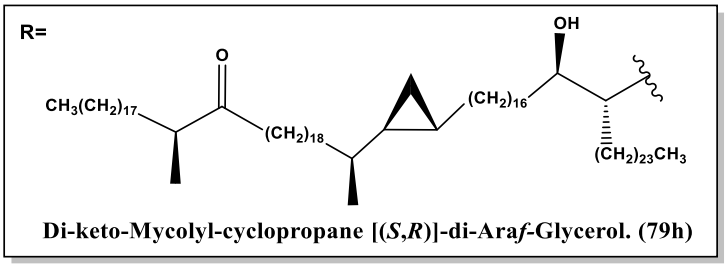
R=



Di-keto-Mycolyl-cyclopropane [(*S*,*R*)]-di-Ara*f*-Glycerol. (79f)

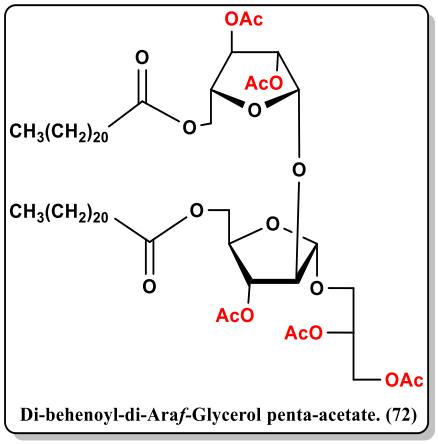


Di-α-Mycolyl-cyclopropane [(*S*,*R*), (*S*,*R*)]-di-Ara*f*-Glycerol. (79g)



Di-keto-Mycolyl-cyclopropane [(*S*,*R*)]-di-Ara*f*-Glycerol. (79h)

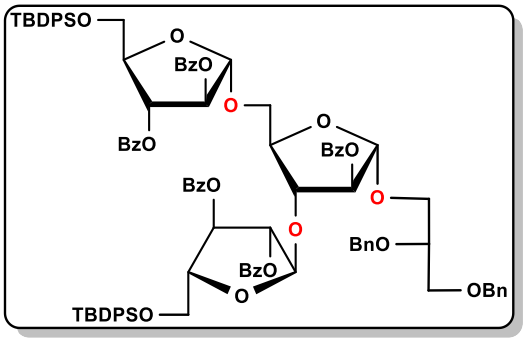
<u>The main target</u> of this part was to compare the NMR data for the synthesised compound with that reported for the naturally occurring compound. The NMR data for the penta-acetate derivative of compound **(72)** was compared directly with that in the literature for the per-acetate of the natural mixture. This comparison gave a very good agreement between the signals for the di-arabino-glycerol fragments of natural and synthetic molecules, and confirmed the structure and stereochemistry of this novel sugar of the DMAG glycolipid to be α-β-di-arabino-furanosyl glycerol.



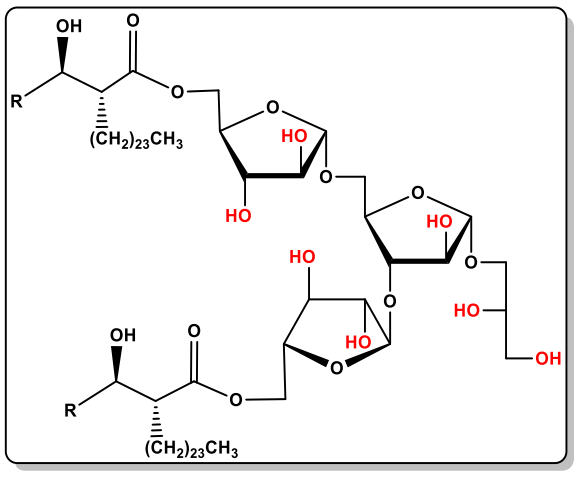
Di-behenoyl-di-Ara*f*-Glycerol penta-acetate. (72)

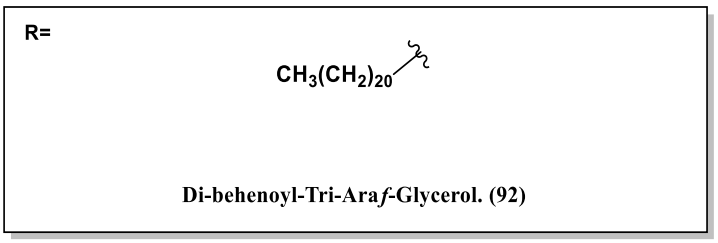
Natural DMAG has been found in the cell wall of *M. tuberculosis* in a high quantity and has been shown to be biologically active. Testing the effects of the synthetic DMAG glycolipids on a range of cytokines involved in the immune system, together with ELISA assays for the detection of TB, are expected to be carried out, which will give a further insight into the initial results discussed above.

The second part of this project involved the first synthesis of DMTAG, which is an important component in the cell wall of mycobacteria as a potential stimulator of some pro-inflammatory cytokines (*e.g* TNF-α). This glycolipid was reported in the literature;[80] however, it has never been synthesised. This part started by preparing the tri-Ara*f*-glycerol **(86)**, which involved preparing the donor **(84)** according to the literature methods with slight modifications in some of the steps, while the acceptor **(85)** has been synthesised for the first time by an efficient route and in a large quantity. Coupling the donor and the acceptor to prepare the desired glycan was carried out using known conditions. One type of tri-Ara*f*-glycerol, which contained three α-glyosidic linkages in its form, was prepared in high yield.

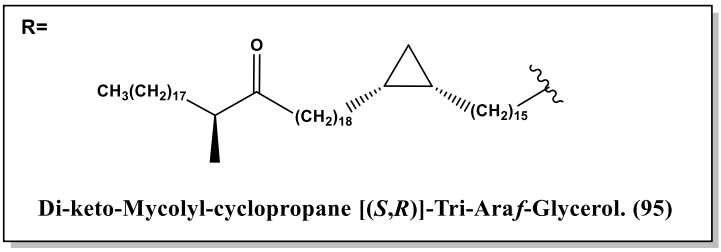


Having the glycan core, the preparation of a model through esterifying with commercially available behenic acid was carried out. A series of three DMTAG compounds were then prepared based on the three common classes of MAs. The following compounds were successfully synthesised:

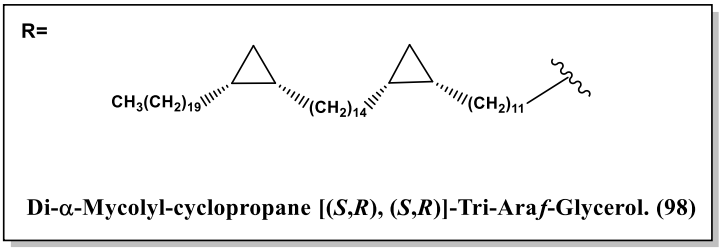




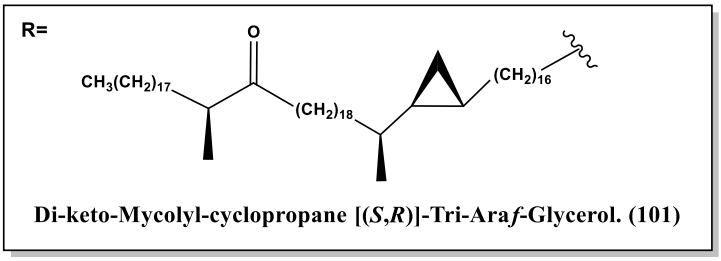
Di-behenoyl-Tri-Ara*f*-Glycerol. (92)



Di-keto-Mycolyl-cyclopropane [(*S*,*R*)]-Tri-Ara*f*-Glycerol. (95)



Di-α-Mycolyl-cyclopropane [(*S*,*R*), (*S*,*R*)]-Tri-Ara*f*-Glycerol. (98)

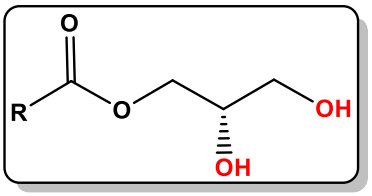


Di-keto-Mycolyl-cyclopropane [(*S*,*R*)]-Tri-Ara*f*-Glycerol. (101)

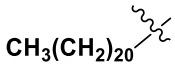
Methyl Tri-Ara*f*-Di-Mycolate (MTADM) compounds derived from a natural mixture of MAs have been reported in the literature and showed a very high response in the stimulation of TNF-α cytokines. The synthesis of the above set of DMTAG glycolipids, from single enantiomers of MAs, will therefore allow the effects of the individual components to be investigated. The assessment of the ELISA assay for detection of TB employing these compounds will also be carried out.

The final part of the project entailed the synthesis of GroMM, which required synthesis of the glycerol with (*S*) stereochemistry, prior to it being coupled to different MAs to produce a single enantiomer of GroMM compounds. According to the lietrature,[197] glycerol esters of complex mixtures of natural mycolic acids have strong effects in the immune system. GroMM has been observed to have adjuvant activity in murine models, and showed an ability to induce high levels of some pro-inflammatory cytokines (*e.g* IL-12 and TNF-α).[174]

In this part, a model of GroMM was synthesised, a series of five compounds was also prepared based on the three common classes of synthetic MAs. The following compounds were successfully synthesised:

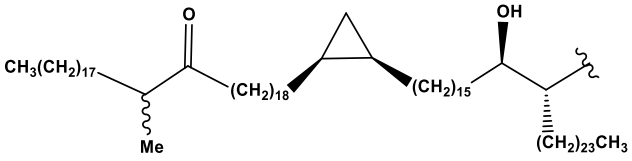


R=



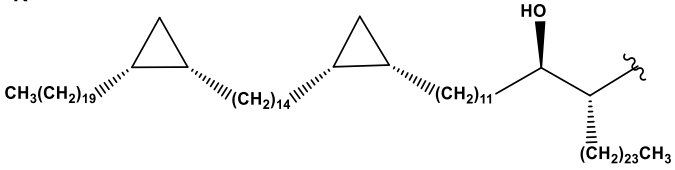
Glycerol behenate (105a)

R=

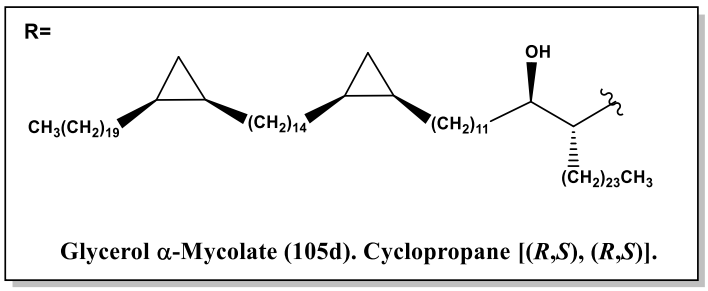


Glycerol-keto-Mycolate (105b). Cyclopropane [(*R*,*S*)].

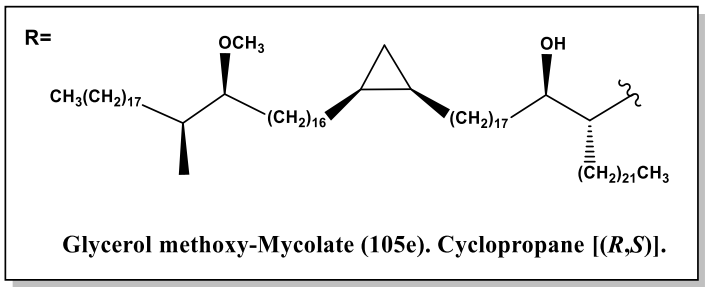
R=



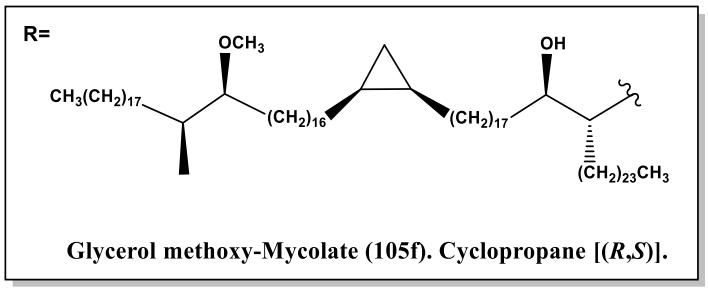
Glycerol α-Mycolate (105c). Cyclopropane [(*S*,*R*), (*S*,*R*)].



Glycerol α-Mycolate (105d). Cyclopropane [(*R*,*S*), (*R*,*S*)].



Glycerol methoxy-Mycolate (105e). Cyclopropane [(*R*,*S*)].



Glycerol methoxy-Mycolate (105f). Cyclopropane [(*R*,*S*)].

The above series of compounds were prepared from single synthetic enantiomers of MAs to investigate the differences in their biological activity, in contrast with that of the *R*-GroMM esters.

3.2 Further Work:

Further work which needs to be investigated, or could be undertaken:

1. Studies on the natural DMAG glycolipid that is obtained from the cell wall of several mycobacterial species, including *M. tuberculosis*, showed that it possesses very high biological activity. Preparing a large amount of the DMAG glycan with the L-glycerol component, and finding an efficient way of coupling it with synthetic MAs has been carried out. Hence it would be interesting to synthesise the DMAG with D-glycerol in order to obtain a range of compounds and compare their biological activities.

2. Selective esterification with different acids at each primary alcohol position was carried out to produce mono or symmetrical di-glycolipids similar to synthetic TDM and TMM which have shown promising results in the ELISA assays for the detection of TB. Therefore, synthesising a series of mono and symmetrical or unsymmetrical DMAG glycolipids would be valuable to use them in different assays to test their biological activity.

3. Natural Arabino-mycolates extracted from the cell wall of mycobacteria contain a number of different components with very complex mixtures of MAs, hence preparing DMTAG with different MAs within the same compound will be valuable, as it is unlikely that the two MAs will be the same, in the same compound, in nature. This will then allow the biological activity of these mixed compounds to be studied.

4. Finally, the synthesis of Penta-Ara*f* Tetra-Mycolates (PATM) is another item of further work which must be done. Again, penta-Ara*f* coupled with a natural mixture of MAs has been reported in the literature and was shown to have a high biological activity. Preparing the glycan unit and esterifying it with different synthetic MAs, followed by testing their biological activities using the previously discussed assays, will be valuable. This will also complete the set of different fragments of the mAGP.

Chapter 4

Experimental

Chapter 4

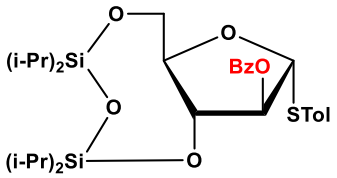
Experimental

4.1 General considerations:

All chemicals were purchased from commercial suppliers. THF was distilled over sodium and benzophenone under nitrogen, while dichloromethane was distilled over calcium hydride. Petrol refers to the fraction b.p 40–60 °C. Organic solutions were dried over anhydrous magnesium sulfate ($MgSO_4$). All glassware used in anhydrous reactions was dried for not less than 6 h in a 250 °C oven. Reactions carried out under inert conditions were under a slow stream of nitrogen. Those carried out at low temperatures were cooled using a bath of methylated spirits and liquid nitrogen. Silica gel and silica gel plates used for column chromatography and thin layer chromatography (TLC) were obtained from Aldrich. Infra-red (IR) spectra were carried out on a Perkin-Elmer 1600 F.T.I.R. spectrometer as liquid films or KBr disc (solid). Optical rotations were measured as solutions in chloroform of known concentration using a Polar 2001 automatic polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 500 and 400 spectrometer in $CDCl_3$ or CD_3OD if not differently indicated; ^1H spectra were normally run at 400MHz, and ^{13}C spectra were run at 101 MHz. Chemical shifts are quoted in δ relative to the trace resonance of proton chloroform (δ_H 7.27 ppm, δ_C 77.0 ppm), and the resonances of methanol (δ_H 4.87 and 3.31 ppm, δ_C 49.00 ppm). Mass spectrometry data was obtained from the EPSRC UK National Mass Spectrometry Facility at Swansea University and Dr Paul Gates (Bristol University). A laboratory book was filled in including chemical safety information following COSHH regulations.

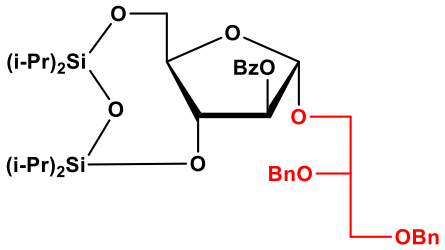
4.2 Experiments:

***p*-Cresyl 2-*O*-benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (51):**[239]



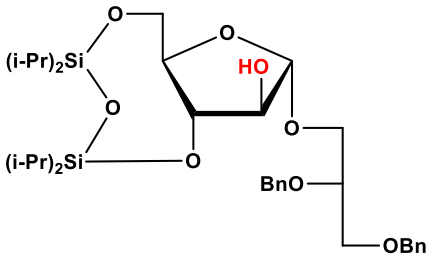
Benzoyl chloride (5.0 g, 4.2 mL, 0.035 mol) was added dropwise to a stirred solution of *p*-cresyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(50)** (16.2 g, 0.0324 mol) in anhydrous pyridine (25 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 6 h, when TLC showed no starting material was left. The solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (100 mL), washed with water (2×50 mL), 1 M aqueous HCl (2×50 mL), sat. aq. $NaHCO_3$ (1×50 mL) and brine (1×50 mL). The organic layer was dried over ($MgSO_4$), then the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to give the title compound **(51)** as a colourless thick oil (17 g, 89%) [MALDI–Found $(M+Na)^+$: 625.2; $C_{31}H_{46}NaO_6SSi_2$, requires: 625.2]; $[\alpha]_D^{22}$ +20 (*c* 4.2, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 8.08 – 8.01 (2H, d, *J* 7.9 Hz), 7.59 (1H, t, *J* 7.4 Hz), 7.52 – 7.42 (4H, m), 7.10 (2H, d, *J* 7.9 Hz), 5.59 (1H, br.dd, *J* 3.8, 5.3 Hz), 5.47 (1H, br.d, *J* 3.7 Hz), 4.57 (1H, dd, *J* 5.3, 7.9 Hz), 4.23 (1H, m), 4.10 (1H, dd, *J* 3.2, 12.7 Hz), 4.03 (1H, dd, *J* 4.4, 12.7 Hz), 2.32 (3H, s), 1.21 – 0.86 (28H, m); δ_C (101 MHz, $CDCl_3$): 165.4, 137.5, 133.3, 132.2, 129.7, 129.6, 129.4, 128.4, 89.6, 83.2, 80.9, 75.5, 61.4, 31.6, 22.6, 21.1, 17.4, 17.3, 17.0, 16.9, 16.85, 13.5, 13.2, 12.8, 12.5; ν_{max}: 3445, 3022, 2947, 2869, 1718,1468, 1045, 861 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-Benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (52):



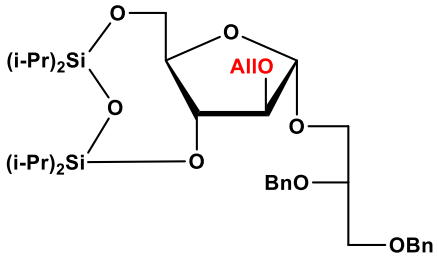
Molecular sieves 4 Å (5.6 g) were added to a stirred solution of α-D-arabinofuranoside **(51)** (15.4 g, 0.0255 mol) and 2',3'-di-*O*-benzyl-L-glycerol **(51G)** (6.9 g, 0.025 mol) in dry CH_2Cl_2 (25 mL) at room temperature under nitrogen. The reaction mixture was stirred for 30 min then cooled to -35 ºC and *N*-iodosuccinimide (9.38 g, 0.0383 mol) was added, followed by the addition of silver trifluoromethanesulfonate (1.17 g, 0.00460 mol). The mixture was stirred at the same temperature until the colour turned a red/dark brown colour and TLC showed no starting material was left. The reaction mixture was quenched by the addition of triethylamine (2 mL) until became yellow. The mixture was diluted with CH_2Cl_2 (50 mL) and filtered through celite and the solvent was evaporated. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) affording the title compound **(52)** as a colourless thick oil (17 g, 91%) [MALDI–Found $(M+Na)^+$: 773.3; $C_{41}H_{58}NaO_9Si_2$, requires: 773.3]; $[\alpha]_D^{22}$ +2.6 (*c* 4.3, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 8.01 – 7.97 (2H, m), 7.55 (1H, t, *J* 7.4 Hz), 7.41 (2H, t, *J* 7.7 Hz), 7.35 – 7.15 (10H, m), 5.41 (1H, br.dd, *J* 1.4, 4.9 Hz), 4.98 (1H, br.d, *J* 1.0 Hz), 4.67 (2H, br.s), 4.50 (2H, br.s), 4.45 (1H, dd, *J* 5.0, 7.4 Hz), 4.04 – 3.95 (2H, including a broad double doublet *J* 3.0, 9.9 Hz at 3.99), 3.92 (1H, dd, *J* 5.5, 13.2 Hz), 3.86 – 3.76 (2H, m), 3.67 – 3.59 (2H, including a broad double doublet *J* 4.2, 10.0 Hz at 3.63), 3.58 (1H, dd, *J* 5.0, 10.2 Hz), 1.32 – 0.75 (28H, m); δ_C (101 MHz, $CDCl_3$): 165.5, 138.7, 138.3, 133.2, 129.7, 128.4, 128.3, 128.2, 127.8, 127.6, 127.5, 127.4, 105.6, 84.4, 81.2, 76.2, 73.4, 72.3, 70.3, 67.7, 61.8, 31.6, 22.6, 17.5, 17.4, 17.3, 17.0, 16.9, 13.4, 13.2, 12.8, 12.5; ν_{max}: 3065, 3031, 2945, 2868,1717, 1105, 884, 712 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (53):



Sodium methoxide in methanol (10 mL, 0.1 M) was added to a stirred solution of compound **(52)** (15.6 g, 0.0207 mol) in dry CH_3OH:CH_2Cl_2 (25 mL, 1:1) at room temperature and the mixture was stirred for 0.5 h then TLC showed no starting material was left. The mixture was neutralized with Amberlite IR-120 (H^+), the resin was filtered off and the solvent was removed under reduced pressure to give a residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:1) to afford the title compound **(53)** as a thick colourless oil (12 g, 89%) [MALDI–Found $(M+Na)^+$: 669.3, $C_{34}H_{54}NaO_8Si_2$, requires 669.3], $[\alpha]_D^{20}$ -40 (*c* 0.10, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.35 – 7.17 (10H, m), 4.79 (1H, br.d, *J* 2.4 Hz), 4.63 (2H, br.s), 4.49 (2H, br.s), 4.14 – 4.04 (2H, m), 3.89 (1H, dd, *J* 3.1, 12.7 Hz), 3.86 (1H, br.d, *J* 3.7 Hz), 3.84 – 3.79 (1H, m), 3.77 (1H, br.dd, *J* 3.7, 7.2 Hz), 3.72 (1H, p, *J* 4.8 Hz), 3.57 (2H, d, *J* 4.8 Hz), 3.54 (1H, dd, *J* 4.4, 10.5 Hz), 1.12 – 0.72 (28H, m); δ_C (101 MHz, $CDCl_3$): 138.5, 138.4, 128.3, 128.3, 127.8, 127.7, 127.6, 107.5, 82.6, 80.8, 76.9, 73.4, 72.2, 70.2, 67.9, 61.4, 31.6, 22.6, 17.4, 17.3, 17.1, 17.05, 17.0, 13.5, 13.1, 12.8, 12.5; ν_{max}: 3402,3062, 2946, 2867, 1467, 1035, 884, 695 cm^{-1}.

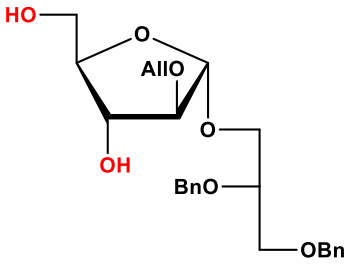
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (54):



A solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(53)** (11.9 g, 0.0183 mol) in dry DMF (20 mL) was added dropwise to a stirred suspension of NaH (0.88 g, 0.036 mol, 60% w/w, dispersion in mineral oil) at 0 ºC

under nitrogen. The mixture was stirred for 10 min then allyl bromide (2.66 g, 1.90 mL, 0.0220 mol) was added. The mixture was stirred at 0 ºC for 2 h then TLC showed no starting material was left. The mixture was quenched by slow addition of CH_3OH (1 mL) and the solvent was evaporated under reduced pressure to give an oily residue which was diluted with ethyl acetate (100 mL), and washed with water (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:1) to give the title compound **(54)** as a colourless thick oil (9.5 g, 75%) [MALDI–Found $(M+Na)^+$: 709.3, $C_{37}H_{58}NaO_8Si_2$, requires:709.3], $[\alpha]_D^{22}$ +72 (*c* 0.10, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.44 – 7.05 (10H, m), 5.81 (1H, ddt, *J* 5.4, 10.6, 17.3 Hz), 5.20 (1H, dd, *J* 1.6, 17.3 Hz), 5.09 (1H, dd, *J* 1.4, 10.6 Hz), 4.84 (1H, br.d, *J* 2.4 Hz), 4.63 (2H, br.s), 4.49 (2H, br.s), 4.14 (1H, dd, *J* 6.0, 8.3 Hz), 4.05 – 3.93 (2H, m), 3.92 – 3.81 (3H, m), 3.80 – 3.77 (2H, including a broad double doublet *J* 3.5, 8.5 Hz at 3.78), 3.76 – 3.70 (1H, m), 3.64 – 3.55 (2H, including a broad double doublet *J* 4.1, 10.7 Hz at 3.58), 3.54 (1H, dd, *J* 3.4, 9.3 Hz), 1.11 – 0.83 (28H, m); δ_C (101 MHz, $CDCl_3$): 138.6, 138.3, 134.3, 128.3, 128.2, 127.7, 127.6, 127.55, 127.5, 116.8, 106.0, 89.5, 80.5, 77.1, 76.1, 73.4, 72.1, 71.4, 70.4, 67.5, 61.5, 17.5, 17.3, 17.2, 17.1, 17.05, 17.0, 13.5, 13.1, 12.8, 12.5; ν_{max}: 3082, 3069, 2927, 2867, 1465, 1036, 885, 696 cm^{-1}.

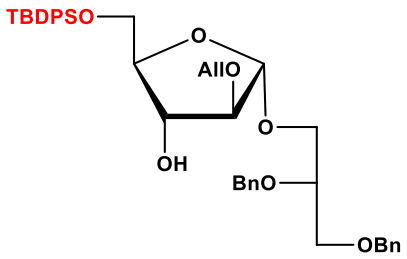
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-α-D-arabinofuranoside (55):



Tetrabutylammonium fluoride (26.2 mL, 0.0904 mol, 1.0 M) was added dropwise to a stirred solution of α-D-arabinofuranoside **(54)** (9.0 g, 0.01 mol) in anhydrous THF (25 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 2 h then TLC showed no starting material was left, then diluted with ethyl acetate (100 mL), washed with sat. aq. NH_4Cl (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), and concentrated to give a residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (3:1) to give to the title compound **(55)** as a colourless thick oil (5.5 g, 95%) [MALDI–Found $(M+Na)^+$: 467.2, $C_{25}H_{32}NaO_7$, requires: 467.2], $[\alpha]_D^{20}$ +80 (*c* 0.10, $CHCl_3$)

which showed δ_H (400 MHz, $CDCl_3$): 7.33 – 7.19 (10H, m), 5.81 (1H, ddt, *J* 5.6, 10.8, 17.2 Hz), 5.22 (1H, dd, *J* 1.5, 17.2 Hz), 5.14 (1H, dd, *J* 1.3, 10.8 Hz), 5.00 (1H, br.s), 4.61 (1H, d, *J* 11.9 Hz), 4.57 (1H, d, *J* 11.9 Hz), 4.48 (2H, br.s), 4.04 – 3.92 (4H, m), 3.84 – 3.77 (2H, including a broad double doublet *J* 5.7, 10.3 Hz at 3.81), 3.72 (1H, br.dd, *J* 4.8, 9.7 Hz), 3.68 (1H, br.d, *J* 3.1 Hz), 3.63 (1H, dd, *J* 3.7, 11.8 Hz), 3.58 – 3.54 (1H, m), 3.53 – 3.49 (2H, including a broad doublet *J* 5.1 Hz at 3.52); δ_C (101 MHz, $CDCl_3$): 138.1, 137.9, 133.6, 128.4, 128.3, 127.8, 127.75, 127.7, 117.9, 105.6, 86.9, 86.5, 76.5, 75.3, 73.5, 72.1, 70.6, 69.6, 66.7, 62.4; ν_{max}: 3437, 3031, 2940, 2867, 1651, 1454, 1055, 668 cm^{-1}. The experiment was repeated on a large scale.

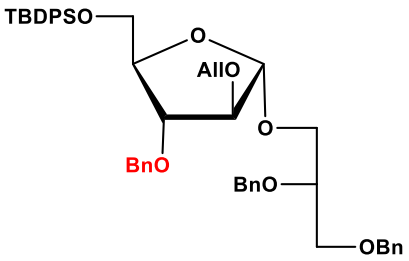
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranoside (56):



tert-Butylchlorodiphenylsilane (9.2 g, 0. 033 mol) was added to a stirred solution of α-D-arabinofuranoside **(55)** (15 g, 0.033 mol) in dry DMF (100 mL), followed by the addition of imidazole (5.7 g, 0.084 mol) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 25 min, when TLC showed no starting material was left. The mixture was diluted with ethyl acetate (100 mL) and water (25 mL). The organic layer was separated, the aqueous layer was re-extracted with ethyl acetate (2×100 mL). The combined organic layers were washed with water (100 mL), brine (100 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (4:1) affording the title compound **(56)** as a colourless thick oil (15 g, 65%) [NSI–Found $(M+NH_4)^+$: 700.3661; $C_{41}H_{54}O_7SiN$, requires: 700.3664]; $[\alpha]_D^{22}$ +26.5 (*c* 1.27, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.66 – 7.55 (4H, m), 7.41 – 7.17 (16H, m), 5.77 (1H, ddt, *J* 5.5, 10.7, 17.2 Hz), 5.18 (1H, dd, *J* 1.0, 17.2 Hz), 5.11 (1H, dd, *J* 0.5, 10.7 Hz), 4.95 (1H, br.s), 4.62 (1H, d, *J* 12.1 Hz), 4.58 (1H, d, *J* 12.1 Hz), 4.48 (2H, br.s), 4.11 – 3.98 (2H, including a broad double doublet *J* 3.5, 9.6 Hz at 4.03), 3.97– 3.87 (2H, including a broad doublet *J* 5.4 Hz at 3.93), 3.83 – 3.75 (3H, including a broad double doublet *J* 5.5, 10.5 Hz at 3.79), 3.74 – 3.69 (1H, m), 3.66 (1H, dd, *J* 6.5, 10.2 Hz), 3.60 – 346 (3H, including a broad quartet *J* 4.7 Hz at 3.54), 1.02 (9H, s); δ_C (101 MHz,

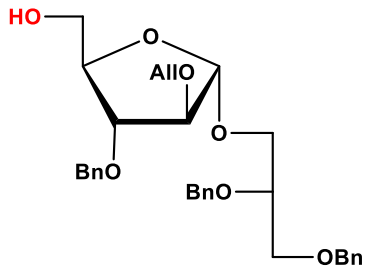
$CDCl_3$): 138.3, 138.0, 135.6, 135.5, 134.0, 133.3, 133.2, 129.7, 128.4, 128.3, 127.75, 127.7, 127.65, 127.6, 127.55, 117.3, 106.0, 87.8, 84.9, 76.6, 76.5, 73.4, 72.0, 70.6, 69.9, 66.8, 64.3, 26.8, 19.2; ν_{max}: 3445, 3069, 3031, 2930, 2859,1590, 1471, 1110, 858, 740 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-3-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranoside (57):



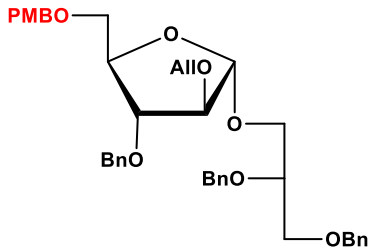
A solution of α-D-arabinofuranoside **(56)** (9.7 g, 0.014 mol) in dry DMF (100 mL) was added dropwise to a stirred suspension of NaH (0.68 g, 0.028 mol, 60% w/w, dispersion in mineral oil) at 0 ºC under nitrogen atmosphere. The mixture was stirred for 30 min. then benzyl bromide (2.5 mL, 3.6 g, 0.021 mol) in dry DMF (5 mL) was added. The mixture was stirred at room temperature for 10 h then quenched slowly with CH_3OH (10 mL) and H_2O (15 mL). The mixture was diluted with ether (200 mL). The organic layer was separated and the aqueous layer was extracted with ether (2×100 mL). The combined extracts were washed with water (100 mL), brine (100 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:1) to give the title compound **(57)** as a colourless thick oil (8.1 g, 72%) [NSI–Found $(M+NH_4)^+$: 790.4132; $C_{48}H_{60}O_7SiN$, requires: 790.4134]; $[\alpha]_D^{22}$ +28 (*c* 3.9, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.79 – 756 (4H, including a broad double doublet *J* 3.9, 10.8 Hz at 7.66), 7.47 – 7.14 (21H, m), 5.84 (1H, ddt, *J* 5.5, 10.7, 17.2 Hz), 5.24 (1H, dd, *J* 1.3, 17.2 Hz), 5.16 (1H, dd, *J* 0.9, 10.7 Hz), 5.01 (1H, br.s), 4.70 (1H, d, *J* 12.0 Hz), 4.66 (1H, d, *J* 12.0 Hz), 4.59 (1H, d, *J* 11.9 Hz), 4.54 – 4.48 (3H, m), 4.13 (1H, br.q, *J* 4.6 Hz), 4.03 – 3.89 (4H, m), 3.85 (1H, dd, *J* 5.1, 10.2 Hz), 3.82 – 3.73 (3H, including a broad double doublet *J* 4.8, 8.1 Hz at 3.79), 3.67 – 3.56 (3H, m), 1.04 (9H, s); δ_C (101 MHz, $CDCl_3$): 138.6, 138.3, 138.0, 135.7, 135.6, 134.1, 133.5, 133.4, 129.6, 129.5, 128.3, 128.2, 127.7, 127.65, 127.6, 127.55, 127.5, 127.45, 127.4, 117.2, 106.4, 88.0, 77.0, 73.3, 72.1, 72.0, 70.6, 70.3, 67.0, 63.7, 26.8, 19.3; ν_{max}: 3068, 3031, 2929, 2859, 1588, 1454, 1027, 823, 738 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-3-*O*-benzyl-α-D-arabinofuranoside (58):



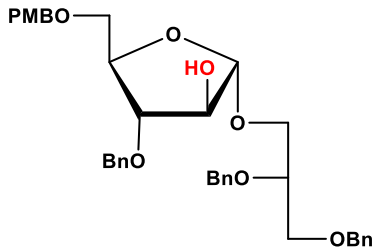
Tetrabutylammonium fluoride (7.0 mL, 7.0 mmol, 1.0 M) was added dropwise to a stirred solution of α-D-arabinofuranoside **(57)** (5.2 g, 0.0067 mol) in anhydrous THF (50 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The mixture was diluted with ethyl acetate (100 mL) and water (50 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with sat. aq. NH_4Cl (50 mL), brine (50 mL), dried ($MgSO_4$) and the solvent was concentrated. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:1) to give the title compound **(58)** as a colourless thick oil (3.3 g, 91%) [NSI–Found $(M+NH_4)^+$: 552.2948; $C_{32}H_{42}O_7N$, requires: 552.2956]; $[\alpha]_D^{22}$ +36 (*c* 3.3, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.28 (15H, m), 5.87 (1H, ddt, *J* 5.6, 10.7, 17.2 Hz), 5.27 (1H, dd, *J* 1.5, 17.2 Hz), 5.20 (1H, dd, *J* 1.1, 10.7 Hz), 5.03 (1H, br.s), 4.70 (2H, br.s), 4.66 (1H, d, *J* 11.8 Hz), 4.58 – 4.49 (3H, m), 4.13 (1H, br.p, *J* 3.4 Hz), 4.01 (1H, br.dd, *J* 4.4, 11.8 Hz), 3.99 – 3.95 (2H, including a broad doublet *J* 10.6 Hz at 3.98), 3.94 (1H, br.dd, *J* 2.6, 6.2 Hz), 3.86 (1H, dd, *J* 5.2, 10.3 Hz), 3.82 (1H, dd, *J* 5.3, 9.6 Hz), 3.79 (1H, br.d, *J* 9.7 Hz), 3.67 – 3.63(2H, including a broad double doublet *J* 6.3, 7.4 Hz at 3.64), 3.63 – 3.59 (2H, including a broad doublet *J* 10.5 Hz at 3.62); δ_C (101 MHz, $CDCl_3$): 138.6, 138.2, 137.8, 133.9, 128.4, 128.35, 128.3, 127.8, 127.75, 127.7, 127.6, 127.5, 117.6, 106.4, 87.6, 82.8, 82.2, 76.9, 73.4, 72.3, 72.2, 70.7, 70.1, 67.1, 62.2; ν_{max}: 3453, 3063, 3031, 2923, 2870, 1603, 1453, 1064, 850, 739 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (59):



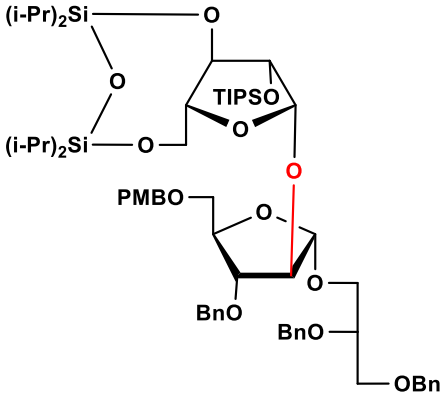
A solution of α-D-arabinofuranoside **(58)** (3.1 g, 0.0057 mol) in dry DMF (10 mL) was added dropwise to a stirred suspension of NaH (0.25 g, 0.010 mol, 60% w/w, dispersion in mineral oil) at 0 ºC under nitrogen. The mixture was stirred for 30 min. then, freshly prepared, *p*-methoxybenzyl bromide (1.4 g, 0.0069 mol) was added. The mixture was stirred at 0 ºC for 2 h then TLC showed no starting material was left. The mixture was quenched with slow addition of CH_3OH (1 mL) and the solvent was evaporated under reduced pressure to give an oily residue which was diluted with ethyl acetate (50 mL). The organic layer was washed with water (25 mL), brine (25 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:1) to give the title compound **(59)** as a colourless thick oil (2.9 g, 76%) [NSI–Found $(M+NH_4)^+$: 672.3526; $C_{40}H_{50}O_8N$, requires: 672.3531]; $[\alpha]_D^{22}$ +41 (*c* 1.6, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.26 (15H, m), 7.24 (2H, d, *J* 8.7 Hz), 6.86 (2H, d, *J* 8.6 Hz), 5.86 (1H, ddt, *J* 5.5, 10.7, 17.2 Hz), 5.25 (1H, dd, *J* 1.6, 17.2 Hz), 5.18 (1H, dd, *J* 1.3, 10.7 Hz), 5.03 (1H, br.s), 4.71 (1H, d, *J* 12.1 Hz), 4.68 (1H, d, *J* 12.1 Hz), 4.61 (1H, d, *J* 11.9 Hz), 4.54 (3H, br.s), 4.51 (1H, d, *J* 11.7 Hz), 4.47 (1H, d, *J* 11.7 Hz), 4.22 – 4.15 (1H, m), 4.03 – 3.91 (3H, m), 3.88 (1H, dd, *J* 5.2, 10.5 Hz), 3.86 (1H, br.d, *J* 6.5 Hz), 3.84 – 3.75 (4H, including a singlet at 3.8 for O*CH*$_3$), 3.66 – 3.58 (4H, m), 3.55 (1H, dd, *J* 5.2, 10.7 Hz); δ_C (101 MHz, $CDCl_3$): 138.7, 138.3, 138.0, 134.1, 130.2, 129.4, 128.8, 128.4, 128.3, 127.75, 127.7, 127.6, 127.5, 127.45, 117.4, 113.7, 106.4, 88.1, 83.7, 80.8, 73.4, 73.0, 72.2, 70.8, 70.4, 69.3, 67.2, 55.3; ν_{max}: 3064, 3030, 2912, 2864, 1612, 1513, 1454, 1106, 820, 738 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (60):



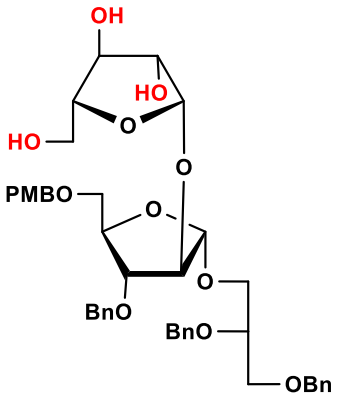
Palladium (II) chloride (0.30 g, 0.0017 mol) was added to a stirred solution of α-D-arabinofuranoside **(59)** (5.7 g, 0.0087 mol) in dry CH_2Cl_2:MeOH (0.6:5, 5 mL) at room temperature. The mixture was stirred for 16 h then TLC showed no starting material was left. The mixture was quenched with triethylamine (1 mL) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (4:1) to give the title compound **(60)** as a pale yellow thick oil (4.5 g, 84%) [NSI–Found $(M+NH_4)^+$: 632.3209; $C_{37}H_{46}O_8N$, requires: 632.3218]; $[\alpha]_D^{22}$ +60 (*c* 4.6, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.26 (15H, m), 7.23 (2H, d, *J* 8.5 Hz), 6.89 (2H, d, *J* 8.6 Hz), 5.03 (1H, br.s), 4.74 (1H, d, *J* 12.1 Hz), 4.68 (1H, d, *J* 12.1 Hz), 4.66 (1H, d, *J* 11.9 Hz), 4.58 (1H, d, *J* 11.9 Hz), 4.51 (2H, br.s), 4.49 (1H, d, *J* 11.7 Hz), 4.44 (1H, d, *J* 11.7 Hz), 4.26 (1H, br.d, *J* 2.4 Hz), 4.18 (1H, d, *J* 10.8 Hz), 3.89 (1H, dd, *J* 5.4, 10.4 Hz), 3.87 (1H, br.d, *J* 3.1 Hz), 3.85 – 3.79 (4H, including a singlet at 3.82 for O*CH*$_3$), 3.68 – 3.63 (3H, m), 3.61 (1H, dd, *J* 5.5, 10.2 Hz), 3.49 (1H, dd, *J* 2.1, 10.4 Hz), 3.39 (1H, d, *J* 10.8 Hz); δ_C (101 MHz, $CDCl_3$): 159.5, 138.8, 138.4, 137.8, 129.5, 129.1, 128.4, 128.3, 128.2, 127.75, 127.7, 127.65, 127.55, 127.45, 127.4, 113.9, 109.4, 85.4, 83.6, 77.5, 76.9, 73.4, 73.3, 72.2, 71.9, 70.4, 69.4, 67.4, 55.2; ν_{max}: 3433, 3063, 3031, 2912, 2867, 1611, 1513, 1454, 1248, 1098, 820, 738, 699 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-(triisopropylsilyl)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (62):



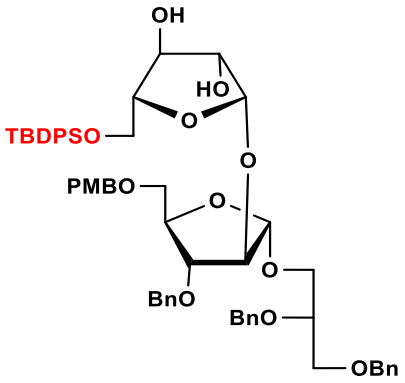
Molecular sieves 4 Å (5 g) was added to a stirred solution of -D-arabinofuranoside **(60)** (4.3 g, 0.0069 mol) and *p*-tolyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-2-*O*-triisopropylsilyl-α-D-arabinofuranoside **(61)**[3] (11.4 g, 0.0174 mol) in dry CH_2Cl_2 (50 mL) at room temperature under nitrogen. The mixture was stirred for 30 min then cooled to -78 ºC and *N*-iodosuccinimide (6.4 g, 0.026 mol) was added followed by the addition of silver trifluoromethanesulfonate (0.71 g, 0.0028 mol). The mixture was stirred until the colour turned red/dark brown at -60 ºC, when TLC showed no starting material was left. The mixture was quenched with triethylamine (4 mL) until the colour turned yellow. The mixture was diluted with CH_2Cl_2 (100 mL) and filtered through celite and the solvent was evaporated. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (4:1) affording the title compound **(62)** as a yellow thick oil (6.9 g, 86%) [NSI–Found $(M+NH_4)^+$:1162.6495; $C_{63}H_{100}O_{13}Si_3N$, requires: 1162.6497]; $[\alpha]_D^{22}$ +4.5 (*c* 0.97, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.28 – 7.18 (15H, m), 7.15 (2H, d, *J* 8.5 Hz), 6.78 (2H, d, *J* 8.5 Hz), 4.96 (1H, br.s), 4.79 (1H, br.d, *J* 4.3 Hz), 4.63 (1H, d, *J* 12.0 Hz), 4.59 (2H, d, *J* 12.0 Hz), 4.44 (2H, br.s), 4.43 (1H, d, *J* 12.0 Hz), 4.39 (2H, br.s), 4.34 (1H, br.dd, *J* 5.7, 7.3 Hz), 4.20 – 4.17 (1H, br.m), 4.15 (1H, dd, *J* 4.7, 9.2 Hz), 4.12 (1H, br.t, *J* 5.1 Hz), 3.87 (1H, d, *J* 5.7 Hz), 3.86 (1H, d, *J* 6.8 Hz), 3.84 – 3.76 (3H, m), 3.75 – 3.70 (4H, including a singlet at 3.73 for OC*H*$_3$), 3.56 (1H, dd, *J* 3.9, 9.7 Hz), 3.53 (1H, br.d, *J* 5.3 Hz), 3.51 – 3.47 (2H, including a broad double doublet *J* 4.6, 10.5 Hz at 3.49), 3.45 (1H, dd, *J* 5.9, 10.8 Hz), 1.04 – 0.92 (49H, m); δ_C (101 MHz, $CDCl_3$): 159.1, 138.8, 138.4, 138.0, 130.2, 129.3, 128.3, 128.25, 128.2, 127.7, 127.65, 127.6, 127.5, 127.4, 127.3, 113.7, 106.2, 100.6, 85.9, 84.4, 82.0, 81.5, 79.4, 79.1, 77.05, 73.3, 72.9, 72.2, 72.15, 70.6, 69.8, 67.4, 66.6, 55.2, 18.0, 17.95, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 16.9, 13.4, 13.3, 13.0, 12.7, 12.4; ν_{max}: 3064, 3031, 2943, 2867, 1513,1248, 736, 695 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (63):



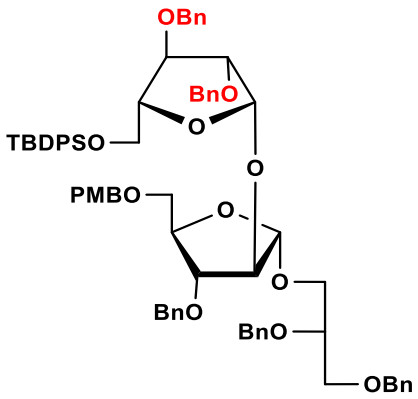
Tetrabutylammonium fluoride (17.1 mL, 0.0202 mol, 1.0 M) was added dropwise to a stirred solution of α-D-arabinofuranoside **(62)** (6.5 g, 0.0056 mol) in dry THF (100 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 6 h. When TLC showed no starting material was left, the mixture was diluted with ethyl acetate (100 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with sat. aq. NH_4Cl (25 mL), brine (25 mL), dried ($MgSO_4$) and the solvent was concentrated to give a residue which was purified by column chromatography on silica eluting with dichloromethane /methanol (20:1) to give the title compound **(63)** as a colourless thick oil (4.0 g, 95%) [NSI–Found $(M+NH_4)^+$: 764.3639; $C_{42}H_{54}O_{12}N$, requires: 764.3641]; $[\alpha]_D^{24}$ +16 (*c* 0.50, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.28 – 7.17 (15H, m), 7.15 (2H, d, *J* 8.6 Hz), 6.79 (2H, d, *J* 8.6 Hz), 4.96 (1H, br.s), 4.94 (1H, br.d, *J* 4.7 Hz), 4.60 (2H, br.s), 4.56 (1H, d, *J* 11.9 Hz), 4.44 (3H, br.s), 4.40 (1H, d, *J* 11.6 Hz), 4.33 (1H, d, *J* 11.6 Hz), 4.23 (1H, m), 4.06 (1H, br.p, *J* 3.6 Hz), 4.01 (1H, dd, *J* 2.7, 6.1 Hz), 3.95 (1H, t, *J* 7.2 Hz), 3.87 (1H, br.dd, *J* 5.9, 10.6 Hz), 3.79 (1H, br.dd, *J* 4.8, 10.5 Hz), 3.76 (1H, br.dd, *J* 3.3, 6.8 Hz), 3.73 – 3.69 (4H, including a singlet at 3.7 for O*CH*$_3$), 3.61 – 3.47 (6H, m), 3.39 (1H, dd, *J* 3.9, 10.9 Hz); δ_C (101 MHz, $CDCl_3$): 159.3, 138.5, 138.2, 137.8, 129.8, 129.7, 128.4, 128.35, 128.3, 127.8, 127.7, 127.6, 127.55, 127.5, 113.8, 106.2, 101.0, 86.5, 82.9, 82.4, 81.2, 78.1, 76.9, 75.0, 73.4, 73.1, 72.3, 72.2, 70.1, 68.6, 67.3, 62.3, 55.3; ν_{max}: 3430, 3063, 3031, 2923, 2868, 1612, 1514, 1100, 740, 699 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-*tert*-butyldiphenylsilyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (64):



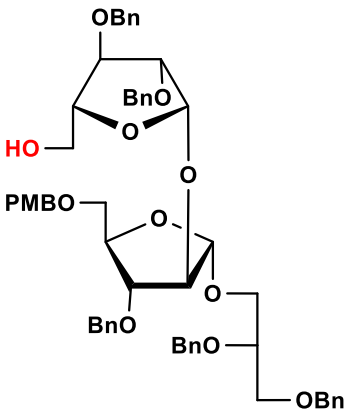
tert-Butylchlorodiphenylsilane (1.39 mL, 1.47 g, 0.00535 mol) was added to a stirred solution of α-D-arabinofuranoside **(63)** (4.0 g, 0.005 mol) in dry DMF (5 mL), followed by the addition of imidazole (0.73 g, 0.010 mol) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 30 min, when TLC showed no starting material was left. The mixture was diluted with ethyl acetate (25 mL) and water (5 mL). The organic layer was separated, and the aqueous layer was re-extracted with ethyl acetate (2×25 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting chloroform/methanol (20:1) affording the title compound **(64)** as a colourless thick oil (4.1 g, 77%) [NSI–Found $(M+NH_4)^+$: 1002.4816; $C_{58}H_{72}O_{12}SiN$, requires: 1002.4818]; $[\alpha]_D^{22}$ -6.3 (*c* 0.38, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.69 – 7.63 (4H, m), 7.46 – 7.16 (21H, m), 7.11 (2H, dd, *J* 2.9, 6.5 Hz), 6.85 (2H, d, *J* 8.6 Hz), 5.03 (1H, br.d, *J* 4.5 Hz), 5.02 (1H, br.s), 4.69 (1H, d, *J* 12.0 Hz), 4.66 (1H, d, *J* 12.0 Hz), 4.56 – 4.48 (4H, m), 4.45 (1H, d, *J* 11.6 Hz), 4.40 (1H, d, *J* 11.6 Hz), 4.31 – 4.29 (1H, m), 4.25 (1H, d, *J* 11.7 Hz), 4.14 (1H, br.p, *J* 5.1 Hz), 4.01 – 3.84 (4H, m), 3.82 (1H, br.dd, *J* 3.8, 9.1 Hz), 3.79 – 3.75 (4H, including a singlet at 3.77 for O*CH*$_3$), 3.71 (1H, dd, *J* 6.6, 10.0 Hz), 3.65 – 3.60 (3H, including a broad double doublet *J* 4.9, 8.9 Hz at 3.63), 3.54 (1H, dd, *J* 3.3, 10.8 Hz), 3.46 (1H, dd, *J* 4.9, 10.8 Hz), 2.43 (1H, d, *J* 9.4 Hz), 2.16 (1H, d, *J* 2.7 Hz), 1.07 (9H, s); δ_C (101 MHz, $CDCl_3$): 159.2, 138.6, 138.3, 137.8, 135.8, 135.7, 135.6, 135.5, 133.1, 133.0, 130.0, 129.95, 129.9, 129.6, 129.5, 128.4, 128.35, 128.3, 128.2, 128.0, 127.9, 127.85, 127.8, 127.7, 127.65, 127.6, 127.55, 127.5, 113.8, 106.3, 100.8, 85.9, 83.8, 81.6, 81.4, 78.2, 77.4, 77.0, 73.4, 73.0, 72.3, 72.2, 70.2, 69.1, 67.3, 66.1, 55.3, 26.9, 19.2; ν_{max}: 3438, 3067, 3031, 2930, 2859, 1612, 1513, 1248, 739, 700 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (65):



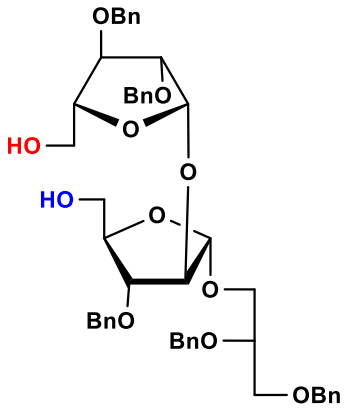
A solution of α-D-arabinofuranoside **(64)** (4.0 g, 0.0040 mol) in dry DMF (5 mL) was added dropwise to a stirred suspension of NaH (0.39 g, 0.016 mol, 60% w/w, dispersion in mineral oil) at 0 ºC under nitrogen. The mixture was stirred for 0.5 h then benzyl bromide (1.44 mL, 2.08 g, 0.012 mol) in dry DMF (5 mL) was added. The mixture was stirred at room temperature for 10 h and then quenched with CH_3OH (1 mL) and H_2O (5 mL). The mixture was diluted with ether (25 mL). The organic layer was separated and the aqueous layer was extracted with ether (2×25 mL). The combined extracts were washed with water (25 mL), brine (25 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (4:1) to give the title compound **(65)** as a colourless thick oil (4.3 g, 90%) [NSI–Found $(M+NH_4)^+$: 1182.5751; $C_{72}H_{84}O_{12}SiN$, requires: 1182.5757]; $[\alpha]_D^{22}$ -11 (*c* 0.38, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.68 – 7.64 (4H, m), 7.42 – 7.16 (31H, m), 7.08 (2H, dd, *J* 1.6, 7.5 Hz), 6.85 (2H, d, *J* 8.6 Hz), 5.08 (1H, d, *J* 4.4 Hz), 5.04 (1H, br.s), 4.70 (1H, d, *J* 12.1 Hz), 4.66 (1H, d, *J* 12.1 Hz), 4.64 (2H, br.s), 4.56 (1H, d, *J* 11.7 Hz), 4.52 (2H, br.s), 4.48 (1H, d, *J* 11.7 Hz), 4.44 (2H, d, *J* 11.5 Hz), 4.41 (2H, d, *J* 11.5 Hz), 4.29 (1H, br.d, *J* 1.9 Hz), 4.20 (1H, br.d, *J* 5.9 Hz), 4.17 (2H, br.dd, *J* 5.0, 6.1 Hz), 4.11 (1H, br.q, *J*, 6.5 Hz), 4.05 (1H, br.dd, *J* 4.5, 6.1 Hz), 3.89 (1H, dd, *J* 5.2, 10.4 Hz), 3.84 – 3.78 (6H, including a singlet at 3.79 for O*CH*$_3$), 3.61 (3H, br.dd, *J* 4.8, 9.4 Hz), 3.53 (1H, br.dd, *J* 2.8, 9.5 Hz), 3.49 (1H, br.dd, *J* 4.7, 9.5 Hz), 1.05 (9H, s); δ_C (101 MHz, $CDCl_3$): 159.1, 138.7, 138.4, 138.2, 137.9, 137.7, 135.6, 135.5, 133.2, 133.1, 130.3, 129.8, 129.3, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.75, 127.7, 127.6, 127.55, 127.5, 127.4, 113.7, 106.0, 100.3, 85.4, 84.6, 84.1, 84.0, 82.0, 81.6, 77.1, 73.3, 72.8, 72.4, 72.3, 72.25, 72.2, 70.4, 69.9, 67.2, 66.2, 55.2, 26.8, 19.2; ν_{max}: 3065, 3031, 2930, 2860, 1612, 1513, 1248, 738, 699 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (66):



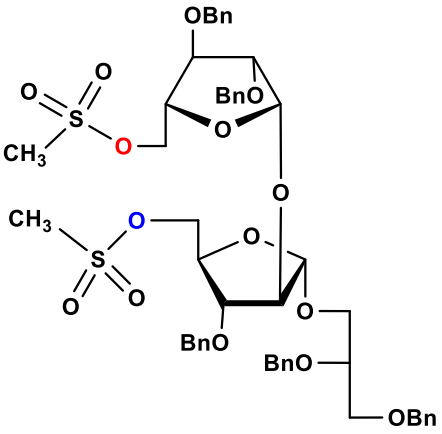
Tetrabutylammonium fluoride (3.5 mL, 0.0038 mol, 1.0 M) was added dropwise to a stirred solution of α-D-arabinofuranoside **(65)** (4.1 g, 0.0035 mol) in dry THF (25 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 6 h. When TLC showed no starting material was left, the mixture was diluted with ethyl acetate (15 mL) and water (5 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with sat. aq. NH_4Cl (25 mL), brine (25 mL), dried ($MgSO_4$) and the solvent was concentrated to give a residue which was purified by column chromatography on silica eluting with petrol /ethylacetate (5:2) to give the title compound **(66)** as a colourless thick oil (3.0 g, 93%) [NSI–Found $(M+NH_4)^+$: 944.4574; $C_{56}H_{66}O_{12}N$, requires: 944.4580]; $[\alpha]_D^{22}$ -7.1 (*c* 0.79, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.29 – 7.18 (25H, m), 7.16 (2H, d, *J* 8.7 Hz), 6.78 (2H, d, *J* 8.7 Hz), 4.97 (1H, d, *J* 4.5 Hz), 4.95 (1H, br.d, *J* 1.1 Hz), 4.64 (1H, d, *J* 11.7 Hz), 4.60 (2H, br.s), 4.53 (1H, d, *J* 11.5 Hz), 4.51 (1H, d, *J* 11.5 Hz), 4.47 – 4.43 (4H, m), 4.39 (1H, d, *J* 11.9 Hz), 4.38 (1H, d, *J* 11.9 Hz), 4.35 (1H, d, *J* 11.7 Hz), 4.21 (1H, br.dd, *J* 1.4, 3.5 Hz), 4.18 (1H, d, *J* 6.8 Hz), 4.09 (1H, br.p, *J* 4.1 Hz), 4.02 (1H, br.dd, *J* 3.5, 6.5 Hz), 3.95 (1H, dd, *J* 4.5, 7.3 Hz), 3.93 – 3.88 (1H, m), 3.79 (1H, dd, *J* 5.2, 10.4 Hz), 3.74 – 3.68 (4H, including a singlet at 3.71 for O*CH*$_3$), 3.59 – 3.50 (5H, m), 3.48 (1H, br.d, *J* 3.7 Hz), 3.43 (1H, dd, *J* 4.9, 10.8 Hz), 2.22 (1H, br.dd, *J* 5.1, 7.8 Hz); δ_C (101 MHz, $CDCl_3$): 159.2, 138.6, 138.3, 138.1, 137.9, 137.6, 130.0, 129.5, 128.5, 128.45, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 113.8, 106.1, 100.1, 86.3, 84.1, 83.3, 82.0, 81.0, 80.7, 77.0, 73.4, 73.0, 72.6, 72.4, 72.2, 70.3, 69.1, 67.4, 63.4, 55.2; ν_{max}: 3491, 3063, 3031, 2925, 2869, 1612, 1513, 1454, 1248, 738, 699 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-α-D-arabinofuranoside (67):



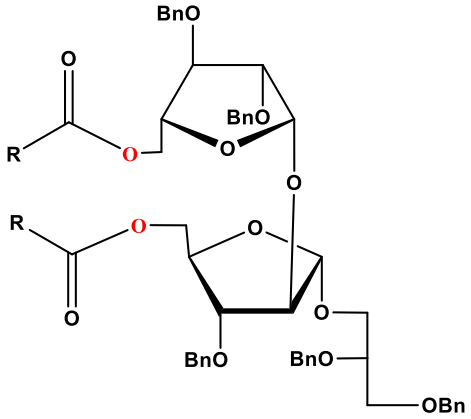
Cerium ammonium nitrate (CAN) (3.5 g, 0.0064 mol) was added to a stirred solution of α-D-arabinofuranoside **(66)** (2.0 g, 0.002 mol) in CH_3CN:H_2O (9:1,15 mL) at 0 ºC. The mixture was allowed to reach room temperature and stirred at ambient temperature for 1 h then TLC indicated the conversion had finished. The mixture was diluted with chloroform (25 mL), washed with aq. $NaHCO_3$ (15 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:2) to give the title compound **(67)** as a colourless thick oil (1.5 g, 89%) [NSI–Found $(M+Na)^+$: 829.4; $C_{48}H_{54}NaO_{11}$, requires: 829.4]; $[\alpha]_D^{21}$ -4.3 (*c* 0.83, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.64 – 6.67 (25H, m), 4.97 (1H, br.d, *J* 4.6 Hz), 4.95 (1H, br.s), 4.67 (1H, d, *J* 11.6 Hz), 4.64 (2H, br.s), 4.62 (1H, d, *J* 11.6 Hz), 4.55 (1H, d, *J* 11.6 Hz), 4.50 – 4.46 (5H, m), 4.2 – 4.17 (2H, broad double doublet *J* 5.6, 8.1 Hz), 4.16 (1H, br.dd, *J* 2.2, 5.6 Hz), 4.09 – 4.04 (1H, m), 4.00 (1H, dd, *J* 4.6, 7.3 Hz), 3.97 – 3.92 (1H, m), 3.80 (1H, dd, *J* 5.2, 10.2 Hz), 3.76 (1H, br.d, *J* 6.4 Hz), 3.73 (1H, br.dd, *J* 3.9, 8.3 Hz), 3.63 (1H, dd, *J* 2.9, 10.2 Hz), 3.60 – 3.48 (5H, m); δ_C (101 MHz, $CDCl_3$): 138.5, 138.2, 137.9, 137.8, 137.4, 128.5, 128.4, 128.35, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.65, 127.6, 127.55, 127.5, 106.3, 100.5, 86.4, 84.0, 83.0, 82.7, 81.9, 80.4, 76.9, 73.3, 72.6, 72.5, 72.3, 72.2, 70.1, 67.2, 63.2, 62.0; ν_{max}: 3463, 3063, 3031, 2922, 2872, 1454, 1107, 738, 698 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-methanesulfonyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-methanesulfonyl-α-D-arabinofuranoside (68):



Methanesulfonyl chloride (1.98 g, 1.36 mL, 0.0171 mol) and DMAP (0.10 g, 0.86 mmol) were added to a stirred solution of α-D-arabinofuranoside **(67)** (1.4 g, 0.0017 mol) in dry pyridine (10 mL) under nitrogen at room temperature. The mixture was stirred for 16 h then TLC showed no starting material was left. The mixture was quenched by the addition of H_2O (3 mL), the organic layer was separated by decanting and diluted with CH_2Cl_2 (10 mL). The mixture was washed with 1N aq. HCl (4×10 mL), sat. aq. $NaHCO_3$ (4×10 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (4:1) to afford the title compound **(68)** as a colourless thick oil (1.4 g, 85%) [NSI–Found $(M+Na)^+$: 985.3109; $C_{50}H_{58}NaO_{15}S_2$, requires: 985.3115]; $[\alpha]_D^{22}$ +2.8 (*c* 1.3, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.36 – 7.16 (25H, m), 5.01 (1H, br.d, *J* 4.4 Hz), 4.93 (1H, br.s), 4.70 (1H, d, *J* 11.7 Hz), 4.67 (1H, d, *J* 11.7 Hz), 4.63 (2H, br.s), 4.58 (1H, d, *J* 11.7 Hz), 4.51 (1H, d, *J* 11.7 Hz), 4.46 (4H, br.s), 4.32 (1H, br.q, *J* 4.6 Hz), 4.25 – 4.13 (5H, m), 4.13 – 4.07 (3H, m), 4.00 (1H, br.dd, *J* 4.4, 6.9 Hz), 3.80 (1H, dd, *J* 5.2, 10.3 Hz), 3.74 (1H, br.p, *J* 5.1 Hz), 3.60 – 3.50 (3H, including a broad double doublet *J* 4.6, 7.2 at 3.56), 2.85 (3H, s), 2.84 (3H, s); δ_C (101 MHz, $CDCl_3$): 138.5, 138.2, 137.7, 137.6, 137.2, 128.6, 128.5, 128.4, 128.35, 128.3, 128.1, 128.0, 127.95, 127.9, 127.8, 127.75, 127.7, 127.6, 127.55, 127.5, 106.4, 101.2, 85.9, 83.5, 81.1, 80.9, 78.4, 76.9, 73.3, 72.7, 72.6, 72.4, 72.3, 69.9, 69.8, 69.0, 67.3, 37.5, 37.4; ν_{max}: 3087, 3031, 2929, 2867, 1606, 1454, 1046,738, 697 cm^{-1}.

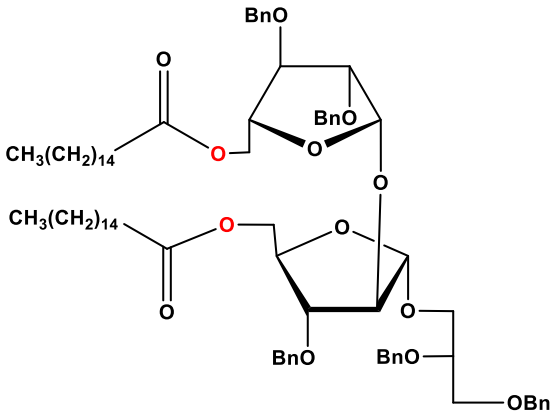
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-alkanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-alkanoate-α-D-arabinofuranoside (70a-d):



General procedure:

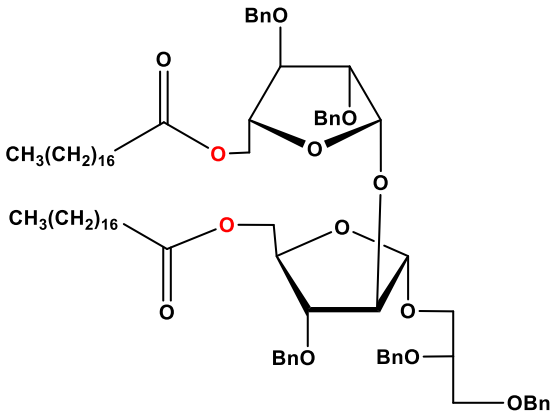
Cesium hydrogencarbonate was added to a stirred solution of α-D-arabinofuranoside **(68)** and fatty acids **(69a-d)** in dry THF:DMF (5:1, 1 mL) at room temperature under nitrogen . The mixture was stirred at 70 ºC for 4 days then TLC showed no starting material was left. The suspension was diluted with ethyl acetate (25 mL) and water (5 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$) and filtered. The filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to afford the title compounds **(70a-d)**.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-palmitate-β-D-arabinofuranosyl-(1→2) -3-*O*-benzyl-5-*O*-palmitate-α-D-arabinofuranoside (70a):



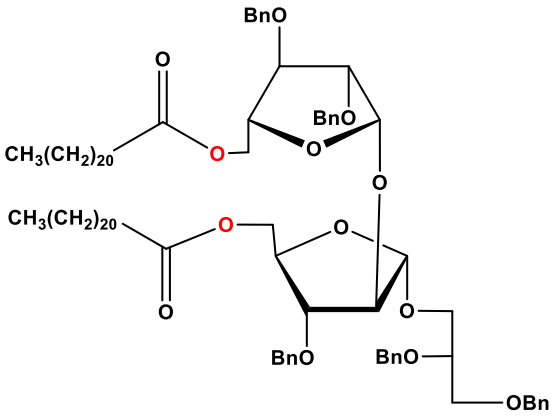
Cesium hydrogencarbonate (66 mg, 0.34 mmol), α-D-arabinofuranoside **(68)** (33.0 mg, 0.034 mmol) and plamitic acid **(69a)** (22 mg, 0.085 mmol); to give **(70a)** as a colourless thick oil (41 mg, 92%) [NSI–Found $(M+Na)^+$: 1305.8; $C_{80}H_{114}NaO_{13}$, requires: 1305.8]; $[\alpha]_D^{22}$ -7.6 (*c* 0.58, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.16 (25H, m), 5.01 (1H, d, *J* 4.2 Hz), 4.95 (1H, br.s), 4.66 (1H, d, *J* 11.6 Hz), 4.63 (3H, br.s), 4.56 (1H, d, *J* 11.6 Hz), 4.48 (1H, d, *J* 11.6 Hz), 4.47 (2H, br.s), 4.45 (1H, d, *J* 11.8 Hz), 4.41 (1H, d, *J* 11.8 Hz), 4.30 (1H, br.d, *J* 2.0 Hz), 4.25 – 4.15 (3H, m), 4.11 (1H, br.dd, *J* 3.1, 6.6 Hz), 4.09 – 4.01 (3H, m), 3.99 (1H, dd, *J* 4.3, 6.6 Hz), 3.90 (1H, br.dd, *J* 2.5, 5.8 Hz), 3.82 (1H, dd, *J* 5.2, 10.4Hz), 3.74 (1H, br.p, *J* 5.0 Hz), 3.61 – 3.50 (3H, including a broad double doublet *J* 4.7, 8.5 Hz at 3.56), 2.33 – 2.21 (2H, m), 2.18 (2H, dt, *J* 2.1, 7.4 Hz), 1.64 – 1.01 (52H, m), 0.84 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.5, 173.4, 138.6, 138.3, 137.8, 137.7, 137.4, 128.5, 128.4, 128.35, 128.3, 128.0, 127.8, 127.75, 127.7, 127.65, 127.6, 127.5, 106.1, 100.5, 85.6, 84.3, 83.8, 82.5, 80.1, 78.9, 73.4, 72.6, 72.5, 72.4, 72.3, 70.2, 67.3, 66.0, 63.7, 34.0, 31.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.35, 29.3, 29.25, 29.2, 29.1, 24.9, 24.8, 22.7, 14.1; ν_{max}: 3065, 3031, 2924, 2853, 1741, 1732, 1455, 1114, 737, 698 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-stearate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-stearate-α-D-arabinofuranoside (70b):



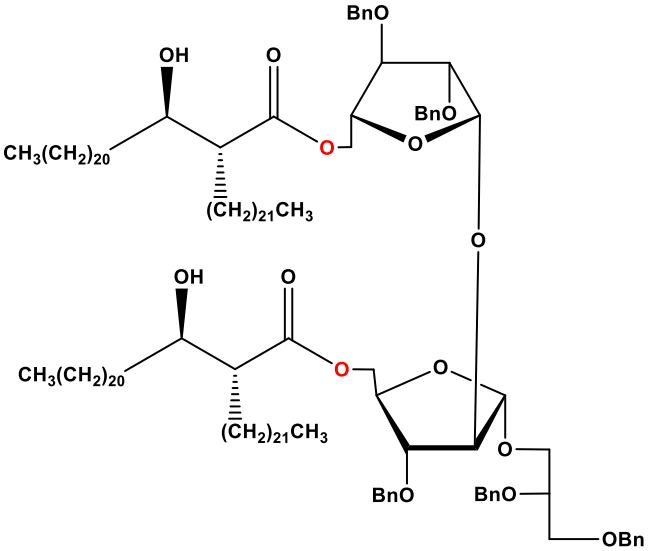
Cesium hydrogencarbonate (80 mg, 0.41 mmol), α-D-arabinofuranoside **(68)** (40 mg, 0.041 mmol) and stearic acid **(69b)** (30 mg, 0.10 mmol); to give **(70b)** as a colourless thick oil (49 mg, 89%) [NSI–Found $(M+NH_4)^+$: 1356.9227; $C_{84}H_{126}O_{13}N$, requires: 1356.9224]; $[\alpha]_D^{22}$ -4.6 (*c* 0.44, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.36 – 7.14 (25H, m), 5.01 (1H, d, *J* 4.2 Hz), 4.95 (1H, br.s), 4.66 (2H, d, *J* 11.5 Hz), 4.63 (2H, br.s), 4.56 (1H, d, *J* 11.5 Hz), 4.52 – 4.43 (4H, m), 4.41 (1H, d, *J* 11.6 Hz), 4.31 (1H, br.d, *J* 1.8 Hz), 4.25 – 4.16 (3H, m), 4.14 – 4.02 (4H, m), 3.99 (1H, dd, *J* 4.3, 6.6 Hz), 3.90 (1H, br.dd, *J* 2.4, 5.7 Hz), 3.82 (1H, dd, *J* 5.2, 10.4 Hz), 3.74 (1H, br.p, *J* 4.9 Hz), 3.62 – 3.52 (3H, including a broad double doublet *J* 4.8, 7.6 Hz at 3.56), 2.23 (2H, t, *J* 7.6 Hz), 2.18 (2H, dt, *J* 1.9, 7.4 Hz), 1.31 – 1.14 (60H, m), 0.85 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.5, 173.3, 138.6, 138.3, 137.8, 137.7, 137.4, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.75, 127.7, 127.65, 127.6, 127.5, 106.1, 100.4, 85.6, 84.3, 83.7, 82.5, 80.1, 78.8, 77.0, 73.3, 72.6, 72.5, 72.3, 72.2, 70.2, 67.3, 65.9, 63.7, 34.0, 31.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.35, 29.3, 29.25, 29.2, 29.1, 24.9, 24.8, 22.7, 14.1; ν_{max}: 3065, 3032, 2925, 2854, 1740, 1731, 1454, 1116, 734, 697 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-behenate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-behenate-α-D-arabinofuranoside (70c):



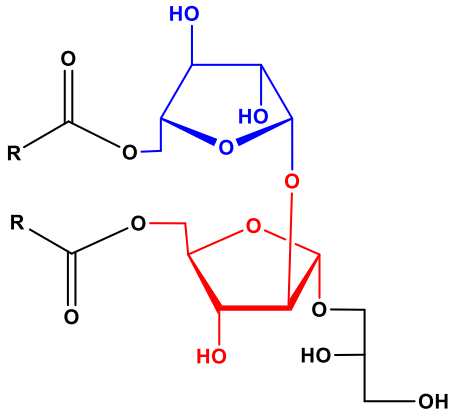
Cesium hydrogencarbonate (161 mg, 0.830 mmol), α-D-arabinofuranoside **(68)** (80 mg, 0.083 mmol) and behenic acid **(69c)** (71 mg, 0.20 mmol); to give **(70c)** as a colourless thick oil (0.11 g, 87%) [NSI–Found $(M+Na)^+$: 1474.0; $C_{92}H_{138}NaO_{13}$, requires: 1474.0]; $[\alpha]_D^{22}$ -4.2 (*c* 1.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.30 – 7.15 (25H, m), 4.97 (1H, br.d, *J* 4.2 Hz), 4.91 (1H, br.s), 4.62 (1H, d, *J* 11.5 Hz), 4.61 (1H, d, *J* 11.5 Hz), 4.59 (2H, br.s), 4.52 (1H, d, *J* 11.6 Hz), 4.44 (3H, br.s), 4.41 (1H, d, *J* 11.6 Hz), 4.37 (1H, d, *J* 11.6 Hz), 4.27 (1H, br.d, *J* 2.0 Hz), 4.20 (1H, *J* 3.5 Hz), 4.16 (1H, *J* 4.6 Hz), 4.13 (1H, br.dd, *J* 3.6, 5.8 Hz), 4.10 – 4.04 (2H, including a broad double doublet *J* 4.1, 8.2 Hz at 4.06), 4.03 (1H, dd, *J* 3.7, 9.7 Hz), 3.99 (1H, d, *J* 5.2 Hz), 3.95 (1H, dd, *J* 4.3, 6.7 Hz), 3.86 (1H, dd, *J* 2.4, 5.9 Hz), 3.78 (1H, dd, *J* 5.2, 10.4 Hz), 3.70 (1H, br.p *J* 4.8 Hz), 3.57 – 3.48 (3H, including a broad double doublet *J* 4.7, 8.3 Hz at 3.52), 2.19 (2H, t, *J* 7.6 Hz), 2.14 (2H, dt, *J* 2.7, 7.5 Hz), 1.53 – 1.03 (76H, m), 0.80 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.5, 173.4, 138.6, 138.3, 137.8, 137.7, 137.4, 128.5, 128.4, 128.35, 128.3, 128.0, 127.8, 127.75, 127.7, 127.6, 127.55, 127.5, 106.1, 100.4, 85.6, 84.3, 83.8, 82.5, 80.1, 78.9, 77.2, 73.4, 72.5, 72.4, 72.35, 72.3, 70.2, 67.3, 66.0, 63.7, 34.0, 31.9, 29.7, 29.65, 29.6, 29.55, 29.5, 29.4, 29.3, 29.25, 29.2, 29.1, 24.9, 24.8, 22.7, 14.1; ν_{max}: 3063, 3031, 2917, 2850, 1740, 1732, 1467, 1110, 735, 697 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxydocosyl) hexacosanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxydocosyl) hexacosanoate-α-D-arabinofuranoside (70d):



Cesium hydrogencarbonate (134 mg, 0.691 mmol), α-D-arabinofuranoside **(68)** (33 mg, 0.034 mmol) and (*R*)-2-((*R*)-1-hydroxydocosyl) hexacosanoic acidc **(69d)** (50 mg, 0.069 mmol); to give **(70d)** as a colourless thick oil (60 mg, 55%) [NSI–Found $(M+Na)^+$: 2178.7; $C_{140}H_{234}NaO_{15}$, requires: 2178.7]; $[\alpha]_D^{24}$ -3 (*c* 0.9, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.43 – 7.11 (25H, m), 5.01 (1H, br.d, *J* 4.2 Hz), 4.97 (1H, br.s), 4.71 (2H, d, *J* 11.5 Hz), 4.66 (2H, m), 4.61 (1H, d, *J* 11.5 Hz), 4.59 – 4.47 (4H, m), 4.45 (1H, d, *J* 11.6 Hz), 4.32 (1H, br.d, *J* 1.8 Hz), 4.30 – 4.21 (4H, m), 4.17 – 4.08 (3H, including a broad pentet *J* 5.7 Hz at 4.12), 4.04 – 3.99 (1H, m), 3.98 – 3.95 (1H, m), 3.84 (1H, dd, *J* 5.3, 10.2 Hz), 3.79 (1H, br.p, *J* 5.1 Hz), 3.72 – 3.52 (5H, including a broad double doublet *J* 4.5, 10.8 Hz at 3.59), 2.41 (2H, ddt, *J* 4.7, 9.4, 12.7 Hz), 1.80 – 1.04 (174H, m), 0.89 (12H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 138.5, 138.2, 137.7, 137.3, 128.5, 128.4, 128.35, 128.3, 128.25, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 105.9, 100.4, 85.5, 84.4, 83.7, 82.8, 80.3, 78.9, 73.4, 72.6, 72.5, 72.4, 72.2, 72.1, 70.2, 67.2, 66.1, 63.7, 51.9, 51.6, 35.4, 35.3, 31.9, 31.6, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 27.5, 27.4, 25.7, 25.6, 22.7, 22.6, 14.1; ν_{max}: 3491, 3065, 3031, 2918, 2850, 1732, 1725, 1468, 1115, 735, 697 cm^{-1}.

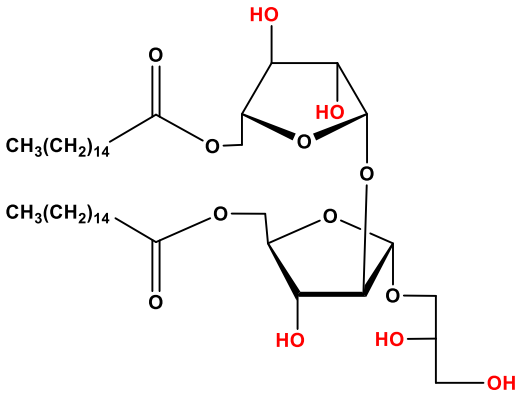
L-Glycerol-(1'→1)-5-*O*-alkanoate-β-D-arabinofuranosyl-(1→2)-5-*O*-alkanoate-α-D-arabinofuranoside (71a-d):



General procedure:

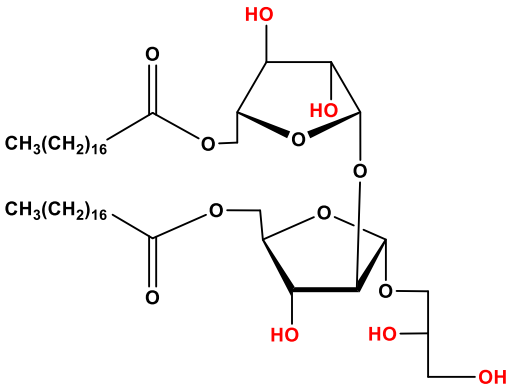
Palladium hydroxide on activated charcoal was added to a stirred solution of α-D-arabinofuranoside **(70a-d)** in CH_2Cl_2:MeOH (1:1, 1 mL) at room temperature under hydrogen. The mixture was stirred for 36 h then TLC showed no starting material was left. The mixture was filtered off through celite and the solvent was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (10:1) affording L-glycerol-(1'→1)-5-*O*-alkanoyl-β-D-arabinofuranosyl-(1→2)-5-*O*-alkanoyl-α-D-arabinofuranoside **(71a-d)**.

L-Glycerol-(1'→1)-5-*O*-palmitate-β-D-arabinofuranosyl-(1→2)-5-*O*-palmitate-α-D-arabinofuranoside (71a):



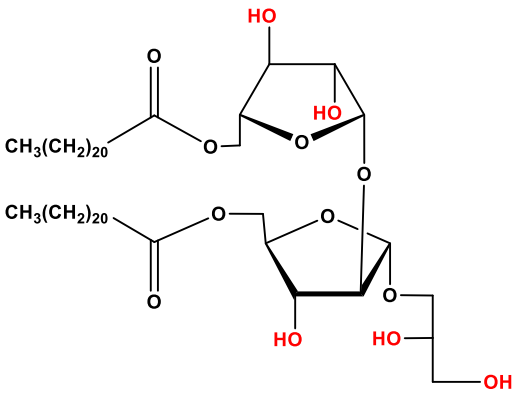
($Pd(OH)_2$-C/20%, 25 mg, 0.75 fold by weight) and α-D-arabinofuranoside **(70a)** (33 mg, 0.025 mmol); to give **(71a)** as a colourless thick oil (18 mg, 82%) [NSI–Found $(M+Na)^+$: 855.5804; $C_{45}H_{84}NaO_{13}$, requires: 855.5810]; $[\alpha]_D^{21}$ +14 (*c* 0.30, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 5.06 (1H, br.d, *J* 4.5 Hz), 5.02 (1H, br.d, *J* 1.9 Hz), 4.45 (1H, dd, *J* 7.2, 11.9 Hz), 4.38 – 3.30 (1H, m), 4.28 (1H, br.dd, *J* 4.1, 6.8 Hz), 4.26 – 4.21 (1H, m), 4.20 – 4.15 (2H, including a broad double doublet, *J* 1.9, 9.6 Hz at 4.18), 4.15 – 4.10 (2H, m), 4.07 (2H, including a broad double doublet, *J* 5.4, 8.3 Hz at 4.07), 4.04 (1H, br.d, *J* 7.0 Hz), 4.00 (1H, dd, *J* 4.8, 10.3 Hz), 3.91 – 3.85 (2H, m), 3.77 (2H, dd, *J* 6.0, 10.7 Hz), 3.71 (1H, br.d, *J* 2.9 Hz), 3.67 (2H, br.t, *J* 6.0 Hz), 2.36 (4H, t, *J* 7.6 Hz), 1.46 – 1.08 (53H, m), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$+few drops CD_3OD): 173.5, 173.4, 138.6, 138.3, 137.8, 137.7, 137.4, 128.5, 128.4, 128.35, 128.3, 128.0, 127.8, 127.75, 127.7, 127.65, 127.6, 127.5, 106.1, 100.5, 85.6, 84.3, 83.8, 82.5, 80.1, 78.9, 77.0, 73.4, 72.6, 72.5, 72.4, 72.3, 70.2, 67.3, 66.0, 63.7, 34.0, 31.9, 29.7, 29.65, 29.6, 29.5, 29.45, 29.4, 29.3, 29.25, 29.2, 29.1, 24.9, 24.8, 22.7, 14.1; ν_{max}: 3436, 2918, 2850, 1738, 1643, 1469, 1219, 1116, 1041, 927 cm^{-1}.

L-Glycerol-(1'→1)-5-*O*-stearate-β-D-arabinofuranosyl-(1→2)-5-*O*-stearate-α-D-arabinofuranoside (71b):



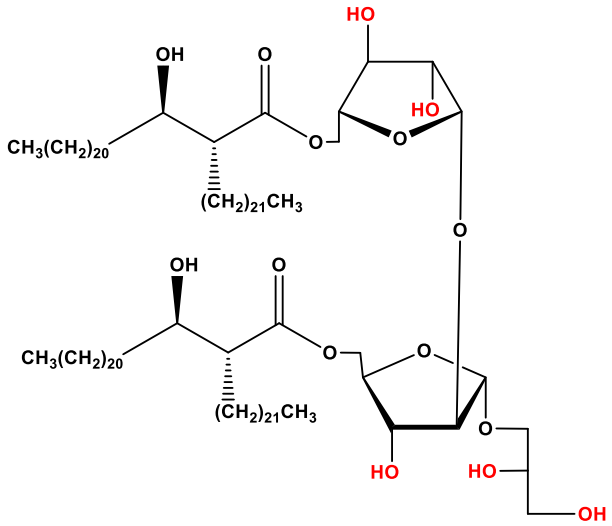
($Pd(OH)_2$-C/20%, 34 mg, 0.75 fold by weight) and α-D-arabinofuranoside **(70b)** (45 mg, 0.033 mmol); to give **(71b)** as a colourless thick oil (24 mg, 81%) [NSI–Found $(M+Na)^+$: 911.6430; $C_{49}H_{92}NaO_{13}$, requires: 911.6436]; $[\alpha]_D^{25}$ -3.4 (*c* 0.71, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 5.01 (1H, br.d, *J* 4.3 Hz), 5.00 (1H, br.s), 4.33 – 4.29 (1H, m), 4.27 (1H, br.d, *J* 5.7 Hz), 4.20 – 4.15 (2H, m), 4.13 (1H, br.d, *J* 7.0 Hz), 4.04 (1H, dd, *J* 5.9, 10.6 Hz), 4.00 (1H, br.dd, *J* 2.6, 6.6 Hz), 3.98 – 3.88 (3H, including a broad double doublet *J* 4.9, 9.0 Hz at 3.96), 3.86 – 3.78 (1H, m), 3.74 (1H, dd, *J* 5.8, 10.6 Hz), 3.63 (1H, br.dd, *J* 3.9, 11.8 Hz), 3.6 – 3.53 (2H, including a broad double doublet *J* 3.0, 10.8 Hz at 3.58), 2.33 (4H, t, *J* 7.5 Hz), 1.36 – 1.17 (65H, m), 0.86 (6H, t, *J* 6.3 Hz); δ_C (101 MHz, $CDCl_3$): 174.3, 174.0, 138.6, 138.3, 137.8, 137.7, 137.4, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.65, 127.6, 127.55, 127.5, 106.1, 100.4, 85.6, 84.3, 83.8, 82.5, 80.1, 78.8, 77.0, 73.4, 72.5, 72.4, 72.3, 72.2, 70.2, 67.3, 65.9, 63.7, 34.0, 31.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.35, 29.3, 29.2, 29.15, 29.1, 24.9, 24.8, 22.7, 14.1; ν_{max}: 3430, 2917, 2849, 1737, 1643, 1467, 1214, 1172, 1041, 719 cm^{-1}.

L-Glycerol-(1'→1)-5-*O*-behenate-β-D-arabinofuranosyl-(1→2)-5-*O*-behenate-α-D-arabinofuranoside (71c):



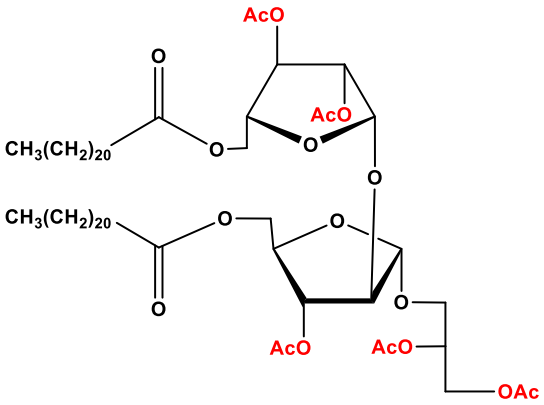
($Pd(OH)_2$-C/20%, 75 mg, 0.75 fold by weight); (100 mg, 0.0688 mmol) of α-D-arabinofuranoside **(70c);** to give **(71c)** as a colourless thick oil (60 mg, 87%) [NSI–Found $(M+Na)^+$: 1023.7682; $C_{57}H_{108}NaO_{13}$, requires: 1023.7688]; $[\alpha]_D^{22}$ -2.3 (*c* 0.44, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 4.97 (1H, br.d, *J* 4.7 Hz), 4.96 (1H, br.s), 4.30 – 4.21 (2H, including a broad double doublet *J* 8.4, 11.2 Hz at 4.25), 4.16 (1H, dd, *J* 3.2, 11.9 Hz), 4.14 – 4.06 (2H, m), 4.03 – 3.97 (2H, m), 3.96 – 3.88 (3H, m), 3.78 (1H, br.p, *J* 5.1 Hz), 3.71 (1H, dd, *J* 6.0, 10.4 Hz), 3.63 – 3.59 (1H, m), 3.57 (1H, dd, *J* 4.3, 11.5 Hz), 3.53 (1H, dd, *J* 4.8, 11.2 Hz), 2.31 (4H, t, *J* 7.6 Hz), 1.34 – 1.14 (81H, m), 0.83 (6H, t, *J* 6.6 Hz); δ_C (126 MHz, $CDCl_3$+few drops CD_3OD): 174.2, 173.9, 105.9, 101.9, 88.5, 80.3, 80.1, 75.8, 75.5, 70.4, 69.5, 65.5, 63.6, 63.2, 34.0, 33.9, 31.8, 29.5, 29.45, 29.4, 29.3, 29.2, 29.15, 29.1, 29.0, 24.7, 22.5, 13.8; ν_{max}: 3419, 2956, 2917, 1738, 1732, 1464, 1215, 1171, 1048, 881, 720 cm^{-1}.

L-Glycerol-(1'→1)-5-*O*-(*R*)-2-((*R*)-1-hydroxydocosyl)hexacosanoate-β-D-arabinofuranosyl-(1→2)-5-*O*-(*R*)-2-((*R*)-1-hydroxydocosyl)hexacosanoate-α-D-arabinofuranoside (71d):



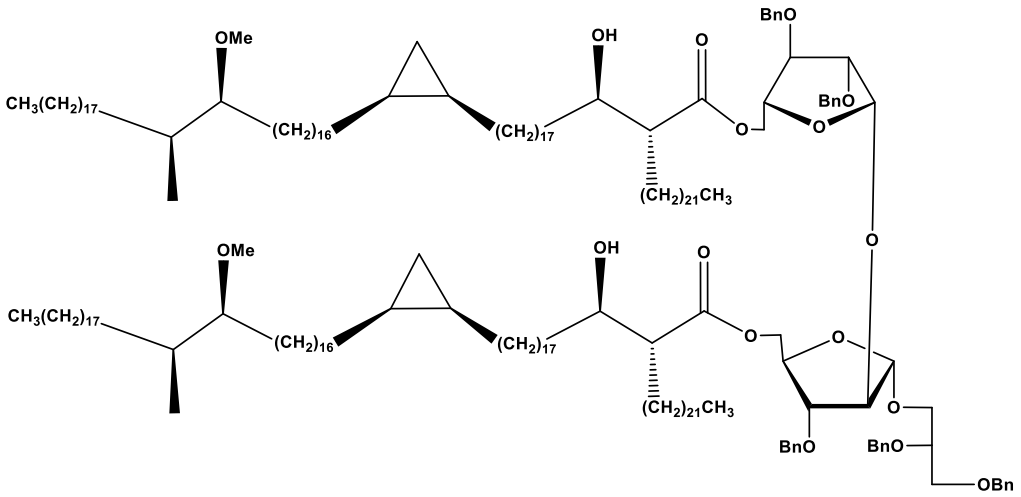
(Pd(OH)$_2$-C/20%, 23 mg, 0.75 fold by weight) and α-D-arabinofuranoside **(70d)** (30 mg, 0.013 mmol); to give **(71d)** as a colourless thick oil (17 mg, 74%) [NSI–Found $(M+Na)^+$: 1728.5; $C_{105}H_{204}NaO_{15}$, requires: 1728.5]; $[\alpha]_D^{22}$ +8 (*c* 0.3, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 4.98 (1H, br.d, *J* 5.7 Hz), 4.97 (1H, br.s), 4.40 (1H, dd, *J* 4.7, 11.6 Hz), 4.34 (1H, dd, *J* 4.8, 11.4 Hz), 4.22 (1H, dd, *J* 5.6, 11.6 Hz), 4.20 (1H, dd, *J* 6.4, 12.0 Hz), 4.13 (1H, dd, *J* 6.1, 10.7 Hz), 4.10 (1H, br.q, *J* 6.9 Hz), 4.05 – 3.98 (4H, including a broad doublet *J* 11.2 Hz at 4.02), 3.85 – 3.76 (1H, m), 3.71 (1H, dd, *J* 6.4, 10.6 Hz), 3.68 – 3.62 (2H, br.m), 3.61 (1H, d, *J* 4.1 Hz), 3.57 (1H, dd, *J* 4.2, 9.6 Hz), 3.54 (1H, br.dd, *J* 3.2, 10.3 Hz), 2.46 – 2.37 (2H, m), 1.64 – 1.01 (179H, m), 0.86 (12H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$+few drops CD_3OD): 175.1, 175.0, 105.8, 101.5, 87.6, 80.7, 79.5, 77.2, 76.4, 76.1, 72.8, 72.5, 70.4, 69.5, 65.4, 63.7, 63.3, 63.2, 53.3, 52.6, 34.8, 34.7, 31.9, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 29.1, 27.4, 27.3, 25.4, 25.2, 22.6, 14.0; ν_{max}: 3416, 2927, 2854, 1728, 1719, 1466, 1215, 1121, 1044, 759, 669 cm^{-1}.

2',3'-Di-*O*-acetyl-L-glycerol-(1'→1)-2,3-di-*O*-acetyl-5-*O*-behenate-β-D-arabinofuranosyl-(1→2)-3-*O*-acetyl-5-*O*-behenate-α-D-arabinofuranoside (72):



Acetic anhydride (0.02 g, 0.20 mmol, 0.02 mL) was added to a stirred solution of α-D-arabinofuranoside **(71c)** (20 mg, 0.019 mmol) in dry pyridine (2 mL) at room temperature and the mixture was stirred at room temperature for 18 hours under nitrogen. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (2:1) to afford the title compound **(72)** (20 mg, 83%) [NSI–Found $(M+Na)^+$: 1233.8210; $C_{67}H_{118}NaO_{18}$, requires: 1233.8216]; $[\alpha]_D^{23}$ -13 (*c* 0.62, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 5.40 (1H, br.d, *J* 4.7 Hz), 5.34 (1H, dd, *J* 5.3, 6.3 Hz), 5.21 (1H, br.p, *J* 4.8 Hz), 4.95 (2H, br.dd, *J* 4.7, 6.6 Hz), 4.91 (1H, br.s), 4.37 (1H, dd, *J* 4.6, 11.6 Hz), 4.29 (1H, dd, *J* 4.4, 7.6 Hz), 4.27 – 4.10 (7H, m), 3.82 (1H, dd, *J* 5.2, 11.0 Hz), 3.60 (1H, dd, *J* 4.5, 10.9 Hz), 2.42 – 2.29 (4H, m), 2.11 – 2.09 (12H, m), 2.08 (3H, s), 1.70 – 0.99 (76H, m), 0.89 (6H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 173.4, 173.3, 170.6, 170.4, 170.2, 170.1, 169.9, 105.0, 99.4, 83.9, 80.6, 79.1, 77.5, 75.6, 69.8, 65.3, 65.2, 63.6, 62.5, 34.1, 34.0, 31.9, 31.6, 29.7, 29.65, 29.5, 29.35, 29.3, 29.2, 24.9, 24.8, 22.7, 22.6, 21.0, 20.8, 20.7, 20.6, 20.4, 14.1; ν_{max}: 2918, 2850, 1742, 1736, 1466, 1224, 1167, 1045, 755, 721 cm^{-1}.

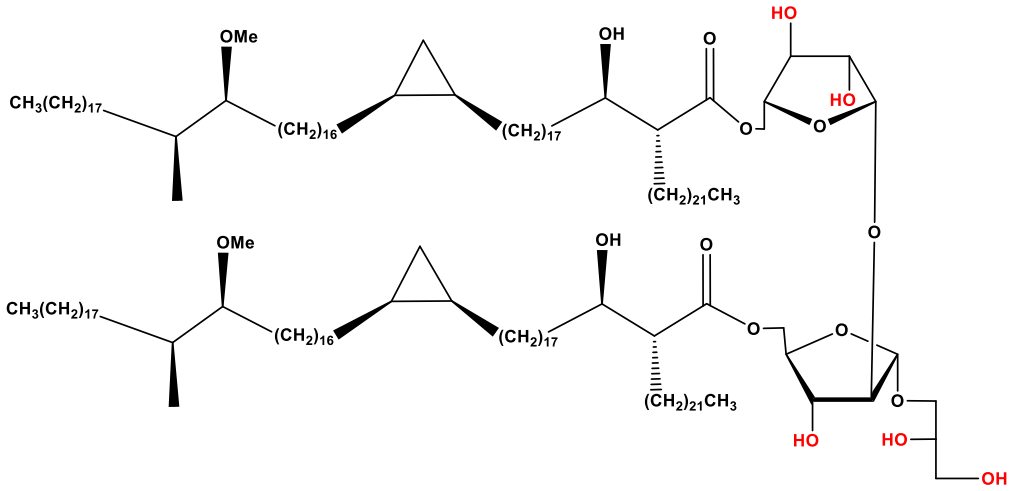
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl) cyclopropyl) octadecyl) tetracosanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate-α-D-arabinofuranoside (74):



Cesium hydrogencarbonate (79 mg, 0.40 mmol) was added to a stirred solution of α-D-arabinofuranoside **(68)** (20 mg, 0.020 mmol) and (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl) octadecyl)tetracosanoic acid **(73)** (50 mg, 0.040 mmol) in dry THF:DMF (1mL, 5:1) at room temperature under nitrogen. The mixture was stirred at 70 ºC for 4 days then TLC showed no starting material was left. The suspension was diluted with ethyl acetate (25 mL) and water (5 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to give **(74)** as a colourless thick oil (70 mg, 54%) [NSI–Found $(M+Na)^+$: 3243.9; $C_{214}H_{378}NaO_{17}$, requires: 3243.9]; $[\alpha]_D^{24}$ -4.4 (*c* 0.73, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.35 – 7.16 (25H, m), 4.97 (1H, d, *J* 4.3 Hz), 4.93 (1H, br.s), 4.67 (2H, d, *J* 11.7 Hz), 4.63 (2H, m), 4.58 (1H, d, *J* 11.7 Hz), 4.52 – 4.44 (4H, m), 4.41 (1H, d, *J* 11.6 Hz), 4.28 (1H, br.d, *J* 1.6 Hz), 4.26 – 4.16 (4H, m), 4.12 – 4.04 (2H, including a broad pentet *J* 6.5 Hz at 4.08), 4.00 – 3.95 (1H, m), 3.93 (1H, br.d, *J* 2.9 Hz), 3.80 (1H, dd, *J* 5.3, 10.3 Hz), 3.78 – 3.74 – 3.69 (5H, including a broad triplet *J* 6.6 Hz at 3.72), 3.58 – 3.53 (4H, including a broad double doublet *J* 4.5, 10.7 Hz at 3.55), 3.31 (6H, s), 2.92 (2H, dt, *J* 4.1, 7.1 Hz), 2.42 – 2.32 (2H, m), 1.56 – 0.97 (286H, m), 0.85 (12H,

t, *J* 6.8 Hz), 0.82 (6H, d, *J* 6.9 Hz), 0.65 – 0.58 (4H, m), 0.56 – 0.49 (2H, dt, *J* 4.0, 8.4 Hz), -0.36 (2H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.04, 175.03, 138.5, 138.3, 137.8, 137.3, 128.5, 128.45, 128.4, 128.35, 128.3, 128.2, 128.1, 127.9, 127.8, 127.75, 127.7, 127.6, 127.5, 105.9, 100.4, 85.5, 85.4, 84.4, 83.7, 82.8, 80.3, 78.9, 77.1, 73.4, 72.6, 72.5, 72.45, 72.4, 72.2, 72.1, 70.2, 68.0, 67.2, 66.1, 63.7, 57.7, 51.8, 51.6, 35.3, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.7, 29.65, 29.6, 29.5, 29.45, 29.4, 29.3, 28.7, 27.6, 27.4, 26.2, 25.7, 25.6, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3522, 3063, 3031, 2922, 2852, 1744, 1737, 1464, 1101,733, 698 cm^{-1}.

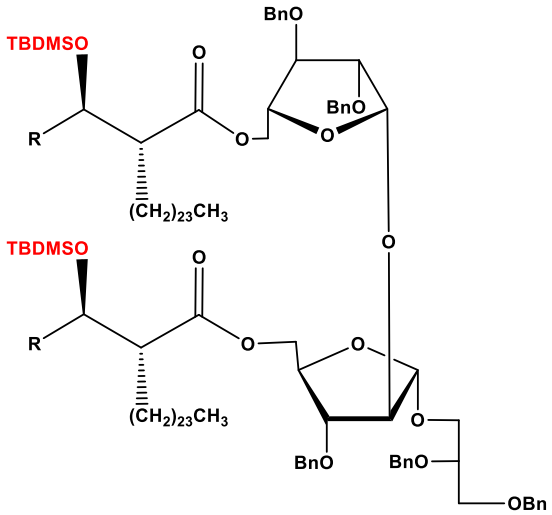
L-Glycerol-(1'→1)-5-*O*-(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate-β-D-arabinofuranosyl-(1→2)-5-*O*-(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl) cyclopropyl)octadecyl)tetracosanoate-α-D-arabinofuranoside (75):



Palladium hydroxide on activated charcoal ($Pd(OH)_2$-C/20%, 33 mg, 0.75 fold by weight) was added to a stirred solution of compound **(74)** (43 mg, 0.013 mmol) in CH_2Cl_2:MeOH (1:1, 1 mL) at room temperature under hydrogen. The mixture was stirred for 36 h then TLC showed no starting material was left. The mixture was filtered off through celite and the solvent was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (10:1) to give **(75)** as a colourless thick oil (27 mg, 73%) [NSI–Found $(M+Na)^+$: 2793.6; $C_{179}H_{348}NaO_{17}$, requires: 2793.6]; $[\alpha]_D^{22}$ +13 (*c* 0.36, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 4.98 (1H, br.d, *J* 4.7 Hz), 4.82 (1H, br.s), 4.40 (1H, dd, *J* 4.4, 12.2 Hz), 4.37 – 4.31 (2H, including a broad double doublet *J* 4.7, 11.6 Hz at 4.34), 4.22 (1H, dd, *J* 5.6, 11.5 Hz), 4.15 (1H, br.dd, *J* 5.6, 11.7 Hz), 4.10 (1H, br.dd, *J* 4.1, 9.0 Hz), 4.07 – 3.94 (6H, br.m), 3.89 (1H, br.dd, *J* 2.6, 4.7 Hz), 3.85 –

3.77 (1H, m), 3.72 (1H, dd, *J* 5.5, 11.5 Hz), 3.69 – 3.51 (6H, m), 3.38 (1H, dd, *J* 4.2, 8.5 Hz), 3.32 (6H, s), 2.99 – 2.90 (2H, m), 2.47 – 2.37 (2H, m), 1.66 – 0.96 (288H, m), 0.86 (12H, t, *J* 6.9 Hz), 0.83 (6H, d, *J* 6.9 Hz), 0.66 – 0.58 (4H, m), 0.53 (2H, dt, *J* 4.1, 8.6 Hz), -0.36 (2H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$+few drops CD_3OD): 175.1, 175.0, 105.8, 101.5, 87.7, 85.5, 80.6, 79.5, 77.2, 76.4, 76.0, 72.6, 72.4, 70.3, 69.3, 65.4, 63.3, 63.1, 57.6, 53.2, 52.6, 35.2, 32.2, 31.8, 30.4, 30.1, 29.8, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 29.0, 28.6, 27.4, 27.35, 27.3, 26.0, 25.3, 25.2, 22.6, 15.6, 14.7, 13.9, 10.8; ν_{max}: 3397, 2920, 2851, 1730, 1467, 1171, 1099, 1046, 721 cm^{-1}.

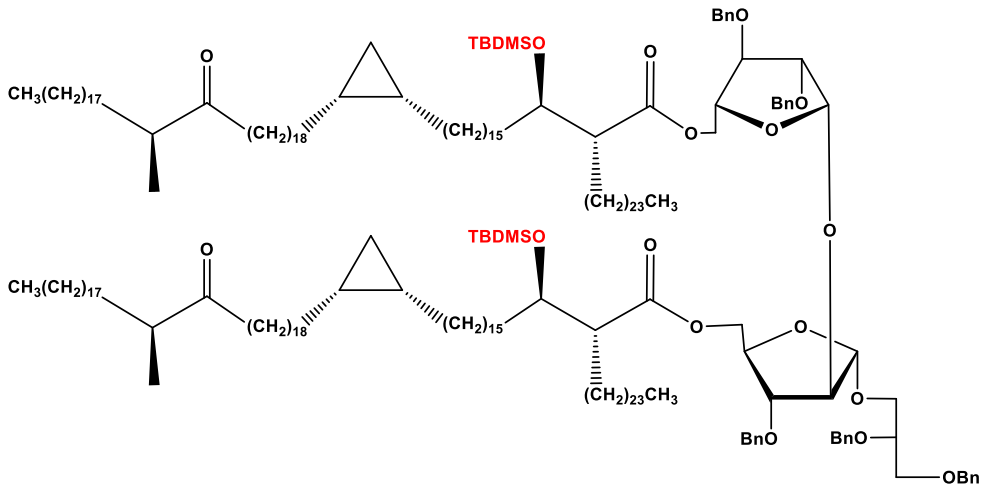
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-mycolate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-mycolate-α-D-arabinofuranoside (77f-h):



General procedure:

A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) in dry CH_2Cl_2 (1 mL) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-α-D-arabinofuranoside **(67)**; molecular sieves 4 Å, DMAP and mycolic acids **(76f-h)** in dry CH_2Cl_2 (1 mL) at room temperature under nitrogen. The mixture was stirred for 5 days. When TLC showed no starting material was left. The precipitate was filtered off and washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethylacetate (5:1) to afford the compounds **(77f-h).**

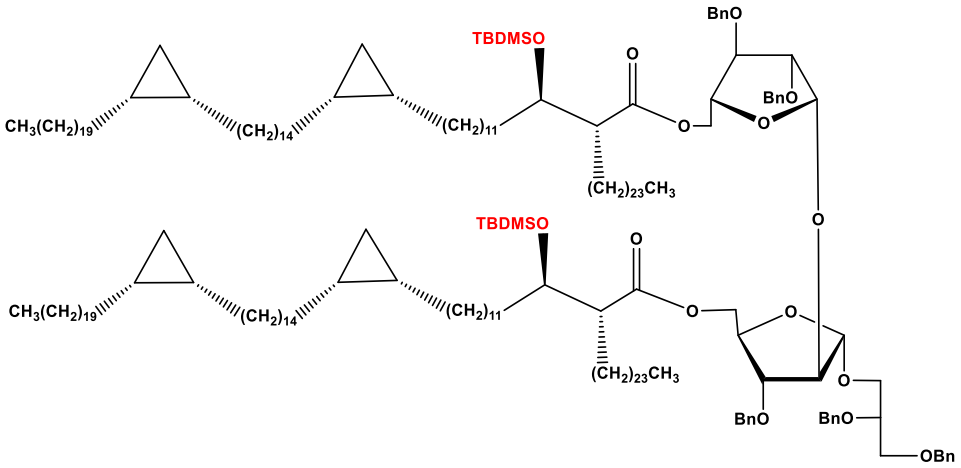
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl) oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate-α-D-arabinofuranoside (77f):



1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (77 mg; 0.40 mmol), molecular sieves 4 Å (50 mg), α-D-arabinofuranoside **(67)** (33 mg, 0.040 mmol), DMAP (49 mg; 0.40 mmol) and (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl) hexacosanoic acid **(76f)** (108 mg, 0.0790 mmol); to give **(77f)** as a colourless thick oil (0.13 g, 97%) [NSI–Found $(M+Na)^+$: 3496.1; $C_{228}H_{406}NaO_{17}Si_2$, requires: 3496.1]; $[\alpha]_D^{21}$ +4.2 (*c* 0.38, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.18 (25H, m), 5.03 (1H, br.d, *J* 4.2 Hz), 4.97 (1H, br.s), 4.72 (1H, d, *J* 11.6 Hz), 4.68 (3H, d, *J* 11.6 Hz), 4.62 (1H, d, *J* 11.7 Hz), 4.56 – 4.48 (4H, m), 4.43 (1H, d, *J* 11.7 Hz), 4.37 (1H, br.d, *J* 2.0 Hz), 4.29 – 4.11 (6H, m), 4.06 (1H, t, *J* 6.0 Hz), 4.00 (1H, br.dd, *J* 4.3, 6.5 Hz), 3.96 – 3.81 (4H, m), 3.78 (1H, br.p, *J* 4.7 Hz), 3.67 – 3.54 (3H, including a broad double doublet *J* 4.4, 10.4 Hz at 3.60), 2.53 (4H, including a sextet *J* 6.8 Hz at 2.53), 2.42 (4H, dt, *J* 1.0, 7.2 Hz), 1.61 – 1.12 (288H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.71 – 0.62 (4H, m), 0.57 (2H, dt, *J* 4.1, 8.4 Hz), 0.04 (3H, s), 0.02 (3H, s), 0.01 (3H, s), -0.01 (3H, s), -0.32 (2H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 174.3, 174.1, 138.6, 138.3, 137.9, 137.7, 137.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.65, 127.6, 127.5, 127.4, 105.9, 100.2, 84.9, 84.6, 83.6, 83.3, 80.1, 79.1, 77.1, 73.4, 73.2, 73.1, 72.5, 72.4, 72.2, 70.3, 67.2, 66.3, 64.3, 51.5, 51.4, 46.3, 41.1, 33.7, 33.0, 31.9, 30.2, 29.9, 29.85, 29.8, 29.75, 29.7, 29.65, 29.6,

29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 28.7, 27.8, 27.7, 27.4, 27.3, 25.9, 25.8, 24.0, 23.9, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9, -4.4, -4.5, -4.7, -4.8; ν_{max}: 3088, 3063, 2922, 2852, 1739, 1713, 1465, 1115, 758, 698 cm^{-1}.

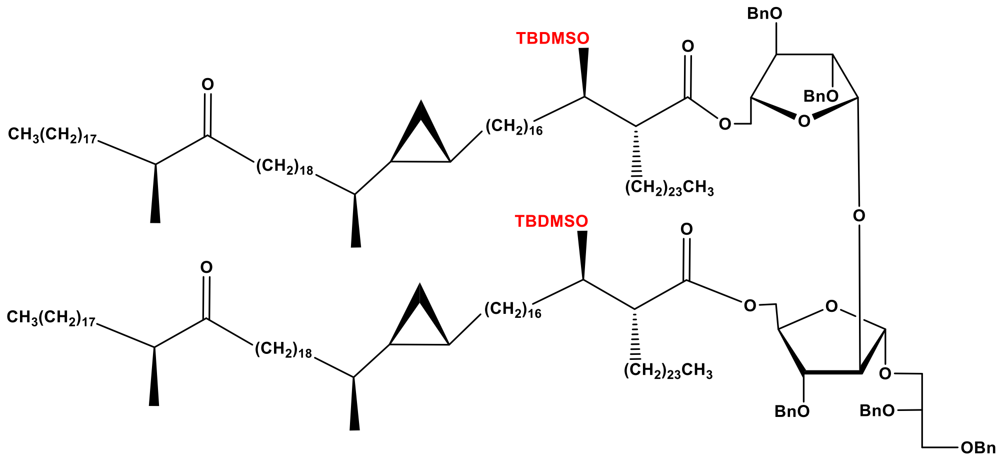
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl) oxy)-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl) cyclopropyl)dodecyl) hexacosanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl) oxy)-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl) tetradecyl) cyclopropyl)dodecyl) hexacosanoate-α-D-arabinofuranoside (77g):



1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (83 mg; 0.43 mmol), molecular sieves 4 Å (50 mg), α-D-arabinofuranoside **(67)** (35 mg, 0.043 mmol), DMAP (52 mg; 0.42 mmol) and (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl) tetradecyl) cyclopropyl)dodecyl)hexacosanoic acid **(76g)** (113 mg, 0.0900 mmol); to give **(77g)** as a colourless thick oil (0.12 g, 84%) [NSI–Found $(M+Na)^+$: 3295.9; $C_{216}H_{382}NaO_{15}Si_2$, requires: 3295.9]; $[\alpha]_D^{22}$ -10.2 (*c* 0.47, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.20 (25H, m), 5.04 (1H, br.d, *J* 4.2 Hz), 4.97 (1H, br.s), 4.73 (1H, d, *J* 11.6 Hz), 4.69 (3H, d, *J* 11.6 Hz), 4.62 (1H, d, *J* 11.7 Hz), 4.56 – 4.48 (4 H, m), 4.43 (1H, d, *J* 11.7 Hz), 4.37 (1H, br.d, *J* 1.9 Hz), 4.30 – 4.11 (6H, m), 4.07 (1H, t, *J* 6.0 Hz), 4.00 (1H, br.dd, *J* 4.3, 6.5 Hz), 3.89 (4H, m), 3.78 (1H, br.p, *J* 4.9 Hz), 3.70 – 3.52 (3H, including a broad double doublet *J* 4.4, 10.3 Hz at 3.61), 2.62 – 2.48 (2H, m), 1.65 – 1.06 (268H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.86 (9H, s), 0.85 (9H, s), 0.71 – 0.61 (8H, m), 0.57 (4H, dt, *J* 4.1, 8.5 Hz), 0.04 (3H, s), 0.02 (3H, s), 0.01 (3H, s), -0.01 (3H, s), -0.32 (4H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 174.3, 174.1, 138.6, 138.3, 137.9, 137.7, 137.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8,

127.7, 127.65, 127.6, 127.5, 127.4, 105.9, 100.2, 84.9, 84.6, 83.6, 83.3, 80.1, 79.1, 77.1, 73.4, 73.2, 73.1, 72.5, 72.4, 72.2, 70.3, 67.2, 66.3, 64.3, 51.5, 51.4, 33.7, 31.9, 30.3, 30.2, 29.9, 29.85, 29.8, 29.75, 29.7, 29.65, 29.6, 29.5, 29.4, 28.7, 27.8, 27.7, 27.4, 27.3, 25.9, 25.8, 24.0, 23.9, 22.7, 18.0, 17.9, 15.8, 14.1, 11.0, 10.9, -4.4, -4.5, -4.7, -4.8; ν_{max}: 3062, 3032, 2922, 2853, 1740, 1717, 1465, 1116, 733, 698 cm^{-1}.

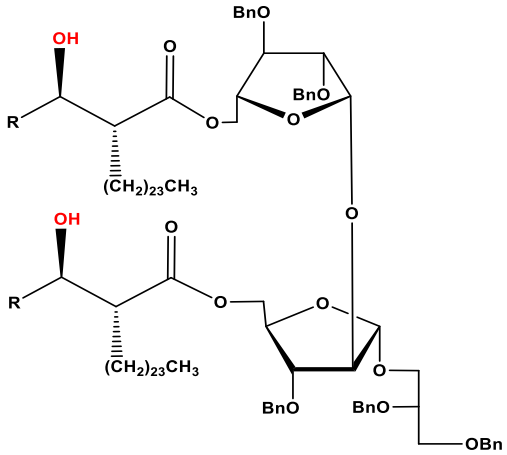
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl) hexacosanoate-α-D-arabinofuranoside (77h):



1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (71 mg; 0.37 mmol), molecular sieves 4 Å (50 mg), α-D-arabinofuranoside **(67)** (30 mg, 0.037 mmol)**,** DMAP (45 mg; 0.36 mmol) and (2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoic acid **(76h)** (103 mg, 0.0730 mmol); to give **(77h)** as a colourless thick oil (12 mg, 91%) [NSI–Found $(M+Na)^+$: 3580.2; $C_{234}H_{418}NaO_{17}Si_2$, requires: 3580.2]; $[\alpha]_D^{22}$ +2.3 (*c* 1.4, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.22 (25H, m), 5.03 (1H, br.d, *J* 4.2 Hz), 4.97 (1H, br.s), 4.72 (1H, d, *J* 11.7 Hz), 4.68 (3H, d, *J* 11.7 Hz), 4.62 (1H, d, *J* 11.6 Hz), 4.56 – 4.47 (4H, m), 4.43 (1H, d, *J* 11.6 Hz), 4.37 (1H, br.d, *J* 1.8 Hz), 4.28 – 4.10 (6H, m), 4.06 (1H, br.t, *J* 5.6 Hz), 4.00 (1H, br.dd, *J* 4.3, 6.4 Hz), 3.96 – 3.81 (4H, m), 3.77 (1H, br.p, *J* 4.9 Hz), 3.66 – 3.55 (3H, including a broad double doublet *J* 4.4, 10.4 Hz at 3.60), 2.60 – 2.46 (4H, including a sextet *J* 6.8 Hz at 2.53), 2.42 (4H, dt, *J* 1.0, 7.5 Hz), 1.67 – 1.12 (300H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.91 – 0.87 (18H, including a triplet *J* 7.5 Hz at 0.89), 0.85 (9H, s), 0.84 (9H, s), 0.75 – 0.62 (2H, m), 0.51 – 0.40 (2H, m), 0.24 – 0.08

(6H, m), 0.04 (3H, s), 0.02 (3H, s), 0.01 (3H, s), -0.01 (3H, s); δ_C (101 MHz, $CDCl_3$): 215.2, 174.3, 174.1, 138.6, 138.3, 137.9, 137.7, 137.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.65, 127.6, 127.5, 127.4, 105.9, 100.1, 84.9, 84.6, 83.6, 83.3, 80.1, 79.1, 77.2, 73.4, 73.2, 73.1, 72.5, 72.4, 72.2, 70.3, 67.2, 66.3, 64.3, 51.5, 51.4, 46.3, 41.1, 38.1, 37.4, 34.5, 33.7, 33.0, 31.9, 31.6, 30.1, 30.0, 29.9, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 27.8, 27.7, 27.4, 27.3, 26.1, 25.9, 25.8, 24.0, 23.9, 23.7, 22.7, 22.6, 19.7, 18.6, 18.0, 17.9, 16.4, 14.1, 10.5, -4.4, -4.5, -4.7, -4.8; ν_{max}: 3064, 3032, 2922, 2852, 1740, 1714, 1465, 1117, 733, 698 cm^{-1}.

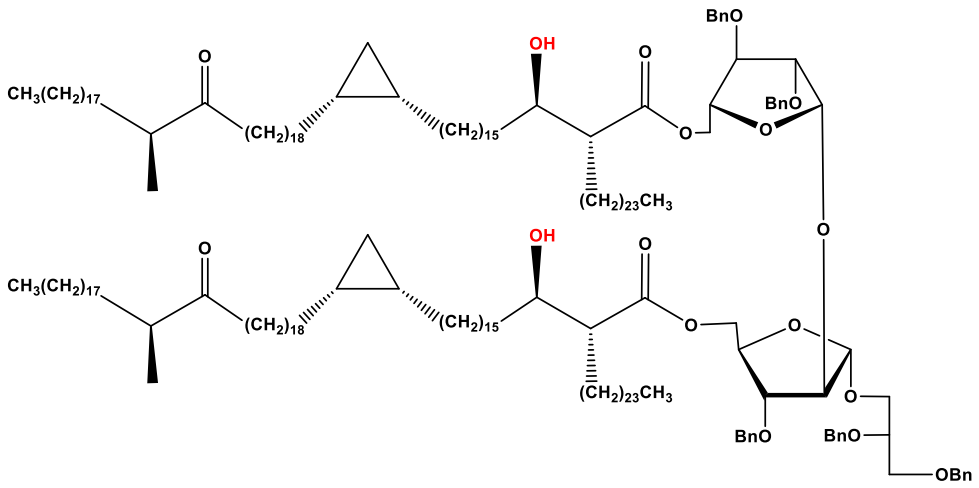
De-protection of silyl group in mycolic acid (78f-h):



General procedure:

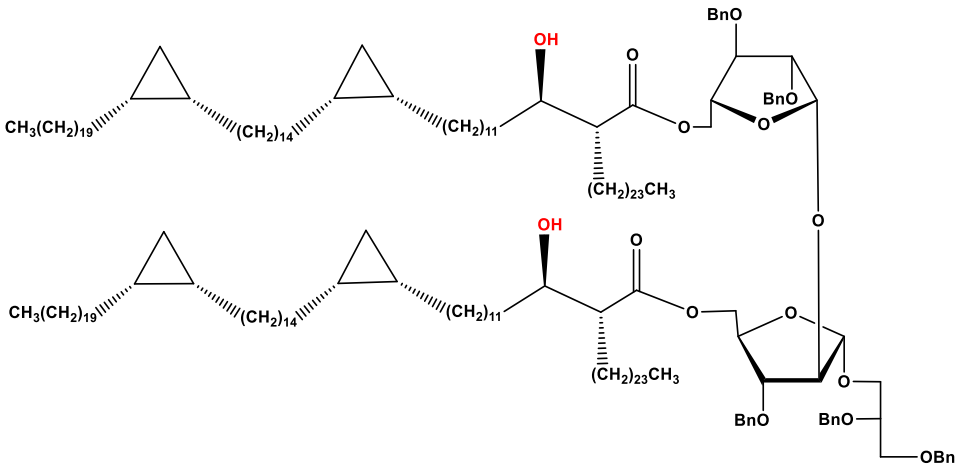
Tetrabutylammonium fluoride 1.0 M solution in THF was added dropwise to a stirred solution of compounds **(77f-h)** in dry THF (1 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 16 h. When TLC showed no starting material was left, the mixture was diluted with ethyl acetate (10 mL) and water (1 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with sat. aq. NH_4Cl (5 mL), brine (5 mL), dried ($MgSO_4$) and the solvent was concentrated to give the residue which was purified by column chromatography on silica eluting with hexane /ethyl acetate (10:1) affording the compounds **(78f-h)**.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl) hexadecyl)hexacosanoate-α-D-arabinofuranoside (78f):



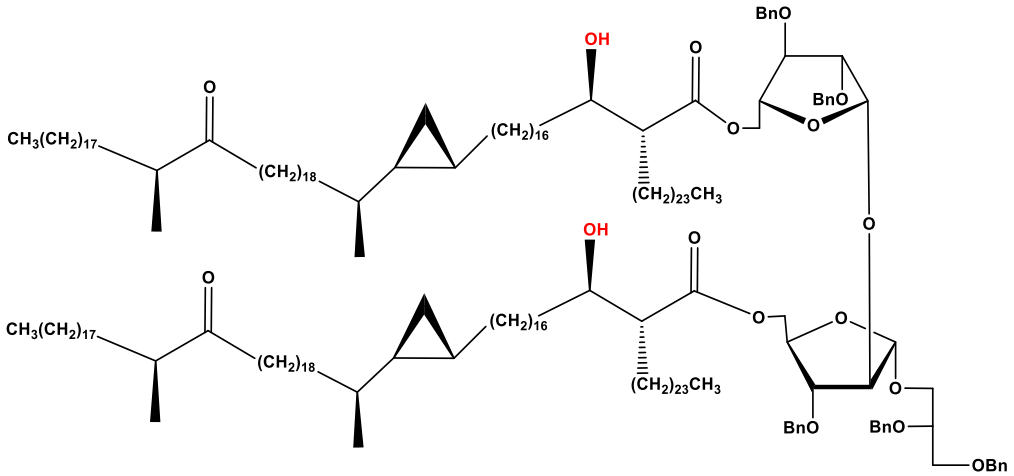
Tetrabutylammonium fluoride (0.56 mL, 1.9 mmol, 1.0 M) and α-D-arabinofuranoside **(77f)** (98 mg, 0.028 mmol); to give **(78f)** as a colourless thick oil (45 mg, 38%) [NSI–Found $(M+Na)^+$: 3267.9; $C_{216}H_{378}NaO_{17}$, requires: 3267.9]; $[\alpha]_D^{22}$ +7.1 (*c* 0.34, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.22 (25H, m), 5.01 (1H, br.d, *J* 4.3 Hz), 4.97 (1H, br.s), 4.71 (2H, d, *J* 11.5 Hz), 4.67 (2H, m), 4.62 (1H, d, *J* 11.5 Hz), 4.56 – 4.50 (3H, m), 4.51 (1H, d, *J* 11.6 Hz), 4.45 (1H, d, *J* 11.6 Hz), 4.32 (1H, br.d, *J* 1.7 Hz), 4.30 – 4.21 (5H, m), 4.17 – 4.08 (2H, including a broad pentet *J* 6.0 Hz at 4.12), 4.02 (1H, br.dd, *J* 4.4, 6.3 Hz), 3.99 – 3.93 (1H, m), 3.84 (1H, dd, *J* 5.3, 10.3 Hz), 3.81 – 3.76 (1H, br.p, *J* 5.3 Hz), 3.59 (5H, including a broad double doublet *J* 4.5, 10.8 Hz at 3.59), 2.56 – 2.46 (4H, including a sextet *J* 6.8 Hz at 2.51), 2.42 (4H, dt, *J* 1.1, 7.6 Hz), 1.65 – 1.11 (290H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8 Hz), 0.71 – 0.61 (4H, m), 0.60 – 0.53 (2H, dt, *J* 4.0, 8.5 Hz), -0.32 (2H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.0, 138.5, 138.3, 137.7, 137.3, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.75, 127.7, 127.6, 127.5, 105.9, 100.4, 85.5, 84.4, 83.7, 82.8, 80.3, 78.9, 77.2, 73.4, 72.7, 72.6, 72.5, 72.4, 72.2, 72.1, 70.2, 67.2, 66.1, 63.7, 51.9, 51.6, 46.3, 41.1, 35.4, 35.3, 33.0, 31.9, 30.3, 30.2, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 29.2, 28.7, 27.5, 27.4, 27.3, 25.7, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: 3501, 3063, 2920, 2852, 1736, 1714, 1465, 1116, 757, 698 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(R)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate-α-D-arabinofuranoside (78g):



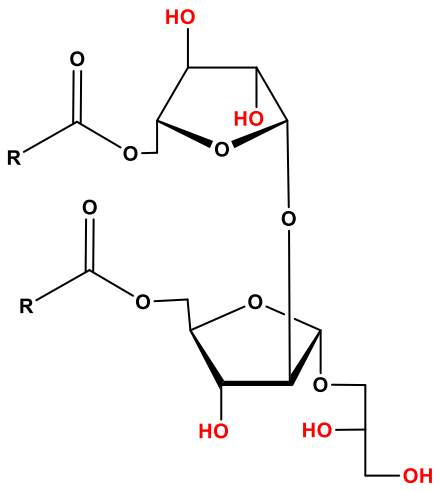
Tetrabutylammonium fluoride (0.40 mL, 1.4 mmol, 1.0 M) and α-D-arabinofuranoside **(77g)** (112 mg, 0.0340 mmol); to give **(78g)** as a colourless thick oil (66 mg, 64%) [NSI–Found $(M+Na)^+$: 3067.7; $C_{204}H_{354}NaO_{15}$, requires: 3067.7]; $[\alpha]_D^{24}$ -18.3 (*c* 0.24, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.42 – 7.21 (25H, m), 5.02 (1H, br.d, *J* 4.3 Hz), 4.97 (1H, br.s), 4.71 (2H, d, *J* 11.4 Hz), 4.67 (2H, m), 4.62 (1H, d, *J* 11.4 Hz), 4.56 – 4.50 (4H, m), 4.45 (1H, d, *J* 11.6 Hz), 4.32 (1H, br.d, *J* 1.6 Hz), 4.31 – 4.21 (5H, m), 4.16 – 4.09 (2H, including a broad pentet *J* 5.9 Hz at 4.13), 4.02 (1H, br.dd, *J* 4.6, 6.2 Hz), 3.97 (1H, br.d, *J* 2.8 Hz), 3.85 (1H, dd, *J* 5.3, 10.3 Hz), 3.79 (1H, br.p, *J* 5.0 Hz), 3.66 – 3.54 (5H, including a broad double doublet *J* 4.5, 10.7 Hz at 3.6), 2.46 – 2.35 (2H, m), 1.68 – 1.03 (270H, m), 0.89 (12H, t, *J* 6.7 Hz), 0.71 – 0.61 (8H, m), 0.57 (4H, dt, *J* 4.0, 8.4 Hz), -0.32 (4H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 175.0, 138.5, 138.3, 137.8, 137.3, 128.5, 128.4, 128.35, 128.3, 128.25, 128.2, 128.1, 127.8, 127.75, 127.7, 127.65, 127.60, 127.5, 105.9, 100.4, 85.5, 84.4, 83.7, 82.8, 80.3, 78.9, 77.1, 73.4, 72.6, 72.5, 72.4, 72.3, 72.2, 72.1, 70.2, 67.2, 66.1, 63.7, 51.9, 51.6, 41.3, 36.1, 35.4, 35.3, 33.7, 31.9, 30.3, 30.2, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 29.1, 28.9, 28.7, 27.7, 27.5, 27.4, 25.7, 25.6, 22.7, 22.6, 22.3, 20.4, 19.4, 18.8, 15.8, 14.3, 14.1, 11.4, 11.0, 10.9; ν_{max}: 3516, 3061, 2920, 2851, 1734, 1728, 1465, 1116, 737, 696 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl) hexacosano-ate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoate-α-D-arabinofuranoside (78h):



Tetrabutylammonium fluoride (0.35 mL, 1.2 mmol, 1.0 M) and α-D-arabinofuranoside **(77h)** (105 mg, 0.0295 mmol); to give **(78h)** as a colourless thick oil (30 mg, 31%) [NSI–Found $(M+Na)^+$: 3352.0; $C_{222}H_{390}NaO_{17}$, requires: 3352.0]; $[\alpha]_D^{24}$ +5.0 (*c* 0.32, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.22 (25H, m), 5.01 (1H, br.d, *J* 4.2 Hz), 4.97 (1H, br.s), 4.71 (2H, d, *J* 11.7 Hz), 4.68 – 4.65 (2H, m), 4.62 (1H, d, *J* 11.7 Hz), 4.56 – 4.49 (4H, m), 4.45 (1H, d, *J* 11.6 Hz), 4.32 (1H, br.d, *J* 1.3 Hz), 4.29 – 4.22 (4H, m), 4.17 – 4.08 (3H, including a broad pentet *J* 5.5 Hz at 4.13 m), 4.04 – 3.99 (1H, m), 3.97 (1H, br.d, *J* 2.3 Hz), 3.85 (1H, dd, *J* 5.3, 10.2 Hz), 3.79 (1H, br.p, *J* 5.3 Hz), 3.60 (5H, including a broad double doublet *J* 4.4, 10.6 Hz at 3.6), 2.57 – 2.47 (4H, m), 2.42 (4H, dt, *J* 1.0, 7.5 Hz), 1.66 – 1.09 (294H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.88 – 0.85 (18H, including a triplet *J* 6.8 Hz at 0.87), 0.77 – 0.61 (2H, m), 0.52 – 0.40 (2H, m), 0.26 – 0.07 (6H, m); δ_C (101 MHz, $CDCl_3$): 215.2, 175.1, 175.0, 138.5, 138.3, 137.8, 137.3, 128.5, 128.45, 128.4, 128.35, 128.3, 128.2, 128.1, 127.9, 127.8, 127.75, 127.7, 127.6, 127.5, 105.9, 100.4, 85.5, 84.4, 83.7, 82.8, 80.3, 78.9, 77.1, 73.4, 72.6, 72.5, 72.4, 72.3, 72.2, 72.1, 70.2, 67.2, 66.1, 63.8, 60.4, 51.9, 51.6, 46.3, 41.3, 41.1, 38.1, 37.4, 36.1, 35.4, 35.3, 34.5, 33.7, 33.0, 31.9, 30.1, 29.9, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 29.1, 28.9, 27.7, 27.5, 27.4, 27.35, 27.3, 26.1, 25.7, 25.6, 23.8, 22.7, 22.6, 22.3, 21.0, 20.4, 19.7, 19.4, 18.7, 18.6, 16.4, 14.3, 14.1, 11.4, 10.5; ν_{max}: 3472, 3063, 2920, 2851, 1735, 1714, 1465, 1116, 757, 698 cm^{-1}.

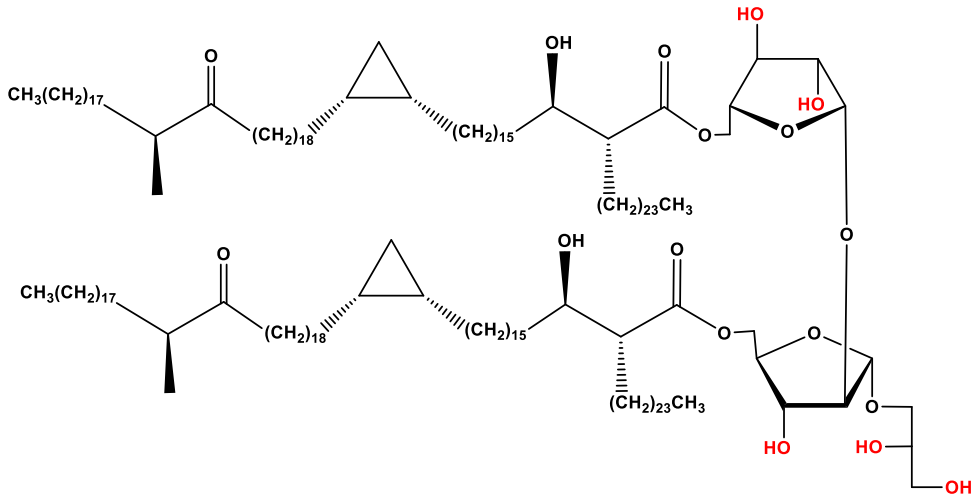
L-Glycerol-(1'→1)-5-*O*-mycolate-β-D-arabinofuranosyl-(1→2)-5-*O*-mycolate-α-D-arabinofuranoside (79f-h):



General procedure:

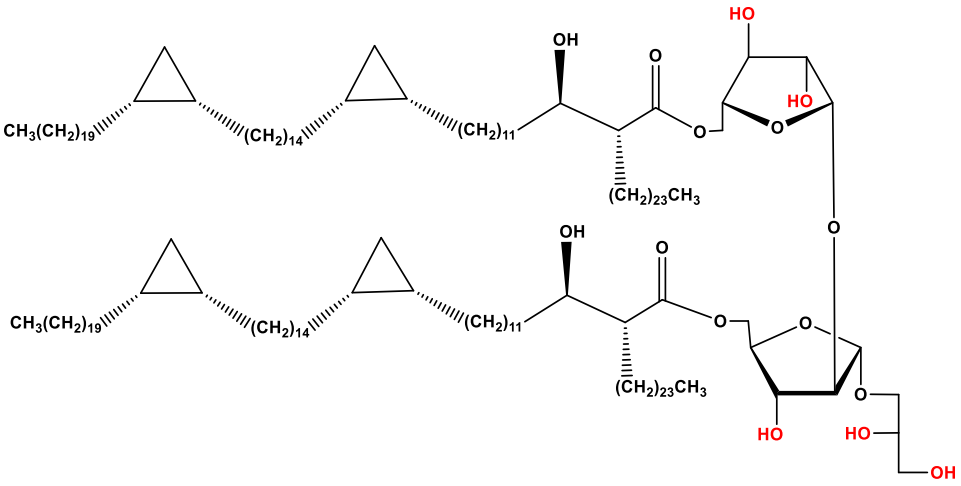
Palladium hydroxide on activated charcoal was added to a stirred solution of α-D-arabinofuranoside **(78f-h)** in CH_2Cl_2:MeOH (1:1, 1 mL) at room temperature under hydrogen. The mixture was stirred for 36 h then TLC showed no starting material was left. The mixture was filtered off through celite and the solvent was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (10:1) affording the title compounds **(79f-h)**.

L-Glycerol-(1'→1)-5-*O*--(*R*)-2-((*R*)-1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxoocta-triacontyl)cyclopropyl)hexadecyl)hexacosanoate-β-D-arabinofuranosyl -(1→2)-5-*O*-(*R*)-2-((*R*)-1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl) hexacosanoate-α-D-arabinofuranoside (79f):



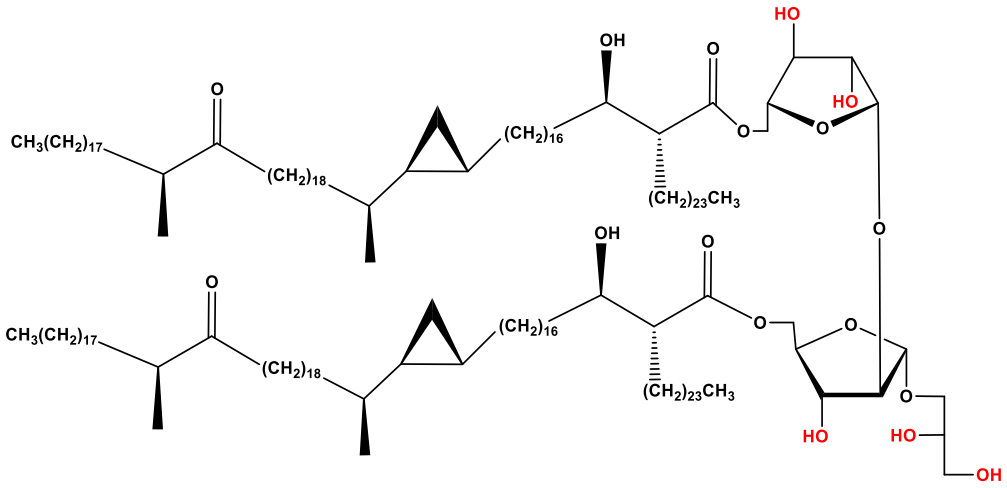
($Pd(OH)_2$-C/20%, 26 mg, 0.75 fold by weight) and α-D-rabinofuranoside **(78f)** (35 mg, 0.010 mmol); to give **(79f)** as a colourless thick oil (22 mg, 71%) [NSI–Found $(M+Na)^+$: 2817.6; $C_{181}H_{348}NaO_{17}$, requires: 2817.6]; $[\alpha]_D^{22}$ +7.4 (*c* 0.38, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 4.97 (1H, br.d, *J* 4.4 Hz), 4.96 (1H, br.s), 4.38 (1H, dd, *J* 4.7, 11.6 Hz), 4.33 (1H, br.dd, *J* 6.9, 11.4 Hz), 4.20 (1H, dd, *J* 6.0, 11.5 Hz), 4.18 (1H, dd, *J* 5.4, 11.5 Hz), 4.11 (1H, br.q, *J* 5.5 Hz), 4.08 – 3.92 (5H, m), 3.82 – 3.75 (1H, br.m), 3.69 (1H, br.dd, *J* 6.3, 10.5 Hz), 3.66 – 3.62 (3H, m), 3.57 (1H, br.dd, *J* 4.5, 11.4 Hz), 3.52 (1H, dd, *J* 3.4, 10.4 Hz), 2.60 – 2.40 (4H, including sextet *J* 6.8 Hz at 2.48), 2.38 (4H, br.t, 7.3 Hz), 1.67 – 1.05 (295H, m), 1.01 (6H, d, *J* 6.9 Hz), 0.84 (12H, t, *J* 6.8 Hz), 0.65 – 0.56 (4H, m), 0.52 (2H, dt, *J* 4.1, 8.5 Hz), -0.37 (2H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$+few drops CD_3OD): 215.9, 175.1, 175.0, 105.8, 101.5, 87.6, 80.7, 79.5, 77.2, 76.4, 76.1, 72.7, 72.5, 70.3, 69.4, 65.4, 63.2, 53.3, 52.6, 46.3, 41.1, 34.8, 34.7, 32.9, 31.8, 30.2, 30.1, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 29.25, 29.2, 29.1, 29.0, 28.6, 27.4, 27.3, 27.2, 25.3, 25.2, 23.6, 22.6, 16.2, 15.7, 14.0, 10.8; ν_{max}: 3420, 2919, 2851, 1733, 1714, 1467, 1120, 1046, 721 cm^{-1}.

L-Glycerol-(1'→1)-5-*O*-(*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl) tetradecyl)cyclopropyl)dodecyl)hexacosanoate-β-D-arabinofuranosyl-(1→2)-5-*O*-(*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl) dodecyl)hexacosanoate-α-D-arabinofuranoside (79g):



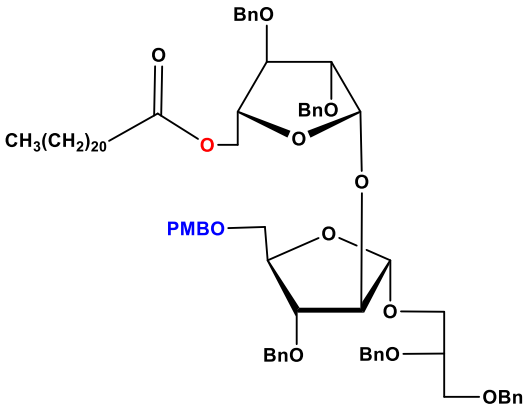
($Pd(OH)_2$-C/20%, 46 mg, 0.75 fold by weight) and α-D-arabinofuranoside **(78g)** (62 mg, 0.020 mmol); to give **(79g)** as a colourless thick oil (36 mg, 70%) [NSI–Found $(M+Na)^+$: 2617.5; $C_{169}H_{324}NaO_{15}$, requires: 2617.5]; $[\alpha]_D^{24}$ +2.7 (*c* 1.9, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 4.99 (1H, br.d, *J* 6.2 Hz), 4.98 (1H, br.s), 4.42 (1H, dd, *J* 4.5, 11.6 Hz), 4.37 (1H, br.dd, *J* 6.5, 11.1 Hz), 4.23 (1H, dd, *J* 5.2, 11.2 Hz), 4.20 (1H, br.dd, *J* 4.8, 10.3 Hz), 4.14 (1H, br.q, *J* 5.3 Hz), 4.10 – 3.97 (5H, m), 3.85 – 3.79 (1H, m), 3.72 (1H, br.dd, *J* 7.0, 11.2 Hz), 3.69 – 3.63 (2H, br.m), 3.62 (1H, br.d, *J* 3.6 Hz), 3.58 (1H, dd, *J* 4.2, 9.7 Hz), 3.55 (1H, dd, *J* 3.2, 10.4 Hz), 2.47 – 2.39 (2H, m), 1.61 – 1.05 (275H, m), 0.86 (12H, t, *J* 6.8 Hz), 0.69 – 0.58 (8H, m), 0.54 (4H, dt, *J* 4.1, 8.5 Hz), -0.35 (4H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$+few drops CD_3OD): 175.1, 175.0, 105.8, 101.5, 87.6, 80.8, 79.5, 77.5, 77.2, 76.5, 76.2, 72.8, 72.6, 70.3, 69.6, 65.4, 63.3, 63.2, 53.3, 52.6, 34.8, 34.7, 31.9, 30.3, 30.2, 29.8, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 29.1, 28.7, 27.5, 27.4, 25.4, 25.3, 22.6, 15.8, 15.7, 14.0, 10.9, 10.8; ν_{max}: 3417, 2920, 2851, 1729, 1723, 1466, 1215, 1116, 1045, 761, 669 cm^{-1}.

L-Glycerol-(1'→1)-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoate-β-D-arabinofuranosyl-(1→2)-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl) heptadecyl)hexacosanoate-α-D-arabinofuranoside (79h):



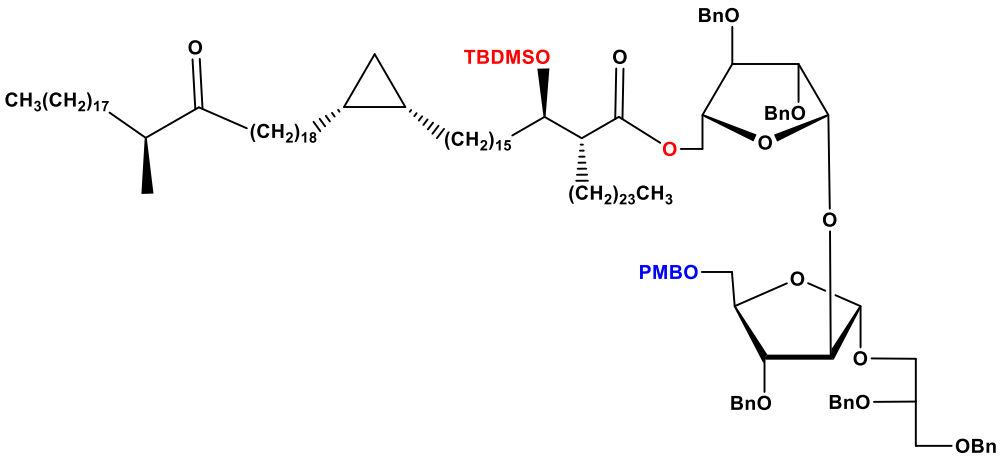
($Pd(OH)_2$-C/20%, 25 mg, 0.75 fold by weight) and α-D-arabinofuranoside **(78h)** (25 mg, 0.0075 mmol); to give **(79h)** as a colourless thick oil (16 mg, 72%) [NSI–Found $(M+Na)^+$: 2901.7; $C_{187}H_{360}NaO_{17}$, requires: 2901.7]; $[\alpha]_D^{22}$ -7.1 (*c* 0.34, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 5.04 (1H, br.d, *J* 7.7 Hz), 5.03 (1H, br.s), 4.49 (1H, dd, *J* 5.6, 12.7 Hz), 4.46 (1H, br.dd, *J* 3.8, 11.6 Hz), 4.29 (1H, dd, *J* 5.1, 11.6 Hz), 4.24 – 4.20 (1H, m), 4.18 (1H, br.q, *J* 6.1 Hz), 4.13 (1H, d, *J* 7.2 Hz), 4.11– 4.03 (4H, including a broad doublet *J* 6.8 Hz at 4.09), 3.90 – 3.81 (1H, m), 3.79 – 3.60 (6H, m), 2.51 (4H, including a sextet *J* 6.9 Hz at 2.51), 2.42 (2H, t, *J* 7.6 Hz), 2.41 (2H, t, *J* 7.1 Hz), 1.65 – 1.10 (299H, m), 1.05 (6H, d, *J* 6.9 Hz), 0.93 – 0.84 (18H, including a broad triplet *J* 7.4 Hz at 0.89), 0.72 – 0.62 (2H, m), 0.50 – 0.40 (2H, m), 0.25 – -0.06 (6H, m); δ_C (101 MHz, $CDCl_3$+few drops CD_3OD): 215.3, 175.0, 174.8, 105.8, 101.6, 88.2, 80.5, 79.6, 77.7, 77.2, 76.7, 76.6, 73.1, 72.7, 70.4, 70.0, 69.9, 65.2, 63.7, 63.2, 60.4, 53.4, 52.5, 46.3, 41.1, 38.1, 37.4, 35.0, 34.8, 34.5, 33.0, 31.9, 30.1, 29.8, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 27.4, 27.3, 27.25, 26.1, 25.5, 25.4, 23.7, 22.7, 19.7, 18.6, 16.4, 14.1, 10.5; ν_{max}: 3421, 2920, 2852, 1735, 1715, 1466, 1119, 1045, 733 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-behenate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (80a):



A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (13.6 mg, 0.070 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise to a stirred solution of α-D-arabinofuranoside **(66)** (13.2 mg, 0.0142 mmol), DMAP (8.6 mg, 0.070 mmol) and behenic acid **(69c)** (7.1 mg, 0.020 mmol) in dry CH_2Cl_2 (1 mL) at 0 ºC under nitrogen. The mixture was stirred for 48 h. When TLC showed no starting material was left. The precipitate was filtered off and washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to afford the title compound **(80a)** as a colourless thick oil (15 mg, 85%) [NSI–Found $(M+NH_4)^+$: 1266.7800; $C_{78}H_{108}O_{13}N$, requires: 1266.7815]; $[\alpha]_D^{22}$ -2.2 (*c* 0.92, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.30 – 7.17 (25H, m), 7.15 (2H, d, *J* 8.6 Hz), 6.77 (2H, d, *J* 8.6 Hz), 5.00 (1H, br.d, *J* 4.1 Hz), 4.95 (1H, br.s), 4.61 (1H, d, *J* 12.1 Hz), 4.60 (2H, br.s), 4.58 (1H, d, *J* 12.1 Hz), 4.51 (1H, d, *J* 11.6 Hz), 4.46 – 4.41 (3H, m), 4.37 (3H, br.d, *J* 11.7 Hz), 4.36 (1H, d, *J* 11.7 Hz), 4.25 (1H, br.d, *J* 1.9 Hz), 4.17 – 4.10 (2H, m), 4.07 – 3.98 (3H, m), 3.95 (1H, br.q, *J* 6.6 Hz), 3.89 (1H, br.dd, *J* 2.4, 6.0 Hz), 3.80 (1H, dd, *J* 5.1, 10.4 Hz), 3.74 – 3.68 (4H, including a singlet at 3.72 for O*CH*$_3$), 3.57 – 3.50 (3H, including a broad double doublet *J* 5.0, 8.5 Hz at 3.53), 3.49 – 3.42 (2H, including a broad double doublet *J* 4.2, 11.1 Hz at 3.47), 2.13 (2H, dt, *J* 3.6, 7.7 Hz), 1.53 – 1.02 (38H, m), 0.81 (3H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 173.4, 159.2, 138.7, 138.3, 138.0, 137.8, 137.5, 130.2, 129.4, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.65, 127.6, 127.55, 127.5, 127.4, 113.7, 106.0, 100.5, 85.9, 84.4, 83.8, 82.6, 81.5, 78.9, 77.0, 73.4, 72.9, 72.5, 72.4, 72.3, 72.2, 70.3, 69.6, 67.2, 66.0, 55.2, 34.0, 31.9, 29.8, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 29.1, 24.8, 22.7, 14.1; ν_{max}: 3062, 3031, 2924, 2859, 1741, 1612, 1513, 1454, 1248, 1110,738, 699 cm^{-1}.

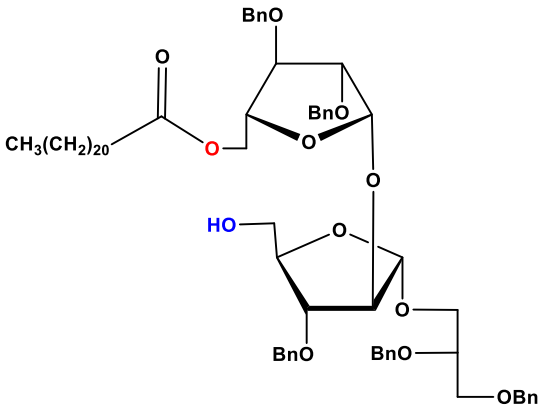
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-(1-((*tert*-butyldimethylsilyl) oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl) hexadecyl) hexacosanoate)-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*p*-methoxybenzyl-α-D-arabinofuranoside (80b):



A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (17.7 mg, 0.0923 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise to a stirred solution of α-D-arabinofuranoside **(66)** (17.2 mg, 0.0185 mmol), DMAP (11.3 mg, 0.0924 mmol) and (2*R*)-2-(1-((*tert*-butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl) hexadecyl) hexacosanoic acid **(76f)** (37.5 mg, 0.0277 mmol) in dry CH_2Cl_2 (1 mL) at 0 ºC under nitrogen atmosphere. The reaction mixture was stirred for 48 h. When TLC showed no starting material was left. The reaction mixture was worked up and purified as above affording the title compound **(80b)** (36 mg, 86%) [NSI–Found $(M+Na)^+$: 2282.8; $C_{146}H_{238}NaO_{15}Si$, requires: 2282.8]; $[\alpha]_D^{22}$ +12 (*c* 0.22, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.34 – 7.24 (25H, m), 7.22 (2H, d, *J* 8.6 Hz), 6.84 (2H, d, *J* 8.6 Hz), 5.06 (1H, br.d, *J* 4.2 Hz), 5.02 (1H, br.s), 4.69 (2H, d, *J* 11.7 Hz), 4.68 (2H, d, *J* 11.7 Hz), 4.61 (1H, d, *J* 11.6 Hz), 4.54 – 4.49 (3H, m), 4.48 – 4.41 (4H, m), 4.32 (1H, br.d, *J* 2.0 Hz), 4.21 (3H, br.dd, *J* 3.8, 8.4 Hz), 4.13 (1H, dd, *J* 6.1 Hz), 4.07 – 4.03 (1H, m), 4.01 (1H, dd, *J* 4.3, 6.6 Hz), 3.95 (1H, dd, *J* 2.5, 6.0 Hz), 3.87 (2H, br.dd, *J* 5.3, 10.4 Hz), 3.82 – 3.75 (4H, including a singlet at 3.8 for O*CH*$_3$), 3.61 (3H, br.dd, *J* 4.8, 9.4 Hz), 3.53 (2H, dd, *J* 6.8, 12.1 Hz), 2.52 (2H, including a sextet *J* 6.2 Hz at 2.52) 2.42 (2H, dt, *J* 1.0, 7.0 Hz), 1.45 – 1.11 (144H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.7 Hz), 0.84 (9H, br.s), 0.69 – 0.65 (2H, m), 0.57 (1H, dt, *J* 4.0, 7.7 Hz), 0.02 (3H, s), -0.01 (3H, s), -0.33 (1H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 174.1, 159.1, 138.7, 138.3, 138.0, 137.9, 137.6, 130.2, 129.4, 128.5, 128.4, 128.3, 128.25,

128.2, 127.9, 127.8, 127.75, 127.7, 127.6, 127.55, 127.5, 127.4, 113.7, 106.0, 100.4, 85.6, 84.4, 83.7, 83.4, 81.6, 79.2, 77.1, 73.3, 73.1, 72.9, 72.5, 72.4, 72.3, 72.2, 70.4, 69.7, 67.2, 66.3, 60.4, 55.2, 51.5, 46.3, 41.1, 33.7, 33.0, 31.9, 30.2, 29.8, 29.75, 29.70, 29.6, 29.55, 29.5, 29.4, 29.35, 29.3, 28.7, 27.8, 27.3, 25.8, 24.0, 23.7, 22.7, 22.6, 22.3, 21.0, 18.0, 16.4, 15.8, 14.2, 14.1, 14.0, 10.9, -4.4, -4.7; ν_{max}: 3088, 3065, 2923, 2856, 1739, 1614, 1456, 1247, 1115, 739, 698 cm^{-1}.

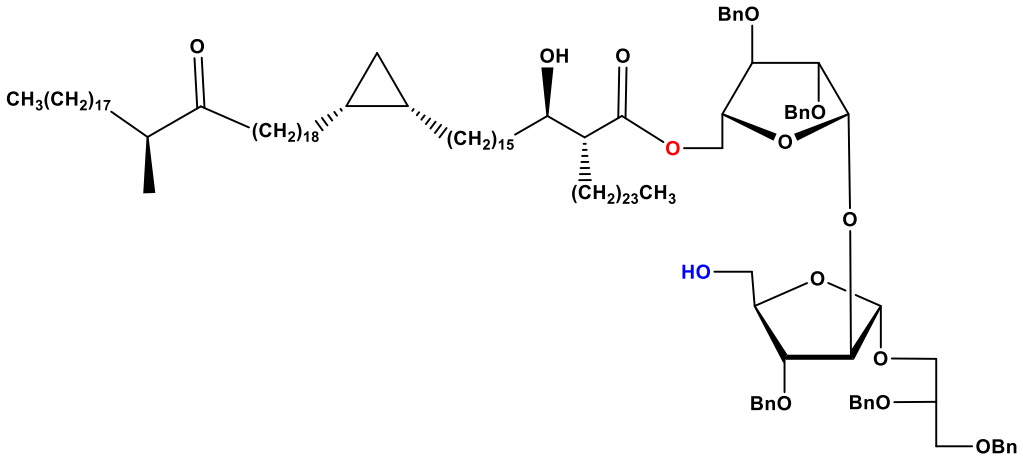
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-behenate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-α-D-arabinofuranoside (81a):



Cerium ammonium nitrate (13 mg, 0.023 mmol) was added to a stirred solution of compound **(80a)** (15 mg, 0.012 mmol) in CH_3CN:H_2O:THF (9:1:0.2, 1 mL)at 0 ºC. The mixture was allowed to reach room temperature and stirred at ambient temperature for 16 h then TLC showed no starting material was left. The reaction mixture was diluted with chloroform (20 mL), washed with aq. $NaHCO_3$ (10 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (4:1) to give the title compound **(81a)** as a colourless thick oil (8.4 mg, 62%) [NSI–Found $(M+Na)^+$: 1151.7; $C_{70}H_{96}NaO_{12}$, requires: 1151.7]; $[\alpha]_D^{22}$ -11 (*c* 0.27, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.25 (25H, m), 5.04 (1H, br.d, *J* 4.1 Hz), 4.98 (1H, br.s), 4.71 (1H, d, *J* 11.5 Hz), 4.69 (3H, br.s), 4.62 (1H, d, *J* 11.5 Hz), 4.54 – 4.49 (5H, m), 4.33 (1H, br.d, *J* 1.3 Hz), 4.21 (2H, including a broad doublet *J* 6.0 Hz at 4.21), 4.18 – 4.12 (2H, including a broad double doublet *J* 4.3, 7.2 Hz at 4.15), 4.11 (1H, br.dd, *J* 3.6, 7.9 Hz), 4.08 (1H, br.d, *J* 2.5 Hz), 4.05 (1H, dd, *J* 4.2, 6.6 Hz), 3.86 (1H, dd, *J* 5.2, 10.3 Hz), 3.82 – 3.76 (2H, including a broad double doublet *J* 4.3, 9.7 Hz at 3.8), 3.66 – 3.58 (4H, including a broad double doublet *J* 4.7, 11.8 Hz at 3.6), 2.25 (2H, dt, *J* 0.9, 7.3 Hz), 1.36 – 1.18 (39H, m), 0.90 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.5, 138.3, 137.9,

137.7, 137.4, 136.9, 128.5, 128.4, 128.3, 128.25, 128.1, 128.0, 127.9, 127.75, 127.7, 127.6, 127.55, 127.5, 106.0, 100.4, 85.1, 83.8, 83.5, 83.4, 82.5, 78.9, 73.3, 72.6, 72.4, 72.2, 70.1, 67.2, 66.0, 62.3, 34.0, 31.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 29.1, 24.8, 22.7, 14.1; ν_{max}: 3414, 3062, 3032, 2915, 2852, 1737, 1467, 735, 697 cm^{-1}.

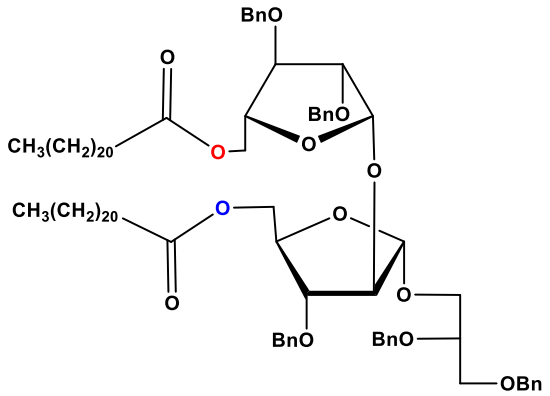
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(*R*)-2-((*R*)-1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate)-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-α-D-arabinofuranoside (81b):



Cerium ammonium nitrate (31 mg, 0.057 mmol) was added to a stirred solution of compound **(80b)** (32 mg, 0.014 mmol) in CH_3CN:H_2O:THF (9:1:0.2, 1 mL) at 0 ºC. The mixture was allowed to reach room temperature and stirred at ambient temperature for 16 h then TLC showed no starting material was left. The mixture was worked up and purified as above giving the title compound **(81b)** as a colourless thick oil (25 mg, 83%) [NSI–Found $(M+Na)^+$: 2048.7; $C_{132}H_{216}NaO_{14}$, requires: 2048.7]; $[\alpha]_D^{23}$ -8.5 (*c* 0.21, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.23 (25H, m), 5.01 (1H, br.d, *J* 4.3 Hz), 4.97 (1H, br.s), 4.70 (1H, d, *J* 11.6 Hz) 4.68 (1H, d, *J* 11.6 Hz), 4.67 (2H, br.s), 4.61 (1H, d, *J* 11.6 Hz), 4.56 – 4.46 (5H, m), 4.30 (2H, br.dd, *J* 8.1, 12.0 Hz), 4.22 (1H, dd, *J* 3.9, 11.4 Hz), 4.15 (1H, br.dd, *J* 3.3, 6.7 Hz), 4.10 (3H, br.dd, *J* 5.9, 12.1 Hz), 4.02 (1H, dd, *J* 4.4, 6.5 Hz), 3.85 (1H, dd, *J* 5.2, 10.3 Hz), 3.79 (1H, br.dd, *J* 5.1, 9.4 Hz), 3.77 (1H, br.dd, *J* 3.7, 6.1 Hz), 3.66 – 3.55 (5H, including a broad double doublet *J* 4.6, 12.1 Hz at 3.6), 2.51 (1H, sextet, *J* 6.7 Hz), 2.44 – 2.38 (3H, including a triplet *J* 7.2 Hz at 2.42), 2.08 – 1.07 (146H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.8 Hz), 0.69 – 0.62 (2H, m), 0.57 (1H, dt *J* 4.1, 8.6 Hz), -0.33 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.3, 175.0, 138.6, 138.1, 138.0, 137.7, 137.4, 128.5, 128.4, 128.35, 128.3, 128.1, 128.0,

127.9, 127.8, 127.7, 127.6, 127.5, 106.1, 100.7, 85.7, 83.8, 83.5, 83.4, 82.5, 78.9, 73.4, 72.6, 72.4, 72.3, 72.2, 70.2, 67.2, 66.0, 62.2, 51.8, 46.3, 41.1, 35.4, 33.0, 31.9, 30.2, 29.7, 29.6, 29.5, 29.4, 28.7, 27.4, 27.3, 25.7, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: 3496, 3062, 2921, 2850, 1738, 1465, 1116, 758, 699 cm^{-1}.

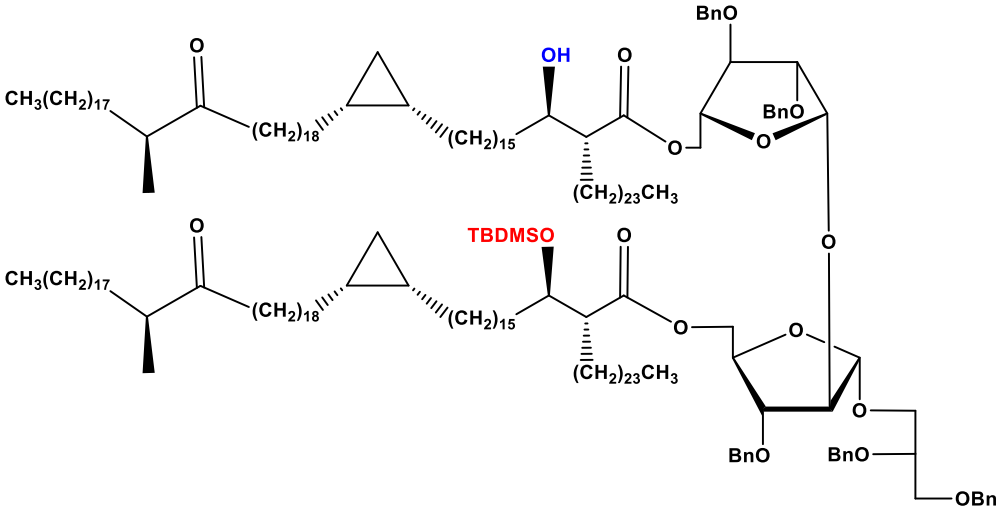
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-behenate-β-D-arabinofuranosyl-(1→2)-3-*O*-benzyl-5-*O*-behenate-α-D-arabinofuranoside (82a):



A solution of (EDCI) (5.4 mg, 0.028 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise to a stirred solution of α-D-arabinofuranoside **(81a)** (6.4 mg, 0.0056 mmol), DMAP (3.4 mg, 0.027 mmol) and behenic acid (2.9 mg, 0.0085 mmol) in dry CH_2Cl_2 (1 mL) at 0 ºC under nitrogen. The mixture was stirred for 72 h. When TLC showed no starting material was left. The precipitate was filtered off and washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to afford the title compound **(82a)** as a colourless thick oil (4.2 mg, 50%) [NSI–Found $(M+Na)^+$: 1474.0; $C_{92}H_{138}NaO_{13}$, requires: 1474.0]; $[\alpha]_D^{22}$ -4.2 (*c* 1.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.30 – 7.15 (25H, m), 4.97 (1H, br.d, *J* 4.2 Hz), 4.91 (1H, br.s), 4.62 (1H, d, *J* 11.5 Hz), 4.61 (1H, d, *J* 11.5 Hz), 4.59 (2H, br.s), 4.52 (1H, d, *J* 11.6 Hz), 4.44 (3H, br.s), 4.41 (1H, d, *J* 11.6 Hz), 4.37 (1H, d, *J* 11.6 Hz), 4.27 (1H, br.d, *J* 2.0 Hz), 4.20 (1H, *J* 3.5 Hz), 4.16 (1H, *J* 4.6 Hz), 4.13 (1H, br.dd, *J* 3.6, 5.8 Hz), 4.10 – 4.04 (2H, including a broad double doublet *J* 4.1, 8.2 Hz at 4.06), 4.03 (1H, dd, *J* 3.7, 9.7 Hz), 3.99 (1H, d, *J* 5.2 Hz), 3.95 (1H, dd, *J* 4.3, 6.7 Hz), 3.86 (1H, dd, *J* 2.4, 5.9 Hz), 3.78 (1H, dd, *J* 5.2, 10.4 Hz), 3.70 (1H, br.p *J* 4.8 Hz), 3.57 – 3.48 (3H, including a broad double doublet *J* 4.7, 8.3 Hz at 3.52), 2.19 (2H, t, *J* 7.6 Hz), 2.14 (2H, dt, *J* 2.7, 7.5 Hz), 1.53 – 1.03 (76H, m), 0.80 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.5, 173.4, 138.6, 138.3, 137.8, 137.7, 137.4, 128.5, 128.4, 128.35, 128.3, 128.0, 127.8, 127.75, 127.7, 127.6, 127.55, 127.5, 106.1, 100.4, 85.6, 84.3, 83.8, 82.5,

80.1, 78.9, 77.2, 73.4, 72.5, 72.4, 72.35, 72.3, 70.2, 67.3, 66.0, 63.7, 34.0, 31.9, 29.7, 29.65, 29.6, 29.55, 29.5, 29.4, 29.3, 29.25, 29.2, 29.1, 24.9, 24.8, 22.7, 14.1; ν_{max}: 3063, 3031, 2917, 2850, 1740, 1732, 1467, 1110, 735, 697 cm^{-1}.

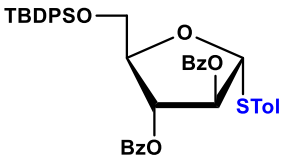
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)- 5-*O*-(*R*)-2-((*R*)-1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl) hexacosanoate)-β-D-arabinofuranosyl-(1→2)-5-*O*-(2*R*)-2-(1-((*tert*-butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl)hexadecyl)hexacosanoate)-α-D-arabinofuranoside (82b):



A solution of (EDCI) (6.1 mg, 0.032 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise to a stirred solution of α-D-arabinofuranoside **(81b)** (13.5 mg, 0.00630 mmol), DMAP (3.8 mg, 0.032 mmol) and (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl) hexadecyl)hexacosanoic acid (12.8 mg, 0.00945 mmol) in dry CH_2Cl_2 (1 mL) at 0 ºC under nitrogen. The mixture was stirred for 72 h. then worked up and purified as above to afford the title compound **(82b)** (10 mg, 50%) [NSI–Found $(M+Na)^+$: 3381.9; $C_{222}H_{392}NaO_{17}Si$, requires: 3381.9]; $[\alpha]_D^{23}$ -7 (*c* 0.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.21 (25H, m), 5.02 (1H, br.d, *J* 4.3 Hz), 4.97 (1H, br.s), 4.70 (2H, d, *J* 11.8 Hz), 4.66 (2H, br.s), 4.61 (1H, d, *J* 11.8 Hz), 4.55 – 4.46 (4H, m), 4.42 (1H, d, *J* 11.7 Hz), 4.34 (1H, br.d, *J* 1.4 Hz), 4.26 – 4.21 (2H, including a broad double doublet *J* 5.4, 9.0 Hz at 4.24), 4.20 (1H, br.d, *J* 5.6 Hz), 4.17 – 4.09 (4H, including a broad quartet *J* 7.1 Hz at 4.13), 4.10 – 4.05 (1H, m), 4.00 (1H, br.dd, *J* 4.4, 6.8 Hz), 3.96 – 3.88 (2H, m), 3.84 (1H, dd, *J* 4.8, 10.3 Hz), 3.79 – 3.75 (1H, m), 3.64 – 3.58 (3H, including a broad double doublet *J* 4.5, 10.2 Hz at 3.6), 2.55 – 2.46 (4H, including a sextet *J* 6.7 Hz at 2.51), 2.41 (4H, dt, *J* 0.9, 7.1 Hz), 1.73 – 1.10 (289H, m), 1.05 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8 Hz), 0.85 (9H, s), 0.70 – 0.61 (4H, m), 0.56 (2H, dt, *J*

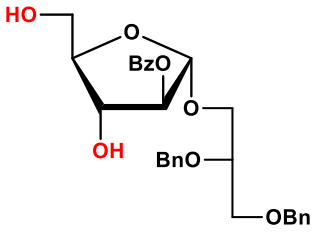
4.2, 8.3 Hz), 0.04 (3H, s), 0.01 (3H, s), -0.33 (2H, br.q, 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.0, 174.4, 138.6, 138.5, 137.8, 137.75, 137.7, 137.4, 136.1, 128.5, 128.4, 128.35, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.65, 127.6, 127.5, 127.4, 125.0, 105.9, 100.2, 85.2, 84.5, 83.7, 82.9, 80.1, 78.9, 73.4, 73.2, 72.5, 72.45, 72.4, 72.2, 72.1, 70.3, 67.2, 66.2, 64.3, 51.5, 46.3, 41.1, 35.5, 33.7, 33.0, 32.2, 31.9, 30.2, 28.7, 27.7, 27.4, 27.3, 26.4, 25.8, 23.7, 23.4, 22.7, 16.4, 15.8, 14.1, 10.9, -4.4, -4.8; ν_{max}: 3415, 3067, 3032, 2957, 2920, 1740, 1731, 1463, 1217, 1171, 1048, 881, 721 cm^{-1}.

***p*-Cresyl 2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-arabinofuranoside (84):**[239]



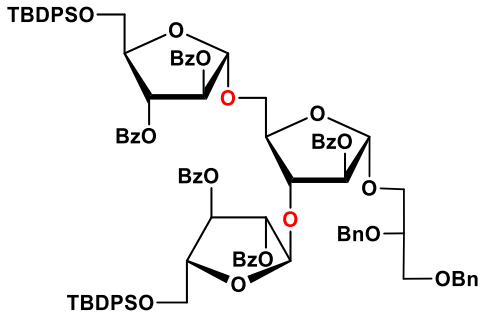
Benzoyl chloride (21.8 g, 18.1 mL, 155 mmol) was added dropwise to a stirred solution of α-D-arabinofuranoside **(83)** (22 g, 4.4 mmol) in anhydrous pyridine (50 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 6 h, when TLC showed no starting material was left. The solvent was evaporated under reduced pressure, to give the residue which was diluted with ethyl acetate (250 mL), and washed with water (2×50 mL), 1 M aq. HCl (2×50 mL), sat. aq. $NaHCO_3$ (1×50 mL) and brine (1×50 mL). The organic layer was dried ($MgSO_4$), then the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to give the title compound **(84)** as a colourless thick oil (22 g, 85%) [MALDI–Found $(M+Na)^+$: 702.1, $C_{42}H_{42}NaO_6SSi$, requires: 702.1], $[\alpha]_D^{20}$-16 (*c* 1.0, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.76 – 7.25 (24H, m), 5.74 (2H, br. d, *J* 5.4 Hz), 5.68 (1H, br. s), 4.64 (1H, br. q, *J* 4.5 Hz), 4.06 (2H, br. d, *J* 4.4 Hz), 2.35 (3H, s), 1.08 (9H, s); δ_C (101 MHz, $CDCl_3$): 165.4, 165.3, 135.7, 135.65, 135.6, 135.5, 133.4, 133.1, 133.0, 132.8, 132.7, 132.4, 129.9, 129.8, 129.7, 129.5, 128.4, 128.3, 128.2, 127.6, 91.3, 83.1, 82.3, 77.6, 63.5, 26.7, 21.1; ν_{max}: 3069, 2929, 2858, 1725, 1493, 1109, 708 cm^{-1}. All data were identical to the authentic sample.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-benzoyl-α-D-arabinofuranoside (85):



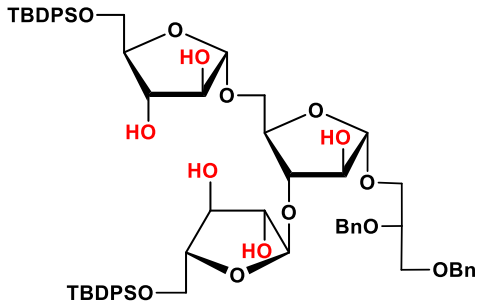
Tetrabutylammonium fluoride (17.8 mL, 0.0616 mol, 1.0 M in THF) was added dropwise to a stirred solution of α-D-arabinofuranoside **(52)** (6.7 g, 0.0089 mol) in anhydrous THF (50 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 4 h, when TLC showed no starting material was left. The mixture was diluted with ethyl acetate (100 mL) and water (50 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with sat. aq. NH_4Cl (50 mL), brine (50 mL), dried ($MgSO_4$) and the solvent was concentrated. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (1:1) to give the title compound **(85)** as a colourless thick oil (4.3 g, 95%) [MALDI–Found $(M+Na)^+$: 531.2; $C_{29}H_{32}NaO_8$, requires: 531.2]; $[\alpha]_D^{23}$ +53 (*c* 4.0, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.98 – 7.93 (2H, m), 7.53 (1H, t, *J* 7.4 Hz), 7.39 (2H, t, *J* 7.7 Hz), 7.33 – 7.17 (10H, m), 5.20 (1H, br.s), 5.04 (1H, br.s), 4.65 (1H, d, *J* 12.0 Hz), 4.61 (1H, d, *J* 12.0 Hz), 4.51 (1H, d, *J* 12.2 Hz), 4.48 (1H, d, *J* 12.2 Hz), 4.10 (2H, br.s), 3.85 (1H, dd, *J* 5.6, 10.3 Hz), 3.80 (1H, m), 3.76 (1H, br.p, *J* 5.2 Hz), 3.72 – 3.66 (1H, m), 3.63 (1H, dd, *J* 4.3, 10.3 Hz), 3.60 – 3.52 (2H, including a broad doublet *J* 4.8 Hz at 3.58), 2.59 – 2.31 (2H, including 2 x OH groups); δ_C (101 MHz, $CDCl_3$): 166.6, 138.3, 138.0, 133.6, 129.8, 129.0, 128.5, 128.4, 128.3, 127.8, 127.6, 105.3, 85.9, 84.2, 76.6, 76.4, 73.4, 72.2, 69.7, 67.1, 61.9; ν_{max}: 3405, 3065, 3031, 2945, 2868,1715, 1465, 1105, 884, 712 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzoyl-α-D-arabinofuranoside (86):



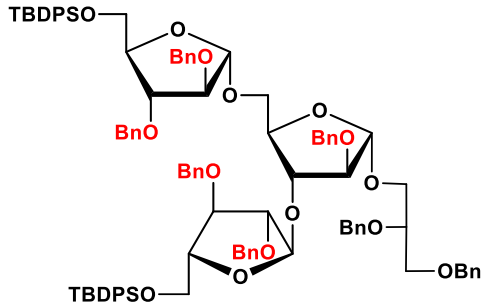
Molecular sieves 4 Å (1.5 g) was added to a stirred solution of the donor *p*-cresyl 2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-arabinofuranoside **(84)** (16.7 g, 28.9 mmol) and the acceptor 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-benzoyl-α-D-arabinofuranoside **(85)** (4.2 g, 8.2 mmol) in dry CH_2Cl_2 (50 mL) at room temperature under nitrogen. The mixture was stirred for 30 min., then cooled to -36 ºC and *N*-iodosuccinimide (9.1 g, 0.037 mol) was added followed by the addition of silvertriflate (2.1 g, 8.2 mmol). The mixture was stirred at the same temperature until the colour turned red/dark brown. When TLC showed no starting material was left, the mixture was quenched by the addition of triethylamine (2 mL) until the colour turned yellow, then diluted with CH_2Cl_2 (100 mL) and filtered through celite. The filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:2) to give the title compound **(86)** as a colourless thick oil (13 g, 91%) [MALDI–Found $(M+Na)^+$: 1682.6679, $C_{99}H_{104}NO_{20}Si_2$, requires: 1682.6685], $[\alpha]_D^{17}$ -1.4 (*c* 2.8, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 8.01 – 7.92 (10H, m), 7.70 – 7.67 (4H, m), 7.65 – 7.61 (4H, m), 7.58 – 7.53 (2H, m), 7.50 – 7.44 (3H, m), 7.41 – 7.21 (32H, m), 5.66 – 5.62 (2H, including a broad doublet *J* 4.8 Hz at 5.63), 5.61 (1H, br.s), 5.55 (1H, br.d, *J* 1.3 Hz), 5.51 (1H, br.d, *J* 0.9 Hz), 5.43 (1H, br.d, *J* 0.9 Hz), 5.31 (1H, br.s), 5.22 (1H, br.s), 4.68 (2H, br.s), 4.53 (1H, d, *J* 12.0 Hz), 4.49 (1H, d, *J* 12.0 Hz), 4.47 (1H, br.s), 4.40 (1H, dd, *J* 5.2, 9.7 Hz), 4.38 – 4.32 (2H, including a broad double doublet *J* 5.8, 10.5 Hz at 4.36), 4.04 (1H, dd, *J* 4.9, 11.4 Hz), 3.95 – 3.89 (5H, m), 3.85 (1H, dd, *J* 2.2, 11.6 Hz), 3.81 (1H, dd, *J* 5.1, 10.1 Hz), 3.68 (1H, dd, *J* 4.7, 10.4 Hz), 3.65 – 3.59 (2H, including a broad triplet *J* 4.7 Hz at 3.63), 1.00 (9H, s), 0.96 (9H, s); δ_C (101 MHz, $CDCl_3$): 165.5, 165.4, 165.2, 165.1, 138.6, 138.3, 135.7, 135.6, 135.5, 133.3, 133.2, 133.15, 133.1, 133.0, 130.0, 129.9, 129.8, 129.75, 129.7, 129.6, 129.35, 129.3, 129.25, 129.2, 128.4, 128.35, 128.3, 128.25, 128.2, 127.8, 127.7, 127.6, 127.55, 127.5, 127.4, 126.3, 106.1, 105.2, 83.7, 83.4, 82.3, 82.2, 82.1, 81.8, 80.5, 77.2, 76.6, 73.3, 72.2, 70.1, 67.2, 66.1, 63.3, 26.7, 26.6, 19.3, 19.2; ν_{max}: 3069, 3010, 2932, 2857, 1723, 1602, 1452, 1072, 706 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-α-D-rabinofuranoside (87):



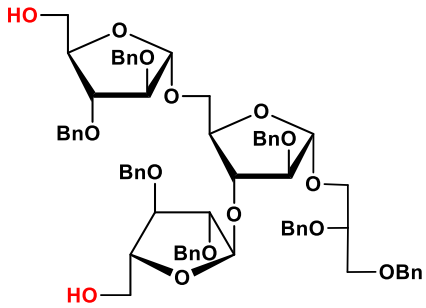
A solution of sodium methoxide (25 mL, 1M, in methanol) was added to a stirred solution of α-D-arabinofuranoside **(86)** (9.1 g, 5.4 mmol) in dry CH_2Cl_2:MeOH (1:1, 50 mL) at room temperature until a PH of 11 was obtained. The mixture was stirred at room temperature for 90 min. When TLC showed no starting material was left, the mixture was neutralized by the addition of acetic acid. The solvent was evaporated under reduced pressure to give an oily residue. The residue was purified by column chromatography on silica eluting with chloroform/methanol (1:1) to give the title compound **(87)** as a colourless thick oil (5.1 g, 83%) [MALDI–Found $(M+Na)^+$: 1167.4913, $C_{64}H_{80}NaO_{15}Si_2$, requires: 1167.4928], $[\alpha]_D^{17}$ +35 (*c* 6.7, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.69 – 7.62 (7H, m), 7.51 – 7.18 (23H, m), 5.18 (1H, br.s), 5.12 (1H, br.s), 4.93 (1H, br.s), 4.67 (1H, d, *J* 12.1 Hz), 4.63 (1H, d, *J* 12.1 Hz), 4.55 (1H, d, *J* 12.1 Hz), 4.51 (1H, d, *J* 12.1 Hz), 4.18 (1H, br.d, *J* 3.4 Hz), 4.16 (1H, br.dd, *J* 3.7, 6.1 Hz), 4.12 (1H, br.d, *J* 3.8 Hz), 4.07 (2H, br.s), 4.03 – 3.98 (2H, br.m), 3.98 – 3.93 (2H, including a broad doublet *J* 2.0 Hz at 3.96), 3.80 (1H, dd, *J* 3.3, 8.8 Hz), 3.78 – 3.68 (4H, m), 3.67 – 3.64 (1H, m), 3.63 – 3.59 (2H, including a broad double doublet *J* 3.0, 8.1 Hz at 3.60), 3.57 (1H, dd, *J* 5.2, 9.9 Hz), 3.02 – 2.55 (5H, br.m), 1.05 (9H, s), 1.03 (9H, s); δ_C (101 MHz, $CDCl_3$): 138.4, 138.2, 135.6, 135.5, 131.9, 131.8, 131.7, 131.6, 130.2, 130.1, 130.0, 128.4, 128.35, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.55, 127.5, 108.6, 108.4, 108.3, 87.8, 87.4, 83.7, 82.3, 79.5, 78.8, 78.5, 77.8, 77.7, 76.7, 76.6, 73.3, 71.9, 69.7, 67.0, 66.0, 64.0, 63.8, 26.7, 26.6, 19.0, 18.9; ν_{max}: 3418, 3071, 2933, 2858, 1454, 1053, 822 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (88):



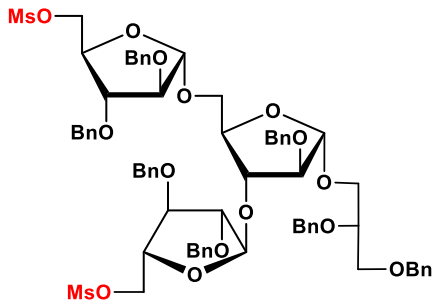
A solution of α-D-rabinofuranoside **(87)** (5.0 g, 4.0 mmol) in dry DMF was added dropwise to a stirred suspension of NaH (1.0 g, 43 mmol) (60% w/w, dispersion in mineral oil) at room temperature under nitrogen. The mixture was stirred for 10 min then benzyl bromide (5.2 g, 3.6 mL, 30 mmol) in dry DMF (2 mL) was added. The mixture was stirred at room temperature for 6 h. When TLC showed no starting material was left. The reaction mixture was quenched by slow addition of methanol (2 mL), and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×50 mL). The combined organic layers were washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:1) to give the title compound **(88)** as a colourless thick oil (4.5 g, 65%) [MALDI–Found $(M+Na)^+$: 1617.7, $C_{99}H_{110}NaO_{15}Si_2$, requires: 1617.7], $[\alpha]_D^{17}$ +43 (*c* 1.9, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.71 – 7.57 (8H, m), 7.48 – 7.13 (47H, m), 5.18 (2H, br.d, *J* 2.2 Hz), 5.07 (1H, br.s), 4.70 (1H, d, *J* 12.0 Hz), 4.66 (1H, d, *J* 12.0 Hz), 4.58 – 4.50 (7H, m), 4.47 (2H, d, *J* 11.9 Hz), 4.46 (1H, d, *J* 11.9 Hz), 4.39 (1H, d, *J* 11.9 Hz), 4.38 (1H, d, *J* 11.9 Hz), 4.30 (1H, br.dd, *J* 2.8, 6.8 Hz), 4.18 (2H, br.m), 4.13 – 4.07 (4H, m), 4.06 – 3.99 (2H, including a broad double doublet *J* 4.2, 10.6 Hz at 4.03), 3.95 (1H, dd, *J* 4.7, 11.9 Hz), 3.87 (1H, dd, *J* 5.0, 10.5 Hz), 3.84 – 3.80 (2H, including a broad double doublet *J* 4.0, 11.0 Hz at 3.82), 3.79 – 3.74 (4H, m), 3.68 – 3.58 (3H, m), 1.03 (18H, s); δ_C (101 MHz, $CDCl_3$): 138.6, 138.3, 138.2, 138.0, 137.9, 137.6, 137.5, 135.75, 135.67, 135.65, 135.6, 133.55, 133.5, 133.4, 133.3, 129.6, 129.55, 129.5, 128.4, 128.35, 128.3, 128.25, 128.2, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 127.45, 127.4, 106.6, 106.4, 105.4, 88.6, 88.5, 88.0, 83.0, 82.8, 82.3, 81.8, 81.3, 80.3, 73.4, 72.2, 72.0, 71.9, 71.8, 71.7, 71.6, 70.4, 67.0, 66.0, 63.4, 63.2, 26.8, 26.7, 19.3, 19.2; ν_{max}: 3067, 3031, 2929, 2857, 1495,1455, 1111, 698 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (89):



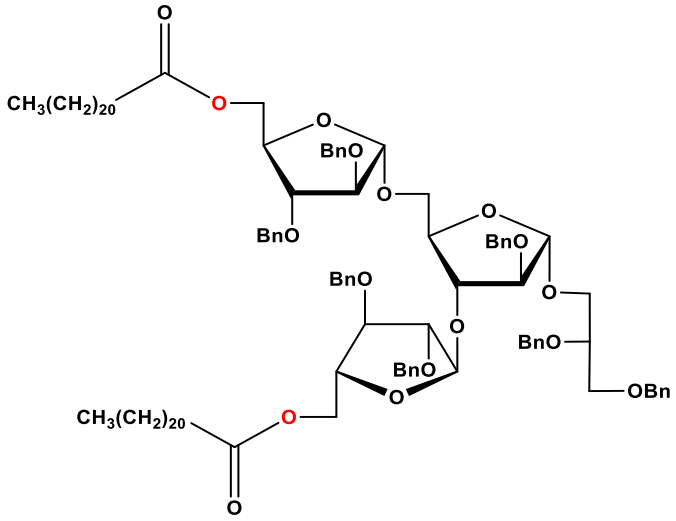
Tetrabutylammonium fluoride (14.3 mL, 0.0493 mol, in 1.0 M THF) was added dropwise to a stirred solution of α-D-arabinofuranoside **(88)** (3.8 g, 2.3 mmol) in anhydrous THF (25 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 8 h then TLC showed no starting material was left, the mixture was diluted with ethyl acetate (100 mL) washed with sat. aq. NH_4Cl (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated to give the residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to give to the title compound **(89)** as a colourless thick oil (2.3 g, 87%) [MALDI–Found $(M+Na)^+$: 1141.5, $C_{67}H_{74}NaO_{15}$, requires: 1141.5], $[\alpha]_D^{17}$+53 (*c* 4.7, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.34 – 7.16 (35H, m), 5.10 (1H, br.s), 5.07 (1H, br.s), 5.04 (1H, br.d, *J* 0.7 Hz), 4.64 (2H, br.s), 4.55 – 4.39 (11H, m), 4.31 (1H, d, *J* 11.7 Hz), 4.27 (1H, dd, *J* 3.8, 7.4 Hz), 4.23 – 4.17 (1H, m), 4.07 (1H, br.d, *J* 2.2 Hz), 4.06 (1H, br.dd, *J* 4.0, 7.6 Hz), 4.02 (1H, br.dd, *J* 2.2, 5.9 Hz), 3.98 (1H, br.dd, *J* 1.3, 3.8 Hz), 3.96 (1H, br.dd, *J* 1.2, 3.6 Hz), 3.88 (1H, br.dd, *J* 4.1, 12.3 Hz), 3.85 (1H, br.dd, *J* 3.2, 6.5 Hz), 3.82 (1H, d, *J* 5.2 Hz), 3.78 (1H, dd, *J* 3.7, 7.4 Hz), 3.76 – 3.72 (2H, m), 3.71 – 3.64 (2H, including a broad double doublet *J* 12.3, 2.4 Hz at 3.68), 3.61 (1H, dd, *J* 4.8, 7.3 Hz), 3.59 – 3.55 (3H, including a broad doublet *J* 5.1 Hz at 3.58), 3.53 (1H, dd, *J* 5.9, 12.3 Hz); δ_C (101 MHz, $CDCl_3$): 138.5, 138.2, 137.7, 137.6, 137.5, 137.4, 137.2, 128.5, 128.45, 128.4, 128.35, 128.3, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 127.5, 106.1, 106.0, 105.9, 88.7, 88.3, 87.4, 83.0, 82.9, 82.4, 81.9, 80.8, 79.8, 73.4, 72.3, 72.25, 72.2, 72.0, 71.9, 71.8, 70.2, 67.2, 64.8, 62.7; ν_{max}: 3459, 3064, 3030, 2921, 2860, 1605, 1496, 1115, 820 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-methanesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-methanesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (90):



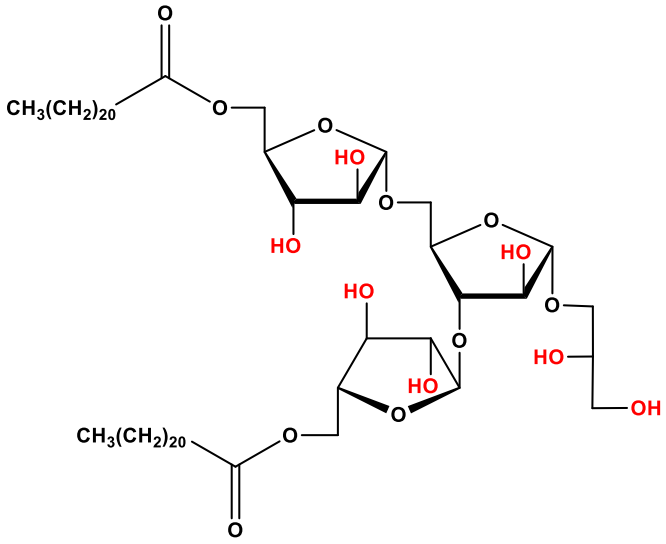
Methanesulfonyl chloride (0.57 g, 0.40 mL, 0.0050 mol) and DMAP (0.05g, 0.43 mmol) were added to a stirred solution of α-D-arabinofuranoside **(89)** (0.56 g, 0.50 mmol) in dry pyridine (5 mL) under nitrogen at room temperature. The mixture was stirred for 16 h then TLC showed no starting material was left. The mixture was quenched by the addition of water (1 mL), the organic layer was separated by decanting and diluted with CH_2Cl_2 (10 mL). The mixture was washed with 1N aq. HCl (2×10 mL), sat. aq. $NaHCO_3$ (2×10 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (3:1) to afford the title compound **(90)** as a colourless thick oil (0.55 g, 87%) [MALDI–Found $(M+Na)^+$: 1297.4; $C_{69}H_{78}NaO_{19}S_2$, requires: 1297.4]; $[\alpha]_D^{17}$+59 (*c* 0.60, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.15 (35H, m), 5.16 (1H, br.s), 5.13 (1H, br.s), 5.07 (1H, br.s), 4.68 (2H, br.s), 4.57 – 4.43 (11H, m), 4.40 (1H, d, *J* 11.3 Hz), 4.38 – 4.34 (1H, m), 4.33 (1H, br.dd, *J* 3.9, 7.3 Hz), 4.30 – 4.26 (2H, including a broad double doublet *J* 3.4, 7.3 Hz at 4.29), 4.24 (1H, d, *J* 5.4 Hz), 4.22 – 4.12 (2H, m), 4.11 – 4.07 (2H, m), 4.02 – 3.97 (2H, m), 3.91 – 3.84 (4H, m), 3.80 (1H, br.p, *J* 4.8 Hz), 3.73 (1H, br.dd, *J* 1.5, 11.5 Hz), 3.67 – 3.59 (3H, including a broad double doublet *J* 4.8, 8.7 Hz at 3.64), 2.94 (3H, s), 2.89 (3H, s); δ_C (101 MHz, $CDCl_3$): 138.5, 138.2, 137.4, 137.3, 137.2, 137.0, 128.6, 128.55, 128.5, 128.45, 128.4, 128.3, 128.1, 128.05, 128.0, 127.95, 127.9, 127.7, 127.65, 127.6, 127.55, 106.5, 106.1, 105.8, 88.3, 87.7, 87.5, 82.9, 82.8, 80.3, 80.2, 79.3, 79.2, 77.2, 73.4, 72.35, 72.3, 72.2, 72.1, 72.0, 71.9, 70.2, 68.8, 68.5, 67.2, 65.2, 37.6, 37.5; ν_{max}: 3088, 3065, 3031, 2933, 2871, 1586, 1454, 1177, 745 cm^{-1}.

2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-behenate-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-behenate-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (91):



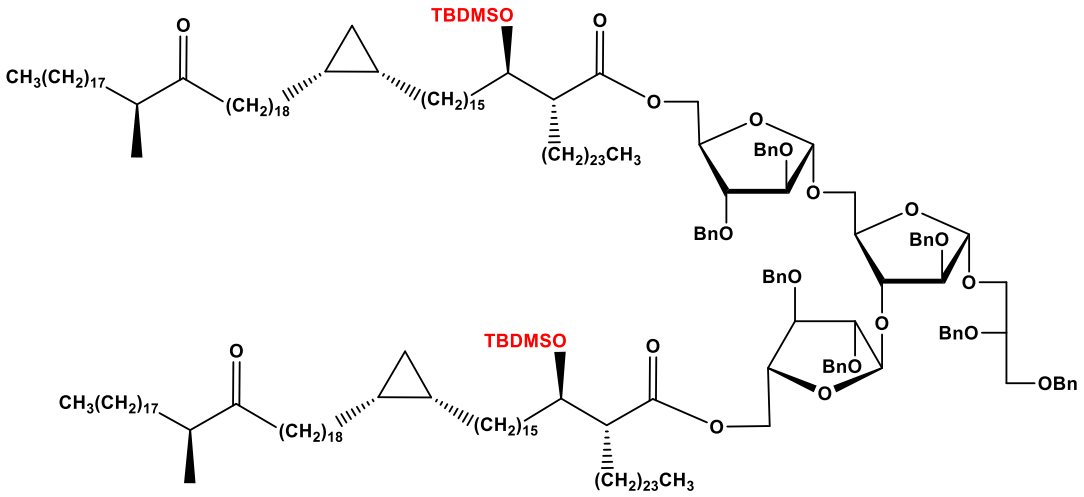
Cesium hydrogencarbonate (0.076 g, 0.39 mmol) was added to a stirred solution of α-D-arabinofuranoside **(90)** (0.05 g, 0.03 mmol) and behenic acid (0.03 g, 0.09 mmol) in dry DMF:THF (1:5, 1 mL) at room temperature under nitrogen. The mixture was stirred at 70 ºC for 3 days, then TLC showed no starting material was left. The suspension was diluted with ethyl acetate (25 mL) and water (5 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with water (5 mL) and brine (5 mL), dried ($MgSO_4$) and filtered. The filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (3:1) to afford the title compound **(91)** as a colourless thick oil (55 mg, 80%) [MALDI–Found $(M+Na)^+$: 1786.1, $C_{111}H_{158}NaO_{17}$, requires: 1786.1], $[\alpha]_D^{22}$ +36 (*c* 1.0, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.31 – 7.11 (35H, m), 5.09 (1H, br.s), 5.06 (1H, br.s), 4.97 (1H, br.s), 4.59 (2H, br.s), 4.51 – 4.35 (10H, m), 4.33 (1H, d, *J* 11.8 Hz), 4.26 (1H, d, *J* 11.8 Hz), 4.20 (1H, br.dd, *J* 3.3, 7.3 Hz), 4.12 (6H, br.m), 4.04 (1H, br.dd, *J* 2.8, 6.9 Hz), 4.00 (1H, br.d, *J* 2.5 Hz), 3.95 – 3.89 (2H, m), 3.84 (1H, dd, *J* 4.3, 11.8 Hz), 3.81 – 3.76 (2H, including a broad double doublet *J* 4.1, 8.5 Hz at 3.78), 3.75 (1H, br.d, *J* 3.4 Hz), 3.74 – 3.68 (1H, p, *J* 5.0 Hz), 3.66 (1H, br.dd, *J* 2.1, 11.7 Hz), 3.58 – 3.49 (3H, including a broad quartet *J* 4.6 Hz at 3.53), 2.17 (4H, t, *J* 7.6 Hz), 1.56 – 1.00 (76H, m), 0.81 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 173.5, 138.6, 138.3, 137.7, 137.6, 137.5, 137.4, 137.3, 128.5, 128.4, 128.35, 128.3, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.5, 106.5, 106.2, 105.5, 88.3, 88.2, 88.1, 83.4, 83.3, 80.8, 80.3, 79.2, 78.9, 76.9, 73.4, 72.3, 72.2, 72.1, 72.0, 71.9, 71.7, 70.3, 67.1, 65.6, 63.3, 63.2, 34.1, 34.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.35, 29.3, 29.2, 24.8, 22.7, 14.1; ; ν_{max}: 3064, 3031, 2923, 2853, 1740, 1718, 1455, 1066, 698 cm^{-1}.

L-glycerol-(1'→1)-5-*O*-behenate-α-D-arabinofuranosyl-(1→3)-5-*O*-behenate-α-D-arabinofuranosyl-(1→5)-α-D-arabinofuranoside (92):



Palladium hydroxide on activated charcoal ($Pd(OH)_2$-C/20%, 60 mg, 1.1 fold by weight) was added to a stirred solution of α-D-arabinofuranoside **(91)** (55 mg, 31 mmol) in CH_2Cl_2:MeOH (2:1, 2 mL) at room temperature under hydrogen. The mixture was stirred for 36 h. When TLC showed no starting material was left, the mixture was filtered off through celite and the solvent was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (10:1) to give **(92)** as a colourless thick oil (29 mg, 82%) [MALDI–Found $(M+Na)^+$: 1155.8; $C_{62}H_{116}NaO_{17}$, requires: 1155.8]; $[\alpha]_D^{18}$-21 (*c* 1.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 5.01 (1H, br.s), 4.97 (1H, br.s), 4.90 (1H, br.s), 4.28 – 4.20 (2H, including abroad double doublet *J* 3.2, 11.7 Hz at 4.24), 4.19 – 4.13 (3H, including abroad double doublet *J* 5.0, 11.8 Hz at 4.17), 4.12 (1H, br.d, *J* 4.3 Hz), 4.11 – 4.08 (1H, m), 4.04 (1H, br.q, *J* 5.5 Hz), 4.01 – 3.95 (3H, br.m), 3.94 (1H, dd, *J* 3.6, 11.5 Hz), 3.83 – 3.76 (3H, m), 3.73 (1H, br.dd, *J* 4.8, 10.1 Hz), 3.67 – 3.62 (1H, m), 3.62 – 3.57 (2H, including a broad doublet *J* 3.1 Hz at 3.60), 3.56 – 3.51 (1H, m), 2.30 (4H, t, *J* 7.6 Hz), 1.64 – 1.02 (83H, m), 0.83 (6 H, t, *J* 6.5 Hz); δ_C (101 MHz, $CDCl_3$): 174.05, 174.0, 108.0, 107.7, 83.3, 83.0, 82.4, 81.8, 81.3, 80.7, 79.0, 77.6, 75.8, 69.9, 69.1, 66.4, 63.9, 63.7, 63.5, 34.0, 33.95, 31.8, 29.6, 29.5, 29.45, 29.4, 29.3, 29.25, 29.2, 29.1, 29.0, 24.8, 24.7, 22.6, 13.9.; ν_{max}: 3374, 2920, 2852, 1730, 1723, 1180, 757 cm^{-1}.

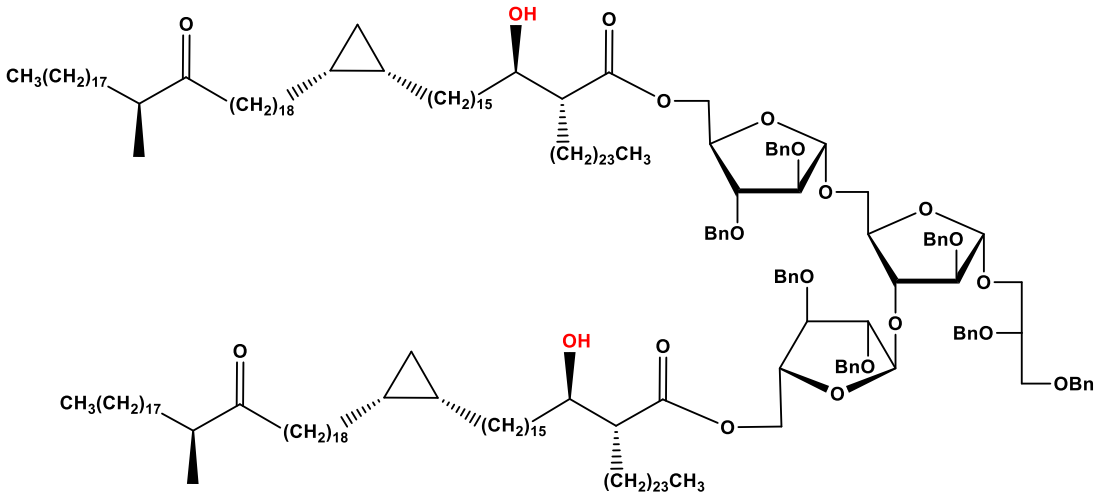
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-(1-((*tert*-butyldimethyl-silyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl) hexadecyl) hexacosanoate-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-(1-((*tert*-butyl -dimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl) hexadecyl)hexacosanoate-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (93):



A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (60 mg, 0.31 mmol), in dry CH_2Cl_2 (1 mL) was added to a stirred solution of α-D-arabinofuranoside **(89)** (35 mg, 0.031 mmol), molecular sieves 4 Å (50 mg), DMAP (38 mg, 0.31) (2*R*)-2-(1-((*tert*-butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl) hexadecyl) hexacosanoic acid **(76f)** (85 mg, 0.062 mmol) in dry CH_2Cl_2 (1 mL) at room temperature under nitrogen. The mixture was stirred for 4 days. When TLC showed no starting material was left. The precipitate was filtered off and washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethylacetate (10:1) to afford the title compound **(93)** as a colourless thick oil (60 mg, 51%) [MALDI–Found $(M+Na)^+$: 3808.2; $C_{247}H_{426}NaO_{21}Si_2$, requires: 3808.2]; $[\alpha]_D^{20}$ +18 (*c* 5.0, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.69 – 6.95 (35H, m), 5.16 (1H, br.s), 5.13 (1H, br.s), 5.04 (1H, br.s), 4.68 (1H, d, *J* 12.1 Hz), 4.65 (1H, d, *J* 12.1 Hz), 4.58 – 4.45 (9H, m), 4.45 (1H, d, *J* 11.9 Hz), 4.38 (1H, d, *J* 11.9 Hz), 4.33 (1H, d, *J* 11.8 Hz), 4.29 – 4.22 (5H, including a broad double doublet *J* 4.0, 9.1 Hz at 4.25), 4.21 – 4.16 (2H, m), 4.13 (1H, br.dd, *J* 5.7, 8.2 Hz), 4.06 (1H, br.d, *J* 2.7 Hz), 4.01 – 3.96 (2H, m), 3.94 – 3.87 (5H, m), 3.85 (1H, br.dd, *J* 4.8, 10.7 Hz), 3.78 (1H, br.p, *J* 5.1 Hz), 3.76 – 3.71 (1H, m), 3.65 – 3.56 (3H, including a broad double doublet *J* 4.8, 8.2 Hz at 3.60), 2.53 (4H, including sextet, *J* 5.4 Hz),

2.42 (4H, t, *J* 7.6 Hz), 1.68 – 1.11 (288H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.71 – 0.61 (4H, m), 0.57 (2H, dt, *J* 4.0, 8.5 Hz), 0.03 (6H, s), 0.01 (6H, s), -0.32 (2H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 174.3, 174.2, 138.5, 138.2, 137.8, 137.7, 137.6, 137.4, 137.3, 128.5, 128.45, 128.4, 128.35, 128.3, 128.2, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 106.6, 106.3, 105.2, 88.3, 88.2, 87.8, 83.7, 83.6, 81.2, 80.2, 79.4, 78.9, 77.2, 73.4, 73.1, 72.3, 72.2, 72.0, 71.9, 71.6, 70.3, 67.0, 65.9, 63.0, 62.7, 51.6, 46.3, 41.1, 33.6, 33.5, 33.0, 31.9, 30.3, 30.2, 29.9, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 28.7, 27.9, 27.8, 27.3, 27.2, 27.1, 25.8, 24.0, 23.7, 22.7, 22.6, 18.0, 16.4, 15.8, 14.1, 10.9, -4.4, -4.5, -4.7, -4.8; ν_{max}: 3063,3031, 2920, 2851, 1741, 1714, 1467, 1106, 836, 699 cm^{-1}.

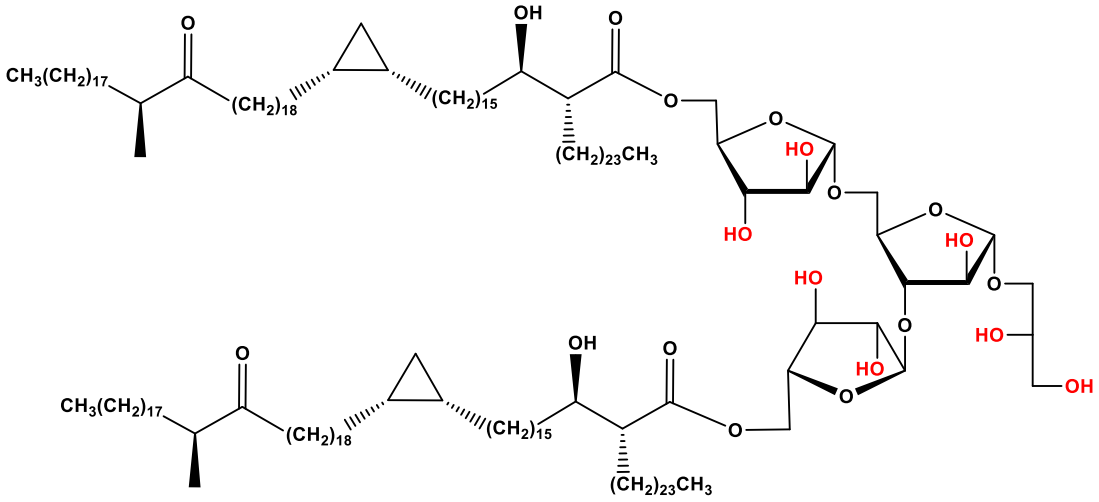
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-(1-hydroxy-16-((1*S*, 2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-((2*R*)-2-(1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl) hexadecyl) hexacosanoate-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (94):



The protected glycolipid α-D-arabinofuranoside **(93)** (53 mg, 0.014 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial equipped with an acid proof rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The mixture was cooled to 0 °C, and then hydrogen fluoride-pyridine complex as (70% w, 1.5 mL) was added dropwise. The mixture was stirred at 43 °C for 24 h. When TLC showed no starting material was left, the mixture was neutralized by pouring it slowly into saturated aqueous solution of $NaHCO_3$ and stirred until no more CO_2 was liberated. The organic layer was

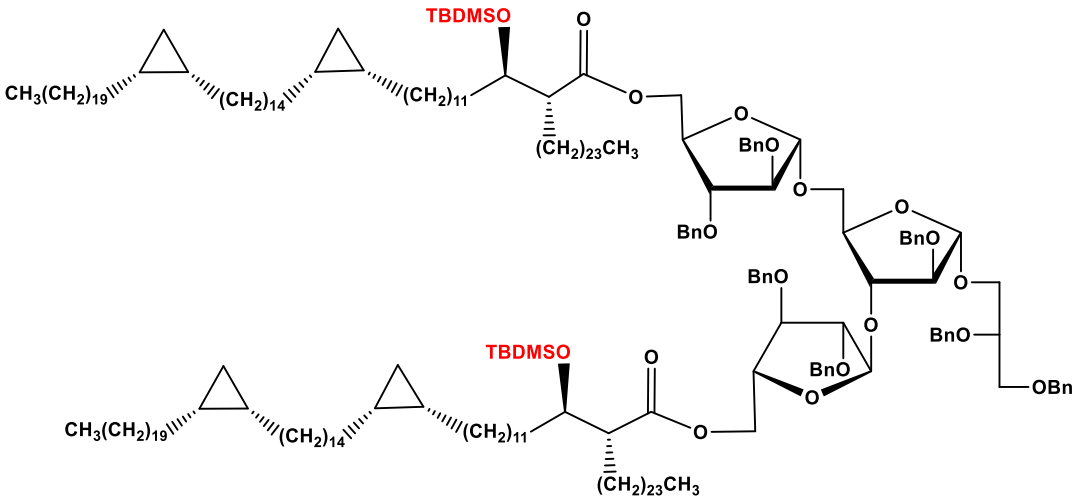
separated and the aqueous layer was re-extracted with chloroform (3×10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated to give the residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) affording compound **(94)** as a colourless thick oil (38 mg, 76%) [MALDI–Found $(M+Na)^+$: 3579.9; $C_{235}H_{398}NaO_{21}$, requires: 3579.9]; $[\alpha]_D^{21}$+19 (*c* 1.2, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.36 – 7.15 (35H, m), 5.16 (1H, br.s), 5.13 (1H, br.s), 5.05 (1H, br.s), 4.67 (2H, br.s), 4.55 (1H, d, *J* 11.8 Hz), 4.53 – 4.47 (7H, m), 4.45 (3H, d, *J* 11.8 Hz), 4.40 (1H, d, *J* 11.8 Hz), 4.36 – 4.22 (7H, m), 4.17 (1H, br.dd, *J* 3.7, 6.9 Hz), 4.11 (1H, br.dd, *J* 3.3, 6.3 Hz), 4.07 (1H, br.d, *J* 2.1 Hz), 4.00 – 3.96 (2H, m), 3.94 – 3.88 (2H, m), 3.88 – 3.82 (2H, including a broad double doublet *J* 4.5, 9.8Hz at 3.86), 3.81 – 3.75 (2H, m), 3.73 (1H, br.d, *J* 11.7 Hz), 3.67 – 3.51 (5H, including a broad doublet *J* 5.3 Hz at 3.61), 2.51 (2H, sextet, *J* 6.7 Hz), 2.42 (6H, including a triplet *J* 7.2 Hz), 2.05 – 1.10 (288H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.7 Hz), 0.69 – 0.61 (4H, m), 0.57 (2H, dt, *J* 4.1, 8.5 Hz), -0.33 (2H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.1, 175.0, 138.6, 138.2, 137.6, 137.5, 137.45, 137.4, 137.2, 128.5, 128.4, 128.35, 128.3, 128.2, 128.1, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 127.5, 106.3, 106.2, 105.5, 88.2, 88.0, 87.9, 83.6, 80.7, 80.3, 79.3, 79.2, 77.2, 73.4, 72.4, 72.2, 72.1, 72.0, 71.7, 70.2, 68.0, 67.1, 65.4, 63.0, 62.9, 51.9, 51.7, 46.3, 41.1, 35.3, 35.2, 33.0, 31.9, 30.3, 30.2, 29.8, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 28.7, 27.5, 27.4, 27.3, 25.7, 25.6, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: 3511, 3063,3030, 2918, 2851, 1737, 1714, 1467, 1105, 754, 698 cm^{-1}.

L-glycerol-(1'→1)-5-*O*-(2*R*)-2-(1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl) cyclopropyl)hexadecyl)hexacosanoate-α-D-arabinofuranosyl-(1→3)-[5-*O*-((2*R*)-2-(1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)cyclopropyl) hexadecyl) hexacosanoate-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (95):



Palladium hydroxide on activated charcoal ($Pd(OH)_2$-C/20%, 40 mg, 1.1 fold by weight) was added to a stirred solution of α-D-arabinofuranoside **(94)** (36 mg, 0.010 mmol) in CH_2Cl_2:MeOH (2:1, 2 mL) at room temperature under hydrogen. The mixture was stirred for 36 h. When TLC showed no starting material was left, the mixture was filtered off through celite and the solvent was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (10:1) affording compound **(95)** as a colourless thick oil (25 mg, 86%) [MALDI–Found $(M+Na)^+$: 2949.7; $C_{186}H_{356}NaO_{21}$, requires: 2949.7]; $[\alpha]_D^{18}$ +19 (*c* 2.2, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 5.00 (1H, br.s), 4.96 (1H, br.s), 4.90 (1H, br.s), 4.41 (1H, dd, *J* 4.2, 11.6 Hz), 4.36 (1H, dd, *J* 5.1, 11.9 Hz), 4.26 (1H, dd, *J* 11.9, 5.4 Hz), 4.21 (1H, d, *J* 4.0 Hz), 4.17 (1H, dd, *J* 3.8, 10.3 Hz), 4.12 (1H, br.d, *J* 4.8 Hz), 4.08 (1H, br.q, *J* 6.6 Hz), 4.04 (1H, d, *J* 5.1 Hz), 4.01 – 3.96 (3H, m), 3.92 (1H, dd, *J* 3.8, 8.0 Hz), 3.90 – 3.86 (2H, m), 3.77 (1H, dd, *J* 2.7, 8.4 Hz), 3.72 (1H, d, *J* 5.1 Hz), 3.62 (5H, br.m), 3.54 (1H, dd, *J* 2.6, 10.5 Hz), 2.52 – 2.44 (2H, m), 2.38 (6H, including a triplet, *J* 7.5 Hz), 1.65 – 1.05 (207 H, m), 1.01 (6H, d, *J* 6.9 Hz), 0.84 (12H, t, *J* 6.8 Hz), 0.66 – 0.57 (4H, m), 0.52 (2H, dt, *J* 4.2, 8.2 Hz), -0.37 (2H, br.q, *J* 4.7 Hz); δ_C (101 MHz, $CDCl_3$): 216.2, 175.05, 174.9, 109.2, 108.7, 81.95, 81.2, 79.4, 78.0, 77.6, 77.2, 76.5, 72.5, 71.9, 65.0, 63.5, 63.4, 61.5, 52.6, 35.0, 32.8, 31.8, 30.1, 29.6, 29.55, 29.5, 29.4, 29.3, 29.25, 29.2, 28.9, 28.6, 27.3, 27.2, 26.1, 25.3, 23.5, 22.6, 16.1, 15.7, 14.0, 10.8.; ν_{max}: 3511, 3063,3030, 2918, 2851, 1737, 1714, 1467, 1105, 754, 698 cm^{-1}.

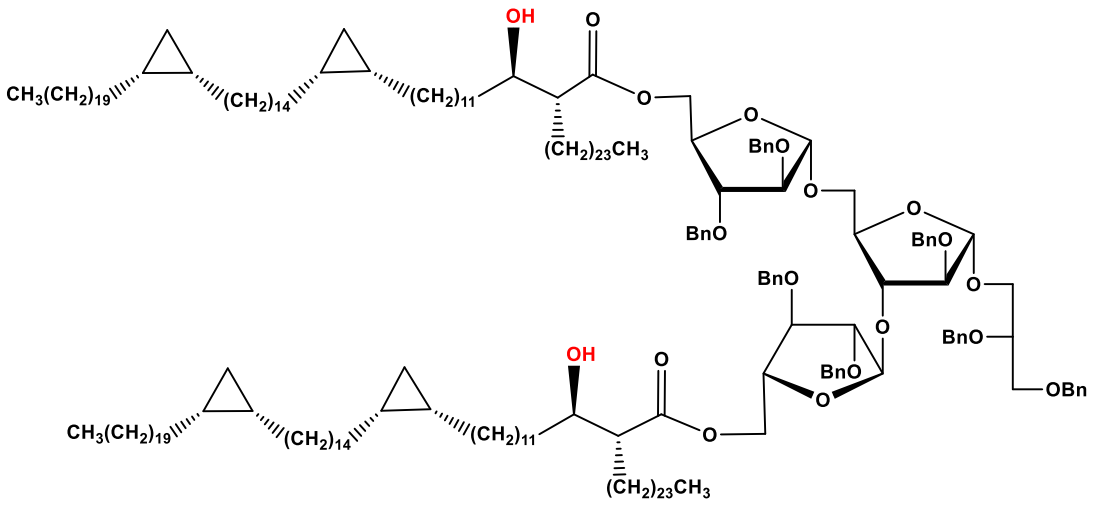
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-((*tert*-butyl dimethyl silyl)oxy)-12-((2*R*)-2-(14-((2*R*)-2-icosylcyclopropyl)tetradecyl) cyclopropyl) dodecyl) hexacosanoate-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-((2*R*)-2-(14-((2*R*)-2-icosylcyclopropyl)tetradecyl) cyclopropyl) dodecyl) hexacosanoate-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (96):



A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (48 mg, 0.25 mmol) in dry CH_2Cl_2 (1 mL) was added to a stirred solution of α-D-arabinofuranoside **(89)** (28 mg, 0.025 mmol), molecular sieves 4 Å (50 mg), DMAP (30 mg, 0.24 mmol) and (2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-((2*R*)-2-(14-((2*R*)-2-icosylcyclopropyl) tetradecyl) cyclopropyl) dodecyl)hexacosanoic acid **(76g)** (62 mg, 0.049 mmol) in dry CH_2Cl_2 (1 mL) at room temperature under nitrogen. The mixture was stirred for 4 days. When TLC showed no starting material was left. The precipitate was filtered off and washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethylacetate (10:1) to afford the title compound **(96)** as a colourless thick oil (75 mg, 84%) [MALDI–Found $(M+Na)^+$: 3607.9; $C_{235}H_{402}NaO_{19}Si_2$, requires: 3607.9]; $[\alpha]_D^{22}$ +20 (*c* 0.90, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.94 – 6.85 (35H, m), 5.16 (1H, br.s), 5.13 (1H, br.s), 5.04 (1H, br.s), 4.68 (1H, d, *J* 12.1 Hz), 4.65 (1H, d, *J* 12.1 Hz), 4.57 – 4.45 (9H, m), 4.45 (1H, d, *J* 11.8 Hz), 4.38 (1H, d, *J* 11.8 Hz), 4.32 (1H, d, *J* 11.8 Hz), 4.28 – 4.20 (5H, m), 4.18 (1H, br.dd, *J* 3.6, 7.4 Hz), 4.15 – 4.09 (1H, m), 4.06 (1H, br.d, *J* 2.9 Hz), 4.01 – 3.96 (2H, m), 3.90 (3H, br.m), 3.87 – 3.81 (2H, including a broad double doublet *J* 4.8, 10.7 Hz at 3.85), 3.78 (1H, br.p, *J* 5.1 Hz), 3.76 – 3.71 (1H, m), 3.64 – 3.49 (3H, including a broad double doublet *J* 4.8, 8.3 Hz at 3.60), 2.60 – 2.47 (2H, m), 1.67 – 1.06

(270H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.74 – 0.62 (8H, m), 0.57 (4H, td, *J* 4.1 Hz), 0.02 (6H, s), 0.00 (6H, s), -0.33 (4H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$): 174.3, 174.2, 138.5, 138.3, 137.8, 137.7, 137.6, 137.4, 137.3, 128.5, 128.45, 128.4, 128.35, 128.3, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 106.6, 106.3, 105.2, 88.3, 88.2, 87.8, 83.7, 83.6, 81.2, 80.2, 79.4, 78.9, 77.2, 73.4, 73.1, 72.3, 72.2, 71.9, 71.6, 70.3, 67.0, 65.9, 63.0, 62.7, 51.6, 33.6, 33.5, 31.9, 30.3, 30.2, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.7, 27.9, 27.8, 27.2, 27.1, 25.9, 25.8, 24.0, 22.7, 18.0, 15.8, 14.1, 11.0, 10.9, -4.4, -4.5, -4.7, -4.8; ν_{max}: 3063, 2925, 2854, 1737, 1456, 1101, 770 cm^{-1}.

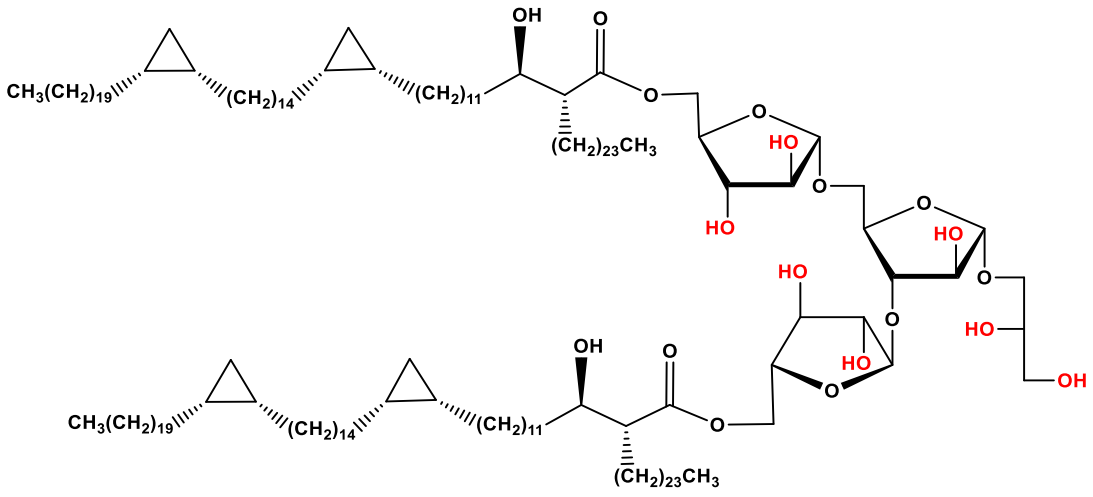
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-12-((2 *R*)-2-(14-((2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-12-((2*R*)-2-(14-((2*R*)-2-icosylcyclopropyl)tetradecyl) cyclopropyl) dodecyl)hexacosanoate-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (97):



The protected glycolipid α-D-arabinofuranoside **(96)** (70 mg, 0.019 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial equipped with an acid proof rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The mixture was cooled to 0 °C, and then hydrogen fluoride-pyridine complex as (70% w, 1.5 mL) was added dropwise. The mixture was stirred at 43 °C for 24 h. When TLC showed no starting material was left, the mixture was neutralized by pouring it slowly into sat. aq. $NaHCO_3$ and stirred until no more CO_2 was liberated. The organic layer was separated and the aqueous layer was re-extracted with chloroform (3×10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated to give the residue which was purified by column

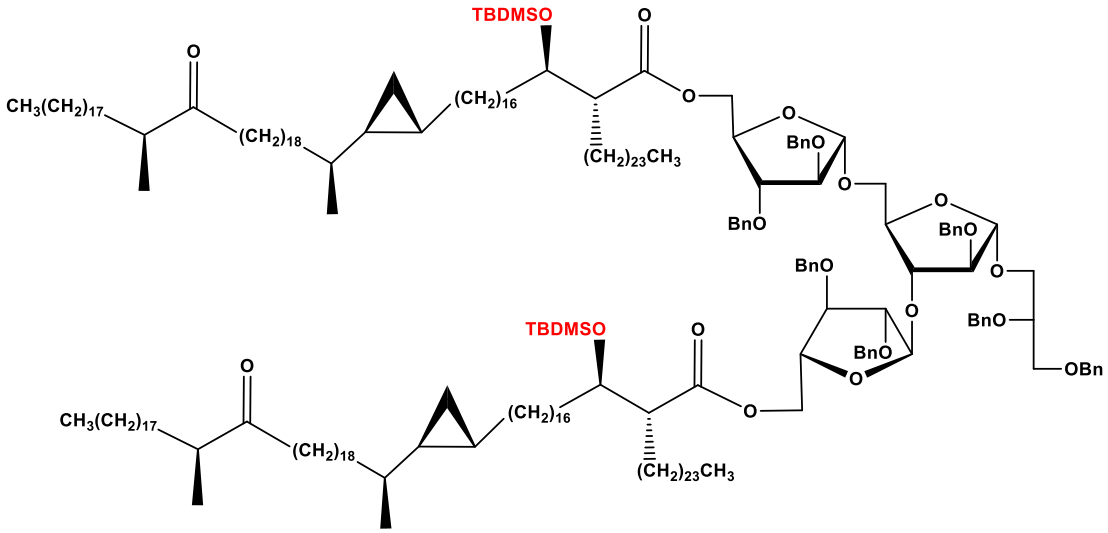
chromatography on silica eluting with hexane/ethyl acetate (10:1) affording compound **(97)** as a colourless thick oil (33 mg, 51%) [MALDI–Found $(M+Na)^+$: 3379.8; $C_{223}H_{374}NaO_{19}$, requires: 3379.8]; $[\alpha]_D^{21}$ +22 (*c* 0.90, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.17 (35H, m), 5.16 (1H, br.s), 5.13 (1H, br.s), 5.05 (1H, br.s), 4.67 (2H, br.s), 4.55 (1H, d, *J* 11.9 Hz), 4.53 – 4.47 (8H, m), 4.46 (2H, d, *J* 11.8 Hz), 4.41 (1H, d, *J* 11.9 Hz), 4.35 – 4.23 (6H, m), 4.17 (1H, br.dd, *J* 3.7, 6.9 Hz), 4.15 – 4.08 (3H, including a broad triplet *J* 7.0 Hz at 4.12), 4.07 (1H, br.d, *J* 2.1 Hz), 4.00 – 3.95 (2H, m), 3.94 – 3.87 (2H, m), 3.95 – 3.87 (2H, including a broad double doublet *J* 4.6, 10.3 Hz at 3.86), 3.79 (1H, p, *J* 4.8 Hz), 3.75 – 3.69 (1H, m), 3.67 – 3.55 (5H, including a broad doublet *J* 5.3 Hz at 3.61), 2.44 – 2.37 (2H, m), 1.67 – 1.03 (271H, m), 0.89 (12H, t, *J* 7.1 Hz), 0.69 – 0.61 (8H, m), 0.57 (4H, dt, *J* 4.2, 8.5 Hz), -0.32 (4H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 175.0, 138.5, 138.2, 137.6, 137.5, 137.4, 137.4, 137.2, 128.5, 128.4, 128.35, 128.3, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 127.5, 106.3, 106.2, 105.5, 88.2, 88.0, 87.9, 83.6, 80.7, 80.3, 79.3, 79.2, 77.2, 73.4, 72.4, 72.2, 72.0, 71.9, 71.7, 70.3, 67.1, 65.4, 63.1, 63.0, 62.0, 51.9, 51.8, 35.3, 35.2, 34.2, 31.9, 30.3, 30.2, 29.8, 29.75, 29.7, 29.65, 29.6, 29.5, 29.4, 28.7, 27.5, 27.4, 25.8, 25.7, 24.9, 22.7, 22.6, 20.4, 15.8, 14.1, 10.9; ν_{max}: 3511, 3062, 2921, 2854, 1733,1725, 1456, 1115, 754 cm^{-1}.

L-glycerol-(1'→1)-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-12-((2*R*)-2-(14-((2*R*)-2-icosylcyclopropyl) tetradecyl) cyclopropyl)dodecyl)hexacosanoate-α-D-arabinofuranosyl-(1→3)-[5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-12-((2*R*)-2-(14-((2*R*)-2-icosylcyclopropyl)tetradecyl) cyclopropyl) dodecyl) hexacosanoate-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (98):



Palladium hydroxide on activated charcoal ($Pd(OH)_2$-C/20%, 33 mg, 1.1 fold by weight) was added to a stirred solution of α-D-arabinofuranoside **(97)** (30 mg, 0.0089 mmol) in CH_2Cl_2:MeOH (2:1, 2 mL) at room temperature under hydrogen. The mixture was stirred for 36 h. When TLC showed no starting material was left, the mixture was filtered off through celite and the solvent was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (10:1) affording compound **(98)** as a colourless thick oil (22 mg, 88%) [MALDI–Found $(M+Na)^+$: 2749.5; $C_{174}H_{332}NaO_{19}$, requires: 2749.5]; $[\alpha]_D^{18}$ +18 (*c* 2.2, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 5.00 (1H, br.s), 4.96 (1H, br.s), 4.90 (1H, br.s), 4.41 (1H, dd, *J* 4.6, 11.7 Hz), 4.37 (1H, br.dd, *J* 5.1, 11.9 Hz), 4.26 (1H, dd, *J* 5.0, 11.7 Hz), 4.20 (1H, dd, *J* 4.7, 11.8 Hz), 4.16 (1H, br.q, *J* 4.8 Hz), 4.12 (1H, br.d, *J* 6.2 Hz), 4.10 – 4.06 (2H, including a broad triplet *J* 7.4 Hz at 4.08), 4.05 (1H, br.d, *J* 5.6 Hz), 4.02 – 3.96 (3H, including a broad doublet *J* 8.5 Hz at 3.99), 3.95 – 3.86 (3H, m), 3.77 (1H, dd, *J* 4.2, 7.4 Hz), 3.72 (1H, d, *J* 5.4 Hz), 3.67 – 3.57 (4H, including a broad doublet *J* 10.5 Hz at 3.63), 3.54 (1H, dd, *J* 2.6, 9.7 Hz), 2.42 – 2.35 (2H, m), 1.64 – 1.04 (277H, m), 0.84 (12H, t, *J* 6.7 Hz), 0.64 – 0.56 (8H, m), 0.52 (4H, dt, *J* 4.1, 8.4 Hz), -0.37 (4H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$+few drops CD_3OD): 175.0, 174.9, 108.7, 108.0, 81.7, 81.3, 79.7, 79.4, 77.9, 77.5, 77.2, 76.3, 72.4, 71.9, 69.7, 69.15, 68.9, 67.6, 65.1, 63.4, 62.2, 61.4, 57.8, 52.6, 34.9, 31.8, 30.1, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 29.15, 29.1, 28.6, 27.3, 25.3, 22.5, 20.5, 15.6, 13.9, 10.7.; ν_{max}: 3399, 2920, 2851, 1734, 1467, 1043 cm^{-1}.

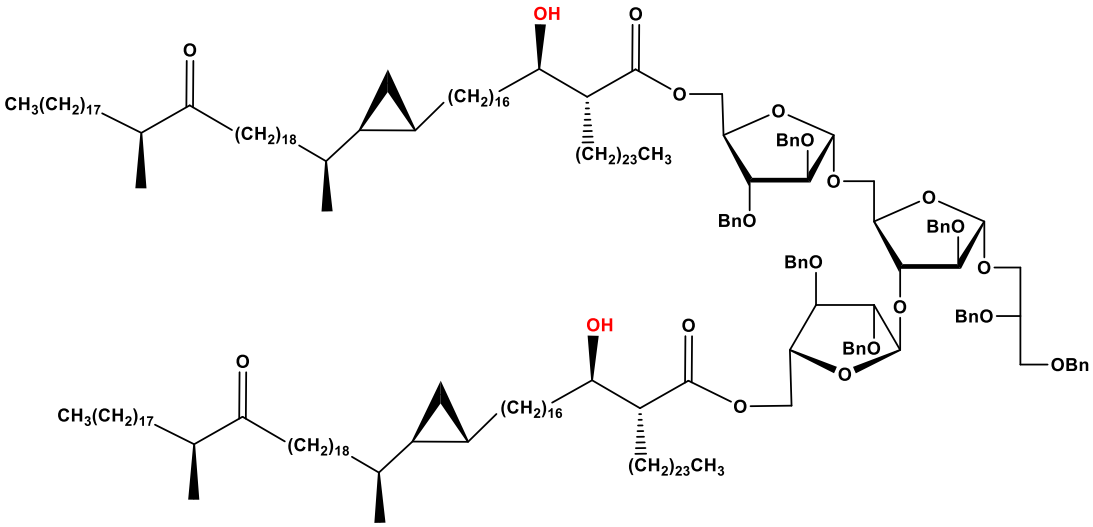
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-((*tert*-butyl dimethyl silyl)oxy)-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl) cyclopropyl) heptadecyl) hexacosanoate-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl) cyclopropyl) heptadecyl)hexacosanoate-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (99):



A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (35 mg, 0.18 mmol) in dry CH_2Cl_2 (1 mL) was added to a stirred solution of α-D-arabinofuranoside **(89)** (22 mg, 0.019 mmol), molecular sieves 4 Å (25 mg), DMAP (23 mg, 0.18 mmol) and (2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2yl) cyclopropyl) heptadecyl) hexacosanoic acid **(76h)** (50 mg, 0.035 mmol) in dry CH_2Cl_2 (1 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 4 days. When TLC showed no starting material was left. The precipitate was filtered off and washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethylacetate (10:1) to afford the title compound **(99)** as a colourless thick oil (35 mg, 46%) [MALDI–Found $(M+Na)^+$: 3892.2; $C_{253}H_{438}NaO_{21}Si_2$, requires: 3892.2]; $[\alpha]_D^{20}$ +14 (*c* 3.0, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.54 – 7.00 (35H, m), 5.16 (1H, br.s), 5.13 (1H, br.s), 5.04 (1H, br.s), 4.68 (1H, d, *J* 12.1 Hz), 4.65 (1H, d, *J* 12.1 Hz), 4.58 – 4.47 (9H, m), 4.46 (1H, d, *J* 11.9 Hz), 4.39 (1H, d, *J* 11.8 Hz), 4.33 (1H, d, *J* 11.9 Hz), 4.30 – 4.22 (5H, including a broad double doublet *J* 3.9, 10.4 Hz at 4.26), 4.22 – 4.16 (2H, m), 4.13 (1H, br.dd, *J* 5.6, 8.4 Hz), 4.06 (1H, br.d, *J* 2.9 Hz), 4.01 – 3.97 (2H, m), 3.95 – 3.87 (5H, m), 3.85 (1H, br.dd, *J* 3.8, 9.4 Hz), 3.78 (1H, br.p,

J 4.9 Hz), 3.76 – 3.72 (1H, m), 3.65 – 3.55 (3H, including a broad double doublet *J* 4.8, 8.3 Hz at 3.60), 2.53 (4H, including sextet, *J* 5.6 Hz), 2.42 (4H, t, *J* 7.5 Hz), 1.62 – 1.14 (295 H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8 Hz), 0.88 (6H, d, *J* 6.8 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.75 – 0.62 (2H, m), 0.51 – 0.40 (2H, m), 0.24 – 0.08 (6H, m), 0.03 (6H, s), 0.01 (6H, s); δ_C (101 MHz, $CDCl_3$): 215.1, 174.2, 174.1, 138.6, 138.3, 137.8, 137.7, 137.6, 137.55, 137.5, 128.45, 128.4, 128.35, 128.3, 128.25, 128.2, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 106.6, 106.3, 105.2, 88.3, 88.2, 87.8, 83.8, 83.7, 81.2, 80.2, 79.5, 78.9, 77.2, 73.4, 73.2, 72.3, 72.2, 72.0, 71.9, 71.6, 70.3, 67.0, 65.9, 63.0, 62.7, 51.6, 46.3, 41.1, 38.1, 37.4, 34.5, 33.7, 33.6, 33.0, 31.9, 30.1, 29.9, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 27.8, 27.4, 27.3, 27.2, 27.1, 26.1, 25.9, 25.8, 24.1, 23.7, 22.7, 22.6, 19.7, 18.6, 18.0, 16.4, 14.1, 10.5, -4.4, -4.5, -4.7, -4.8; ν_{max}: 3063,3032, 2919, 2851, 1739, 1714, 1467, 1105, 836, 698 cm^{-1}.

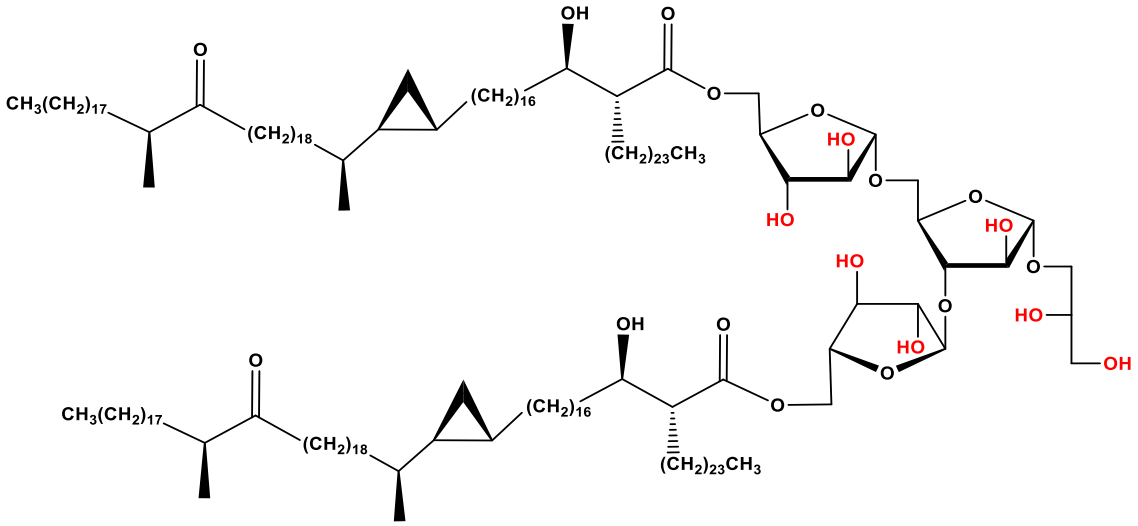
2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*, 2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl) hexacosanoate-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*, 2*R*)-2-((22*S*) -22-methyl-21-oxotetracontan-2-yl)cyclopropyl) heptadecyl) hexacosanoate-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (100):



The protected glycolipid α-D-arabinofuranoside **(99)** (31 mg, 0.0080 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial equipped with an acid proof rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The mixture was cooled to 0 °C, and then hydrogen fluoride-pyridine complex as (70% w, 1.5 mL) was added dropwise. The mixture was stirred at 43 °C for 24 h. When TLC showed no starting

material was left, the mixture was neutralized by pouring it slowly into sat. aq. $NaHCO_3$ and stirred until no more CO_2 was liberated. The organic layer was separated and the aqueous layer was re-extracted with chloroform (3×10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated to give the residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) affording compound **(100)** as a colourless thick oil (19 mg, 66%) [MALDI–Found $(M+Na)^+$: 3664.1; $C_{241}H_{410}NaO_{21}$, requires: 3664.1]; $[\alpha]_D^{18}$ +25 (*c* 1.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.18 (35H, m), 5.16 (1H, br.s), 5.13 (1H, br.s), 5.04 (1H, br.s), 4.67 (2H, br.s), 4.55 (1H, d, *J* 11.9 Hz), 4.53 – 4.47 (8H, m), 4.45 (2H, d, *J* 11.8 Hz), 4.40 (1H, d, *J* 11.8 Hz), 4.29 (6H, br.m), 4.17 (1H, br.dd, *J* 3.8, 6.9 Hz), 4.11 (1H, br.dd, *J* 3.4, 6.2 Hz), 4.07 (1H, br.d, *J* 2.5 Hz), 3.99 – 3.95 (2H, m), 3.94 – 3.87 (2H, m), 3.87 – 3.82 (2H, including abroad double doublet *J* 4.6, 10.3 Hz at 3.85), 3.79 (1H, br.p, *J* 4.9 Hz), 3.72 (1H, br.dd, *J* 1.7, 11.6 Hz), 3.64 – 3.57 (5H, m), 2.51 (2H, sextet, *J* 6.8 Hz), 2.42 (6H, including a triplet, *J* 7.5 Hz), 1.87 – 1.08 (300H, m), 1.05 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 7.3 Hz), 0.72 – 0.62 (2H, m), 0.50 – 0.38 (2H, m), 0.25 – 0.05 (6H, m); δ_C (101 MHz, $CDCl_3$): 215.2, 175.1, 175.0, 138.6, 138.3, 137.7, 137.5, 137.45, 137.4, 137.2, 128.5, 128.4, 128.35, 128.3, 128.2, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 106.3, 106.2, 105.4, 88.2, 88.0, 87.9, 83.6, 80.7, 80.3, 79.3, 79.2, 77.2, 73.4, 72.4, 72.2, 72.1, 72.0, 71.9, 71.7, 70.3, 67.1, 65.4, 63.0, 62.9, 51.9, 51.7, 46.3, 41.1, 38.1, 37.4, 35.3, 35.2, 34.5, 33.0, 31.9, 30.1, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 27.5, 27.4, 27.35, 27.3, 26.1, 25.7, 23.7, 22.7, 19.7, 18.6, 16.4, 14.1, 10.5; ν_{max}: 3457, 3063,3031, 2919, 2852, 1737, 1715, 1464, 1104,734, 698 cm^{-1}.

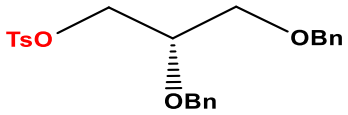
L-glycerol-(1'→1)-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl) hexacosanoate-α-D-arabinofuranosyl-(1→3) -[-5-*O*-(2*R*)-2-((1*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((22*S*)-22-methyl-21-oxotetracontan-2-yl) cyclopropyl)heptadecyl)hexacosanoate-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (101):



Palladium hydroxide on activated charcoal ($Pd(OH)_2$-C/20%, 12 mg, 1.1 fold by weight) was added to a stirred solution of α-D-arabinofuranoside **(100)** (11 mg, 0.0030 mmol) in CH_2Cl_2:MeOH (2:1, 2 mL) at room temperature under hydrogen. The mixture was stirred for 36 h. When TLC showed no starting material was left, the mixture was filtered off through celite and the solvent was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (10:1) affording compound **(101)** as a colourless thick oil (9.0 mg, 82%) [MALDI–Found $(M+Na)^+$: 3033.8; $C_{192}H_{368}NaO_{21}$, requires: 3033.8]; $[\alpha]_D^{18}$ +26 (*c* 0.90, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops CD_3OD): 5.01 (1H, br.s), 4.96 (1H, br.s), 4.91 (1H, br.s), 4.42 (1H, dd, *J* 4.9, 12.1 Hz), 4.37 (1H, dd, *J* 4.6, 11.8 Hz), 4.27 (1H, dd, *J* 4.7, 11.1Hz), 4.22 (1H, br.d, *J* 4.7 Hz), 4.18 (1H, dd, *J* 4.2, 9.0 Hz), 4.13 (1H, br.q, *J* 5.4 Hz), 4.09 (1H, t, *J* 7.0 Hz), 4.06 (1H, d, *J* 5.1 Hz), 4.02 – 3.97 (3H, br.m), 3.93 (1H, br.dd, *J* 3.3, 7.3 Hz), 3.89 (2H, br.m), 3.80 – 3.72 (2H, m), 3.67 – 3.58 (5H, m), 3.55 (1H, dd, *J* 2.4, 9.6 Hz), 2.53 – 2.44 (2H, sextet, *J* 6.6 Hz), 2.39 (6H, including a triplet, *J* 7.4 Hz), 1.57 – 1.06 (210 H, m), 1.01 (6H, d, *J* 6.9 Hz), 0.85 (12H, t, *J* 7.5 Hz), 0.67 – 0.57 (2H, m), 0.47 – 0.34 (2H, m), 0.21 – 0.02 (6H, m); δ_C (101 MHz, $CDCl_3$): 215.8, 175.0, 174.75, 109.3, 108.7, 87.1, 82.95, 82.9, 80.9, 79.4, 78.8, 78.2, 77.9, 77.2, 72.6, 71.7, 68.8, 65.0, 64.0, 63.3, 61.8, 52.5, 46.3, 41.1, 38.1, 37.4, 35.1,

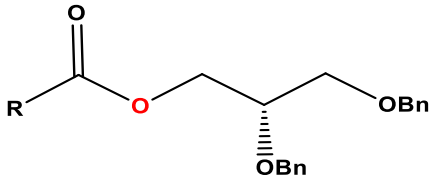
34.4, 33.0, 32.9, 31.9, 30.0, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 27.4, 27.35, 27.3, 27.25, 27.2, 26.1, 25.45, 25.4, 23.6, 23.55, 22.6, 19.6, 18.6, 16.3, 16.2, 14.1, 10.4.; ν_{max}: 3434, 2919, 2852, 1736, 1717, 1467, 1104,735, 699 cm^{-1}.

1-*O*-*p*-toluene-sulfonyl-(*S*)-2,3-di-*O*-benzyl glycerol (102):



p-Toluenesulfonyl chloride (4.86 g, 0.0255 mol) was added to a stirred solution of (*S*)-2,3-di-*O*-benzyl propanol **(51G)** [247] (3.2 g, 0.011 mol), pyridine (5.85 g, 6.20 mL, 73.9 mmol) and DMAP (0.71 g, 5.8 mmol) in dry CH_2Cl_2 (25 mL) at 0 ºC under nitrogen. The mixture was allowed to reach room temperature and stirred for 24 h, then TLC showed no starting material was left. The mixture was diluted with ethyl acetate (100 mL) and water (40 mL), the organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with water (25 mL), brine (25 mL) and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give an oily residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) affording the title compound **(102)** as a colourless thick oil (3.1 g, 62%) [MALDI–Found $(M+Na)^+$: 449.1; $C_{24}H_{26}O_5NaS$, requires: 449.1]; $[\alpha]_D^{22}$ -5.6 (*c* 0.71, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.77 (2H, d, *J* 8.3 Hz), 7.52 – 7.12 (12H, m), 4.58 (2H, br.s), 4.47 (2H, br.s), 4.22 (1H, dd, *J* 4.2, 10.4 Hz), 4.12 (1H, dd, *J* 5.8, 10.4 Hz), 3.85 – 3.76 (1H, m), 3.55 – 3.51 (2H, m), 2.43 (3H, s); δ_C (101 MHz, $CDCl_3$): 129.8, 128.4, 128.3, 128.0, 127.8, 127.75, 127.7, 127.6, 75.5, 73.4, 72.4, 69.5, 68.8, 21.6; ν_{max}: 3032, 2867, 1598, 1362, 1496, 1177, 1097, 921, 814 cm^{-1}. All data were identical to the authentic sample[248].

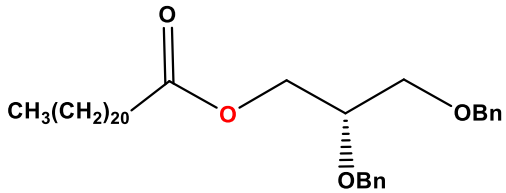
(*S*)-2,3-Bis(benzyloxy)propyl alkanoate (104a-f):



Genera procedure:

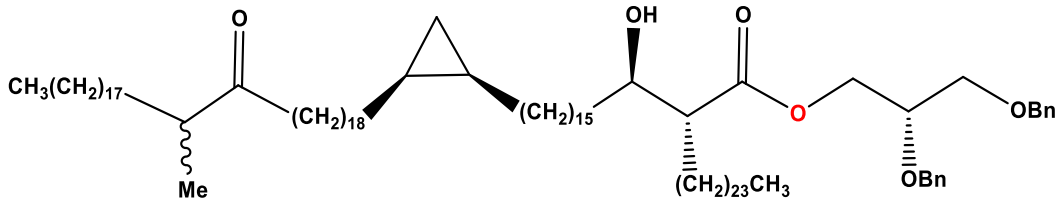
Cesium hydrogencarbonate was added to a stirred solution of 1-*O*-*p*-toluene-sulfonyl-(*S*)-2,3-di-*O*-benzyl glycerol **(102)** and fatty acids **(103a-f)** in dry DMF:THF (1:5, 2 mL) at room temperature. The mixture was stirred at 70 ºC for 2 days. The suspension was diluted with ethyl acetate (10 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×10 mL). The combined organic layers were washed successively with water (15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and evaporated to give a thick oil residue. The residue was purified by column chromatography.

(*S*)-2,3-Bis(benzyloxy)propyl docosanoate (104a):



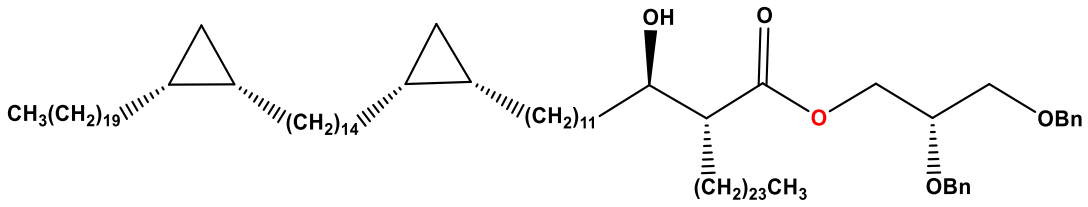
Cesium hydrogencarbonate (0.284 g, 1.46 mmol), tosylate **(102)** (0.137 g, 0.323 mmol) and behenic acid **(103a)** (0.10 g, 0.29 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to afford the title compound (**104a**) (59 mg, 83%) [MALDI–Found $(M+Na)^+$: 617.4; $C_{39}H_{62}NaO_4$, requires: 617.4]; $[\alpha]_D^{22}$ +7 (*c* 0.6, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.31 – 7.19 (10H, m), 4.61 (2H, br.s), 4.49 (2H, br.s), 4.25 (1H, dd, *J* 4.2, 11.7 Hz), 4.12 (1H, dd, *J* 5.8, 11.7 Hz), 3.80 – 3.70 (1H, m), 3.55 – 3.51 (2H, m), 2.23 (2H, t, *J* 7.6 Hz), 1.66 – 0.97 (38H, m), 0.83 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 138.2, 138.0, 128.4, 128.3, 127.7, 127.65, 127.6, 75.8, 73.4, 72.1, 69.6, 63.6, 34.2, 31.9, 29.7, 29.65, 29.6, 29.4, 29.35, 29.3, 29.1, 24.9, 22.7, 14.1; ν_{max}: 2917, 2850, 1740, 1467 cm^{-1}.

(*S*)-2,3-Bis(benzyloxy)propyl(2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl) cyclopropyl)hexadecyl)hexacosanoate (104b):



Cesium hydrogencarbonate (0.0572 g, 0.295 mmol), tosylate **(102)** (0.0277 g, 0.0649 mmol) and (2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl) hexadecyl) hexacosanoic acid **(103b)** (0.0731 g, 0.0590 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to afford the title compound **(104b)** (52 mg, 59%) [MALDI–Found $(M+Na)^+$: 1514.3829, $C_{101}H_{182}NaO_6$, requires: 1514.3834]; $[\alpha]_D^{23}$ +6.5 (*c* 0.55, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.31 – 7.05 (10H, m), 4.60 (1H, d, *J* 11.8 Hz), 4.57 (1H, d, *J* 11.8 Hz), 4.47 (2H, br.s), 4.35 (1H, dd, *J* 4.0, 11.7 Hz), 4.14 (1H, dd, *J* 5.5, 11.7 Hz), 3.80 – 3.71 (1H, m), 3.58 – 3.48 (3H, including br dd, *J* 1.4, 5.4 Hz at 3.52), 2.48 – 2.38 (2H, including OH proton at 2.43), 2.38 – 2.29 (3H, including dt, *J* 5.4, 7.9 Hz at 2.35), 1.75 – 1.01 (144H, m), 0.98 (3H, d, *J* 6.9 Hz), 0.81 (6H, t, *J* 6.7 Hz), 0.62 – 0.53 (2H, m), 0.49 (1H, dt, *J* 4.0, 8.5 Hz), -0.40 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.0, 175.7, 138.0, 128.4, 128.3, 127.7, 127.6, 127.5, 75.7, 73.4, 72.2, 72.0, 69.5, 63.4, 51.3, 46.2, 41.0, 35.4, 33.0, 31.8, 30.1, 29.6, 29.55, 29.5, 29.4, 29.35, 29.3, 29.2, 28.6, 27.4, 27.2, 25.7, 23.7, 23.6, 22.6, 16.3, 15.7, 14.0, 10.8; ν_{max}: 2918, 2850, 1717, 1467 cm^{-1}.

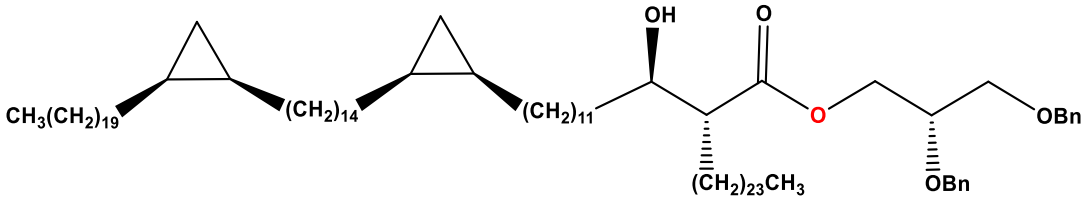
(*S*)-2,3-Bis(benzyloxy)propyl(*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosyl-cyclopropyl) tetradecyl)cyclopropyl)dodecyl)hexacosanoate (104c):



Cesium hydrogencarbonate (0.0605 g, 0.312 mmol), tosylate **(102)** (0.0293 g, 0.0687 mmol) and (*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl) cyclopropyl)

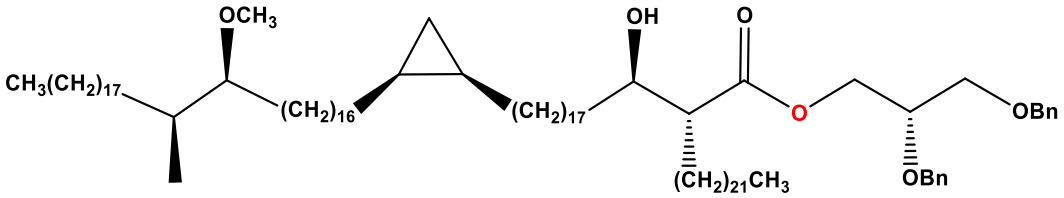
dodecyl) hexacosanoic acid **(103c)** (0.0711 g, 0.0624 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to afford the title compound **(104c)** (51 mg, 58%) [MALDI–Found $(M+Na)^+$: 1414.2940, $C_{95}H_{170}NaO_5$, requires: 1414.2946]; $[\alpha]_D^{23}$ +3.6 (*c* 0.88, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.37 – 7.26 (10H, m), 4.68 (1H, d, *J* 11.8 Hz), 4.65 (1H, d, *J* 11.9 Hz), 4.55 (2H, br.s), 4.43 (1H, dd, *J* 4.0, 11.7 Hz), 4.22 (1H, dd, *J* 5.5, 11.7 Hz), 3.86 – 3.80 (1H, m), 3.67 – 3.56 (3H, including br.dd *J* 1.6, 5.4 Hz at 3.60), 2.45 (1H, d, *J* 7.9 Hz), 2.43 (1H, br.dd, *J* 3.5, 7.4 Hz) 1.85 – 0.97 (134H, m), 0.89 (6H, t, *J* 7.2 Hz), 0.70 – 0.61 (4H, m), 0.60 – 0.57 (2H, dt, *J* 4.0, 8.5 Hz), -0.32 (2H, br q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.0, 137.8, 128.4, 128.3, 127.7, 127.6, 127.5, 121.9, 75.7, 73.4, 72.3, 72.0, 69.5, 63.4, 51.3, 35.5, 31.9, 30.1, 29.7, 29.6, 29.5, 29.45, 29.4, 29.3, 28.6, 27.4, 25.7, 22.6, 15.7, 14.0, 10.8; ν_{max}: 3435, 2917, 2850, 1732, 1468 cm^{-1}.

(*S*)-2,3-Bis(benzyloxy)propyl(2*R*)-2-(1-hydroxy-12-((1*R*,2*S*)-2-(14-((2*S*)-2-icosylcyclo-propyl) tetradecyl)cyclopropyl)dodecyl)hexacosanoate (104d):



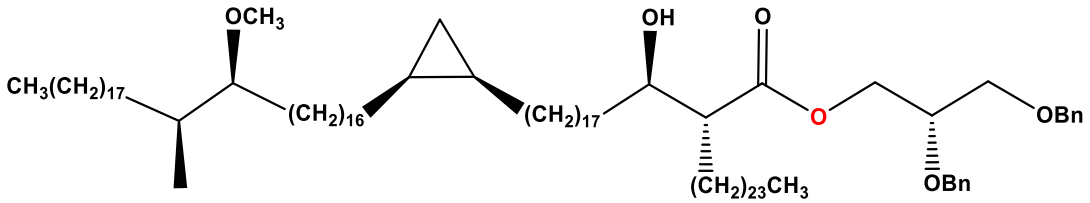
Cesium hydrogencarbonate (0.0616 g, 0.317 mmol), tosylate **(102)** (0.0298 g, 0.0698 mmol) and (2*R*)-2-(1-hydroxy-12-((1*R*,2*S*)-2-(14-((2*S*)-2-icosylcyclopropyl) tetradecyl) cyclopropyl) dodecyl) hexacosanoic acid **(103d)** (0.0724 g, 0.0636 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to afford the title compound **(104d)** (62 mg, 71%) [MALDI–Found $(M+Na)^+$: 1414.3; $C_{95}H_{170}NaO_5$, requires: 1414.3]; $[\alpha]_D^{22}$ +3.8 (*c* 0.31, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.35 – 7.27 (10H, m), 4.68 (1H, d, *J* 11.8 Hz), 4.65 (1H, d, *J* 11.8 Hz), 4.55 (2H, br.s), 4.43 (1H, dd, *J* 4.1, 11.7 Hz), 4.22 (1H, dd, *J* 5.5, 11.7 Hz), 3.86 – 3.80 (1H, m), 3.67 – 3.55 (3H, including br dd *J* 1.6, 5.4 Hz at δ 3.6), 2.45 (1H, d, *J* 8.0 Hz), 2.43 (1H, br.dd, *J* 3.8, 10.5 Hz), 1.80 – 1.00 (134H, m), 0.89 (6H, t, *J* 6.7 Hz), 0.71 – 0.61 (4H, m), 0.57 (2H, dt, *J* 3.9, 8.4 Hz), -0.32 (2H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.0, 137.9, 128.4, 128.3, 127.8, 127.7, 127.6, 75.8, 73.5, 72.3, 72.1, 69.6, 63.5, 51.4, 35.5, 31.9, 30.2, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 27.5, 25.8, 23.0, 15.8, 14.1, 10.9; ν_{max}: 2991, 2917, 2850, 1732, 1599, 1469 cm^{-1}.

(*S*)-2,3-Bis(benzyloxy)propyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (104e):



Cesium hydrogencarbonate (0.0436 g, 0.225 mmol), tosylate **(102)** (0.0211 g, 0.0495 mmol) and (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl) cyclopropyl) octadecyl)tetracosanoic acid **(103e)** (0.0552 g, 0.0450 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to afford the title compound **(104e)** (41 mg, 62%) [MALDI–Found $(M+Na)^+$: 1502.4, $C_{100}H_{182}NaO_6$, requires: 1502.4]; $[\alpha]_D^{22}$ +9 (*c* 0.3, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.32 – 7.19 (10H, m), 4.62 (1H, d, *J* 11.8 Hz), 4.58 (1H, d, *J* 11.8 Hz), 4.48 (2H, br s), 4.36 (1H, dd, *J* 4.1, 11.7 Hz), 4.15 (1H, dd, *J* 5.5, 11.7 Hz), 3.81 – 3.72 (1H, m), 3.60 – 3.49 (3H, including br dd, *J* 1.6, 5.4 Hz, at 3.53), 3.28 (3H, s), 2.90 (1H, br.p, *J* 4.1 Hz), 2.39 (1H, d, *J* 7.9 Hz), 2.36 (1H, br.dd, *J* 3.5, 7.4 Hz), 1.75 – 0.93 (143H, m), 0.83 (6H, t, *J* 6.8 Hz), 0.79 (3H, d, *J* 6.9 Hz), 0.65 – 0.54 (2H, m), 0.50 (1H, dt, *J* 4.0, 8.4 Hz), -0.39 (1H, br q, *J* 5.2 Hz); δ_C (101MHz, $CDCl_3$): 175.3, 137.9, 137.8, 128.3, 128.2, 127.7, 127.6, 127.5, 85.3, 75.7, 73.4, 72.2, 72.0, 69.5, 63.4, 57.6, 51.3, 38.6, 35.4, 35.2, 32.3, 31.8, 30.4, 30.3, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 28.6, 27.5, 27.4, 26.0, 25.7, 23.6, 22.9, 22.6, 15.7, 14.8, 14.0, 13.9, 10.9, 10.8; ν_{max}: 2923, 2853, 1737, 1465 cm^{-1}.

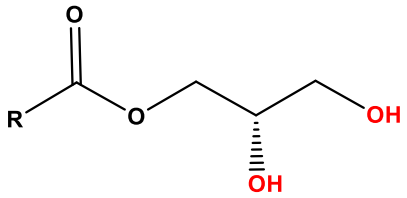
(*S*)-2,3-Bis(benzyloxy)propyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (104f):



Cesium hydrogencarbonate (0.0351 g, 0.181 mmol), tosylate **(102)** (0.0170 g, 0.0399 mmol) and (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl) cyclopropyl) octadecyl) hexacosanoic acid **(103f)** (0.0455 g, 0.0362 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with

hexane/ethyl acetate (10:1) to afford the title compound **(104f)** (40 mg, 72%) [MALDI–Found $(M+Na)^+$: 1530.4142, $C_{102}H_{186}NaO_6$, requires: 1530.4147]; $[\alpha]_D^{23}$ +6.4 (*c* 0.50, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.39 – 7.26 (10H, m), 4.70 (1H, d, *J* 11.8 Hz), 4.66 (1H, d, *J* 11.8 Hz), 4.57 (2H, br.s), 4.44 (1H, dd, *J* 4.1, 11.6 Hz), 4.23 (1H, dd, *J* 5.5, 11.7 Hz), 3.85 (1H, m), 3.69 – 3.57 (3H, including br dd, *J* 1.6, 5.4 Hz at 3.62), 3.37 (3H, s), 2.98 (1H, br.p, *J* 3.8 Hz), 2.47 (1H, d, *J* 7.9 Hz), 2.44 (1H, br.dd, *J* 4.5, 8.5 Hz), 1.88 – 1.03 (147H, m), 0.91 (6H, t, *J* 7.1 Hz), 0.89 (3H, d, *J* 6.8 Hz), 0.71 – 0.63 (2H, m), 0.58 (1H, dt, *J* 4.0, 8.3 Hz), -0.31 (1H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 128.4, 128.3, 127.7, 127.6, 127.5, 85.3, 75.7, 73.4, 72.2, 72.0, 69.5, 68.0, 63.4, 57.6, 51.3, 38.6, 35.4, 35.2, 32.3, 31.8, 30.4, 30.3, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.8, 28.6, 27.5, 27.4, 26.0, 22.6, 15.7, 14.8, 14.0, 10.8; ν_{max}: 3030, 2923, 2853, 1733, 1496 cm^{-1}.

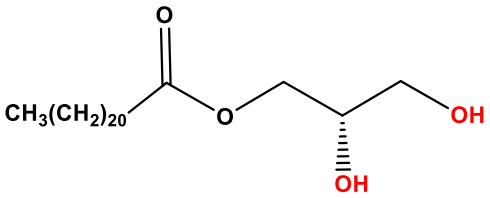
(*S*)-2,3-Dihydroxypropyl alkanoate (105a-f):



Genera procedure:

Palladium hydroxide on activated charcoal (20% Pd, 0.15 fold by weight) was added to a stirred solution of compounds **(104a-f)** in dry CH_2Cl_2:MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The mixture was stirred for 24 h, when TLC showed no starting material was left. The mixture was filtered through celite and the precipitate was washed with CH_2Cl_2 (10 mL), the filtrate was evaporated and the residue was purified by column chromatography to give compounds **(105a-f)**.

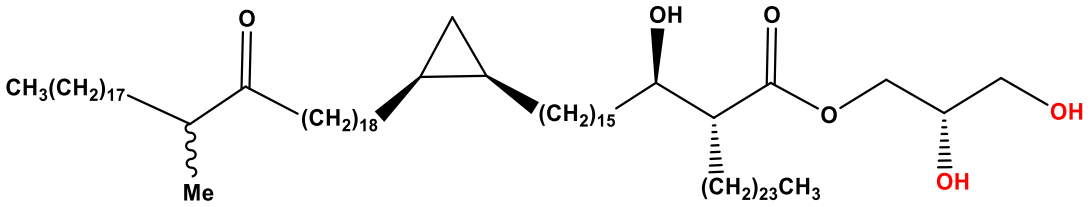
(*S*)-2,3-Dihydroxypropyl docosanoate (105a):



Palladium hydroxide on activated charcoal (0.0085 g) and compound **(104a)** (0.0573 g, 0.0963 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to afford the title compound

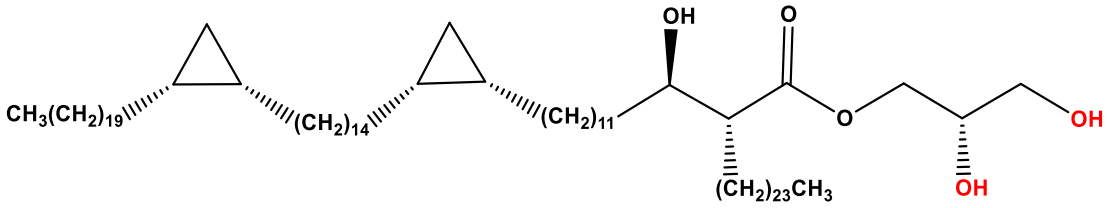
(**105a**) (28 mg, 70%) [MALDI–Found $(M+Na)^+$: 437.3601, $C_{25}H_{50}NaO_4$, requires: 437.3607]; $[\alpha]_D^{22}$ -24 (*c* 0.25, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops of CD_3OD): 4.07 (1H, dd, *J* 3.6, 9.9 Hz), 4.03 (1H, dd, *J* 4.4, 10.0 Hz), 3.80 (1H, m), 3.57 (1H, dd, *J* 4.1, 11.5 Hz), 3.48 (1H, dd, *J* 6.1, 11.5 Hz), 2.28 (2H, t, *J* 7.6 Hz), 1.63 – 0.92 (40H, m), 0.81 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$+few drops of CD_3OD): 174.4, 69.9, 65.0, 63.1, 34.1, 31.8, 29.6, 29.55, 29.5, 29.4, 29.3, 29.2, 29.0, 24.8, 22.6, 14.0; ν_{max}: 3342, 2955, 2918, 2849, 1733, 1472 cm^{-1}.

(*S*)-2,3-Dihydroxypropyl(2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl) cyclopropyl)hexadecyl)hexacosanoate (105b):



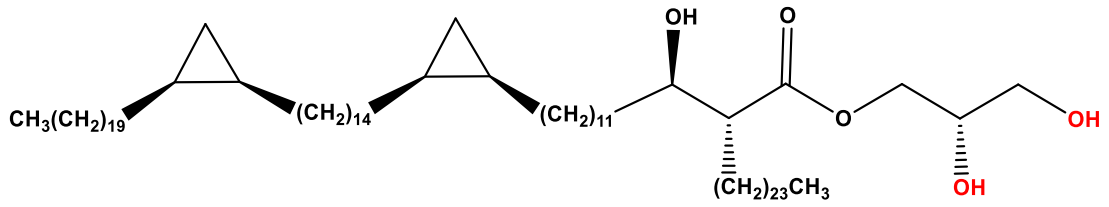
Palladium hydroxide on activated charcoal (0.0075 g) and compound **(104b)** (0.0505 g, 0.0338 mmol); gave a thick colourless oil which was purified by column chromatography on silica eluting with (chloroform/methanol, 40:1) to afford the title compound **(105b)** (38 mg, 87%) [MALDI–Found $(M+Na)^+$: 1334.2890, $C_{87}H_{170}NaO_6$, requires: 1334.2895]; $[\alpha]_D^{23}$ +2.8 (*c* 3.6, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$+few drops of CD_3OD): 4.22 (1H, dd, *J* 4.2, 11.4 Hz), 4.11 (1H, dd, *J* 6.5, 11.4 Hz), 3.89 – 3.81 (1H, m), 3.68 – 3.57 (2H, including br dd *J* 4.3, 11.5 Hz at δ 3.61), 3.54 (1H, dd, *J* 5.8, 11.6 Hz), 2.53 – 2.44 (1H, m), 2.38 (3H, including br t, *J* 2.38), 1.68 – 1.05 (147H, m), 1.01 (3H, d, *J* 6.9 Hz), 0.84 (6H, t, *J* 6.7 Hz), 0.65 – 0.57 (2H, m), 0.52 (1H, dt, *J* 4.1, 8.4 Hz), -0.37 (1H, br.q, *J* 5.2 Hz); δ_C (126 MHz, $CDCl_3$+few drops of CD_3OD): 216.0, 175.5, 72.6, 69.7, 65.1, 63.0, 52.5, 46.3, 41.1, 35.0, 32.9, 31.8, 30.1, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 29.25, 29.2, 29.15, 29.1, 28.6, 27.4, 27.2, 25.3, 23.6, 22.6, 16.2, 15.7, 14.0, 10.8; ν_{max}: 3396, 3017, 2922, 2853, 1713, 1467 cm^{-1}.

(*S*)-2,3-Dihydroxypropyl(*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl) tetradecyl)cyclopropyl)dodecyl)hexacosanoate (105c):



Palladium hydroxide on activated charcoal (0.0063 g) and compound **(104c)** (0.0425g, 0.0305 mmol); gave a thick clourless oil residue which was purified by column chromatography on silica eluting with (chloroform/methanol, 20:1) to afford the title compound **(105c)** (27 mg, 74%) [MALDI–Found $(M+Na)^+$: 1234.2001, $C_{81}H_{158}NaO_5$, requires: 1234.2007]; $[\alpha]_D^{23}$ +2.3 (*c* 5.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$+few drops of CD_3OD): 4.22 (1H, dd, *J* 4.4, 11.5 Hz), 4.12 (1H, dd, *J* 6.4, 11.5 Hz), 3.89 – 3.83 (1H, m), 3.67 – 3.58 (2H, including br dd, *J* 4.0, 11.1 Hz at 3.61), 3.55 (1H, dd, *J* 5.8, 11.6 Hz), (1H, ddd, *J* 4.4, 7.9, 10.0 Hz), 1.79 – 0.96 (137H, m), 0.85 (6H, t, *J* 6.8 Hz), 0.66 – 0.57 (4H, m), 0.53 (2H, dt, *J* 4.4, 8.4 Hz), -0.36 (2H, br.q, *J* 5.1 Hz); δ_C (126 MHz, $CDCl_3$+few drops of CD_3OD): 175.4, 72.4, 69.6, 65.0, 62.8, 52.4, 31.7, 30.0, 29.4, 29.1, 29.0, 28.5, 27.2, 25.1, 22.4, 15.5, 13.8, 10.6; ν_{max}: 3400, 3017, 2917, 2850, 1733, 1468 cm^{-1}.

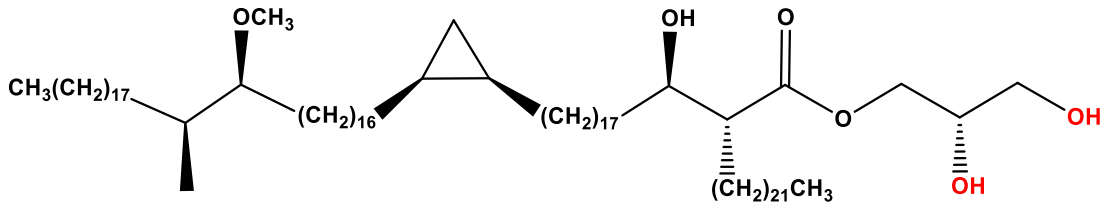
(*S*)-2,3-Dihydroxypropyl(*2R*)-2-(1-hydroxy-12-((1*R*,2*S*)-2-(14-((2*S*)-2-icosylcyclopropyl) tetradecyl) cyclopropyl)dodecyl)hexacosanoate (105d):



Palladium hydroxide on activated charcoal (0.0077 g) and compound **(104d)** (0.0515 g, 0.0369 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with (chloroform/methanol, 10:1) to afford the title compound (**105d**) (40 mg, 92%) [MALDI–Found $(M+Na)^+$: 1234.2001, $C_{81}H_{158}NaO_5$, requires: 1234.2007]; $[\alpha]_D^{22}$ +1.8 (*c* 0.34, $CHCl_3$); δ_H (400 MHz, $CDCl_3$+few drops of CD_3OD): 4.21 (1H, dd, *J* 4.3, 11.4 Hz), 4.10 (1H, dd, *J* 6.4, 11.5 Hz), 3.89 – 3.80 (1H, m), 3.60 (2H, br dd, *J* 4.1, 11.5 Hz), 3.54 (1H, dd, *J* 5.8, 11.6 Hz), 2.39 (1H, ddd, *J* 4.8, 7.6, 10.0 Hz), 1.86 – 0.94 (137H, m), 0.84 (6H, t, *J* 6.8 Hz),

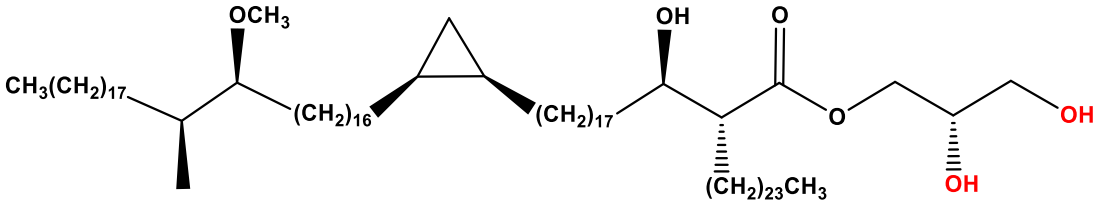
0.65 – 0.56 (4H, m), 0.52 (2H, dt, *J* 3.9, 8.5 Hz), -0.37 (2H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$+few drops of CD_3OD): 175.5, 72.6, 69.8, 65.1, 63.0, 52.5, 37.0, 35.0, 31.8, 30.1, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 28.6, 27.4, 25.3, 22.6, 15.7, 14.0, 10.8; ν_{max}: 3585, 2917, 2849, 1734, 1468 cm^{-1}.

(*S*)-2,3-Dihydroxypropyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (105e):



Palladium hydroxide on activated charcoal (0.0054 g) and compound **(104e)** (0.0364 g, 0.0245mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with (chloroform/methanol, 20:1) to afford the title compound **(105e)** (27 mg, 85%) [MALDI–Found $(M+Na)^+$: 1322.2890, $C_{86}H_{170}NaO_6$, requires: 1322.2895]; $[\alpha]_D^{23}$ +1.6 (*c* 2.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$+few drops of CD_3OD): 4.21 (1H, dd, *J* 4.3, 11.4 Hz), 4.11 (1H, dd, *J* 6.4, 11.4 Hz), 3.88 – 3.82 (1H, m), 3.66 – 3.57 (2H, including br dd, *J* 4.3, 11.4 Hz at δ 3.6), 3.54 (1H, dd, *J* 5.8, 11.6 Hz), 3.31 (3H, s), 2.94 (1H, br.p, *J* 4.4 Hz), 2.40 (1H, ddd, *J* 4.8, 7.4, 10.2 Hz), 1.81 – 0.94 (146H, m), 0.84 (6H, t, *J* 7.0 Hz), 0.81 (3H, d, *J* 6.9 Hz), 0.65 – 0.57 (2H, m), 0.52 (1H, dt, *J* 4.0, 8.5 Hz), -0.37 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$+few drops of CD_3OD): 175.5, 85.5, 72.6, 69.8, 65.1, 63.0, 57.6, 52.4, 35.3, 35.0, 32.3, 31.8, 30.4, 30.1, 29.9, 29.8, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 28.6, 27.5, 27.4, 26.0, 25.3, 22.6, 15.7, 14.7, 14.0, 10.8; ν_{max}: 3368, 2918, 2850, 1731, 1467 cm^{-1}.

(*S*)-2,3-Dihydroxypropyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (105f):



Palladium hydroxide on activated charcoal (0.0032g) and compound **(104f)** (0.0215g, 0.0142 mmol); gave a thick colourless oil residue which was purified by column chromatography on silica eluting with (chloroform/methanol, 20:1) to afford the title compound **(105f)** (18 mg, 92%) [MALDI–Found $(M+Na)^+$: 1350.3203, $C_{88}H_{174}NaO_6$, requires: 1350.3208]; $[\alpha]_D^{23}$ +1.9 (*c* 0.74, $CHCl_3$); δ_H (400 MHz, $CDCl_3$+few drops of $CD0_3OD$): 4.22 (1H, dd, *J* 4.2, 11.5 Hz), 4.12 (1H, dd, *J* 6.4, 11.5 Hz), 3.89 – 3.83 (1H, m), 3.71 – 3.58 (2H, including br dd, *J* 4.1, 11.5 Hz at δ 3.61), 3.55 (1H, dd, *J* 5.8, 11.5 Hz), 3.31 (3H, s), 2.94 (1H, br.p, *J* 3.7 Hz), 2.40 (1H, ddd, *J* 4.8, 7.4, 10.4 Hz), 1.68 – 0.94 (150H, m), 0.85 (6H, t, *J* 6.9 Hz), 0.82 (3H, d, *J* 6.9 Hz), 0.65 – 0.58 (2H, m), 0.52 (1H, dt, *J* 4.0, 8.1 Hz), -0.37 (1H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$+few drops of CD_3OD): 175.5, 85.5, 72.6, 69.7, 65.1, 63.0, 58.0, 52.5, 35.2, 35.0, 33.0, 31.8, 30.4, 30.1, 29.8, 29.7, 29.6, 29.55, 29.5, 29.4, 29.3, 29.2, 29.1, 28.6, 27.4, 27.3, 26.0, 25.3, 22.6, 15.7, 14.7, 14.0, 10.8; ν_{max}: 3389, 3017, 2919, 2850, 1733, 1467 cm^{-1}.

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ELISA protocol from Mr. Paul Mason

0.5% casein w/v PBS buffer - KCl (0.4 g), KH_2PO_4 (0.4 g), anhydrous Na_2HPO_4 (2.3 g), NaCl (16 g) was dissolved in 1800 mL d H_2O, stirred and heated to 37 ºC. Casein (10 g) was added slowly and the solution was stirred at 37°C and 400 rpm for 2 hours. It was then adjusted to pH 7.4 with NaOH (1 M) and made up to 2000 mL.

Citrate Buffer (0.1 M) - Citric acid monohydrate (2.365 g) and tri-sodium citrate (2.925 g) were dissolved in separate beakers with d H_2O 112.5 mL. Citric acid monohydrate was added to trisodium citrate until pH 4.5 was reached, then was made up to 250 mL with d H_2O. *OPD (5 mg) and* H_2O_2 *(4 mg)* were weighed out in separate black eppendorfs.
Dilute antigen (50 µl) was centrally pipetted into each well of the ELISA plate (96 well, flat bottomed, polystyrene, sterile, gamma irradiated), to G,H-11,12 only hexane was added. The plates were allowed to evaporate before being sealed.

The plates were unsealed and 0.5% casein PBS (350 µl) was dispensed using a 96 well plate washer, then incubated at 25°C for 30 minutes. Blood product (6 µL) were diluted to 1:40 with 0.5% casein PBS and resuspended by pipette. Casein was aspirated off the plates and tapped dry, serum or plasma (50 µl) was added to the wells. Remaining serum or plasma was pooled and added (50 µl) to C,D-11,12 and G,H-11,12 for a pooled and negative control respectively, 0.5% casein PBS was added to E-F 11-12 for a negative control. The plates were then sealed with a self-adhesive plate seal and incubated at 25 ºC for 60 minutes. The plates were washed three times using 0.5% casein PBS and tapped dry, before adding IgGFcHRP (50 µl, 2.9 µg/mL) using a multichannel pipette and then incubated at 25 ºC for 30 minutes. The plates were washed three times using 0.5% casein PBS and tapped dry, the OPD (5 mg) and H_2O_2 (4 mg) were dissolved in citrate buffer (25 mL) and added (50 µl) to the plates using a multichannel pipette. Plates were incubated at 25 ºC for 30 minutes. H_2SO_4 (50 µl, 3.0 M) was added to the plates and the results read on a UV-visible ELISA plate reader at 450, 492, 620nm.