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Synthesis of glycolipid fragments from Myobacterium tuberculosis

Mohammed, Moshin Omar

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Synthesis of Glycolipid Fragments from *Mycobacterium Tuberculosis*

A thesis submitted to the Bangor University

for the degree of Doctor of Philosophy

by

Mohsin Omar Mohammed

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Abbreviation and acronyms

[α]	Specific rotation
Ac	Acetyl
Å	Angstrom
Ac	Acetyl
AIDS	Acquired immunodeficiency syndrome
AG	Arabinogalactan
Aq	Aqueous
All	Allyl
br	Broad
Bn	Benzyl
BMDC	Bone marrow-derived dendritic cell
BSA	*N,O*-bis(trimethylsilyl)-acetamide
Bu	Butyl
Bz	Benzoyl
CAN	Ceric ammonium nitrate
°C	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
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DHP	2,3-Dihydro-2*H*-pyran
DMAP	4-(*N,N*-dimethylamino)pyridine
DMAG	Di-Mycolyl-Di-Ara*f*-Glycerol
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DOTS	Directly Observed Treatment Short course

DTBBP	4,4`-Di-*tert*-butylbiphenyl
ELISA	Enzyme-linked immunosorbent assay
Ether	Diethyl ether
EDCI	*N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide
Et	Ethyl
equiv	Equivalents
EPTB	Extrapulmonary TB
h	Hours
HIV	Human immunodeficiency virus
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
mAG	Mycolyl-arabinogalactan
MAC	*Mycobacterium avium* complex
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MDR-TB	Multiple Drug Resistant tuberculosis
Me	Methyl
MHz	Megahertz
min	Minute(s)
mL	Milliters
mmol	Millimol

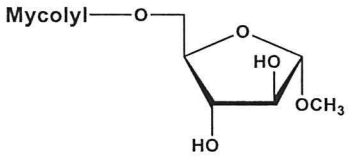
m/z	mass to charge ratio
Mincle	Macrophage-inducible C-type lectin
mol eq.	Molar equivalents
MAM	Methyl Arabino-Mycolates
GAM	Glycerol-Arabino-Mycolates
m.p.	Melting Point
mDAP	*meso*-Diaminopimelic
MS	Mass Spectroscopy
M. tuberculosis	*Mycobacterium tuberculosis*
MTT	3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NAG	*N*-acetylglucosamine
NAM	*N*-acetylmuramic acid
NBS	*N*-bromosuccinimide
NAD	Nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NIS	*N*-iodosuccinimide
NMR	Nuclear Magnetic Resonance
PAMPs	Pathogen-associated molecular patterns
Ph	Phenyl
PIMs	Phosphatidylinositol mannosides
PMB	*p*-Methoxybenzyl
ppm	Parts per million
Py	Pyridine
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*p*-Toluenesulfonic acid monohydrate
q	Quartet
R_f	Retention factor
R.T.	Room temperature
s	Singlet
sat.	Saturated
S_N2	Nucleophilic substitution, bimolecular
t	Triplet
T-cells	T Lymphocites

TB	Tuberculosis
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDMSCl	Tetrabutyldimethylsilyl chloride
TBDPSCl	Tetrabutyldiphenylsilyl chloridc
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluraniumtetrafluoroborate
TDM	Trehalose dimycolate
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TIR	Total internal reflection
TIPDS	1,3-(1,1,3,3)-Tetraisopropyldisiloxanylidene
TLC	Thin-Layer Chromatography
MTADM	Tri-Arabino-Di-Mycolates
TMM	Trehalose monomycolate
TMS	Tetramethylsilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNF-α	Tumor Necrosis Factor-α
TADM	*Tri*-arabino-*di*-mycolates
Ts	*p*-Toluene sulfonyl
trityl	Triphenylmethyl
p-TsCl	*p*-Toluene sulfonyl chloride
p-TsOH	*p*-Toluene sulfonic acid
WHO	World Health Organisation
XDR-TB	Extensively Drug Resistant tuberculosis
w/w	weight to weight ratio

Abstract

The objectives of this thesis may be divided into four parts:

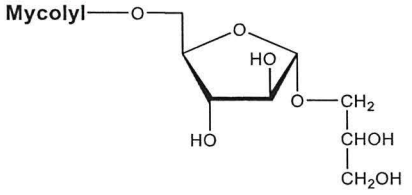
[1] Preparation of Methyl Arabino-Mycolates (MAM) **(I)** via the esterification of methyl-α-D-arabinofuranoside with seven classes of synthetic mycolic acids, and also esterifying with four fatty acids as model systems:



I

The initial biological and statistical analysis studies carried out on these compounds afforded promising results in ELISA assays for detection of TB. Also, most of these compounds showed a good response in the stimulation of the bone marrow-derived dendritic cells derived from mice.

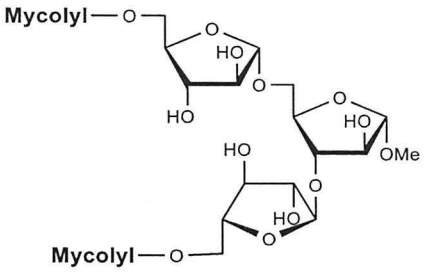
[2] Preparation of Glycerol-Arabino-Mycolates (GAM). In this part, the synthesis of both D- and L-Glycerol-α-D-arabinofuranoside was undertaken, and subsequent esterification of each glycan with three different synthetic mycolic acids **(II)** was carried out. Also, two models were prepared from the D-glycan, derived from simple fatty acids.



II

The main aim of this part was to prove the stereochemistry of the GAM (*i.e.* whether the desymmetrised glycerol unit resides in either the D or L configuration), and this was achieved by comparing the NMR data for both synthetic isomers (L and D-GAM) with those reported for the natural extract. This exercise confirmed the natural stereochemistry to be L-GAM. Some of the compounds produced in this part of the project were shown, via biological testing, to possess a good capacity for the induction of Bone marrow-derived dendritic cells (BMDCs) to stimulate a variety of pro-inflammatory cytokines (*e.g.*, TNF-α, IL-β, IL-6), the latter being essential to control TB disease.

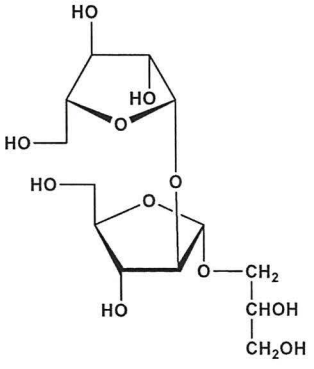
[3] The third part entailed preparation of Methyl Tri-Arabino-Di-Mycolates (MTADM) **(III)**, which involved synthesis of both the donor and the acceptor according to literature methods with slight modifications. The coupling of the donor and the acceptor to prepare the desired glycan was carried out using known coupling conditions. A model of this glycan was prepared through esterification with a normal fatty acid. A series of five compounds from MTADM was prepared, based on the three common classes of MAs.



III

The ELISA assay for detection of TB employing these as the antigenic compounds is in progress. This series of compounds was subjected to stimulation assays, however, the compounds were found to engender low responses compared with the members of the other two series.

[4] The final part of this project involved synthesis of Di-Mycolyl-Di-Ara*f*-Glycerol (DMAD) (**IV**). The glycan moiety of DMAG was successfully prepared for both stereochemistries of the glycerol component. A study to explore the effect of the protecting group on the yield and the β-selectivity of the glycosylation was carried out, and we were successful in the development of efficient route to prepare the DMAG glycan with an excellent β-selectivity and in excellent yield. The attempted esterification of this glycan with MAs was found, however, to be unsuccessful.



IV

The testing and assay of the effects of the DMAG glycan on a range of cytokines involved in the immune system, in addition to an assessment of their antigenic capacity, are expected to be carried out in the near future.

Chapter 1

Introduction

Mycolyl-arabinogalactan (mAG) complex is the largest component in the mycobacterial cell wall and acts as a permeability barrier that prevents the passage of antibiotics. Therefore, blocking mAG biosynthesis is an important strategy for developing new anti-TB drugs. The work described in this thesis sets out to synthesise different *mono-*, *di-* and *tri-*arabino-mycolates and to allow their biological effects to be determined and applied. The following introduction provides background information on Tuberculosis (TB), *Mycobacterium tuberculosis* and its components, a brief introduction regarding the roles of carbohydrates and their conformations and finally a review of arabino-mycolates and their biological activities.

1.1 Tuberculosis

1.1.1 History

Tuberculosis (TB) is a devastating disease caused by *M. tuberculosis* and several closely related mycobacterial species belonging to the so-called *M. tuberculosis* complex. This disease is usually transferred when a person breathes in air that contains species of *M. tuberculosis* from an infected person.[1] The lung is the main place of infection in the body, however any other organ can be infected, such as the lymph nodes, kidneys, central nervous system.[2,3,4] TB is believed to date back more than one hundred and fifty million years,[5,6] and is known by many names, such as: 'The king of diseases' in India; 'The captain of all these men of death';[7] and 'phthisis' by the Greek physician, Hippocrates.[8] The earliest specific discoveries of TB were in the remains of a bison from 18,000 years ago.[9] Investigations in Egyptian mummies, over 5,000 years old have also shown signs of death as a result of TB.[10] There is evidence which shows the presence of TB in China 2300 years ago and in India 3300 years ago.[11,12] It is believed that TB in the Americas was present before the arrival of European explorers, with a similarity to that found in Egypt.[13,14] There is much archaeological evidence of the incidence of TB during the middle ages.[14] TB was well documented in ancient Greece and its treatment was devised by the physician Clarissimus Galen as sea voyages, fresh air and milk.[15] In 1865, surgeon Jean-Antoine Villemin, established the nature of TB infection by injecting a rabbit with liquid from the lung of a patient who died due to infection with TB.[16] In 1882, when Robert Koch finished a presentation on the discovery of the causative agent

of tuberculosis (*M. tuberculosis*), silence enveloped the room at the Berlin Physiological Society.[17] Koch, who received the Nobel Prize in Medicine and Physiology in 1905, described his discoveries, in a manuscript published in the Berliner Klinische Wochenschrift: "In the future the fight against this terrible plague of mankind will deal no longer with an undetermined something, but with a tangible parasite, whose living conditions are for the most part known and can be investigated further." (transl.)[18] In 1921, Albert Calmette and Camelle Guérin developed a vaccine from *Mycobacterium Bovis* called Bacillus Calmette-Guérin (BCG).[19] In the 1940's the BCG vaccination had become more widespread, being used in Scandinavia, France, Spain, Russia, Latin America and some Eastern European countries.[20,21] Currently, the vaccination is a freeze-dried form of BCG and more than 115 million units are dispersed annually in 172 countries on average.[22] Since Koch's discovery, a dependable vaccine has never been developed because the complexity of TB immunity differs from the immunity of other microbial diseases. According to some sources, in the 19th century approximately one fourth of all deaths in major American and European cities was caused by TB.[23] TB came to be considered a public health problem, particularly when the populations increased in cities after the Industrial Revolution, with poor living conditions causing a shortage in health services, thus leading to an increase in TB infection.[24] Although largely a curable disease, TB is among the top ten causes of death and disability worldwide.[25] A significant rise in the number of deaths of TB patients was reported in 1990, in contrast to the number of deaths due to other illnesses, for instance malaria, AIDS and leprosy.[26] In 1993, the World Health Organization (WHO) announced TB as a major health problem and declared a global health emergency.[27] 9.4 million new cases of TB were recorded in 2009, with most being concentrated in South Africa, China, India, Indonesia and Nigeria.[28] In 2010 this reduced to 9 million new cases, leading to 1.4 million deaths, equal to approximately 3,800 deaths per day, and a reduction in the estimated global incidence rate to 128 cases per 100,000 population. **Figure 1.1** shows the estimated number of new cases of TB per 100,000 population in 2010.[2,30] The WHO estimates that nearly one third of the world's population is currently infected with *M. tuberculosis* and about 15% of these are believed to be co-infected with HIV **Figure 1.2**.[29,30,31]

In 2012, The WHO South-East Asia Region with an estimated 3.4 million incident cases and about 4.8 million prevalent cases and 450,000 deaths, carried about 48% mortality and 39.5% morbidity of the global burden of tuberculosis.[32]

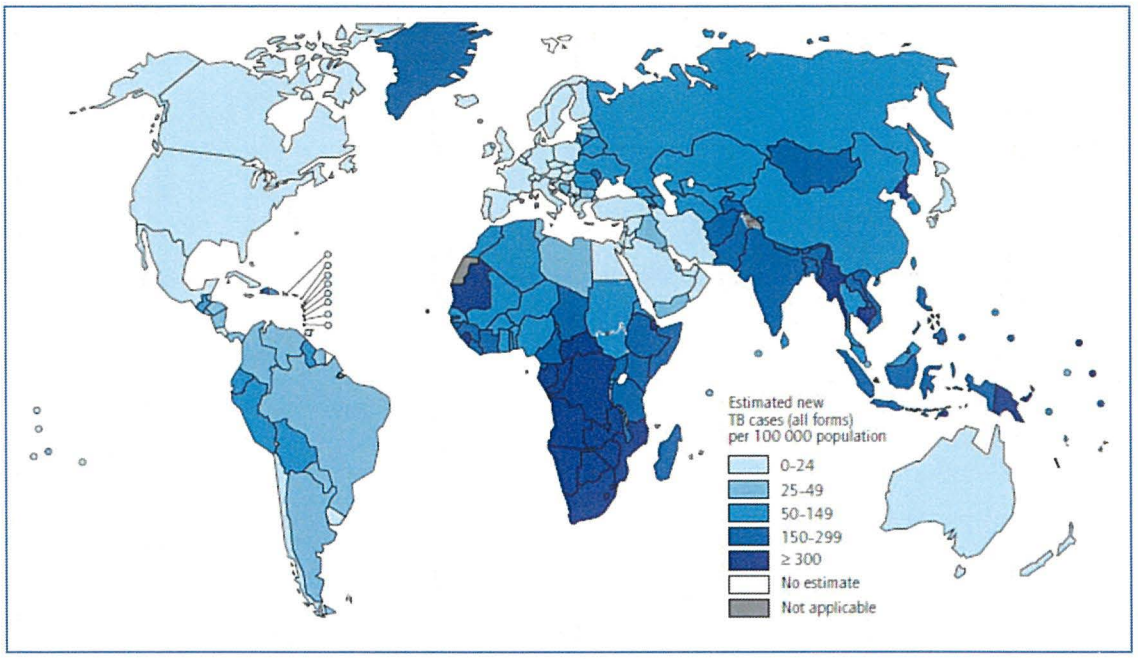


Figure 1.1: Map showing the estimated number of new TB cases in 2010 [30]

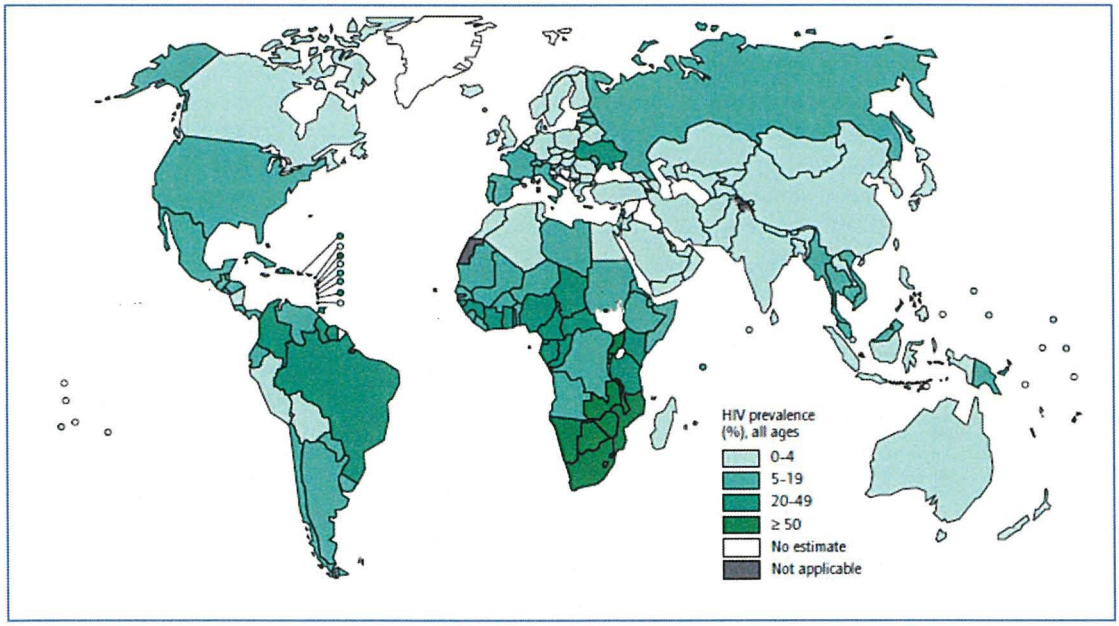


Figure 1.2: Map showing the estimated number of TB patients co-infected with HIV in 2010 [30]

TB and HIV work in conjunction with each other and form 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 noteworthy rise in the universal rate of TB by causing a weakening in individual immunity systems. The risk of developing active TB for individuals infected

with HIV is 20-37 times greater than for uninfected persons. Although HIV and TB are both preventable and treatable, they continue to increase in developing nations in which TB infection and HIV are prevalent and resources are limited.[33,34] Similarly, TB may also destructively affect the natural progress of HIV infection. Numerous researchers have shown that TB co-infection increases the danger of HIV development and death, mainly in individuals with untreated HIV disease.[35,36] The effect of TB on HIV illness development is assumed to be assignable to increased immune system activation.[37]

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. tuberculosis* showing resistance to the anti-TB drug streptomycin (SM) were developed during early attempts to treat TB.[38] Presently, strains of *M. tuberculosis* 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 (INH), Rifampicin (RIF), Pyrazinamide (PZA), Ethambutol (EMB) and Streptomycin (STM), it is considered to be multiply drug resistant (MDR).[39] In 2008, 29,423 new cases of TB were reported worldwide in 127 countries and about 7% from those cases represented MDR-TB. The WHO estimates that about 630,000 new cases of MDR-TB in 2013 and their last report in 2012 estimated that 3.7% of new cases and 20% of previously treated cases are multidrug-resistant.[28, 40] More recently, extensively drug-resistant tuberculosis (XDR-TB) cases have shown that *M. tuberculosis* is resistant to RIF, INH, a Fluoroquinolone and a second-line injectable drug (Capreomycin, Kanamycin or Amikacin). This type of resistance was reported for the first time in KwaZulu-Natal in South Africa.[41] In 2008, 963 infections of XDR-TB were reported globally.[28] Resistance of *M. tuberculosis* to the treatment makes this disease more problematic and an estimated budget of approximately $4.4 billion was promised in 2012 in order to address and control, by diagnosis and treatment, the TB problem.[31]

1.1.2 Pulmonary *M. tuberculosis* Infection

TB is an airborne disease, and the lung is the main site of TB infection (Pulmonary TB, PTB). There are many symptoms of PTB such as fever, loss of appetite, weight loss, chest pain and prolonged coughing. Extra-pulmonary TB (EPTB) in the lymphatic system affects bones, joints and the central nervous system.[42,43] Miliary TB is a

dangerous type of EPTB in which several organs are infected by the bacterium after it enters the circulatory system, and it causes approximately 100% mortality.[44,45] 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 by the host, where the bacilli are killed after being inhaled into the lung, no TB infection will appear. The bacilli could similarly advance to the alveoli when they are swallowed by macrophages followed by the activating of both the innate and an adaptive immune response sequentially. Activation of the immune system of the host starts the process of forming a granuloma which characterises an interaction between a variety of types of immune system cells such as dendritic cells (DCs), T cells and macrophages. In the case of a weak immune response system, the bacilli can be suspended in granulomas producing latent TB infection, which shows no symptoms; however, any change in the immune system due to another illness, will cause the bacteria to break the granulomas leading to reactivation of the TB.[46] An individual infected with active TB will produce small droplets of *M. tuberculosis*, which are spread as aerosolised drops when an infected patient sneezes.[47,48]

"Allergies" or immune system responses can be defined as an over reaction from the host's body against any foreign substance that enters it. Usually these substances may be harmful, in some cases they are not. In medical terms, allergies are described as "abnormal reactions of the immune system that occur in response to otherwise harmless substances".[49] Any substance that induces an immune response will be considered an allergen. Approximately half the world's population suffer from allergies and there is a budget of $5.9 billion to spend on their treatment.[50]

Immune system components are located in different organs in the body, namely, bone marrow, spleen, lymph nodes and thymus. These organs are the source of the white cell or lymphocytes; therefore they are called "lymphoid organs". Depending on the specificity and the speed of the interaction between the substance and the immune system, immunity can be divided in two parts: innate responses and adaptive responses, however, practically there is much interaction between them. Innate immunity involves the elements of the immune system, macrophages, complement, neutrophils, monocytes, cytokines and acute phase proteins, which provide immediate host defence. However, innate immunity is sometimes used to include chemical, physical and microbiological walls. Adaptive immunity can be regarded as the hallmark of the immune system, which consists of antigen-specific reactions through T lymphoctyes and B lymphocytes.[51] The role of the immune system is to harness its cellular and molecular components to work

together to destroy antigens. Its functions include four key general roles: recognising antigens introduced in the body as distinct from 'self' cells; eliminating the source of the foreign antigen; retaining a memory of immunological encounters which is referred to as sensitisation; and acting as an exclusion barrier.[52]

Toll-like receptors (TLRs) can recognize pathogen-associated molecular patterns (PAMPs) of *M. tuberculosis* and initiate signalling pathways that lead to the activation of the innate immune response, cytokines and formation of the adaptive immune response. The major receptors for *M. tuberculosis* are TLRs-1, -2, -4, -6 and -9; among them TLR2 and TLR4 play a key role in the reorganization process. TLR2 can form heterodimers with either TLR1 or TLR6 to sense the PAMPs of *M. tuberculosis* and activate macrophages and dendritic cells through adaptor proteins, myeloid differentiation primary response gene (88) (MyD88) and an adapter molecule associated with toll-like receptors (TIRAP).[53]

1.2 The role of Leukocytes in the immune response to *M. tuberculosis* infection

Theoretically, lung resident, alveolar macrophages are the first cells to encounter *M. tuberculosis* upon inhalation of infectious droplets. The primary homes of *M. tuberculosis* within the host are monocyte-derived macrophages and phagocytes within the lung, and ultimately the granuloma is formed by surrounding the infected macrophages with T lymphocytes and other leukocytes.[54] Infectious droplets of *M. tuberculosis* are thought to be phagocytised by lung macrophages or resident alveolae through a variety of receptors.[55,56] Mycobacteria are also phagocytised by dendritic cells; however these cells lose their phagocytic potential.[57] Dendritic cells and *M. tuberculosis* infected macrophages produce inflammatory cytokines and chemokines.[57,58] As a result, additional leukocytes can migrate to the infected site. Infected dendritic cells migrate to the lymph nodes to present mycobacterial antigens to prime naïve T lymphocytes.[59,60] Primarily lymphocytes and macrophages combine to produce the granuloma; however they may present other types of cell from the immune system such as neutrophils.[61,62] The granuloma provides a central focus for cell to cell interactions, such as CD4 and CD8 T cell-mediated induction of apoptosis of infected macrophages, or activation of macrophages through IFN-γ and TNF cytokine signalling.[63] Furthermore, the granuloma is also believed to provide a barrier to bacterial dissemination.[64]

1.2.1 Macrophages

Macrophages can be activated upon uptake of the mycobacterium, but it depends upon the route of entry. Signalling by pro-inflammatory cytokines such as IFN-γ and TNF, also activates the macrophages.[58,65] IFN-γ is essential for macrophage control, and can act in combination with TNF on a macrophage to result in the optimal activation.[66] During *M. tuberculosis* infection, dendritic cells are far more effective than macrophages at stimulating IFN-γ production or T lymphocyte proliferation.[57] This is supposed to be because of some factors stimulated by *M. tuberculosis* infection, which decrease the capability of macrophages to efficiently present antigens through mechanisms that are not yet understood.[67]

1.2.2 Dendritic Cells

Steinman, about thirty years ago, was the first to define Dendritic Cells (DCs). He described DCs as striking dendritic-shaped cells in the spleen. Soon, it became known that these cells are present in all lymphoid and most non-lymphoid tissues. Dendritic cells are antigen-presenting cells, which play a serious role in the regulation of the adaptive immune response. Through the 1970s, macrophages were believed to be the principal antigen-presenting cells in the immune system. The abundance of macrophages was more than DCs. Since the 1980s it has been shown that DCs act as "professional antigen-presenting cells". DCs can be transmitted from blood and bone marrow using various mixtures of growth factors, for example TNF-α and interleukin IL-4.[68]

DCs infected with *M. tuberculosis,* increase co-stimulatory molecules, signifying that they are capable of priming an adaptive immune response.[60,69] Dendritic cells, like macrophages, are capable of supporting *M. tuberculosis* growth,[69] and are activated with lipopolysaccharide (LPS), IFN-γ or TNF during the infection of *M. tuberculosis*.[57]

1.3 Cytokines in the immune response to *M. tuberculosis* infection

Dendritic cells and macrophages start to produce cytokines like IL-12[70,71] and TNF,[58] sequentially upon infection with *M. tuberculosis*. These cytokines help to shape the immune response. TNF is responsible for recruitment of macrophage chemokine production.[58] IL-12 is also made by macrophages or dendritic cells during T cell priming.[71]

1.3.1 Tumour Necrosis Factor

Tumour necrosis factor (TNF) is a pro-inflammatory cytokine produced by dendritic cells, macrophages, activated T cells and natural killer cells.[72] Coley, more than 100 years ago, found that a crude bacterial extract was able to stimulate tumour necrosis. The TNF gene was isolated and characterized in 1984. Two genes were found to belong to the same family, therefore TNF was named ''TNF-α'', while TLα was called ''TNF-β.[73] The significance of TNF as a modulator of the host response to certain infections, and the different roles of the two TNF receptors, has been confirmed by studies using TNF receptor-deficient mice.[74]

1.3.2 Interleukin 6

Interleukin-6 (IL-6) is an interleukin which acts as a pro-inflammatory cytokine. It is secreted by macrophages and T cells to stimulate immune response. This cytokine plays a significant role in the coordinated systemic host defence response to injury. There are many functions for IL-6, such as regulating immune responses and acute-phase protein response. The role of IL-6 in active TB is mainly negative. This was confirmed when it was shown that IL-6 inhibits the production of TNF-α and IL-1β, which may promote intracellular killing of microorganisms and development of granulomata, and also promotes the growth of mycobacteria in peripheral blood monocytes.[75]

1.4 Latent TB infection and active TB disease

As mentioned before, TB is an airborne transmissible disease. Individuals with active TB disease spread the bacteria to other people directly, and, upon inhalation, *M. tuberculosis* starts to grow in the lung. It has been established, that only a person with active TB can transmit TB, and if it is untreated an average of between 10 and 15 individuals can be infected annually.[76] Not everybody infected with TB bacteria becomes ill. Consequently, different TB-related conditions exist: active TB disease and latent TB infection **(Table 1.1)**. It is believed that approximately 5-10% of infected individuals will develop active TB in their lifetime, and half of those persons will develop active TB disease within the first two years of infection.[77,78,53]

Table: 1.1 Active disease vs. latent TB infection

Active TB disease	Latent TB infection
May present symptoms.	Does not feel ill.
May present abnormalities in chest X-ray. May present positive sputum smear or culture. Positive tuberculin skin test. Positive Quanti FERON-TB[a] Gold test	Normal chest X-ray. Negative sputum test. Positive tuberculin skin test. Positive Quanti FERON-TB Gold test
The organism is active.	The organism is inactive, dormant.
Treatment to treat active TB disease.	Chemoprophylaxis.

a The Quanti FERON-TB is a whole blood assay based on the detection of INF-γ released by T cells in response to *M. tuberculosis* – specific antigens.

1.5 Tuberculosis Treatment

TB requires a long and complex treatment regimen. This may be due to the pathogen's unusual method of survival within the host's macrophage cells. A number of anti TB drugs are currently used, and the treatment of TB is different from most other diseases because the course of chemotherapy takes a longer time, normally six to eight months as a standard short treatment, and consists of a mixture of different antibiotics which are usually two classes, first and second line drugs.[79,80,81] First line drugs include INH **(1)**, PZA **(2)**, EMB **(3)**, STM **(9)** and RIF **(10)**, while the second line drugs, which are more expensive than the first line drugs and sometimes less effective, include Fluoroquinolone and *p*-Aminosalicylic acid (PAS) **(4)**.[82] Most drugs used for the treatment of TB are effective in restricting the growth of the bacilli.[83] STM was the first antibiotic used (since 1946) and reformed the chemotherapy of TB. PZA and INH have been used since 1955, and EMB since the 1960s. RIF, a natural compound extracted from *Streptomyces mediterranei*, became available in the 1970s. **Figure 1.3** shows the structures.[84,85] Today, there are more than thirty different types of anti TB drugs being used, however most of them are analogues of the above products. Mycobacterial cell wall biosynthetic pathways are a common target of these drugs as illustrated in **Table 1.2**.[86]

Table 1.2: General TB drugs and their mechanisms [86]

Drug	Mechanisms
PZA	Energy depletion and disruption of membrane transport.
EMB	Cell wall arabinogalactan biosynthesis inhibition.
Kanamycin	Protein biosynthesis inhibition.
Ethionamide	MA biosynthesis inhibition.
INH	Cell wall MA biosynthesis inhibition; effects on metabolism of NAD, carbohydrates, lipids, and DNA.
RIF	RNA biosynthesis inhibition.
SM	Protein biosynthesis inhibition.
PAS	Inhibition of folic acid and iron metabolism.
DCS	Peptidoglycan biosynthesis Inhibition.
STM	Protein biosynthesis inhibition.

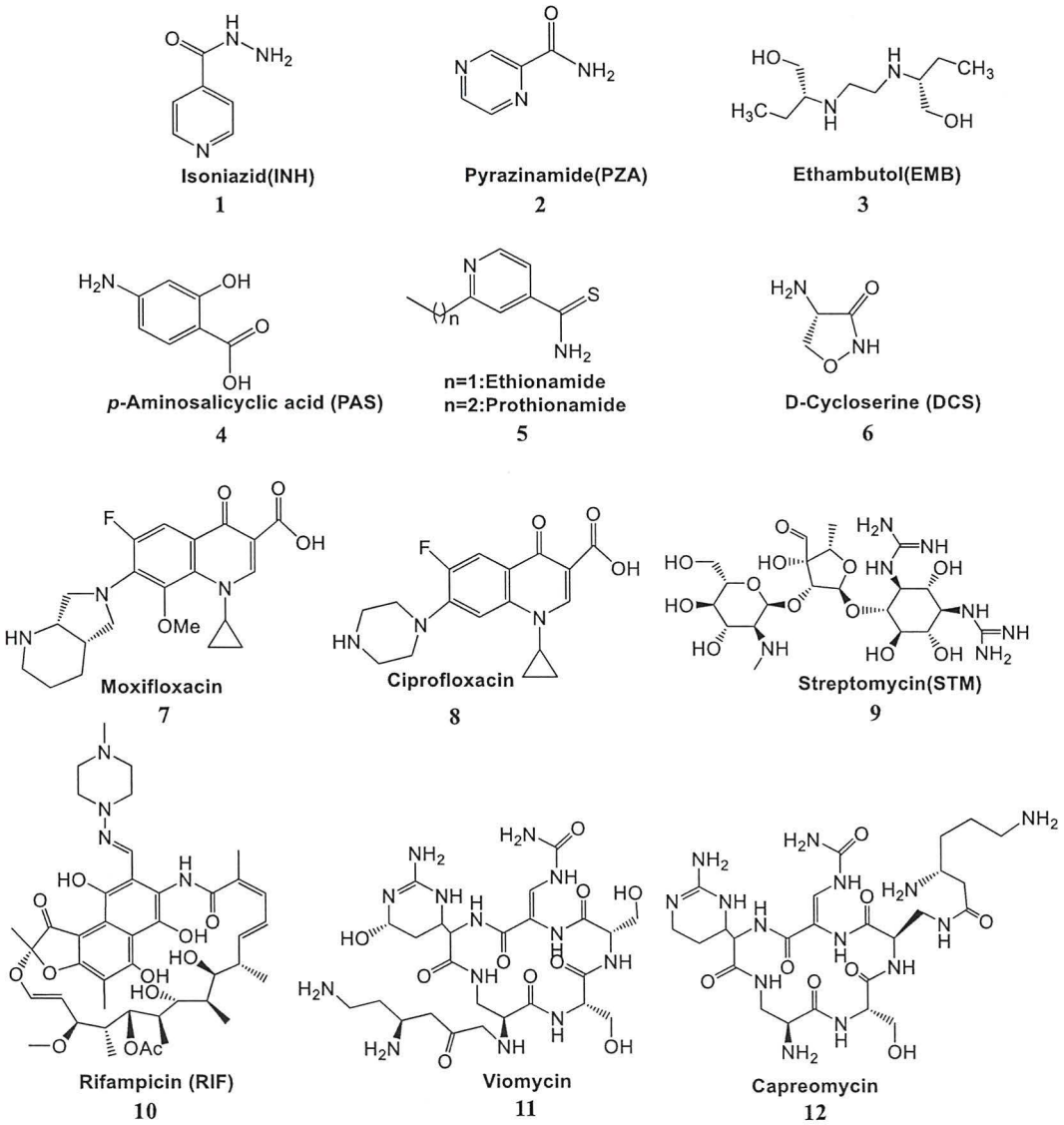


Figure 1.3: Structures of the main first and second line anti-TB drugs [85]

1.6 *Mycobacterium Tuberculosis*

The German physician, Robert Koch, in 1882, was the first scientist to identify and isolate *M. tuberculosis*, a scanning electron micrograph image of which is shown in **Figure 1.4**, and he was subsequently awarded the Nobel Prize in Physiology and Medicine in 1905. *M. tuberculosis* and *Mycobacterium leprae* are two of the most well-known species of Mycobacteria, a varied group that contains more than 70 different species. Some species such as *M. tuberculosis* cause TB in humans only, while some, such as *M. bovis,* can cause TB in both humans and animals.[87,88,89,90] Mycobacteria, are non-motile, aerobic, non-encapsulated, non-spore forming, acid-fast (do not retain the methyl violet stain well), weak Gram-positive bacilli.[91] However, it was reported recently that they have features of both Gram-positive and Gram-negative bacteria. *M. tuberculosis* grows most successfully in tissues with oxygen content, such as the lungs, and appears under the microscope as straight or slightly curved rods approximately 1-4 x 0.3-0.6 μm in size, and divides aerobically every 16 to 20 hours,[7,92,93,94] an extremely slow rate compared with other bacteria, such as *Escherichia coli* that can divide roughly every 20 minutes.[95] *M. tuberculosis* is resistant to very harsh conditions, for example chemical sanitizers and drying; this is due to its unique cell wall composition compared with the cell walls of other bacteria. The cell wall is rich in mycolic acids (MAs) which will be discussed forthwith; these lipids are very important for survival and growth of *M. tuberculosis* inside the infected organism.[96]

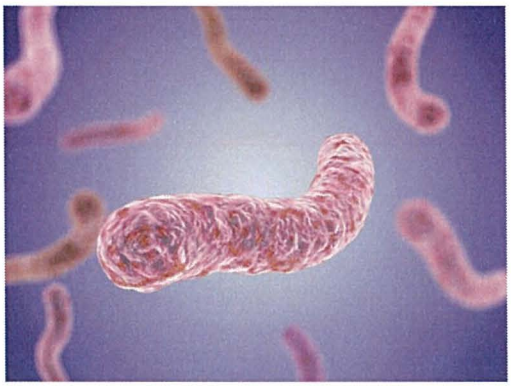


Figure 1.4: Scanning electron micrograph of M. tuberculosis [97]

1.6.1 The Mycobacterial Cell Wall

The cell wall in *M. tuberculosis* has an unusual structure, containing a thick, multi-layered and extremely hydrophobic cell envelope, and this is important for the organism because it prevents the passage of antibiotics inside the cell and protects it from the immune system of the host by allowing it to survive in macrophages, acting as a permeability barrier. The cell wall biosynthetic pathway in mycobacteria has attracted attention for various studies developing new classes of anti-TB drugs.[98] For example, INH targets MA biosynthesis[99,100] and EMB targets the biosynthesis of cell wall arabinan by inhibition of the enzyme arabinosyltransferase, which is involved in the pathway of the biosynthesis.[101] The general structure of the mycobacterial cell envelope is now well understood **(Figure 1.5)**, and it was basically proposed by Minnikin with its complex architecture of lipids, glycolipids, polysaccharides and proteins.[102, 103] Generally speaking, the *M. tuberculosis* cell wall is made up of four significant components as shown in **Figure 1.5**, namely, the plasma membrane (PM) (inner membrane), peptidoglycan (PG), mycolyl-arabinogalactan (mAG), and an outer capsule-like layer.[81,103,104,105,106]

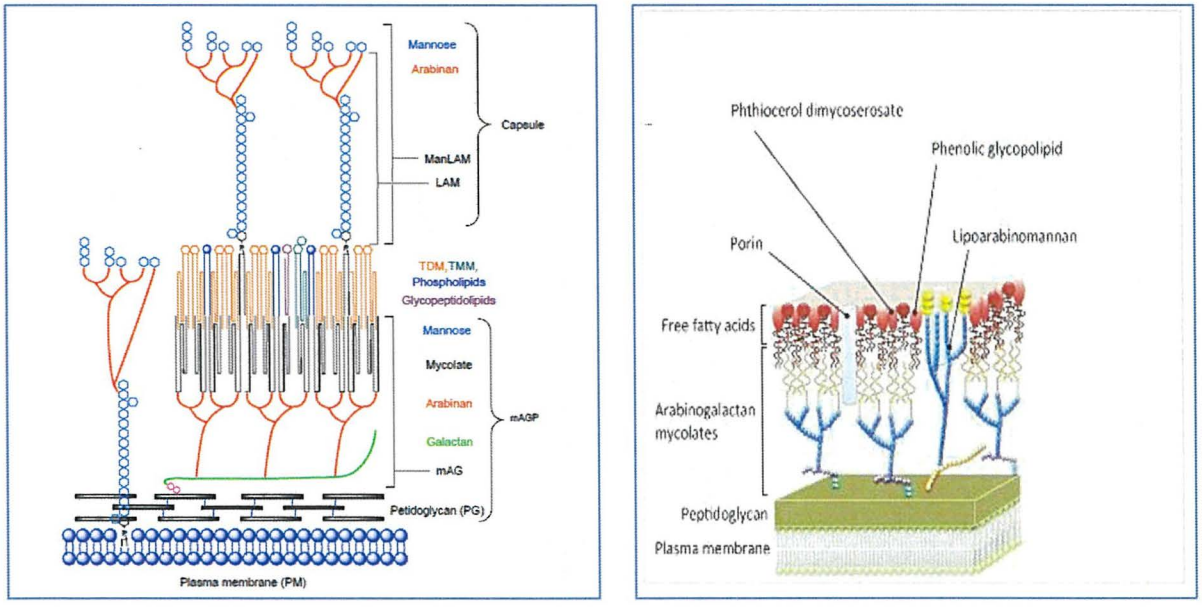


Figure 1.5: General structural of M. tuberculosis cell wall [107,108]

1.6.2 Peptidoglycan

Peptidoglycan (PG) is a polymer that forms the backbone of the cell wall skeleton, a porous layer between the plasma membrane and the cell wall, and is similar to that of other bacteria. PG consists of a polysaccharide with β-(1,4) linked chains of alternating

units of *N*-acetyl-α-D-glucosamine (NAG) and *N*-acetylmuramic acid residues. The *N*-glycolylmuramic acid units in the polysaccharide are esterified at *O*3 with tetrapeptide motifs of L- and D-alanine, D-glutamic acid and *meso*-diaminopimelic acid (mDAP) **(Figure 1.6)**.[92,109,110] In Mycobacteria, two molecules of mDAP are cross-linked or mDAP and D-alanine are linked.[111] The PG biosynthesis pathway in *E. coli* is believed to be comparable to that in Mycobacteria.[112,113]

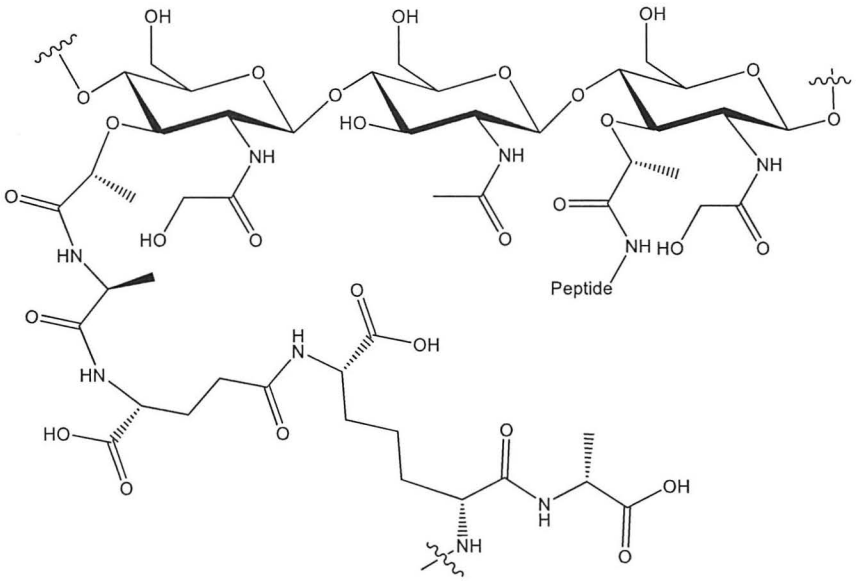


Figure 1.6: Structures motifs present in mycobacterial PG [114]

1.6.3 Mycolyl-arabinogalactan complex

The mycolyl-arabinogalactan complex (mAG) complex **(Figure 1.7)**, is the largest component structure in the cell wall of mycobacteria and is located directly outside the PG. It is believed that mAG 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 a long chain (C70-C90), α-alkyl branched β-hydroxylated fatty acid, 'mycolic acid' (MA). Carbohydrate (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 in the wall by a unique 'linker disaccharide', α-L-Rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranos-ylphosphate. 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 unit of linear (1→5) (α-D-Ara*f*) residues and branches to form a 3,5-α-D-Ara*f* linked fork.[115,116,117,118] Galactan and arabinan are bonded from the C-5

position in the galactan core.[107,119,120,121,122] 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.[103,105] Both galactosyl and arabinosyl units in mAG are in a furanose form which is less stable thermodynamically than the pyranose form.[79] It is believed that this plays a large role in raising the flexibility of the polysaccharide and making the MAs pack strongly by van de Waals interactions. Thereby, the structure of the cell wall has extremely low permeability and this 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 an intact mAG complex.[98] Therefore, mAG biosynthesis is an important strategy for developing new anti-TB drugs because it is important for the growth and survival of mycobacteria. Indeed, isoniazid and ethambutol, two of the standard antibiotics, target mAG biosynthesis; ethambutol inhibits arabinosyltransferases which contribute to the biosynthesis of the arabinan part of the polysaccharides, while isoniazid inhibits MA biosynthesis.[123]

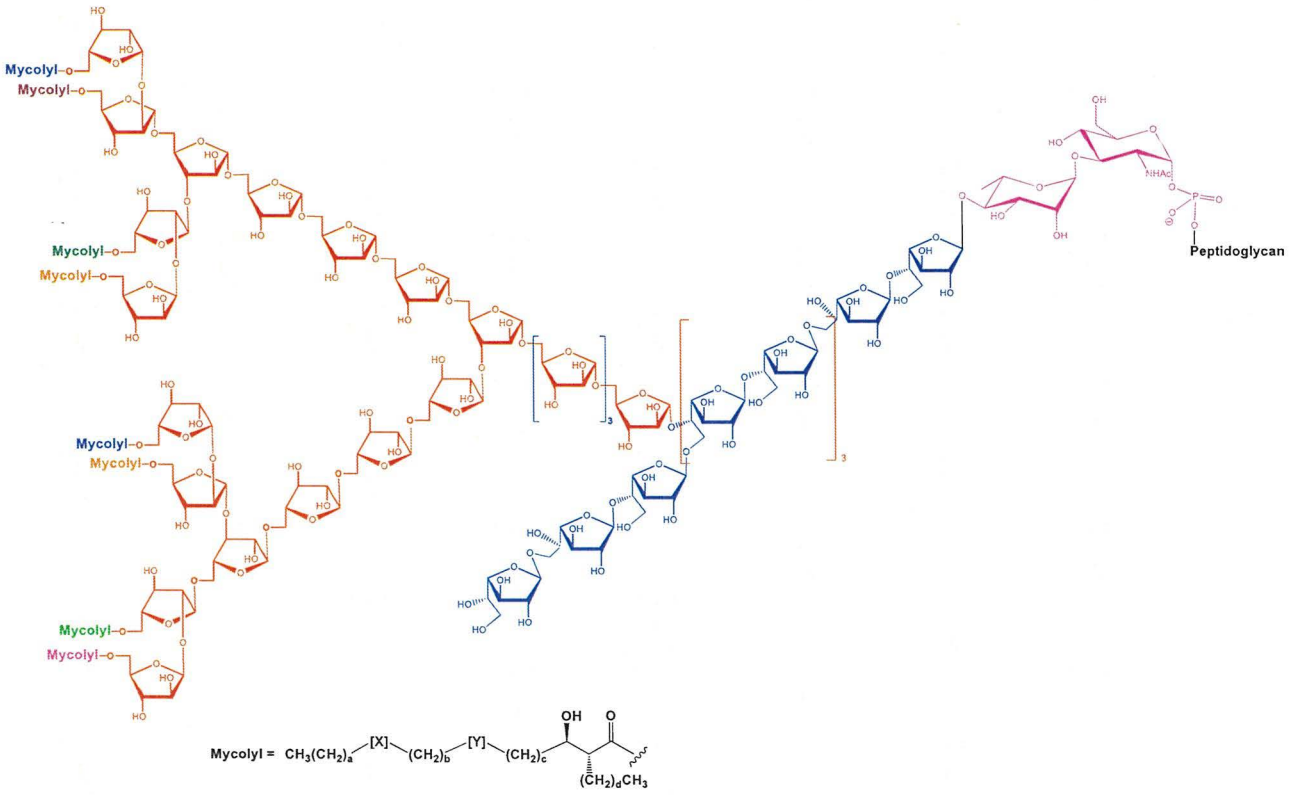


Figure 1.7: Primary structure of the mAG

1.6.4 Biosynthesis of arabinogalactan (AG)

The biosynthesis of AG has been understood in the last few years.[102,124,125,126,127,128,129] Brennan[114] suggested the route for the first time, **Figure 1.8**, and later on all the enzymes and genes which contribute in the biosynthetic pathway were identified. [115,124,130]

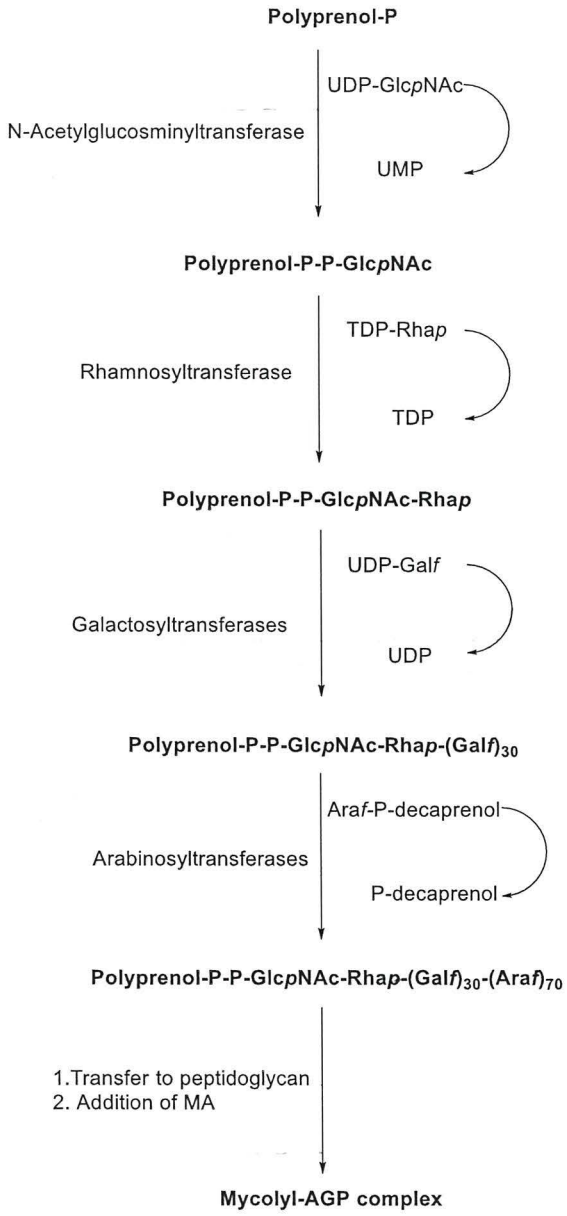


Figure 1.8: Biosynthetic pathway for the association of the mAG complex [114]

As shown in **Figure 1.8**, the biosynthesis includes the consecutive addition of glycan moieties to a polyprenol. After the completion of the polysaccharide and transfer to peptidoglycan, MAs are then added. Since this thesis is related to the arabinan portion of the cell wall, the biosynthesis of this part of the mAG complex will be discussed.

The biosynthesis of the arabinan units of mAG involves the contribution of many enzymes, arabinosyltransferases (AraT's), and most of these have not been isolated successfully; however, assays of the enzymes in crude membrane fractions have been reported.[131,132,133] There are many studies on the AraT's particularly on two types that play important roles in the synthesis of mycobacterial arabinan, namely EmbA (Rv3794) and EmbB (Rv3795); however they are not clearly understood.[134] An inhibitor of these enzymes is EMB, which is one of the important anti-TB drugs that targets the biosynthesis of the cell wall. Recently, three other types of AraT's were recognized which were not inhibited by ethambutol, namely AftA, AftB and AftC. They are also derivatives of EmbA and EmbB.[130] Finally all these enzymes use the same donor substrate, β-D-arabinofuranosyl-1-monophosphodeca-prenol (DPA) as the building block of the arabinan moieties, while the latter is the only known donor of Ara*f*.[135,136] The carbon skeleton of the arabinosyl residues **(Figure 1.9)** is derived from the non-oxidative pentose shunt.[137]

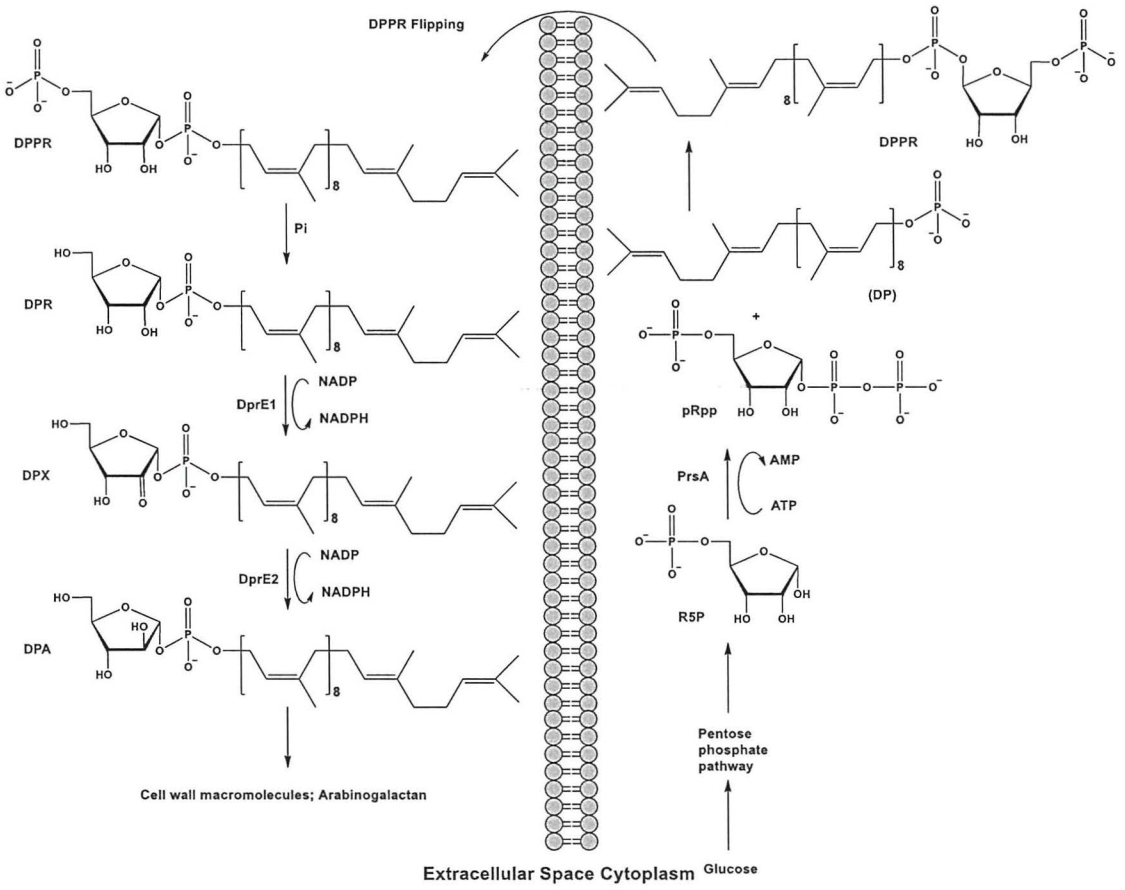


Figure 1.9: Formation of arabinan precursor DPA

The carbon atoms of the Ara*f* residues are derived from the pentose shunt pathway. α-D-Ribose-5-phosphate (R5P) is then acted on by PrsA, a pRpp synthetase, which catalyses the addition of a diphosphate moiety from ATP to the carbon-1-OH. The formation of decaprenylphosphoryl-D-arabinose (DPA) then proceeds with the transfer of ribose-5-phosphate from pRpp to decaprenylphosphate to form decaprenylphosphoryl-5-phosphoribose (DPPR). DPPR then undergoes dephosphorylation to decaprenol-1-monophosphoribose (DPR) and epimerisation of the ribosyl unit at carbon 2-OH position. DprE1, a FAD-containing oxidoreductase is responsible for oxidising the ribosyl carbon-2-OH producing the keto sugar decaprenol-1-monophosphoryl-2-keto-β-*erythro*-pentofuranose (DPX). This is further reduced to DPA DprE2, a decaprenylphosphoryl-2-keto-D-erythropentose reductase.[138,139]

1.6.5 Lipoarabinomannan

Lipoarabinomannan (LAM) and phosphatidylinositol mannosides (PIMs), are the main lipoglycans found in the mycobacterial cell wall and they play a significant role in the survival of mycobacteria by protecting them from their environment. Furthermore, it is found that they have an important role in the interaction between bacteria and the host by modulating the host response during an infection.[140,141] LAM is attached to the cell wall membrane non-covalently and also may be bound to the MA layer in the diacylglycerol of the phosphatidyl-*myo*-inositol (PI) moiety and/or attached to the plasma membrane **(Figure 1.10)**. LAM has been classified into three diverse classes depending on the cap motifs existing on the non-reducing terminal. Namely, a mannose-capped LAM (Man LAM), is found in the cell wall of slow growing mycobacteria like *M. leprae*, *M. bovis* and *M. tuberculosis*; a phospho-*myo*-inositol capped LAM (PI LAM), is present in the cell wall of fast growing mycobacteria like *Mycobacterium tuitum* and *Mycobacterium smegmatis;* finally, LAM devoid of capping (Ara LAM), is present in *M. chelonae*.[107,140]

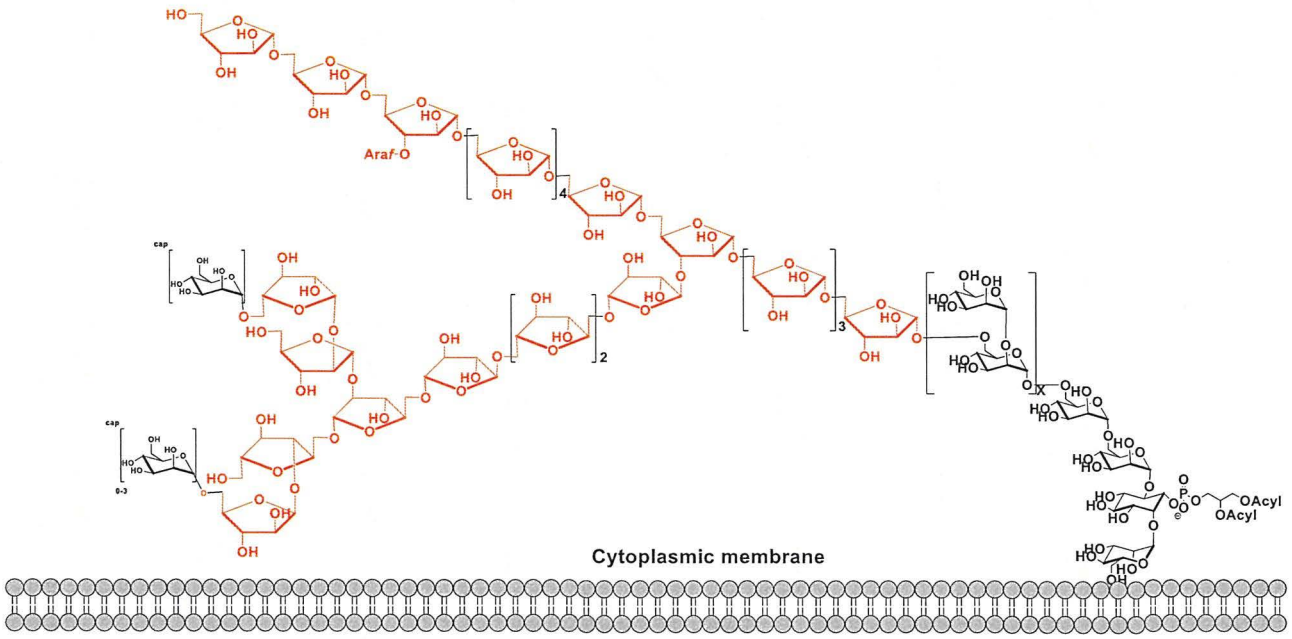
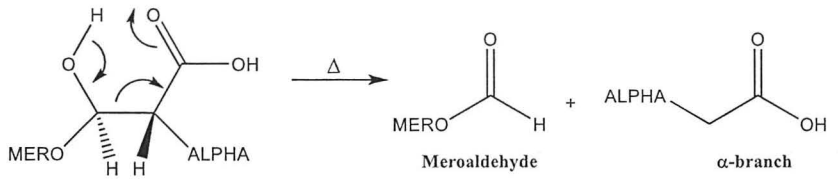


Figure 1.10: Model structure of LAM

1.7 Mycolic Acids

Mycolic Acids (MAs) are one of the main components of the cell wall, and can be found bonded either to AG or as components of other free lipids, such as trehalose *mono*-mycolates (TMM) and *di*-mycolates (TDM), glycerol *mono*-mycolate (GroMM) and glucose *mono*-mycolate (GMM). The cell envelope of *M. tuberculosis* is believed to contain a mixture of over 500 different mycolic acids with a varying combination of functional group type and chain length. 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.[81,156] MAs are a homologous series of high molecular weight (C60-C90) α-alkyl, β-hydroxy fatty acids; and are characteristic components of the cell wall of all the strains of mycobacteria. In 1938, Anderson *et al.* first isolated MA as unsaponifable ether-soluble hydroxy acids from the human tubercle bacillus and believed it to have a formula of either $C_{88}H_{172}O_4$ or $C_{88}H_{176}O_4$. During the purification of MA it was apparent that it was very difficult to purify and not possible to crystallize.[142,143] Based on the pyrolysis of MA **(13)**, Asselineau *et al.* confirmed the positions of the hydroxyl group (-β-) and a long alkyl chain (-α-) in relation to the carboxylic acid **(Scheme 1.1)**.[144]



13 14 15

Scheme 1.1: Thermally induced cleavage of the β-hydroxy group of MA [81]

MAs exist in the cell wall of different mycobacteria and differ in both the functional groups and also the number of carbon atoms present in the molecules.[93] On the whole, the structure of MA has two portions: meromycolate and corynomycolate. **Figure 1.11** on the next page illustrates the general structure of MAs **(16)**. Corynomycolate is the same in all types of MAs with the β-hydroxyl group and the α-alkyl chain in the (*R*,*R*) configuration. The meromycolate main chain normally possesses two functional groups in the distal (X) and proximal (Y) positions and can contain different chain lengths, as illustrated in **Figure 1.11**. The type of MA present can vary between different species **(Table 1.3)**.[81] MAs in *M. tuberculosis* have 80-90 carbons and are longer than those from *Corynebacterium* (30-36 carbons), *Rhodococcus* (34-38 carbons) and *Nocardia* (46-60 carbons).[145,146] Extraction of the mycobacterial cell wall yielded a mixture of different types of MAs which was separated using different analytical techniques such as gas chromatography (GC),[147] high-performance liquid chromatography (HPLC),[148,149,150] and thin layer chromatography (TLC).[151,152,153] Recently MALDI-TOF mass spectrometry was used for the analysis of MAs and other lipids.[154] The determination of the stereochemistry of full MAs was very complex and it could be achieved by degrading the MA into smaller parts and comparing them with known compounds.[155] Today, over 500 related chemical structures of MAs, isolated from *M. tuberculosis*, are recognized. These MAs are one of the most important components in the cell wall, and this large number of different structures have significant biological properties.[81]

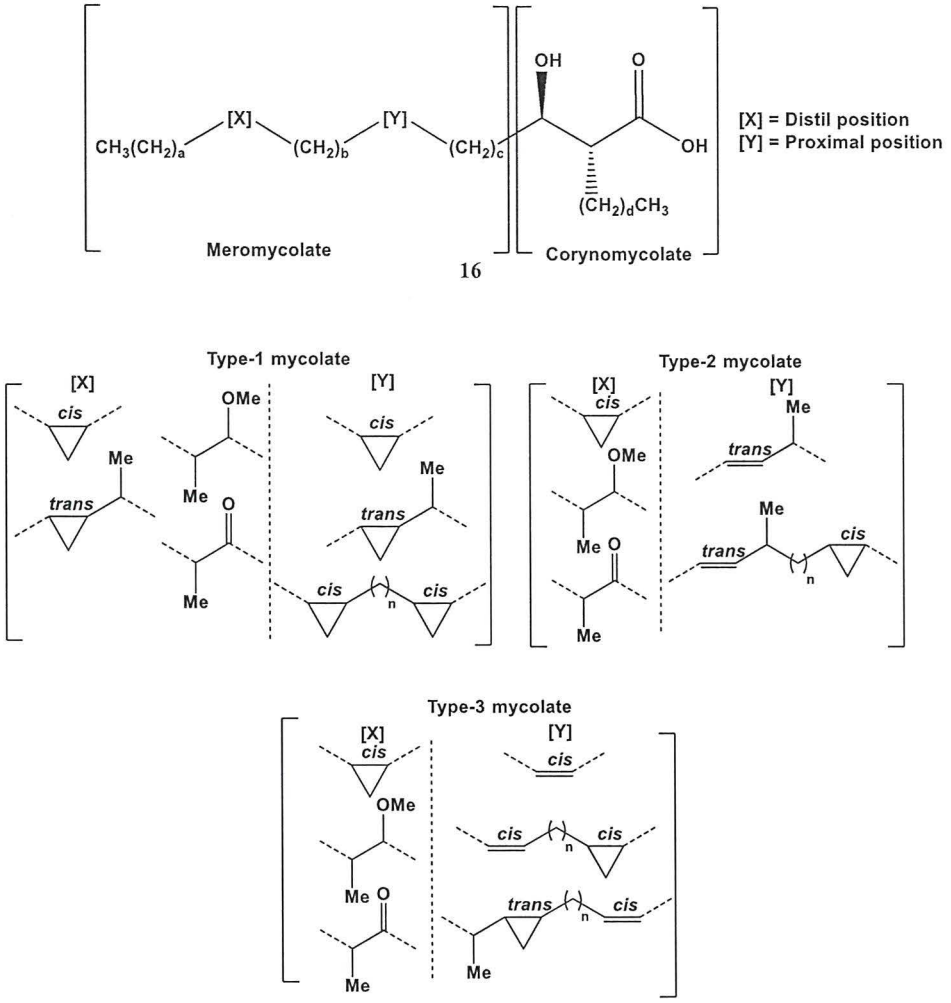


Figure 1.11: Generalized MA structures and common functionality [156]

Table 1.3: MAs classes in Mycobacterium strains [81]

Species strains	*MA classes*					
	α	*α'*	*k*	*E*	*M*	*W*
M. tuberculosis	*		*		*	
BCG	*		*		*	
M. bovis	*		*		*	
M. avium	*		*			*
M. kansasii	*		*		*	
M. marinum	*		*		*	
M. smegmatis	*	*		*		

α (X=alkene or cyclopropane; Y= alkene or cyclopropane) and α' (X=alkane; Y= alkene) absent oxygen functions, K-keto, M-methoxy, E-epoxy, W-wax ester.

Watanabe *et al.* suggested a comprehensive classification method because of the variability of the functional groups in the MAs. They are separated into three kinds: the

first (**17**) where the proximal position, labelled [Y] in **Figure 1.11** above, can be either a *cis* or *trans*-cyclopropane ring; the second **(18)** where the proximal position is a *trans* double bond; and finally **(19)** where the proximal position is a *cis* double bond. **Figure 1.12** shows these three different types.[156] It has been reported that when the double bond or the cyclopropane are in the *trans* configuration there is a methyl group on the adjacent distal carbon while in the *cis* configuration it is not present.[81,93] There are three main types of MA which are present in *M. tuberculosis*: α-MA **(20)** (containing two cyclopropane rings, usually in a *cis*-configuration) **(Figure 1.13)**; keto-MA (containing a carbonyl group with a *trans* cycloprpane **(21)** or a *cis* cyclopropane **(22)** **(Figure 1.14)**; methoxy-MA **(23)** (containing a methoxy group) **(Figure 1.15)**.

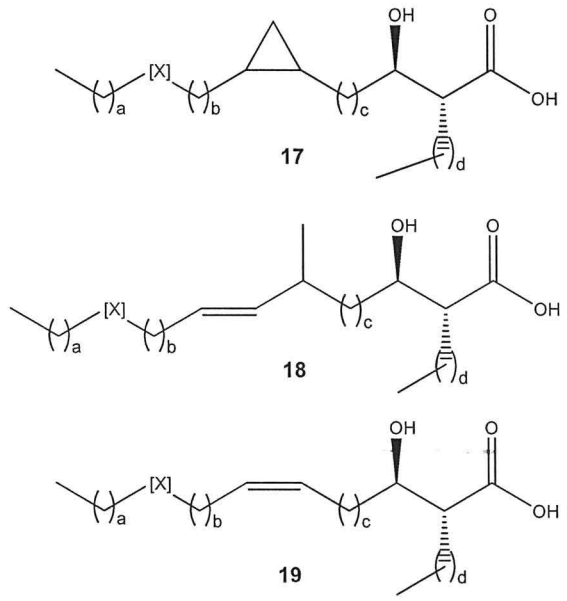


Figure 1.12: Types of MAs structures according to Watanabe et al. classification [156]

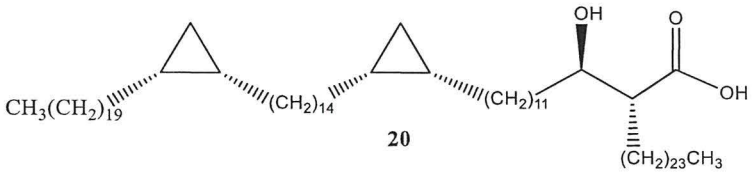


Figure 1.13: Structure of α-MA

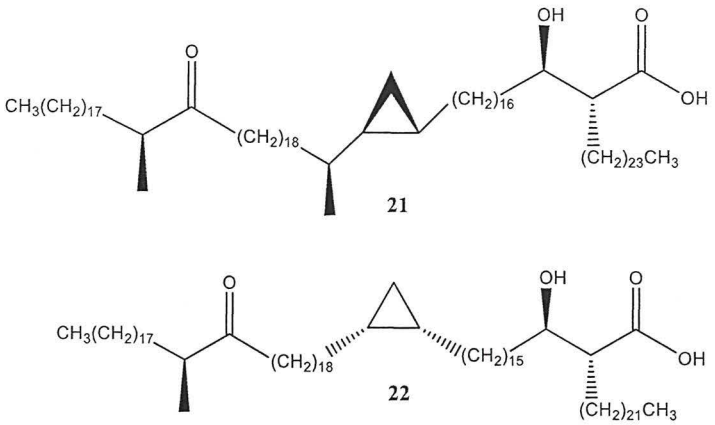


Figure 1.14: Keto-MAs with a cyclopropane ring in the trans-configuration (up) (A); cis-configuration (down) (B)

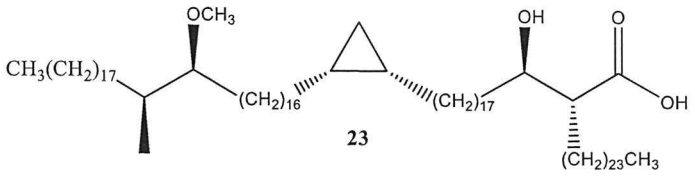


Figure 1.15: Methoxy-MA with a cyclopropane ring in the cis-configuration

In 2003, Al-Dulayymi *et al.* reported the synthesis of a single enantiomer of an α-MA from *M. tuberculosis.*[157] Various types of single enantiomers of MAs have also been prepared by the same group such as methoxy,[158] keto-[159,160] and α-MAs[161] which are major MAs present in *M. tuberculosis*.[162]

1.8 Cord Factor

Trehalose *di*-mycolate (TDM) also known as 'Cord Factor', is trehalose that has been esterified at both primary alcohol positions with MAs.[163] It is one of the most interesting and potentially valuable glycolipids found in the cell wall of *M. tuberculosis*, As previously mentioned, the inner layer of the mycobacterial cell wall is made up of MAs bound to the terminal part of the AG, while the outer layer which forms 60% of the cell wall, is extractable glycolipids,[103] that consist of MAs esterified with trehalose, creating (TDM) **(24)** and (TMM) **(25) (Figure 1.16)**.

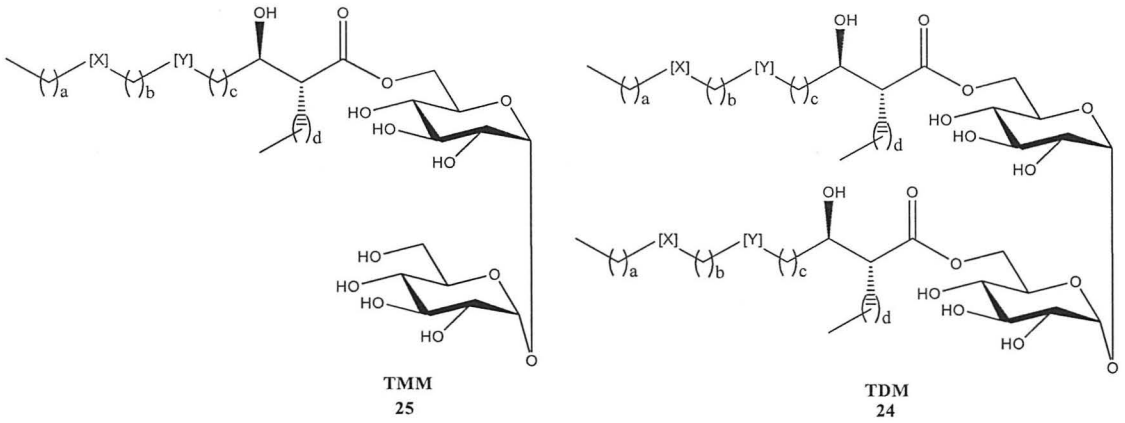


Figure 1.16: Structures of TMM and TDM

Koch, in 1884, was the first to discover that certain *tubercle bacilli* formed long strands or 'cords'.[164] Middlebrook *et al.* discovered that the formation of 'serpentine cords' was typical of contagious strains of *tubercle bacilli*.[165] For the first time, in 1950, Bloch isolated the glycolipid from the *tubercle bacilli* cells and he referred to it as a 'Cord factor'.[166] The toxicity of cord factors in mycobacteria was reported in 1953 by Bloch *et al.* when they extracted four diverse strains of cord factors and they tested them on mice. It was proven that cord factors caused inhibition of respiration and inflammation.[167,168] It has also been confirmed that cord factors have anti-bacterial, anti-tumour and anti-parasitic characteristics.[169] Purified TDM induces a wide range of cytokines and chemokines which contributes in its immunomodifying function.[170] A study on guinea pigs showed that cord factors act as immunomodulators by inducing the immune system and antibody production.[171] Finally TMM, TDM and mAG 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.

1.9 Tests Available for TB Detection

As mentioned before, *M. tuberculosis* is one of several related bacilli, therefore diagnosing TB is complicated. A summary of the tests presently commercially available is given below:

- **Bacterial Culture**: This method is considered the 'gold standard' for detecting active TB,[172] and it has one of the highest sensitivities (the percentage of positive samples detected as such) among TB diagnostics. It can also distinguish TB patients with drug resistant strains from those with non-drug resistant strains,[173]

providing valuable information for the treatment of the disease. This method does however take 3-8 weeks to obtain a result.[174,175]

- **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.173 An advantage of this method over bacterial culture is that the time required for the result is much less, the staining process being performed in less than 1 hour, [176] making this valuable as a quick screening method for mycobacterial disease.[177,178]
- **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.[173] One drawback is that it can detect both latent and active TB; moreover, it cannot distinguish an active TB patient from the one that was previously infected by the disease.[179] False negative samples include patients co-infected with HIV;[174,181,182,180,183,184] while false positive samples include patients vaccinated with BCG.[174,181 ,182 ,180 ,183 ,184]
- **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. tuberculosis*.[185] This method also detects both latent and active TB and again cannot distinguish between them.[179,186] It is also reasonably fast, with results available in 24 h;[187,188 ,189] no false positive samples are seen due to BCG vaccination.[187,188 ,189] One disadvantage however is there is only a limited amount of clinical and laboratory experience with this assay.[187,188 ,189]
- **Nucleic Acid Amplification Test**: Detects the presence of *M. tuberculosis* complex, but cannot distinguish the individual mycobacteria.[190] This method has low limits of detection,[191] and the results are obtained in 2.5 to 3.5 h.[190,192]
- **Serodiagnostic assays**: ELISA assays for the detection of TB are used to detect antibodies produced against *M. tuberculosis*;[193 , 194] however, the WHO has strongly recommended that the current assays are not to be used for the detection of TB.[193] It indicates that an assay which does meet its criteria would be valuable.

1.10 ELISA in TB Diagnosis

The enzyme-linked immunosorbent assay (ELISA) is a method widely utilized in clinical medicine and designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. The test is a rapid and simple screening method using chemical and parts from the immune system to detect the response of the immune system.[195] This basic principle of ELISA was derived from the radioimmuno-assay (RIA) which used for the first time by Berson and Yalow. [196] In the early 1970's this assay was developed as a high throughput screening procedure for many illnesses. ELISA tests have been used of TB detection because *M. tuberculosis* has many different antigens such as A60 or 38 kDa antigen which were widely used for detection; however, it suffers from low sensitivity and specificity (the percentage of negative samples detected as such).[197] A number of natural mixtures of cord factors and MAs have been used in ELISA tests to investigate their both biological activity and diagnosis applications. Both types were very potent signalling agents in the serodiagnosis of TB on account of their antigenic activities.[198,199,200]

Yano's group in Japan produced a series of papers in the 1990's using MA sugar ester antigens as surrogate markers of TB infection using ELISA assay.[201,202,203] Schleicher *et al.,* used ELISA to try and detect anti-mycolic acid antibodies from *M. tuberculosis* in serum samples from patients infected with TB and HIV, and from patients infected with TB alone. They used natural MAs, isolated from *M. tuberculosis*, 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 MAs are also preserved in patients who have been tested HIV-positive.[204]

Beukes *et al.* used synthetic MAs made by Al Dulayymi *et al.* and natural MA extracted from *M. tuberculosis* to compare the antibody responses, in addition to examining their corresponding methyl esters.[205] They proved that in the case of free MAs the antibody recognition is much higher than their corresponding methyl esters, signifying that the carboxylic acid unit of the MAs either has a large contribution to the binding of the MAs to the antibodies, or that they stabilise a particular antigen conformation. They also established that oxygenated MAs are more antigenic than α-MAs but none of the synthetically produced MAs could reproducibly distinguish TB positive sera from TB negative sera.[205]

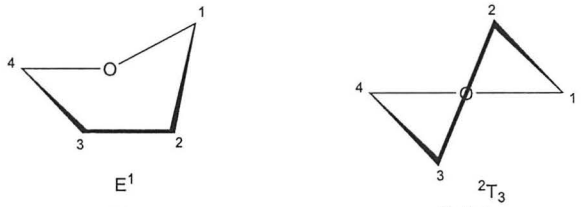
1.11 Carbohydrates

Carbohydrates are the most plentiful biomolecules 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.[206] 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.[207] 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. [208] 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, for example, just three different monosaccharides (hexopyranoses) can form nearly 28,000 different structures. In addition to that, the hydroxyl groups in glycans can be modified through different reactions such as esterification, oxidation and methylation.[209] 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 of glycans 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.[210,211]

1.11.1 Carbohydrate Conformations

The overall structure of an oligosaccharide is determined by many factors: (i) the stereochemistry of the glycosidic bond (α- or β-anomer); (ii) pseudo-rotation of the furanose rings; (iii) stereoelectronic effects; and (iv) 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.[209]

Because this study deals with furanose glycans, the conformation of this ring will be briefly discussed. There are two main conformations of the furanose ring: twist (T) **(26)**, in this form, two atoms are outside the plane and the other three atoms are in the plane; and envelope (E) **(27)**, in which four adjacent atoms are all in one plane and only one atom is out of the plane (above or below) **(Figure 1.17)**.



E^1 Envelope **27**

$^2T_3$ Twist **26**

Figure 1.17: Envelope and twist conformations in furanose rings

The energy difference between the T and E 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 ($^0T_1$, $^1T_0$, $^1T_2$, $^2T_1$, $^2T_3$, $^3T_2$, $^3T_4$, $^4T_3$, $^4T_0$, and $^0T_4$) and ten different envelope conformations (^1E, E_1, ^2E, E_2, ^3E, E_3, ^4E, E_4, ^0E, and E_0). These conformations are represented on the pseudorotational wheel **(Figure 1.18)**.[212]

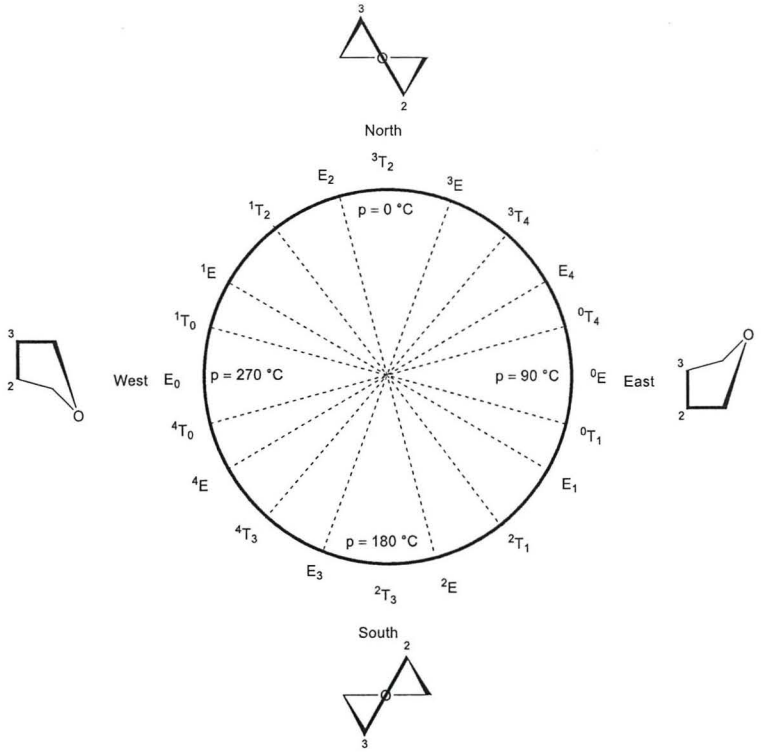


Figure 1.18: Pseudorotational wheel for a-D-aldofuranose ring [212]

This wheel is for the D-aldofuranose ring, which describes the puckered ring conformation through two parameters, *P* [Altona-Sundaralingam (AS) pseudorotational phase angle], and Φ_m [pseudorotational puckering amplitude]; both parameters are represented by the circle radius. *P* shows the puckered part of the ring and Φ_m shows the degree of deviation from the plane **(Figure 1.19)**.[213] For example, the positions ^1E and E_1 represent the interchanged conformations of the ring in which the value of *P* is constant and that means the conformation is located at 180 ° across the ring **(Figure 1.18)**. In solution, furanose rings are present as a mixture of conformers. Therefore analysis of NMR data such as measuring the $^3J_{H\text{-}H}$ coupling constant or the chemical shifts is more complicated because all the data is an average from more than one conformation.[213]

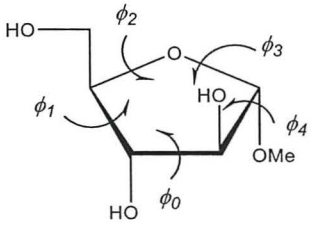


Figure 1.19: Endocyclic torsion angles definition of α-D-Araf

Finally the value of P and Φ_m can be calculated from the two equations.[212]

$$\tan P = \frac{(\Phi 2 + \Phi 4) + (\Phi 1 + \Phi 3)}{3.077\,\Phi 0}$$

$$\Phi m = \frac{\Phi 0}{cos\ P}$$

"Stereoelectronic effects", which can be 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 (Edward-Lemieux) 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 **(29)** compared to the corresponding β-anomers of this compound **(Figure 1.20)**.[214,215]

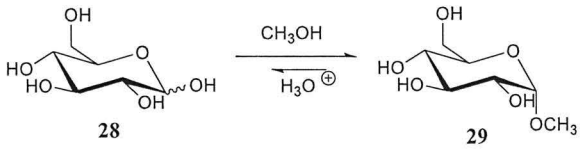


Figure 1.20: Glycosylation of D-glucose

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 (C1) on the ring typically favour the axial α-anomer. A reasonable explanation for this is hyperconjugation between the non-bonding electron pair on endo oxygen (O) and the empty anti-bonding orbital (σ*) of the C1-X substituent atom which leads to stabilization of the axial substituent compared to the equatorial substituent. This interpretation is based on bond polarizable dipolar moieties (C1-X) that can be stabilized by electron transfer from an electron rich moiety (non-bonding electron on oxygen) **(Figure 1.21)**.[216,217]

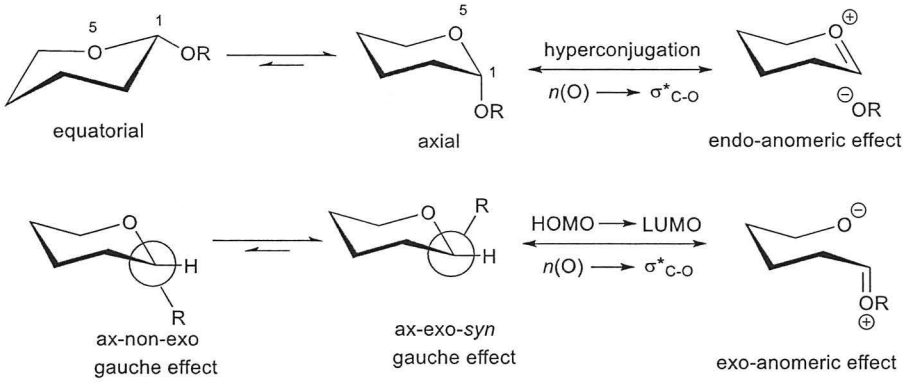


Figure 1.21: Stereoelectronic effects

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 1.21** and **Figure 1.22)**.[218]

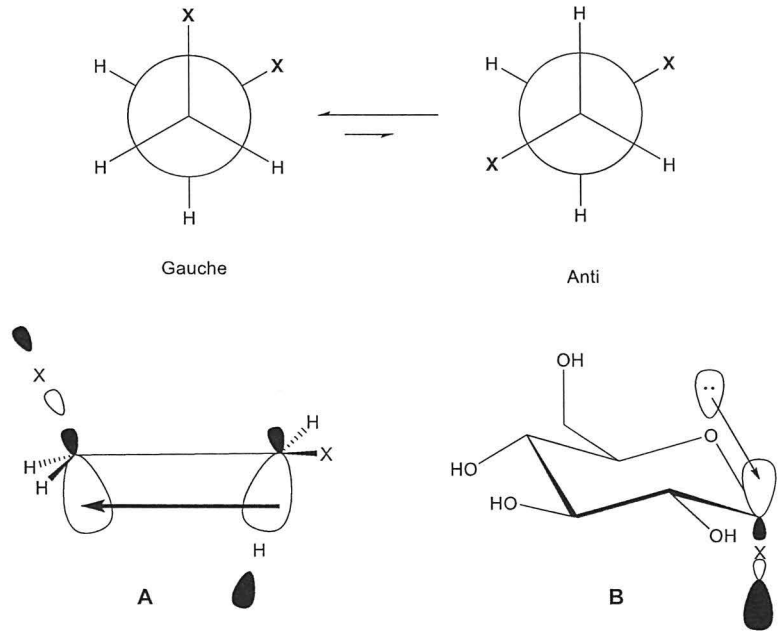


Figure 1.22: Stereoelectronic effects: A. Gauche effect; B. Anomeric effect

1.11.2 Preparation of Glycosidic Linkages

As previously mentioned, glycosidic bonds between two *mono*-saccharides form disaccharides or higher molecules. There are two main methods used for their synthesis:

chemical or enzymatic. Glycosidic linkages in nature originate through a biosynthesis reaction involving an enzyme (glycosyltransferase). Enzymatic preparation is a highly stereo- and regiospecific method to synthesise significant carbohydrates. [219] Nevertheless, it is high in cost and requires a specific enzyme (not available sometimes) which limits its application. Therefore, chemical methods are usually utilized. Glycosylation is the reaction between the glycosyl acceptor **(31)**, which has a free hydroxyl group (nucleophile), with a glycosyl donor **(30)**, having a leaving group (electrophile). The coupling between these two glycans is done in the presence of an activator to give a disaccharide **(32)** 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 1.23)**.[220,221,222,223,224,225,226,227,228]

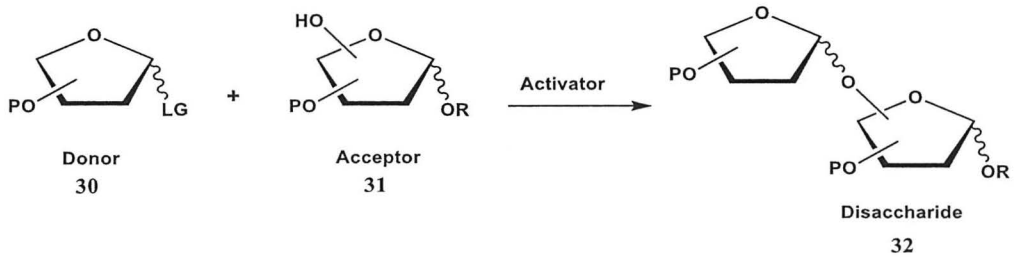


Figure 1.23: Glycosidic linkage formation

Mostly, glycosidic linkages exist in two types, 1,2-*cis* **(33)** and 1,2-*trans* **(34)** glycosides **(Figure 1.24)**. *Cis* and *trans* refers to the stereochemistry of the substituents at the C1 and C2 positions.

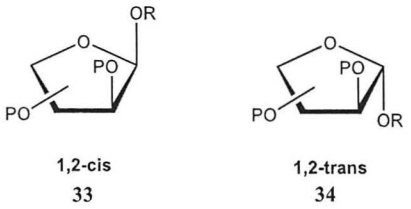


Figure 1.24: Types of the glycosidic linkages

1.11.2.1 Strategies that Lead to 1,2-*trans* Glycosidic Linkages

1,2-*trans* Glycosidic bonds, can be realized straightforwardly by using a donor protected at C2-*O*-acyl which allows for neighboring group participation. As illustrated in **Figure 1.25**, losing the leaving group from the anomeric centre through activation by the promoter Lewis acid, led to the formation of an oxocarbenium ion which is attacked directly by the 2-*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.

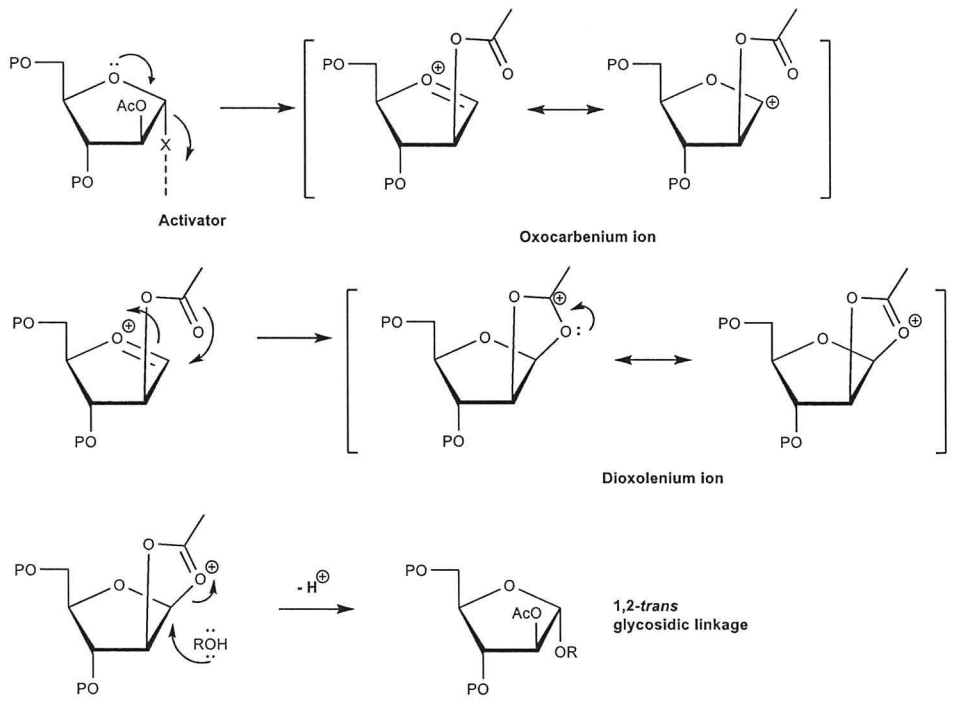


Figure 1.25: Proposed formation of stabilized cation [229]

When the 2-*O*-acyl bears an electron-withdrawing substituent, the stereoselectively 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.[230]

In some cases, the participating group can be an electronegative atom, for example nitrogen, or a chiral auxiliary **(Figure 1.26)**. However the application of chiral auxiliaries is limited, due to the difficulty of installation 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.[231,232]

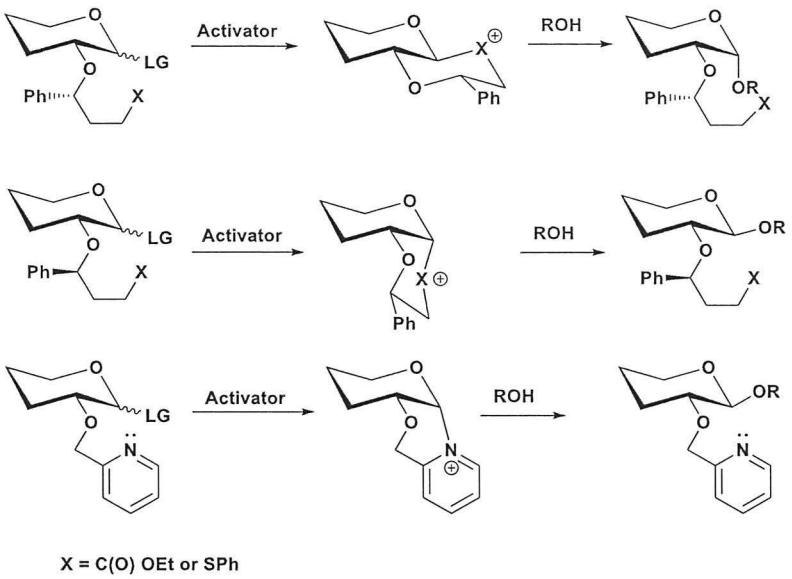


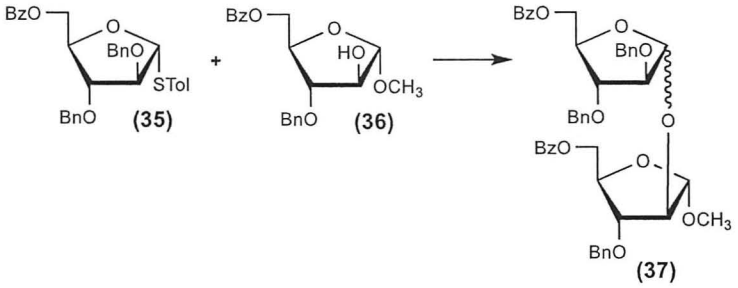
Figure 1.26: Neighbouring group participating approaches

1.11.2.2 Strategies that Lead to 1,2-*cis* Glycosidic Linkages

In contrast, a general route for the synthesis of β-arabinofuranoside (1,2-*cis*) 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.[233] In the literature, numerous strategies for the preparation of β- glycosidic linkages have been reported, however most of these studies focus on pyranose glycan classes.

Lowary and co-workers[288] reported a study to find optimal conditions to use in the glycosylation between the donor and the acceptor to improve β-selectivity. They reacted the donor **(35)** and the acceptor **(36)** in CH_2Cl_2 **(Scheme 1.1)**, using the promoter silver trifluoromethanesulfonate (NIS-AgOTf) and *N*-iodosuccinimide 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 1.4)**. They developed an approach where the reaction was initiated at a temperature of - 60 °C and then gradually warmed to - 40 °C over 2 h. The reaction gave improved yield and stereoselectivity.[288] Studying the effect of the reactant concentration (entries 6-8) was also carried out, and established that using a low concentration, a slight increase in the β-selectivity was observed, and the 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 1.4)**, gave a low yield and β-selectivity.



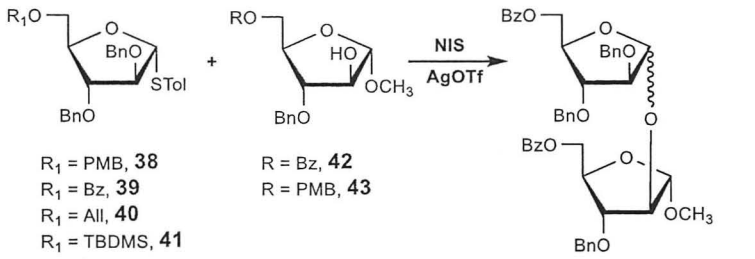
Scheme 1.1: Synthesis of di-saccharides[288]

Table 1.4: Optimization of β-Arabinofuranosylation[288]

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)

[a]Acceptor 16 (1 equiv), donor 13 (1.2 equiv), NIS (1.2 equiv), AgOTf (0.1 equiv). [b]Acceptor 16 (1 equiv), donor 13 (1.2 equiv), NIS (1.2 equiv), TMSOTf (0.1 equiv). [c]Acceptor 16 (1 equiv), donor 13 (1.2 equiv), Ph_2SO (3 equiv), TTBP (6 equiv), Tf_2O (1.1 equiv). All reactions were carried out in CH_2Cl_2

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 1.2**. The best (α/β) ratio of (1:7.7) was achieved (entry 4), and the two anomers are inseparable.



R_1 = PMB, **38**
R_1 = Bz, **39**
R_1 = All, **40**
R_1 = TBDMS, **41**

R = Bz, **42**
R = PMB, **43**

R_1 = Bz, R = Bz, **44**
R_1 = All, R = Bz, **45**
R_1 = PMB, R = PMB, **46**
R_1 = PMB, R = Bz, **47**
R_1 = All, R = PMB, **48**
R_1 = TBDMS, R = PMB, **49**

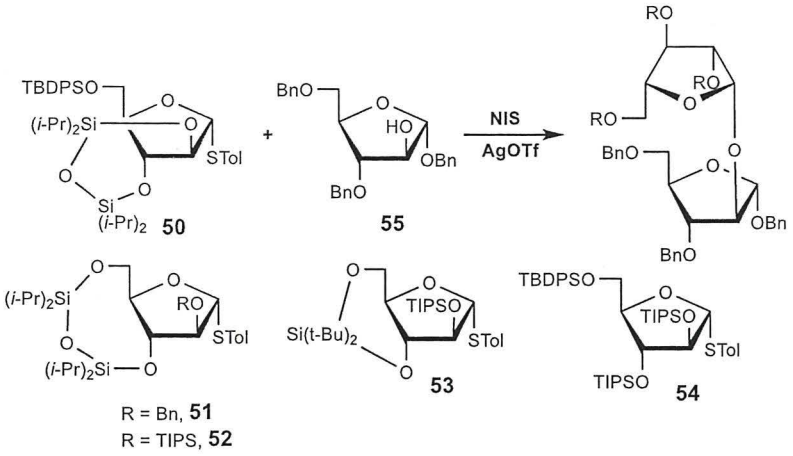
Entry	Acceptor	Donor	Product	Yield (%) (α/β)
1	**42**	**38**	a	96 (1:3)
2	**42**	**39**	**44**	93 (3:1)
3	**42**	**40**	**45**	96 (1:2.5)
4	**43**	**38**	**46**	89 (1:7.7)
5	**43**	**39**	**47**	93 (1:4.4)
6	**43**	**40**	**48**	93 (1:6.3)
7	**43**	**41**	**49**	91 (1:2)

a The ratio of product could not be separated

Scheme 1.2: Reaction of different donors and acceptors[288]

In conclusion, the best (α/β) ratio of the products was obtained by utilising PMB as a protecting group at the C1 position on both the donor and the acceptor **(Scheme 1.2**, entry 4**)**.

Ishiwata and co-workers, [234] reported a new strategy for conducting β-selective glycosylation using donors protected with 3,5-TIDPS. An enhancement of β-selectivity was achieved by utilising a donor with an eight membered ring **(51)**. The best (α/β) ratio of (1:20) from the disaccharide was realised **(**entry 3, **Scheme 1.3)**. On the other hand, using a donor with eight-membered ring 3,5-*O*-protection **(51)** in comparison with those being six-membered ring 3,5-*O*-protection **(53)**, with the same acceptor **(55)** showed marked differences in β-selectivity (entry 2 and 3). In the case of α-attack to the anomeric carbon, there seems to be a large steric repulsion from the α-hydrogen atom at C2 **(Figure 1.27)**.[234]



Entry	Acceptor	Donor	Product	Yield (%) (α/β)
1	**55**	**50**	**56**	96 (1:2.45)
2	**55**	**51**	**57**	94 (1:12.5)
3	**55**	**52**	**58**	93 (1:20.1)
4	**55**	**53**	**59**	70 (1:5.36)
5	**55**	**54**	**60**	99 (5.26:1)

Scheme 1.3: Effect of protection of the glycosyl donor in Arabinofuranosylation[234]

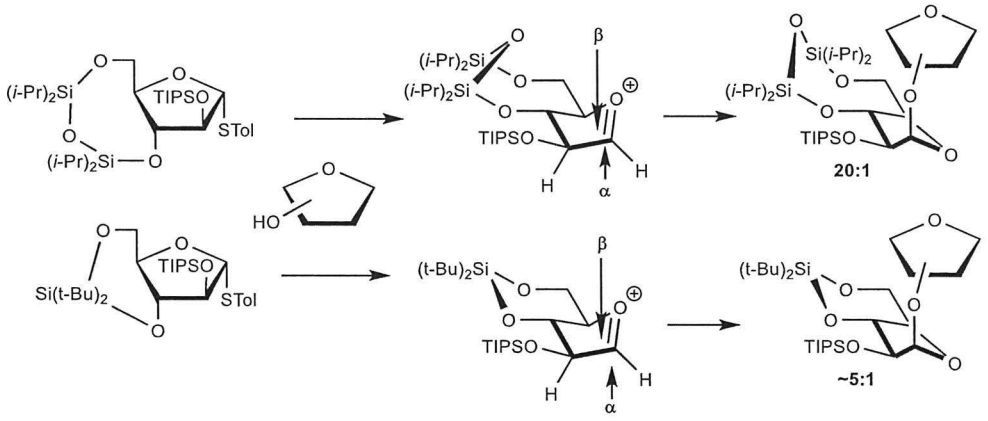


Figure 1.27: A reasonable explanation for the β-selective addition to the activated donor [234]

1.12 Significance of Natural Arabino-Mycolate

As previously mentioned, 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.[121,235,122] It is believed that this plays a large role in raising the flexibility of the polysaccharide, causing the structure of the cell wall to have extremely low permeability, which provides the organism with high protection from drugs and from its environment.[236]

Anderson & Geiger in 1937 reported the first extraction of arabino-mycolate from the cell wall of *M. bovis* using natural organic solvent.[237] Some fifty years ago, the isolation of arabinose 5-mycolate by extraction of the cell walls of various mycobacteria under acidic conditions was reported.[238,239] More recently, mass spectrometry and NMR have provided powerful tools for the analysis of such molecules.[116] Azuma and Yamamura in 1962 isolated D-arabinose-5-mycolate and proved it was toxic to mice.[240] Inflammatory reactions similar to that observed after inoculation of live BCG were induced in the lungs by TMM, TDM or GMM isolated from BCG. However, the toxic reactions caused by GroMM and MAM were characterized by an acute inflammatory process.[241] In 2005, the preparation of a *tetra*-mycolyl *penta*-arabinose using a complex natural mixture of MAs was described.[242] However, only in 2010 were structural studies of the composition of the arabinose mycolates of the cell wall of *M. bovis* reported.[243] Hydrolysis gave a number of fractions. One of these was a *penta*-arabinose *tetra*-mycolate, one was an arabinose *mono*-mycolate, while the others were *hexa*-arabinose, *hepta*-arabinose and *octa*-arabinose *tetra*-mycolates. The MA methyl esters released from each of these showed a mass spectrometric pattern almost identical to the methyl esters obtained by hydrolysis of the original cell wall. A comparison of the *penta*-arabinose *tetra*-mycolate and arabinose *mono*-mycolate with samples prepared by combining a mixture of natural mycolic acids with arabinose was reported. **Figure 1.28** shows the MALDI-TOF MS spectrum of the synthesized *mono*-arabino-*mono*-mycolate from natural mixed MAs.[243]

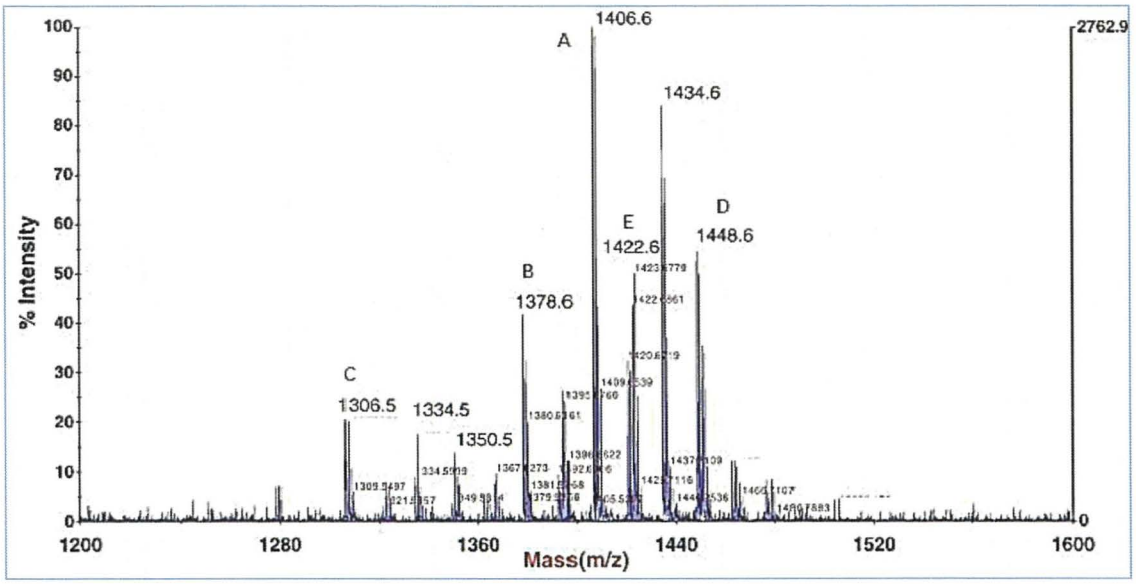


Figure 1.28: MALDI-TOF MS spectrum of mono-arabino-mono-mycolate synthesized from natural mixed MAs [243]

A: keto-*cis*-24-α-alkyl chain; **B**: keto-*cis*-22-α-alkyl chain; **C**: α-MA; **D**: keto-*trans*-24-α-alkyl-chain; **E**: methoxy-*cis*-24-α-alkyl chain

Ishiwata *et al* reported the synthesis of a series of *mono*-, *di*- and *tetra*-arabino-mycolates found at the terminal position of the cell wall skeleton of BCG from *M. bovis,* by using natural MA mixtures extracted from the cell wall. All the compounds showed strong TNF-α inducing activity *in vitro*. The mechanism of the activity of arabino-mycolate is not clear.[242] Synthetic arabino-mycolates induce the production of TNF-α in murine macrophage cell lines at an intensity similar to BCG-cell wall skeletons. However the immunological activity of natural arabino-mycolates isolated from BCG has not been investigated; this is probably due to the complexity of the molecule. Arabino-mycolates obtained by acid hydrolysis from CWS (SMP-105) of *M. bovis* BCG Tokyo 172 strain consisted mainly of *mono*-arabinose *mono*-mycolate, *penta*-arabinose *tetra*-mycolate and *hexa*-arabinose *tetra*-mycolate fractions.[122]

Arabino-mycolates significantly induced TNF-α production with an intensity comparable to that of CWS and enhanced delayed type hypersensitivity (DTH) reactions against inactivated tumour cells. Arabino-mycolate-induced TNF-α production was completely dependent on TLR2 and MyD88 pathways. Thus isolated natural arabino-mycolates possess potent adjuvant immunostimulatory activity.[122,244]

Intratumour injections of extracts of Re mutant *Salmonella typhimurium* in combination with TDM or arabinose mycolate were highly effective in producing regression of tumours in guinea pigs. Similar extracts from *M. bovis* strain BCG strain AN5 in combination with TDM also possessed tumour-regressive activity. The activity was reduced when the arabinose mycolate was substituted for the TDM.

An extract of *Coxiella burnetii*, in combination with either TDM or arabinose mycolate was also active. Intracutaneous administration of Re glycolipid or aqueous extracts from BCG, in combination with trehalose or arabinose mycolates, did not produce life-threatening clinical signs of toxicity in young mice. If additional toxicity studies demonstrate that adverse side effects can be satisfactorily controlled, these water soluble extracts may prove beneficial in the treatment of spontaneous tumours in humans and other animals.[245,246]

The adjuvant activity of cell wall skeletons (mycolic acid-arabino-galactan-mucopeptide, CWS) prepared from the cells of mycobacteria, nocardia and corynebacteria was examined *in vivo* in mice and guinea pigs. The cell wall skeletons of *M. bovis* BCG (BCG-CWS), *Nocardia asteroides* 131 and *Corynebacterium diphtheriae* PWC suspended in Freund's incomplete adjuvant (FIA) as water-in-oil emulsions showed potent adjuvant activity on the formation of circulating antibody and cell-mediated immunity to bovine serum albumin (BSA), sheep erythrocytes (SRBC) and sulfanylazo-bovine serum albumin (SA-BSA) in mice and guinea pigs. After acetylation or acid treatment, BCG-CWS retained its adjuvant activity, but the activity of BCG-CWS was destroyed completely by alkaline treatment. The cell wall constituents, arabinose-mycolate and arabino-galactan, prepared from BCG-CWS showed no adjuvant activity.[247] Few studies of arabinose mycolates have been carried out and little is known of the effect of structure on the immunostimulatury their activity in activating macrophages.[122] However, D-arabinose-5-mycolate, purified from bound lipids of the cell-wall skeleton of *M. bovis* BCG may be a prominent structure for recognition by host immunity.[122]

Mycolylated glycolipids like GMM, TDM, or GroMM, 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.[248,249] Components such as triacylglycerols (TAGs), C70–90 *mono*-mycolyl glycerol (C70–90 MMG) 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.[250]

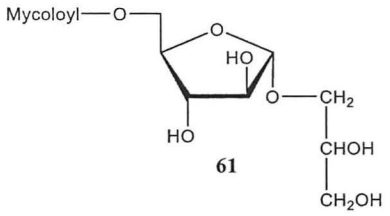
An antigenic glycolipid, 5-mycoloyl-β-arabinofuranosyl-(1→2)-5-mycoloyl-α-arabino-furanosyl-(1→1`)-glycerol (DMAG) was isolated for the first time by Watanabe and co-workers in all 12 strains of the *M. avium-M. intracellulare* complex (MAI) and reacted immunologically with antisera from rabbits.[251] DMAG also showed applicability for serodiagnosis of MAI infection by ELISA.[252,253,254] In 1997 DMAG was obtained from *M. Kansasii* among glycolipid fractions of the cell wall.[255] Recently, Rombouts and co-workers identified DMAG in large quantities, in slow growing pathogenic species, including *M. tuberculosis*, *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 α-, keto- and methoxy-mycolates in *M. marinum* while only α- and keto-mycolates are found in *M. bovis* BCG. Furthermore, the cyclopropane ring in *M. marinum* seems more likely to be in *trans* stereochemistry.[248]

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. tuberculosis* and is not synthesized along with other lipids/ glycolipids. It is considered a surface-exposed immunogenic molecule, suggesting that the molecule 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.[249,252]

TNF-α has been proven as a significant inflammatory mediator, that can affect different kinds of cells.[256,257,258] 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.[248,259,260]

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 pertinent to mycobacterial pathogenesis, for example, formation of granuloma and tissue-destructive lesions and proinflammatory cytokine production.[261]

A new glycolipid from MAI was obtained by Watanabe and co-workers,[262] 5-mycoloyl-α-arabinofuranosyl (1→1′)-glycerol (MAG) **(61) (Figure 1.29)**.



61

Figure 1.29: Structure of MAG [262]

The biological activity, particularly the antigenicity, of MAG was not investigated. Furthermore, the stereochemistry of the glycerol, *i.e.* whether it is D- or L- configuration, has not been proven.[262]

1.13 Overall Aim of the Research Projects

The aim of this study was to synthesis a series of glycolipids related to the mAG complex present in the cell wall of mycobacteria. In particular, the synthesis of:

1- Methyl Arabino-Mycolates (MAM) **(62)**.
2- Glycerol-Arabino-Mycolates (GAM) **(63)**, this include synthesis of both stereochemistries of the glycerol component (D and L), to prove the stereochemistry of the GAM.
3- Methyl Tri-Arabino-Di-Mycolates (MTADM) **(64)**.
4- Di-Mycolyl-Di-Arabino-Glycerol (DMAG) **(65)**.

The object of this exercise was to ascertain if these fragments **(Figure 1.31)** have any capacity for the stimulation of the production of costimulatory molecules and certain proinflammatory cytokines (*e.g.*, TNF-α, IL-1β, IL-6), in addition to probing their antigenicity for detection of TB.

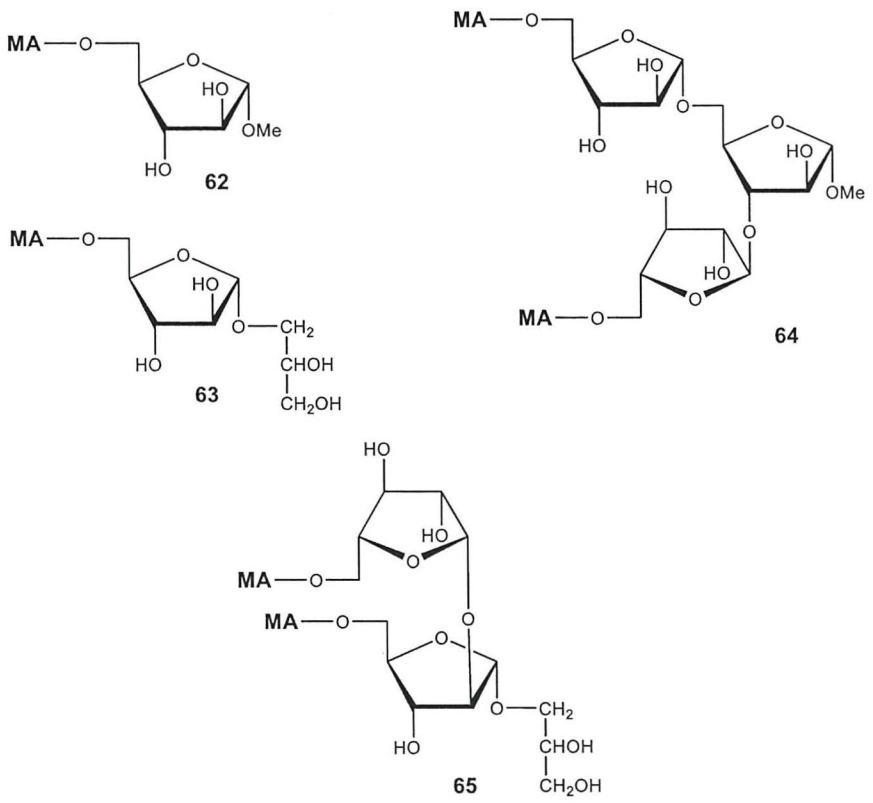


Figure 1.31: Structures of target molecules

Chapter 2

Results and Discussion

2.1 Synthesis of Methyl Arabino-Mycolates (MAM)

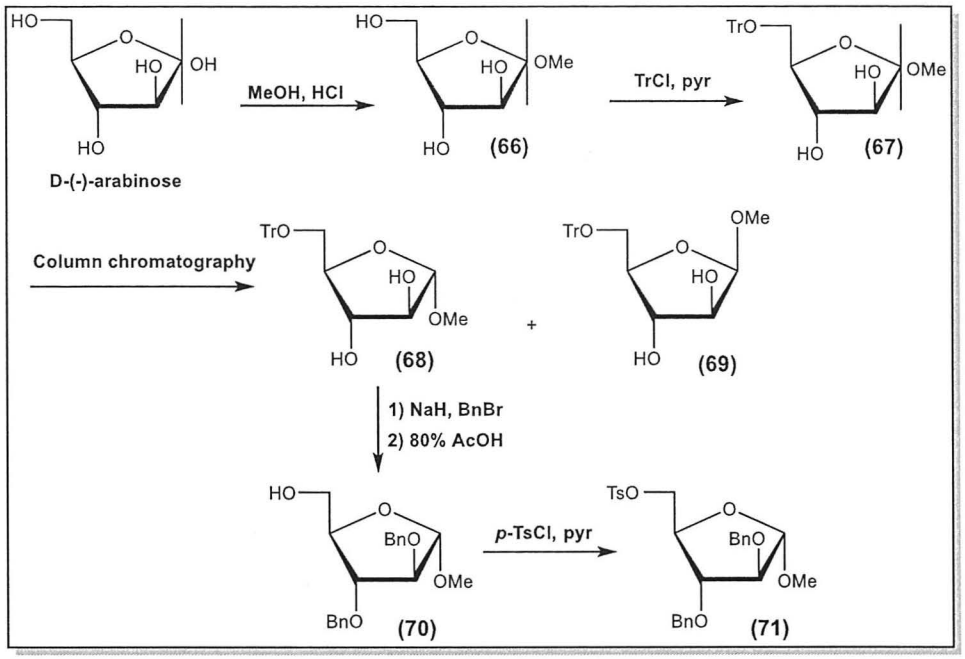
2.1.1 The aims of this part:

- Synthesis of the glycan methyl-α-D-arabinofuranoside (methyl-α-D-Ara*f*).
- Synthesis of a model of the glycolipids through esterification with normal fatty acids.
- Synthesis of a series of arabino-mycolates through esterification of the glycan with different synthetic MAs.
- Investigate the biological activity of the synthetic compounds, particularly their antigenicity.

2.1.2 Synthesis of methyl 5-*O*-trityl-α-D-Ara*f*

The overall aim of this part of the work was the preparation of methyl-α-D-Ara*f*, which would then be esterified with different fatty acids as models, then with different synthetic MAs.

According to literature procedures, D-(-)-arabinose was treated with freshly prepared HCl (0.22 N), generated *in situ* by addition of acetyl chloride to anhydrous methanol at 0 °C, and then worked-up with pyridine rather than ammonium bicarbonate,[263] to give methyl-α, β-D-Ara*f* **(66) (Scheme 2.1)** with predominant formation of the α-anomer (α/β, 3:2).[106,264] Separation of the two anomers of **(66)** was carried out by tritylation of the mixture followed by column chromatography to give methyl 5-*O*-trityl-α-D-Ara*f* **(68)** (42%) and methyl 5-*O*-trityl-β-D-Ara*f* **(69)** (29%). Compound **(68)** was perbenzylated to protect the two secondary hydroxyl groups using benzyl bromide and sodium hydride in dry DMF, then the primary hydroxyl group was deprotected by hydrolysis in 80% AcOH, affording compound **(70) (Scheme 2.1)**.[264,242]



Scheme 2.1: Synthesis of the mono glycan target

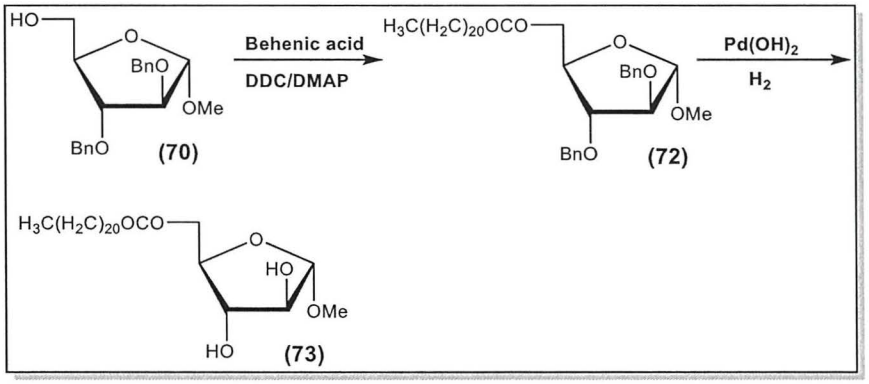
Having the sugar moieties of the glycolipid targets in hand, the exploration of their coupling with fatty acid counterparts to obtain model analogues to the target glycolipids, was carried out. For the initial series of compounds, we chose fatty acids that were commercially available, which we anticipated would be easy to connect to the sugar alcohol by esterification. Two methods were used, direct coupling of the glycan with the fatty acid using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), and an alkylative coupling using $CsHCO_3$ after tosylation of the primary hydroxyl group in the glycan.

2.1.3 Coupling the sugar moiety with different fatty acids

Surprisingly, esters of arabinose with simple fatty acids do not appear to have been reported.

Firstly, the direct condensation of compound **(70)** with behenic acid was investigated using DCC as an activating agent and DMAP as catalyst in dry CH_2Cl_2,[265,266] to give compound **(72)** in 80% yield **(Scheme 2.2)**. Confirmation of the formation of **(72)** was achieved by ^1H NMR spectroscopy. A downfield signal at δ 4.95 corresponded to the proton attached to the C1 of the sugar. The three protons in the terminal position of the acid appeared as a triplet up-field at δ 0.89. The ^{13}C NMR spectrum showed a carbonyl at δ 173.6, C1 at δ 107.3 and the remaining sugar carbons in the region of 87.9 – 63.5. The CH_2 chain ranged from δ 34 – 22 and the CH_3 came up-field at δ 14.0. Mass

spectrometry of the products confirmed the structures ($[M+Na]^+$ peak at *m/z* 689 as expected) as did the I.R. spectrum which gave a band for the carbonyl group at 1741 cm^{-1}. This compound was then subjected to hydrogenolysis,[242] by stirring it 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 the compound **(73) (Scheme 2.2)**. The formation of compound **(73)** was confirmed by the proton NMR spectrum which gave a singlet at 4.92 ppm for the proton at the anomeric centre, and a singlet at 3.42 ppm for the methoxy group in the sugar moiety. The CH_2 adjacent to the carbonyl group showed a multiplet between 2.38 – 2.32 ppm. The ^{13}C NMR spectrum for **(73)** gave a signal at 108.8 ppm for the carbon at the anomeric centre, which indicated that the α-anomer had been obtained,[294] a signal at 173.5 ppm for the carbonyl group and a signal at 55.1 ppm for the methoxy group. The specific rotation was $[\alpha]_D^{22}$ + 46 (c 0.1, $CHCl_3$) and the I.R. spectrum of **(73)** gave a broad absorbance at 3416 cm^{-1} for the two hydroxyl groups and a band for the carbonyl group at 1739 cm^{-1}.

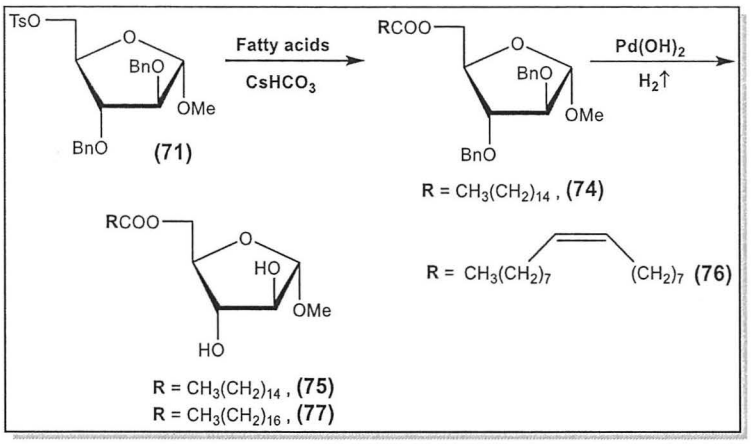


Scheme 2.2: Synthesis of glycolipid model

The above coupling method seemed to be work well with a simple fatty acid but not with the complex acid also, 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, it was found that by activating the sugar as a tosylate, the yield was raised to 79%.[242] Therefore, the hydroxyl group in the compound **(70)** was tosylated by reaction with *p*-toluenesulfonyl chloride (TsCl) in dry pyridine and catalytic DMAP in dry CH_2Cl_2 at 0 °C, to afford the tosylate **(71) (Scheme 2.1)** in 90% yield. The synthesis of this compound was confirmed as all the data obtained were consistent with **(71)** the literature.[242] Compound **(71)** was reacted with palmitic acid via the alkylative

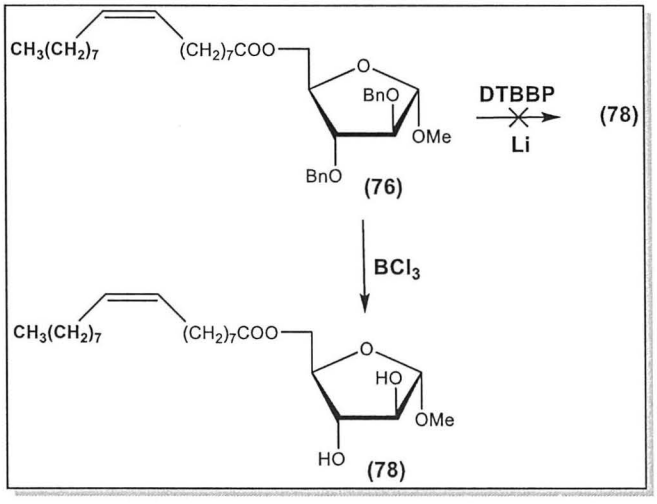
esterification strategy, using cesium hydrogen carbonate in dry DMF: THF at 70 °C, to give compound **(74)** in 76% yield **(Scheme 2.3** on the following page**)**. Confirmation of the formation of **(74)** was achieved by ^{1}H NMR. A downfield signal at δ 4.95 corresponds to the proton attached to C1 of the sugar. The remaining 12 protons on the sugar core appeared in the range from δ 3.4 – 4.6. The signal corresponding to the CH_2 adjacent to the carbonyl group in the acid occurred at δ 2.3 as a multiplet. The three protons in the terminal position of the acid chain appeared as an up-field triplet at δ 0.89. The ^{13}C NMR spectrum showed a carbonyl at δ 173.6, C1 at δ 107.3 and the remaining sugar carbons in the region of δ 87.9 – 63.5. The CH_2 chain ranged from δ 34 – 22 and the CH_3 came up-field around δ 14.0. Debenzylation of **(74)** was achieved by stirring it 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 compound **(75)** in 90% yield **(Scheme 2.3)**. Once again, the formation of this compound was proved by NMR (^{1}H and ^{13}C), which showed clearly the disappearance of those signals corresponding to the benzyl groups between δ 4.6 – 4.5. A downfield signal at δ 4.9 corresponds to the proton attached to C1 of the sugar. The ^{13}C NMR spectrum showed a carbonyl at δ 173.5, C1 at δ 108.8 and the remaining sugar carbons in the region of δ 83.8 – 55.0. The CH_2 chain ranged from δ 34 – 22 and the CH_3 came up-field around δ 14. The specific rotation of **(74)** was $[\alpha]_D^{22}$ + 37 (*c* 0.1, $CHCl_3$), changing for the deprotected compound **(75)** to $[\alpha]_D^{18}$ + 1.1 (*c* 0.1, $CHCl_3$).

In the same way, tosylate **(71)** was reacted with oleic acid to give compound **(76)** in 83% yield **(Scheme 2.3)**. Peaks could be seen representing the olefin at 5.41 – 5.30 ppm in the ^{1}H NMR spectrum and at 128.5 and 130.7 ppm in the ^{13}C NMR spectrum. A peak could also be seen in the ^{13}C NMR spectrum representing the carbonyl of the ester at 173.6 ppm. Hydrogenolysis in dry CH_2Cl_2: MeOH (1:1) in the presence of $Pd(OH)_2$ under a hydrogen atmosphere gave compound **(77)** in 80% yield **(Scheme 2.3)**, in which the double bond was saturated in tandem with the removal of the benzyl groups. The most significant change seen in the ^{1}H NMR spectrum, was that the doublet representing the protons of the benzylic groups disappeared in addition to the two proton multiplet for the double bond. Confirmation of the regeneration of the alcohol could be seen in the I.R. spectrum, where a broad signal was seen at 3468 cm^{-1}. This model proved that arabino-mycolates containing a double bond in their structures cannot be debenzylated selectively by the above method.



Scheme 2.3: Synthesis of glycolipid models

An alternative method was used for deprotecting compound **(76)** by dissolving it in dry THF, cooling to - 78 °C and adding a solution of freshly activated lithium wire and 4,4`-di-*tert*-butylbiphenyl (DTBBP) in dry THF, whereupon a green solution was observed; however, no product was observed.[267] Another procedure, treating compound **(76)** with BCl_3 at - 78 °C, did give **(78)** in 64% yield **(Scheme 2.4)**.[268] This debenzylation is optimal with 10 equivalents of BCl_3. The ^1H NMR spectrum of this compound showed the disappearance of the doublet for the methylene protons of the benzyl groups, while the two proton multiplet for the double bond was still present.



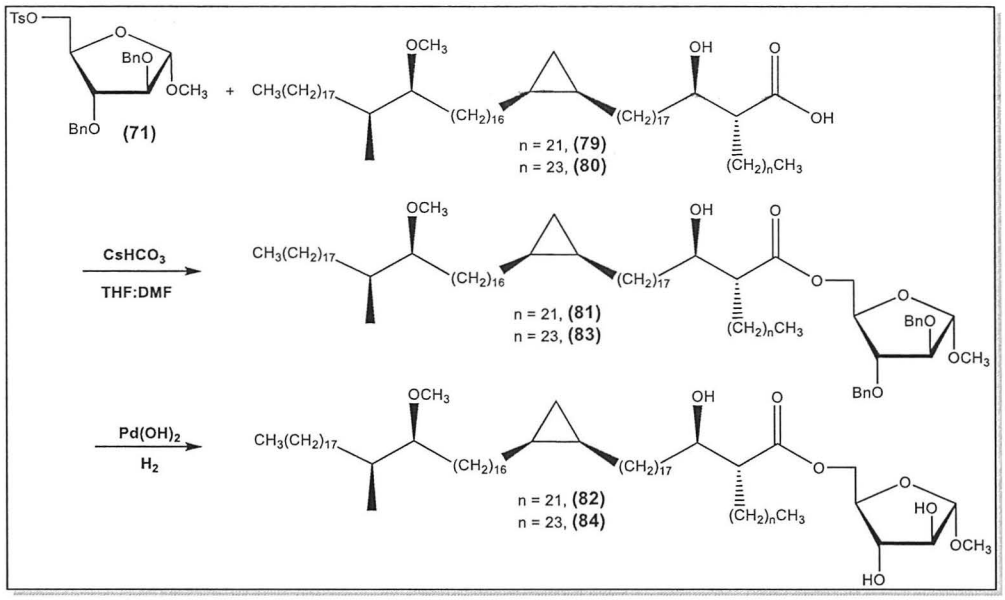
Scheme 2.4: De-protecting of compounds (11)

2.1.4 Esterification of synthetic MAs with the glycan (71).

2.1.4.1 Esterification of (71) with the methoxy-MA (79).

As mentioned in Chapter 1, the two stereo-centres in the structure of mycobacterial MAs in the positions α- and β- relative to the carboxylic group are both in the *R*-configuration. The two branches are separated by these two stereogenic centres: the shorter chain is called the α-branch, which is a saturated aliphatic chain with a 24 carbon chain in *M. tuberculosis* species and with a 22 carbon chain in *M. kansasii* species. The mero-chain which is longer, contains functionalities such as cyclopropane, methoxy, keto and alkene groups. In *M. tuberculosis,* based on the presence of different functional groups in the mero-chain, there are three main types of MAs, namely α-, keto- and methoxy-MAs. The absolute stereochemistry of the *cis*-cyclopropane in the MA has not been proved, *i.e.* the [(*S*)-(*R*)] or [(*R*)-(*S*)] configurations respectively, therefore the two possible structures had been prepared in Bangor. Synthesising MAs provides valuable information for determining the stereochemistry of naturally occurring MAs, which subsequently may provide further understanding of the biosynthetic pathway. Synthetic MAs may also be significant in tuberculosis therapy and diagnosis.

In this part of the study, methoxy-MAs with 24 and 22 carbon α-chains, were used, in addition to methoxy–MAs being cyclopropanes with both the [(*S*)-(*R*)] and [(*R*)-(*S*)] configurations. Firstly, the structurally defined synthetic MA reported by Al Dulayymi *et al.*,[158] a methoxy-*cis*-cyclopropane with 22 carbon α-alkyl chain **(79)**, which is present in nature in *M. kansasii*, was reacted with compound **(71)**, using cesium hydrogen carbonate (5eq.) in dry DMF : THF (1:5) at 70 °C for 2 days giving compound **(81)** in 75% yield **(Scheme 2.5, Figure 2.1)**.



Scheme 2.5: Synthesis of methyl arabino-methoxy-mycolates

The compound showed characteristic NMR signals (**Table 2.1**). Hydrogenolysis of **(81)** in dry CH_2Cl_2: MeOH (1:1) in the presence of $Pd(OH)_2$ under a hydrogen atmosphere gave compound **(82)** **(Figure 2.4)**. This showed NMR signals corresponding to the cyclopropane protons and the remaining protons in the MA similar to those of compound **(81)** and the signals corresponding to the glycan protons were shifted slightly down-field **(Table 2.2)**. The specific rotation of **(81)** was $[\alpha]_D^{23}$ + 18 (*c* 0.1, $CHCl_3$), changing for the deprotected compound **(82)** to $[\alpha]_D^{16}$ + 10 (*c* 0.1, $CHCl_3$).

2.1.4.2 Esterification of the glycan (71) with methoxy-MA (80).

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.[269] Methoxy-MA **(80)** is present in *M. tuberculosis*. In order to probe the biological effects of varying the α-alkyl chain length, compound **(71)** was coupled with **(80)**[158] by the same method as above to prepare compound **(83)** **(Scheme 2.5, Figure 2.1)** in 80% yield; this showed NMR signals which were approximately the same to those of compound **(81)**. Mass spectroscopy confirmed the formation of the compound which gave an $[M+Na]^+$ peak at *m/z* 1602.4304 ($C_{105}H_{190}NaO_8$, requires: 1602.4353). Compound **(83)** was hydrogenolysed by the same method as above to afford **(84)** **(Figure 2.4)**. Once again, compound **(84)** showed characteristic NMR (^1H and ^{13}C) signals similar to those of compound **(82)**. The structures of these compounds were confirmed by two dimensional

NMR; **Figures 2.2** and **2.3** show the HSQC and COSY spectra respectively for compound **(81)**.

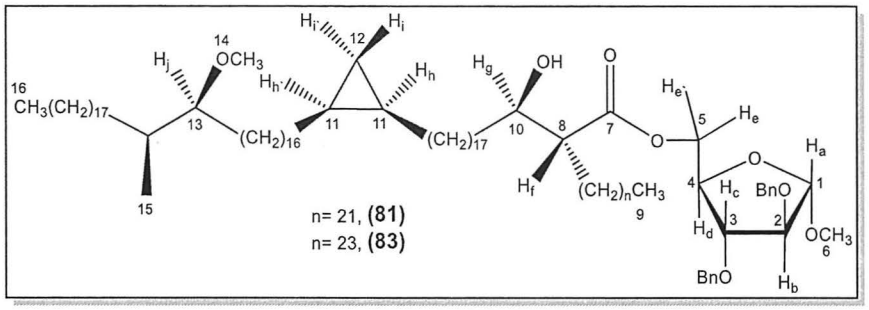


Figure 2.1: Structures of arabino-methoxy mycolates

Table 2.1: The ^{1}H and ^{13}C NMR data analysis of (81)

H_X	Shift	H's	Class	*J*/Hz	Cn	δ/ppm
H_a	4.92	1	s	-	C_1	107.2
H_b	3.99	1	dd	1.0, 2.7	C_2	87.9
H_c	3.84	1	dd	2.7, 6.4	C_3	83.7
H_d	4.22	1	m	-	C_4	79.4
$H_{e, e'}$	4.30	2	m	-	C_5	63.5
H_f	2.43	1	dt	5.5, 9.0	C_8	51.5
H_g	3.63	1	m	-	C_{10}	72.2
$H_{h, h'}$	0.66	2	m	-	C_{11}	15.8
H_i	-0.32	1	br. q	5.2	C_{12}	10.9
$H_{i'}$	0.57	1	dt	3.9, 7.9	C_{13}	85.4
H_j	2.96	1	m	-	$(OCH_3)_6$	54.9
$(OCH_3)_6$	3.38	3	s	-	$(OCH_3)_{14}$	57.7
$(OCH_3)_{14}$	3.35	3	s	-	$(CH_3)_{9, 16}$	14.1
$(CH_3)_{9, 16}$	0.89	6	t	6.9	$(CH_3)_{15}$	14.9
$(CH_3)_{15}$	0.86	3	d	6.8	$C_{\text{-Bn}}$	72.4
$H_{a\text{-Bn}}$	4.58	1	d	12	$C_{\text{-Bn}}$	72.1
$H_{a`\text{-Bn}}$	4.56	1	d	12	C_7	175
$H_{b\text{-Bn}}$	4.51	1	d	12		
$H_{b`\text{-Bn}}$	4.48	1	d	12		

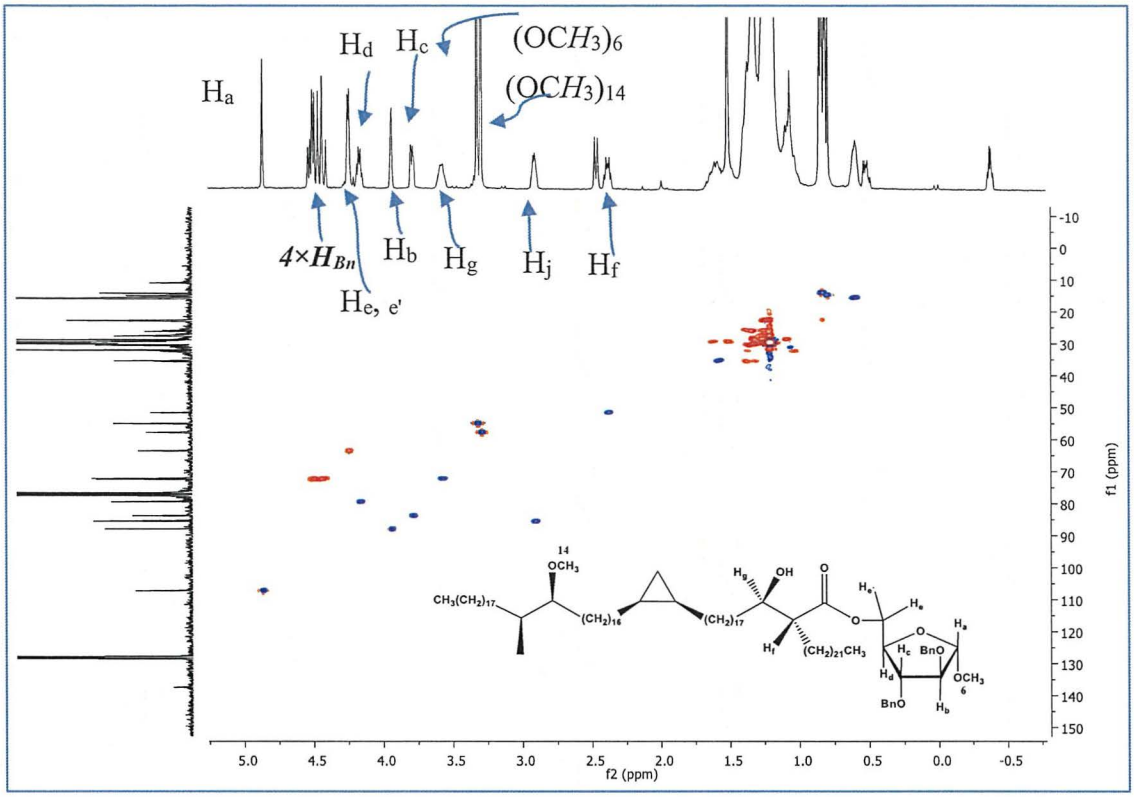


Figure 2.2: HSQC spectrum of compound (81)

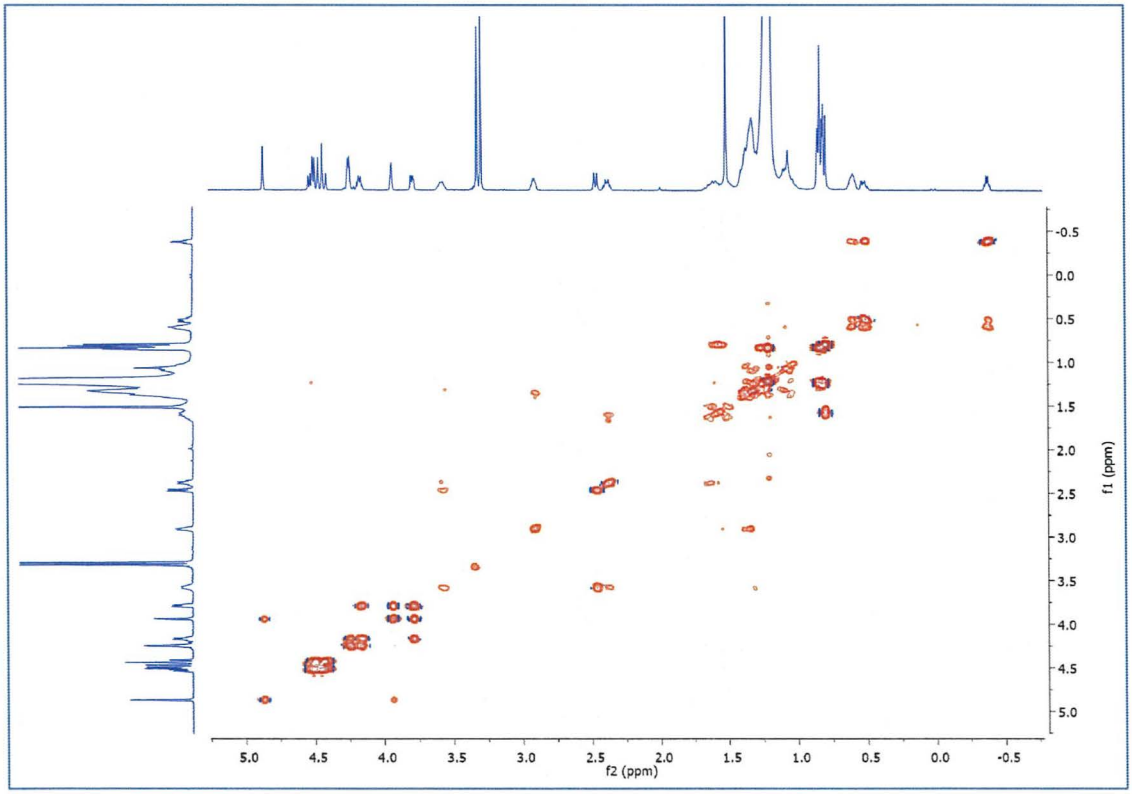


Figure 2.3: COSY spectrum of compound (81)

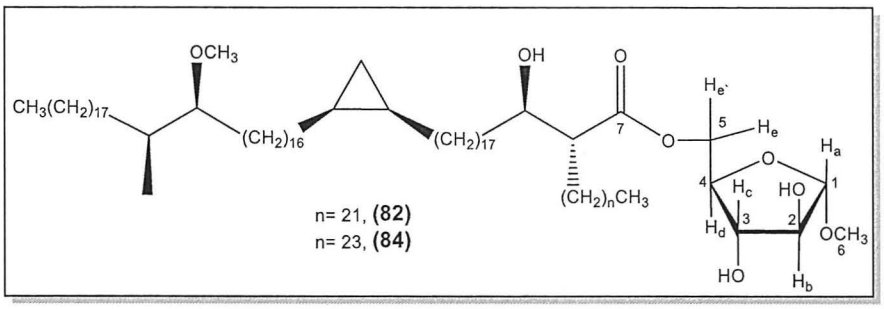


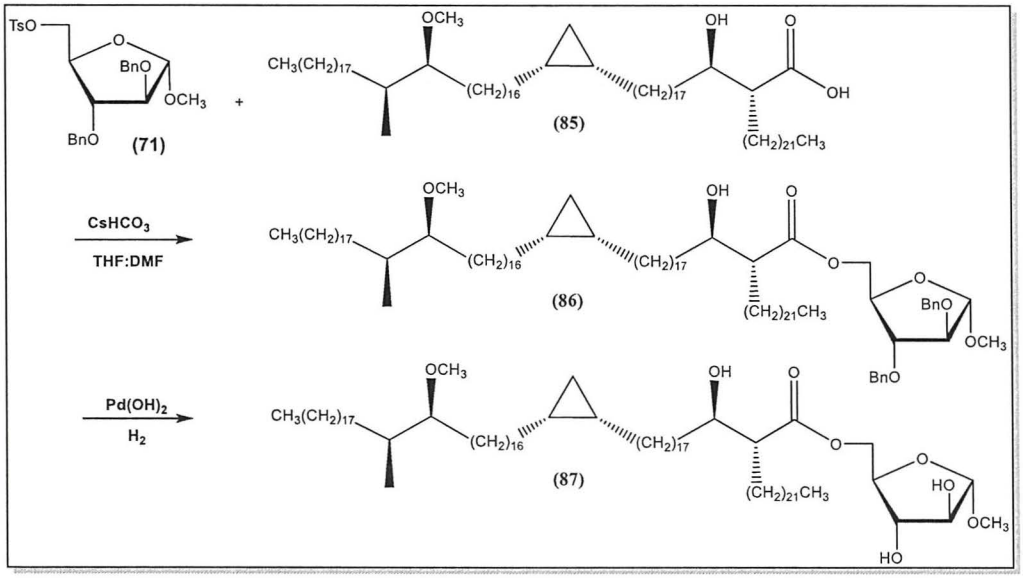
Figure 2.4: Structures of compounds (82) and (84)

Table 2.2: The ^{1}H and ^{13}C NMR data analysis of the glycan moiety of compound (82)

Hx	Shift	H's	Class	J/Hz	Cn	δ/ppm
H_a	4.9	1	s	-	C_1	108.7
H_b	4.07	1	br. d	4.7	C_2	78.4
H_c	3.98	1	br. d	7.5	C_3	77.3
H_d	4.18	1	m	-	C_4	83.8
H_e	4.52	1	dd	3.9, 12.0	C_5	63.3
$H_{e`}$	4.32	1	dd	4.0, 12.0	C_7	174.9
$(OCH_3)_6$	3.35	3	s	-		

2.1.4.3 Esterification of the glycan (71) with methoxy-MA (85).

As mentioned before, the configuration of the *cis*-cyclopropane in the MA has not been proven to be either [(*S*)-(*R*)] or [(*R*)-(*S*)]. After synthesising compound **(82)**, where the cyclopropane was in [(*R*)-(*S*)] configuration, compound **(71)** was used again via the same method with **(85)**[158] to prepare compound **(86)**, **(Scheme 2.6)**.



Scheme 2.6: Synthesis of methyl arabino-methoxy-mycolates

This compound showed characteristic NMR signals which were similar to those of compound **(81)**. The region of the ^{1}H NMR spectrum of **(86)** of most interest is between δ 0.65 and - 0.33, which corresponds to the four protons of the *cis*-cyclopropane ring. The proton H_a **(Figure 2.5)** gave a doublet of triplets; the broadness of this signal shown at δ 0.6 to 0.52 is possibly because H_c and $H_{c'}$ 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_b should show a doublet of triplets; however, due to the difference in magnetism of H_c and $H_{c'}$ the signal at δ - 0.3 to - 0.36 is distorted and it appears as a broad quartet. Protons H_c and $H_{c'}$ again showed a distorted multiplet at δ 0.71 to 0.61 for the same reason **(Figure 2.5)**. A singlet at δ 3.35 corresponded to the methoxy group in the MA. The methoxy group in the glycan gave a singlet at δ 3.37. The α-proton H_m exhibits a doublet of triplets seen at δ 2.47 – 2.40, which is consistent with the expected splitting pattern. H_v and H_i are seen at δ 2.99 – 2.94 and 3.67 – 3.59, due to their adjacency to the β-hydroxy and methoxy respectively. As the hydrogens of the methylene groups adjacent to H_v and H_i are diastereotopic, the splitting patterns observed are slightly distorted.

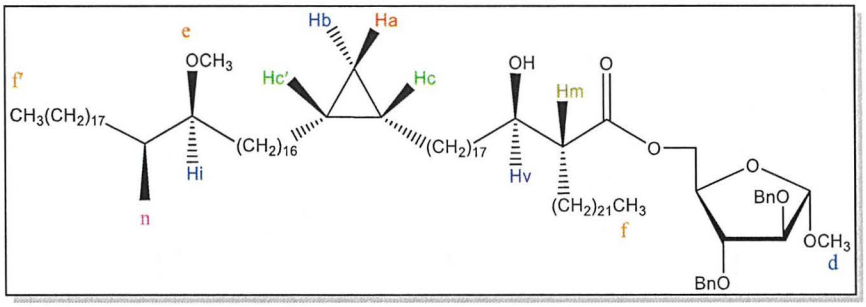


***Figure 2.5: Structure of compound* (86)**

Debenzylation of **(86)** was achieved by the method described previously to give compound **(87)** in 77% yield **(Scheme 2.6)**. Once again, compound **(87)** showed NMR signals similar to those of compound **(84)**.

2.1.4.4 Esterification of the glycan (71) with keto-MA (88)

Keto-MAs are the major oxygenated MAs in the cell wall of mycobacteria, such as *M. bovis*. Synthetic keto-MA **(88)** **(Scheme 2.7)** is present in nature in *M. kansasii*, and synthetic material was provided by Dr. Al Dulayymi.[159] This compound was esterified with the tosylate **(71)** in order to obtain the corresponding arabino-mycolate **(90)**

(Scheme 2.7), by the same procedure as above. The ^{1}H NMR spectrum **(Figure 2.6)** showed characteristic signals in the keto-MA illustrated in **Table 2.3**. Hydrogenolysis of **(90)** was undertaken by stirring it in dry CH_2Cl_2 : MeOH (1:1) in the presence of $Pd(OH)_2$ and under a hydrogen atmosphere to give compound **(91)** in 78% yield **(Scheme 2.7)**. Compound **(91)** showed characteristic NMR signals corresponding to the cyclopropane protons. The specific rotation of **(90)** was $[\alpha]_D^{21}$ + 20 (*c* 0.1, $CHCl_3$), changing for the deprotected compound **(91)** to $[\alpha]_D^{21}$ + 10 (*c* 0.1, $CHCl_3$).

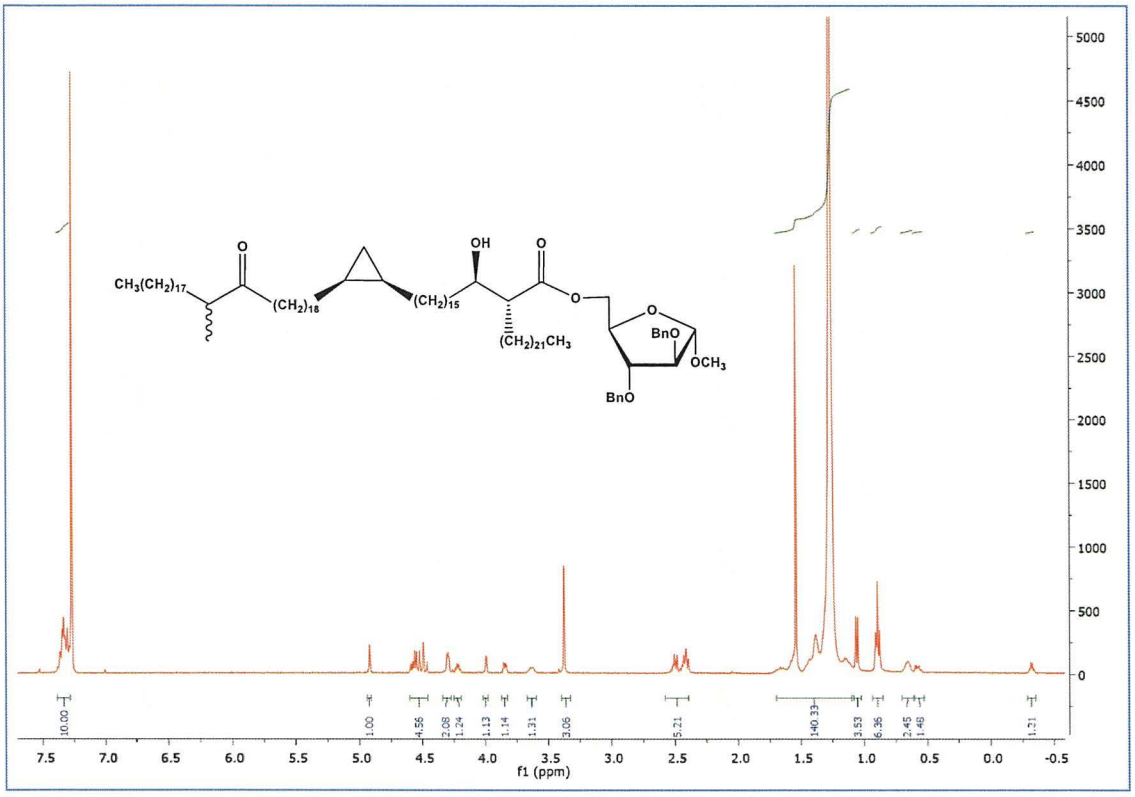
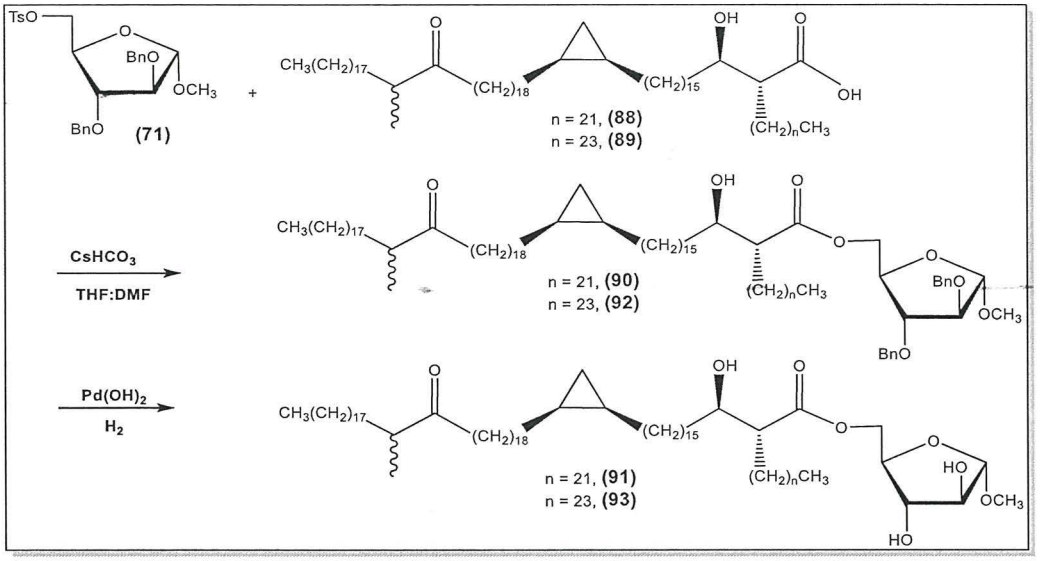


Figure 2.6: ^{1}H NMR of compounds **(90)**



Scheme 2.7: Synthesis of methyl arabino-keto-mycolates

2.1.4.5 Esterification of the glycan (71) with keto-MA (89)

Keto-MAs encourage the growth of the bacterial cell. Their formation in the cell increases during the growth in macrophages and at low oxygen concentrations.[269] The α-chain in keto-MA in *M. tuberculosis* has 24 carbons. The keto-MA in the natural extract of arabino-mycolate is the highest percentage component compared with the other MAs (see **Figure 1.28** in Chapter 1, p. 38). Compound **(71)** was coupled with the synthetic keto-MA **(89)**[159] by the same method as above to prepare compound **(92)** **(Scheme 2.7)** in 71% yield. Compound **(92)** showed characteristic NMR signals similar to those of compound **(90)**. Hydrogenolysis of **(92)** was achieved by the same method described above to afford **(93)**. Compound **(93)** showed NMR signals similar to those of compound **(91)**.

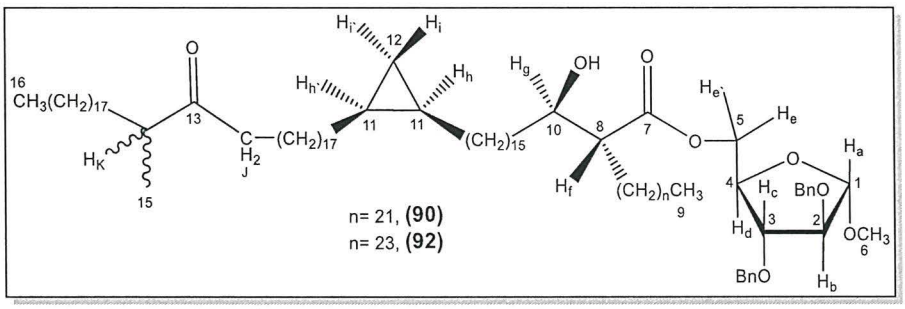


Figure 2.7: Structures of arabino-keto-mycolates

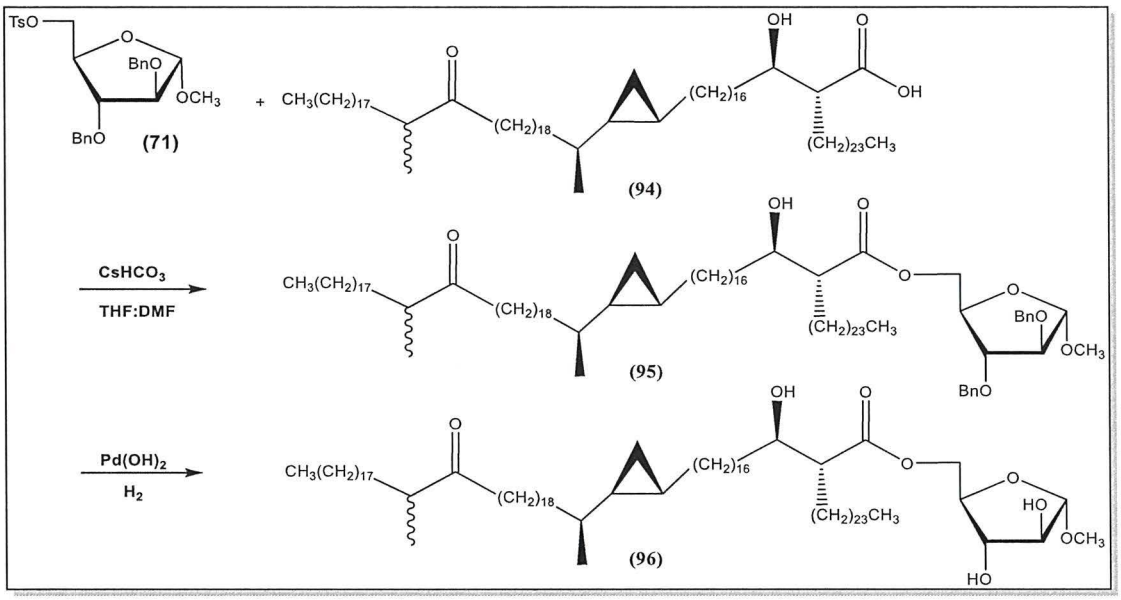
Table 2.3: The ^{1}H and ^{13}C NMR data analysis of (90)

Hx	Shift	H's	Class	*J*/Hz	Cn	δ/ppm
H_a	4.92	1	s	-	C_1	107.2
H_b	3.99	1	br. d	2.2	C_2	87.8
H_c	3.84	1	dd	2.6, 6.4	C_3	83.7
H_d	4.22	1	m	-	C_4	79.4
$H_{e, e'}$	4.30	2	m	-	C_5	63.4
$H_{f, J, K}$, *OH*	2.46	5	m	-	C_8	51.5
H_g	3.63	1	m	-	C_{10}	72.4
$H_{h, h'}$	0.66	2	m	-	C_{11}	15.7
H_i	-0.32	1	br. q	5.2	C_{12}	10.9
$H_{i'}$	0.57	1	dt	3.9, 7.9	$(OCH_3)_6$	54.9
$(OCH_3)_6$	3.38	3	s	-	$(CH_3)_{9, 16}$	14.1
$(CH_3)_{9, 16}$	0.89	6	t	6.7	$(CH_3)_{15}$	15.7
$(CH_3)_{15}$	1.06	3	d	6.8	C_{Bn}	72.4
$H_{a\text{-}Bn}$	4.58	1	d	12	C_{Bn}	72.1
$H_{a'\text{-}Bn}$	4.56	1	d	12	C_7	175
$H_{b\text{-}Bn}$	4.51	1	d	12	C_{13}	215.2
$H_{b'\text{-}Bn}$	4.48	1	d	12		

2.1.4.6 Esterification of the glycan (71) with keto-MA (94)

There are three major classes of MAs found in *M. tuberculosis*. These include ones containing *cis* or *trans*–cyclopropanes and unsaturation. Keto-MAs with *trans*-cyclopropanes have been found in the cell wall of *M. tuberculosis*. Compound **(71)** was coupled with synthetic keto-MA **(94)**[159] by the method described above to prepare compound **(95) (Scheme 2.8)** in 74% yield. An area of the ^{1}H NMR spectrum which is of particular interest that between δ 0.50 and δ 0.05, corresponds to the four protons directly bound to the *trans*-cyclopropane. The region between δ 0.22 – 0.05 contains signals for three different hydrogens. This is because H_a and $H_{a'}$, **(Figure 2.8)**, 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 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 0.5 – 0.4, 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 of the overlapping of peaks. H_e

and the CH_2 group adjacent to the distal position appeared as a multiplet at δ 2.42. The β-chiral centre proton H_g gave a doublet of triplets at 3.63 ppm (*J* 7.6, 5.2 Hz). The region between 0.91 – 0.89 (9H, including a triplet at 0.89 with *J* 7.5 Hz) for the three terminal methyl groups and a doublet at 1.06 (*J* 6.9 Hz) for the other α-methyl groups. Compound **(95)** showed characteristic ^{13}C NMR signals showed in **Table 2.4**. Hydrogenolysis of compound **(95)** was achieved as above to afford **(96) (Scheme 2.8)**. Once again, compound **(96)** was showed characteristic NMR signals between δ 0.51 to 0.05 for the four protons of the *trans*-cyclopropane. The signal for proton on the anomeric centre appeared as a singlet at δ 4.89. The remaining 6H on the glycan moieties appeared at δ 4.49 - 3.75.



Scheme 2.8: Synthesis of methyl arabino-keto-trans-mycolates

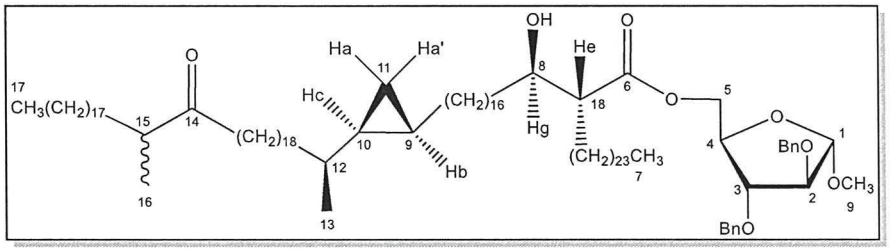


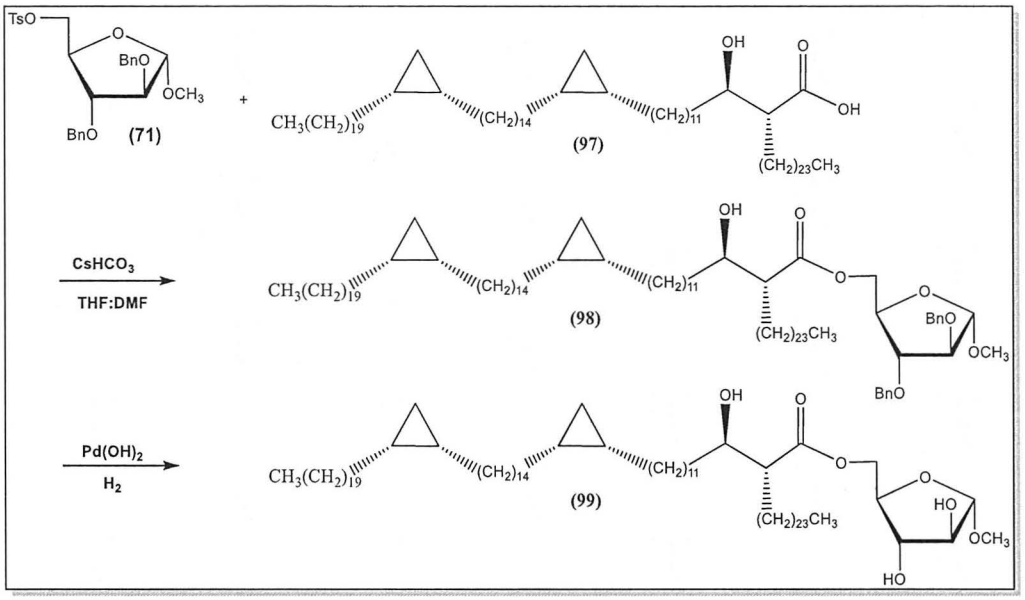
Figure 2.8: Structure of compound (95)

Table 2.4: The ^{13}C NMR data analysis of (95)

Cn	C_1	C_2	C_3	C_4	C_5	C_8	C_{11}	C_{12}	C_{18}	C_{13}	C_9
δ	107.2	87.8	83.7	79.4	63.5	72.4	10.5	38.1	51.5	19.7	18.6
Cn	C_{10}	C_{15}	C_{16}	$C_{17,7}$	C_{18}	C_6	C_{14}				
δ	26.1	46.3	16.3	14.1	51.5	175	215.2				

2.1.4.7 Esterification of the glycan (71) with α-MA (97)

The α-MA type is the most abundant among MAs in *M. tuberculosis*, and contains two *cis*-cyclopropanes. This acid was reported by Minnikin and Polgar to be the major mycolic acid of *M. tuberculosis* var *hominis*.[270] Synthetic α-MA **(97)**,[157] prepared in Bangor, was esterified with compound **(71)** by the method above to give **(98) (Scheme 2.9)**. The ^1H NMR spectrum of this compound showed a multiplet at δ 3.63 for 1 proton which corresponds to the proton at the β-position from the carboxylic group in the MA. The signal corresponding to the proton at the α-position in the MA appeared as a doublet of triplets at δ 2.44 (*J* 9.3, 5.5 Hz). Protons corresponding to the cyclopropane were revealed as a broad quartet for 2H at δ - 0.32 (*J* 5.2 Hz), a doublet of triplets for 1H at δ 0.57 (*J* 4.0, 8.0 Hz) and multiplet for 4H at δ 0.70 – 0.62. Signals corresponding to the glycan moieties were approximately similar to those of the previously prepared arabino-mycolates. Formation of the compound **(98)** was proved by ^{13}C NMR spectrum which showed a signal for the carbonyl ester at δ 175.0, followed by the carbon on the β-hydroxy acid position resonating at δ 72.1. This compound was then subjected to hydrogenolysis to afford **(99) (Scheme 2.9)**. Confirmation of the formation of **(99)** was achieved by ^1H NMR. A downfield signal at δ 4.89 corresponds to the 1 proton attached to the C1 of the sugar core. Signals for the cyclopropane appeared at approximately the same chemical shift as those in the spectrum of the compound **(98)**. The ^{13}C NMR spectrum showed a carbonyl at δ 175, a signal corresponding to the C1 for the glycan at δ 108.7, and the remaining sugar carbons in the region of δ 83 – 54. The β-hydroxy carbon appeared at δ 72.8. The CH_2 chain ranged from δ 35 – 25 and the CH_3 came up-field to around δ 14.0. The MA α-carbon resonated at δ 52.4. 2D NMR was used to provide further proof of the structure. **Figure 2.9** shows the HSQC spectrum of the glycan part of compound **(98)** which showed the signals of the two protons at C5 position on the glycan, as a double doublet for each proton, and also all the proton signals were correlated to their carbons.



Scheme 2.9: Synthesis of methyl arabino-α-mycolates

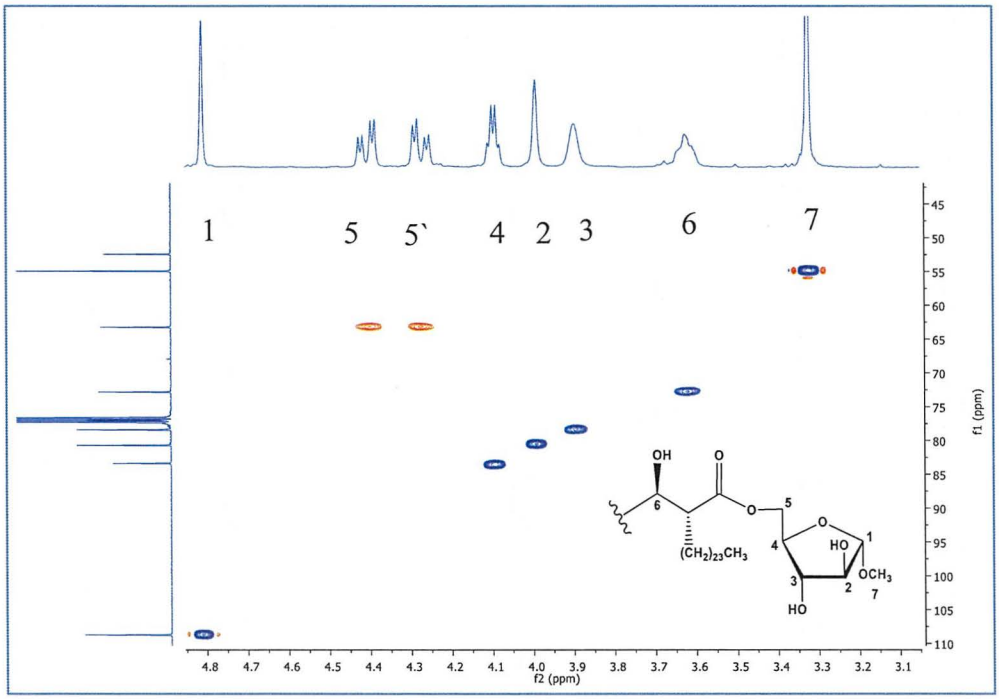


Figure 2.9: HSQC spectrum of the glycan part of compound (99)

2.1.5 Summary

In this part of this thesis, seven examples from MAM were prepared based on the three common classes of MAs, and also four linear alkyl acids as models:

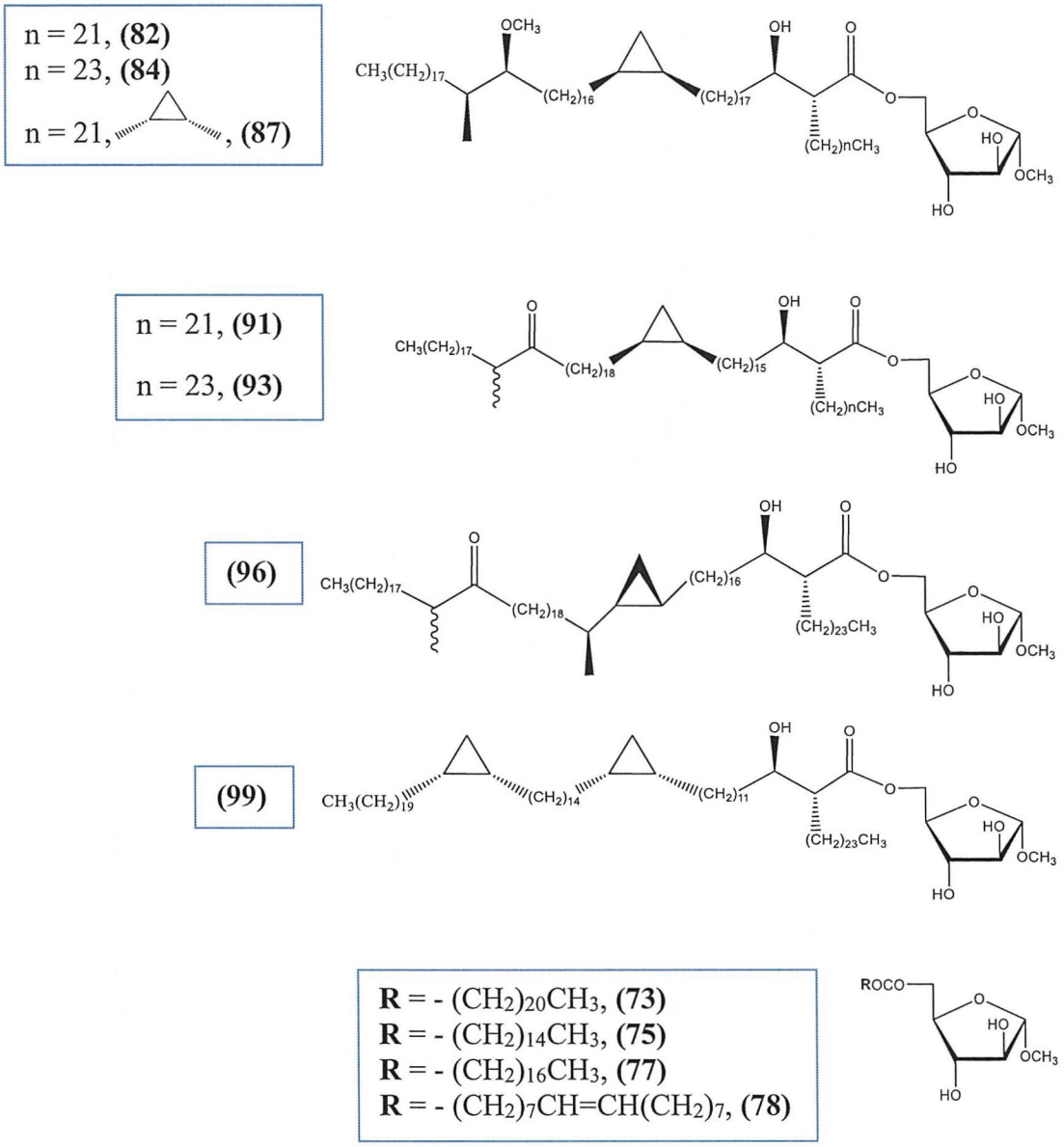


Figure 2.10: Structures of the target compounds

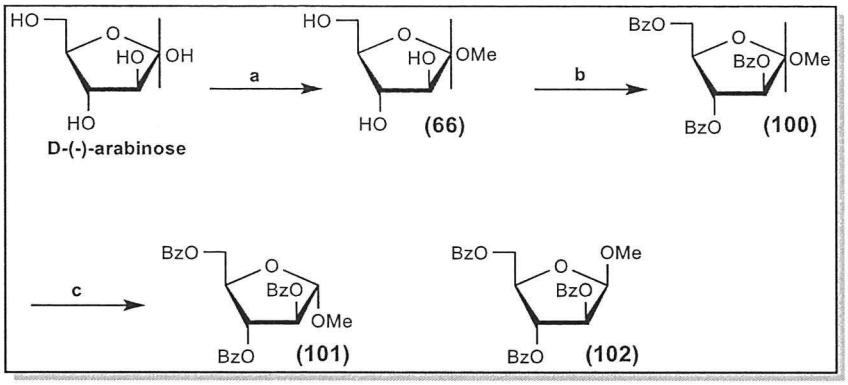
2.2 Synthesis of Glycerol-Arabino-Mycolates (GAM)

2.2.1 The aims of this part:

- To prepare the glycan α-D-arabinofuranosyl-(1→1′)-glycerol with the two configurations of the glycerol (D and L).
- To prepare models of those glycolipids formed by the esterification of the glycerol-glycan with normal fatty acids.
- To prepare a series of glycerol-arabino-mycolates through esterification of the two types of the glycerol-glycan with different synthetic MAs.
- To prepare a penta-acetate from the glycerol-arabino-mycolates in order to compare their NMR data with those reported for the naturally occurring compounds, which would allow for the elucidation of the correct stereo-chemistry of this glycolipid.
- To investigate the biological activity of the synthetic compounds, particularly their antigenicity.

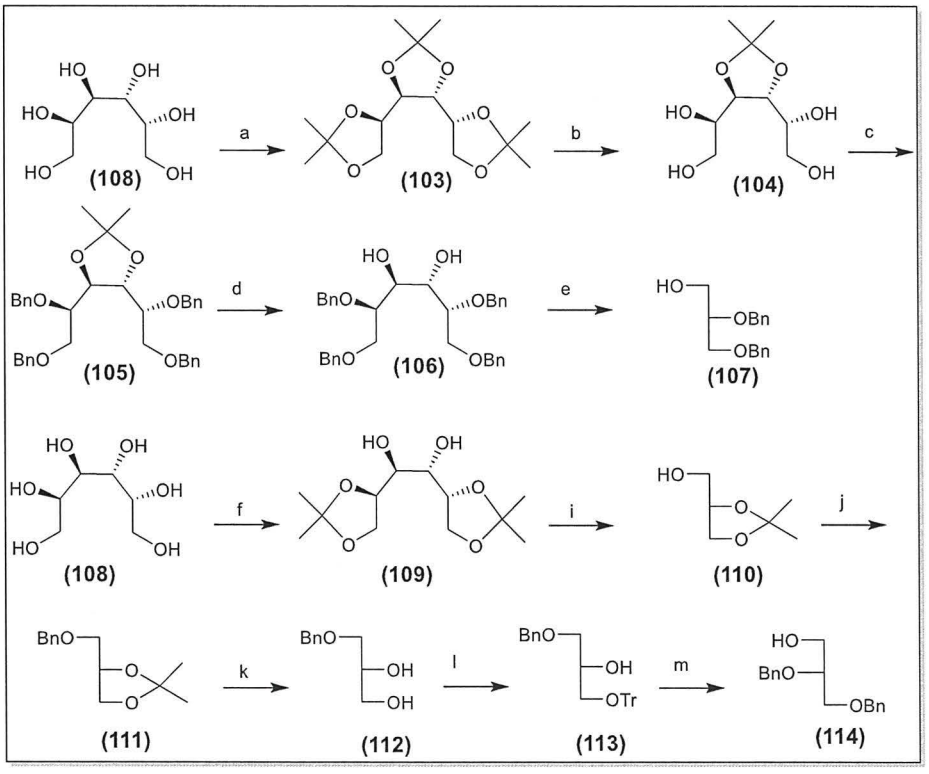
2.2.2 Synthesis of the glycan moieties

As mentioned in the introduction, Watanabe and co-workers extracted a new glycolipid from MAI, 5-mycoloyl-α-arabinofuranosyl-(1→1′)-glycerol (MAG).[262] The stereo-chemistry of the glycerol, *i.e.* the D- or L- configuration, was not proven, and also the biological activity, particularly the antigenicity of MAG, was not examined. In this part of the study we prepared both D and L-glycerol derivatives and introduced them to the α-D-Ara*f* ring in order to determine the stereochemistry of the glycolipid, as well as studying their biological activity. The initial step in the synthetic route was the separation of the two anomers of methyl-arabinoside **(66)**, and this was carried out by esterification of **(66)** under standard conditions to give *tri*-benzoate **(100)**. The pure α-anomer **(101)** was separated from the β-anomer **(102)** by precipitating it in ethanol. Recrystallization from ethanol afforded the α-anomer **(101)** in 50% yield **(Scheme 2.10)**.[271]



Scheme 2.10: Reagents and conditions: (a) HCl, CH_3OH; (b) BzCl, pyridine, 0 °C/R.T., then 40 °C 1.5 h, 50% from α-anomer; (c) precipitation by ethanol, recrystallization from ethanol.

The protected D-glycerols **(107)**, **(110)** and **(114)** **(Scheme 2.11)** were synthesized from D-mannitol as reported in the literature with some modification in the methods (see appendix, page 265).[272,273,274,275,276,277]

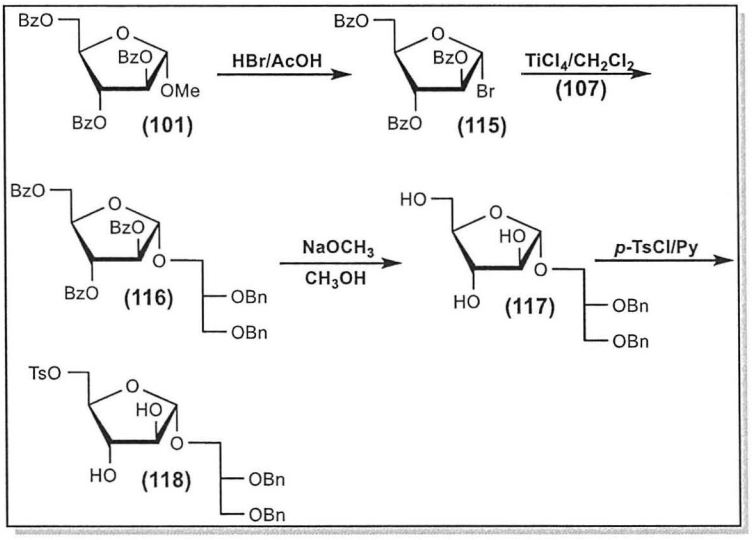


Scheme 2.11: Synthesis of protected glycerols[278,279,280,281,282,283]

(a) Acetone, H_2SO_4, 66%; (b) aqua AcOH 70%, 76%; (C) NaH, BnBr, DMF, 78% (d) dioxane:methanol:1N HCl, 91%; (e)$NaIO_4$, methanol and $NaBH_4$,89%.(f) $ZnCl_2$, acetone, 67%; (i) Lead acetate, THF and $NaBH_4$, 60%; (j) NaH, BnBr, DMF, 79%; (k) aqua. AcOH 70%, 54%; (l) TrCl, DMAP, pyridine, 51%; (m) NaH, BnBr, DMF, then aqua AcOH 80%, 77%.

The methoxy group in benzoylated methyl arabinoside **(101)** was converted to a bromide by reacting it with hydrogen bromide in acetic acid to give bromide **(115)** as a white

foam in 90% yield **(Scheme 2.12)**, which was used in the next step without further purification.[284] The next step entailed the introduction of a protected glycerol at the C1 position of compound **(115)**. In an initial attempt, bromide **(115)** was treated with tin (IV) chloride in dichloromethane, followed by the addition of alcohol **(110)** under basic conditions by adding ethyldiisopropylamine as reported in the literature, with a modification in the method,[285] but the product was obtained as a mixture of anomers. Also, the isopropylidene protecting group was not stable in acidic conditions. An alternative glycosidation was therefore used, in which compound **(107)** was prepared and, using the same coupling condition as above, compound **(116)** was obtained exclusively as one anomer (α-anomer). This showed in the ^{1}H NMR a broad singlet at 5.34 ppm for one proton at the anomeric centre while the ^{13}C NMR showed a peak at 105.9 ppm due to the carbon at position 1 **(Figure 2.11)**, both indicating that only the α-anomer was present. Deprotection of compound **(116)** under Zemplén conditions, thus the removal of the *O*-acyl protecting groups of carbohydrates under transesterification conditions using methanol and a catalytic amount of sodium methoxide, gave **(117)** in 87% yield **(Scheme 2.12)**. The primary hydroxyl group (C5) in the compound **(117)** was tosylated directly without prior protection of the two secondary hydroxyl groups, by reacting it with TsCl in dry pyridine in the presence of a catalytic amount of DMAP at 0 °C to afford the tosylate **(118)** in 72% yield **(Scheme 2.12)**.



Scheme 2.12: Synthesis of D-glycerol-Araf target

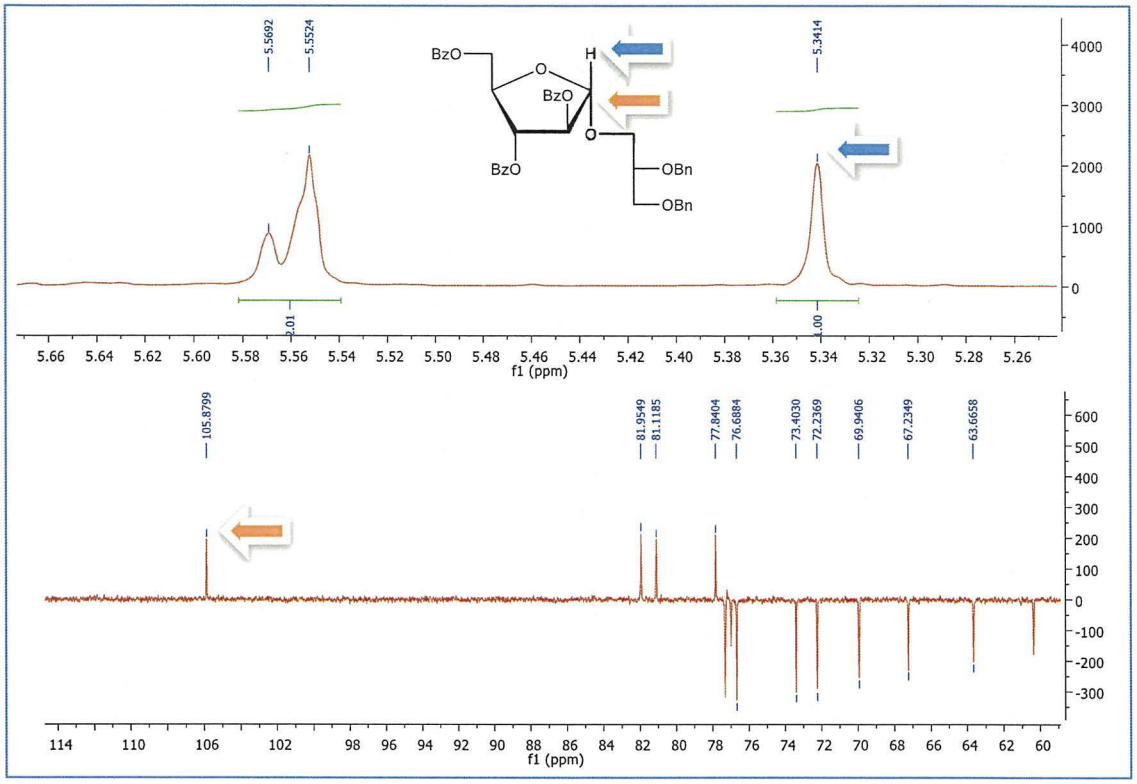


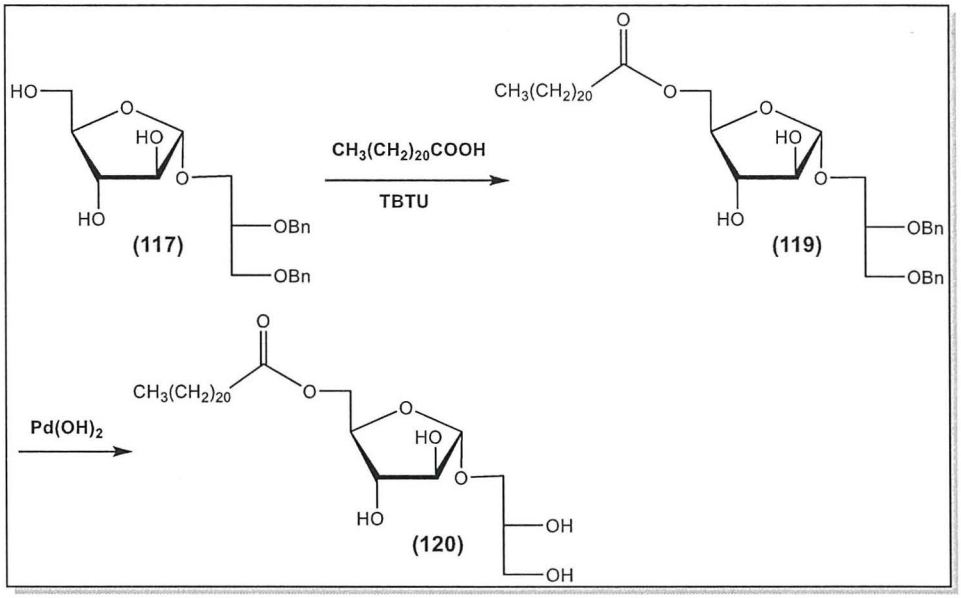
Figure 2.11: ^{1}H and DEPT NMR of compound (116)

Formation of the α-anomer (1,2-*trans*) as the principal product is due to the participation of the acyl group at the C2 position on the glycan ring,[286] as illustrated in the **Figure 1.25** (Chapter 1 p. 32).

2.2.3 Direct esterification of the glycan (117) with fatty acids

Having the glycerol-sugar moiety of the glycolipid targets in hand, we now explored their esterification with various fatty acids to obtain model analogues. Esterification was carried out by using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluraniumtetrafluoro-borate (TBTU)[287] as a coupling reagent with behenic acid and compound **(117)** in dry pyridine at 60 °C for 4 days. This gave the expected ester **(119)**, which was then subjected to hydrogenolysis to afford **(120)** in 74% yield (over two steps) **(Scheme 2.13)**. The ^{1}H NMR spectrum of **(120)** showed a signal as a doublet at δ 4.91 (*J* 1.1 Hz), corresponding to the proton at the anomeric centre. The two protons at the C5 position on the glycan ring appeared as two doublets of doublets at δ 4.27 (*J* 3.2, 12.0 Hz) and 4.17 (*J* 6.2, 11.5 Hz) respectively, the large coupling constant corresponding to germinal coupling between the two protons, with the small *J* value relating to coupling between each of the protons with the proton at C4 on the ring. The methylene group adjacent to the carbonyl group of the acid was observed as a triplet at δ 2.32 (*J* 7.6 Hz). The signal

for the terminal methyl group was shifted to up field and appeared as a triplet at δ 0.84 (*J* 6.8 Hz). The ^{13}C NMR spectrum showed a carbonyl group at δ 174.1, C1 for the glycan at 108.4 and signals at 83.8, 78.4 and 77.3 corresponding to C4, C2 and C3 in the ring respectively. The CH_2 chain ranged from δ 34.0 – 22.6 and the CH_3 came up-field at δ 14.0.



Scheme 2.13: Synthesis of D-glycerol--arabino-lipids

Having a successful direct method to prepare a model compound from glycerol-glycolipid, an attempt to prepare the desired D-glycerol-α-D-Ara*f*-mycolate by the method above was undertaken. Methoxy-MA **(79)**[158] and compound **(117)** were stirred in pyridine at 60 °C for 4 days, but no signs of product were detected. The advantage of the (TBTU) method is it is avoid protecting groups and gives a good yield but unfortunately it is not work with a complex fatty acids. Therefore, the primary hydroxyl group of compound **(117)** was converted into a tosylate by reaction with TsCl in dry pyridine and catalytic DMAP at room temperature to afford the tosylate **(118)** **(Figure 2.12)**, in 72% yield **(Scheme 2.12)**. The synthesis of this compound was confirmed by NMR, which gave the signals illustrated in **Table 2.5**.

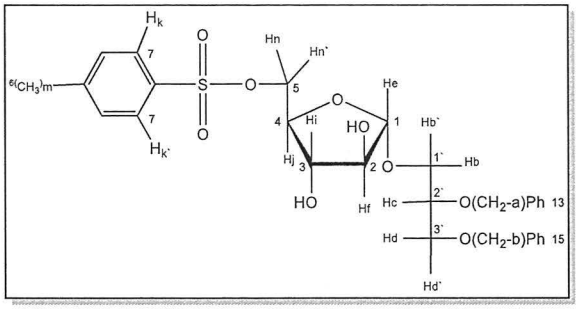


Figure 2.12: Structure of compound (118)

Table 2.5: The ^{1}H and ^{13}C NMR data analysis of (118)

H_X	Shift	H's	Class	*J*/Hz	Cn	δ/ppm
H_e	4.94	1	s	-	C_1	107.6
H_f	4.01	1	br. d	5.6	C_2	79.9
H_i	3.81	1	br. d	8.8	C_3	77.6
H_j	4.09	1	m	-	C_4	83.3
H_n	3.87	1	dd	4.0, 10.4	C_5	69.1
$H_{n`}$	3.53	1	dd	5.1, 10.6	-	-
H_b	4.18	1	dd	3.9, 10.4	$C_{1`}$	69.5
$H_{b`}$	4.13	1	dd	2.9, 7.3	-	-
H_c	3.73	1	br. p	4.8	$C_{2`}$	76.4
$H_{d,d`}$	3.57	2	br. d	5.3	$C_{3`}$	66.5
$(CH_{2\text{-}a})_{13}$	4.64	1	d	12.1	C_{Bn}	73.4
$(CH_{2\text{-}a`})_{13}$	4.59	1	d	11.9	-	-
$(CH_{2\text{-}b})_{15}$	4.54	1	d	12.0	C_{Bn}	72.0
$(CH_{2\text{-}b`})_{15}$	4.51	1	d	12.2	-	-
$H_{k,k`}$	7.8	2	d	8.2	C_7	128
CH_3m	2.44	3	s	-	C_6	21.6

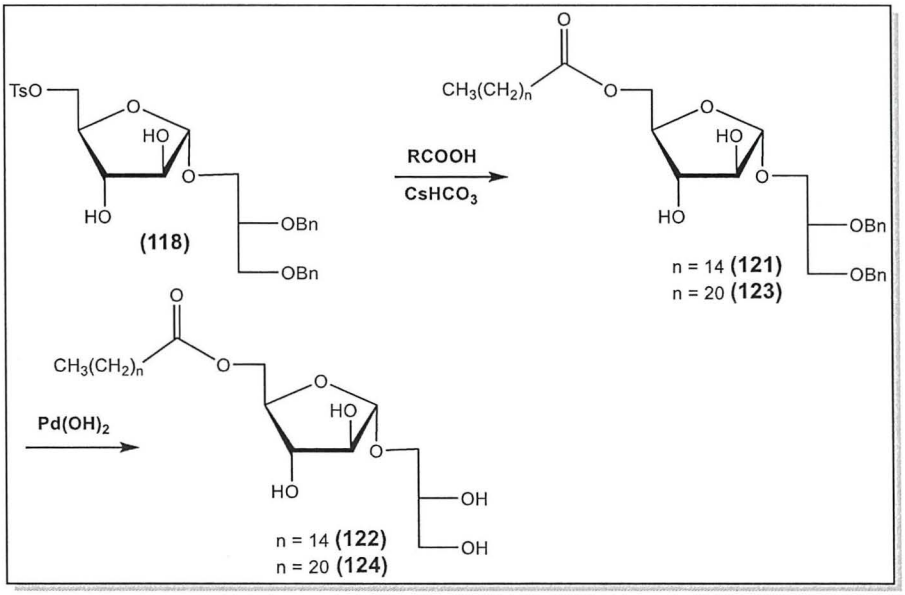
2.2.4 Esterification of the glycan (118) with fatty acids

Compound **(118)** was reacted with palmitic acid through the alkylative esterification strategy, by using cesium hydrogen carbonate in dry DMF : THF at 70 °C, to give compound **(121)** in 72% yield **(Scheme 2.14)**. Confirmation of the formation of **(121)** was achieved by proton ^{1}H NMR. A downfield signal at δ 5.02 corresponding to theproton attached to the C1 of the sugar. Protons corresponding to the CH_2 of the two benzyl groups appeared as four doublets with coupling constants of 12 Hz. The remaining 10 protons on the sugar appeared in the range from δ 3.5 – 4.2. Protons corresponding to the CH_2 adjacent to the carbonyl group in the acid came around δ 2.3 as a triplet (*J* 7.6). The ^{13}C NMR spectrum showed a carbonyl at δ 173.4, C1 at δ 107.5

and the remaining sugar carbons in the region of δ 83.9 – 63.9. The CH_2 chain ranged from δ 34 - 22 and the CH_3 came up-field around δ 14.1.

Debenzylation of **(121)** was now undertaken, by dissolving it in dry CH_2Cl_2 : MeOH (1:1) in the presence of $Pd(OH)_2$ (0.15 eq. fold by weight) and stirring under a hydrogen atmosphere, which gave compound **(122)** in 79% yield **(Scheme 2.14)**. Once again, the formation of **(122)** was proved by NMR, which showed the disappearance of those signals corresponding to the benzyl groups between δ 4.6 – 4.5, and the signals of the aromatic protons. The ^{13}C NMR spectrum showed a carbonyl at δ 174, C1 was shifted slightly downfield at δ 108.3, and the remaining sugar carbons occurred in the region of δ 82 – 63. The specific rotation of **(121)** was $[\alpha]_D^{23}$ + 40.2 (*c* 0.1, $CHCl_3$), changing for the deprotected compound **(122)** to $[\alpha]_D^{21}$ + 5.1 (*c* 0.1, $CHCl_3$).

In the same way, tosylate **(118)** was reacted with behenic acid to give compound **(123)** **(Scheme 2.14)**. This showed NMR signals similar to those obtained for compound **(121)**. Hydrogenolysis of **(123)** by the method described above gave **(124)** **(Scheme 2.14)**, which showed characteristic NMR (^1H and ^{13}C) signals which were identical to those of compound **(120)** prepared by a different method.



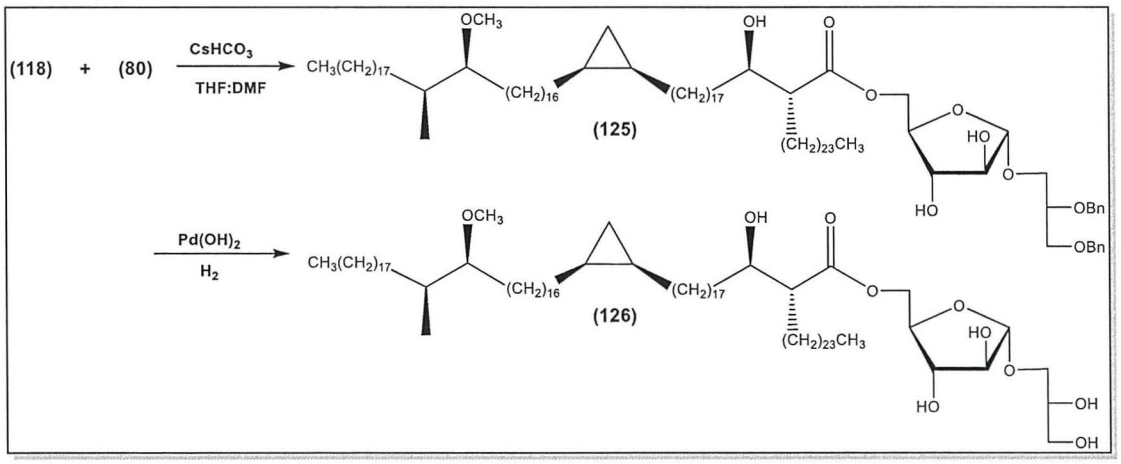
Scheme 2.14: Synthesis of D-glycerol-arabino-lipids

2.2.5 Esterification of the glycan (118) with synthetic MAs

2.2.5.1 Esterification of the glycan (118) with the methoxy-MA (80)

Having secured a successful method for synthesising models of the glycerol-arabino-lipids, the coupling the glycerol arabinose with synthetic MAs was now undertaken.

Firstly, methoxy-MA **(80)**,[158] was reacted with compound **(118)** to afford compound **(125)** in 66% yield **(Scheme 2.15)**, by using cesium hydrogen carbonate (7 eq.) in dry DMF : THF (1:5) at 70 °C for 2 days. The success of the esterification was demonstrated by the ^{1}H NMR spectrum, where the characteristic signal corresponding to the proton at the anomeric centre appeared as a singlet at δ 5.0. The proton at C2 appeared as a broad singlet at δ 4.0. A signal appeared as a broad doublet of doublets at δ 3.9, corresponding to the proton at C3 and the proton at C3` on the glycerol core. The proton at C4 showed a signal as a multiplet at δ 4.1, which should, in principle, appear as a doublet of doublets of doublets, but due to the complexity of the ring the signal was distorted. The two protons at C5 in the glycan part were seen as two doublets of doublets at δ 4.2 and 4.4 respectively. A signal appeared as a multiplet at δ 3.5 integrated for three protons, and corresponding to the 2 protons at C1` and the proton at C3` on the glycerol. Finally, the benzylic protons were seen as four doublets at δ 4.53, 4.56, 4.61 and 4.67, the coupling constant for each signal being 12 Hz. All the signals corresponding to the glycan described above are shown in the HSQC spectrum **(Figure 2.13)**. Signals corresponding to the MA were similar to those discussed before.



Scheme 2.15: Synthesis of D-glycerol arabino-methoxy-mycolates

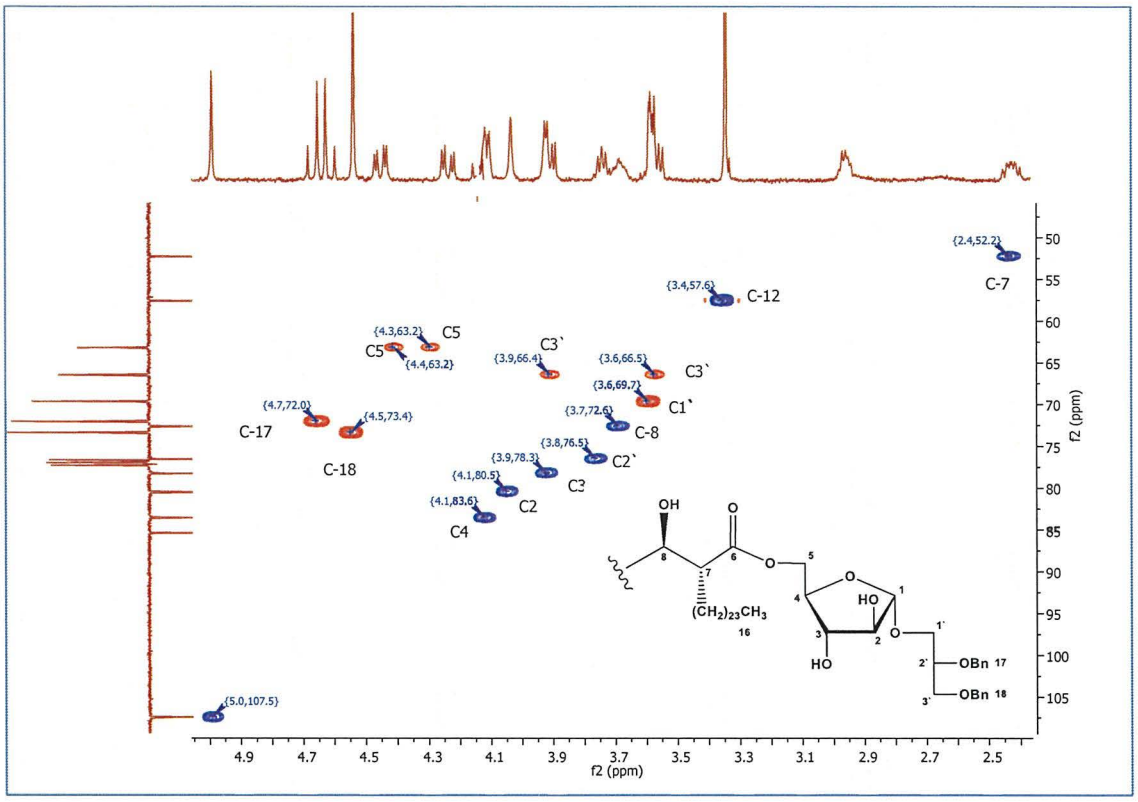


Figure 2.13: The HSQC spectrum of the glycan part of the compound (125)

Compound **(125)** was debenzylated by stirring in hexane : ethyl acetate (1:1) in the presence of $Pd(OH)_2$ under a hydrogen atmosphere to give compound **(126)** in 89% yield **(Scheme 2.15, Figure 2.14)**. Once again, compound **(126)** showed characteristic NMR signals for the glycerol glycan illustrated in **Table 2.6**, and the MA signals are similar to those observed before.

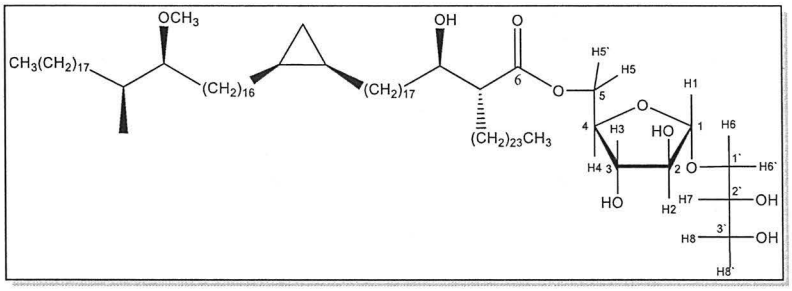


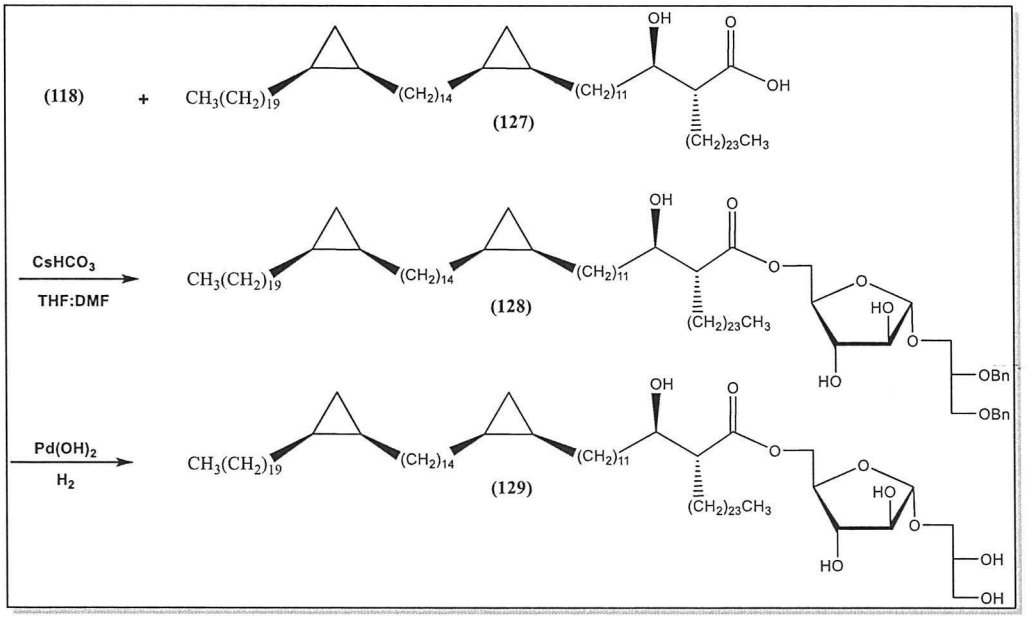
Figure 2.14: Structure of compound (126)

Table 2.6: The ^{1}H and ^{13}C NMR data analysis of (126)

H_X	Shift	H's	Class	*J*/Hz	Cn	δ/ppm
H_1	4.88	1	s	-	C_1	108.0
H_2	3.99	1	dd	1.2, 2.8	C_2	81.0
H_3	3.84	1	dd	2.8, 5.4	C_3	78.3
H_4	4.09	1	br. dd	5.5, 9.9	C_4	81.4
H_5	4.33	1	dd	4.2, 11.6	C_5	63.2
$H_{5'}$	4.22	1	dd	5.7, 11.6	$C_{1'}$	68.5
$H_{7,6}$	3.76	2	m	-	$C_{2'}$	70.2
$H_{8,8'}$	3.54	2	m	-	$C_{3'}$	63.2
$H_{6'}$	3.44	1	dd	7.1, 11.3	C_6	174.6

2.2.5.2 Esterification of the glycan (118) with α-MA (127)

The α-MAs constitute the most abundant class among the MAs of *M. tuberculosis*, and are characterised by their containing two *cis*-cyclopropanes. Synthetic α-MA **(127)**,[157] was esterified with tosylate **(118)** as before to give **(128)** in 84% yield **(Scheme 2.16)**. The resulting glycerol ester **(128)** was characterised by ^{1}H NMR, which gave signals corresponding to α-MAs similar to those discussed before, and signals corresponding to the protons in the glycan extremely similar to those found for compound **(125)**. The ^{13}C NMR spectrum for **(128)** included the ester carbonyl signal at δ 174.9, and signals for the cyclopropane carbons between δ 15.8 and 10.9. Hydrogenolysis of compound **(128)**, was achieved as before, affording **(129)** in 18% yield **(Scheme 2.16, Figure 2.15)**.



Scheme 2.16: Synthesis of D-glycerol arabino-α-mycolates

Confirmation of the formation of **(129)** was achieved by ^{1}H NMR **(Figure 2.16)**. Compound **(129)** showed characteristic ^{13}C NMR signals, given in **Table 2.7**.

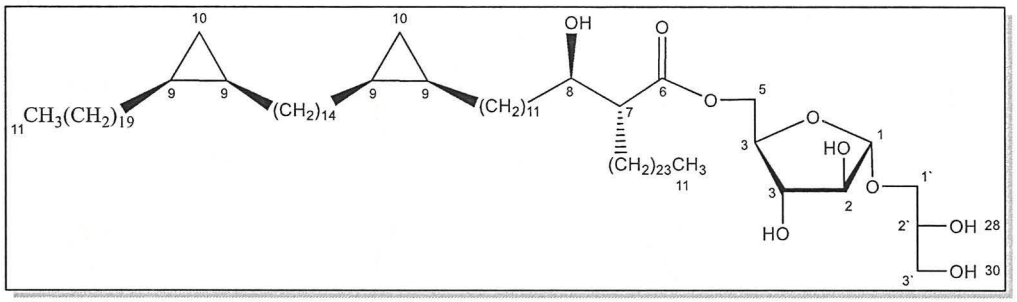


Figure 2.15: Structure of compound (129)

Table 2.7: The ^{13}C NMR data analysis of (129)

Cn	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}
δ	108.2	81.2	77.8	82.1	63.3	175	52.8	72.5	15.7	10.8	13.9
Cn	$C_{1`}$	$C_{2`}$	$C_{3`}$								
δ	69.2	70.6	63.3								

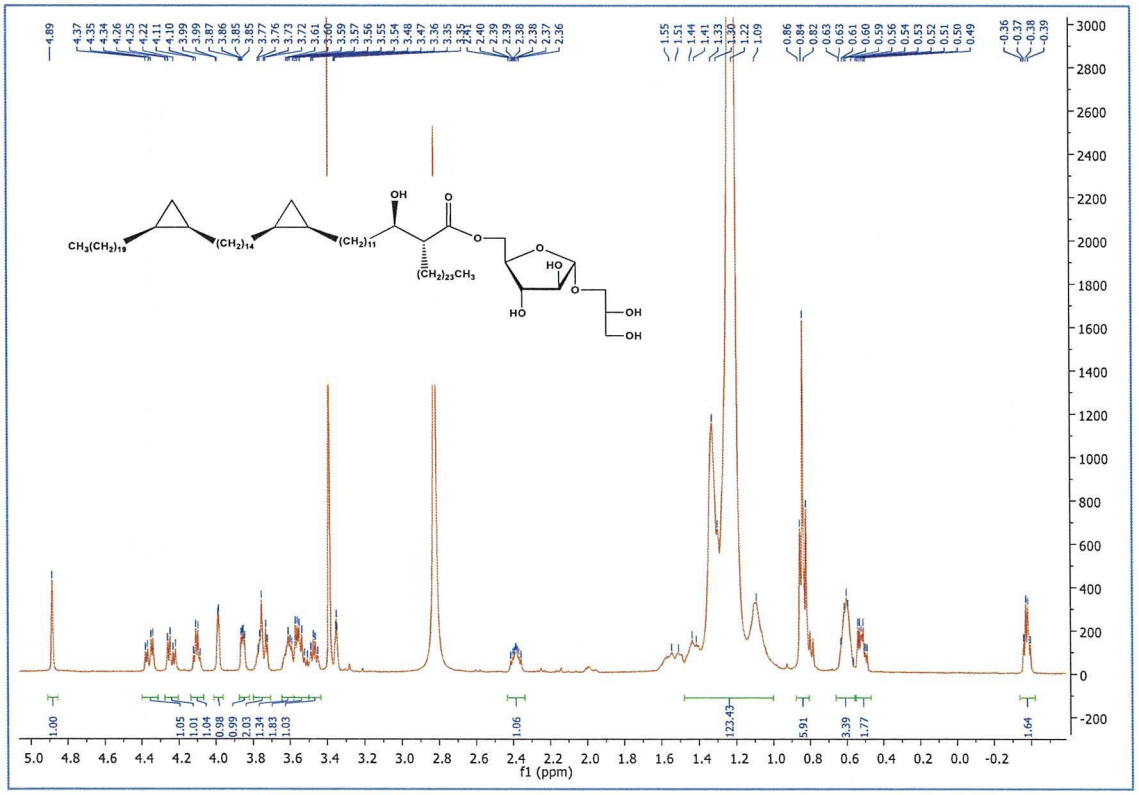
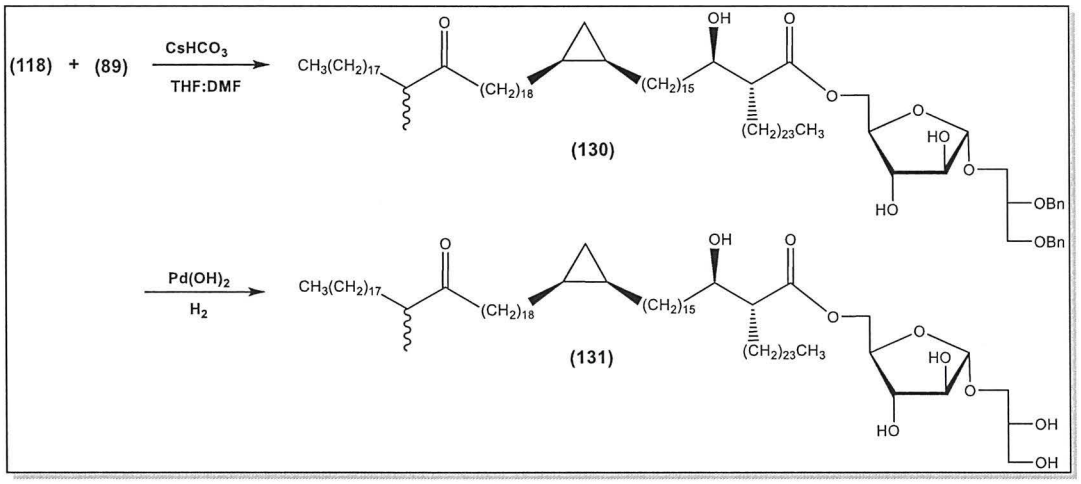


Figure 2.16: ^{1}H NMR of compound (129)

2.2.5.3 Esterification of the glycan (118) with keto-MA (89)

Compound **(118)** was coupled with the synthetic keto-MA **(89)**[159] by the same method to prepare compound **(130)** **(Scheme 2.17)** in 76% yield. This showed characteristic ^{1}H NMR (**Figure 2.17**) and ^{13}C NMR signals; all the signals corresponding to the glycerol glycan part were similar to those of compound **(128)** and the signals belonging to the mycolic acid were identical to those discussed before for the keto-MA. Debenzylation of **(130)** was achieved by the method given previously, and afforded **(131)** in 75% yield **(Scheme 2.17)**. Compound **(131)** showed characteristic NMR signals for the glycerol glycan part, again similar to those for compound **(129)** and signals of the mycolic acid also similar to those discussed before.



Scheme 2.17: Synthesis of D-glycerol arabino-keto-mycolates

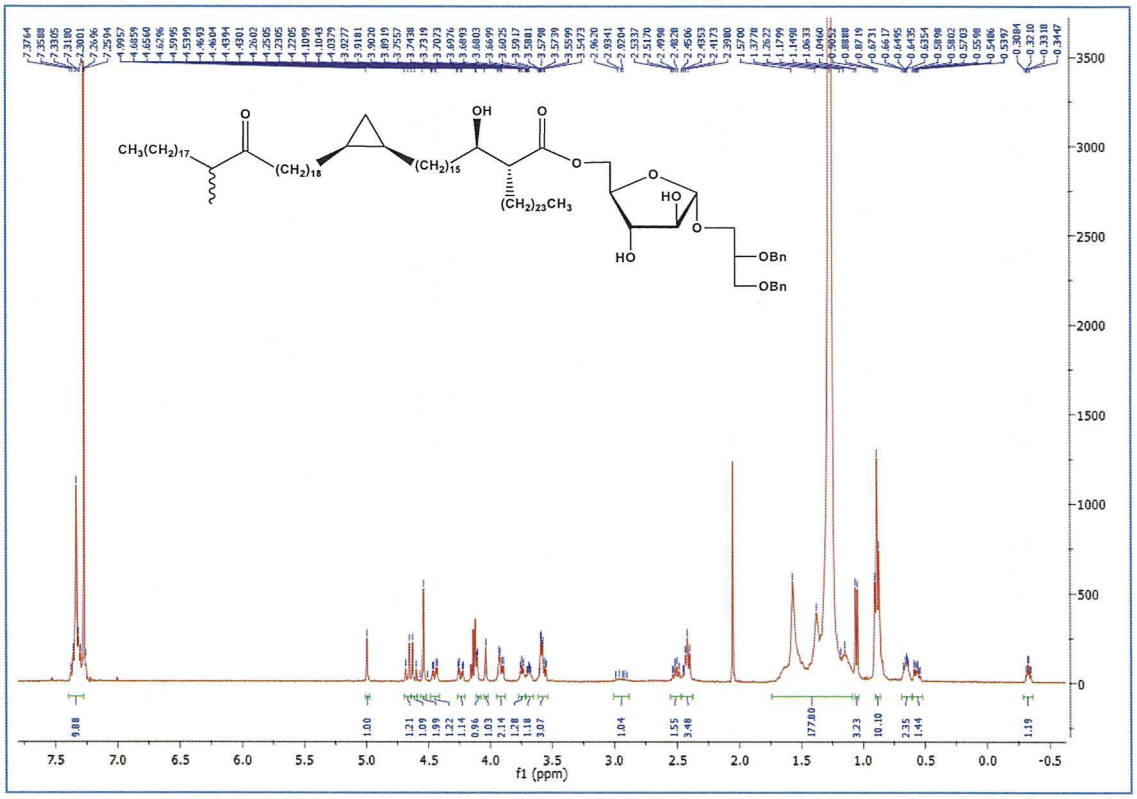


Figure 2.17: ^{1}H NMR of compound (130)

As mentioned before, Watanabe and co-workers[262] reported the NMR data (^{1}H and ^{13}C) for the glycerol-arabino-mycolate which was extracted as a penta-acetate from the cell wall of *M. avium*. In order to compare the NMR data of our synthetic compound with the literature data, compound **(126)** was reacted with acetic anhydride in dry pyridine at 0 °C to give the penta-acetate **(132)** in 82% yield **(Figure 2.18)**.

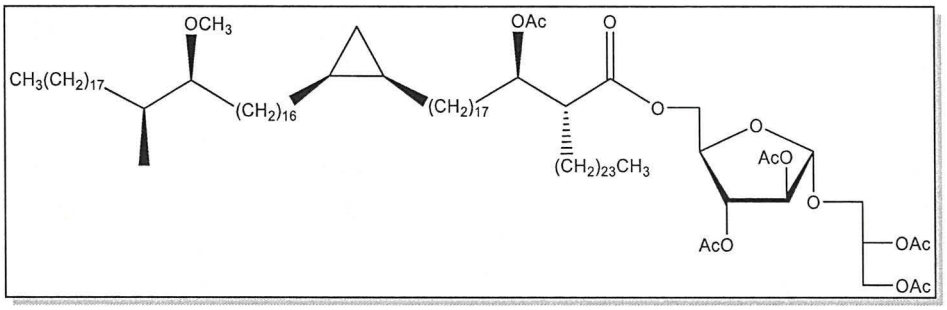


Figure 2.18: Structure of compound (132)

The NMR data of compound **(132)** were identical to the reported data, except for one signal at 5.08 ppm in the ^{1}H NMR spectrum, which corresponds to the proton at C2 on the arabinose ring. **Figure 2.19** shows a comparison between the ^{1}H NMR of the natural glycerol-arabino-mycolate (containing a mixture of mycolic acids) (upper spectrum) and

the synthetic single enantiomer D-glycerol-arabino-mycolate (lower spectrum). This result suggested that the natural material might actually contain the L-configuration of glycerol.

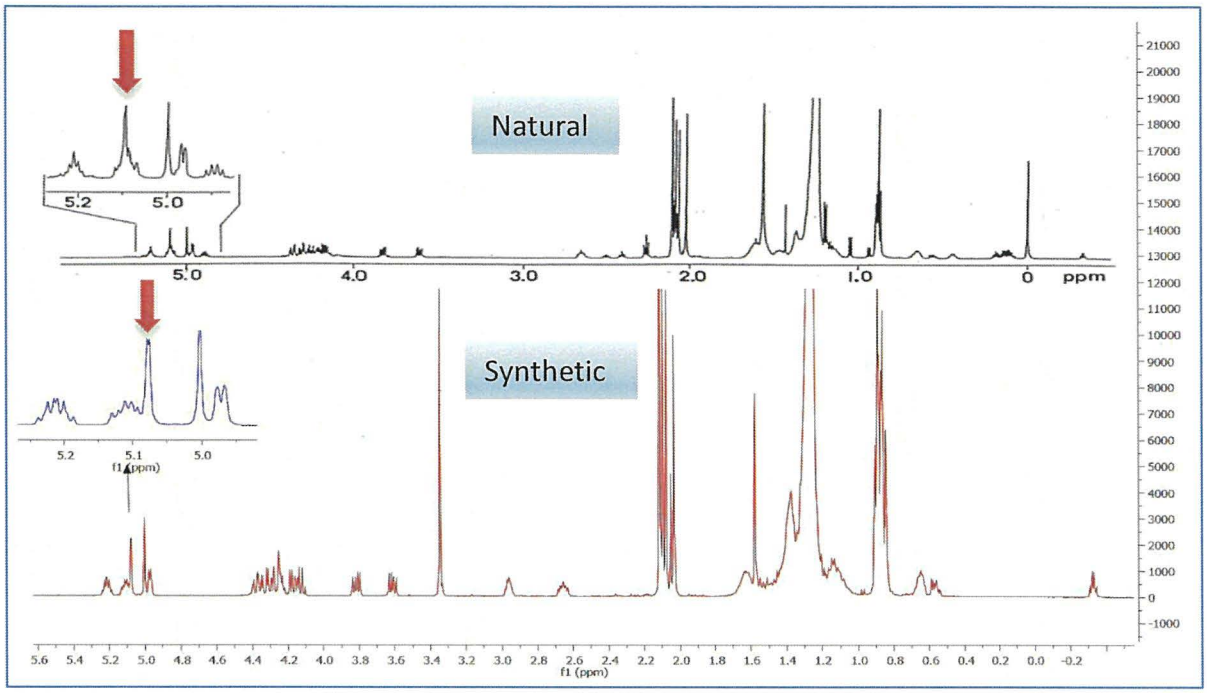
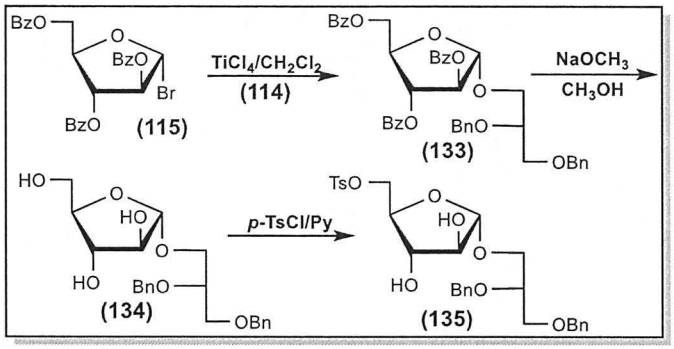


Figure 2.19: A comparison of ^{1}H NMR spectra of synthetic D-glycerol arabino mycolate (132) (lower) with that reported for the natural material (upper) [262]

2.2.6 Synthesis of L-glycerol-α-D-Ara*f*

L-Glycerol **(114)** was prepared according to the literature method, with the modification of some steps see **(Scheme 2.11**, p. 63).[277] The same method used to synthesise compound **(116)** (see **Scheme 2.12**, p. 63) was employed. Compound **(133)** was prepared by reacting the bromide **(115)** with L-glycerol **(114)** **(Scheme 2.18)**. The formation of **(133)** was confirmed by its ^{1}H NMR spectrum, which was similar to that of the compound **(116)**, except for the difference caused by the glycerol core, which is demonstrated in **Figure 2.20**. The superimposition of the ^{1}H NMR for both D- and L-glycerol arabinose shows that the signal corresponding to the first proton at C3` in the L-glycerol is shifted slightly up-field in comparison to its counterpart on the D-glycerol, while the second proton at C3` in the L-glycerol is shifted slightly downfield. Signals corresponding to the two protons at C1` in the L-glycerol are shifted slightly downfield relative to those in the D-glycerol. The final difference was the signal for the proton at C2` in the L-glycerol, which is shifted slightly downfield. The ^{13}C NMR spectrum of

compound **(133)** showed signals essentially identical to those for compound **(116)**. **Figure 2.21** shows the HSQC for compound **(133)** in which all the proton signals were correlated to their carbons. Hydrolysis of **(133)** was achieved by the method given previously, and afforded **(134)** **(Scheme 2.18)** in 84% yield.



Scheme 2.18: Synthesis of the L-glycerol-Araf

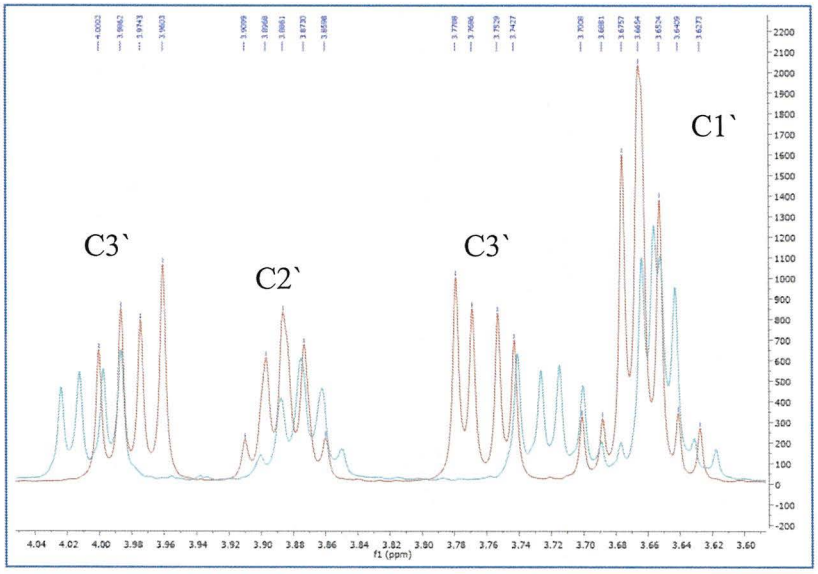


Figure 2.20: Superimposition of a part of the ^{1}H NMR spectra of D and L- glycerol-arabinose, red line (L); green line (D)

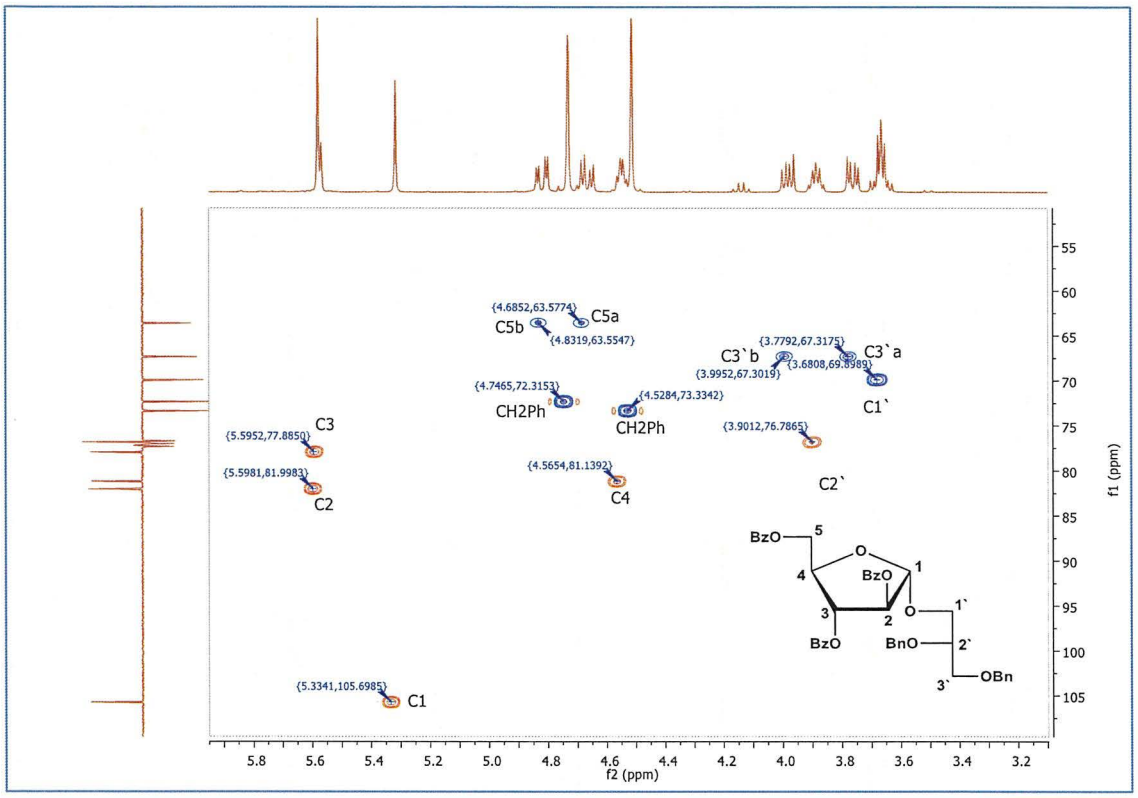


Figure 2.21: The HSQC spectrum of compound (133)

The synthesis of compound **(134)** was confirmed by NMR (^{1}H and ^{13}C). Tosylation of compound **(134)** was undertaken as before to afford **(135) (Scheme 2.18)** in 62% yield. All the ^{1}H NMR signals belonging to the glycerol core were slightly different between the two configurations of glycerol; in addition, the signal corresponding to the proton at C2 in the L-glycerol appeared as a broad doublet at δ 4.04 (*J* 0.6 Hz), which was shifted slightly downfield compared to the same proton in D-glycerol, which appeared as a broad doublet at δ 4.01 (*J* 5.6 Hz).

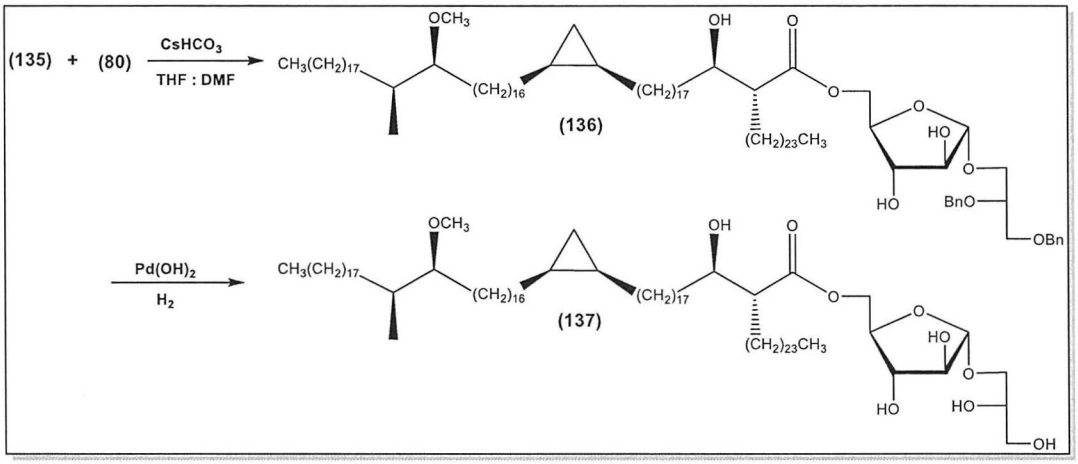
2.2.6.1 Esterification of (135) with the methoxy-MA (80)

As before, compound **(135)** was coupled with the methoxy-MA **(80)**[158] to prepare the glycolipid **(136) (Scheme 2.19)** in 74% yield, by using the same conditions as in the synthesis of compound **(125)**. This compound was then hydrolysed by the method given previously, which afforded **(137) (Scheme 2.19)** in 90% yield. Confirmation of the formation of **(137)** was achieved by ^{1}H NMR. A downfield signal at δ 4.88 corresponds to the proton attached to the C1 position of the sugar core. The two protons at C5 on the glycan ring appeared as two double doublets at δ 4.35 (*J* 11.8, 4.4 Hz) and δ 4.23 (*J* 11.5, 5.2 Hz). A signal belonging to the proton at C4 appeared as a multiplet at δ 4.10. The

remaining signals of the glycan and the glycerol appeared in the range from δ 4.01 – 3.48. The region of the ^{1}H NMR spectrum between δ 0.65 to - 0.33 showed a signal corresponding to the four protons of the *cis*-cyclopropane ring as discussed before. Compound **(137)** showed characteristic ^{13}C NMR signals, given in **Table 2.8** and **Figure 2.22**.

Table 2.8: The ^{13}C NMR data analysis of (137)

C_n	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}
δ	107.9	81.2	77.8	82.1	63.2	175	52.7	72.4	15.6	10.7	57.5
C_n	C_{12}	$C_{15,16}$	C_{14}	$C_{1`}$	$C_{2`}$	$C_{3`}$					
δ	85.5	13.9	14.6	69.0	70.4	63.2					



Scheme 2.19: Synthesis of L-glycerol arabino-methoxy-mycolates

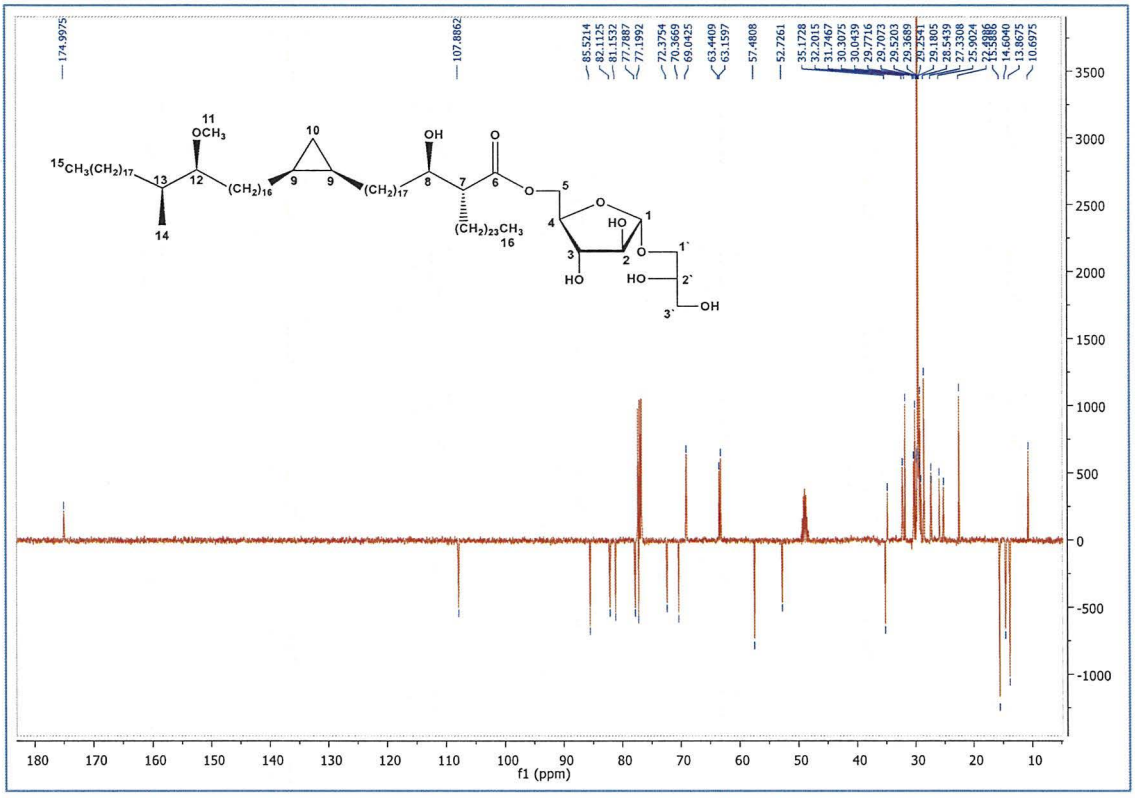


Figure 2.22: ^{13}C NMR spectrum of compound (137)

The five free hydroxyl groups in compound **(137)** were acetylated by reaction with acetic anhydride in anhydrous pyridine at 0 °C to give the penta-acetate **(137)** in 80% yield **(Figure 2.23)**. This gave the signals illustrated in **Table 2.9**, in comparison with the data for the natural material. The NMR data for **(137)** was found to be identical to the natural glycerol-arabino-mycolate (mixture of MAs) as shown in **Figure 2.24**.

This proved that the glycerol in the natural material is of the L configuration. **Figures 2.25 A** and **B** show the ^{1}H NMR spectrum for the compound **(73)**.

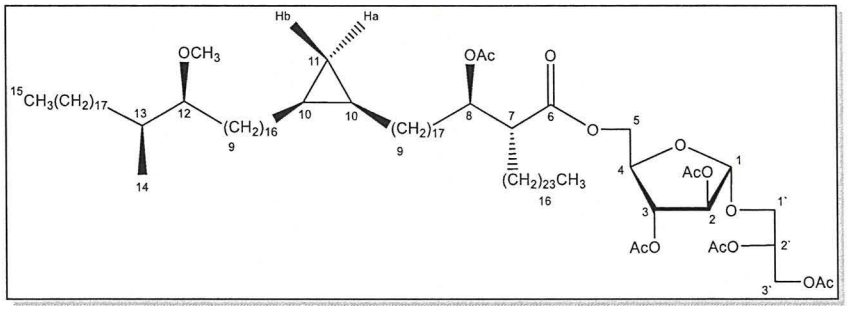


Figure 2.23: Structure of compound (138)

Table 2.9: The ^{1}H and ^{13}C NMR data analysis of (138) and the natural reported.[262]

	Synthetic		Natural[262]	
$H_{X(Araf)}$	^{1}H/Hz	^{13}C	^{1}H/Hz	^{13}C
H_1	5.0(s)	105.5	4.99 (s)	105.5
H_2	5.09 (d *J* 1.3)	81.1	5.09 (d *J* 1.3)	81.1
H_3	4.96 (dd *J* 1.0,4.8)	77.1	4.96 (dd *J* 1.3,4.0)	77.2
H_4	4.21 (ddd *J* 5.5,5.5,3.5)	80.7	4.21 (ddd *J* 4.0,5.7,3.5)	80.7
H_5	4.26 (dd *J* 5.7,11.8) 4.37 (dd *J* 3.5,11.8)	63.3	4.26 (dd *J* 5.7,11.7) 4.37 (dd *J* 3.5,11.7)	63.3
$H_{X(Glycerol)}$				
$H_{1'}$	3.61 (dd *J* 4.7,10.8) 3.83 (dd *J* 5.2,10.8)	65.2	3.60 (dd *J* 4.8,10.8) 3.83 (dd *J* 5.2,10.8)	65.2
$H_{2'}$	5.21(dddd *J* 4.8,6.5,5.0,4.5)	69.8	5.22 (dddd *J* 4.8,5.2,6.3,4.0)	69.8
$H_{3'}$	4.17 (dd *J* 6.3,11.9) 4.31 (dd *J* 4.0,11.9)	62.5	4.16 (dd *J* 6.3,11.8) 4.30 (dd *J* 4.0,11.8)	62.5
$H_{X(MA)}$				
C_6	-	172.8	-	172.8
H_7	2.65 (ddd *J* 10.4, 7.0, 4.4)	49.6	2.65 (ddd *J* 10.6,6.8,4.5)	49.6
H_8	5.10 (ddd *J* 11.0, 4.0, 3.8)	73.8	5.10 (ddd *J* 10.6,4.0,4.0)	73.8
H_9	1.10 (m), 1.37 (m)	29.0	1.15 (m), 1.38 (m)	29.0
H_a	- 0.34 (ddd *J* 4.0,5.5,5.5)	10.9	- 0.34 (ddd *J* 4.2,5.3,5.3)	10.9
H_b	0.56 (ddd *J* 8.5,8.5,4.0)	-	0.56 (ddd *J* 8.2,8.2,4.2)	-
H_{10}	0.65 (m)	15.7	0.65 (m)	15.8
CH_3 (Terminal)	0.88 (t *J* 7.0)	14.1	0.88 (t *J* 6.9)	14.1

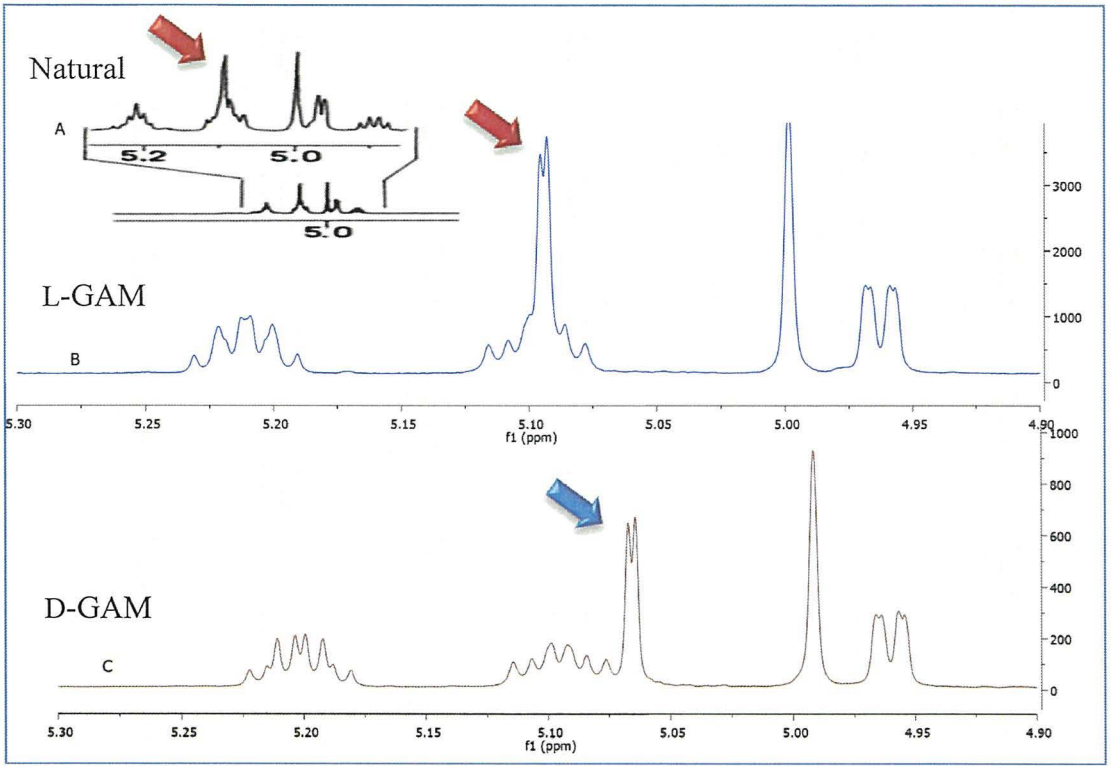


Figure 2.24: A comparison of the 1H NMR spectra of A: natural glycerol-arabino-mycolate[262] ; B: synthetic L-glycerol-arabino-mycolate **(138)**; C: synthetic D-glycerol-arabino-mycolate **(132)**

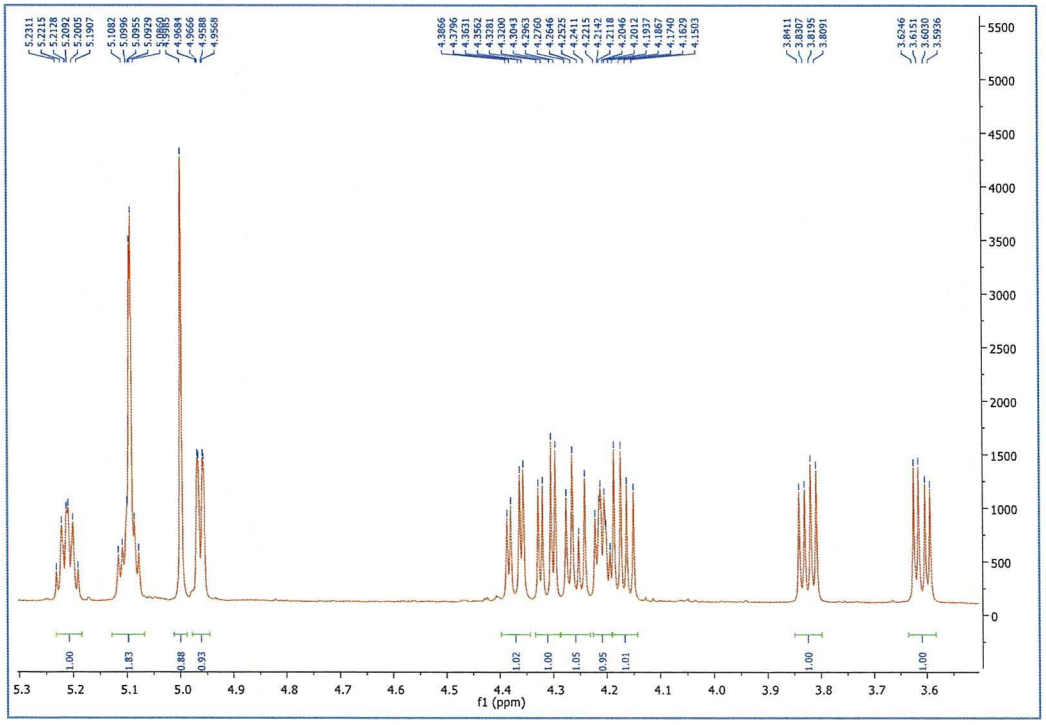


Figure 2.25: A- ^{1}H NMR spectrum of compound (138)

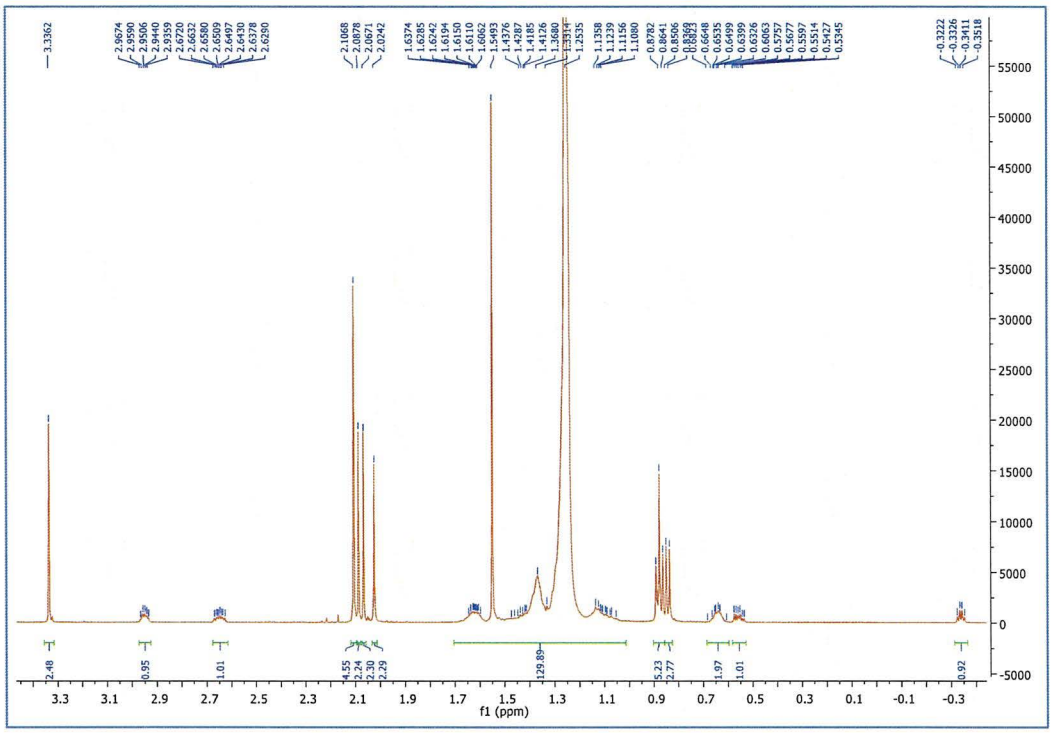
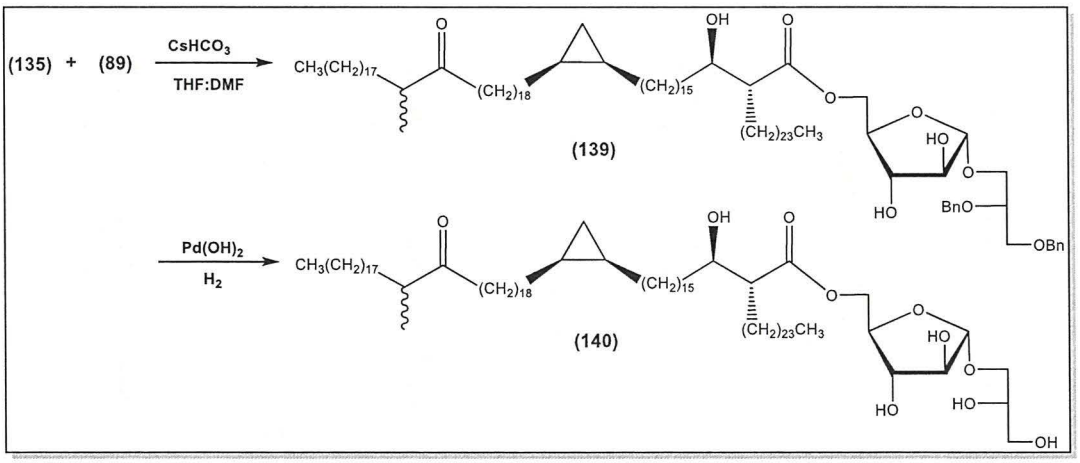


Figure 2.25: B- ^{1}H NMR spectrum of compound (138)

2.2.6.2 Esterification of (135) with the keto-MA (89)

Having proven the stereochemistry of the glycerol-arabino-mycolate, the tosylate **(135)** was esterified with the keto-MA **(89)**[159] by the same method as before, and afforded compound **(139)** in 71% yield **(Scheme 2.20)**. All the ^{1}H NMR signals belonging to the mycolic acid appeared in the same area as discussed above, as did the signals for the glycerol glycan. The ^{13}C NMR spectrum for the compound **(139)** is shown in **Figure 2.26**. Compound **(139)** was debenzylated by a similar method to that discussed before, to give **(140)** in 80% yield **(Scheme 2.20)**.



Scheme 2.20: Synthesis of L-glycerol arabino-keto-mycolates

The disappearance of the ^{1}H NMR signals corresponding to the benzylic protons and signals in the aromatic area confirmed the debenzylation of the compound. Compound **(140)** showed ^{1}H and ^{13}C NMR signals for the glycerol glycan and the keto-MA, similar to those obtained before.

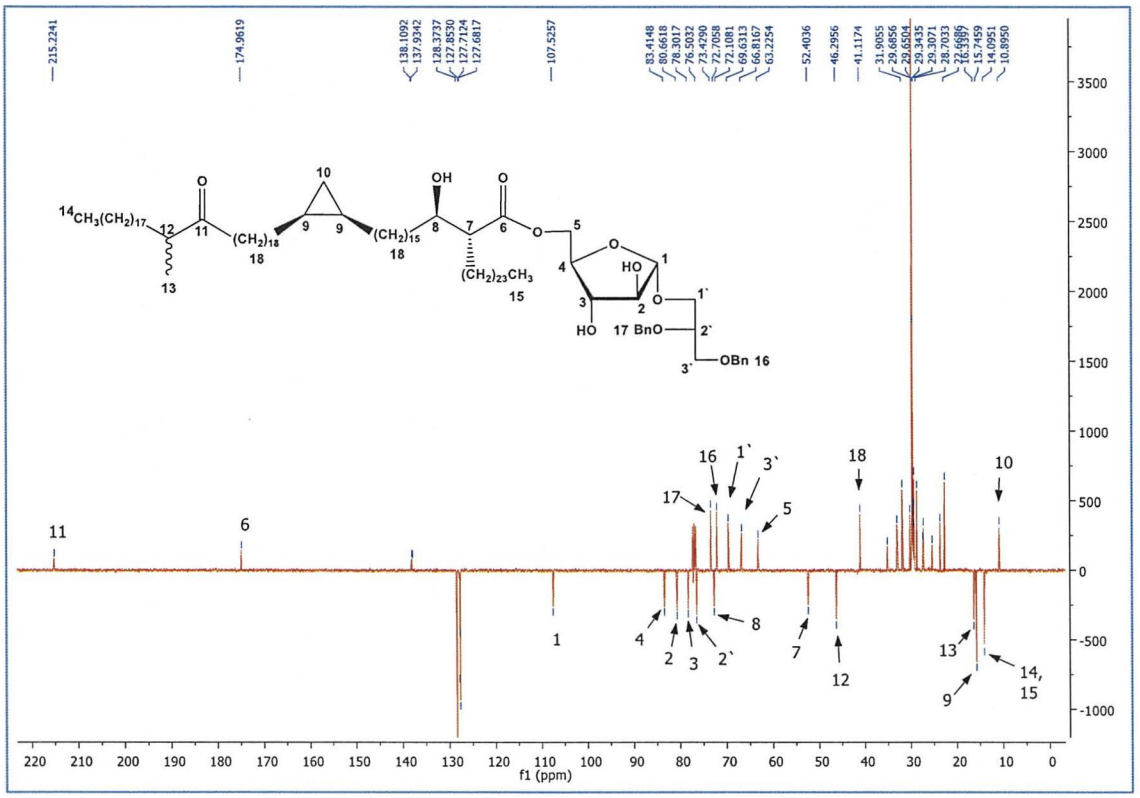
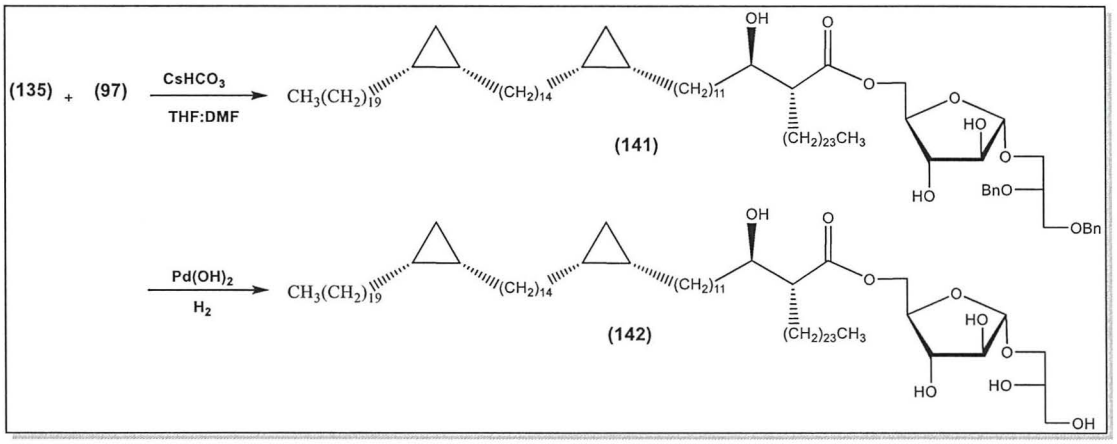


Figure 2.26: ^{13}C NMR spectrum for compound (139)

2.2.6.3 Esterification of the glycan (135) with α-MA (97)

In order to produce a series of L-glycerol-arabino-mycolates from the most common classes of mycolic acids, synthetic α-MA **(97)**,[157] was esterified with tosylate **(135)** by the same method described before to give **(141)** in 71% yield **(Scheme 2.21)**. The formation of **(141)** was established by the ^{1}H NMR spectrum, which gave signals for the α-MA and for the glycan similar to those described before. Hydrogenolysis of this compound was achieved by the method employed previously, and afforded **(142)** **(Scheme 2.21)** in 81% yield. Confirmation of the formation of **(142)** was achieved by NMR (^{1}H and ^{13}C).



Scheme 2.21: Synthesis of L-glycerol-arabino-α-mycolates

2.2.7 Summary

In this part of this thesis, both isomers L and D-GAM were prepared and each glycan was esterified with three different classes of MAs. Two models from D-GAM with linear alkyl acids were prepared.

Penta-acetate from the L and D-GAM of the methoxy-MA were prepared to prove the stereochemistry of this novel glycolipid, and this confirmed the stereochemistry to be L-GAM not D-GAM.

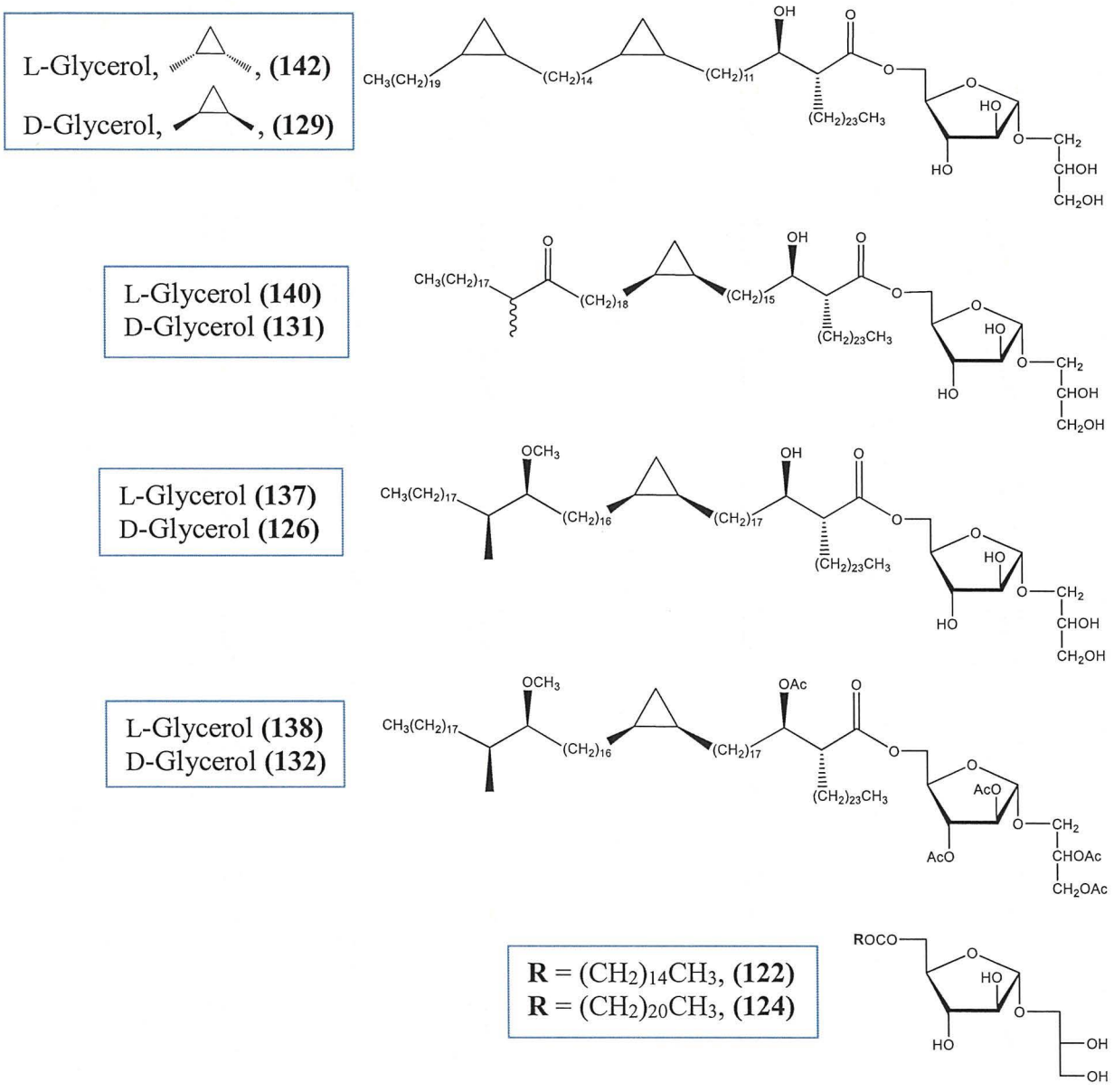


Figure 2.27: Structures of the target compounds.

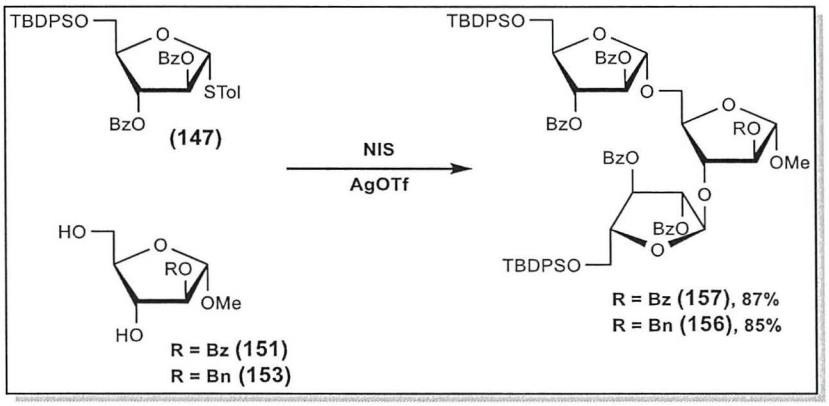
2.3 Synthesis of Methyl *Tri*-Ara*f*-*Di*-Mycolates (MTADM)

2.3.1 The aims of this part:

- To prepare the glycan methyl 2,3-di-*O*-benzyl-α-D-a Ara*f*-yl-(1→3)-[2,3-di-*O*-benzyl-α-Ara*f*-yl-(1→5)]-2-*O*-benzyl-α-Ara*f*.
- To prepare model glycolipids formed by the esterification of the *tri*-Ara*f* with normal fatty acids.
- To prepare a series of *tri*-Ara*f*-*di*-mycolates through esterification of the glycan with different synthetic MAs.
- To investigate the biological activity of the synthetic compounds, particularly their antigenicity.

2.3.2 Synthesis of *tri*-Ara*f*

Although the methyl *tri*-saccharide of Ara*f* had been prepared and esterified with different fatty acids, such as behenic, palmatic and butyric acids earlier,[288] there is no report of the synthesis of Ara*f* oligosaccharides esterified with synthetic structurally defined MAs. The target trisaccharide structure **(157)** has two α-glycosidic linkages, and can be assembled readily from known building blocks, the donor **(147)**[90] and the diol acceptor **(151)**[289] or **(153)** **(Scheme 2.22)**.

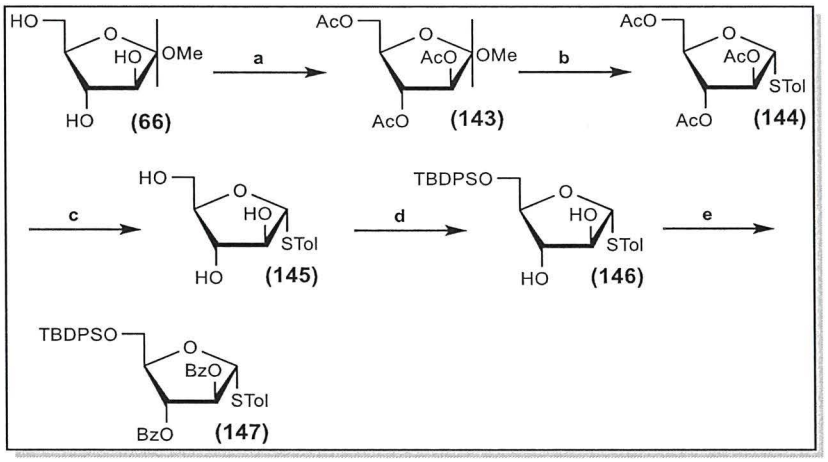


Scheme 2.22: Synthesis of tri-α-D-Araf

2.3.2.1 Synthesis of the donor (147)

The synthetic route to the 2,3-*O*-benzoyl protected thioglycosyl donor **(147)** was slightly modified compared to that reported in the literature **(Scheme 2.23)**.[90] Fischer glycosylation of commercially available D-arabinose in methanol under kinetic control, provided a mixture of methyl-α, β-Ara*f* **(66)** as described before on p. 44 **(Scheme 2.1)**.[263] The crude product was suspended in dry pyridine without further purification,

before acetic anhydride was added rather, than it being esterified as benzoate, to give triacetate **(143)** in 80% yield.[90] Methyl glycoside **(143)** was stereoselectively transformed to the α-thioglycoside **(144)** as the principal product upon reaction with *p*-thiotoluene in the presence of boron trifluoride diethetate in CH_2Cl_2 afforded **(144)** in 65% yield. The NMR data for this were identical to those reported **(Scheme 2.23)**.[290] The ^1H NMR spectrum for compound **(144)** gave a singlet at δ 5.47 corresponding to the proton at the C1 position, while the ^{13}C NMR spectrum showed a signal at δ 91.0, both indicating the presence of a glycosidic linkage in the Ara*f* as an α-anomer. Subsequent treatment of compound **(144)** with $NaOCH_3$ in methanol afforded thioglycoside triol **(145)** in 89% yield. The primary hydroxyl group was protected by treating **(145)** with *tert*-butylchlorodiphenylsilane in dry DMF in the presence of a catalytic amount of imidazole to afford **(146)** in 82% yield. The NMR data for this compound were identical to the literature data.[289] In order to protect the two secondary hydroxyl groups at the C2 and C3 positions, compound **(146)** was suspended in pyridine before benzoyl chloride was added, to give **(147)** in 83% yield. This compound again showed spectra identical with the literature **(Scheme 2.23)**.[289]



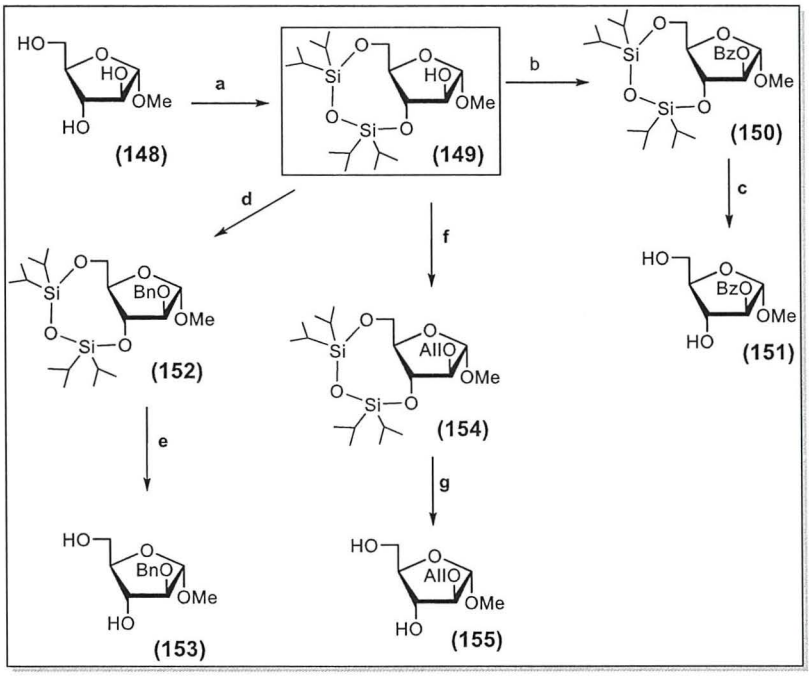
Scheme 2.23: Reagents and conditions: (a) Ac_2O, pyridine, 0 °C, 80%; (b) *p*-$CH_3C_6H_4SH$, $BF_3.Et_2O$ (cat.), CH_2Cl_2, 0 °C/R.T., 8 h, 65%, (c) $NaOCH_3$ (cat.), MeOH : CH_2Cl_2 (1:1), R.T., 3 h, 89%; (d) *t*- $BuPh_2SiCl$, imidazole, DMF, 0 °C/R.T., 16 h, 82%; (e) BzCl, pyridine, 0 °C/R.T., 2 h, 83%.

2.4.2.2 Synthesis of the acceptors

Having the donor target in hand, the exploration of the synthesis of the acceptors was carried out. The initial step in the synthetic route to prepare the acceptors **(153)**,[292] **(151)**[289] and **(155)** was the separation of the two anomers of compound **(66)**; this was achieved by using two methods. The first was tritylation of **(66)** followed by column

chromatography to give compound **(68)** as discussed before in **(Scheme 2.1)**. An efficient protocol for the deprotection of trityl ethers was used to deprotect **(68)**; $NaHSO_4.SiO_2$ was prepared according to the literature and added to a stirred solution of **(68)** in CH_2Cl_2 : MeOH at room temperature to afford triol **(148)** in 95% yield.[291]

The second method was esterification of **(66)** under standard conditions to give the α-anomer of the *tri*-benzoate-Ara*f* **(101)**[271] as in **Scheme 2.10**, p. 62. Hydrolysis of **(101)** by dissolving it in a mixture of CH_2Cl_2 : MeOH at room temperature followed by addition of sodium methoxide, afforded the triol **(148)** in 83% yield.



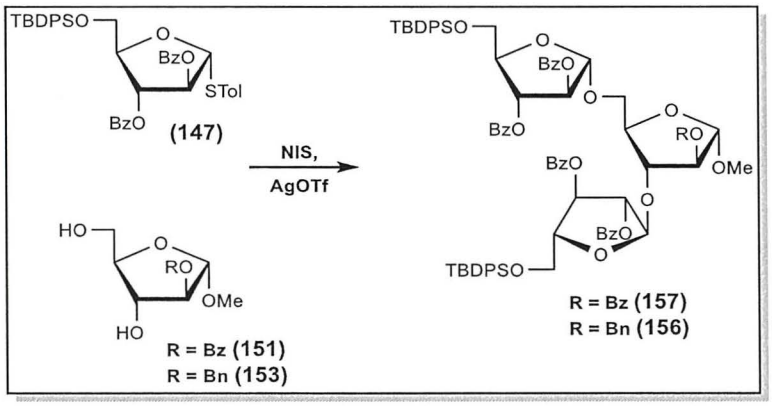
***Scheme 2.24: Reagents and conditions*:** (a) $TIPDSCl_2$, pyridine, 6 h, 0 °C/R.T., 66%; (b) BzCl, pyridine, 0 °C/R.T., 3 h, 72%; (C) TBAF, THF, 0 °C/R.T., 16 h, 74%; (d) NaH, BnBr, DMF, 0 °C/R.T. 2 h, 76%; (e) TBAF, THF, 0 °C/R.T., 16 h, 79%.(f) NaH, AllBr, DMF, 0 °C/R.T., 2 h, 90%; (g) TBAF, THF, 0 °C/R.T., 16 h, 57%.

Reaction of **(148)** with 1,3-dichloro-1,1,3,3-*tetra*-isopropyldisiloxane in dry pyridine at 0 °C afforded the siloxane **(149)** in 66% yield **(Scheme 2.24)**.[289] Intermediates **(149)**, **(150)**[289] and **(152)**[292] were prepared as described in the literature; the hydroxyl group of **(148)** was protected as the benzoyl ester under standard conditions to give **(150)** in 72% yield followed by desilyation mediated by fluoride ion to give the target acceptor **(151)** in 74% yield **(Scheme 2.24)**. To synthesize another acceptor, **(149)** was treated with sodium hydride and benzyl bromide (BnBr) in dry DMF to afford compound **(152)** in 76% yield, followed by desilylation to give **(153)** in 79% yield **(Scheme 2.24)**. The final

acceptor **(155)**, was synthesised by treating **(149)** with sodium hydride and allyl bromide (AllBr) in dry DMF to afford compound **(154)** in 90% yield, followed by desilylation to give **(155)** in 57% yield **(Scheme 2.24)**.

2.4.2.3 Coupling the donor and the acceptors

Lowary *et al.*[288] used the 2-*O*-benzylated diol acceptor **(153)** with the thioglycoside donor **(147)** using NIS/AgOTf in dry CH_2Cl_2 **(Scheme 2.25)**. In order to probe the effect of the protecting group of the C2 position, the 2-*O*-benzoylated acceptor **(151)** and 5-sillylated thioglycoside **(147)** were reacted under the reported conditions to give the glycan **(157)** **(Scheme 2.25)**. Trisaccharide **(157)** was obtained in 87% yield which is similar to that reported when Lowry was using the 2-*O*-benzylated acceptor.[288] The ^1H NMR spectrum of compound **(157)** showed three signals downfield corresponding to the three protons at the anomeric centre of each ring, as a doublet at δ 5.57 (*J* 2.6 Hz), a broad singlet at δ 5.32 and a broad singlet at δ 5.10. The ^{13}C NMR spectrum **(Figure 2.28)** established the presence of glycosidic linkages in the Ara*f*, signals at 107.0, 106.0 and 105.3 ppm belonging to the three carbons at the anomeric centres. Those signals together confirmed the three α-glycosidic linkages in the compound.



Scheme 2.25: Synthesis of tri-saccharides

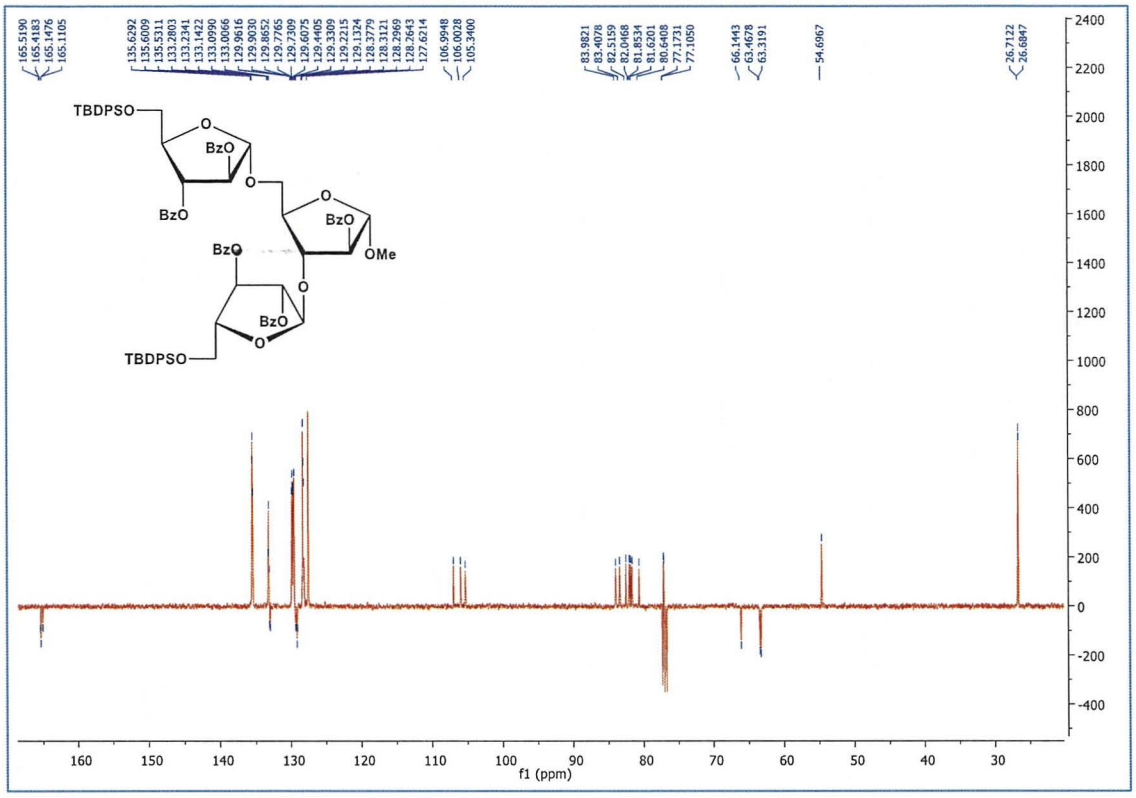
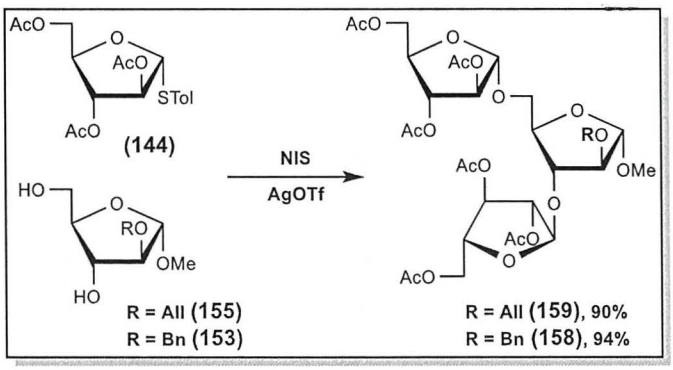


Figure 2.28: ^{13}C NMR spectrum for compound (157)

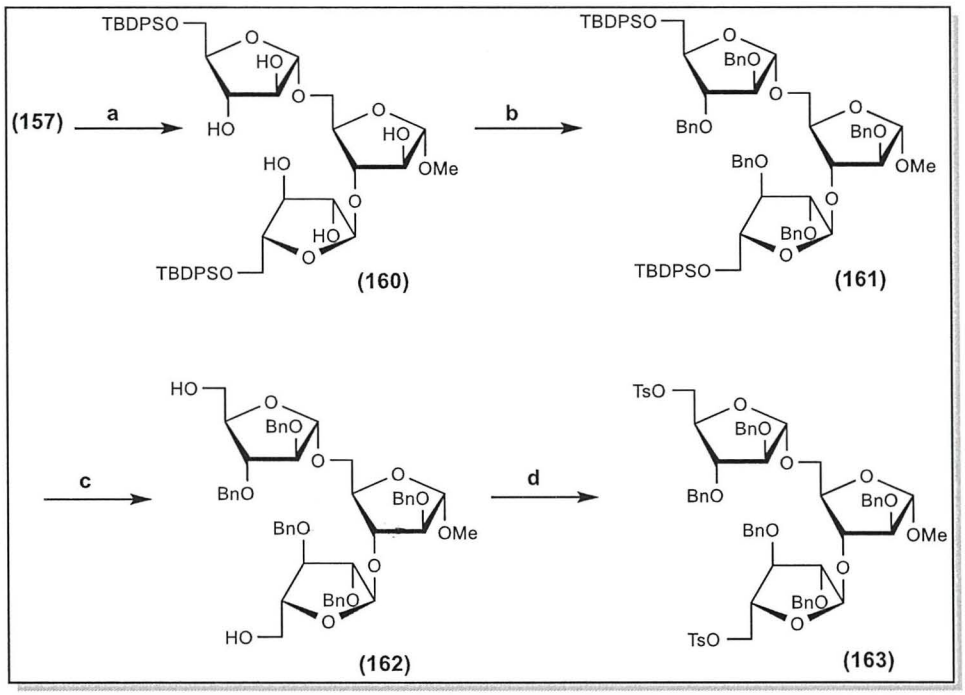
The glycosyl acceptors **(153)** and **(155)** were reacted under the same conditions with the thioglycoside **(144)**, to afford the trisaccharide **(158)** and **(159)** in 94 and 90% respectively **Scheme 2.26**.



Scheme 2.26: Synthesis of tri-Araf

Compound **(157)** was deprotected by sodium methoxide to give **(160)** as a thick oil in 81% yield. Formation of the pentahydroxy saccharide **(160)** 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 for **(160)** showed disappearance of carbonyl signals which indicated the success of the hydrolysis. Compound **(160)** was

benzylated to protect the five secondary hydroxyl groups using benzyl bromide and sodium hydride in dry DMF to give **(161)** in 77% yield, followed by desilylation of the two primary hydroxyl groups to afford **(162)** in 77% yield. These compounds gave data identical with the literature **(Scheme 2.27)**.[288] The two hydroxyl groups in compound **(162)** were activated by treating with TsCl in dry pyridine in the presence of catalytic DMAP in dry CH_2Cl_2 at 0 °C to afford the *di*-tosylate-*tri*-Ara*f* **(163)** in 74% yield **(Scheme 2.27)**. The structure of **(163)** was confirmed by mass spectrometry and NMR. **Figure 2.29** shows the ^{13}C NMR spectrum for compound **(163)**.



Scheme 2.27: Reagents and conditions: (a) $NaOCH_3$ (cat.), CH_3OH, R.T., 2 h, 86% (b) NaH, BnBr, DMF, 0 °C/R.T. 16 h, 77%; (C) TBAF, THF, 0 °C/R.T., 16 h, 77% (d) TsCl, pyridine, DMAP, 74%.

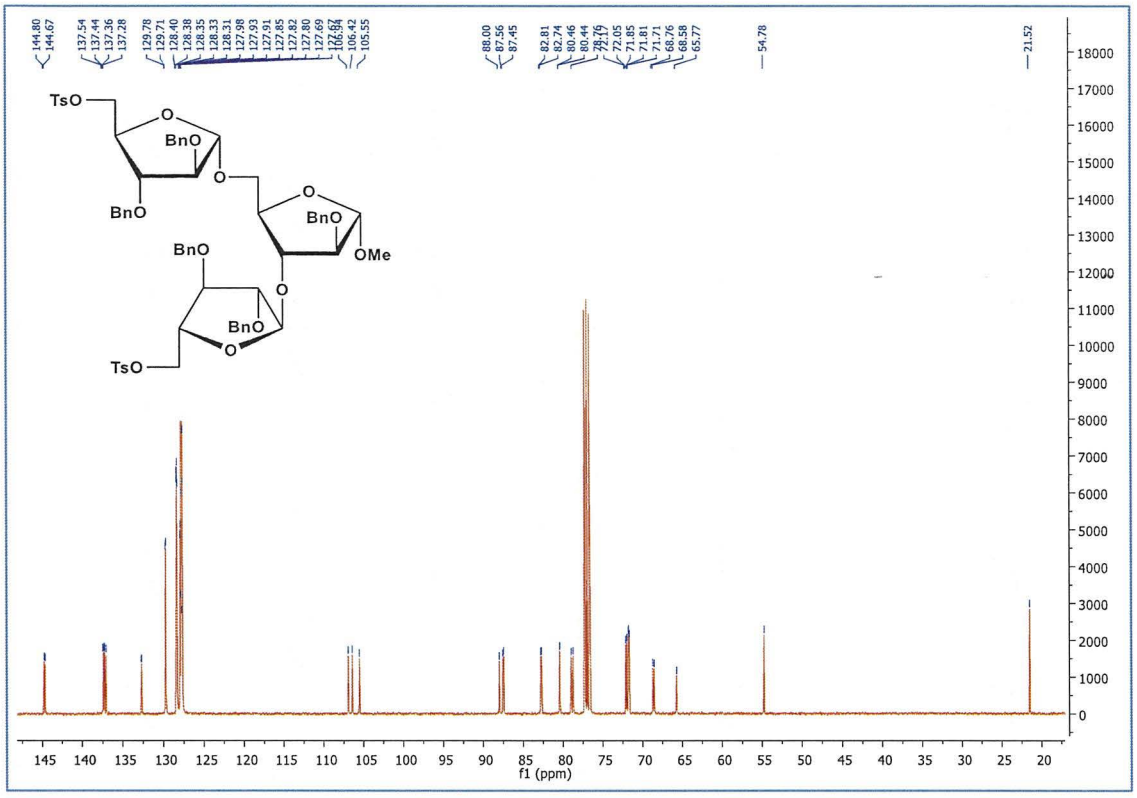
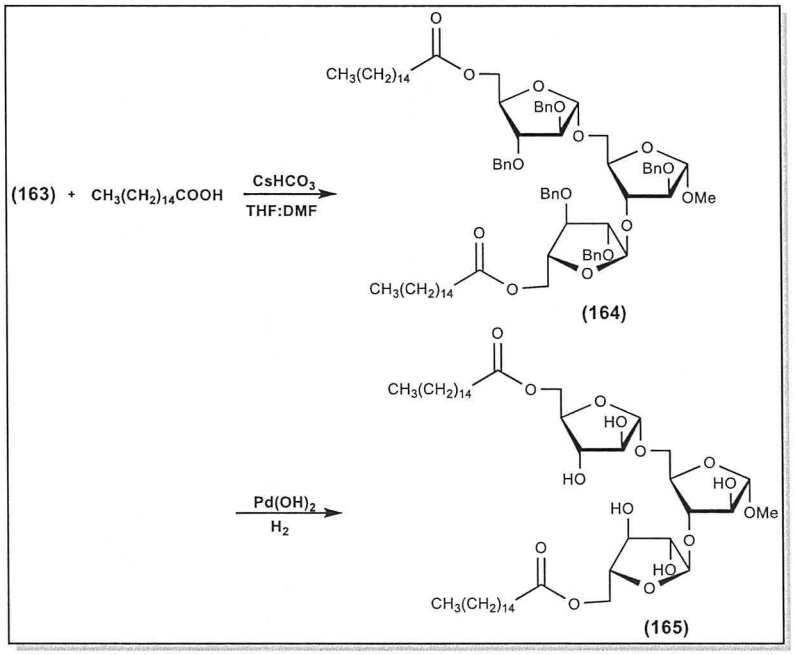


Figure 2.29: ^{13}C NMR spectrum for compound (163)

2.3.3 Esterification of lipids with the *tri*-Araf (163).

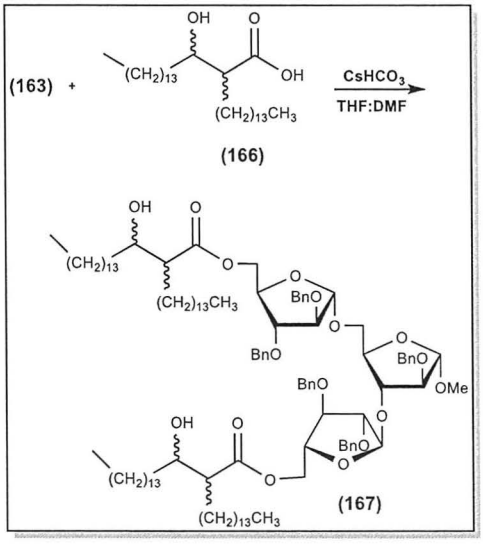
The exploration of coupling of *tri*-saccharide **(163)** with the structurally defined synthetic mycolic acids was now undertaken **(Scheme 2.28)**.



Scheme 2.28: Synthesis of methyl tri-arabino-di-lipids

Firstly, a model *tri*-arabino-*di* mycolate was prepared by coupling the tosylate **(163)** with palmatic acid through the alkylative esterification strategy using cesium hydrogen carbonate in dry DMF : THF at 70 °C for 4 days. This gave compound **(164)**. The synthesis of this glycolipid has been reported[288] but, by using a different method; data obtained for **(164)** were identical to those reported.[288]

Having prepared a model of a simple fatty acid with the glycan **(163)**, compound **(163)** was esterified with the acid **(166)** using the same method, and afforded **(167)** in 38% yield **(Scheme 2.29)**. However, as compound **(167)** is a mixture of four disteroisomers, the product appeared as a two sets of peaks, with one being predominant. The formation of this compound was confirmed by NMR (^{1}H and ^{13}C). The region of the ^{1}H NMR spectrum of **(167)** which was of most interest was between δ 5.2 – 4.8, which corresponds to the three protons at the anomeric centres on the glycan rings. Signals at δ 5.18, 5.13 and 4.92, integrating for one proton each, occurred as broad singlets. The methoxy group in the glycan moiety gave a singlet at δ 3.37 integrating for three protons. The α-proton in the acid part showed a multiplet at δ 2.47 – 2.38. The β-proton in the acid showed a signal as a multiplet at δ 3.8 – 3.7. The terminal methyl group showed a signal as a triplet at δ 0.89 (*J* 6.8 Hz) integrating for 12 protons. The ^{13}C NMR spectrum showed two signals at δ 174.9 and 174.8 for the carbonyl groups. Signals corresponding to the carbon at the anomeric centre for the three rings appeared at δ 107.0, 106.4 and 105.5. The β-hydroxy carbon appeared at δ 72.0. The CH_2 chain ranged from δ 31 - 22 and the CH_3 came up-field, around δ 14.0. The α-carbon in the acid resonated at δ 52.8.

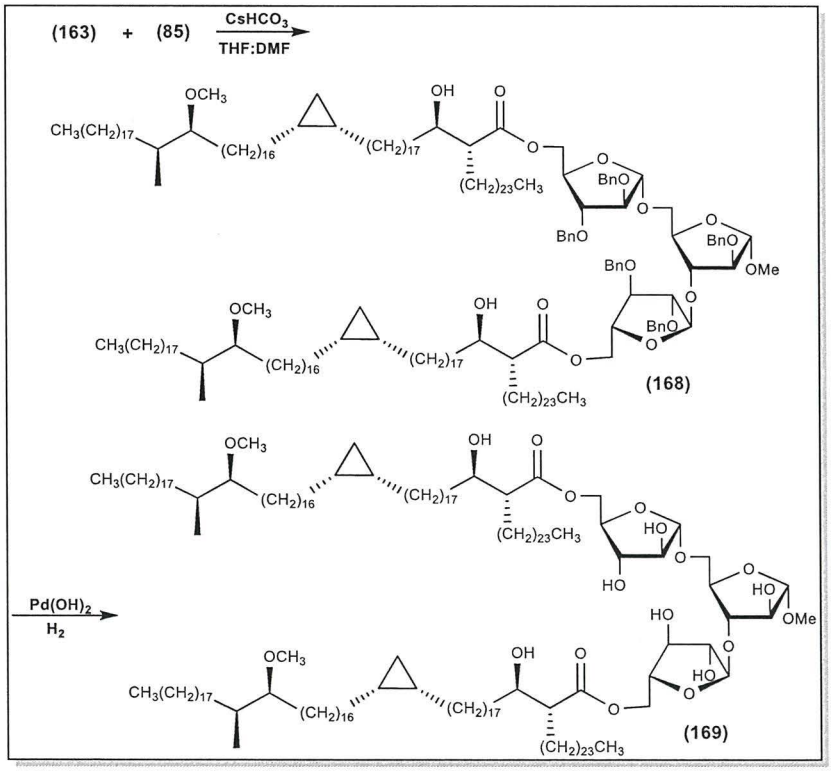


Scheme 2.29: Synthesis of methyl-tri-arabino-lipid model

2.3.4 Esterification of the glycan (163) with MAs

2.3.4.1 Esterification of the glycan (163) with methoxy-MA (85)

Having secured a successful method for preparing models of the *tri*-Ara*f*-*di*-lipid, the coupling of the glycan **(96)** with different synthetic MAs to obtain the target glycolipids, was now undertaken. Methoxy-*cis*-cyclopropane with a 24 α-alkyl chain **(85)**,[158] was reacted with compound **(96)** using cesium hydrogen carbonate (10 eq.) as before, and afforded compound **(101)**, in 45% yield **(Scheme 2.30, Figure 2.30)**, and which gave characteristic NMR signals for the methoxy-MA, similar to those discussed before; the signals for the glycan are given in **Table 2.10**. Hydrogenolysis of **(101)**, by stirring it in dry CH_2Cl_2 : MeOH (1:1) in the presence of $Pd(OH)_2$ and under a hydrogen atmosphere gave compound **(102) (Scheme 2.30, Figure 2.31)**, in 82% yield. Compound **(102)** gave NMR the signal expected for the MA; signals of the glycan are illustrated in **Table 2.11**. The specific rotation of **(101)** was $[\alpha]_D^{23}$ + 35 (*c* 0.1, $CHCl_3$), changing for the deprotected compound **(102)** to $[\alpha]_D^{20}$ + 21 (*c* 0.1, $CHCl_3$).



Scheme 2.30: Synthesis of methyl-tri-arabino-di-methoxy-mycolates

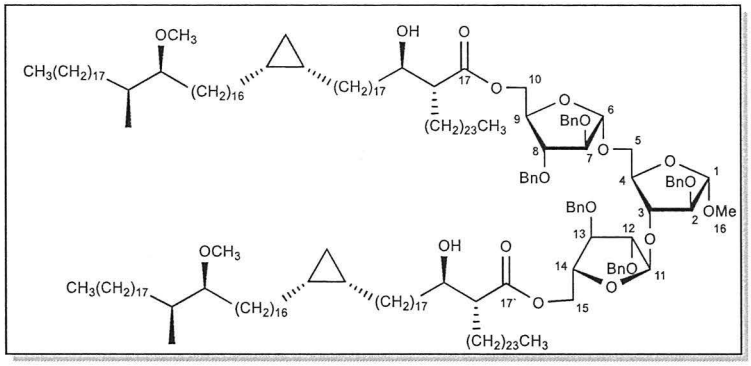


Figure 2.30: Structure of compound (168)

Table 2.10: The ^{1}H and ^{13}C NMR data analysis of the glycan part of compound (168)

H_X	Shift	H's	Class	*J*/Hz	Cn	δ/ppm
H_1	4.91	1	br. s	-	C_1	107.0
H_6	5.18	1	br. s	-	C_6	106.5
H_{11}	5.13	1	br. s	-	C_{11}	105.5
$H_{3,\ 10,\ 10`,\ 15,\ 15`,\ 9}$	4.27	6	m	-	C_2	88.2
H_{14}	4.18	1	ddd	3.0, 6.3, 9.7	C_7	87.9
H_4	4.11	1	dd	2.7, 4.4	C_{12}	87.8
H_7	4.08	1	br. d	2.9	$C_{8,\ 13}$	83.7
H_{12}	4.00	1	dd	0.6, 2.9	C_4	80.6
H_2	3.96	1	br. d	2.3	C_3	80.4
$H_{5`}$	3.93	1	dd	4.8, 12.0	C_{14}	79.3
$H_{13,\ 8}$	3.88	2	m	-	C_9	79.1
H_5	3.76	1	dd	1.8, 11.4	$C_{22} \times 2$	85.4
$(OCH_3)_{16}$	3.37	3	s	-	$PhCH_2$	72.3
$PhCH_2O$	4.49	9	m	-	$PhCH_2 \times 2$	71.98
$PhCH_2O$	4.34	1	d	11.7	$PhCH_2$	72.2
Ph × 5	7.30	25	m	-	$PhCH_2$	71.8
					C_5	65.6
					$C_{15,\ 10}$	63.0
					$(OCH_3)_{16}$	54.8
					C_{17}	175.1
					$C_{17`}$	175.0

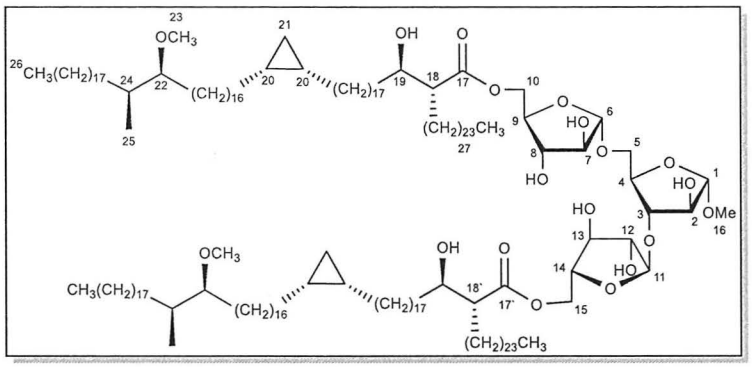


Figure 2.31: Structure of compound (169)

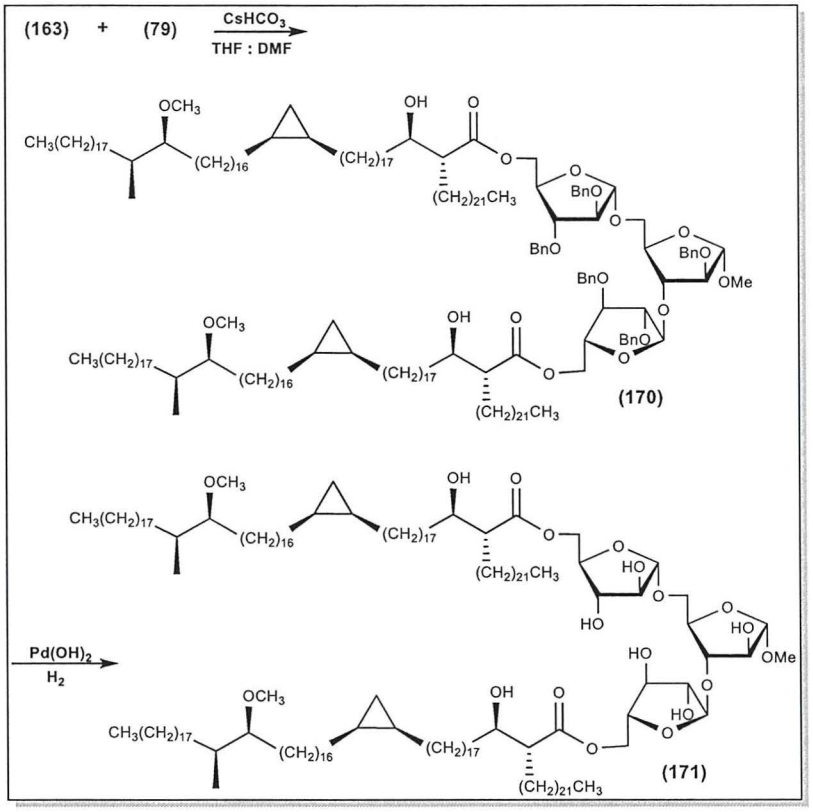
Table 2.11: The ^{1}H and ^{13}C NMR data analysis of the glycan part of compound (169)

Hx	Shift	H's	Class	J/Hz	Cn	δ/ppm
H_1	4.77	1	br. s	-	C_1	109.1
H_6	4.95	1	br. s	-	C_6	107.9
H_{11}	4.98	1	br. s	-	C_{11}	107.2
H_{10}	4.40	1	dd	4.3, 11.8	$C_{2,7}$	80.1
H_{15}	4.33	1	dd	4.5, 11.6	C_{13}	79.7
$H_{15`}$	4.24	1	dd	4.7, 11.6	C_{14}	82.95
$H_{10`}$	4.15	1	dd	4.3, 11.6	C_9	82.4
$H_{4,9}$	4.09	2	m	-	$C_{4,12}$	81.8
$H_{2,3,7,12,14}$	4.02	5	m	-	$C_{22,22`,3}$	85.5
$H_{5`,8,13}$	3.88	3	m	-	C_8	77.9
$H_{5,19,19`}$	3.61	3	m	-	C_5	65.9
$(OCH_3)_{16}$	3.37	3	s	-	C_{10}	63.4
					C_{15}	63.1
					C_{18}	52.8
					$C_{18`}$	49.95
					$(OCH_3)_{16}$	54.8
					C_{19}	72.5
					$C_{19`}$	72.45
					C_{17}	175.1
					$C_{17`}$	175.0

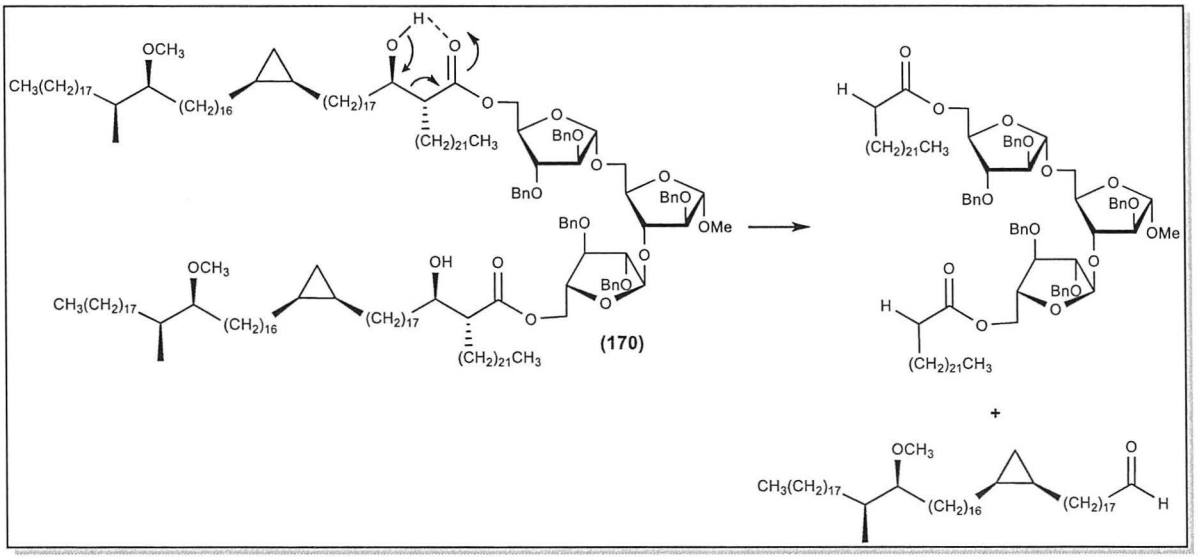
2.3.4.2 Esterification of the glycan (163) with methoxy-MA (79)

Methoxy-*cis*-cyclopropane MA **(79)**,[158] with a 22 carbons α-alkyl chain, was reacted with compound **(163)** as above, which gave compound **(170) (Scheme 2.31)** in 36% yield. In this reaction, after 4 days, TLC showed a small quantity of the starting material; further cesium hydrogen carbonate was added and the reaction left for another day. TLC now showed the appearance of another two spots, and after working up and column chromatography, some cleavage products were found in addition to the required product. The structures of the fragments **(Scheme 2.32)** were studied by NMR and mass

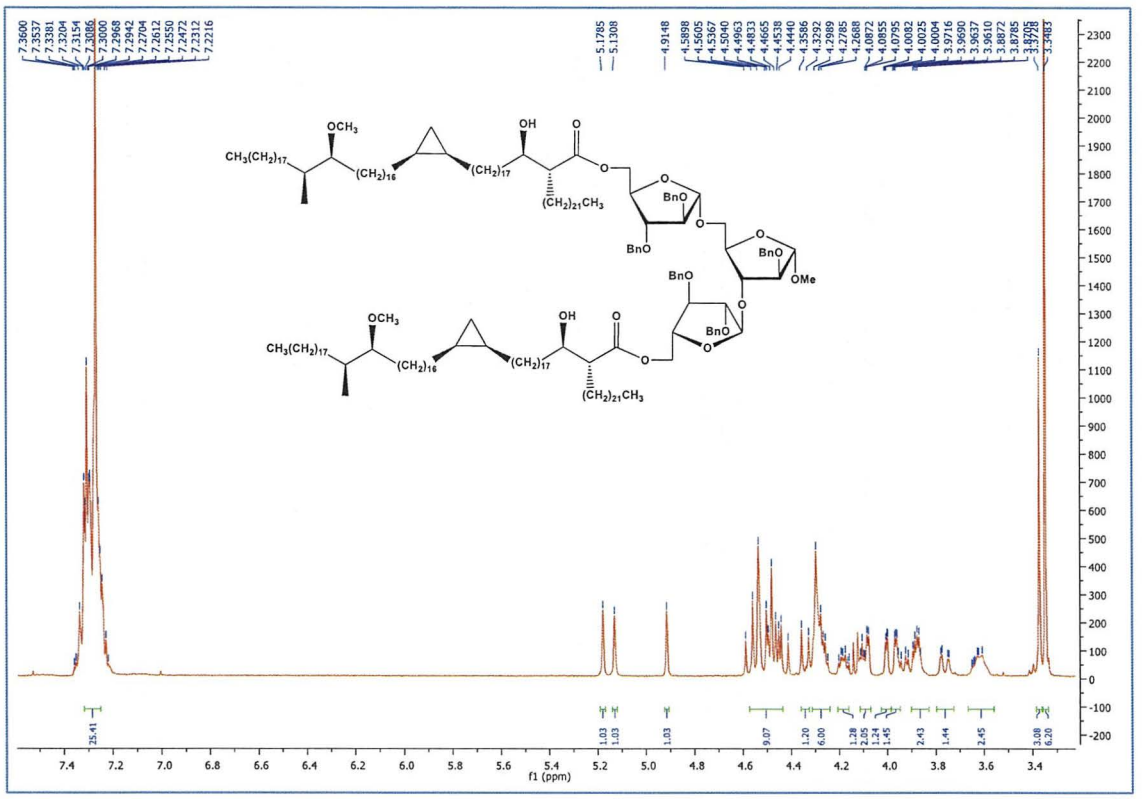
spectrometry. The ^{1}H NMR spectrum for the compound **(170)** **(Figure 2.32)** was identical to that of compound **(168)** **(Figure 2.30)**. The ^{13}C NMR also gave data similar to those for compound **(168).** Compound **(170)** was hydrolysed by the method given previously, and afforded **(171)** **(Scheme 2.31)**. Once again, compound **(171)** showed NMR signals similar to those for compound **(169)**.



Scheme 2.31: Synthesis of methyl-tri-arabino-di-methoxy-mycolates



Scheme 2.32: Fragmentation of compound (170)



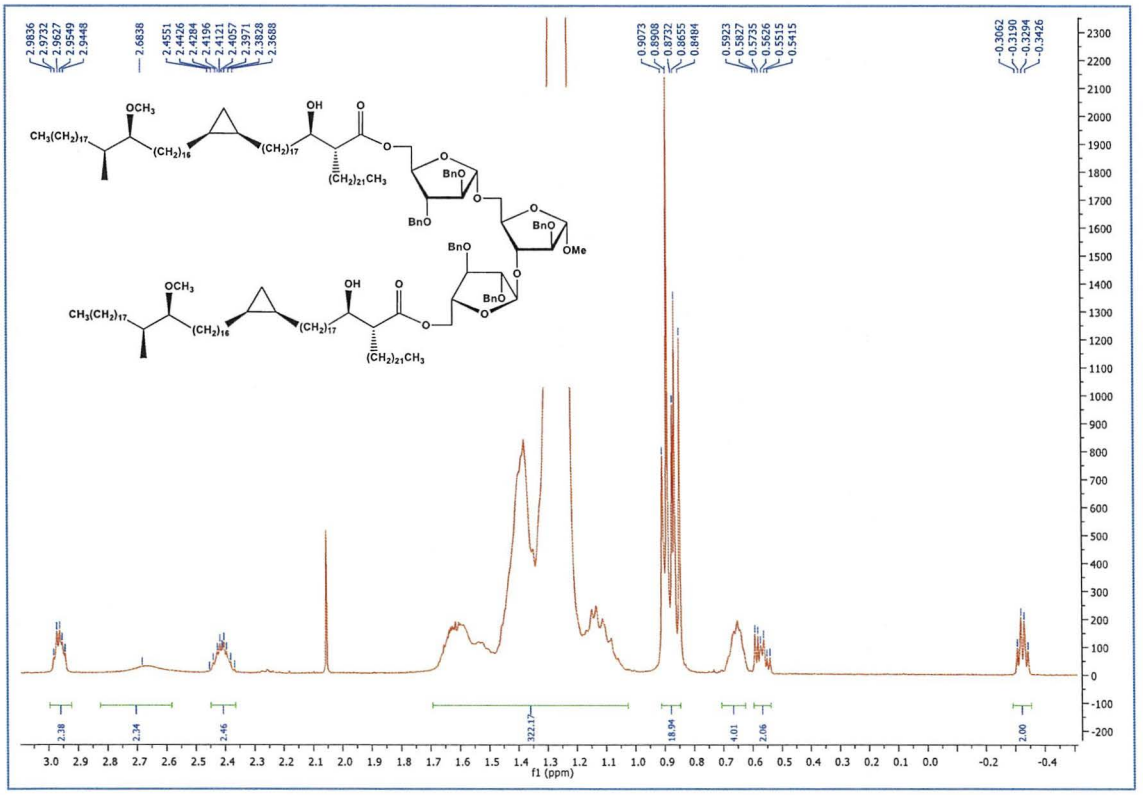
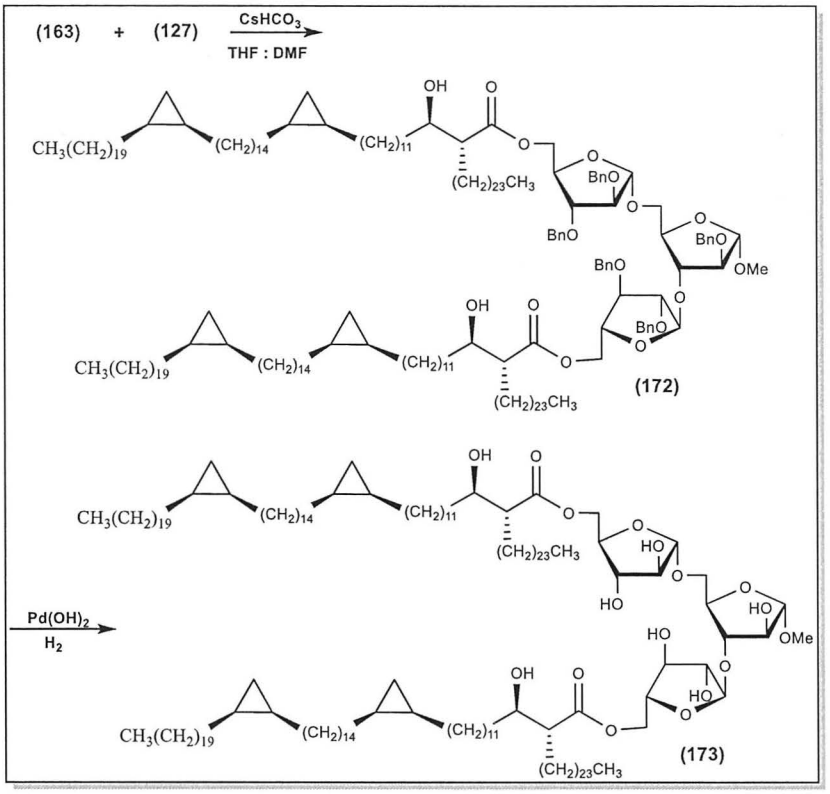


Figure 2.32: ^{1}H NMR spectrum for compound (170)

2.3.4.3 Esterification of the glycan (163) with α-MA (127)

Synthetic α-MA **(127)**[157] was esterified with glycan **(163)** by the same method given previously to give **(172)** in 18% yield **(Scheme 2.33)**.



Scheme 2.33: Synthesis of methyl-tri-arabino-di-α-mycolates

The resulting glycolipid **(172)** gave ^{1}H and ^{13}C NMR signals for the two α-MA similar to those discussed before; the signals for the tri-Ara*f* were also similar to those discussed before. Hydrogenolysis of compound **(172)** was achieved by the method given previously, and afforded **(173)** **(Scheme 2.33, Figure 2.33)** in 64% yield. Confirmation of the formation of **(173)** was achieved by ^{1}H NMR, which gave signals for the two α-MA and the glycan similar to those discussed before. Compound **(173)** showed characteristic ^{13}C NMR signals, which are illustrated in **Table 2.12**.

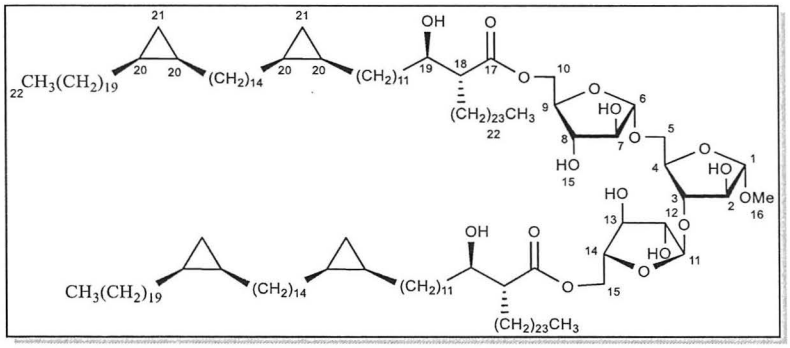


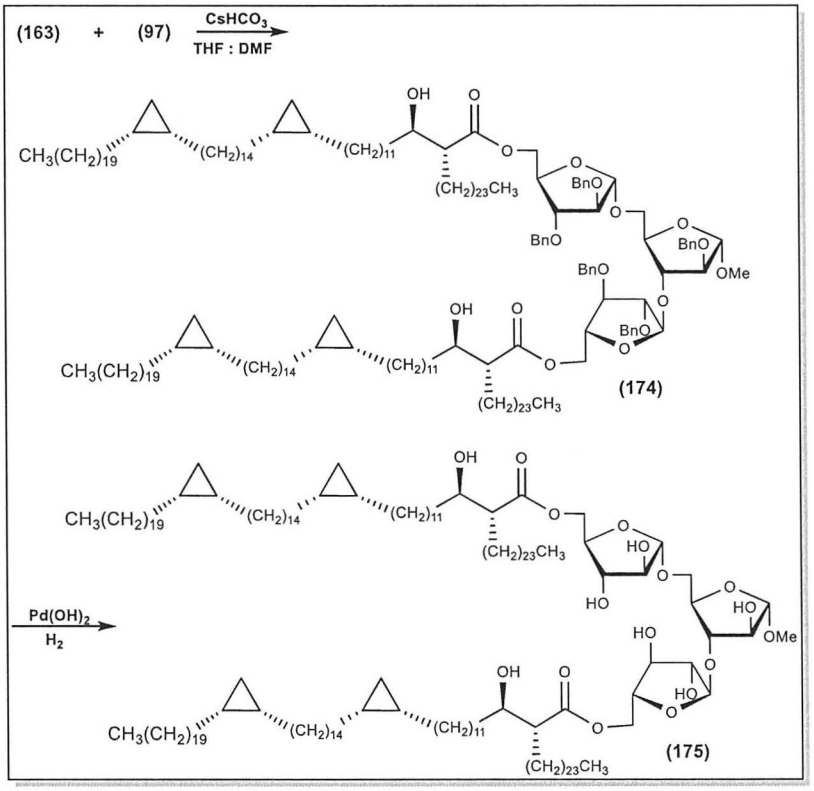
Figure 2.33: Structure of compound (173)

Table 2.12: The ^{13}C NMR data analysis of (173)

Cn	C_1	C_6	C_{11}	$C_{2,7}$	C_{13}	$C_{14,12}$	$C_{9,3}$	C_4	C_8	C_5	C_{10}
δ	109.0	107.8	107.3	81.5	80.0	83.1	82.9	81.1	77.97	65.7	63.6
Cn	C_{15}	C_{18}	$C_{18'}$	C_{16}	C_{22}	C_{19}	$C_{19'}$	C_{17}	$C_{17'}$	C_{21}	C_{20}
δ	63.2	52.8	52.7	54.9	14.1	72.99	72.93	175.2	175.0	10.9	15.8

2.3.4.4 Esterification of the glycan (163) with α-MA (97)

The synthetic α-MA **(97)**,[157] which was prepared in Bangor, was esterified with compound **(163)** by the same method given previously, and afforded **(174)** **(Scheme 2.34)** in a low yield of 11%. Compound **(174)** gave characteristic NMR signals, which were similar to those for compound **(172)**. Compound **(174)** was hydrogenolysed as before affording **(175)** **(Scheme 2.34)** in 97% yield. Again, compound **(175)** showed characteristic NMR signals, which were similar to those obtained for compound **(173)**.

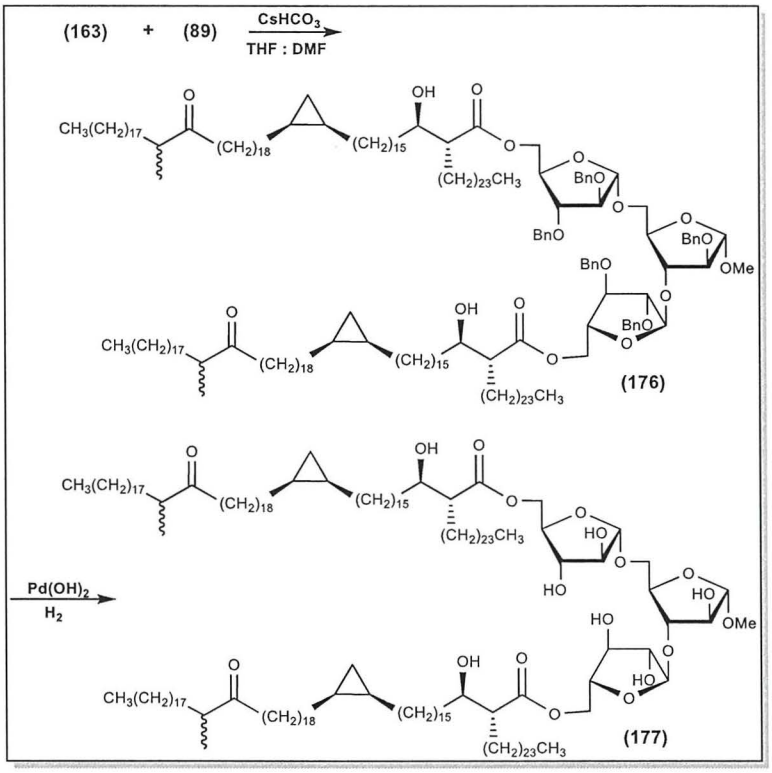


Scheme 2.34: Synthesis of methyl-tri-arabino-di-α-mycolates

2.3.4.5 Esterification of the glycan (163) with keto-MA (89)

Compound **(163)** was coupled with the synthetic keto-MA **(89)**[159] by the same method to prepare compound **(176)** **(Scheme 2.35)** in 36 % yield. Compound **(176)** showed NMR signals, for both the keto-MA and the glycan similar to those discussed before.

Hydrogenolysis of **(176)** by stirring it in dry CH_2Cl_2 : MeOH (1:1) in the presence of $Pd(OH)_2$ and under a hydrogen atmosphere, gave compound **(177)** in 65% yield **(Scheme 2.35)**. Compound **(177)** also gave NMR signals corresponding for both the keto-MA and the glycan similar to those discussed before. The specific rotation of **(176)** was $[\alpha]_D^{23}$ + 62 (*c* 0.1, $CHCl_3$), changing for the deprotected compound **(177)** to $[\alpha]_D^{20}$ + 33 (*c* 0.1, $CHCl_3$). **Figures 2.34** and **2.35** show the ^{13}C spectra of compounds **(176)** and **(177)** respectively.



Scheme 2.35: Synthesis of methyl-tri-arabino-di-keto-mycolates

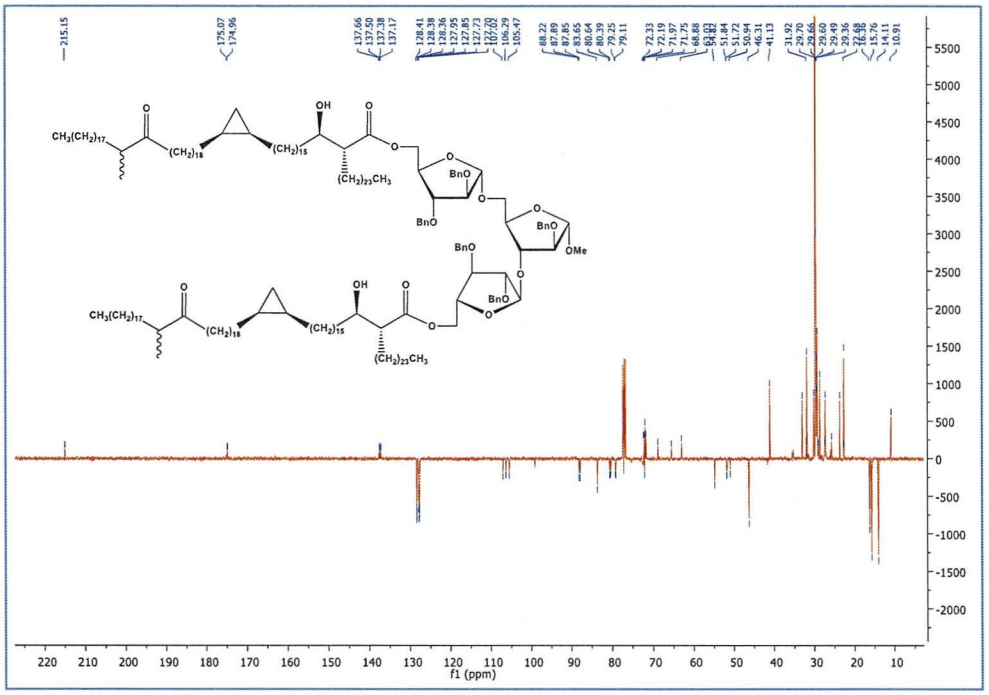


Figure 2.34: ^{13}C NMR spectrum for compound (176)

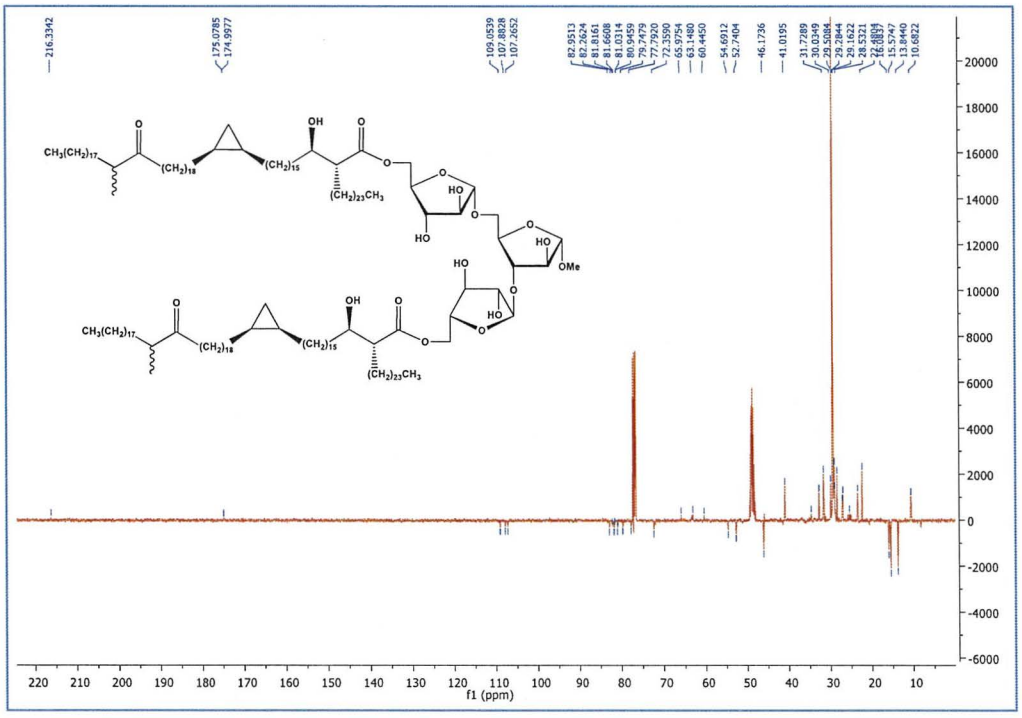


Figure 2.35: ^{13}C NMR spectrum for compound (177)

2.3.5 Summary

In this part, preparation of MTADM was carried out, which included the synthesis of a series of five compounds from MTADM and a model from a normal fatty acid.

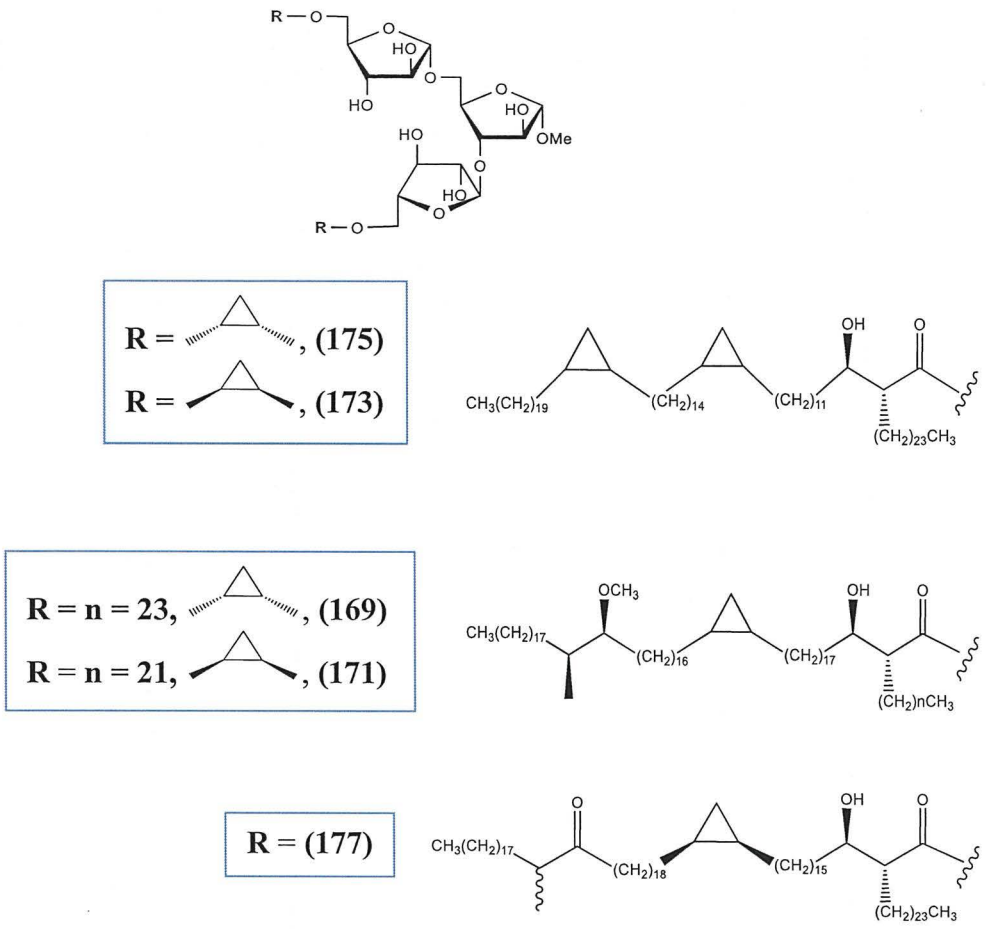


Figure 2.36: Structures of the target compounds

2.4 Synthesis of Di-Mycolyl-Di-Ara*f*-Glycerol (DMAG)

2.4.1 The aims of this part:

- To prepare the glycan 2',3'-di-*O*-benzyl-glycerol-(1'→1)-β-D- Ara*f*-yl-(1→2)-α-D-Ara*f*.
- To prepare a model glycolipid by esterifying the above with a normal fatty acid.
- To prepare a series of *di*-mycolyl-*di*-Ara*f*-glycerol esters through esterifying the glycan with different synthetic MAs.
- To investigate the biological activity of the synthetic compounds.

2.4.2 Synthesis of 2',3'-di-*O*-benzyl-glycerol-(1'→1)-β-D-Ara*f*-yl-(1→2)-α-D-Ara*f*

As mentioned in Chapter 1, in the glycosylation reaction a new stereogenic centre is created at C1 on the ring and two different diasteroisomeric products can be obtained **(Figure 2.37)**. 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.[293]

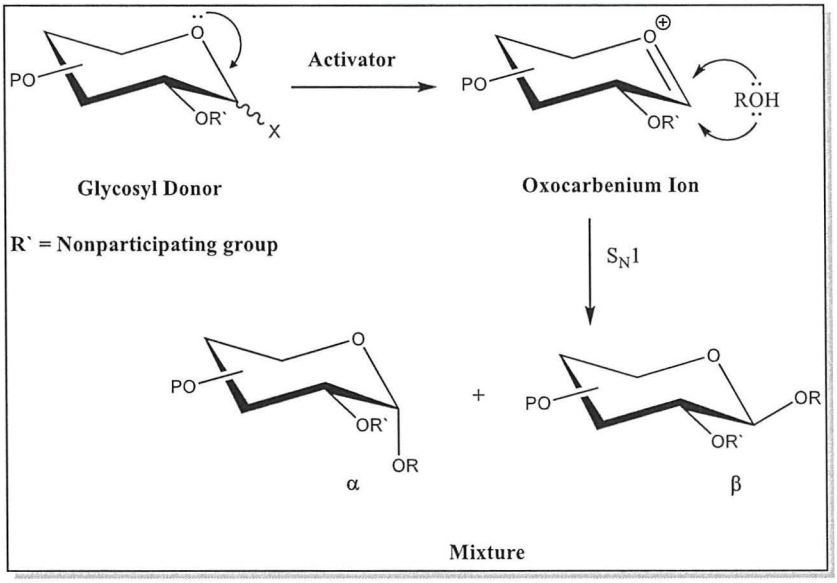


Figure 2.37: Glycosylation using a donor with a nonparticipating group at C-2

In this part of the study, the preparation of the target glycan molecules was undertaken, which contain both α- and β-Ara*f* linkages **(Figure 2.38)**. As discussed in Chapter 1 **(Section 1.11.2.1**, p.31), formation of the α-glycosidic bond can be directly realised 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 2-*O*-acyl protecting group 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 hindered face, through a process that is kinetically favoured. Discrimination between α- and β- isomers was achieved by 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.[294]

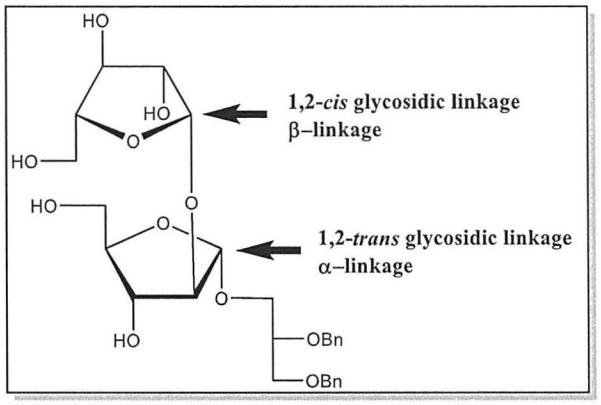
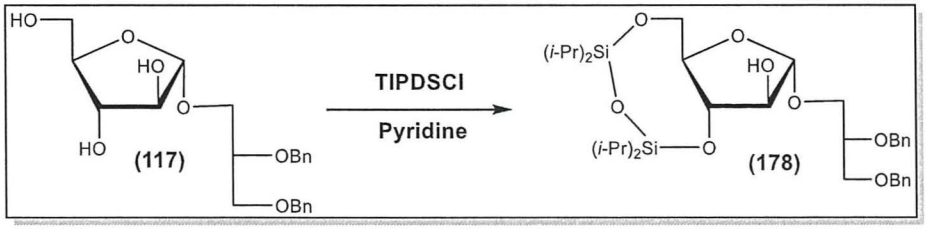


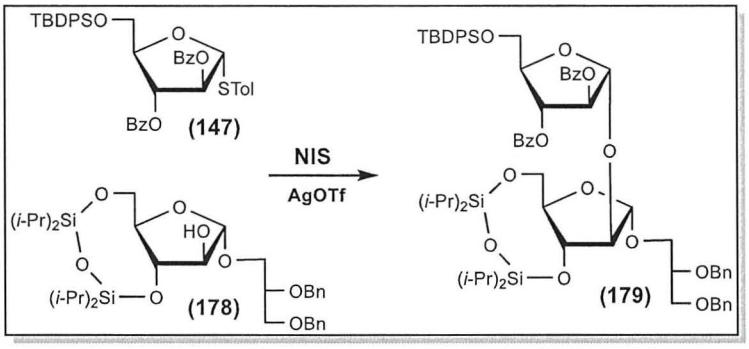
Figure 2.38: Glycosidic linkages in the di-saccharide glycerol target molecules

The acceptor, compound **(117)**, which has been synthesised previously, was protected by reaction with 1,3-dichloro-1,1,3,3-*tetra*-isopropyldisiloxane in dry pyridine at 0 °C which gave the siloxane **(178)** in 81% yield **(Scheme 2.36)**. A downfield signal appeared as a doublet at δ 4.87 (*J* 2.6 Hz), corresponding to the proton attached to the C1 of the sugar core. The protons of each benzyl group appeared as doublets at δ 4.72 (*J* 12.0 Hz), δ 4.69 (*J* 12.3 Hz), δ 4.56 (*J* 11.5 Hz) and δ 4.53 (*J* 11.5 Hz). The ^{13}C NMR spectrum showed a signal at δ 107.4 corresponding to C1 for the glycan.



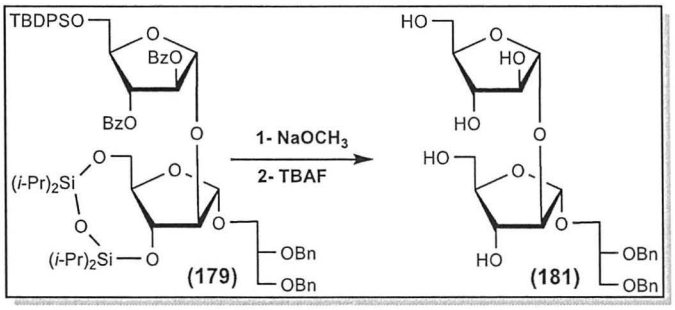
Scheme 2.36: Synthesis of protected D-glycerol-Araf (178)

Reaction of the acceptor **(178)** with the donor **(147)**, which was prepared previously, was now undertaken to afford the *di*-saccharide **(179) (Scheme 2.37)** in 76% yield. ^{1}H NMR spectrum of compound **(179)** showed a predominance of the α-anomer glycosidic linkages, which appeared as the main product, (α/α)-disaccharide, with a ratio of (8.3:1) (α/β). Two downfield signals occurred as a doublet at δ 5.49 (*J* 1.0 Hz) and a singlet at δ 5.41, corresponding to the proton attached to the C1 position on each of the sugar cores. The ^{13}C NMR spectrum showed signals at δ 106.5 and 105.8 corresponding to the C1 position on each of the glycan rings.



Scheme 2.37: Synthesis of the di-saccharide (179)

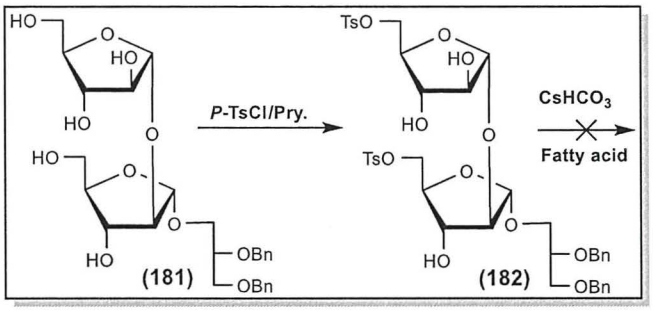
Subsequent treatment of compound **(179)** with $NaOCH_3$ in methanol afforded compound **(180)** in 99% yield, which was followed by the de-protection of the di-silyl group, by stirring the compound **(180)** with *tetra*-butylammonium fluoride in dry THF at 0 °C, which afforded **(181) (Scheme 2.38)** in 77% yield.



Scheme 2.38: Synthesis of compound (181)

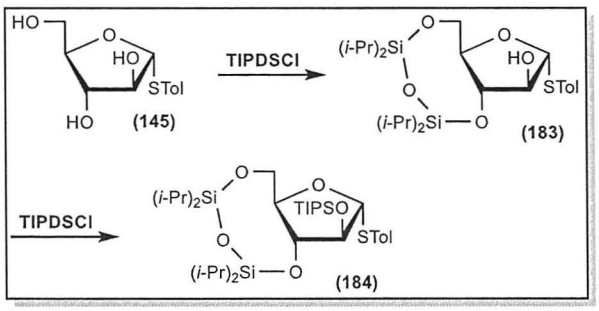
The syntheses of both compounds **(180)** and **(181)** were confirmed by NMR. All the data confirmed the presence of α/α-glycosidic linkages, which was not the target, as illustrated in **Figure 2.49**. Compound **(181)** was subjected to selective tosylation to

convert the two primary hydroxy groups into tosylate, by reaction with TsCl in dry pyridine and catalytic DMAP in dry CH_2Cl_2 at 0 °C, to afford the tosylate **(182) (Scheme 2.39)** in 79% yield. An attempt to esterify the tosylate **(182)** with a commercially available fatty acid was now undertaken using the same coupling conditions which were described previously, but product was not observed **(Scheme 2.39)**.



Scheme 2.39: Synthesis of glycolipid model

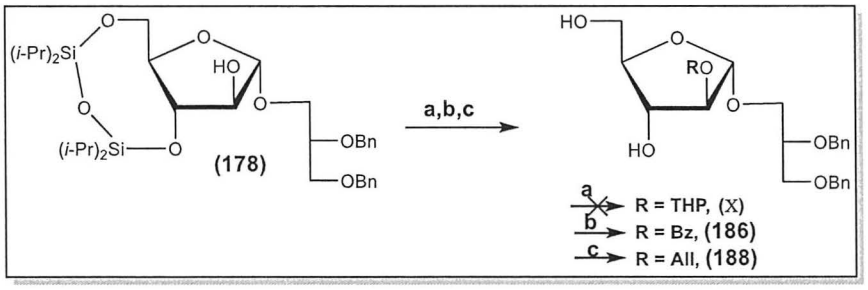
Due to the failure of the above strategy, it was deemed necessary to investigate the effect of the reaction conditions. First, the protecting groups on the donor were changed, **(145)**, which was prepared previously, the hydroxyl groups at C3 and C5 on this compound being protected by *tetra*-isopropyldisiloxane through reaction with 1,3-dichloro-1,1,3,3-*tetra*-isopropyldisiloxane in dry pyridine at 0 °C to afford the siloxane **(183)** in 77% yield **(Scheme 2.40)**. The hydroxyl group at the C2 position on the compound **(183)** was protected by reacting with triisopropylsilyl trifluoromethane-sulfonate and 2,6-lutidine in dry DMF and stirring for 4 h at 90 °C to afford **(184)** in 90% yield **(Scheme 2.40)**, according to the literature method (see page 35).[234] The donor **(184)** was subjected to a glycosylation with the acceptor **(178)** using known conditions, but the product was obtained as a mixture of (α/β)-disaccharides with a ratio of 1:2. It was therefore decided to investigate changing the protecting group on the acceptor.



Scheme 2.40: Synthesis of the donor (184)

Having an 'arming' donor, synthesis of a different acceptor by changing the protect group at the C3 and C5 positions was now undertaken. As discussed at the beginning of this chapter, good β-selectivity can be obtained in the case of glycosylation of 'armed' donors and active acceptors. Armed donors are referred to as those protected with electron-rich ethers, such as compound **(184)**; active acceptors are not sterically hindered and possess electron-donating protecting groups and thus are more nucleophilic. As expected, the best results can be achieved by the combination of active donors and active acceptors.

As mentioned before (p. 35), the presence of PMB protecting group at the C3 and C5 positions on the acceptor is found to give a good β-selectivity; therefore the next step was the protection of the C3 and C5 positions on the acceptor **(178)** by the PMB group. This was achieved by protecting the hydroxyl group at the C2 position first, then de-protection of the siloxane followed by protecting the hydroxyl groups at the C3 and C5 positions by *p*-methoxybenzyl and finally, de-protecting the hydroxyl group at the C2 position. Firstly, an attempt to protect the hydroxyl group at the C2 position on the compound **(178)** by THP was undertaken, but no product was obtained. Secondly, the hydroxyl group of compound **(178)** was protected as the benzoyl ester under standard conditions to give **(185),** followed by desilylation mediated by fluoride ion, to give the target acceptor **(186)** in 83% yield. Compound **(186)** was treated with sodium hydride and freshly prepared *p*-methoxybenzyl bromide (PMBBr) in dry DMF, but we did not obtain the product; apparently the benzoyl undergoes hydrolysis during the reaction. The signal of the benzoyl disappeared in the ^{1}H NMR spectrum of the product. The third protecting group used was the allyl protecting group.

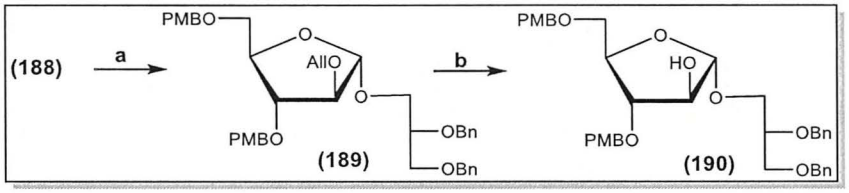


Scheme 2.41: Synthesis of protected D-glycerol-Araf acceptor

Reagents: (a) THP, CH_2Cl_2, 10 °C; (b) BzCl, pyridine, 0 °C/R.T., 88%; (c) AllBr, NaH, DMF 75%.

Compound **(178)** was treated with sodium hydride and allyl bromide in dry DMF, to give **(187),** followed by desilylation mediated by fluoride ion to give the target acceptor

(188) in 82% yield. Compound **(188)** was treated with sodium hydride and freshly prepared PMBBr in dry DMF to afford the desired compound **(189)** in 81% yield **(Scheme 2.41)**. The final step to prepare the acceptor was the removal of the allyl group. First, it was attempted to remove this group by using Wilkinson's catalyst in the presence of triphenylphosphine, but the reaction didn't work.[295] The allyl group was successfully removed by stirring the compound in a mixture of CH_3OH : CH_2Cl_2, and adding 2 equivalents of $PdCl_2$ to afford **(190)** in 95% yield **(Scheme 2.42)**. Formation of compound **(190)** was confirmed by NMR. **Figure 2.39** shows the HSQC of compound **(190)** .



Scheme 2.42: Synthesis of acceptor (190)

Reagents: (a) PMBnBr, NaH, DMF 81%; (b) $PdCl_2$, methanol: CH_2Cl_2 (5:0.6), 95%.

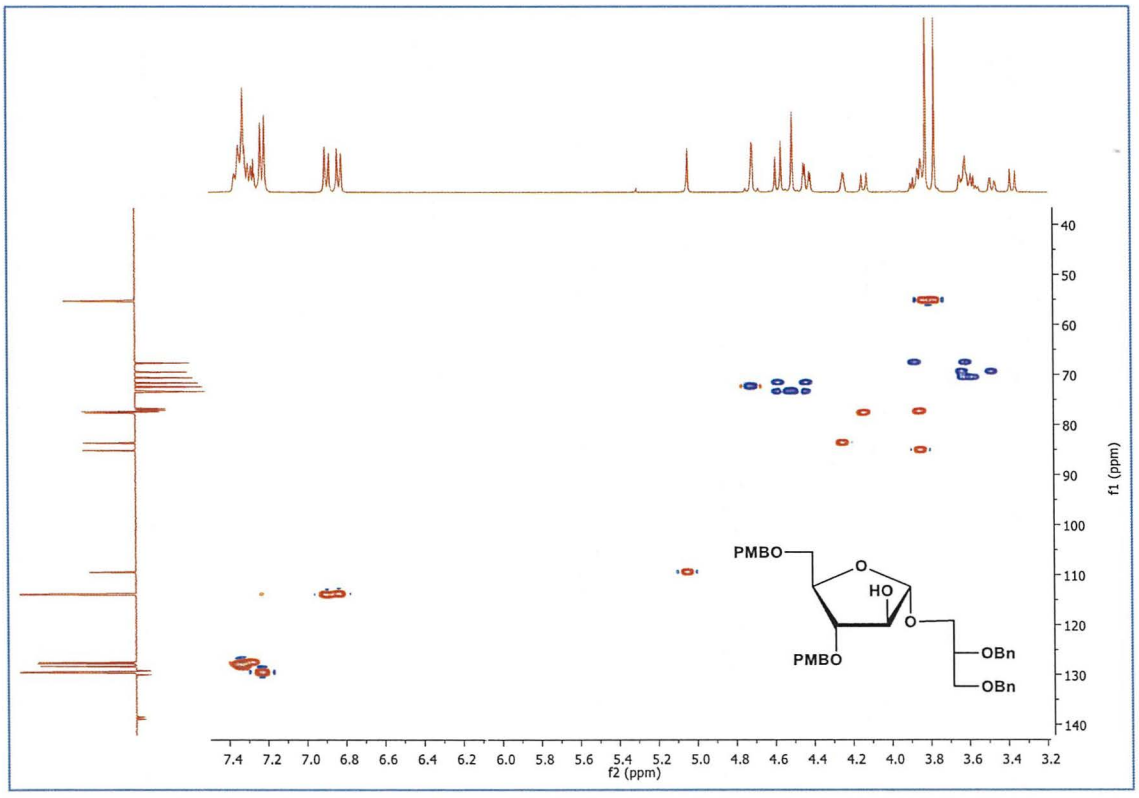
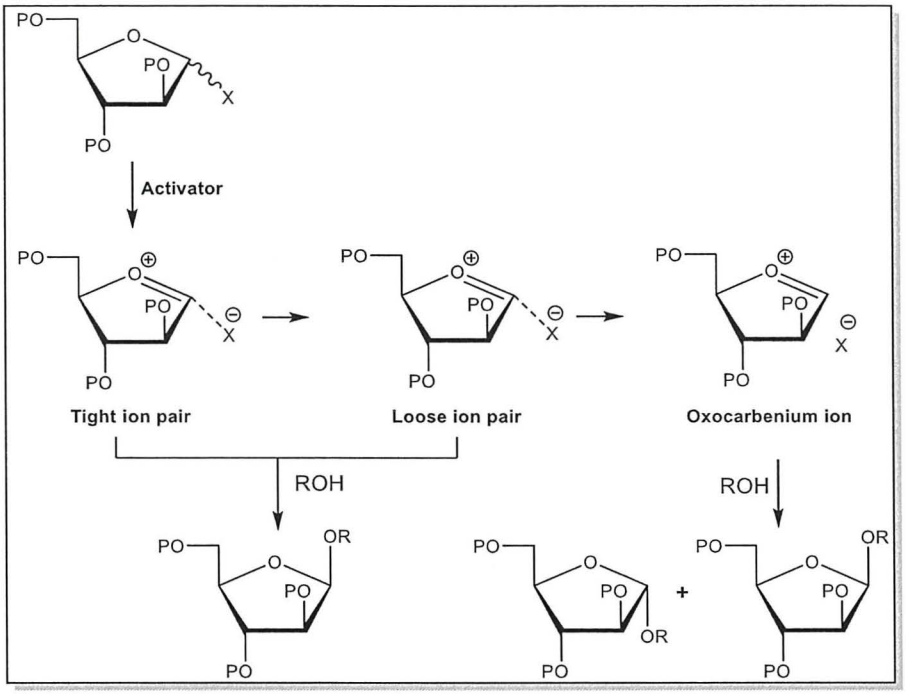


Figure 2.39: The HSQC spectrum of compound (190)

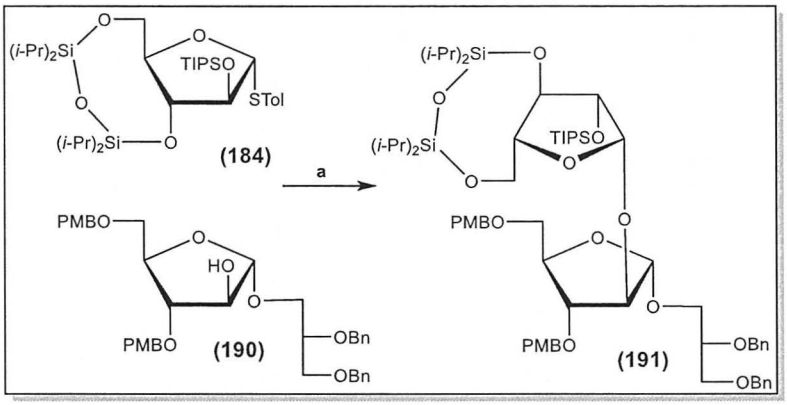
With the donors and acceptors synthesized, next the glycosidation was attempted using those optimized reaction conditions that gave the highest β-selectivity. On the basis of the literature studies, (see **Section 1.11.2.2**, Chapter 1, p. 33), the reaction was carried out at low temperature (- 78 °C). In order to obtain an oxocarbenium ion pair, instead of the free oxocarbenium ion intermediate, it was desirable to carry out the reactions at low temperature. The reason could be that at higher temperatures, this ion pair would dissociate quickly to give the free oxocarbenium ion, which would give mixtures of products. This postulated pathway is consistent with earlier studies on the glycosylation of fully protected arabinofuranosyl chlorides **(Scheme 2.43)**.[296]



Scheme 2.43: Possible mechanism of the glycosylation from the low temperature activation of thioglycosides

The reaction of the 2,5-di-*O*-*p*-methoxybenzylated glycosyl acceptor **(190)** and thioglycoside **(184)** in dry CH_2Cl_2, was carried out by cooling the reaction mixture to - 78 °C. Then NIS and AgOTf were added and the reaction was warmed to - 40 °C over 60 - 90 min. The reaction was quenched with triethylamine. Work-up afforded the disaccharide **(191)**, in 97% yield **(Scheme 2.44)**, with an excellent β-selectivity. The ^1H NMR spectrum indicated the presence of two anomeric hydrogens. A signal downfield at δ 5.05 (br. singlet) and at δ 4.88 (doublet, *J* 4.3 Hz) corresponded to the protons at the anomeric centres of the α-anomer and β-anomer respectively. The ^{13}C NMR spectrum

(Figure 2.40) confirmed the presence of glycosidic linkages in the Ara*f*, signals at δ 106.2 and δ 100.3 ppm belonging to the two carbons at the anomeric centers of the α-anomer and β-anomer respectively.



Scheme 2.44: Synthesis of the disaccharide target

Reagents: (a) NIS/AgOTf, CH_2Cl_2, -78 °C, 97%

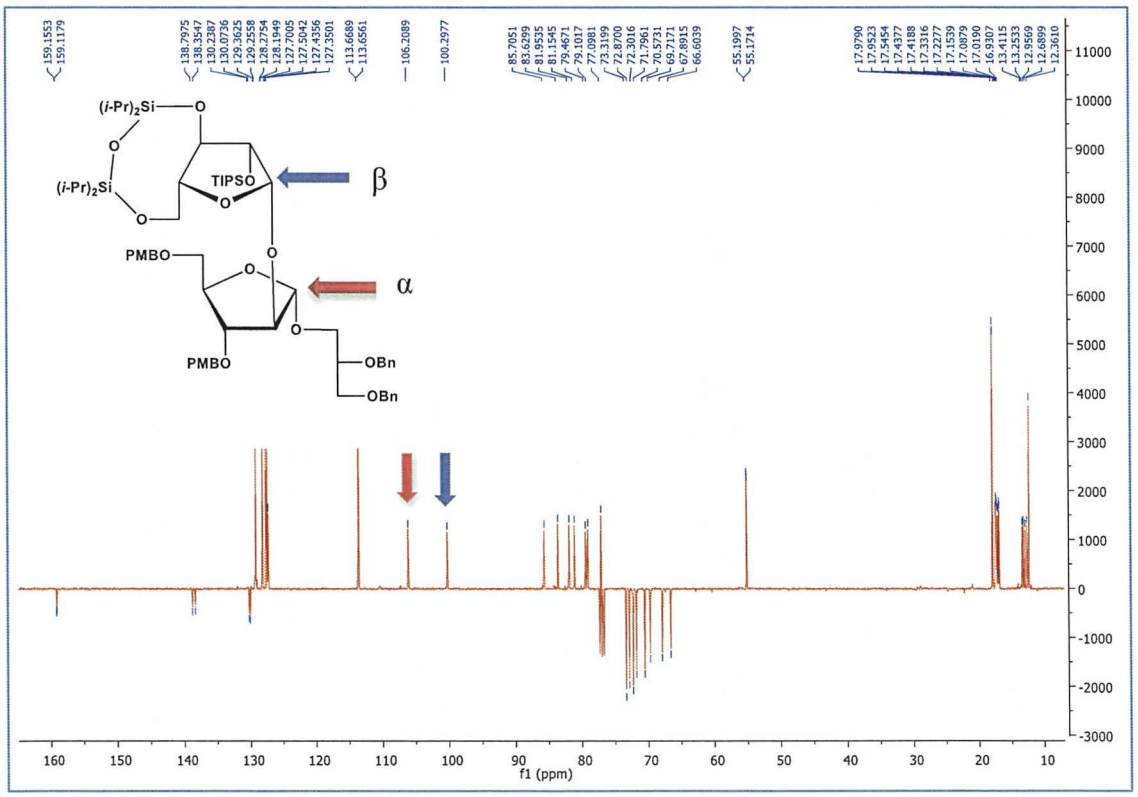
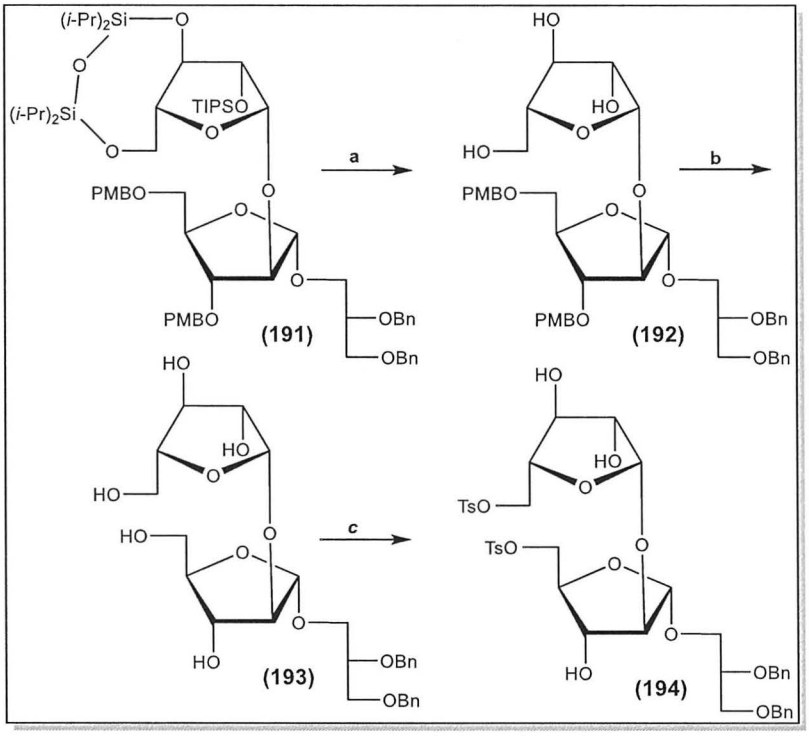


Figure 2.40: The ^{13}C NMR spectrum of compound (191)

With the pure β-anomer **(191)** in hand, the cyclic siloxane protecting group was removed by reacting overnight with $Et_4N^+F^-$ in THF to give the triol **(192) (Scheme 2.45)** in 94% yield. A small amount of the desilylated product obtained was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in a mixture of CH_2Cl_2 : H_2O (20:1) to remove

the PMB groups and after the usual workup the product was purified to afford **(193)** in 62% yield **(Scheme 2.45)**. This reaction was scaled up, on the remaining amount of **(192)**, but was not reproducible, and most of the starting material was lost in this step. The two primary hydroxyl groups of compound **(193)** were converted into tosylate by reaction with TsCl in dry pyridine and catalytic DMAP at room temperature to afford the di-tosylate **(194)** in 34% yield **(Scheme 2.48)**. In this reaction, some of the secondary hydroxyl group also reacted, therefore the product was obtained in a low yield.



Scheme 2.45: Synthesis of the disaccharide target

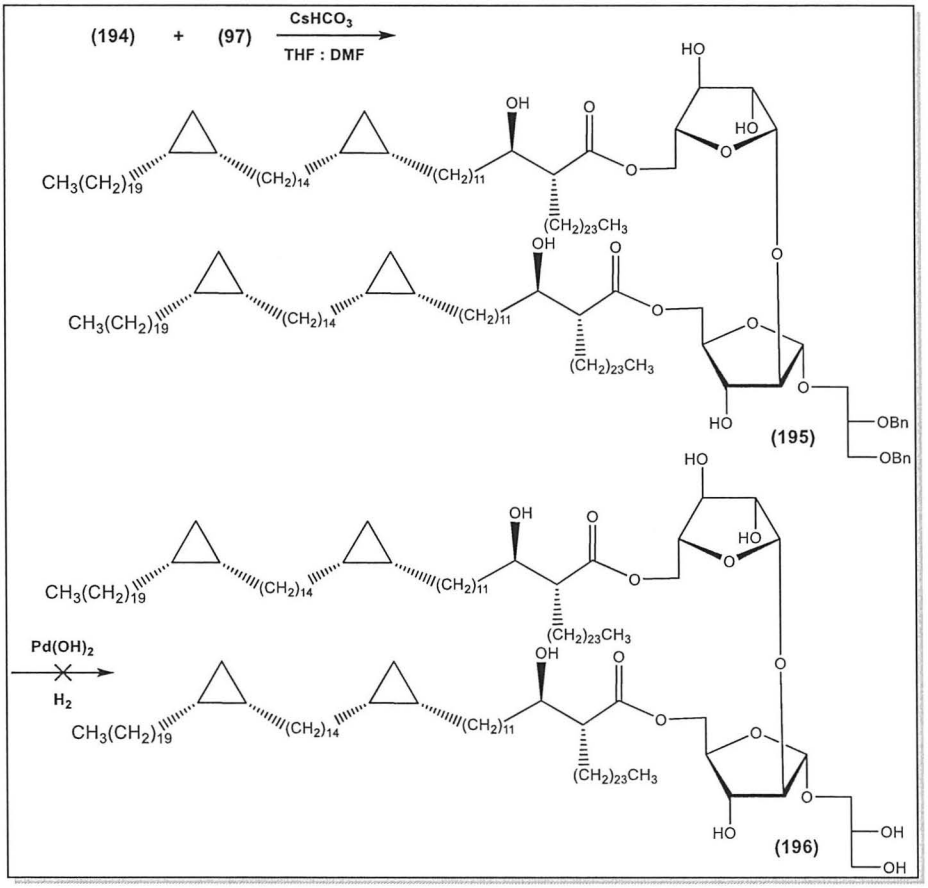
Reagents: (a) TBAF, THF, 0 °C/R.T., 16 h, 94%; (b) DDQ, CH_2Cl_2:H_2O (20:1),62%; (C) *p*-TsCl, DMAP, pyridine, 34%.

2.4.2.1 Esterification of the glycan (194) with α-MA (97)

Synthetic α-MA **(97)**,[157] was esterified with compound **(194)** by the same method given previously to give **(195)** in 15% yield **(Scheme 2.46)**. The ^{1}H NMR spectrum of this compound showed a multiplet at δ 3.7 for the two protons at the β-position on each of the MA molecule. The signals corresponding to the two protons at the α-position on each MA appeared as a multiplet at δ 2.45. Protons corresponding to the cyclopropane ring were revealed as a broad quartet for 4H at δ - 0.33 (*J* 5.2 Hz), a doublet of triplets for 4H at δ 0.57 (*J* 4.0, 8.0 Hz) and a multiplet for 8H at δ 0.71 – 0.60. A signal downfield at δ 4.99 (br. singlet) and at δ 4.95 (doublet, *J* 4.2 Hz) correspond to the protons at the

anomeric centres of the α-anomer and β-anomer respectively. The ^{13}C NMR spectrum confirmed the presence of glycosidic linkages in the Ara*f*, signals at δ 106.0 and 101.6 ppm belonging to the two carbons at the anomeric centres of the α-anomer and β-anomer respectively. The remaining signals corresponding to MA carbons were similar to those of the glycolipids prepared previously.

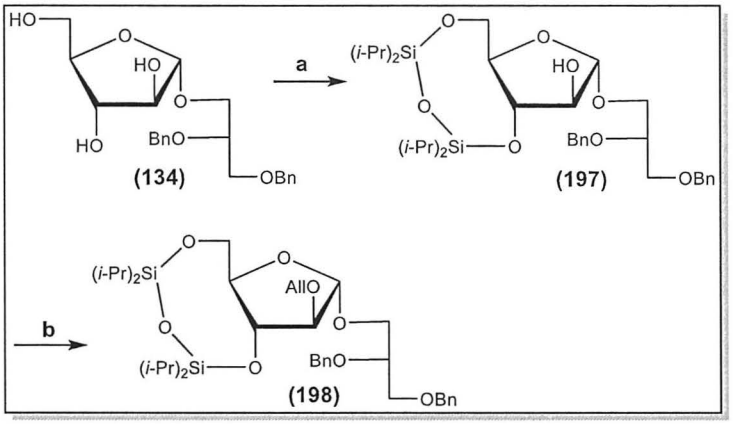
This compound was then subjected to hydrogenolysis by the method given previously **(Scheme 2.46)**. However, after stirring for 16 h, TLC showed that the reaction had not worked; therefore additional $Pd(OH)_2$ was added and the reaction mixture was stirred for another 16 h. Then TLC and 1H NMR showed that the compound had decomposed.



Scheme 2.46: Synthesis of DMAG

At this juncture, the synthetic sequence to prepare a large amount of the glycan was repeated. Having proved the stereochemistry of the glycerol to be in the L-configuration in the arabino-glycerol (see **Section 2.2.6.1**, p.78), the new disaccharide was prepared with the correct stereochemistry.

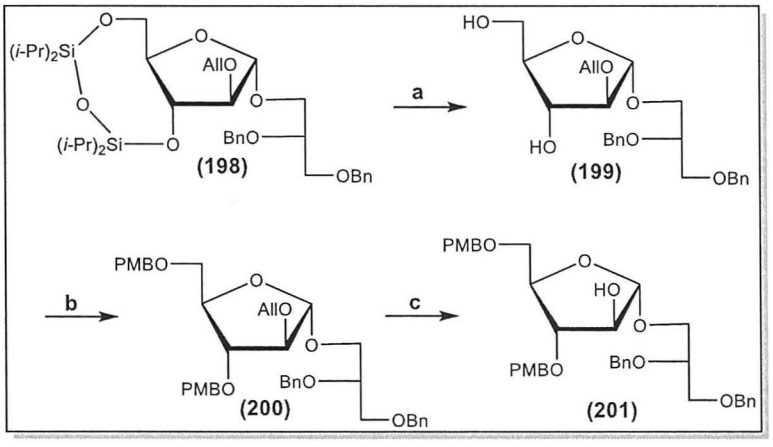
Compound **(134)**, which was prepared before (see **Scheme 2.18**, p.75), and by the same method used to prepare the D-glycerol-Ara*f* acceptor, was protected to prepare compound **(197)** and **(198)** **(Scheme 2.47)** in 81% and 96% yield respectively. The formation of these compounds was proved by NMR (^{1}H and ^{13}C) and mass spectrometry.



Scheme 2.47: Synthesis of the acceptor L-glycerol-Araf

Reagents: (a) TDIPDSCl, pyridine, 0 °C/R.T., 81%; (b) AllBr, NaH, DMF 96%.

The next step was de-protection of compound **(198)** in TBAF to afford compound **(199)** in 90% yield. The hydroxyl groups at the C5 and C3 positions on compound **(199)** were protected by the PMB group by the method described previously to afford **(200)** in 81% yield **(Scheme 2.48)**. Finally, the allyl group on the hydroxyl group at the C2 position was de-protected as before to afford **(201)** in 95% yield **(Scheme 2.48)**.



Scheme 2.48: Synthesis of acceptor (201)

Reagents: (a) TBAF, THF, 0 °C/R.T., 16 h, 90%; (b) PMBnBr, NaH, DMF 81%; (c) $PdCl_2$, methanol: CH_2Cl_2 (5:0.6), 95%.

Once again, the formation of these compounds was proved by NMR (^{1}H and ^{13}C) and mass spectrometry. **Figure 2.41** shows the HSQC spectrum of compound **(201)** which confirmed the structure of the compound, where all the proton signals were correlated to their carbons.

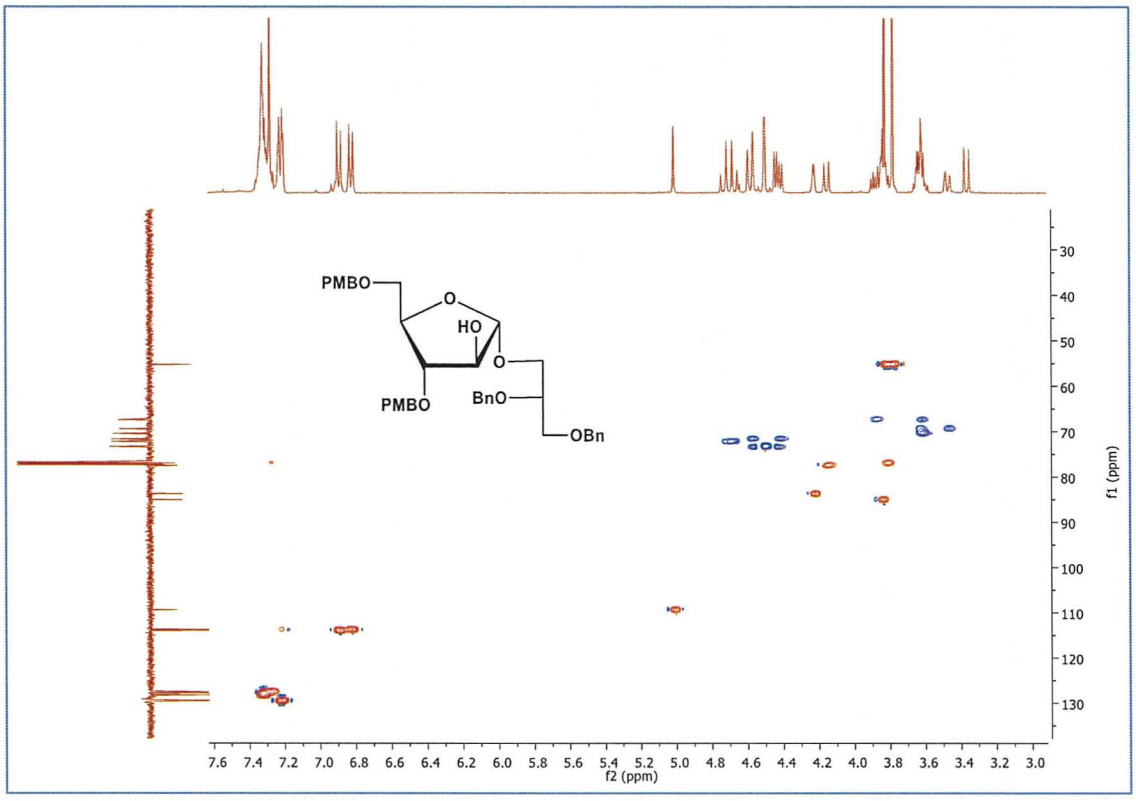
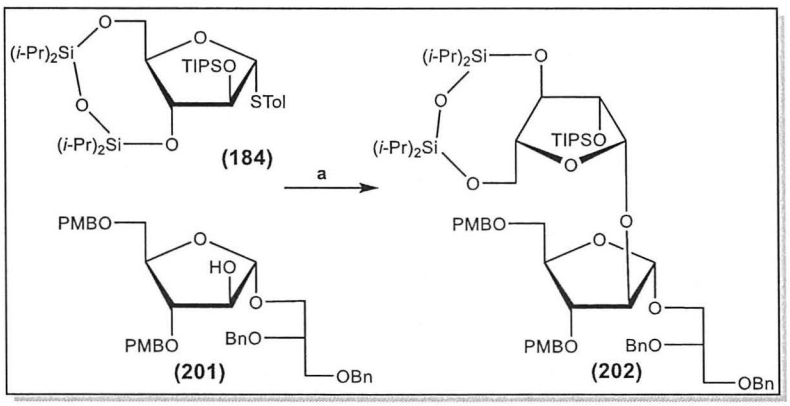


Figure 2.41: HSQC spectrum of compound (201)

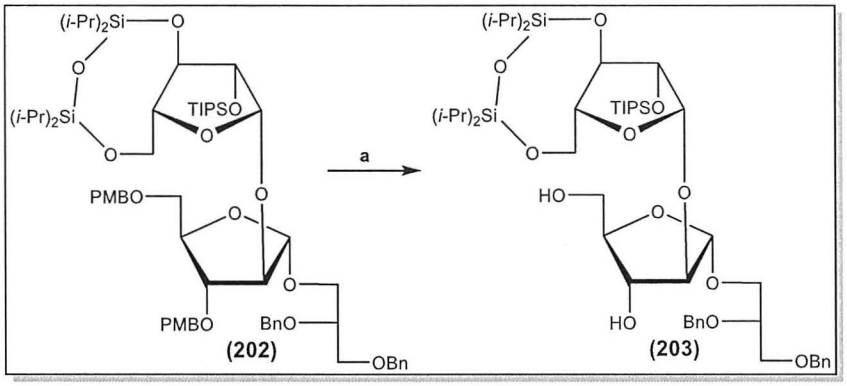
Now, by utilising the same coupling conditions used before, the acceptor **(201)** and the donor **(184)** were coupled to afford **(202)** in 86% yield and with excellent β-selectivity **(Scheme 2.49)**.



Scheme 2.49: Synthesis of the disaccharide (202)

Reagents: (a) NIS/AgOTf, CH_2Cl_2, -78 °C, 86%.

The next step was the de-protection of compound **(202)**, and this time the PMB group was de-protected first. Previously, DDQ had been used to de-protect this group and most of the compound was lost, therefore ceric ammonium nitrate (CAN) was now used in a mixture of water : CH_3CN (1:3) for 3 h. TLC showed no conversion of the starting material. The reaction repeated for the second time, changing the conditions to 1:9 water : CH_3CN, and this afforded **(203)** in 67% yield **(Scheme 2.50)**.

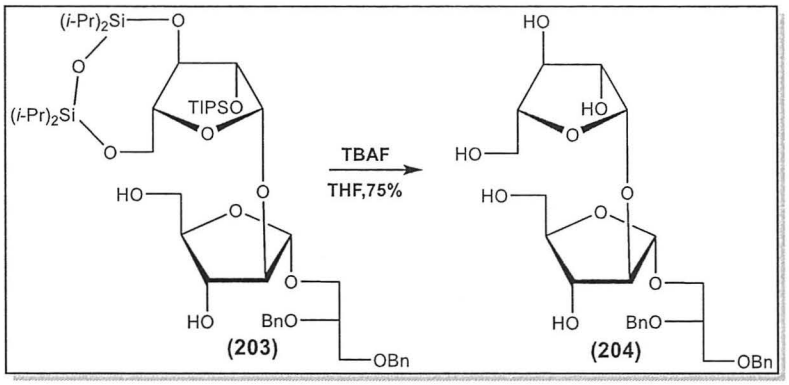


Scheme 2.50: Synthesis of the di-saccharide (203)

Reagents: (a) CAN, CH_3CN:H_2O, 0 °C, 67%.

Before de-silylation of compound **(203)**, a selective esterification on the C5 position of the glycan **(203)** with behenic acid was carried out, by using *N*-(3-dimethylamino-propyl)-*N'*-ethylcarbodiimide (EDCI) and DMAP in dry CH_2Cl_2 (1 eq.). The product was obtained as an intractable mixture of glycans acylated at both the C5 and C3 positions. An attempt to separate the two compounds after de-protecting of the silyl group was unsuccessful.

At this point, the remaining amount of **(203)** was de-protected to afford **(204)** in 75% yield **(Scheme 2.51)**.



Scheme 2.51: Synthesis of the disaccharide (204)

In order to prepare the full, un-protected glycan moieties from DMAG, and to investigate the biological activity for the glycan unit, a small amount of compound **(204)** was de-benzylated by stirring it in H_2O : MeOH (1:4) in the presence of $Pd(OH)_2$ and under a hydrogen atmosphere, and this gave compound **(205)** (**Figure 2.42)** in 72% yield.

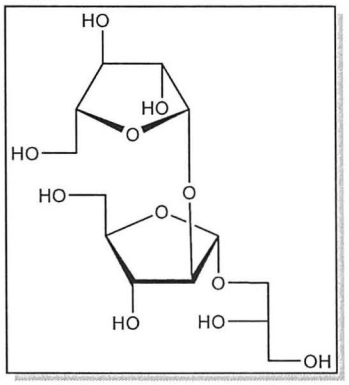
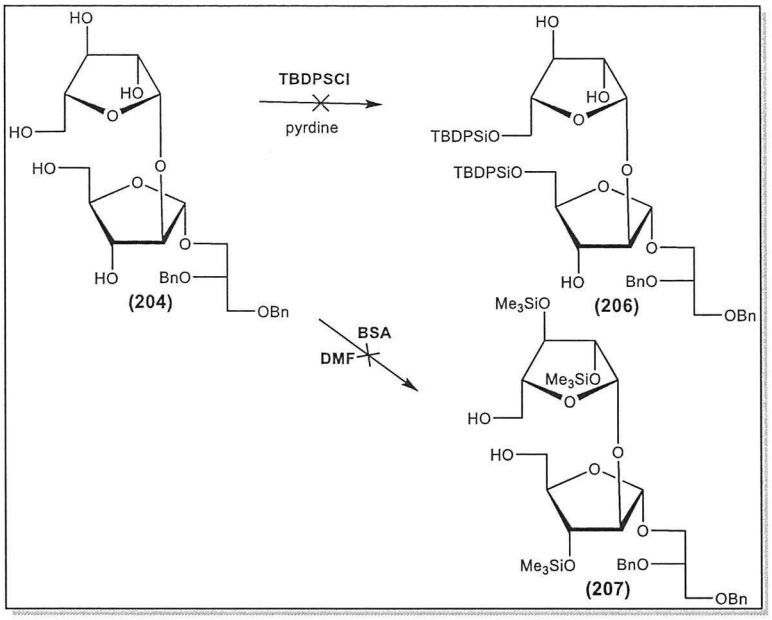


Figure 2.42: Structure of compound (205)

An attempt to protect the secondary hydroxyl groups in compound **(204)** was undertaken, compound **(204)** being stirred in dry DMF in the presence of imidazole and TBDPSCl. However, no sign of the desired compound **(206)** was observed **(Scheme 2.52)**. Compound **(204)** was now stirred in dry DMF and *N,O*-bis(trimethylsilyl)-acetamide (BSA) was added, followed by the addition of TBAF, to protect all the secondary hydroxyl groups to give compound **(207)**. Again the reaction failed **(Scheme 2.52)**.



Scheme 2.52: Synthesis of the disaccharide target

2.4.3 Summary

For this part of our study, the following points can report:

1- Successful synthesis of both anomers (D and L) glycerol of the disaccharide 2',3'-di-*O*-benzyl-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside was achieved for the first time in a good yield and with excellent β-selectivity.

2- A study of the effect of the protecting groups on both the donor and acceptor in the glycosylation reaction was conducted, leading to the preparation of the disaccharide with a good β- selectivity and in good yield.

3- The DMAG glycans moiety prepared may possess a high biological activity. The testing of these glycans is in progress.

4- An unsuccessful attempt to protect the secondary hydroxy groups in the glycan.

5- An unsuccessful attempt to direct esterification of the free hydroxy-glycan with a fatty acid; this suggests that the secondary hydroxy groups in the disaccharide are very active, and thus must be protected prior to the coupling.

6- As future work for this part of study, the preparation of a large amount of the glycan and finding a way to protect all the secondary hydroxy groups prior esterification with fatty acids.

2.5 The Biological Activity

It is significant to know that the analysis of natural arabino-mycolate is fairly complex, as a large number of different MAs can be bound to the glycan moiety, increasing the number of possible structures. Natural arabino-mycolates show a variety of immune related effects as previously described in the introduction **(Section 1.3)**. They are able to stimulate the immune system and the complex mixture produces a range of overall effects on chemokines and cytokines, which are essential in controlling TB disease. In contrast, arabino-mycolates derived from synthetic single enantiomer MAs may show more selective biological activity, in which certain compounds are capable of stimulating specific cytokines more than others.

To study the recognition of the compounds synthesised in this work, by the lipid antibodies present in the serum of patients infected with TB, an ELISA assay was used to measure the response. The second biological test, carried out by Prof. Kris Huygen's group in Brussels, was to test the ability of the synthetic glycolipids to stimulate some essential cytokines *e.g.* TNF-α, IL-6 and IL-1β in different types of cells derived from the bone marrow of mice.

2.5.1 ELISA assay

The use of the ELISA assay as a tool for diagnosing TB is attractive for a number of reasons. Relatively, this technique is simple, cheap and quick. However, the results obtained from the ELISA assay 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.

In ELISA, the surface of the well is coated with the antigen (glycolipid). To block any free non-specific binding sites, casein/PBS buffer is used. The ELISA plate is then treated with serum samples which may contain anti-TB antibodies, depending on whether the samples are TB+ or TB-. The coated surface is washed again with casein/PBS buffer to remove any excess antibodies, leaving only bound antibodies on the ELISA plate. Next, a secondary antibody, containing a peroxidase enzyme, is added to the plate, which will bind to any primary antibodies present in the wells. To remove the excess unbound secondary antibody, the plate is again washed with casein/PBS, followed by the addition of a colour reagent (OPD/H_2O_2 solution). A detectable colour

appears, which depends on the quantity of secondary antibody present, and the absorbance is measured at 492 nm.

'Cut-off' values were used to determine whether a sample was identified as TB+ or TB- by the assay. Samples that gave a value higher than the 'cut-off' were identified as TB+, while those lower were identified as TB-. Two parameters are then calculated in ELISA tests, sensitivity (the proportion of actual TB positive sera which are correctly detected by the antigen) and specificity (the proportion of the TB negatives which are correctly detected as such).

ELISA assays were carried out by Dr. A. Jones in the School of Chemistry at Bangor University using the synthetic glycolipids as antigens and a set of serum samples from the WHO, which were from patients who had been sent to hospital with TB symptoms, all in countries where TB is common. After further testing / monitoring using standard WHO protocols, some were diagnosed as TB+, with the others being diagnosed as TB-.

A blind set of 350 serum samples from the WHO were received. 50 of these samples were unblinded and used to screen a range of antigens. These consisted of 17 TB+ samples and 33 TB- samples and they were from various countries. These were tested using compounds **(82)** (1 in 20), **(84)** (1 in 80), **(91)** (1 in 20), **(96)** (1 in 20), **(87)** (1 in 80) and **(99)** (1 in 80) as antigens **Figure 2.43**. Anti-human IgG Fc specific was used as secondary antibody for all these antigens. Also included in the graph below **Figure 2.44** is the response of the same samples to a synthetic TDM (this was the antigen that performed best in the blind test) and natural human TDM from *M. tuberculosis* (IgG whole molecule was used as secondary antibody in this case), for comparison.

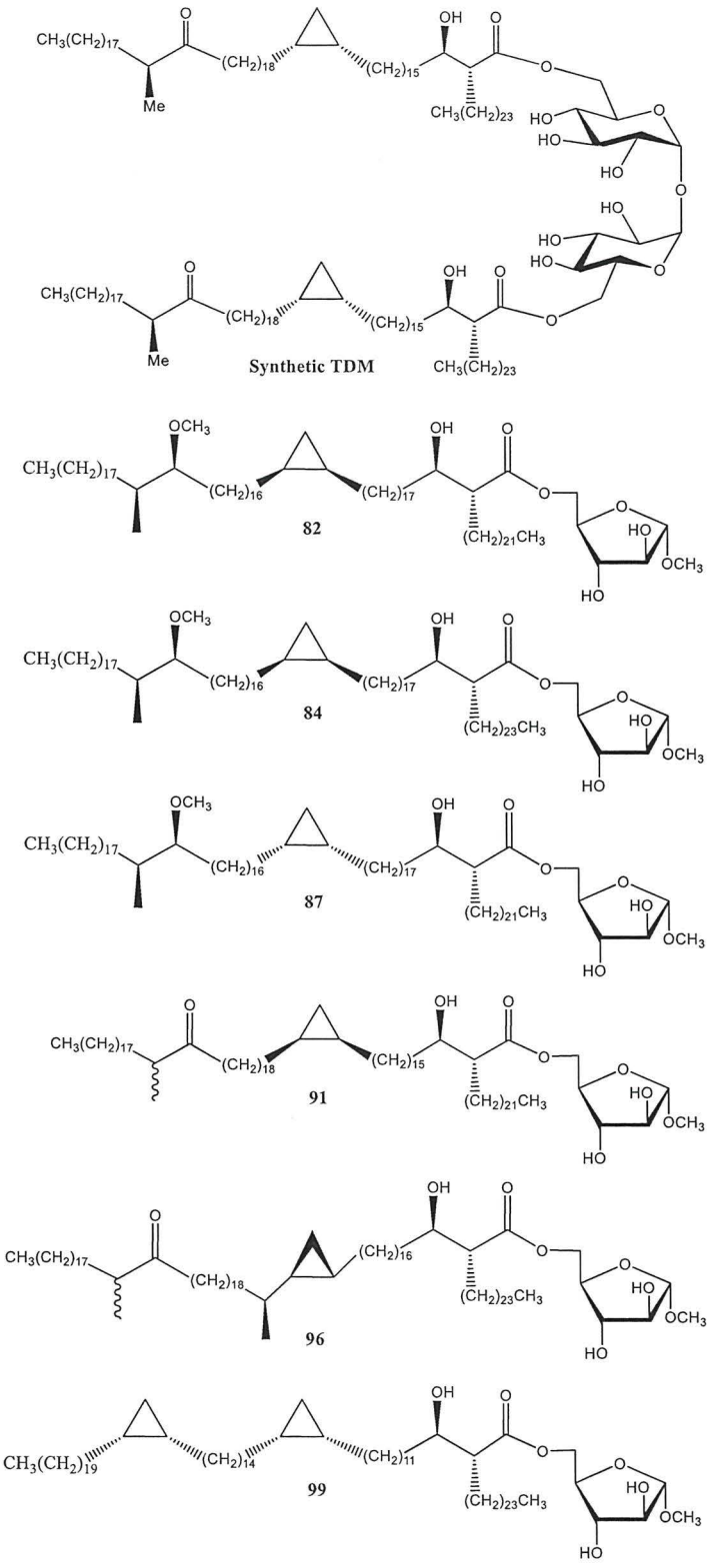


Figure 2.43: Structures of synthetic methyl arabino-mycolates and TDM used in ELISA assays for TB

The results showed a good distinction between the TB+ and TB- serum samples for the synthetic TDM and natural TDM from *M. tuberculosis* **(Figure 2.44)**.

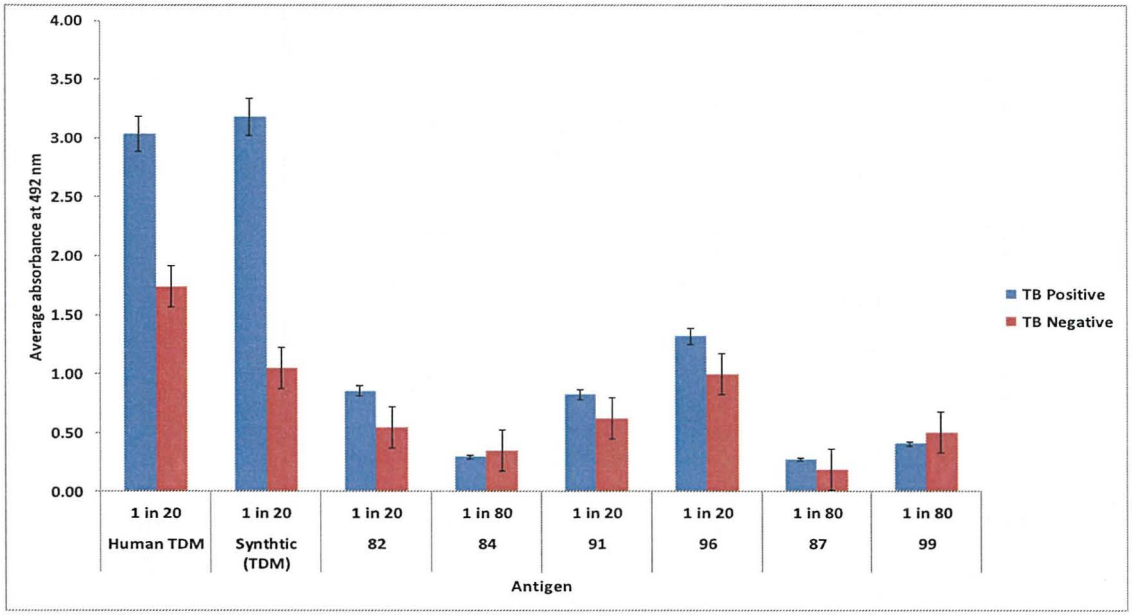


Figure 2.44: The average absorbance of the serum samples (TB+) and (TB-) against glycolipid antigens

On average, the absorbencies of the arabino-mycolates samples were lower than those for the TDM antigens. Compounds **(82)**, **(91)**, **(96)** and **(87)** show a slight distinction between the two sets of serum samples, while for **(84)** and **(99)** the average absorbance for the TB- samples is slightly higher than that for the TB+ samples. Compounds **(82)** and **(91)** are the only antigens that give a relatively good sensitivity and specificity. The sensitivity and specificity for each of the above antigens is shown in the **Table 2.13**.

Table 2.13: The data analysis of the ELISA results when using compounds (82), (84), (91), (96), (87) and (99) as antigens

Antigens	Average absorbance positive	Average absorbance negative	Sensitivity (%)	Specificity (%)
Natural TDM (1 in 20)	3.04	1.74	88	67
Synthetic TDM(1 in 20)	3.18	1.05	100	76
(82) (1 in 20)	0.85	0.54	65	73
(84) (1 in 80)	0.29	0.34	50	66
(91) (1 in 20)	0.82	0.62	71	70
(96) (1 in 20)	1.32	1.00	65	39
(87) (1 in 80)	0.27	0.19	43	78
(99) (1 in 80)	0.40	0.50	86	41

After unblinding the 350 samples from the WHO a sub-set of samples was chosen (all samples from Gambia) and these were tested against a range of antigens. This set of samples consisted of 9 TB+ samples and 55 TB- samples and they were all run at a serum dilution of 1 in 80 with IgG Fc specific being used as the secondary antibody.

The synthetic TDM run with serum samples at a 1 in 20 dilution was the antigen that gave the best results in the blind test. This was subsequently run at a dilution of 1 in 80 for comparison with the previous results and as can be seen from below **(Figure 2.45, Table 2.14)**. Although the absorbencies are slightly lower at a 1 in 80 dilution, a good distinction between the TB+ and TB- samples is observed. Again the absorbencies observed for the arabino-mycolate samples are lower than those observed for the TDM antigens. Compounds **(84)**, **(87)** and **(99)** show a distinction between the TB+ and TB- serum samples, with that for **(99)** being the biggest **(Figure 2.45)**. Compound **(82)** shows a slight distinction, while **(91)** and **(96)** show almost no distinction with the average responses for both the TB+ and TB- samples being almost the same. These results show a different trend to those for the other set of samples, where the average for the TB- samples was higher than that of the TB+ samples for **(84)** and **(99)**. Also, some of the antigens that showed a slight distinction in the previous set of samples show little or no distinction for the Gambia samples. It is however difficult to compare the results directly as the samples were run at different serum concentrations and also the previous set

contains samples from different countries. Further assays with a larger set of samples are therefore required in order to further investigate the potential of these compounds as antigens.

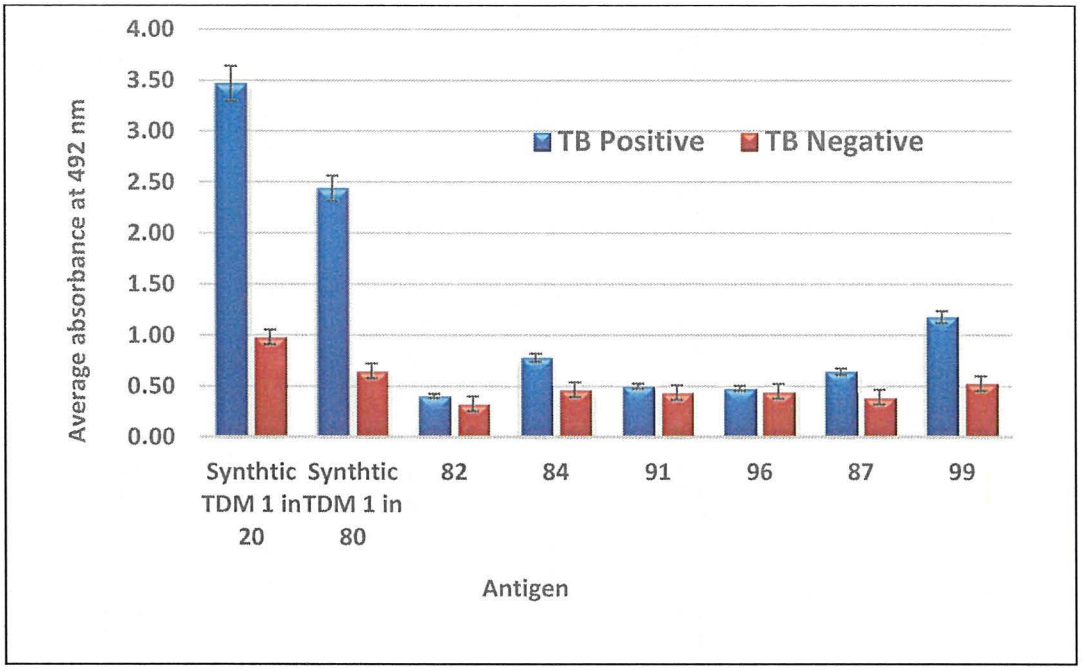


Figure 2.45: The average absorbance of the serum samples (TB+) and (TB-) against glycolipids

Although the absorbance values were lower for **(99)**, compared to the synthetic TDM, a sensitivity and specificity of 100 % and 89 % respectively could be obtained with this antigen. Compound **(87)** also gives a good sensitivity and specificity with values similar to those observed for synthetic TDM. The values observed for **(84)** are also relatively good.

The sensitivity and specificity for each of the antigens is shown in the **Table 2.14**.

Table 2.14: The data analysis of the ELISA results when using compounds (82), (84), (91), (96), (87) and (99) as antigens

various antigens	Average absorbance positive	Average absorbance negative	Sensitivity (%)	Specificity (%)
Synthetic TDM (1 in 20)	3.47	0.98	89	80
Synthetic TDM (1 in 80)	2.44	0.65	89	78
(82) (1 in 80)	0.40	0.33	33	87
(84) (1 in 80)	0.78	0.46	78	71
(91) (1 in 80)	0.50	0.44	67	44
(96) (1 in 80)	0.48	0.45	78	51
(87) (1 in 80)	0.64	0.39	89	75
(99) (1 in 80)	1.18	0.52	100	85

A combination study between compound **(99)** and the synthetic TDM on the same set of samples was carried out. A cut-off of 2.3 was used for the data for the synthetic TDM, and a cut-off of 1.09 was used for compound **(99)**; the values highlighted in pink in the **Table 2.15** below are therefore detected as TB+.

By using a traffic light system, where samples that are detected TB+ by both antigens are found to be TB+, while all the rest are found to be TB-, a sensitivity and specificity of 100% and 94% is observed.

Table 2.15: Average absorbance (492 nm)) values of 64 blind serum samples using synthetic antigens TDM and compound (99). Sample codes with a red background represent TB+ samples, and a blue background represent TB-, as diagnosed by WHO; pink boxes for results are TB+ with a cut-off of 2.3 for the synthetic TDM and 1.09 for compound **(99)**

	Synthtic TDM	99		Synthtic TDM	99
	IgG Fc specific	IgG Fc specific		IgG Fc specific	IgG Fc specific
	1 in 20	1 in 80		1 in 20	1 in 80
1-00010	2.95	0.74	1-00383	0.81	0.52
1-00026	4.41	0.76	1-00384	0.27	0.35
1-00030	3.66	0.99	1-00389	0.41	0.35
1-00033	3.92	0.97	1-00391	0.23	0.66
1-00044	3.31	0.99	1-00394	2.97	0.55
1-00048	4.22	1.39	1-00454	0.50	0.30
1-00316	3.55	1.97	1-00459	2.34	0.98
1-00320	3.88	1.67	1-00464	0.74	0.43
1-00321	1.31	1.09	1-00465	0.32	0.31
1-00001	0.39	0.59	1-00466	0.55	0.56
1-00003	0.71	0.92	1-00495	0.48	0.25
1-00006	4.06	0.84	1-00039	0.36	0.68
1-00009	0.64	0.60	1-00326	1.17	0.71
1-00014	1.25	1.30	1-00327	0.51	1.03
1-00019	0.60	0.64	1-00332	0.55	0.61
1-00022	0.21	0.56	1-00339	2.53	0.66
1-00029	1.54	0.81	1-00342	0.33	0.81
1-00482	0.41	0.41	1-00350	1.66	0.62
1-00484	1.11	0.45	1-00352	0.19	0.21
1-00486	1.16	0.44	1-00353	0.62	0.72
1-00490	0.63	0.41	1-00358	0.55	0.61
1-00493	1.64	0.30	1-00468	0.92	0.32
1-00360	0.30	0.38	1-00469	0.49	0.51
1-00364	0.31	0.41	1-00470	2.37	0.37
1-00365	0.36	0.39	1-00471	1.66	0.21
1-00369	0.45	0.51	1-00472	0.54	0.36
1-00371	1.29	0.91	1-00474	0.87	0.26
1-00372	0.52	0.42	1-00475	2.11	0.27
1-00374	0.46	0.49	1-00476	0.39	0.27
1-00375	0.39	0.38	1-00478	0.65	0.73
1-00376	0.25	0.28	1-00480	1.39	0.37
1-00382	1.32	0.55	1-00498	4.32	0.29

This combination study using both the synthetic TDM and compound **(99)** together may be useful in future assays to give a better sensitivity and specificity than can be obtained by using only one antigen alone.

Further testing and ELISA assays of the effects of these glycolipids and on the other prepared arabino-mycolates are expected to be carried out in the near future.

2.5.2 TNF-α and IL-6 cytokines stimulation

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, for example (LPS). TNF-α has been proved 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*.[297]

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, for instance LPS, through a two-stage set of maturation changes. In the first stage, these cells capture a fragment of circumferential tissue and re-present it to secondary lymphoid organs. This process stimulates the production of co-stimulatory molecules and certain pro-inflammatory cytokines (*e.g.*, TNF-α, IL-12, IL-6).[298]

As described in the Introduction **(Section 1.12)**, natural mixtures of arabino-mycolates show very strong effects on a number of immune system responses.

Experiments using the synthetic glycolipids against the production of co-stimulatory molecules and certain pro-inflammatory cytokines (*e.g.*, TNF-α, IL-1β, IL-6), were carried out by Mr.G. Tima in the group of Prof K. Huygen in the Scientific Institute of Public Health (WIV-ISP), Brussels.

Bone marrow-derived dendritic cells (BMDCs) were generated from mice, by flushing out the femurs of mice into complete medium and pipetting vigorously to make a single-cell suspension. The glycolipids were coated to the bottom of 96 micro-well plates (100 μg/ml). BMDCs were added, and after 6 or 48 hours of incubation the supernatants were harvested. The concentrations of cytokines in the cell-free medium were determined using cytokine-specific ELISA **(Figure 2.46)**.

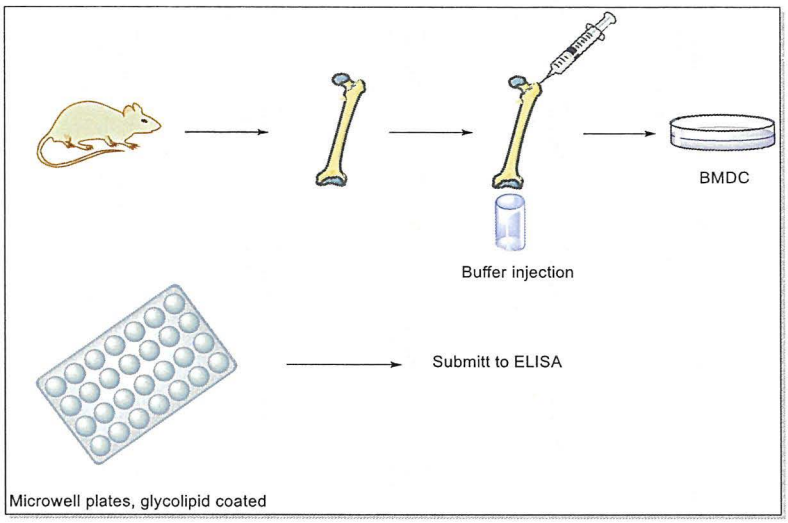
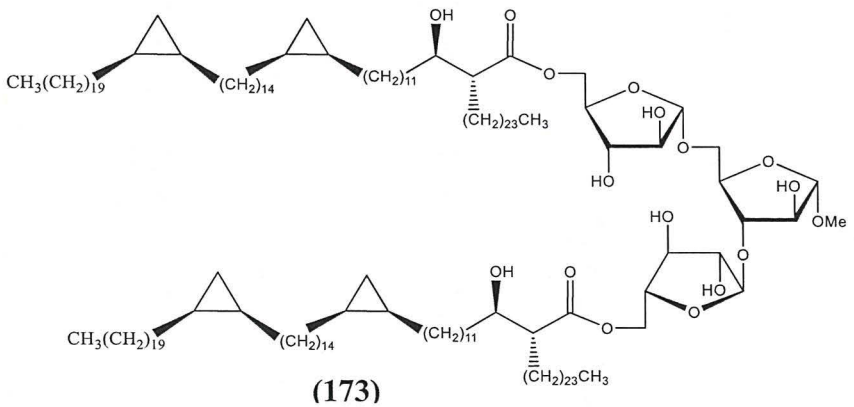


Figure 2.46: Description of the cytokines stimulation method

The first experiment carried out was the IL-6 secretion by wild-type BMDCs from mice using compounds **(84)**, **(96)**, **(91)** and **(173)** as antigens at 100 μg/ml, and isopropyl alcohol (ISO) as a solvent. Commercial TDM, LPS, and trehalose-6,6-dibehenate (TDB) [A synthetic analogue of trehalose-6,6-dimycolate (TDM, which is the most studied immunostimulatory component of *M. tuberculosis*] were used as a controls. **Figure 2.47** illustrates the results. Initial interpretation of these showed that compound **(96)** stimulated the BMDCs to produce IL-6 in a very high level, in comparison to LPS. Compounds **(84)** and **(173)** also showed a good level of stimulation, while for compound **(91)** no significant stimulation was observed.



(173)

Figure 2.48: Structure of compound (173)

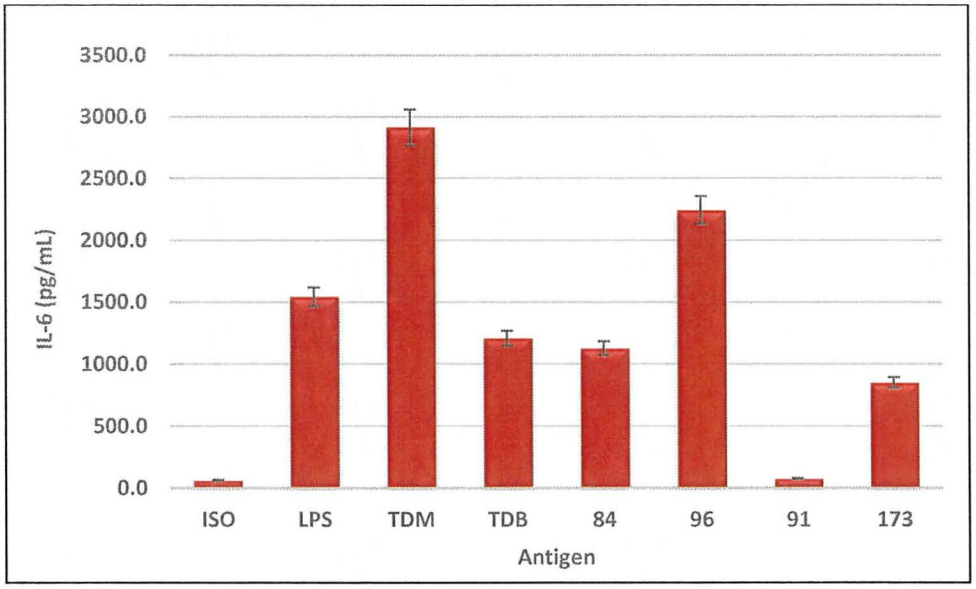


Figure 2.47: IL-6 secretion by wild-type BMDCs, stimulated with different synthetic glycolipids

The second experiment was IL-1β secretion by wild-type BMDCs (un-primed cells), and with an incubation time of 48 h. Compounds **(84)**, **(96)**, **(82)**, **(171)** and **(173)** were used as antigens at 100 μg/ml, and ISO was used as solvent. Commercial TDM, LPS, and TDB were used as controls. **Figure 2.49** illustrates the results.

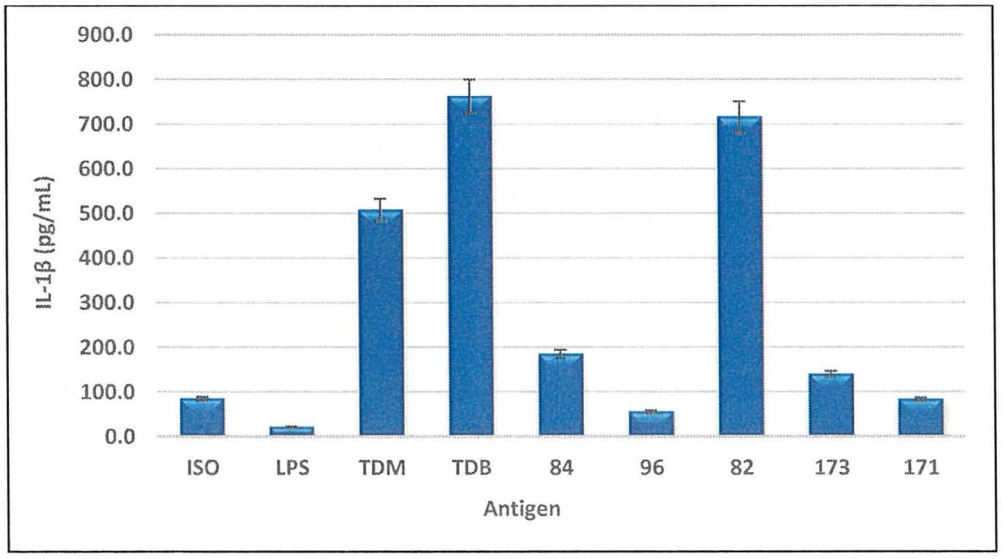


Figure 2.49: IL-1β secretion by un-primed BMDCs, 48 h incubation time

At the same time, some of the above compounds were also used in another experiment to measure the IL-1β secretion by wild-type BMDCs (primed cells), with an incubation time of 6 h. **Figure 2.50** demonstrates the results. In both experiments, there is a marked

difference in response between compounds **(82)** and **(96)**; compound **(82)** in the first experiment showed a very high response, while in the second experiment the response is lower, while for compound **(96)** the response changed in the opposite way.

Compound **(82)** and **(84)** are both arabinose mycolates of methoxy-MA with a difference in the α-branched chain, **(82)** having 22 carbons and **(84)** 24 carbons. The response of these two compounds was different, with a slightly higher level for **(82)**, and this may prove the effect of the α-chain length on the biological activity. The main compounds showed a varied response as shown in the charts. Compounds **(171)** and **(173)** are both tri-arabino-di-mycolates and it can be seen from the results that both compounds showed a lower response compared with the other compounds.

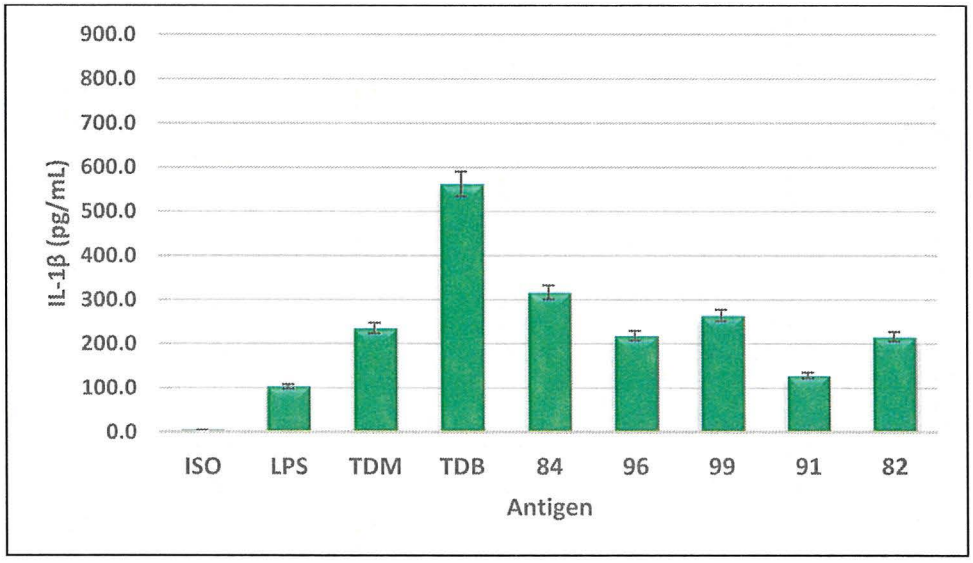


Figure 2.50: IL-1β secretion by primed BMDCs, 6 h incubation time

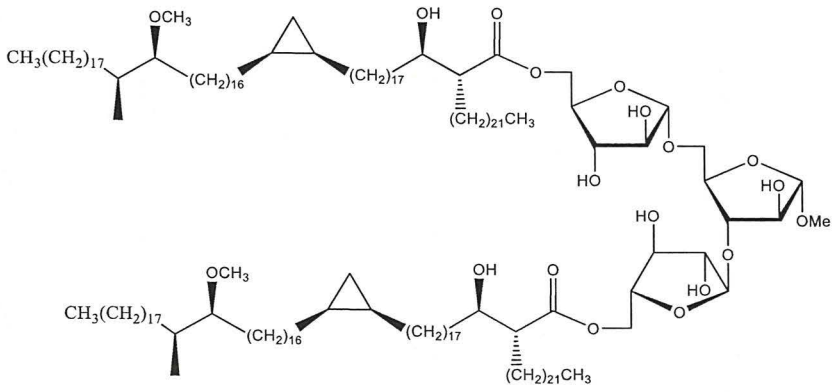


Figure 2.51: Structure of compound (171)

TNF-α secretion by wild-type BMDCs from mice using compounds **(82)**, **(87)**, **(91)**, **(126)** and **(173)** was carried out at 100 μg/ml, with ISO being used as solvent. Commercial TDM and LPS were again used as controls. **Figure 2.52** illustrates the results. Once again initial interpretation of the results showed that compound **(82)** stimulated the BMDCs to produce TNF-α in a very good level, as it did in the IL-1β experiments. Compound **(173)** again showed a low response as did all the other compounds used in the experiment.

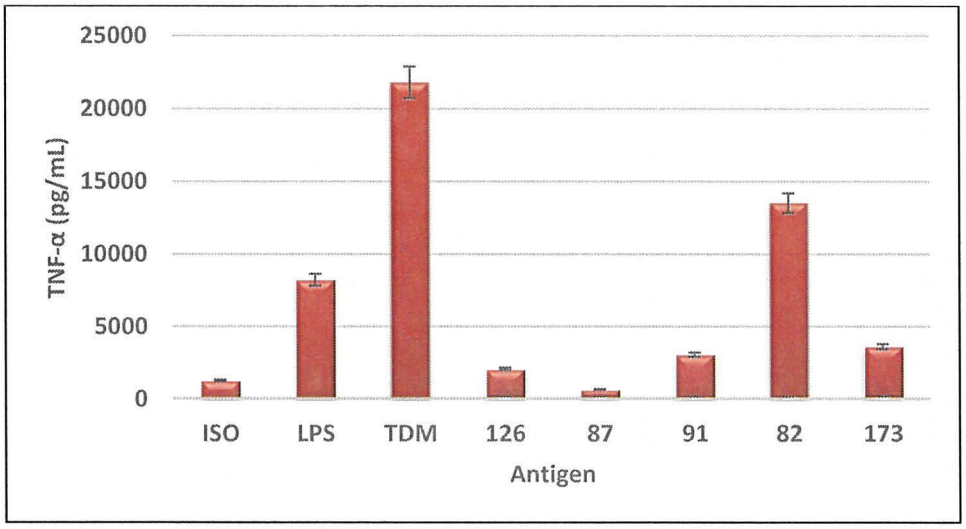


Figure 2.52: TNF-α secretion by wild type BMDCs, 48 h incubation time

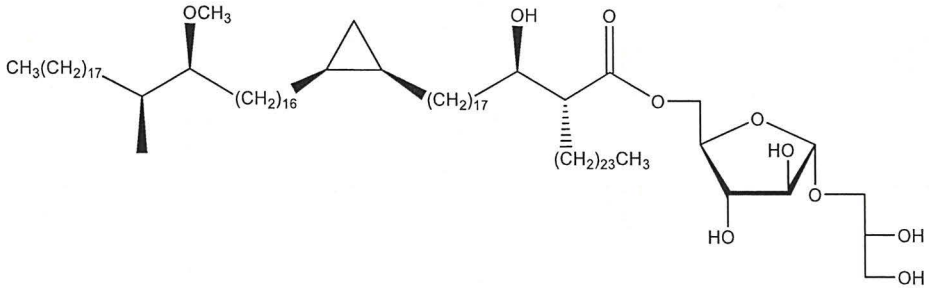


Figure 2.53: Structure of compound (126)

TNF-α secretion by wild-type BMDCs from mice using compounds **(177)**, **(131)**, **(129)** and **(126)** was carried out using 10 μg/ml of the antigens, and ISO as solvent. LPS was used as a control. **Figure 2.54** illustrates the results. All compounds showed a good level of stimulation.

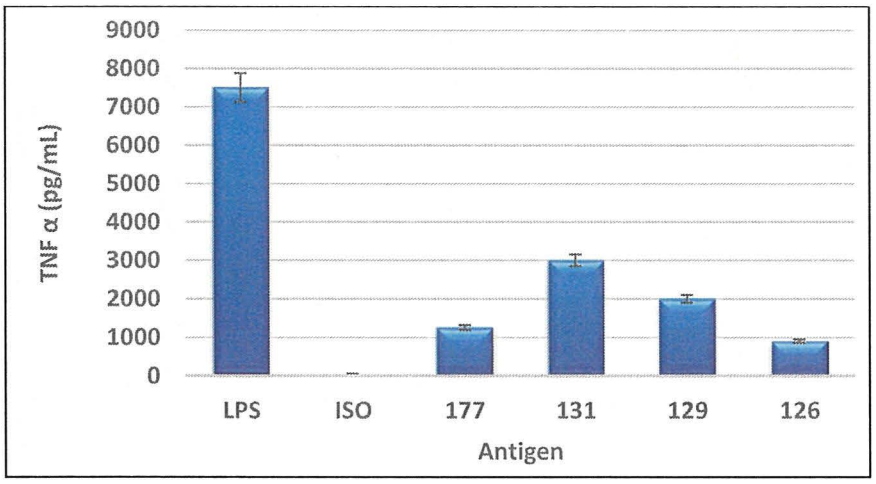


Figure 2.54: TNF-α secretion by wild type BMDCs, 6 h incubation time

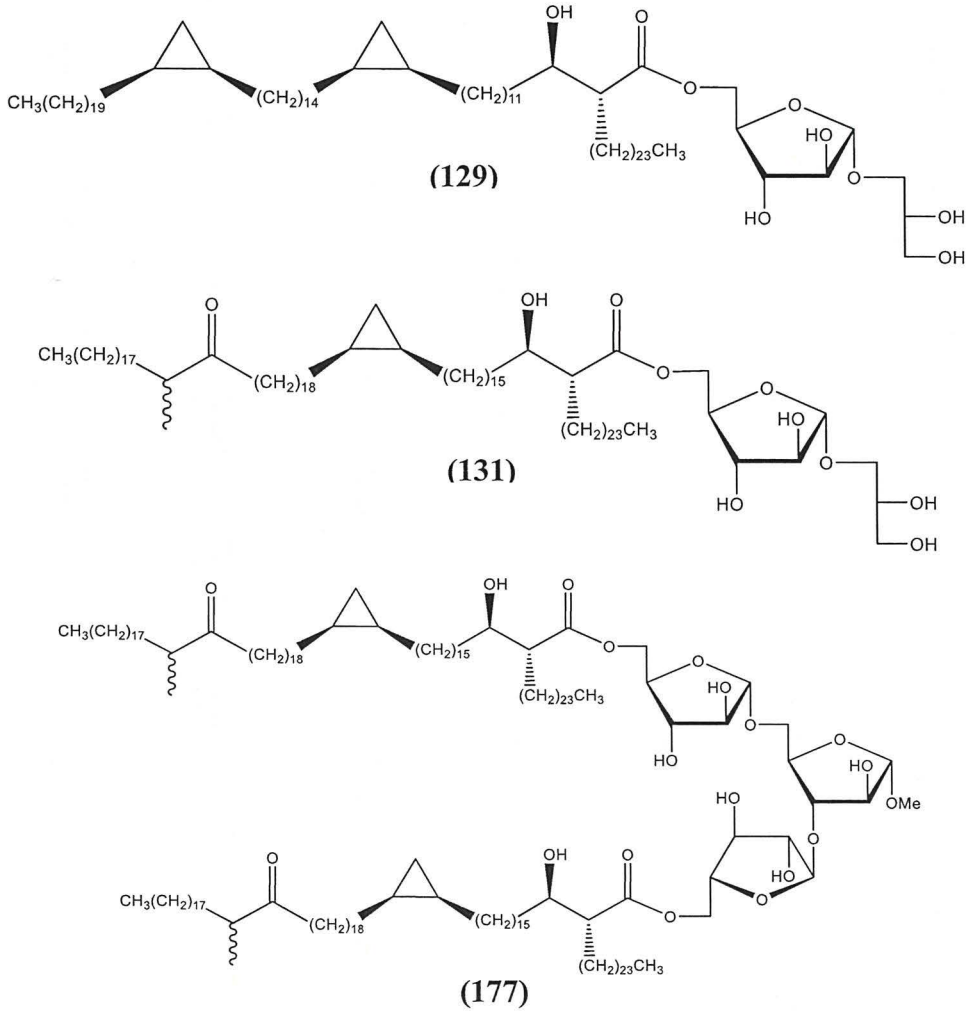


Figure 2.55: Structure of synthetic methyl arabino-mycolate types used for TNF-α secretion by wild-type BMDCs

The last series of compound tested for TNF-α secretion by wild-type BMDCs from mice were compounds **(137)**, **(175)**, **(140)**, **(142)**, **(96)**, **(84)**, **(82)** and **(169)**. The effects of these compounds have been compared to two compounds known to be strongly stimulatory:

(i) Zymosan, prepared from yeast cell wall and consists of protein-carbohydrate complexes. It is used to induce experimental sterile inflammation.

(ii) Curdlan, a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, produced by pure-culture fermentation from a non-pathogenic and non-toxicogenic such as *Agrobacterium biovar*.

Again the experiment were carried out using 100 μg/ml of the antigens. **Figure 2.56** illustrates the results. Some of the compounds showed a good level of stimulation.

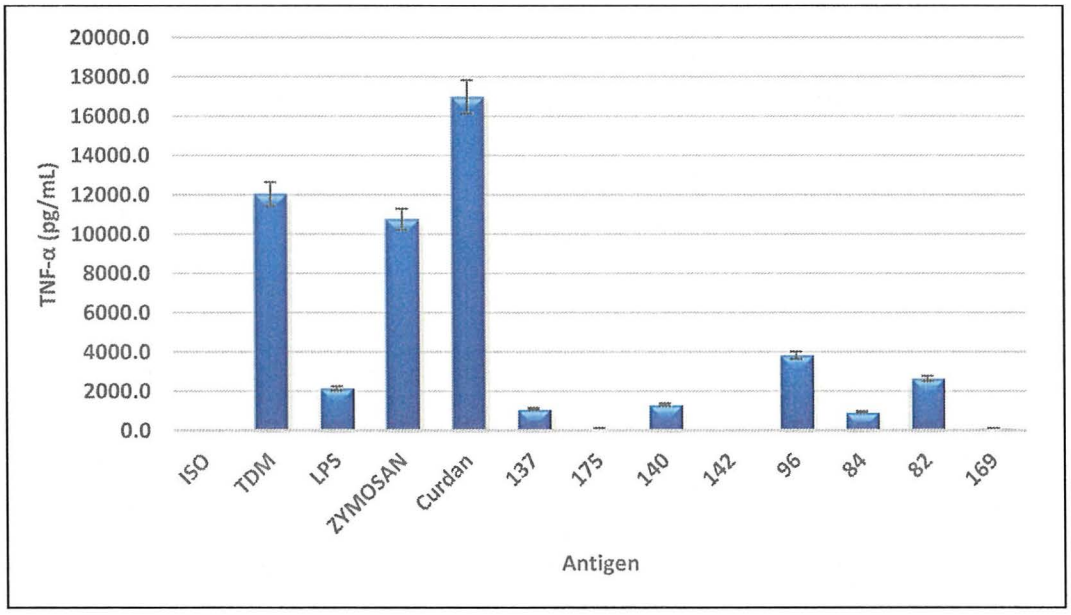


Figure 2.56: TNF-α secretion by wild type BMDCs

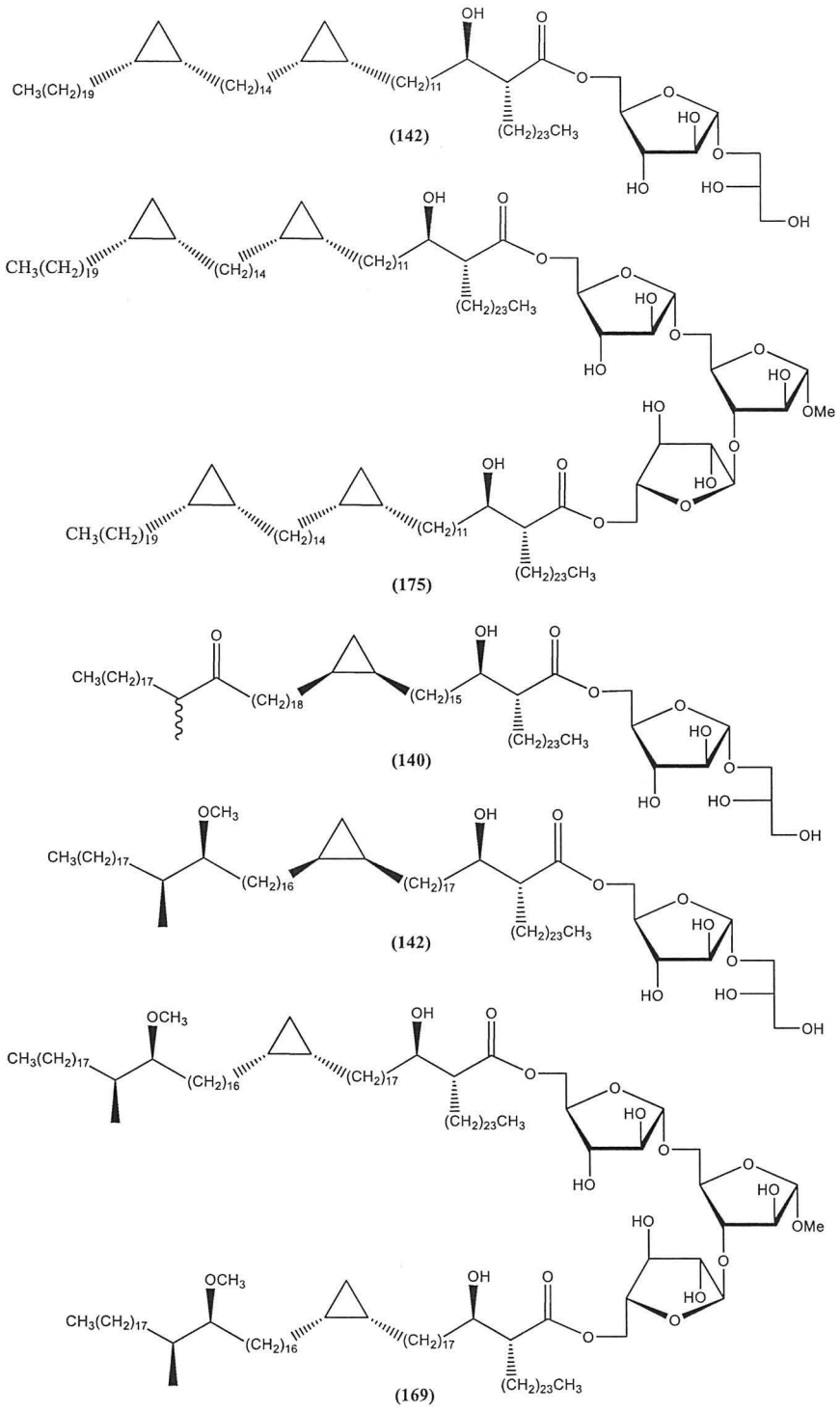


Figure 2.57: Structures of synthetic methyl arabino-mycolate types used for TNF-α secretion by wild-type BMDCs

A series of synthetic compounds **(96)**, **(91)**, **(84)**, **(171)**, **(173)**, **(82)**, **(87)** and **(99)** were also tested on bone marrow derived macrophages stimulation at a concentration of 100 μg/ml. After 24 h culture, supernatants were assessed for TNF-α and IL-6 production.

As can be seen, some of the compounds induce very good TNF levels. A selective stimulation by compound **(96)** was observed; this compound showed a high level response in the TNF-α production, but a low response in the IL-6 production. This result indicates clearly that synthetic single glycolipids shows a selectivity in the inducing of cytokines, while in the case of natural extracted glycolipids from the cell wall, which contain a complex mixture of MAs, this selectivity was not seen. Compound **(91)** showed the opposite activity of inducing TNF-α and IL-6 production **(Figures 2.58** and **2.59)**.

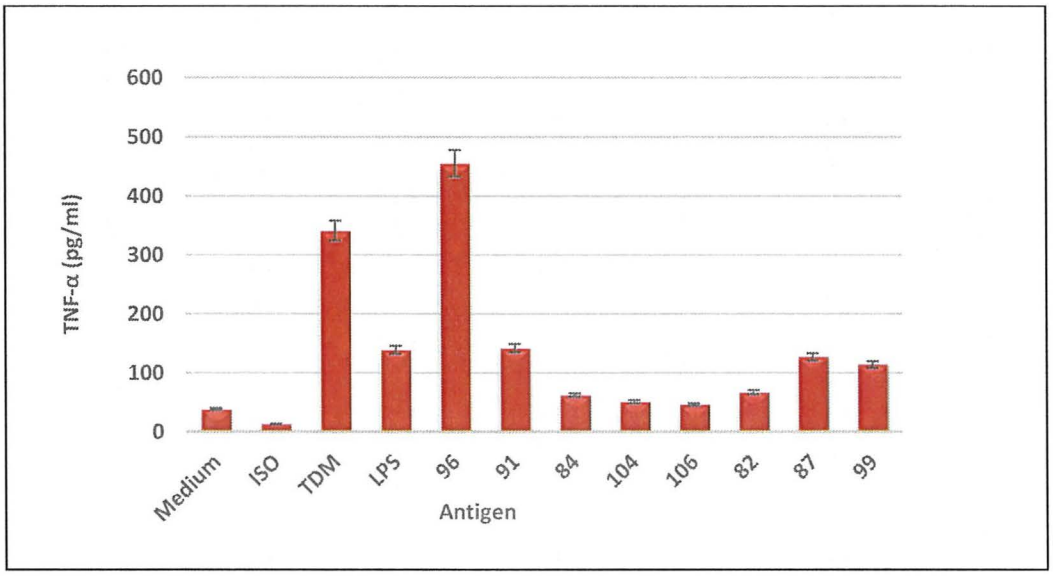


Figure 2.58: TNF-α secretion by un-primed BMDCs 24 h culture supernatants

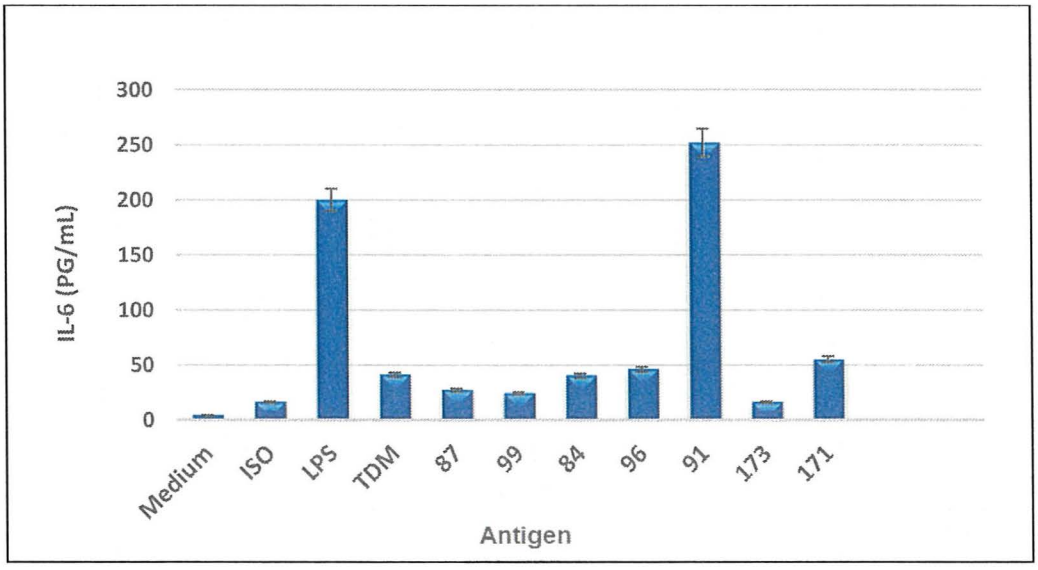


Figure 2.59: IL-6 secretion by un-primed BMDCs 24 h culture supernatants

In order to investigate the effect of the dose of the antigen on the response, one compound, **(96)**, was used to screen TNF-α in wild-type BMDCs. A range of concentrations were used (0.001 μg/mL - 200 μg/mL). **Figure 2.60** shows the results. The result, in comparison with a similar experiment on natural TDM **(Figure 2.61)**, proved that a concentration of 10 μg/mL of the antigen was effective.

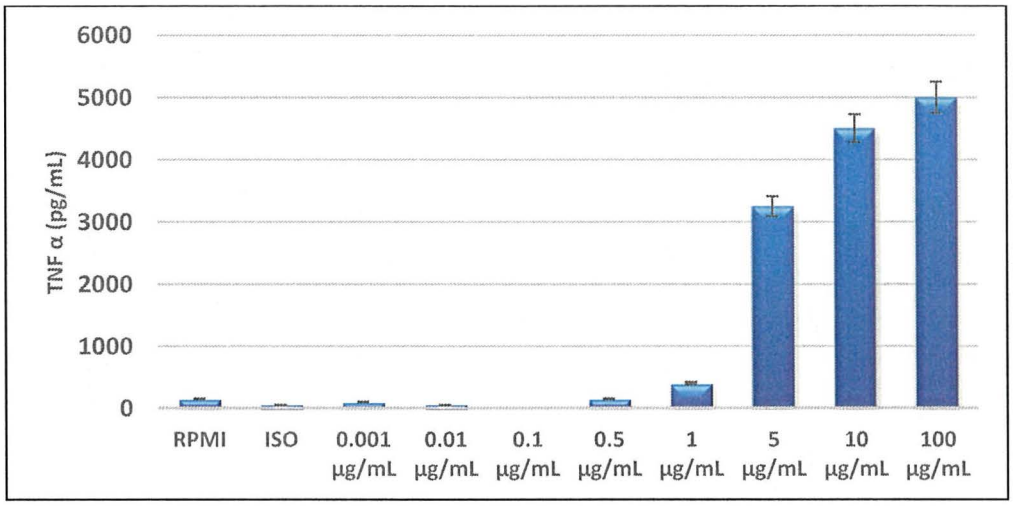


Figure 2.60: TNF-α secretion using a BMDCs, by different dose of compound (96)

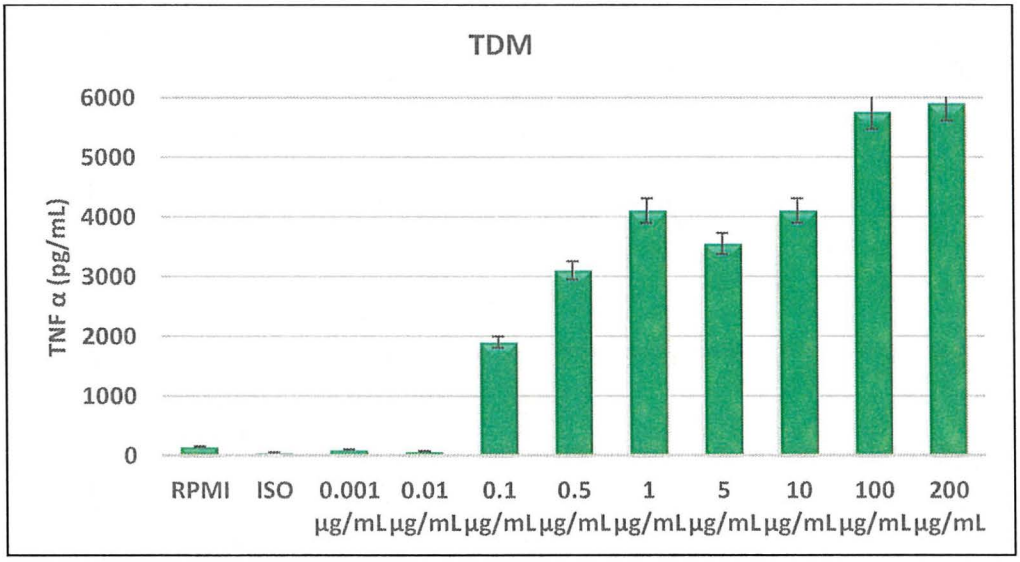


Figure 2.61: TNF-α secretion by BMDCs, using a different dose of TDM

2.5.3 C-type lectin receptors

The C-type lectin receptors (CLRs) include a huge family of receptors that bind to carbohydrates in a calcium-dependent manner. In general there are three main types of CLRs **(Figure 2.62)**.[299]

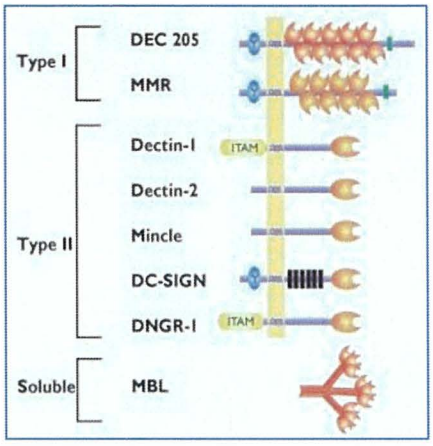


Figure 2.62: CLR Types.[299]

The macrophage-inducible C-type lectin (Mincle) receptor has been described as recognizing the TDM.[300] Analysis of the synthetic glycolipid's inflammatory power in Mincle KO mice compared to the WT BMDCs was tested. **Figure 2.63** illustrates the results. Compound **(96)** shows no significant difference between the WT and KO mice in the TNF-α production. This result proves that the glycolipid **(96)** does not bind to the to the Mincle receptor. This is very interesting, because it could indicate another pathway for this compound **(96)**, which does not involve Mincle receptor.

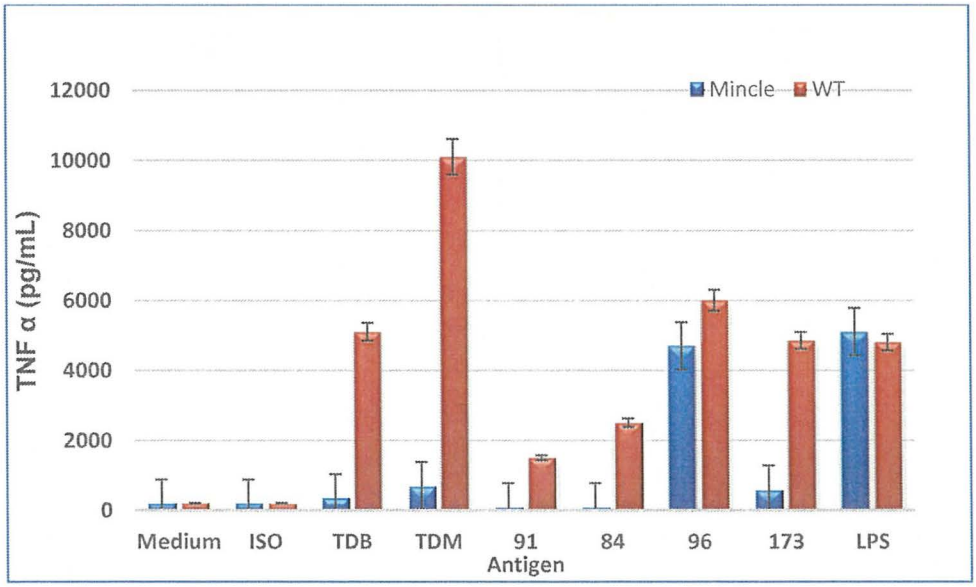


Figure 2.63: TNF-α secretion by Mincle KO mice compared to the WT BMDCs, 6h with 10μg/ml de glycolipids

The potential of the glycolipids in Myd88 KO mice was examined. As can be seen in **Figure 2.64**, responses are comparable in WT and Myd88 KO mice. Again compound **(96)** is active in both cases. As has been reported in the literature, in *M. tuberculosis* the activation pathway of both macrophages and dendritic cells involve the adaptor proteins Myd88.[53] This result is therefore very interesting, because it may indicate another pathway for this compound **(96)**, in a way which does not involve the Myd88 adaptor.

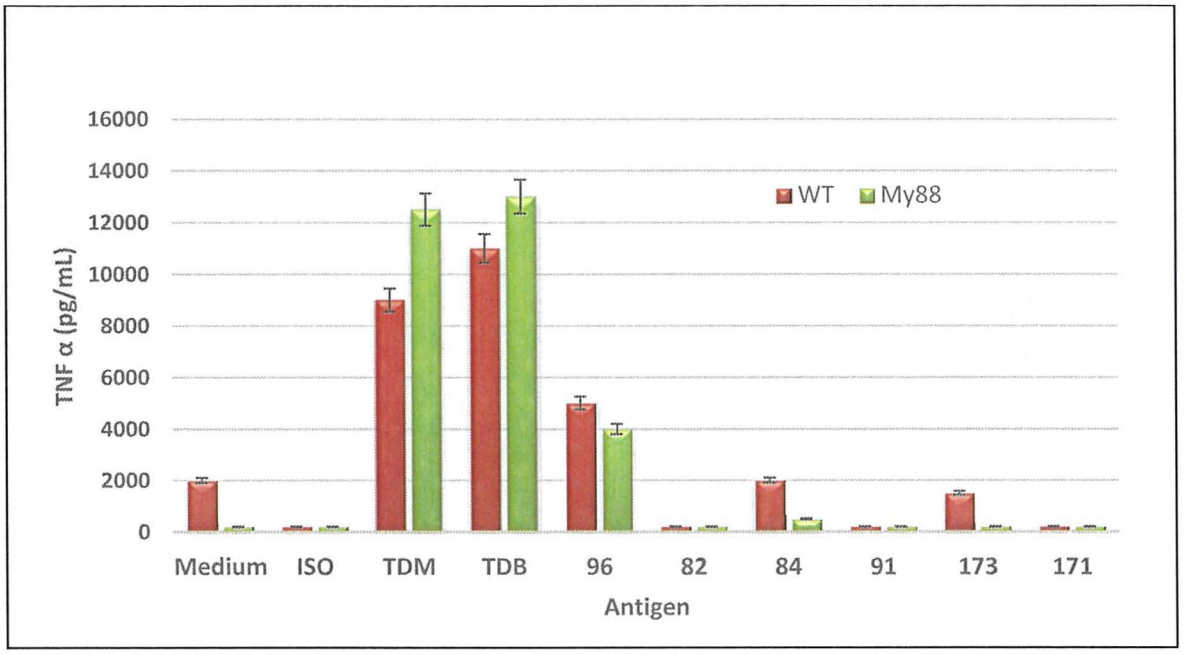


Figure 2.64: TNF-α secretion by MyD88 KO mice compared to the WT BMDCs, 6h with 100μg/ml from glycolipids

A new test (the MTT test) was also carried out, on compounds **(177)**, **(131)**, **(129)** and **(126)**. This allows detection of the toxicity of the glycolipids. As **Figure 2.65** shows, compound **(126)** is very toxic to cells. The MTT test is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (*e.g.* ISO), and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

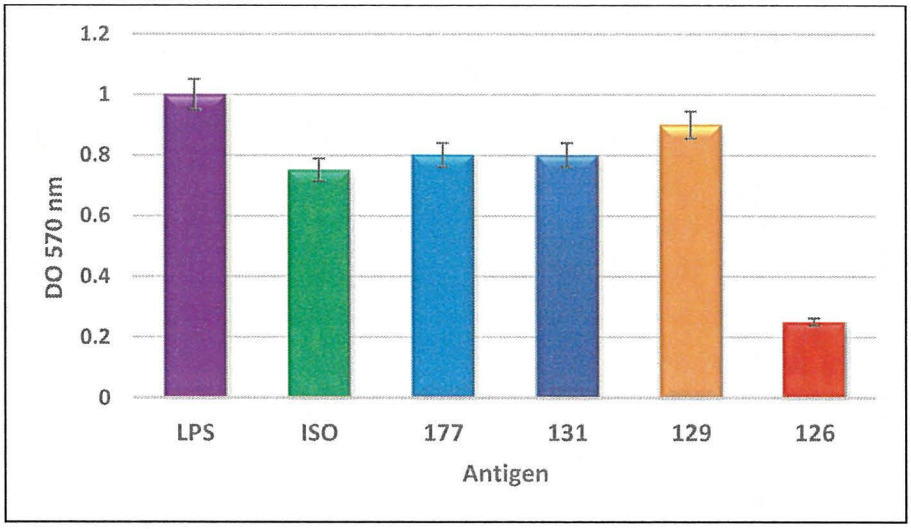


Figure 2.65: MTT experiment

In conclusion:

1- MAM compounds used as antigens in the ELISA assays showed a very good sensitivity and specificity. Further ELISA assays of the other synthetic arabino-mycolates are expected to be carried out in the near future.

2- A combination study using two different synthetic glycolipids, using a traffic light system in the ELISA assays was carried out, and promising results were obtained. This method could be useful to obtain a higher sensitivity and specificity than can be obtained by using one antigen alone. Further assays with more samples will need to be carried out to confirm that these results are true for a larger sample set.

3- Some of the MAM compounds showed a very selective and high level of stimulation of the BMDCs to produce cytokines such as TNF-α, IL-6 and IL-1β. Further testing of the effects of these glycolipids on a range of other cytokines is expected to be carried out.

4- Generally, GAM compounds showed a good stimulation of the BMDCs to produce different types of cytokines. ELISA assays for the detection of TB using these as antigens will be carried out in the future.

5- MTADM compounds generally showed a low stimulation of cytokines, this is in contrast to the other types of synthetic glycolipids, *i.e.* MAM and GAM. ELISA assays using these as antigens will be carried out in the future.

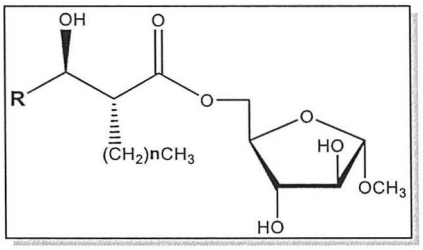
Chapter 3
Conclusion and Further Work

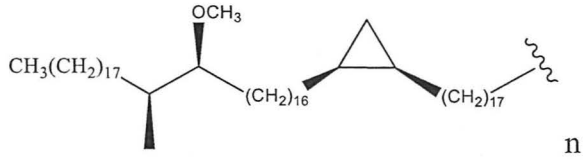
3.1 Conclusions

The aim of this project was the synthesis for the first time of single enantiomers of arabino-mycolates from structurally defined synthetic MAs. These compounds would then 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.

This work involved the successful synthesis of seven Methyl Arabino-Mycolates (MAM); six Glycerol-Arabino-Mycolates (GAM), which involved the two different stereochemistries of the glycerol component (D and L); five Methyl Tri-Arabino-Di-Mycolates (MTADM), and finally, the synthesis of the glycan moiety of Di-Mycolyl-Di-Ara*f*-Glycerol (DMAG) with the two different stereochemistries of the glycerol component, followed by an unsuccessful attempted esterification of the glycan with the MAs. All the synthetic MAs used in this project were provided by researchers within the group of Prof. M. S. Baird.

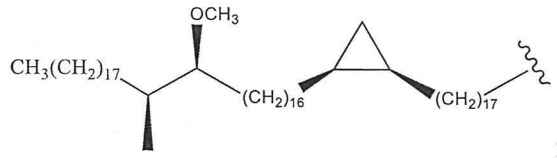
The aim of the first part of this project was the synthesis of MAM, which was achieved by firstly preparing the glycan moiety and using this to prepare four models of MAM. In this part, a series of seven compounds was then prepared based on the three common classes of MAs. The following compounds were successfully synthesised:





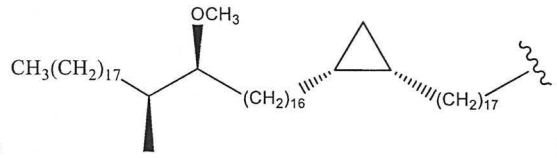
R = n = 21

Methyl Ara*f* Methoxy-MA-(21C) α-branched cyclopropane [(*R*, *S*)]. **(82)**



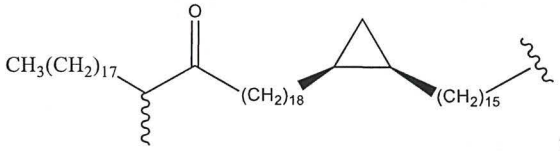
R = n = 23

Methyl Ara*f* Methoxy-MA-(24C) α-branched cyclopropane [(*R*, *S*)]. **(84)**



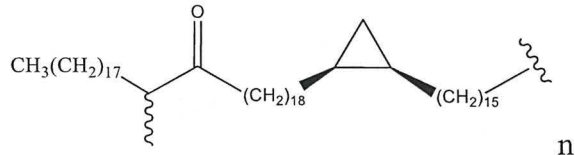
R = n = 21

Methyl Ara*f* Methoxy-MA-(22C) α-branched cyclopropane [(*S*, *R*)]. **(87)**



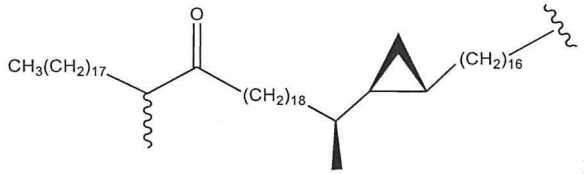
R = n = 21

Methyl Ara*f* Keto-MA-(22C) α-branched cyclopropane [(*R*, *S*)]. **(91)**



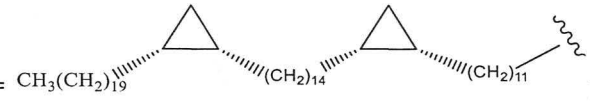
R = n = 23

Methyl Ara*f* Keto-MA-(24C) α-branched cyclopropane [(*R*, *S*)]. **(93)**



R = n = 23

Methyl Ara*f* Keto-MA-(24C) α-branched cyclopropane [(*S*, *R*)]. **(96)**



n = 23

Methyl Ara*f* α-MA-(24C) α-branched cyclopropane [(*S*,*R*), (*S*,*R*)]. **(99)**

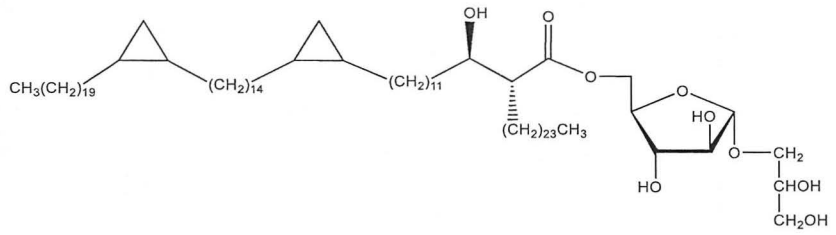
Synthesis of a series of *mono*-arabino-mycolates by using natural MA mixtures extracted from the cell wall has been reported. These glycolipids showed strong TNF-α inducing activity *in vitro*.

The above series of compounds were prepared from single synthetic enantiomers of MAs to investigate the differences in their biological activity, in contrast with the natural compounds. In addition, synthetic TDM and TMM showed a promising result in the ELISA assays for detection of TB. Therefore, the above series of glycolipids were used in different assays to test their biological activity.

As the initial biological and statistical analysis studies carried out on compounds in this part of the project, ELISA assays for detection TB showed significant sensitivity and specificity for some of the synthetic glycolipids antigens. By using a traffic light system, where samples that are detected TB+ by two antigens (a synthetic TDM and **99**) are found to be TB+, while all the rest are found to be TB-, a sensitivity and specificity of 100% and 94% respectively was observed. These values were better than those observed with a single antigen alone; this combination assay, using more than one antigen, may therefore prove useful for the future development of an assay for the detection of TB.

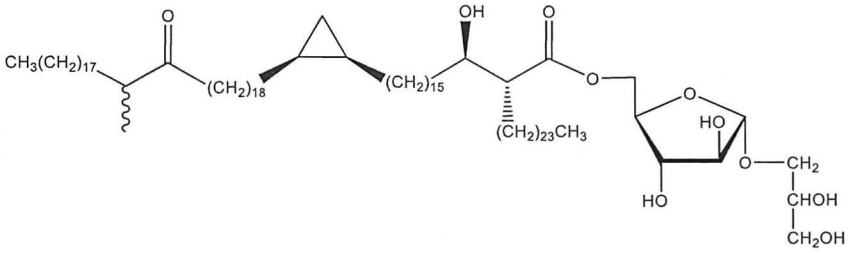
Most of these compounds showed a good response in the stimulation of the BMDCs derived from mice. For instance, compound **(96)** induced the cells to an even greater extent than natural TDM. This suggests that these glycolipids play an important role in the production of cytokines and could be more active than natural TDM against their production.

The second part of the project entailed the synthesis of GAM, through initial preparation of the glycan moieties, which required synthesis of both stereochemistries of the glycerol component (D and L or R and S), prior to their being appended to the arabinose ring to produce a single enantiomer of each arabino-glycerol compound. This was followed by the preparation for the first time of two models of D-GAM. In this part a series of six compounds (three derived from each type of glycerol-arabinose) was prepared based on the three common classes of MAs. The following compounds were successfully synthesised:

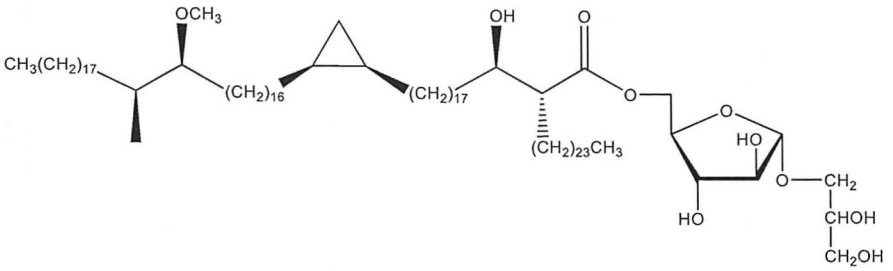


L-Glycerol,  , Araf α-MA-(24C) α-branched cyclopropane [(*S*,*R*), (*S*,*R*)]. **(142)**

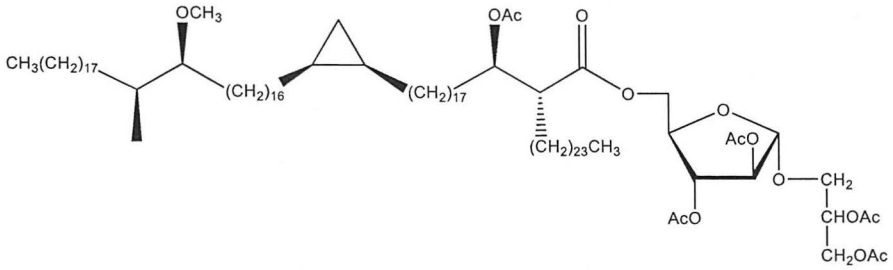
D-Glycerol,  , Araf α-MA-(24C) α-branched cyclopropane [(*R*,S), (*R*,*S*)]. **(129)**



L-Glycerol Araf Keto-MA-(23C) α-branched cyclopropane [(*R*, *S*)]. **(140)**
D-Glycerol Araf Keto-MA-(23C) α-branched cyclopropane [(*R*, *S*)]. **(131)**



L-Glycerol Araf Methoxy-MA-(24C) α-branched cyclopropane [(*R*, *S*)]. **(137)**
D-Glycerol Araf Methoxy-MA-(24C) α-branched cyclopropane [(*R*, *S*)]. **(126)**

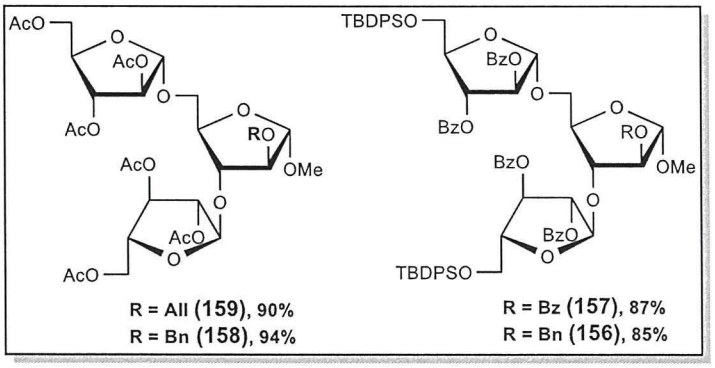


Penta-acetate-L-Glycerol Araf Methoxy-MA-(24C) α-branched cyclopropane [(*R*, *S*)]. **(138)**
Penta-acetate-D-Glycerol Araf Methoxy-MA-(24C) α-branched cyclopropane [(*R*, *S*)]. **(132)**.

The main aim of this part was to compare NMR data for the synthesised compounds with those reported for the naturally occurring compounds, this comparison ultimately proving the stereochemistry of the glycerol moiety in the natural product to be *L* not *D*. The NMR data for the penta – acetate derivatives of compounds **(126)** and **(137)** were compared directly with those in the literature for the per-acetate of the natural GAM.

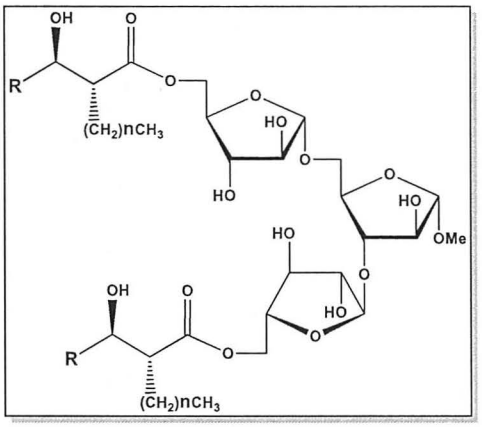
The biological activity of some of the compounds prepared in this part of the project showed a good induction of the BMDCs. Further testing and assays of the effects of these glycolipids on a range of cytokines involved in the immune system, together with ELISA assays for detection of TB, are expected to be carried out in the near future, which will give a further insight into the preliminary results discussed above.

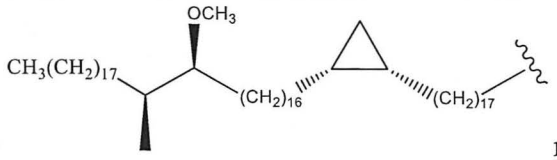
The third part of this project involved the synthesis of MTADM. This part started by preparing the tri-Ara*f*, which involved preparing both the donor and the acceptor according to the literature methods, with slight modifications in some of the steps. Coupling the donor and the acceptor to prepare the desired glycan was carried out using known conditions. Four types of tri-Ara*f*, which contained two α- glycoside linkages in their forms, were prepared, one as in the literature **(156)**, and the others differing in the protecting group on the acceptor **(157)**, in order to investigate the effect of this variation on the yield of the coupling. The yield was found to be similar to that reported in the literature for compound **(156)**; however, for compounds **(158)** and **(159)**, the yields were higher.



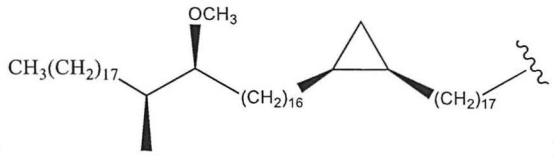
Having the glycan unit, the preparation of a model through esterifying with a commercially available fatty acid was carried out. This glycolipid was reported in the literature, and the data obtained was identical to the authentic sample, however this model was prepared by a different method. A series of five compounds from MTADM

was prepared based on the three common classes of MAs. The following compounds were successfully synthesised:

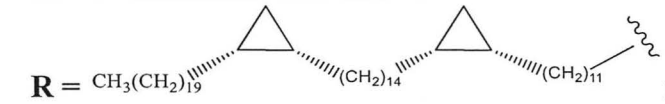


R =  n = 23

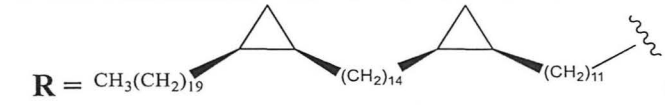
Methyl Tri-Ara*f*-Di-Methoxy-MA-(24C) α-branched cyclopropane [(*S*, *R*)]. **(169)**

R =  n = 21

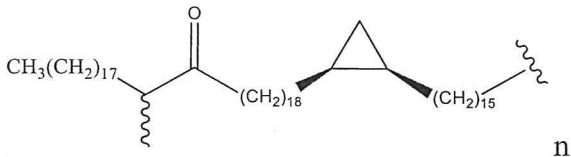
Methyl Tri-Ara*f*-Di-Methoxy-MA-(22C) α-branched cyclopropane [(*R*, *S*)]. **(104)**

R =  n = 23

Methyl Tri-Ara*f* Di-α-MA-(24C) α-branched cyclopropane [(*S*,*R*), (*S*,*R*)]. **(108)**

R =  n = 23

Methyl Tri-Ara*f* Di-α-MA-(24C) α-branched cyclopropane [(*R*,*S*), (*R*,*S*)]. **(106)**



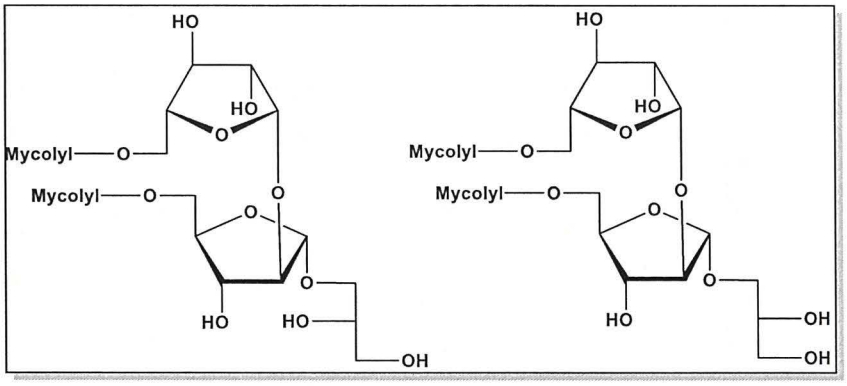
R = n = 23

Methyl Tri-Ara*f* Di-Keto-MA-(24C) α-branched cyclopropane [(*R*, *S*)]. **(110)**

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 compounds, from single enantiomers of MAs, will therefore allow the effects of the individual components to be investigated.

This series of compounds, however, showed low responses in the cytokine stimulation compared with the other two series of synthetic compounds. The appraisal of the ELISA assay for detection of TB employing these compounds is in process.

The final part of this project concerned the synthesis of DMAD:



In this part of the project we successfully prepared the glycan moiety of DMAG with the two possible enantiomers of the desymmetrised glycerol unit. A study to investigate the effect of the protecting group on the yield and the β-selectivity of the glycosylation was carried out, and we successfully developed an efficient route to prepare the DMAG glycan as a single anomer in excellent yield. This was achieved by having a PMB protecting group on the C3 and C5 positions in the acceptor and an 'armed' donor. The synthesis of this compound has not previously been reported.

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 glycans will therefore allow their biological activity to be investigated.

3.2 Further Work

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

1. It has been proved that the DMAG was obtained from the cell wall of several mycobacterial species, including *M. tuberculosis,* and studies on this glycolipid showed that it possess very high biological activity. Initial attempts to couple the DMAG glycan with the MAs was not successful. Hence it will be worth preparing a large amount of the DMAG glycan and finding an efficient way of coupling it with synthetic MAs, in order to obtain a range of compounds and test their biological activity.
2. Natural Arabino-mycolates extracted from the cell wall of mycobacteria were obtained in a very complex mixture of MAs, hence preparing MTADM and DMAG 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.
3. Furthermore, determining the adjuvant activity of Arabino-mycolates is of major importance as, until the present work, no single enantiomers have been synthesised and thus the effects of single structures over a diverse range of chemokines-cytokines could not be appraised. Further synthetic compounds must be prepared and tested in order to have a full picture on how this family of compounds interacts with the human body and immune system. Their activity is not only restricted to immunity, but also with cancer and viral infections due the fact that these compounds are potentially immune system busters.
4. Finally, another item of further work which must be done, is the synthesis of Penta-Ara*f* Tetra-Mycolates. 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
Experiments

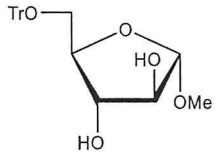
4.1 General considerations

All chemicals were purchased from Aldrich Chemical Co. Ltd, Lancaster Synthesis Ltd, or Avocado Chemical Co. Ltd. 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 sulphate and solvents were removed at 14 mmHg; residual traces of solvent were finally removed at 0.1 mmHg. All glassware used in anhydrous reactions was dried for not less than 6 h in a 250 °C oven.

Column chromatography was conducted under medium pressure using silica gel (BDH, particle size 33–70 mm); TLC was carried out on pre-coated Kieselgel 60 F254 (Art. 5554; Merck) plates. Optical rotations were measured as solutions in chloroform of known concentration using a Polar 2001 automatic polarimeter. Melting points were measured using a Gallenkamp melting point apparatus. Infra-red spectra were recorded as KBr discs (solids) or thin films on NaCl windows or using a Perkin Elmer 1600 series FT-IR spectrometer. NMR spectra were recorded on Bruker Avance (500 and 400) spectrometer in $CDCl_3$ or CD_3OD if not differently indicated. 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 spectra were obtained using a Bruker MicroTOF time of flight mass spectrometer with an ESI source. Matrix assisted laser desorption ionisation (MALDI) were obtained using a Bruker daltonics *Reflex IV*. A laboratory book was filled in including chemical safety information following COSHH regulations.

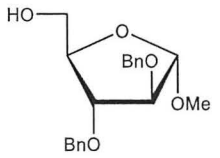
4.2 Experiments

Experiment 1: Methyl 5-*O*-trityl-α-D-arabinofuranoside (68) [263]



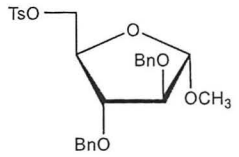
Freshly prepared HCl solution in MeOH (resulting from mixing acetyl chloride (2 mL) in MeOH (30 mL) at 0 °C was added to a stirred solution of D-(-) arabinose (5.0 g, 33 mmol) in anhydrous MeOH (100 mL). Stirring was continued overnight at room temperature, after that a clear solution was obtained. The mixture was neutralized by adding pyridine to pH 7-8. The solvent was evaporated to give a residue. The crude product was purified by column chromatography eluting with chloroform: acetone (3:5) to give a colourless oil of methyl α, β-D-arabinofuranoside **(66)** (4.3 g, 78%) in a ratio (α : β; 3:2). Trityl chloride (6.42 g, 23.0 mmol) and DMAP (2.57 g, 21.0 mmol) were added to a stirred solution of methyl–α,β-D-arabinofuranoside **(66)** (3.12 g, 19.0 mmol) in anhydrous pyridine (60 mL) and the mixture was stirred at room temperature for 16 h then at 70 °C for 4 h The mixture was cooled to room temperature and poured into ice/water (290 mL), after the ice melted the organic phase was separated and the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with aqueous $NaHCO_3$ solution 5% (100 mL), dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting started from 10% to 5% hexane/ethyl acetate to give a colourless oil of 5-*O*-trityl-β-D-arabinofuranoside **(69)** (2.3 g, 29%) and methyl 5-*O*-trityl-α-D-arabinofuranoside **(68)** (3.3 g, 42%); m.p.(α-anomer) 112-113 °C (*lit.*[264] 113-114 °C) [Found (MALDI) $(M+Na)^+$: 429.3, $C_{25}H_{26}NaO_5$, requires: 429.1], $[\alpha]_D^{20}$ + 83 (*c* 0.1, $CHCl_3$) [*lit.*[264] $[\alpha]_D^{24}$ + 85.9 (*c* 1.06, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.42 (6H, br. d, *J* 8.0 Hz), 7.34 (6H, br. t, *J* 7.5 Hz), 7.27 (3H, br. t, *J* 7.1 Hz), 5.01 (1H, s), 4.12 (1H, s), 3.99 (1H, d, *J* 11.0 Hz), 3.89 (1H, d, *J* 11.0 Hz), 3.79 (1H, d, *J* 11.0 Hz), 3.67 (1H, dd, *J* 2.1, 10.5 Hz), 3.42 (3H, s), 3.27 (1H, d, *J* 10.5 Hz), 2.80 (1H, d, *J* 11.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 142.8, 128.8, 128.0, 127.4, 109.3, 78.8, 78.3, 63.4, 54.9; ν_{max}: 3399, 3050, 2927, 2930, 2858, 1445, 633 cm^{-1}. All data were identical to the authentic sample

Experiment 2: Methyl 2,3-di-*O*-benzyl-α-D-arabinofuranoside (70) [264,242]



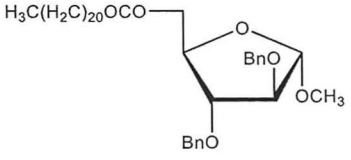
A solution of methyl 5-*O*-trityl-α-D-arabinofuranoside **(66)** (3.20 g, 7.87 mmol) in dry DMF (80 mL) was added dropwise to a stirred suspension solution of NaH (0.84 g, 35 mmol) (60% w/w, dispersion in mineral oil, washed with petrol for three times) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then benzyl bromide (2.5 mL, 3.6 g, 21 mmol) in dry DMF (50 mL) was added. The mixture was stirred at room temperature for 20 h and then quenched by slow addition of water (15 mL) and diluted with ether (25 mL). The organic layer was separated and the aqueous layer was extracted with ether (2×100 mL). The combined extracts were washed with water (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure. Aqueous acetic acid (80%) (30 mL) was added to the crude product and the mixture was stirred and heated at 75 °C for 4 h., the reaction mixture was diluted with water (20 mL) and ether (20 mL) and the organic layer was separated and the aqueous layer was extracted with ether (2×100 mL). The combined organic layer were washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and the solvent evaporated, the residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (7:3) to give methyl 2,3-di-*O*-benzyl-α-D-arabinofuranoside **(70)** as a thick oil (2.1 g, 78%) [Found (MALDI) $(M+Na)^+$: 367.3, $C_{20}H_{24}NaO_5$, requires: 367.1], $[\alpha]_D^{22}$ + 89 (*c* 0.1, $CHCl_3$) [*lit.*[264] $[\alpha]_D^{24}$ + 83.2 (*c* 1.14, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.28 (10H, m), 4.95 (1H, s), 4.61 (1H, d, *J* 12.0 Hz), 4.54 (1H, d, *J* 12 Hz), 4.53 (1H, d, *J* 12 Hz), 4.50 (1H, d, *J* 12 Hz), 4.18 – 4.12 (1H, m), 4.02 – 3.96 (2H, m), 3.85 (1H, dd, *J* 2.8, 12.1 Hz), 3.65 (1H, dd, *J* 4.1, 12.1 Hz), 3.40 (3H, s), 2.93 (1H, br. s); δ_C (101 MHz, $CDCl_3$): 137.6, 137.2, 128.4, 128.37, 127.9, 127.86, 127.8, 127.77, 107.3, 87.7, 82.5, 82.3, 72.3, 71.8, 62.1, 54.8; ν_{max}: br. 3466, 3089, 3064, 3031, 2925, 1725, 1605, 1454, 696, 739 cm^{-1}. All data were identical to the authentic sample.

Experiment 3: Methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabino-furanoside (71) [242]



p-Toluene sulfonyl chloride (0.60 g, 3.15 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-α-D-arabinofuranoside **(70)** (0.50 g, 1.45 mmol), pyridine (1 mL, 12.6 mmol) and DMAP (catalytic amount) in dry CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred and the temperature was allowed to rise to room temperature, the stirring was continued overnight. TLC showed no starting material was left and the reaction mixture was diluted with ethyl acetate (10 mL), the organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×100 mL). The combined organic layers were washed with water (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated, the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (4:1) affording methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(71)** (0.65 g, 90%) [Found (MALDI) $(M+Na)^+$: 521.0, $C_{27}H_{30}NaO_7$, requires: 521.1], $[\alpha]_D^{23}$ + 55 (*c* 0.1, $CHCl_3$) [*lit.*[242] $[\alpha]_D^{27}$ + 57 (*c* 0.4, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.8 (2H, d, *J* 8.2 Hz), 7.41 – 7.25 (12H, m), 4.85 (1H, s), 4.53 (1H, d, *J* 12.0 Hz), 4.47 (1H, d, *J* 12 Hz), 4.44 (1H, d, *J* 12 Hz), 4.41 (1H, d, *J* 12 Hz), 4.22 – 4.15 (1H, m), 4.12 (2H, br. d, *J* 4.6 Hz), 3.94 (1H, br. d, *J* 2.6 Hz), 3.81 (1H, dd, *J* 2.7, 5.9 Hz), 3.33 (3H, s), 2.42 (3H, s); δ_C (101 MHz, $CDCl_3$): 144.8, 137.4, 137.2, 132.8, 129.8, 128.5, 128.0, 127.95, 127.9, 127.87, 127.8, 107.4, 87.5, 82.8, 79.2, 72.3, 71.9, 68.9, 55.1, 21.6; ν_{max}: 3064, 3032, 2916, 1741, 1454, 1365, 1177, 815 cm^{-1}. All data were identical to the authentic sample.

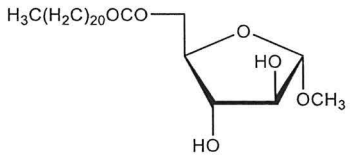
Experiment 4: Methyl 2,3-di-*O*-benzyl-5-*O*-behenoyl-α-D-arabinofuranoside (72)



A solution of *N,N`*-dicyclohexyl carbodimide[288] (0.089 g, 0.432 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise to a stirred solution of methyl 2,3-di-*O*-benzyl-α-D-arabino-furanoside **(70)** (0.10 g, 0.29 mmol), DMAP (0.042 g, 0.034 mmol) and behenic acid

(0.10 g, 0.29 mmol) in dry CH_2Cl_2 (1 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 30 minutes then TLC showed no starting material was left. The precipitated 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 (10:1) to afford methyl 2,3-di-*O*-benzyl-5-*O*-behenoyl-α-D-arabinofuranoside **(72)** (0.15 g, 80%) [Found (MALDI) $(M+Na)^+$: 689.5, $C_{42}H_{66}NaO_6$, requires: 689.4], $[\alpha]_D^{24}$ + 40 (*c* 0.1, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$): 7.41 – 7.28 (10H, m), 4.95 (1H, br. s), 4.59 (1H, d, *J* 12.0 Hz), 4.57 (1H, d, *J* 12 Hz), 4.52 (1H, d, *J* 12 Hz), 4.49 (1H, d, *J* 12 Hz), 4.29 (1H, dd, *J* 2.8,11.3 Hz), 4.21 (1H, ddd, *J* 3.0, 6.0, 11.5 Hz), 4.18 (1H, dd, *J* 5.6, 11.2 Hz), 4.01 (1H, dd, *J* 1.1, 2.9 Hz), 3.84 (1H, dd, *J* 2.9, 6.3 Hz), 3.40 (3H, s), 2.33 – 2.26 (2H, t, *J* 7.5 Hz), 1.64 – 1.52 (2H, m), 1.34 – 1.18 (36H, m), 0.89 (3H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 137.5, 137.4, 128.45, 128.4, 127.9, 127.86, 107.3, 87.96, 83.4, 79.4, 72.3, 72.1, 63.5, 55.0, 34.1, 31.9, 29.7, 29.66, 29.6, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1; ν_{max}: 3034, 2915, 2849, 1741, 1471, 1100, 696, 758 cm^{-1}.

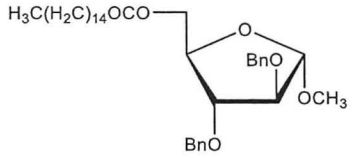
Experiment 5: Methyl 5-*O*-behenoyl-α-D-arabinofuranoside (73)



Palladium hydroxide[288] on activated charcoal (20% $Pd(OH)_2$-C, 2.60 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-behenoyl-α-D-arabinofuranoside **(72)** (0.01 g, 0.01 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h. when TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with CH_2Cl_2 (10 mL), the filtrate was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to afford methyl 5-*O*-behenoyl-α-D-arabinofuranoside **(73)** (6.0 mg, 82%) [Found (MALDI) $(M+Na)^+$: 509.5, $C_{28}H_{54}NaO_6$, requires: 509.3], $[\alpha]_D^{22}$ + 46 (*c* 0.1, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$ + few drops CD_3OD): 4.92 (1H, s), 4.32 – 4.29 (2H, m), 4.22 (1H, dd, *J* 3.9, 7.1 Hz), 4.09 (1H, br. s), 3.90 (1H, br. s), 3.42 (3H, s), 2.81 – 2.67 (1H, m), 2.51 – 2.39 (1H, m), 2.38 – 2.32 (2H, t, *J* 7.5 Hz), 1.62 (2H, p, *J* 7.5 Hz), 1.35 – 1.20 (36H, m), 0.89 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops

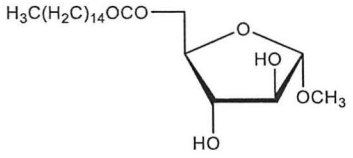
CD_3OD): 173.5, 108.8, 83.8, 79.9, 78.0, 63.8, 55.1, 34.1, 31.9, 29.7, 29.6, 29.58, 29.4, 29.3, 29.2, 29.1, 24.8, 22.7, 14.1; ν_{max}: br. 3500, 2917, 2850, 1739, 1464, 1100, 758 cm^{-1}.

Experiment 6: Methyl 2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranoside (74)



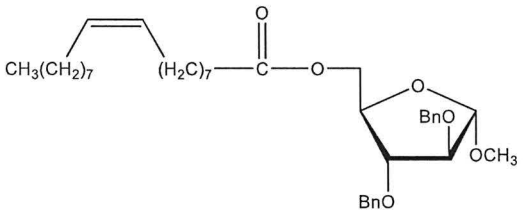
Cesium hydrogencarbonate[242] (0.194 g, 0.001 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(71)** (0.1 g, 0.2 mmol) and palmitic acid (0.061 g, 0.237 mmol) in dry DMF : THF (1:5, 5 mL) at room temperature and the reaction mixture was stirred at 70 °C for two 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 (2×10 mL). The combined organic layers were washed with water (15 mL) and brine (15 mL), dried over $MgSO_4$, filtered and evaporated to give a thick oil residue. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to afford methyl 2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranoside **(74)** (88.6 mg, 76%) [Found (MALDI) $(M+Na)^+$: 605.1, $C_{36}H_{54}NaO_6$, requires: 605.3], $[\alpha]_D^{22}$ + 37 (*c* 0.1, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.95 (1H, s), 4.59 (1H, d, *J* 12.0 Hz), 4.57 (1H, d, *J* 11.7 Hz), 4.52 (1H, d, *J* 12.0 Hz), 4.49 (1H, d, *J* 11.8 Hz), 4.29 (1H, dd, *J* 2.9, 11.3 Hz), 4.21 (1H, ddd, *J* 3.1, 5.7, 11.6 Hz), 4.18 (1H, dd, *J* 5.6, 11.2 Hz), 4.01 (1H, dd, *J* 1.2, 3.0 Hz), 3.84 (1H, dd, *J* 2.9, 6.2 Hz), 3.40 (3H, s), 2.32 – 2.27 (2H, m), 1.64 – 1.54 (2H, p, *J* 7.5 Hz), 1.26 (24H, s), 0.89 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 137.5, 137.4, 128.5, 128.4, 127.9, 127.87, 107.3, 87.97, 83.4, 79.4, 72.3, 72.1, 63.5, 55.0, 34.1, 31.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1; ν_{max} : 3064, 3032, 2924, 2853, 1740, 1455, 1365, 1107, 696, 735 cm^{-1}.

Experiment 7: Methyl 5-*O*-palmitoyl-α-D-arabinofuranoside (75)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 0.01 g , 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranoside (**74**) (0.06 g, 0.11 mmol) in dry CH_2Cl_2 : MeOH, (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h. when TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with CH_2Cl_2 (10 mL), the filtrate was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to afford methyl 5-*O*-palmitoyl-α-D-arabinofuranoside (**75**) (0.04 g, 90%) $[\alpha]_D^{18}$ + 1.15 (*c* 0.1, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 425.5, $C_{22}H_{42}NaO_6$, requires : 425.2] which showed δ_H (500 MHz, $CDCl_3$ + few drops CD_3OD): 4.93 (1H, s), 4.33 – 4.30 (2H, m), 4.22 (1H, dd, *J* 3.9, 6.9 Hz), 4.09 (1H, br. s), 3.91 (1H, br. s), 3.43 (3H, s), 2.35 (2H, t, *J* 7.6 Hz), 1.67 – 1.60 (2H, m), 1.56 (2H, br. s), 1.36 – 1.21 (24H, m), 0.89 (3H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 173.5, 108.8, 83.8, 79.9, 78.0, 63.8, 55.1, 34.1, 31.9, 29.7, 29.64, 29.6, 29.4, 29.3, 29.2, 29.1, 24.8, 22.7, 14.1; ν_{max}: br. 3308, 2916, 2848, 1742, 1472, 1099, 728 cm^{-1}.

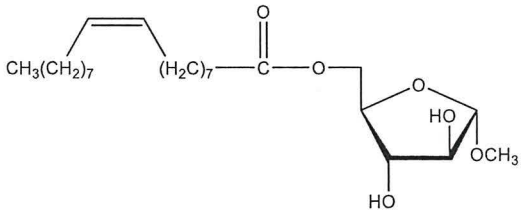
Experiment 8: Methyl 2,3-di-*O*-benzyl-5-*O*-oliyl-α-D-arabinofuranoside (76)



Cesium hydrogencarbonate (0.47 g, 2.47 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (**71**) (0.26 g, 0.53 mmol) and oleic acid (0.10 g, 0.35 mmol) in dry DMF:THF (1:5, 2 mL) at room temperature and the mixture was stirred at 70 °C for two days. The reaction mixture was worked up and purified as before to give methyl 2,3-di-*O*-benzyl-5-*O*-oliyl-α-D-arabinofuranoside (**76**) (0.18 g, 83%) [Found (MALDI) $(M+Na)^+$: 631.3, $C_{38}H_{56}NaO_6$, requires: 631.4], $[\alpha]_D^{22}$ + 45 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 5.41 – 5.30 (2H, m), 4.95 (1H, s), 4.59 (1H, d, *J* 12.0 Hz), 4.57 (1H, d, *J* 12.0 Hz), 4.52 (1H, d, *J* 12 Hz), 4.49 (1H, d, *J* 12 Hz), 4.29 (1H, dd, *J* 2.5,11.0 Hz), 4.21 (1H, ddd, *J* 2.8, 6.0, 12.4 Hz), 4.17 (1H, dd, *J* 5.6, 11.2 Hz), 4.00 (1H, br. d, *J* 2.1 Hz), 3.84 (1H, dd, *J* 2.8, 6.2 Hz), 3.40 (3H, s), 2.30 (2H, t, *J* 7.6 Hz), 2.01 (4H, m), 1.64 – 1.54 (4H, m), 1.28 (18H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 137.5, 137.4, 130.0,

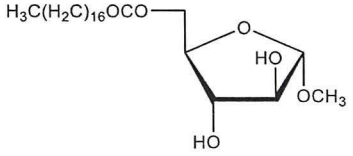
129.7, 128.5, 128.4, 128.0, 127.9, 127.88, 127.87, 107.3, 87.9, 83.3, 79.3, 72.3, 72.1, 63.6, 55.0, 34.1, 31.9, 29.8, 29.7, 29.5, 29.3, 29.2, 29.1, 29.09, 27.2, 27.17, 24.8, 22.7, 14.1; ν_{max}: 3005, 3089, 3031, 2926, 2855, 1740, 1454, 1050, 697, 735 cm^{-1}.

Experiment 9: Methyl 5-*O*-oliyl-α-D-arabinofuranoside (78)



Boron trichloride[268] (0.32 mL, 1M) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-oliyl-α-D-arabinofuranoside **(76)** (0.02 g, 0.03 mmol) in dry CH_2Cl_2 (2 mL) -78 at °C. The reaction mixture was stirred for 2 h. when TLC showed no starting material was left. The reaction mixture was quenched with (1:1, CH_2Cl_2 : MeOH) and the solvent was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to afford methyl 5-*O*-oliyl-α-D-arabino-furanoside **(78)** (9 mg, 64%) [Found (MALDI) $(M+Na)^+$: 451.3, $C_{24}H_{44}NaO_6$, requires : 451.3], $[\alpha]_D^{18}$ + 5.0 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 5.35 – 5.26 (2H, m), 4.77 (1H, d, *J* 4.3 Hz), 4.24 (1H, dd, *J* 2.1, 11.7 Hz), 4.11 – 4.05 (1H, m), 4.02 – 3.97 (1H, m), 3.97 – 3.91 (2H, m), 3.39 (3H, s), 2.32 (2H, t, *J* 7.6 Hz), 2.04 – 1.92 (2H, m), 1.65 – 1.54 (2H, m), 1.35 – 1.15 (24H, m), 0.84 (3H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 174.3, 129.96, 129.7, 102.3, 79.9, 77.7, 75.8, 65.4, 55.2, 49.6, 49.3, 49.1, 48.9, 48.7, 34.1, 31.8, 29.7, 29.6, 29.4, 29.2, 29.1, 29.0, 27.1, 27.09, 24.8, 22.6, 13.98; ν_{max}: br. 3468, 2917, 2850, 1735, 1454, 1050, 824 cm^{-1}.

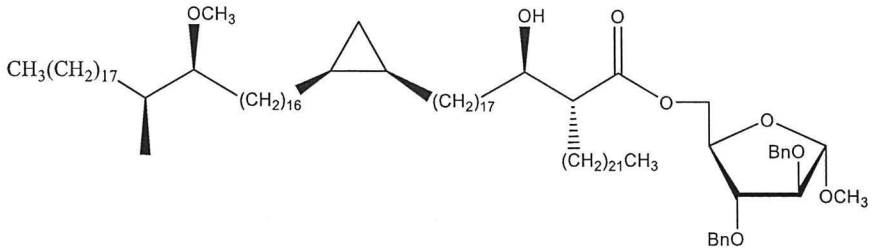
Experiment 10: Methyl 5-*O*-stearyl-α-D-arabinofuranoside (77)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 12.0 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-oliyl-α-D-arabinofuranoside **(76)** (0.08 g, 0.13 mmol) in dry (CH_2Cl_2 : MeOH, 1:1, 3 mL) at room

temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h. when TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with CH_2Cl_2 (15 mL), the filtrate was evaporated and the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to afford methyl 5-*O*-stearyl-α-D-arabinofuranoside **(77)** (45 mg, 80%), [Found (MALDI) $(M+Na)^+$: 453.0, $C_{24}H_{46}NaO_6$, requires: 453.3], $[\alpha]_D^{18}$ + 2.0 (*c* 0.1, $CHCl_3$) δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.92 (1H, s), 4.30 (2H, br. d, *J* 4.0 Hz), 4.21 (1H, dd, *J* 3.8, 7.3 Hz), 4.09 (1H, br. d, *J* 8.9 Hz), 3.90 (1H, br. d, *J* 10.1 Hz), 3.42 (3H, s), 2.77 (1H, d, *J* 10.2 Hz), 2.52 (1H, d, *J* 7.9 Hz), 2.35 (2H, t, *J* 7.6 Hz), 1.69 – 1.58 (2H, m), 1.34 – 1.21 (28H, m), 0.88 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 173.5, 108.8, 83.8, 79.8, 78.0, 63.8, 55.1, 50.8, 34.1, 31.9, 29.7, 29.63, 29.6, 29.4, 29.3, 29.2, 29.1, 24.8, 22.7, 14.1; ν_{max}: br. 3468, 2917, 2850, 1735, 1454, 1050, 824 cm^{-1}.

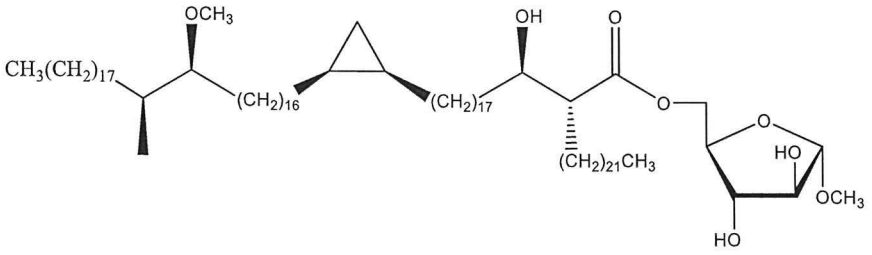
Experiment 11: Methyl 2,3-di-O-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosano-ate)-α-D-arabinofuranoside (81)



Cesium hydrogencarbonate (0.098 g, 0.505 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(71)** (54.6 mg, 0.10 mmol) and (2*R*)-2-[(1*R*)-1-hydroxy-18-[(2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-ethyl-hexatriacontyl]cyclopropyl]octadecyl]tetracosanoic acid **(79)**[158] (100.0 mg, 0.081 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature and the reaction mixture was stirred at 70 °C for two 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 (2×10 mL). The combined organic layers were washed with water (15 mL) and brine (15 mL). The organic layer was dried, filtered and evaporated to give a thick oil residue. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to afford methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-

hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18methyl-hexatriacontyl]cyclopropyl]-octadecyl]tetracosanoate)-α-D-arabinofuranoside **(81)** as a colourless thick oil (94.9 mg, 75%) [Found $(M+Na)^+$: 1574.4085, $C_{103}H_{186}NaO_8$, requires: 1574.4040], $[\alpha]_D^{23}$ + 18 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.92 (1H, s), 4.58 (1H, d, *J* 12.0 Hz), 4.55 (1H, d, *J* 12.0 Hz), 4.51 (1H, d, *J* 12.0 Hz), 4.48 (1H, d, *J* 12.0 Hz), 4.33 – 4.26 (2H, m), 4.21 (1H, m), 3.99 (1H, dd, *J* 1.0, 2.7 Hz), 3.84 (1H, dd, *J* 2.6, 6.4 Hz), 3.67 – 3.59 (1H, m), 3.38 (3H, s), 3.35 (3H, s), 2.99 – 2.93 (1H, m), 2.52 (1H, br. s), 2.43 (1H, dt, *J* 5.5, 9.2 Hz), 1.68 – 1.06 (142H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.70 – 0.61 (2H, m), 0.57 (1H, dt, 3.9, 7.9 Hz), -0.32 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 137.5, 137.3, 128.5, 128.46, 127.94, 127.9, 107.2, 87.9, 85.4, 83.7, 79.4, 72.4, 72.2, 72.1, 63.5, 57.7, 54.9, 51.5, 35.5, 35.3, 31.9, 30.6, 30.56, 30.5, 30.2, 30.16, 30.0, 29.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.2, 28.7, 27.6, 27.4, 27.37, 26.2, 25.8, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max} : 3479, 3064, 2923, 2853, 1733, 1465, 1100, 696, 756 cm^{-1}.

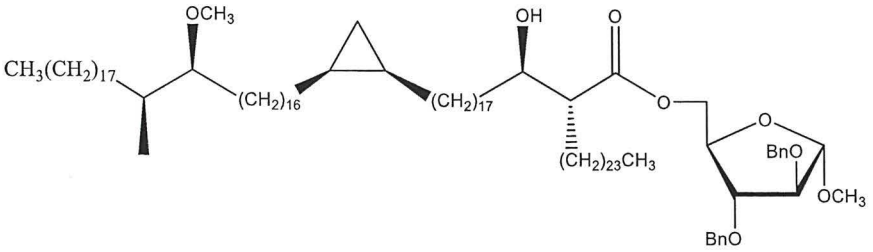
Experiment 12: Methyl 5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranoside (82)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 3.0 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl] octadecyl]tetracosanoate)-α-D-arabinofuranoside **(81)** (0.020 g, 0.012 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The mixture was filtered off and the solvent was evaporated to give a residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to give a colourless oil of methyl 5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosa-noate)-α-D-

arabinofuranoside **(82)** (11 mg, 65%) [Found $(M+Na)^+$: 1394.3138, $C_{89}H_{174}NaO_8$, requires: 1394.3101], $[\alpha]_D^{16}$ + 10 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.90 (1H, s), 4.52 (1H, dd, *J* 3.9, 12.0 Hz), 4.32 (1H, dd, *J* 3.9, 12.0 Hz), 4.18 (1H, m), 4.07 (1H, br. d, *J* 4.7 Hz), 3.98 (1H, br. d, *J* 7.5 Hz), 3.74 – 3.66 (1H, m), 3.41 (3H, s), 3.35 (3H, s), 2.99 – 2.93 (1H, m), 2.79 – 2.66 (2H, m), 2.49 – 2.40 (1H, m), 2.39 – 2.32 (1H, m), 1.62 – 1.05 (143H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.85 (3H, d, *J* 6.9 Hz), 0.70 – 0.61 (2H, m), 0.56 (1H, dt, 3.9, 7.9 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 174.95, 108.8, 85.5, 83.8, 80.4, 78.4, 72.8, 63.3, 57.7, 55.0, 52.2, 35.3, 35.2, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.71, 29.7, 29.6, 29.5, 29.4, 29.36, 28.7, 27.6, 27.4, 26.2, 25.4, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: br. 3436, 2918, 2850, 1732, 1467, 1099, 720 cm^{-1}.

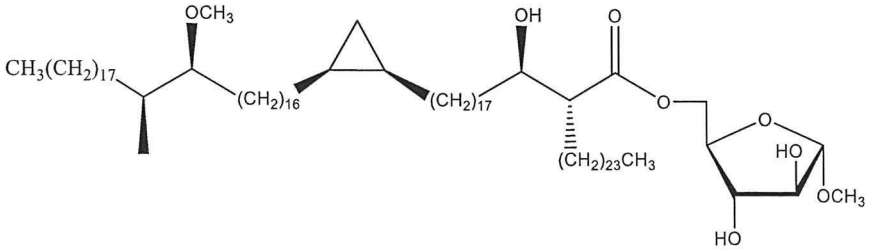
Experiment 13: Methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoat-e)-α-D-arabinofuranoside (83)



Cesium hydrogencarbonate (0.083 g, 0.428 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(71)** (0.045 g, 0.091 mmol) and (2*R*)-2-[(1*R*)-1-hydroxy-18-[(2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoic acid **(80)**[158] (0.076 g, 0.061 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature and the reaction mixture was stirred at 70 °C for two days. The reaction mixture was worked up and purified as before to give methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]-cyclopropyl]octadecyl]hexacosa- noate)-α-D-arabinofuranoside **(83)** as a colourless thick oil (77.8 mg, 80%) [Found $(M+Na)^+$: 1602.4304, $C_{105}H_{190}NaO_8$, requires: 1602.4353], $[\alpha]_D^{22}$ + 17 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$) 7.39 – 7.28 (10H, m), 4.92 (1H, s), 4.58 (1H, d, *J* 11.9 Hz), 4.56 (1H, d, *J* 11.7 Hz), 4.51 (1H, d, *J* 11.8 Hz), 4.48 (1H, d, *J* 11.9 Hz), 4.31 – 4.28 (2H, m), 4.26 – 4.20 (1H, m), 3.99 (1H, br. d, *J* 2.0 Hz), 3.84 (1H, dd, *J* 2.5, 6.3 Hz), 3.68 – 3.58 (1H,

m), 3.37 (3H, s), 3.35 (3H, s), 3.00 – 2.93 (1H, m), 2.50 (1H, d, *J* 8.2 Hz), 2.44 (1H, dt, *J* 5.4, 8.7 Hz), 1.75 – 1.56 (2H, m), 1.58 – 1.04 (145H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.70 – 0.62 (2H, m), 0.57 (1H, dt, 3.9, 7.9 Hz), -0.32 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 137.5, 137.3, 128.5, 128.46, 128.0, 127.97, 127.94, 127.92, 127.9, 127.86, 107.2, 87.9, 85.4, 83.7, 79.4, 72.38, 72.2, 72.1, 63.5, 57.7, 54.9, 51.5, 35.5, 35.3, 32.4, 31.9, 30.8, 30.7, 30.6, 30.59, 30.56, 30.53, 30.5, 30.42, 30.41, 30.4, 30.3, 30.2, 30.1, 30.09, 30.05, 30.0, 29.98, 29.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.24, 29.2, 29.18, 29.13, 29.1, 29.09, 29.05, 29.0, 28.97, 28.9, 28.7, 28.64, 28.6, 28.59, 28.56, 27.57, 27.4, 26.2, 25.8, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: br. 3522, 3064, 2921, 2851 , 1723, 1467, 1027, 695, 732 cm^{-1}.

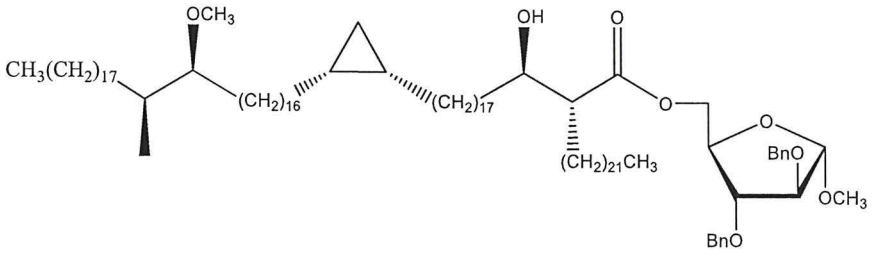
Experiment 14: Methyl 5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside (84)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 3.0 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl] octadecyl]hexacosanoate)-α-D-arabinofuranoside **(83)** (0.020 g, 0.012 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The mixture was worked up as before to give methyl 5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]-hexacosanoate)-α-D-arabinofuranoside **(84)** as a white thick oil (13.5 mg, 76%) [Found $(M+Na)^+$: 1422.3425, $C_{91}H_{178}NaO_8$, requires: 1422.3414], $[\alpha]_D^{21}$ + 10 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.89 (1H, s), 4.51 (1H, dd, *J* 3.9, 11.9 Hz), 4.33 (1H, dd, *J* 4.1, 12.0 Hz), 4.20 – 4.16 (1H, m), 4.07 (1H, br. s), 3.99 (1H, br. s), 3.75 (1H, d, *J* 5.3 Hz), 3.73 – 3.66 (1H, m), 3.41 (3H, s), 3.35 (3H, s), 3.00 – 2.93 (1H, m), 2.85 – 2.70 (2H, m), 2.48 – 2.39 (1H, m), 1.72 – 1.61 (2H, m), 1.60 –

1.03 (145H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.85 (3H, d, *J* 6.8 Hz), 0.70 – 0.61 (2H, m), 0.56 (1H, dt, 3.9, 7.9 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.0, 108.7, 85.5, 83.8, 80.4, 78.4, 72.8, 63.3, 57.7, 55.0, 52.2, 35.3, 35.2, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.36, 28.7, 27.6, 27.4, 26.2, 25.4, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: br. 3436, 2921, 2852, 1732, 1493, 1455, 759 cm^{-1}.

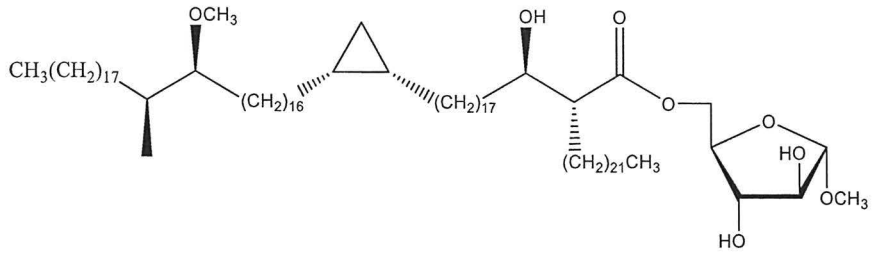
Experiment 15: Methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranoside (86)



Cesium hydrogencarbonate (0.056 g, 0.288 mmol) was added to a solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(71)** (0.031g, 0.062 mmol) 2*R*-2-[(1*R*)-1-hydroxy-18-[(1*S*)-2-[(17*S*,18*S*)-17-methoxy–18–methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoic acid **(85)**[158] (0.051g, 0.041 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature and the reaction mixture was stirred at 70 °C for two days. The reaction was worked up and purified as before to afford methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclo-propyl]octadecyl]tetracosanoate)-α-D-arabinofuranoside **(86)** as a colourless oil (0.05 g, 77%) [Found $(M+Na)^+$: 1574.4044, $C_{103}H_{186}NaO_8$, requires: 1574.4040], $[\alpha]_D^{20}$ + 23 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.92 (1H, s), 4.58 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.0 Hz), 4.51 (1H, d, *J* 12.0 Hz), 4.48 (1H, d, *J* 12.0 Hz), 4.33 – 4.27 (2H, m), 4.25 – 4.19 (1H, m), 3.99 (1H, br. d, *J* 2.0 Hz), 3.84 (1H, dd, *J* 2.6, 6.4 Hz), 3.67 – 3.59 (1H, m), 3.37 (3H, s), 3.35 (3H, s), 2.99 – 2.94 (1H, m), 2.52 (1H, d, *J* 8.3 Hz), 2.43 (1H, dt, *J* 5.4, 9.4 Hz), 1.72 – 1.61 (2H, m), 1.59 – 1.03 (141H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.70 – 0.61 (2H, m), 0.57 (1H, dt, 4.0, 8.1 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 128.5, 128.46, 127.94, 127.9, 107.2, 87.9, 85.4, 83.7, 79.4, 72.4, 72.1, 63.4, 57.7, 54.9, 51.5, 35.5, 35.3, 32.4, 31.9, 30.5, 30.48, 30.4, 30.35, 30.2, 30.1,

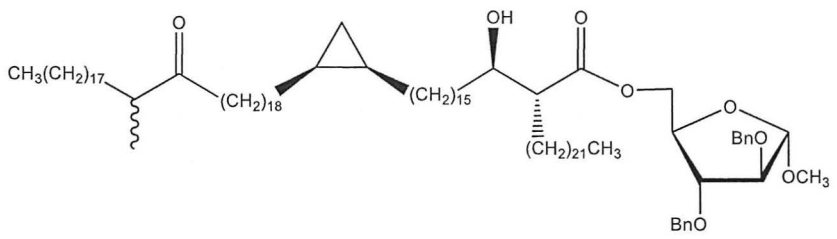
30.0, 29.98, 29.94, 29.9, 29.86, 29.8, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 29.26, 29.21, 29.2, 29.1, 29.06, 29.0, 28.7, 28.67, 27.6, 27.4, 26.2, 25.8, 22.7, 22.6, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3479, 3064, 2924, 2853, 1735, 1494, 1455, 1100 cm^{-1}.

Experiment 16: Methyl 5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranoside (87)



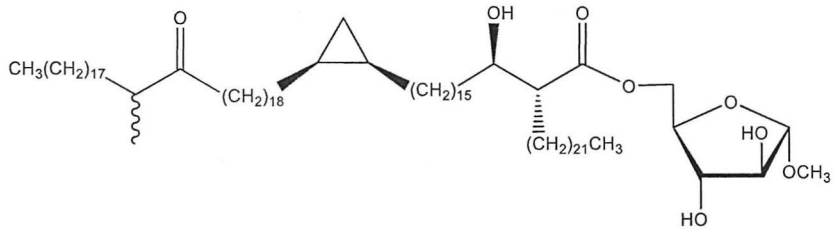
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 3.34 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl] octadecyl]tetracosanoate)-α-D-arabinofuranoside **(86)** (0.022 g, 0.014 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford methyl 5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranoside **(87)** as a thick colourless oil (15.6 mg, 79%) [Found $(M+NH_4)^+$: 1389.3550, $C_{89}H_{178}NO_8$, requires: 1389.3552], $[\alpha]_D^{21}$ + 28 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.89 (1H, s), 4.49 (1H, dd, *J* 4.0, 11.9 Hz), 4.35 (1H, dd, *J* 4.1, 11.9 Hz), 4.20 – 4.15 (1H, m), 4.07 (1H, br. s), 3.98 (1H, br. s), 3.77 – 3.66 (1H, m), 3.41 (3H, s), 3.35 (3H, s), 3.01 – 2.92 (1H, m), 2.85 (1H, br. s, O*H*), 2.45 (1H, ddd, *J* 5.1, 6.9, 10.1 Hz), 1.48 – 1.17 (144H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.70 – 0.61 (2H, m), 0.57 (1H, dt, *J* 4.0, 8.0 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.0, 108.8, 85.5, 83.7, 80.6, 78.4, 72.8, 63.2, 57.7, 55.0, 52.3, 35.3, 35.2, 32.4, 31.9, 30.5, 30.0, 29.9, 29.7, 29.65, 29.6, 29.52, 29.5, 29.4, 29.35, 29.3, 28.7, 27.6, 27.4, 26.2, 25.4, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: br.3435, 2918, 2850, 1732, 1455,1100 cm^{-1}.

Experiment 17: Methyl 2,3-di-*O*-benzyl-5-*O*-{2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]tetracosanoate}-α-D-arabinofuranoside (90)



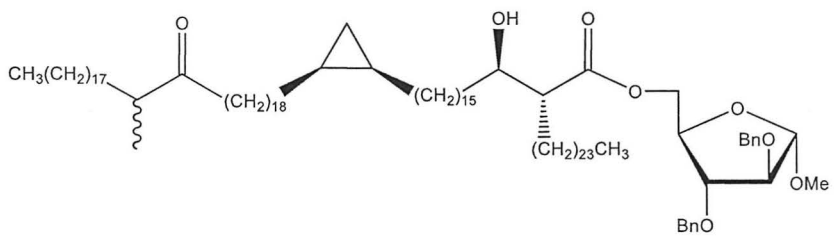
Cesium hydrogencarbonate (0.086 g, 0.443 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(71)** (0.047 g, 0.095 mmol) and (2*R*)–2-[(1*R*)-1-hydroxyl-16-[(2*S*-2-(20-methyl-19-oxooctatriacontyl) cyclopropyl]hexadecyl]tetracosanoic acid **(88)**[159] (0.077 g, 0.063 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature and the mixture was stirred at 70 °C for two days. The reaction mixture was worked up and purified as before to afford methyl 2,3-di-*O*-benzyl-5-*O*-{2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclo-propyl]hexadecyl]tetracosanoate}-α-D-arabinofuranoside **(90)** as a colourless thick oil (77 mg, 80%) [Found $(M+Na)^+$: 1558.3708, $C_{102}H_{182}NaO_8$, requires: 1558.3727], $[\alpha]_D^{21}$ + 20 (c 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.92 (1H, s), 4.58 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12 Hz), 4.51 (1H, d, *J* 12 Hz), 4.48 (1H, d, *J* 12 Hz), 4.32 – 4.28 (2H, m), 4.25 – 4.19 (1H, m), 3.99 (1H, br. d, *J* 2.2 Hz), 3.84 (1H, dd, *J* 2.8, 6.4 Hz), 3.67 – 3.59 (1H, m), 3.38 (3H, s), 2.57 – 2.47 (5H, m, including a singlet for O*H*), 1.75 – 1.09 (140H, m), 1.06 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.7 Hz), 0.70 – 0.62 (2H, m), 0.61 – 0.53 (1H, dt, 3.9, 7.9 Hz), -0.32 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.0, 137.5, 137.3, 128.5, 128.46, 128.0, 127.94, 127.9, 107.2, 87.9, 83.7, 79.4, 72.4, 72.2, 72.1, 63.5, 54.9, 51.5, 46.3, 41.1, 33.0, 31.9, 30.2, 29.7, 29.67, 29.6, 29.5, 29.46, 29.4, 28.7, 27.4, 27.3, 25.8, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: br. 3524, 3030, 3063, 2922, 2851, 1732, 1715, 1465, 1107, 696, 733 cm^{-1}.

Experiment 18: Methyl 5-*O*-{2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxo-octatriacontyl)cyclopropyl]hexadecyl]tetracosanoate}-α-D-arabinofuranoside (91)



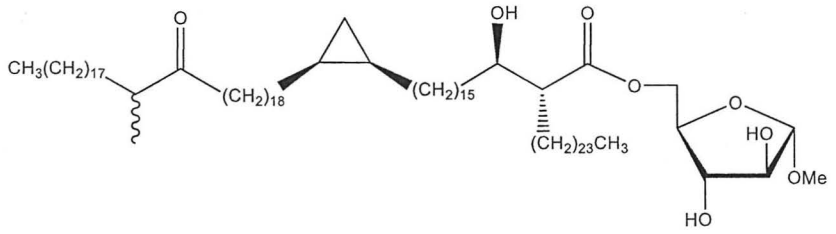
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 1.5 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-{2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]tetra-cosanoate}-α-D-arabinofuranoside **(90)** (0.010 g, 0.006 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford methyl 5-*O*-{2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]tetra-cosanoate}-α-D-arabinofuranoside **(91)** as a white thick oil (6.9 mg, 78%) [Found $(M+Na)^+$: 1378.2803, $C_{88}H_{170}NaO_8$, requires: 1378.2788], $[\alpha]_D^{21}$ + 10 (*c* 0.1, $CHCl_3$ + few drops CD_3OD); which showed δ_H (400 MHz, $CDCl_3$): 4.89 (1H, s), 4.51 (1H, dd, *J* 12.0, 4.0 Hz), 4.33 (1H, dd, *J* 4.1, 12.0 Hz), 4.21 – 4.15 (1H, m), 4.07 (1H, br. s), 4.02 – 3.95 (1H, m), 3.74 – 3.66 (1H, m), 3.41 (3H, s), 2.90 – 2.71 (2H, m), 2.56 – 2.48 (1H, m), 2.48 – 2.37 (4H, m, including a singlet for O*H*), 2.23 – 1.10 (140H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.8 Hz), 0.70 – 0.61 (2H, m), 0.59 – 0.53 (1H, dt, 3.9, 7.9 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 215.0, 174.9, 108.8, 83.8, 80.4, 78.4, 77.2, 72.8, 63.3, 55.0, 52.2, 46.3, 41.1, 35.2, 33.0, 31.9, 30.2, 29.7, 29.6, 29.55, 29.5, 29.4, 29.36, 29.3, 28.7, 27.4, 27.3, 25.4, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: br. 3436, 2918, 2850, 1731, 1708, 1467, 1170 cm^{-1}.

Experiment 19: Methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]hexacosanoate)-α-D-arabinofuranoside (92)



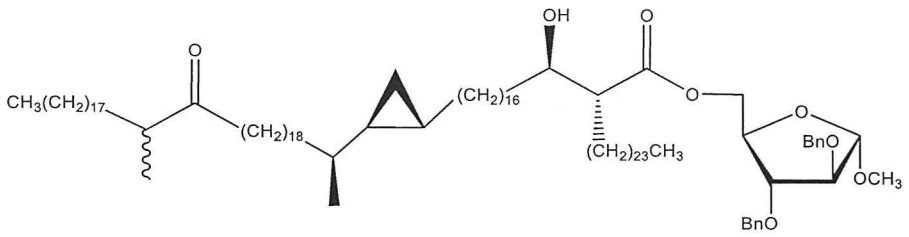
Cesium hydrogencarbonate (0.062g, 0.319 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (**71**) (0.048 g, 0.096 mmol) and (2*R*)-2-[(1*R*)-1-hydroxy-16-[(2*S*)-2-(20-methyl-19-oxooctatriacontyl) cyclopropyl]hexadecyl]hexacosanoic acid **(89)**[159] (0.080 g, 0.064 mmol) in dry DMF : THF (1:5, 4 mL) at room temperature and the mixture was stirred at 70 °C for 2 days. The reaction mixture was worked up and purified as before to afford methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]hexacosanoate)-α-D-arabinofuranoside **(92)** as a colourless thick oil (72 mg, 71%) [Found $(M+NH_4)^+$: 1581.4467, $C_{104}H_{190}NO_8$, requires: 1581.4486], $[\alpha]_D^{23}$ + 25 (c 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.42 – 7.27 (10H, m), 4.92 (1H, s), 4.58 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 11.8 Hz), 4.50 (1H, d, *J* 12.0 Hz), 4.47 (1H, d, *J* 11.8 Hz), 4.34 – 4.26 (2H, m), 4.22 (1H, dt, *J* 4.6, 9.1 Hz), 3.99 (1H, br. d, *J* 2.1 Hz), 3.84 (1H, dd, *J* 2.6, 6.4 Hz), 3.68 – 3.58 (1H, m), 3.38 (3H, s), 2.56 – 2.47 (2H, m, including O*H*), 2.47 – 2.37 (3H, m), 1.76 – 1.09 (144H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.8 Hz), 0.70 – 0.61 (2H, m), 0.56 (1H, dt, 4.0, 8.0 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.0, 137.4, 137.2, 128.5, 128.4, 128.0, 127.9, 127.8, 107.2, 87.8, 83.7, 79.4, 72.4, 72.1, 72.0, 63.4, 54.9, 51.5, 46.3, 41.1, 35.5, 33.0, 31.9, 30.2, 29.7, 29.67, 29.6, 29.57, 29.5, 29.46, 29.4, 29.3, 28.7, 27.4, 27.3, 25.8, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: 3457, 3064, 3032, 2921, 2851, 1717, 1678, 1466, 1101, 755, 697 cm^{-1}.

Experiment 20: Methyl 5-*O*-(2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxo-octatriacontyl)cyclopropyl]hexadecyl]hexacosanoate)-α-D-arabinofuranoside (93)



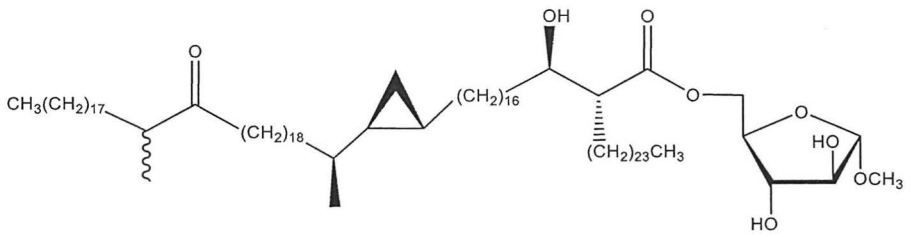
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 0.01g, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]hexa cosanoate)-α-D-arabinofuranoside **(92)** (0.066 g, 0.042 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford methyl 5-*O*-(2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]hexa-cosanoate)-α-D-arabinofuranoside **(93)** as a colourless thick oil (39 mg, 66%) [Found $(M+NH_4)^+$: 1401.3546, $C_{90}H_{178}NO_8$, requires: 1401.3547], $[\alpha]_D^{23}$ + 11 (c 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.89 (1H, s), 4.51 (1H, dd, *J* 3.9, 11.9 Hz), 4.32 (1H, dd, *J* 4.0, 11.9 Hz), 4.21 – 4.15 (1H, m), 4.07 (1H, br. s), 3.98 (1H, br. d, *J* 1.8 Hz), 3.74 – 3.66 (1H, m), 3.41 (3H, s), 2.87 – 2.67 (2H, m), 2.55 – 2.38 (4H, m), 1.71 – 1.12 (145H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.8 Hz), 0.69 – 0.61 (2H, m), 0.56 (1H, dt, *J* 4.0, 8.0 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 215.4, 175.0, 108.7, 83.6, 80.6, 78.4, 72.8, 63.2, 55.0, 52.3, 50.9, 46.3, 41.1, 35.2, 33.0, 31.9, 30.2, 29.7, 29.65, 29.6, 29.5, 29.45, 29.41, 29.40, 29.3, 28.7, 27.4, 27.3, 25.4, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: 3405, 2920, 2851, 1713, 1712, 1466, 1108, 759 cm^{-1}.

Experiment 21: Methyl 2,3-di-*O*-benzyl-5-*O*-{2-[(1*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-[(2S)-22-methyl-21-oxotetracontan-2-yl]cyclopropyl]heptadecyl]hexacosanoate}-α-D-arabinofuranoside (95)



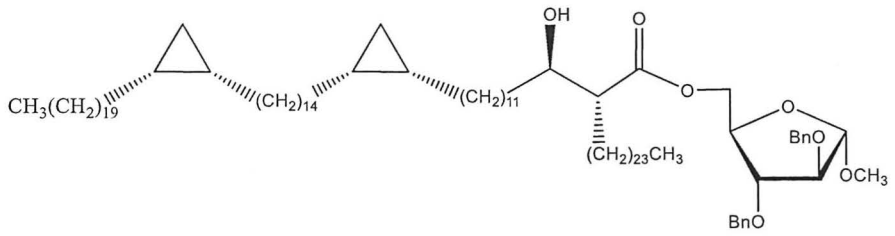
Cesium hydrogencarbonate (0.045 g, 0.232 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(71)** (0.025 g, 0.050 mmol) and (*R*)-2-[(*R*)-1-hydroxy-17-[(1*S*,2*S*)-2-(20-oxononatriacontyl)cyclopropyl]heptadecyl]hexacosanoic acid **(94)**[159] (0.043 g, 0.033 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature and the reaction mixture was stirred at 70 °C for two days. The reaction was worked up and purified as before to afford methyl 2,3-di-*O*-benzyl-5-*O*-{2-[(1*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-[(2*S*)-22-methyl-21-oxotetracontan-2-yl]cycloprop-yl]heptadecyl]hexacosanoate}-α-D-arabinofuranoside **(95)** as a colourless thick oil (40 mg, 74%) [Found $(M+Na)^+$: 1628.4539, $C_{107}H_{192}NaO_8$, requires: 1628.4509], $[\alpha]_D^{21}$ + 16 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.27 (10H, m), 4.92 (1H, s), 4.58 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12 Hz), 4.53 (1H, d, *J* 12 Hz), 4.48 (1H, d, *J* 12 Hz), 4.33 – 4.28 (2H, m), 4.22 (1H, dt, *J* 4.6, 10.7 Hz), 3.99 (1H, br. d, *J* 1.9 Hz), 3.84 (1H, dd, *J* 2.6, 6.4 Hz), 3.68 – 3.58 (1H, dt, *J* 5.2, 7.6 Hz), 3.38 (3H, s), 2.58 – 2.47 (2H, m), 2.42 (3H, m), 1.72 – 1.13 (146H, m), 1.06 (3H, d, *J* 6.9 Hz), 0.91 – 0.89 (9H, including a triplet resonated at 0.89 with *J* 7.5 Hz), 0.72 – 0.61 (1H, m), 0.50 – 0.41 (1H, m), 0.24 – 0.08 (3H, m); δ_C (101 MHz, $CDCl_3$): 215.2, 175.0, 137.5, 137.3, 128.5, 128.4, 128.0, 127.9, 127.8, 107.2, 87.8, 83.7, 79.4, 72.4, 72.1, 63.5, 54.9, 51.5, 46.3, 41.1, 38.1, 37.4, 35.5, 34.5, 33.0, 31.9, 29.7, 29.65, 29.6, 29.4, 29.3, 27.4, 27.32, 27.3, 26.1, 25.7, 23.7, 22.7, 19.7, 18.6, 16.3, 14.1, 10.5 ; ν_{max}: br. 3524, 3064, 3031, 2923, 2853, 1737, 1715, 1465, 1107, 734 cm^{-1}.

Experiment 22: Methyl 5-*O*-{2-[(1*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-[(2*S*)-22-methyl-21-oxotetracontan-2-yl]cyclopropyl]heptadecyl]hexacosanoate}-α-D-arabinofuranoside (96)



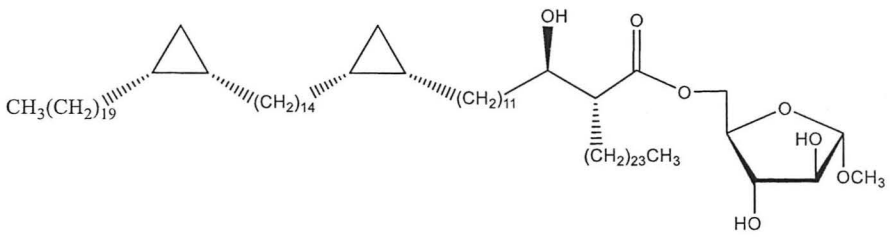
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C , 1.5 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-{2-[(1*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-[(2*S*)-22-methyl-21-oxotetracontan-2-yl]cyclopropyl]heptadecyl]hexacosanoate}-α-D-arabinofuranoside **(95)** (0.010 g, 0.006 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford methyl 5-*O*-{2-[(1*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-[(2*S*)-22-methyl-21-oxotetracontan-2-yl]cyclopropyl]-heptadecyl]hexacosanoate}-α-D-arabinofuranoside **(96)** as a white thick oil (7 mg, 80%), [Found $(M+Na)^+$: 1448.3585, $C_{93}H_{180}NaO_8$, requires: 1448.3570], $[\alpha]_D^{21}$ + 13 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.89 (1H, s), 4.49 (1H, dd, *J* 4.0, 12.0 Hz), 4.33 (1H, dd, *J* 4.2, 12.0 Hz), 4.21 – 4.16 (1H, m), 4.09 (1H, br. d, *J* 6.8 Hz), 4.01 – 3.95 (1H, m), 3.75 (1H, d, *J* 5.4 Hz), 3.73 – 3.65 (1H, m), 3.41 (3H, s), 2.61 – 2.47 (1H, m), 2.46 – 2.28 (5H, m, including a singlet for O*H*), 1.72 – 1.15 (146H, m), 1.06 (3H, d, *J* 6.9 Hz), 0.91 – 0.89 (9H, including a triplet resonating at 0.89 with *J* 7.4 Hz), 0.76 – 0.61 (1H, m), 0.51 – 0.39 (1H, m), 0.26 – 0.05 (3H, m); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 215.4, 174.96, 108.7, 83.8, 80.4, 78.8, 78.4, 72.8, 54.9, 52.2, 46.3, 44.5, 41.1, 38.1, 37.4, 35.2, 34.5, 33.0, 31.9, 30.0, 29.7, 29.6, 29.5, 29.3, 27.3, 26.1, 25.4, 23.71, 22.7, 19.7, 18.6, 16.3, 14.1, 10.5; ν_{max}: br. 3467, 2917, 2849, 1731, 1713, 1467, 1050,720 cm^{-1}.

Experiment 23: Methyl 2,3-di-*O*-benzyl-5-O-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosylcyclo propyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (98)



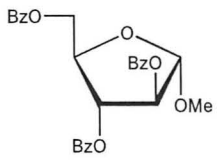
Cesium hydrogencarbonate (0.089 g, 0.458 mmol) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (**71**) (0.049 g, 0.098 mmol) (2*R*)-2-[(1*R*)-1-hydroxy-12-[(2*R*)-2-{14-[(1*S*,2*R*)-2-icosylcyclopropyl]-tetradecyl]cyclopropyl}dodecyl]hexacosanoic acid (**97**)[157] (0.075 g, 0.065 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature and the reaction mixture was stirred at 70 °C for two days. The reaction was worked up and purifies as before to afford methyl 2,3-di-*O*-benzyl-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosylcyclopropyl] tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (**98**) as a colourless oil (84 mg, 87%) [Found $(M+Na)^+$: 1486.3173, $C_{98}H_{174}NaO_7$, requires: 1486.3152], $[\alpha]_D^{21}$ + 31 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.92 (1H, s), 4.58 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 11.8 Hz), 4.51 (1H, d, *J* 12.0 Hz), 4.48 (1H, d, *J* 11.8 Hz), 4.32 – 4.28 (2H, m), 4.25 – 4.19 (1H, m), 3.99 (1H, br. d, *J* 2.3 Hz), 3.84 (1H, dd, *J* 2.6, 6.4 Hz), 3.68 – 3.59 (1H, m), 3.38 (3H, s), 2.52 (1H, d, *J* 8.3 Hz), 2.44 (1H, dt, *J* 5.5, 9.3 Hz), 1.62 – 1.03 (134H, m), 0.89 (6H, t, *J* 6.7 Hz), 0.72 – 0.62 (4H, m), 0.57 (2H, dt, *J* 4.0, 8.0 Hz), -0.32 (2H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 137.5, 137.3, 128.5, 128.48, 128.0, 127.94, 127.92, 127.9, 107.2, 87.9, 83.7, 79.4, 72.4, 72.2, 72.1, 63.5, 54.9, 51.5, 35.5, 31.9, 30.6, 30.54, 30.5, 30.43, 30.39, 30.4, 30.3, 30.24, 30.2, 30.13, 30.1, 30.05, 29.98, 29.7, 29.65, 29.6, 29.5, 29.35, 29.3, 29.2, 29.1, 29.08, 29.0, 28.7, 28.6, 27.4, 25.8, 22.7, 22.674, 15.8, 14.1, 10.9; ν_{max}: 3479, 3065, 2989, 2919, 2849, 1733,1607, 1494, 718 cm^{-1}.

Experiment 24: Methyl 5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosyl cyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (99)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 8.7 mg, 0.15 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside **(98)** (0.058g, 0.039 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford methyl 5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside **(99)** as a colourless oil (0.04 g, 80%) [Found $(M+Na)^+$: 1306.2201, $C_{84}H_{162}NaO_7$, requires: 1306.2213], $[\alpha]_D^{23}$ + 12 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.89 (1H, s), 4.47 (1H, dd, *J* 4.2, 11.8 Hz), 4.37 (1H, dd, *J* 4.1, 11.9 Hz), 4.20 – 4.14 (1H, m), 4.07 (1H, br. s), 3.98 (1H, d, 2.0 Hz), 3.79 – 3.67 (1H, m), 3.40 (3H, s), 3.06 (2H, br. s), 2.45 (1H, td, *J* 5.0, 10.1 Hz), 1.90 – 1.83 (1H, m), 1.63 – 1.07 (134H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.71 – 0.61 (4H, m), 0.57 (2H, dt, *J* 4.0, 8.0 Hz), -0.33 (2H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.0, 108.7, 83.4, 80.7, 78.4, 72.8, 63.2, 55.0, 52.5, 35.2, 31.9, 30.4, 30.24, 30.2, 29.7, 29.65, 29.6, 29.53, 29.5, 29.41, 29.4, 29.3, 28.7, 27.4, 25.4, 22.7, 15.8, 14.1, 10.9; ν_{max}: 3436, 390, 2918, 2850, 1733, 1467, 1455, 1050 cm^{-1}.

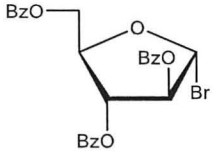
Experiment 25: Methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (101)



Benzoyl chloride (30.0 mL, 0.25 mol) was added dropwise to a stirred solution of methyl–α, β-D-arabinofuranoside **(66)** (5.0 g, 30 mmol) in anhydrous pyridine (40 mL)

at 0 °C. The reaction mixture was allowed to reach room temperature and stirred at 40 °C for 1.5 h then TLC showed no starting material was left, the mixture was cooled to 0 °C and ice chips (5 g) was added. The mixture was diluted with ethyl acetate (50 mL), then the organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×50 mL). The combined organic layers were washed firstly with water (50 mL), sodium bicarbonate (50 mL) and finally with brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure. Traces amount of pyridine was removed by co-evaporation with toluene to give a white solid. The solid residue was recrystallize from ethanol (95%) and kept in a fridge overnight to give methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(101)** as a crystalline solid (7.3 g, 50%) m.p. 100-102 °C (*lit.*[271] 101 °C); $[\alpha]_D^{20}$ - 21 (*c* 0.1, $CHCl_3$) [*lit.*[301] $[\alpha]_D^{21}$ - 19.1 (*c* 2.05, $CHCl_3$)], [Found (MALDI) $(M+Na)^+$: 499.3, $C_{27}H_{24}NaO_8$, requires: 499.1] which showed δ_H (400 MHz, $CDCl_3$): 8.12 – 7.99 (6H, m), 7.64 – 7.56 (2H, m), 7.55 – 7.38 (5H, m), 7.30 (2H, dd, *J* 8.6, 16.3 Hz), 5.60 (1H, d, *J* 5.1 Hz), 5.53 (1H, d, *J* 1.2 Hz), 5.20 (1H, s), 4.86 (1H, dd, *J* 3.4, 11.9 Hz), 4.71 (1H, dd, *J* 4.8, 11.9 Hz), 4.59 (1H, m), 3.51 (3H, s); δ_C (101 MHz, $CDCl_3$): 166.2, 165.8, 165.5, 133.6, 133.5, 133.48, 133.47, 133.02, 133.0, 129.95, 129.9, 129.86, 129.9, 129.8, 129.7, 129.1, 129.0, 128.5, 128.47, 128.46, 128.4, 128.35, 128.3, 106.9, 82.2, 80.8, 77.9, 63.7; 54.98; ν_{max}: 3065, 2936, 2836, 1722, 1451, 1109, 709 cm^{-1}.

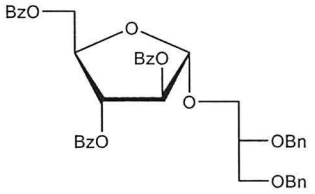
Experiment 26: 2,3,5-Tri-*O*-benzoyl-α-D-arabinfuranosyl bromide (115) [284,302]



A solution of (HBr 30 - 32% in acetic acid, 50 mL) was added to a stirred solution of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(101)** (10 g, 20 mmol) in glacial acetic acid (50 mL). The reaction mixture was stirred at room temperature for 2 h, then TLC showed no starting material was left. The reaction mixture was diluted with dichloro-methane (200 mL) and poured into ice cold water (50 mL), the organic layer was separated quickly and the aqueous layer was re-extracted with dichloromethane (3×50 mL). The combined organic layers were washed with saturated solution of $NaHCO_3$ (3×50 mL), brine (1×50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure to give 2,3,5-tri-*O*-benzoyl-α-D-arabinfuranosyl

bromide **(115)** as a white foam (10.0 g, 90%), which was used in the next step without further purification; $[\alpha]_D^{22}$ + 48 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 8.19 – 8.13 (2H, m), 8.10 – 8.02 (2H, m), 8.02 – 7.94 (2H, m), 7.69 – 7.56 (2H, m), 7.57 – 7.45 (3H, m), 7.44 – 7.35 (2H, m), 7.36 – 7.28 (2H, m), 6.65 (1H, s), 5.98 – 5.96 (1H, m), 5.65 (1H, d, *J* 4.3 Hz), 4.93 (1H, dd, *J* 3.0, 11.6 Hz), 4.89 (1H, dd, *J* 4.5, 8.4 Hz), 4.79 (1H, dd, *J* 4.4, 11.6 Hz); δ_C (101 MHz, $CDCl_3$): 166.0, 165.7, 165.1, 133.9, 133.8, 133.2, 130.0, 129.9, 129.8, 128.7, 128.6, 128.4, 88.5, 85.7, 84.6, 76.6, 62.6.

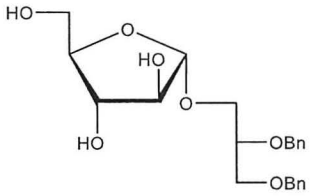
Experiment 27: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (116)



Tin (IV) chloride[285] (4.7 mL, 0.5 M solution in CH_2Cl_2, 1.0 mmol) was added to a stirred solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinfuranosyl bromide **(115)** (5.0 g, 9.5 mmol) in dry CH_2Cl_2 (12 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 10 min, then a solution of 2,3-di-*O*-benzyl-D-glycerol **(107)** (2.8 g, 10 mmol) in CH_2Cl_2 (2 mL) was added following by the addition of ethyldiisopropylamine (0.9 g, 6.9 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 2 h, then TLC showed no starting material was left. The reaction mixture was quenched with aqueous solution of $NaHCO_3$ (50 mL), the organic layer was separated and the aqueous layer was re-extracted with dichloromethane (3×50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure to give the residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (1:1) to give 2',3'-di-*O*-benzyl-D-glycerol(1'→1)-2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(116)** as a thick colourless oil (4.5 g, 65%) $[\alpha]_D^{20}$ - 38 (*c* 0.1, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 739.0, $C_{43}H_{40}NaO_{10}$, requires: 739.2] which showed δ_H (400 MHz, $CDCl_3$): 8.11 – 7.97 (7H, m), 7.64 – 7.26 (18H, m), 5.56 (2H, m), 5.34 (1H, s), 4.79 (1H, dd, *J* 3.3, 12.0 Hz), 4.76 – 4.69 (2H, m), 4.64 (1H, dd, *J* 4.8, 12.0 Hz), 4.57 – 4.49 (3H, m), 4.01 (1H, dd, *J* 4.5, 10.4 Hz), 3.87 (1H, br. p, *J* 5.2 Hz), 3.72 (1H, dd, *J* 5.8, 10.4 Hz), 3.67 (1H, dd, *J* 4.0, 9.3 Hz), 3.64 (1H, dd, *J* 4.5, 9.2 Hz); δ_C (101 MHz, $CDCl_3$): 166.2, 165.7, 165.4, 138.4,

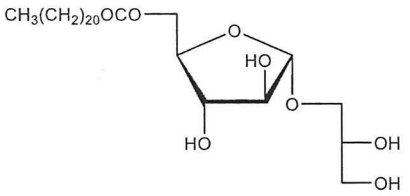
138.1, 133.55, 133.5, 133.0, 129.9, 129.8, 129.7, 128.5, 128.47, 128.33, 128.3, 127.7, 127.6, 127.52, 127.5, 105.9, 81.9, 81.1, 77.8, 76.7, 73.4, 72.2, 69.9, 67.2, 63.7; ν_{max}: 3065, 3033, 2944, 1722, 1268, 1070, 709 cm^{-1}. The experiment was repeated on a large scale.

Experiment 28: 2',3'-Di-O-benzyl-D-glycerol-(1'→1)-α-D-arabinofuranoside (117)



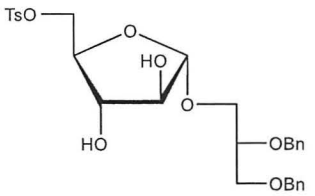
Sodium methoxide (0.1 M, in methanol, 6.0 mL) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(116)** (1.0 g, 1.3 mmol) in dry MeOH : CH_2Cl_2 (1:1, 8 mL) at room temperature and the reaction mixture was stirred for 2 h then TLC showed no starting material was left. The reaction 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 dichloromethane/ methanol (5:1) to afford 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-α-D-arabinofuranoside **(117)** as a thick colourless oil (0.49 g, 87%) [Found (MALDI) $(M+Na)^+$: 427.2, $C_{22}H_{28}NaO_7$, requires: 427.1], $[\alpha]_D^{20}$ + 105 (*c* 0.10, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.39 – 7.28 (10H, m), 5.04 (1H, s), 4.66 (1H, d, *J* 12.0 Hz), 4.62 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.3 Hz), 4.53 (1H, d, *J* 12.3 Hz), 4.10 (1H, br. d, *J* 1.9 Hz), 4.03 – 3.91 (3H, m), 3.86 (1H, dd, *J* 11.6, 2.3 Hz), 3.78 (1H, dd, 2.0, 12.0 Hz), 3.74 (1H, dd, *J* 4.9, 9.8 Hz), 3.63 – 3.54 (3H, m), 3.15 (1H, br. s), 2.51 – 2.22 (1H, br. s), 1.60 (1H, br. s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 138.1, 137.9, 128.5, 128.4, 128.41, 128.3, 127.8, 127.7, 108.0, 87.3, 78.6, 78.2, 76.4, 73.5, 72.0, 69.6, 66.1, 62.1; ν_{max}: v. br. 3392, 3088, 3064, 3030, 2927, 2875, 1721, 1454, 1099, 755 cm^{-1}. The experiment was repeated on a large scale.

Experiment 29: D-glycerol-(1'→1)-5-*O*-behenoyl-α-D-arabinofuranoside (120)



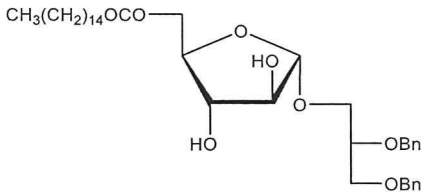
Docosanoic acid (0.08g, 0.23 mmol) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uraniumtetrafluoroborate (TBTU)[287] (0.08 g, 0.24 mmol) were added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-α-D-arabinofuranoside **(117)** (0.1 g, 0.2 mmol) in dry pyridine (6 mL) at room temperature and the mixture was stirred at 60 °C for 4 days. The solvent was evaporated under reduced pressure to give a residue which was diluted with ethyl acetate : THF (3:1, 10 mL). The reaction mixture was washed with saturated solution of $NaHCO_3$ (2×5 mL) dried over $MgSO_4$, filtered and evaporated under reduced pressure to give a thick oil residue. The residue was dissolved in dry CH_2Cl_2 : MeOH (1:1, 2 mL) and palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 15 mg, 0.15 fold by weight) was added at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight, then TLC showed no starting material was left. The reaction mixture was filtered off through a 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 (5:1) to give D-glycerol-(1'→1)-5-*O*-behenoyl-α-D-arabinofuranoside **(120)** as thick white oil (0.1 g, 74%) [Found (MALDI) $(M+Na)^+$: 569.4, $C_{30}H_{58}NaO_8$, requires: 569.4], $[\alpha]_D^{20}$ + 44 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.91 (1H, d, *J* 1.1 Hz), 4.27 (1H, dd, *J* 3.2, 12.0 Hz), 4.17 (1H, dd, *J* 6.2, 11.5 Hz), 4.15 – 4.09 (1H, m), 4.02 (1H, br. d, *J* 1.5 Hz), 3.84 – 3.71 (3H, m), 3.55 (2H, dt, *J* 5.5, 12.0 Hz), 3.47 (1H, dd, *J* 7.0, 12.0 Hz), 2.32 (2H, t, *J* 7.6 Hz), 1.63 – 1.53 (2H, m), 1.22 (40H, s), 0.84 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 174.1, 108.4, 82.0, 80.9, 77.4, 70.6, 69.3, 64.0, 63.2, 34.0, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 24.7, 22.6, 14.0; ν_{max}: v. br. 3399, 2922, 2851, 1733, 1467, 1215, 758 cm^{-1}.

Experiment 30: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (118)



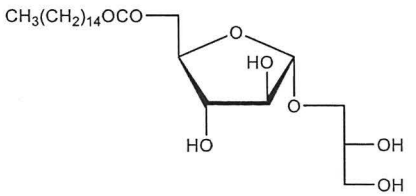
4-Toluenesulfonyl chloride[303] (1.0 g, 5.2 mmol) and DMAP (0.2 g, 1.6 mmol) were added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-α-D-arabinofuranoside (**117**) (1.5 g, 3.7 mmol) in dry pyridine (20 mL) under nitrogen atmosphere at room temperature. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The reaction mixture was quenched with H_2O (20 mL), the organic layer was separated by decanting and diluted with CH_2Cl_2 (50 mL). The organic layer was washed with 1 N aqueous HCl (4×15 mL), saturated aqueous solution of $NaHCO_3$ (4×15 mL), dried over $MgSO_4$, 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 (1:2) to afford 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(118)** as a thick oil (1.5 g, 72%) [Found (MALDI) $(M+Na)^+$: 581.3, $C_{29}H_{34}NaO_9S$, requires: 581.1], $[\alpha]_D^{20}$ + 75 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.8 (2H, d, *J* 8.2 Hz), 7.40 – 7.27 (12H, m), 4.94 (1H, s), 4.64 (1H, d, *J* 12.1 Hz), 4.59 (1H, d, *J* 11.9 Hz), 4.54 (1H, d, *J* 12.0 Hz), 4.51 (1H, d, *J* 12.2 Hz), 4.18 (1H, dd, *J* 3.9, 10.4, Hz), 4.13 (1H, dd, *J* 2.9, 7.3 Hz), 4.15 – 4.07 (1H, m), 4.01 (1H, br. d, *J* 5.6 Hz), 3.87 (1H, dd, *J* 4.0, 10.4 Hz), 3.81 (1H, br. d, *J* 8.8 Hz), 3.73 (1H, br. p, *J* 4.8 Hz), 3.57 (2H, br. d, *J* 5.3), 2.53 (1H, dd, *J* 5.1, 10.6 Hz), 3.17 (1H, br. d, *J* 10.2 Hz), 2.58 (1H, br. d, *J* 6.5 Hz), 2.44 (3H, s); δ_C (101 MHz, $CDCl_3$): 145.1, 138.0, 137.8, 132.4, 130.0, 129.95, 129.9, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 107.6, 83.3, 79.9, 77.6, 76.4, 73.4, 72.0, 69.5, 69.1, 66.5, 21.6; ν_{max}: 3443, 3088, 3064, 3031, 2924, 2854, 1732, 1360, 1190, 736 cm^{-1}.

Experiment 31: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-palmitoyl-α-D-arabino-furanoside (121)



Cesium hydrogencarbonate (0.173 g, 0.892 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(118)** (0.10 g, 0.17 mmol) and palmitic acid (0.05 g, 0.19 mmol) in dry DMF : THF (1:5, 6 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 2 days. The reaction was worked up and purified as before to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-palmitoyl-α-D-arabinofuranoside **121** as a colourless oil (0.08 g, 72%) $[\alpha]_D^{23}$ + 40 (*c* 0.1, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 665.3, $C_{38}H_{58}NaO_8$, requires: 665.4] which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.28 (10H, m), 5.02 (1H, s), 4.67 (1H, d, *J* 12.1 Hz), 4.62 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.3 Hz), 4.53 (1H, d, *J* 12.3 Hz), 4.24 (2H, m), 4.15 (1H, dd, *J* 3.7, 6.8, Hz), 4.05 (1H, br. d, *J* 8.3 Hz), 3.93 (1H, dd, *J* 4.1, 10.4 Hz), 3.85 (1H, br. d, *J* 8.5 Hz), 3.78 – 3.71 (1H, m), 3.61 – 3.54 (3H, m), 3.01 (1H, d, *J* 11.1 Hz), 2.38 (1H, d, *J* 8.3 Hz), 2.34 (2H, t, *J* 7.6 Hz), 1.67 – 1.54 (2H, m), 1.26 (24H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.4, 138.1, 137.9, 128.4, 128.3, 127.73, 127.72, 127.7, 127.6, 107.5, 83.9, 79.8, 77.9, 76.5, 73.4, 72.0, 69.6, 66.5, 63.9, 34.0, 31.9, 29.64, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 24.7, 22.6, 14.1; ν_{max}: 3443, 3088, 3064, 3031, 2924, 2854, 1737, 1454, 1099, 736 cm^{-1}.

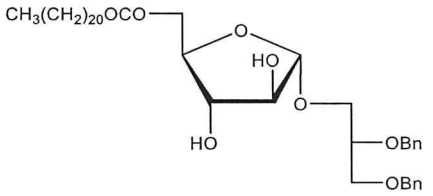
Experiment 32: D-glycerol-(1'→1)-5-*O*-palmitoyl-α-D-arabinofuranoside (122)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 4.2 mg, 0.15 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-palmitoyl-α-D-arabinofuranoside **(121)** (0.028 g, 0.043 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was

stirred for 16 h, then TLC showed no starting material was left. The mixture was worked up and purified as before to give D-glycerol-(1'→1)-5-*O*-palmitoyl-α-D-arabinofuranoside **(122)** as a colourless thick oil (16 mg, 79%) $[\alpha]_D^{21}$ + 5.1 (*c* 0.1, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 485.11, $C_{24}H_{46}NaO_8$, requires: 485.30] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.87 (1H, s), 4.23 (1H, dd, *J* 3.3, 11.5 Hz), 4.16 – 4.06 (3H, m), 3.97 (1H, br. d, *J* 1.6 Hz), 3.80 – 3.71 (4H, m), 3.56 (1H, dd, *J* 4.6, 12.1 Hz), 3.50 (1H, dd, *J* 5.7, 11.4 Hz), 3.42 – 3.38 (1H, m), 2.28 (2H, t, *J* 7.6 Hz), 1.59 – 1.51 (2H, m), 1.19 (26H, m), 0.81 (3H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 174.0, 108.3, 81.9, 80.8, 77.3, 70.5, 69.2, 63.9, 63.1, 33.9, 31.7, 29.44, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 24.6, 22.4, 13.8; ν_{max}: 3400, 2919, 2851, 1736, 1467, 1041, 758 cm^{-1}.

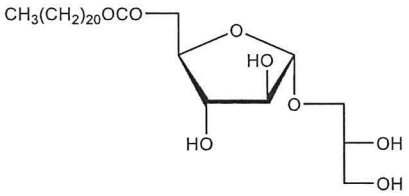
Experiment 33: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-behenoyl-α-D-arabinofuranoside (123)



Cesium hydrogencarbonate (0.173 g, 0.892 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(118)** (0.10 g, 0.17 mmol) and docosanoic acid (0.09 g, 0.27 mmol) in dry DMF : THF (1:5, 4 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C 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 with water (15 mL), brine (15 mL), dried over $MgSO_4$, filtered and evaporated to give a thick oil residue. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-behenoyl-α-D-arabinofuranoside **(123)** as a colourless oil (0.04 g, 30%) [Found (MALDI) $(M+Na)^+$: 749.7, $C_{44}H_{70}NaO_8$, requires: 749.4], $[\alpha]_D^{20}$ + 39 (c 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 5.02 (1H, s), 4.67 (1H, d, *J* 12.0 Hz), 4.62 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.3 Hz), 4.53 (1H, d, *J* 12.3 Hz), 4.25 (2H, m), 4.14 (1H, dd, *J* 4.0, 6.9 Hz), 4.05 (1H, br. d, *J* 8.0 Hz), 4.00 – 3.90 (2H, m), 3.85 (1H, br. d, *J* 10.0

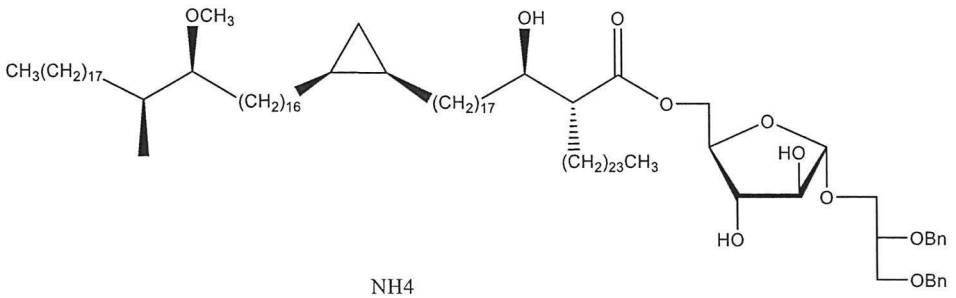
Hz), 3.78 – 3.72 (1H, m), 3.61 – 3.54 (2H, m), 3.06 (1H, d, *J* 10.0 Hz), 2.49 (1H, d, *J* 8.0 Hz), 2.34 (2H, t, *J* 7.6 Hz), 1.67 – 1.57 (2H, m), 1.27 (36H, s), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 128.4, 127.8, 127.7, 107.5, 84.1, 79.7, 78.0, 76.5, 73.5, 72.1, 69.6, 66.5, 63.9, 34.1, 31.9, 29.7, 29.67, 29.6, 29.5, 29.4, 29.2, 29.1, 29.0, 24.8, 22.7, 14.1; ν_{max}: 3446, 3089, 3065, 3032, 2921, 2849, 1739, 1455, 1099, 698, 736 cm^{-1}.

Experiment 34: D-glycerol-(1'→1)-5-*O*-behenoyl-α-D-arabinofuranoside (124)



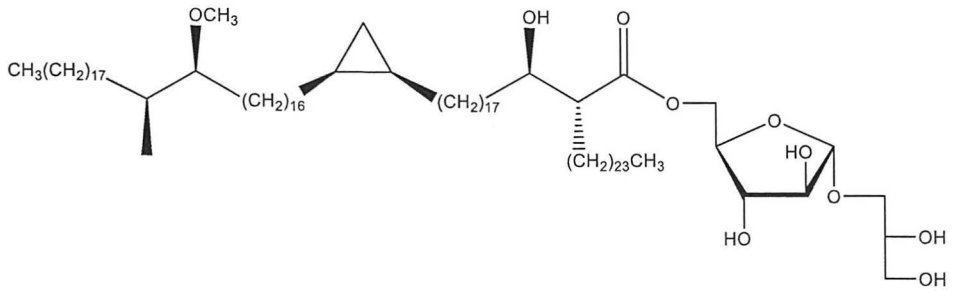
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 4.0 mg, 0.15 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-behenoyl-α-D-arabinofuranoside **(123)** (0.028 g, 0.039 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight then TLC showed no starting material was left. The mixture was filtered off through celite and the filtarate was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:2) gave D-glycerol-(1'→1)-5-*O*-behenoyl-α-D-arabinofurano-side **(124)** as a colourless thick oil (16 mg, 74%). All the data were identical to **(120)**.

Experiment 35: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*, 2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside (125)



Cesium hydrogencarbonate (0.123 g, 0.670 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(118)** (0.051 g, 0.091 mmol) and 2-{(1*R*)-1-hydroxy-18-[2-(17-methoxy-18-methyl hexatriacontyl)cyclopropyl]octadecyl}hexacosanoic acid **(80)**[158] (0.089 g, 0.070 mmol) in dry DMF : THF (1:5, 3 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 3 days then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford 2',3'-di-*O*-benzyl-D-glycerol-(1'-1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside **(125)** as a colourless thick oil (77 mg, 66%) [Found $(M+NH_4)^+$: 1657.5005, $C_{107}H_{198}NO_{10}$, requires: 1657.5010], $[\alpha]_D^{23}$ + 35 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 5.00 (1H, s), 4.67 (1H, d, *J* 12.0 Hz), 4.61 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.0 Hz), 4.53 (1H, d, *J* 12.0 Hz), 4.45 (1H, dd, *J* 3.7, 12.0 Hz), 4.24 (1H, dd, *J* 4.0, 12.0 Hz), 4.11 (1H, m), 4.04 (1H, br. s), 3.91 (2H, br. dd, *J* 4.0, 10.4 Hz), 3.78 – 3.71 (1H, m), 3.69 (1H, m), 3.58 (3H, m), 3.35 (3H, s), 3.00 – 2.93 (1H, m), 2.42 (1H, ddd, *J* 4.9, 7.3, 9.8 Hz), 1.42 – 1.09 (150H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.70 – 0.61 (2H, m), 0.60 – 0.53 (1H, dt, *J* 3.9, 7.9 Hz), -0.33 (1H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 174.9, 138.2, 137.9, 128.4, 127.75, 127.7, 127.6, 107.5, 85.4, 83.6, 80.5, 78.3, 76.6, 73.4, 72.7, 72.1, 69.7, 66.5, 63.3, 57.7, 52.3, 35.3, 35.1, 32.4, 31.9, 30.5, 30.2, 29.96, 29.9, 29.73, 29.7, 29.65, 29.6, 29.54, 29.5, 29.4, 29.35, 29.3, 28.7, 27.5, 27.4, 26.1, 25.4, 22.7, 15.7, 14.9, 14.1, 10.9; ν_{max}: 3419, 3062, 3031, 2920, 2853, 1733, 1456, 1099, 733, 697 cm^{-1}.

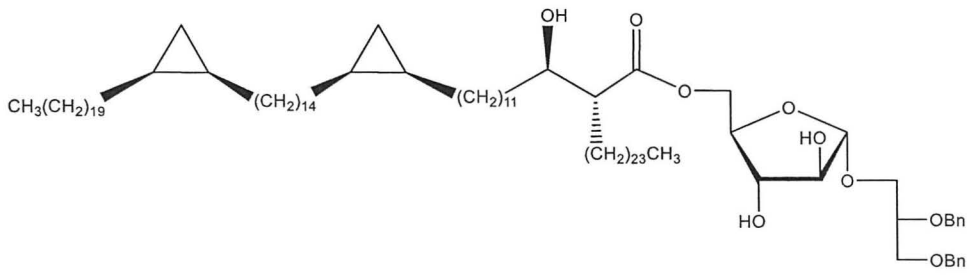
Experiment 36: D-Glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*, 2*S*)-2-[(17*S*, 18 *S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosano-ate)-α-D-arabinofuranoside (126)



Palladium hydroxide on activated charcoal (20 % $Pd(OH)_2$-C, 0.02 g, 0.15 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'-1)-5-*O*-(2-

[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside **(125)** (0.0746 g, 0.0456 mmol) in hexane : ethyl acetate (1:1, 4 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h then TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with (10 mL) CH_2Cl_2, the filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:2) to afford D-glycerol (1'-1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*, 2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside **(126)** as a colourless thick oil (59.6 mg, 89%) $[\alpha]_D^{23}$ + 24 (*c* 0.1, $CHCl_3$), [Found $(M+NH_4)^+$: 1477.4066, $C_{93}H_{186}NO_{10}$, requires: 1477.4071] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.88 (1H, s), 4.33 (1H, dd, *J* 4.2, 11.6 Hz), 4.22 (1H, dd, *J* 5.7, 11.6 Hz), 4.09 (1H, br. dd, *J* 5.5, 9.9 Hz), 3.99 (1H, dd, *J* 1.2, 2.8 Hz), 3.84 (1H, dd, *J* 2.8, 5.4 Hz), 3.76 (2H, m), 3.68 – 3.60 (1H, m), 3.59 – 3.48 (2H, m), 3.44 (1H, dd, *J* 7.1, 11.3 Hz), 3.32 (3H, s), 3.02 – 2.94 (1H, m), 2.46 – 2.35 (1H, m), 1.72 – 0.94 (152H, m), 0.85 (6H, t, *J* 7.2 Hz), 0.82 (3H, d, *J* 6.8 Hz), 0.68 – 0.57 (2H, m), 0.53 (1H, dt, *J* 3.9, 7.9 Hz), -0.37 (1H, br. q, *J*, 5.1 Hz); δ_C (126 MHz, $CDCl_3$ + few drops CD_3OD): 174.6, 108.0, 85.2, 81.4, 81.0, 77.5, 71.8, 70.2, 68.5, 63.2, 62.5, 56.8, 52.3, 34.7, 34.1, 31.8, 31.3, 29.8, 29.6, 29.2, 29.1, 29.0, 28.9, 28.7, 28.4, 28.1, 26.8, 25.3, 24.6, 22.0, 15.2, 14.0, 13.1, 10.1; ν_{max}: 3390, 2919, 2852, 1731, 1467, 1099, 720 cm^{-1}.

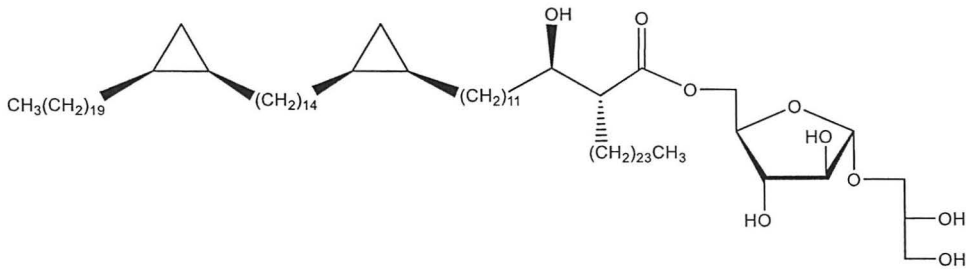
Experiment 37: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (128)



Cesium hydrogencarbonate (0.096 g, 0.465 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(118)** (0.050 g, 0.042 mmol) and (2*R*)-2-{(1*R*)-1-hydroxy-12-[(1*R*)-2-(14-[(2*S*)-2-

icosylcyclopropyl)tetradecyl]cyclopropyl]dodecyl}hexacosanoic acid **(127)**[157] (0.098 g, 0.079 mmol) in dry DMF : THF (1:5, 6 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 3 days then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford 2',3'-di-*O*-benzyl-D-glycerol-(1'-1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside **(128)** as a colourless thick oil (0.11 g, 84%) $[\alpha]_D^{23}$ - 37 (*c* 0.1, $CHCl_3$), [Found $(M+NH_4)^+$: 1541.3777, $C_{100}H_{182}NO_9$, requires: 1541.3809] which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.99 (1H, s), 4.67 (1H, d, *J* 12.0 Hz), 4.63 (1H, d, *J* 12.0 Hz), 4.54 (2H, br. s), 4.43 (1H, dd, *J* 4.0, 12.0 Hz), 4.27 (1H, dd, *J* 4.0, 12.0 Hz), 4.12 (1H, dd, *J* 3.8, 7.4 Hz), 4.05 (1H, dd, *J* 4.5, 8.5 Hz), 3.95 – 3.87 (2H, m), 3.75 (1H, dt, *J* 5.1, 10.0 Hz), 3.69 (1H, br. s), 3.61 – 3.53 (3H, m), 3.17 (1H, s), 3.02 (1H, s), 2.72 (1H, s), 2.48 – 2.38 (1H, m), 2.11 – 1.05 (134H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.73 – 0.61 (4H, m), 0.61 – 0.53 (2H, dt, *J* 4.0, 8.0 Hz), -0.32 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 174.9, 138.2, 137.9, 128.4, 127.7, 127.69, 127.6, 107.5, 83.7, 80.4, 78.3, 76.5, 73.4, 72.7, 72.1, 69.7, 66.5, 63.3, 52.3, 35.2, 31.9, 30.2, 29.7, 29.64, 29.6, 29.5, 29.4, 29.3, 29.1, 28.7, 27.41, 25.4, 23.9, 22.7, 15.8, 14.1, 10.9; ν_{max}: 3414, 3065, 3031, 2921, 2852, 1733, 1466, 1099, 720 cm^{-1}.

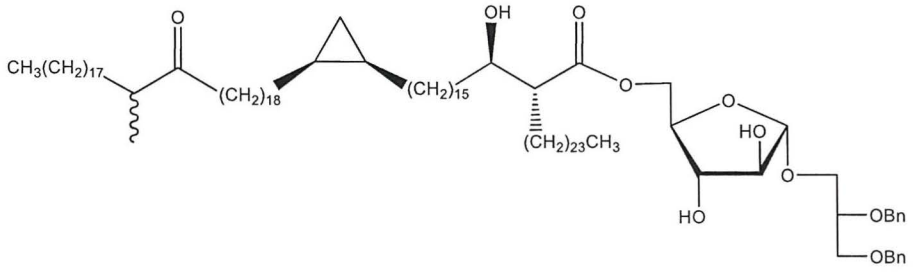
Experiment 38: D-Glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (129)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 11.0 mg, 0.30 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside **(128)** (0.0739 g, 0.0484 mmol) in dry CH_2Cl_2 : MeOH (1:1, 3 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The

mixture was filtered off and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to give D-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosyl-cyclopropyl]tetradecyl)cyclopropyl]do-decyl}hexacosanoate)-α-D-arabinofuranoside **(129)** as a thick oil (11.9 mg, 18%) [Found $(M+NH_4)^+$: 1361.2863, $C_{86}H_{170}NO_9$, requires: 1361.2870], $[\alpha]_D^{23}$ - 15 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.89 (1H, s), 4.36 (1H, dd, *J* 4.5, 11.6 Hz), 4.24 (1H, dd, *J* 5.3, 11.7 Hz), 4.10 (1H, dd, *J* 4.9, 9.9 Hz), 3.99 (1H, br. d, *J* 1.3 Hz), 3.86 (1H, dd, *J* 2.7, 4.9 Hz), 3.74 (2H, m), 3.66 – 3.58 (1H, m), 3.58 – 3.50 (2H, m), 3.47 (1H, dd, *J* 5.4, 9.9 Hz), 2.43 (1H, ddd, *J* 5.0, 7.0, 9.9 Hz), 1.61 – 0.97 (139H, m), 0.84 (6H, t, *J* 6.8 Hz), 0.65 – 0.56 (4H, m), 0.56 – 0.46 (2H, dt, *J* 4.0, 8.0 Hz), -0.38 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.0, 108.2, 82.1, 81.2, 77.8, 72.5, 70.6, 69.2, 63.4, 63.3, 52.8, 50.1, 34.9, 31.8, 30.1, 29.6, 29.5, 29.3, 29.2, 29.1, 28.6, 27.3, 25.2, 22.6, 15.7, 13.9, 10.8; ν_{max}: 3367, 2918, 2850, 1726, 1466, 1045, 759 cm^{-1}.

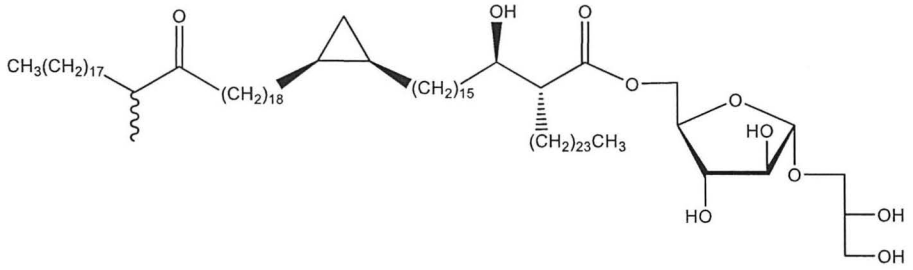
Experiment 39: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-{2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl]hexacosanoyl}-α-D-arabinofuranoside (130)



Cesium hydrogencarbonate (0.126 g, 0.649 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(118)** (0.077 g, 0.137 mmol) and 2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexacosanoic acid **(89)**[159] (0.10 g, 0.08 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 3 days then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranoside **(130)** as a

colourless thick oil (0.1 g, 76%) [Found $(M+NH_4)^+$: 1641.4691, $C_{106}H_{194}NO_{10}$, requires: 1641.4697], $[\alpha]_D^{23}$ + 32 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.29 (10H, m), 5.0 (1H, s), 4.67 (1H, d, *J* 12.0 Hz), 4.61 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.3 Hz), 4.52 (1H, d, *J* 12.3 Hz), 4.45 (1H, dd, *J* 3.6, 12.0 Hz), 4.24 (1H, dd, *J* 3.9, 12.0 Hz), 4.11 (1H, br. d, *J* 2.2 Hz), 4.04 (1H, br. s), 3.91 (2H, br. dd, *J* 3.9, 10.4 Hz), 3.74 (1H, m), 3.71 – 3.64 (1H, m), 3.62 – 3.53 (3H, m), 3.02 – 2.88 (1H, m), 2.51 (1H, m), 2.43 (3H, including a triplet at 2.3 with *J* 7.7 Hz), 1.72 – 1.11 (146H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.7 Hz), 0.69 – 0.61 (2H, m), 0.56 (1H, dt, *J* 4.0, 8.0 Hz), -0.33 (1H, br. q, *J* 5.1 Hz); δ_C (126 MHz, $CDCl_3$): 215.3, 174.9, 128.44, 128.4, 127.8, 127.7, 107.4, 84.1, 80.2, 78.3, 73.5, 72.7, 72.1, 69.6, 66.4, 60.4, 52.1, 46.3, 41.1, 33.0, 31.9, 30.2, 29.7, 29.65, 29.6, 29.5, 29.4, 28.7, 27.4, 27.3, 23.7, 22.7, 21.0, 16.4, 15.8, 14.1, 10.9; ν_{max}: 3419, 3069, 2919, 2851, 1738, 1714, 1467, 1097, 734 cm^{-1}.

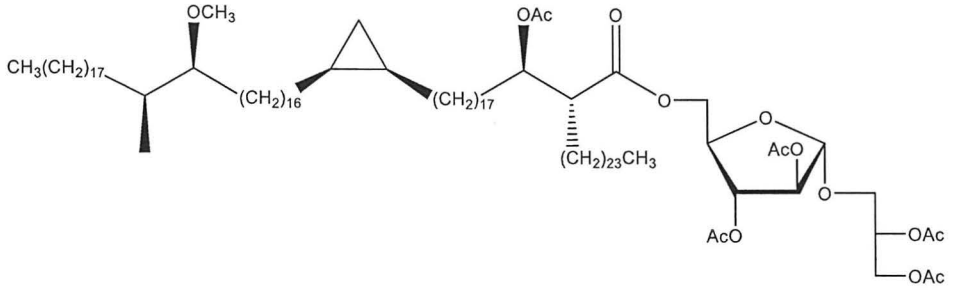
Experiment 40: D-Glycerol (1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabino-furanoside (131)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 18 mg, 0.2 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranoside **(130)** (0.090 g, 0.055 mmol) in dry hexane : ethyl acetate (1:1, 4 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h then TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with (10 mL) CH_2Cl_2, the filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to afford D-glycerol-(1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriaco-ntyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranoside **(131)** as a colour-less thick oil (0.06 g, 75%) $[\alpha]_D^{23}$ + 16 (*c* 0.1, $CHCl_3$), [Found $(M+NH_4)^+$: 1461.3760,

$C_{92}H_{182}NO_{10}$, requires: 1461.3758] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.89 (1H, s), 4.36 (1H, dd, *J* 4.5, 11.7 Hz), 4.24 (1H, dd, *J* 5.3, 11.7 Hz), 4.11 (1H, dd, *J* 4.2, 9.1 Hz), 3.99 (1H, br. d, *J* 1.3 Hz), 3.86 (1H, dd, *J* 2.7, 4.7 Hz), 3.80 – 3.71 (2H, m), 3.65 – 3.59 (1H, m), 3.58 – 3.53 (2H, m), 3.47 (1H, dd, *J* 5.3, 10.0 Hz), 2.48 (1H, sx, *J* 6.8 Hz), 2.42 – 2.35 (3H, including a triplet at 2.3 with *J* 7.7 Hz), 1.64 – 1.04 (149H, m), 1.01 (3H, d, *J* 6.9 Hz), 0.84 (6H, t, *J* 6.8 Hz), 0.65 – 0.56 (2H, m), 0.52 (1H, dt, *J* 4.0, 8.0 Hz), -0.37 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 216.0, 175.0, 108.2, 82.2, 81.2, 77.8, 77.2, 72.5, 70.6, 69.3, 63.4, 63.3, 52.7, 46.2, 41.1, 32.9, 31.8, 30.1, 29.6, 29.5, 29.4, 29.3, 29.24, 29.2, 28.6, 27.3, 27.2, 25.2, 23.6, 22.6, 16.2, 15.7, 13.9, 10.8; ν_{max}: 3378, 2919, 2851, 1731, 1714, 1467, 1044, 735 cm^{-1}.

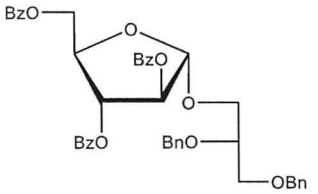
Experiment 41: 2',3'-Di-*O*-acetyl-D-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxyacetyl -18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-2,3-di-*O*-acetyl-α-D-arabinofuranoside (132)



Acetic anhydride (12.3 μL, 0.0130 mmol) was added dropwise to a stirred solution of D-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside **(126)** (0.0192 g, 0.0131 mmol) in anhydrous pyridine (0.0137 mL, 0.1734 mmol), at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was diluted with ethyl acetate (25 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 with water (2×10 mL), 1 M aqueous HCl (2×10 mL), saturated solution of $NaHCO_3$ (1×10 mL), brine (1×10 mL), and dried over ($MgSO_4$). The solvent was evaporated under reduced pressure. The residue was purified by column chromategraphy on silica eluting with hexane/ethyl acetate (5:2) affording 2',3'-di-*O*-acetyl-D-

glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxyacetyl-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-2,3-di-*O*-acetyl-α-D-ara-binofuranoside **(132)** as a colourless oil (18 mg, 82%) [Found $(M+NH_4)^+$: 1687.4598, $C_{103}H_{196}NO_{15}$, requires: 1687.4600], $[\alpha]_D^{20}$ + 52 (*c* 0.1, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$): 5.20 (1H, dddd, *J* 4.5, 4.8, 5.0, 6.5 Hz), 5.10 (1H, td, *J* 3.9, 7.9 Hz), 5.07 (1H, d, *J* 1.4 Hz), 4.99 (1H, s), 4.96 (1H, dd, *J* 1.1, 4.7 Hz), 4.37 (1H, dd, *J* 3.1, 11.4 Hz), 4.32 (1H, dd, *J* 3.6, 12.0 Hz), 4.26 (1H, dd, *J* 5.6, 11.4 Hz), 4.24 – 4.21 (1H, m), 4.16 (1H, dd, *J* 6.0, 12.1 Hz), 3.80 (1H, dd, *J* 5.4, 10.5 Hz), 3.60 (1H, dd, *J* 5.9, 10.5 Hz), 3.33 (3H, s), 2.97 – 2.93 (1H, m), 2.65 (1H, ddd, *J* 4.4, 7.0, 10.4 Hz), 2.10 (6H, s), 2.09 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 1.29 (147H, m), 0.88 (6H, t, *J* 7.0 Hz), 0.84 (3H, d, *J* 8.9 Hz), 0.67 – 0.61 (2H, m), 0.55 (1H, dt, *J* 4.0, 8.0 Hz), -0.34 (1H, q, *J* 5.3 Hz); δ_C (101 MHz, $CDCl_3$): 172.8, 170.5, 170.3, 170.2, 170.1, 169.6, 105.6, 85.4, 81.1, 80.6, 77.2, 73.8, 69.9, 65.2, 63.3, 62.6, 57.7, 49.6, 35.3, 34.1, 32.4, 31.9, 31.7, 31.6, 30.5, 30.2, 29.97, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.0, 28.7, 28.1, 27.6, 27.3, 26.2, 24.9, 22.7, 22.64, 22.6, 22.3, 22.2, 21.0, 20.9, 20.74, 20.7, 20.6, 15.8, 14.9, 14.2, 14.1, 14.0, 11.4, 10.9; ν_{max}: 2924, 2853, 1746, 1464, 1097, 724 cm^{-1}.

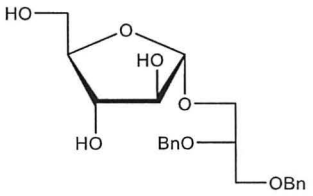
Experiment 42: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (133)



Tin (IV) chloride (4.7 mL, 0.5 M solution in CH_2Cl_2, 1.0 mmol) was added to a stirred solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinfuranosyl bromide **(115)** (5.0 g, 9.5 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C under nitrogen atmosphere. The mixture was stirred for 10 min. then 2,3-di-*O*-benzyl-L-glycerol **(114)** (2.8 g, 10 mmol) in CH_2Cl_2 (2 mL) was added following by the addition of ethyldiisoprpylamine (0.9 g, 6.9 mmol) in CH_2Cl_2 (2 mL). The reaction mixture were stirred for 2 h then TLC showed no starting material was left. The reaction mixture was worked up as before to give the residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (3:1) to give 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(133)** as a thick colourless oil (4.5 g, 65%) $[\alpha]_D^{20}$ - 30 (*c* 0.1, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 739.4, $C_{43}H_{40}NaO_{10}$, requires: 739.2] which showed δ_H (400 MHz, $CDCl_3$):

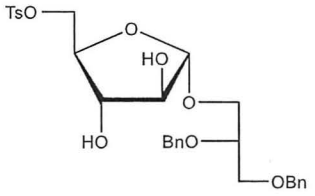
8.10 – 7.99 (6H, m), 7.63 – 7.56 (2H, m), 7.54 – 7.49 (1H, m), 7.45 – 7.38 (4H, m), 7.35 – 7.23 (12H, m), 5.59 – 5.56 (2H, m), 5.32 (1H, s), 4.82 (1H, dd, *J* 3.3, 12.0 Hz), 4.75 (1H, d, *J* 12.5 Hz), 4.71 (1H, d, *J* 12.7 Hz), 4.66 (1H, dd, *J* 4.8, 12.0 Hz), 4.55 (1H, dd, *J* 4.6, 8.3 Hz), 4.51 (2H, br. s), 3.98 (1H, dd, *J* 5.6, 10.4 Hz), 3.92 – 3.85 (1H, m), 3.76 (1H, dd, *J* 4.1, 10.4 Hz), 3.68 (1H, dd, *J* 4.6, 9.6 Hz), 3.65 (1H, dd, *J* 5.3, 9.9 Hz); δ_C (101 MHz, $CDCl_3$): 166.2, 165.7, 165.4, 138.4, 138.1, 133.5, 133.4, 133.0, 129.9, 129.8, 129.7, 129.1, 129.0, 128.5, 128.4, 128.31, 128.3, 128.2, 127.64, 127.6, 127.52, 127.5, 105.7, 82.0, 81.2, 77.9, 76.8, 73.4, 72.3, 69.9, 67.3, 63.6; ν_{max}: 2987, 2935, 2875, 1724, 1070, 845, 712 cm^{-1}. The experiment was repeated on a large scale.

Experiment 43: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-α-D-arabinofuranoside (134)



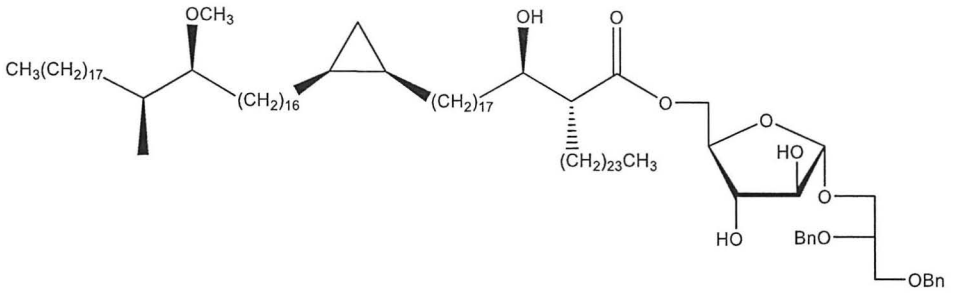
Sodium methoxide (0.1 M, in methanol, 6.0 mL) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(133)** (8.0 g, 11 mmol) in dry MeOH : CH_2Cl_2 (1:1, 8 mL) at room temperature and the reaction mixture was stirred for 2 h then TLC showed no starting material was left. The mixture was neutralized with Amberlite IR-120 (H^+), then the resin was filtered off and the solvent was removed under reduced pressure to give the residue which was purified by column chromatography on silica eluting with dichloromethane/methanol (10:1) to afford 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-α-D-arabinofuranoside **(134)** as a thick colourless oil (3.8 g, 84%) [Found (MALDI) $(M+Na)^+$: 427.325, $C_{22}H_{28}NaO_7$, requires : 427.173], $[\alpha]_D^{20}$ + 95 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, CD_3OD): 7.38 – 7.24 (10H, m), 4.89 (1H, d, *J* 1.4 Hz), 4.69 (1H, d, *J* 11.9 Hz), 4.65 (1H, d, *J* 11.8 Hz), 4.55 (1H, d, *J* 12.2 Hz), 4.52 (1H, d, *J* 12.3 Hz), 3.98 (1H, dd, *J* 1.5, 3.6 Hz), 3.96 – 3.91 (1H, m), 3.86 – 3.79 (3H, m), 3.74 (1H, dd, *J* 3.2, 11.9 Hz), 3.69 – 3.58 (4H, m); δ_C (101 MHz, CD_3OD): 139.9, 139.7, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 109.7, 85.6, 83.5, 78.7, 78.5, 74.3, 73.1, 71.2, 68.2, 62.9; ν_{max}: 3399, 3063, 3030, 2923, 2874, 1454, 1099, 737, 697 cm^{-1}. The experiment was repeated on a large scale.

Experiment 44: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (135)



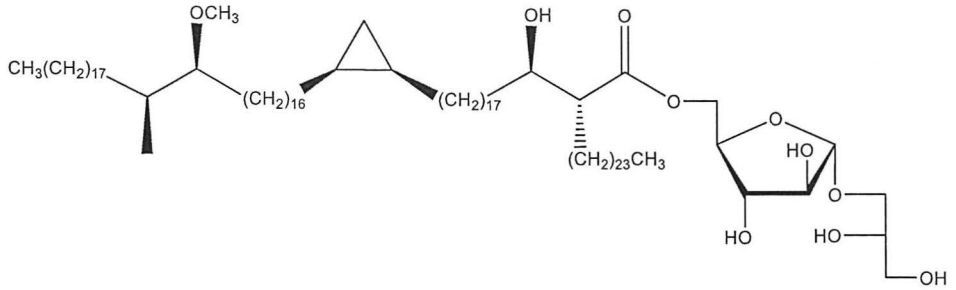
4-Toluenesulfonyl chloride (1.0 g, 5.2 mmol) and DMAP (0.2 g, 1.8 mmol) were added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-α-D-arabino-furanoside **(134)** (1.5 g, 3.7 mmol) in dry pyridine (20 mL) under nitrogen atmosphere at room temperature. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The reaction mixture was quenched by the addition of H_2O (10 mL), the organic layer was separated by decanting and diluted with CH_2Cl_2 (20 mL). The reaction mixture was washed with 1 N aqueous HCl (4×15 mL), saturated aqueous solution of $NaHCO_3$ (4×15 mL), dried over $MgSO_4$, 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 (1:2) to afford 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(135)** as a thick oil (1.3 g, 62%) [Found (MALDI) $(M+Na)^+$: 581.2, $C_{29}H_{34}NaO_9S$, requires: 581.1], $[\alpha]_D^{20}$ + 61 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.80 (2H, d, *J* 8.3 Hz), 7.39 – 7.28 (12H, m), 4.93 (1H, s), 4.65 (1H, d, *J* 11.9 Hz), 4.59 (1H, d, *J* 11.9 Hz), 4.54 (1H, d, *J* 12.3 Hz), 4.51 (1H, d, *J* 12.2 Hz), 4.20 (1H, dd, *J* 10.4, 3.9 Hz), 4.14 (1H, dd, *J* 10.4, 4.5 Hz), 4.11 (1H, m), 4.04 (1H, br. d, *J* 0.6 Hz), 3.84 (1H, br. s), 3.81 (1H, dd, *J* 10.5, 5.7 Hz), 3.78 – 3.70 (1H, m), 3.62 – 3.54 (3H, m), 2.99 (1H, br. s), 2.54 (1H, br. s), 2.44 (3H, s); δ_C (101 MHz, $CDCl_3$):145.1, 138.0, 137.9, 132.5, 129.9, 128.4, 128.0, 127.9, 127.8, 127.7, 107.6, 83.3, 80.0, 77.6, 76.4, 73.4, 72.1, 69.5, 68.98, 66.9, 21.6; ν_{max}: 3435, 3064, 3031, 2925, 2873, 1454, 1176, 740 cm^{-1}.

Experiment 45: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2S)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside (136)



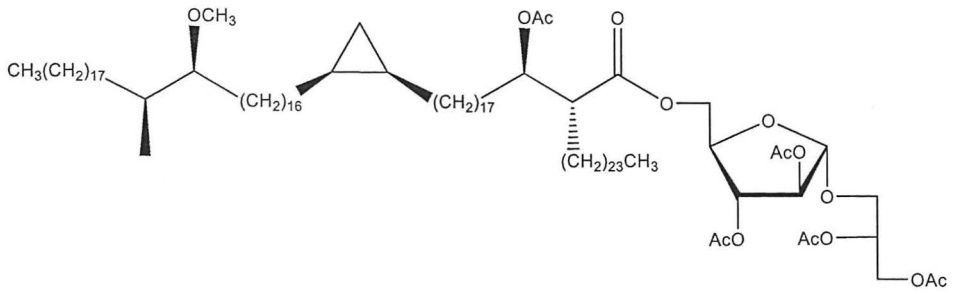
Cesium hydrogencarbonate (0.123 g, 0.634 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(135)** (0.075 g, 0.134 mmol) and 2-{(1*R*)-1-hydroxy-18-[2-(17-methoxy-18-methyl-hexatri-acontyl)cyclopropyl]octadecyl}hexacosanoic acid **(80)**[158] (0.113 g, 0.090 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 2 days then TLC showed no starting material was left. The reaction was worked up and purified as before to give 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexa-triacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside **(136)** (0.11 g, 74%) [Found (MALDI) $(M+Na)^+$: 1662.6, $C_{107}H_{194}NaO_{10}$, requires: 1662.4], $[\alpha]_D^{23}$ + 25 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.97 (1H, s), 4.66 (1H, d, *J* 11.9 Hz), 4.62 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.5 Hz), 4.52 (1H, d, *J* 12.2 Hz), 4.43 (1H, dd, *J* 4.0, 11.9 Hz), 4.28 (1H, dd, *J* 4.1, 12.0 Hz), 4.13 (1H, dd, *J* 3.8, 7.5 Hz), 4.06 (1H, br. s), 3.96 (2H, br. dd, *J* 5.9, 10.3 Hz), 3.80 – 3.73 (1H, m), 3.68 (1H, m), 3.62 – 3.55 (3H, m), 3.35 (3H, s), 3.00 – 2.94 (1H, m), 2.43 (1H, ddd, *J* 5.0, 7.1, 9.9 Hz), 1.75 – 1.05 (150H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.70 – 0.62 (2H, m), 0.57 (1H, dt, *J* 3.9, 7.9 Hz), -0.32 (1H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 174.9, 138.1, 138.0, 128.4, 127.8, 127.7, 127.6, 107.5, 85.4, 83.2, 80.7, 78.3, 76.5, 73.4, 72.7, 72.1, 69.7, 66.8, 63.3, 57.7, 52.4, 35.3, 32.3, 31.9, 30.4, 30.2, 29.9, 29.89, 29.7, 29.6, 29.59, 29.52, 29.5, 29.4, 29.3, 29.27, 28.7, 27.5, 27.4, 26.1, 25.4, 22.7, 15.7, 14.8, 14.1, 10.9; ν_{max}: 3446, 3063, 3030, 2923, 2853, 1733, 1466, 1099, 735 cm^{-1}.

Experiment 46: L-Glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18 *S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside (137)



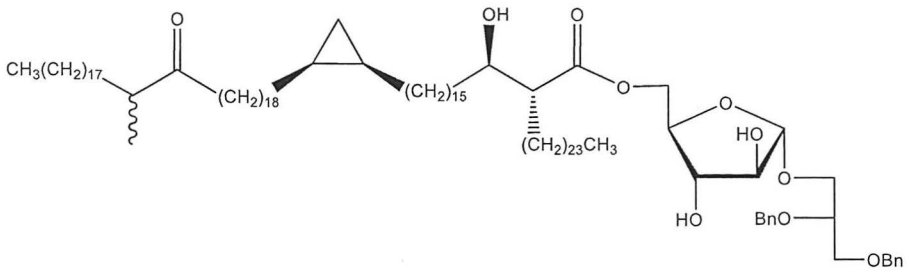
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 20 mg, 0.2 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]-cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofuranoside **(136)** (0.10 g, 0.06 mmol) in hexane : ethyl acetate (1:1, 4 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h then TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with CH_2Cl_2 (10 mL), the filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to afford L-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octa-decyl]hexacosanoate)-α-D-arabinofuranoside **(137)** as a colourless thick oil (80 mg, 90%) $[\alpha]_D^{23}$ + 20 (*c* 0.1, $CHCl_3$), [Found $(M+NH_4)^+$: 1477.4068, $C_{93}H_{186}NO_{10}$, requires: 1477.4071] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.88 (1H, s), 4.35 (1H, dd, *J* 4.4, 11.8 Hz), 4.23 (1H, dd, *J* 5.2, 11.5 Hz), 4.10 (1H, m), 3.98 (1H, br. d, *J* 1.2 Hz), 3.86 (1H, dd, *J* 2.1, 4.6 Hz), 3.82 – 3.75 (1H, m), 3.69 (1H, dd, *J* 5.7, 9.5 Hz), 3.65 – 3.57 (2H, m), 3.57 – 3.48 (2H, m), 3.40 (3H, s), 2.92 (1H, br. s), 2.43 – 2.33 (1H, m), 1.59 – 0.98 (152H, m), 0.83 (6H, t, *J* 6.0 Hz), 0.80 (3H, d, *J* 4.5 Hz), 0.64 – 0.56 (2H, m), 0.54 – 0.48 (1H, m), -0.38 (1H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.0, 107.9, 85.5, 82.1, 81.2, 77.8, 77.2, 72.4, 70.4, 69.0, 63.4, 63.2, 57.5, 52.7, 35.2, 34.8, 32.2, 31.8, 30.3, 30.0, 29.8, 29.7, 29.5, 29.4, 29.3, 29.2, 29.0, 28.5, 27.3, 27.2, 25.9, 25.2, 22.5, 15.6, 14.6, 13.9, 10.7; ν_{max}: 3391, 2919, 2850, 1733, 1467, 1099, 720 cm^{-1}.

Experiment 47: 2',3'-Di-*O*-acetyl-L-glycerol-(1'→1)-5-O-(2-[(*R*)-1-hydroxyacet-yl-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]oct-adecyl]hexacosanoate)-2,3-di-*O*-acetyl-α-D-arabinofuranoside (138)



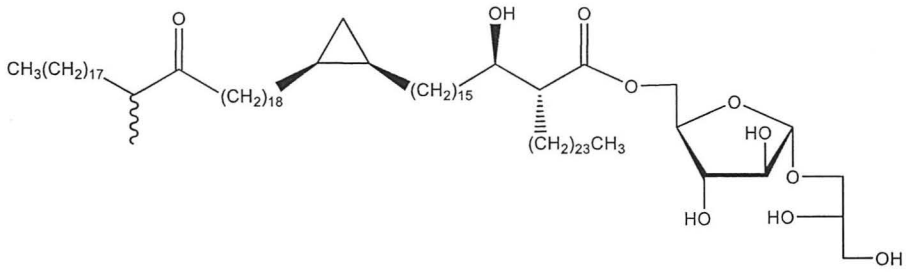
Acetic anhydride (0.01g, 0.01 mL, 0.09 mmol) was added dropwise to a stirred solution of L-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]hexacosanoate)-α-D-arabinofurano-side **(137)** (0.015 g, 0.010 mmol) in anhydrous pyridine (0.01 mL, 13.0 eq., 0.12 mmol), at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before affording 2',3'-di-*O*-acetyl-L-glycerol-(1'→1)-5-*O*-(2-[(*R*)-1-hydroxyacetyl-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclo propyl]octadecyl]hexacosanoate)-2,3-di-*O*-acetyl-α-D-arabinofuranoside **(138)** as a thick oil (12 mg, 80%) [Found (MALDI) $(M+Na)^+$: 1692.4, $C_{103}H_{192}NaO_{15}$, requires: 1692.4], $[\alpha]_D^{20}$ + 43 (*c* 0.1, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$): 5.21 (1H, dddd, *J* 4.5, 4.8, 5.0, 6.5 Hz), 5.09 (1H, d, *J* 1.3 Hz), 5.10 (1H,ddd, *J* 3.8, 4.0, 11.0 Hz), 5.0 (1H, s), 4.96 (1H, dd, *J* 1.0, 4.8 Hz), 4.37 (1H, dd, *J* 3.5, 11.8 Hz), 4.31 (1H, dd, *J* 4.0, 11.9 Hz), 4.26 (1H, dd, *J* 5.7, 11.8 Hz), 4.21 (1H, ddd, *J* 3.5, 5.5, 5.5 Hz), 4.17 (1H, dd, *J* 6.3, 11.9 Hz), 3.83 (1H, dd, *J* 5.2, 10.8 Hz), 3.61 (1H, dd, *J* 4.7, 10.8 Hz), 3.34 (3H, s), 2.98 – 2.93 (1H, m), 2.65 (1H, ddd, *J* 4.4, 7.0, 10.4 Hz), 2.11 (6H, s), 2.09 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 1.37 (2H, m), 1.69 – 1.01 (143H, m), 1.10 (2H,m), 0.88 (6H, t, *J* 7.0 Hz), 0.84 (3H, d, *J* 6.9 Hz), 0.69 – 0.57 (2H, m), 0.56 (1H, ddd, *J* 4.1, 8.2, 8.4, Hz), -0.34 (1H, ddd, *J* 4.0, 5.5, 5.5 Hz); δ_C (101 MHz, $CDCl_3$): 172.8, 170.5, 170.3, 170.2, 170.1, 169.6, 105.5, 85.4, 81.1, 80.7, 77.1, 73.8, 69.8, 65.2, 63.3, 62.5, 57.7, 49.6, 35.3, 32.4, 31.9, 31.8, 30.5, 30.2, 30.0, 29.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 29.0, 28.2, 27.6, 27.3, 26.1, 24.9, 22.7, 21.0, 20.9, 20.75, 20.7, 20.6, 15.7, 14.9, 14.1, 10.9; ν_{max}: 2918, 2850, 1745, 1467, 1049, 721 cm^{-1}.

Experiment 48: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranoside (139)



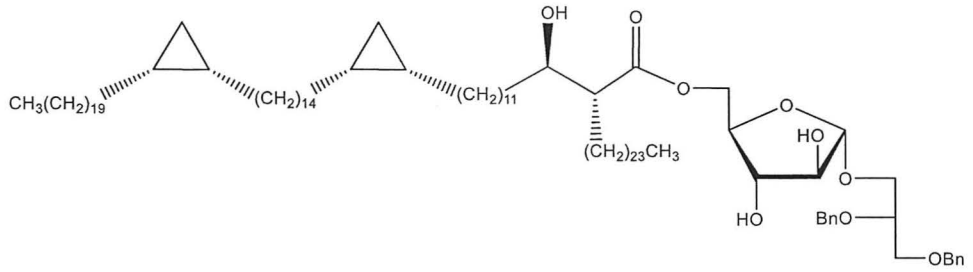
Cesium hydrogencarbonate (0.125 g, 0.644 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(135)** (0.077 g, 0.137 mmol) and 2-{(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl}hexacosanoic acid **(89)**[159] (0.114 g, 0.092 mmol) in dry DMF : THF (1:5, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred at 70 °C for 2 days then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranoside **(139)** as a colourless thick oil (0.1 g, 71%) [Found (MALDI) $(M+Na)^+$: 1646.6, $C_{106}H_{190}NaO_{10}$, requires: 1646.4], $[\alpha]_D^{23}$ + 25 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.98 (1H, s), 4.67 (1H, d, *J* 11.9 Hz), 4.62 (1H, d, *J* 11.9 Hz), 4.56 (1H, d, *J* 12.1 Hz), 4.52 (1H, d, *J* 12.1 Hz), 4.45 (1H, dd, *J* 3.8, 11.9 Hz), 4.27 (1H, dd, *J* 4.0, 11.9 Hz), 4.16 – 4.10 (1H, m), 4.06 (1H, br. s), 3.94 (1H, br. d, *J* 6.7 Hz), 3.86 (1H, dd, *J* 5.8, 10.4 Hz), 3.76 (1H, br. p, *J* 5.2 Hz), 3.68 (1H, m), 3.63 – 3.56 (3H, m), 2.95 (1H, d, *J* 10.1 Hz), 2.84 (1H, br. s), 2.51 (1H, sx, *J* 6.8 Hz), 2.46 – 2.38 (3H, m), 1.70 – 1.09 (145H, m), 1.06 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.8 Hz), 0.70 – 0.61 (2H, m), 0.57 (1H, dt, *J* 4.0, 8.0 Hz), -0.33 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.0, 138.1, 137.9, 128.4, 127.9, 127.7, 127.6, 107.5, 83.4, 80.7, 78.3, 76.5, 73.4, 72.7, 72.1, 69.6, 66.8, 63.2, 52.4, 46.3, 41.1, 35.1, 33.0, 31.9, 30.2, 29.7, 29.6, 29.5, 29.48, 29.44, 29.4, 29.34, 29.3, 28.7, 27.4, 27.3, 25.4, 23.7, 22.7, 16.3, 15.8, 14.1, 10.9; ν_{max}: 3423, 2918, 2850, 1736, 1714, 1467, 1097, 720 cm^{-1}.

Experiment 49: L-Glycerol-(1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabino-furanoside (140)



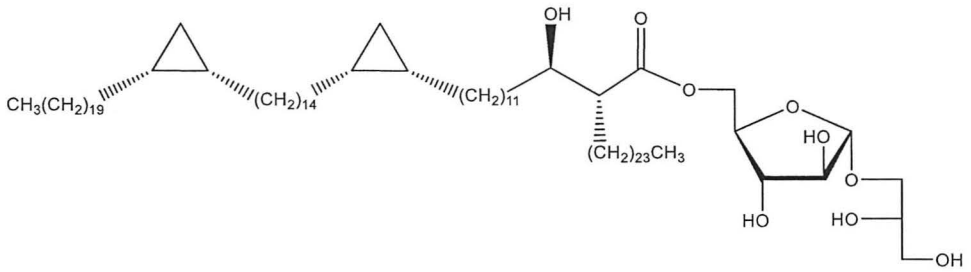
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 18 mg, 0.2 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-{2-[(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]-hexadecyl]hexa cosanoyl}-α-D-arabinofuranoside **(139)** (0.091 g, 0.056 mmol) in hexane : ethyl acetate (1:1, 4 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h then TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with CH_2Cl_2 (10 mL), the filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:2) to afford L-glycerol-(1'→1)-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclo propyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranoside **(140)** as a colourless thick oil (65 mg, 80%) $[\alpha]_D^{23}$ + 10 (*c* 0.1, $CHCl_3$), [Found $(M+NH_4)^+$: 1461.3752, $C_{92}H_{182}NO_{10}$, requires: 1461.3758] which showed δ_H (400 MHz, $CDCl_3$+ few drops CD_3OD): 4.86 (1H, s), 4.33 (1H, dd, *J* 4.5, 11.6 Hz), 4.21 (1H, dd, *J* 5.4, 11.6 Hz), 4.06 (1H, dd, *J* 4.8, 9.6 Hz), 3.96 (1H, br. d, *J* 1.2 Hz), 3.83 (1H, dd, *J* 2.4, 4.8 Hz), 3.79 – 3.71 (1H, m), 3.66 (1H, dd, *J* 3.8, 10.2 Hz), 3.62 – 3.56 (1H, m), 3.53 (2H, br. dd, *J* 5.0, 9.4 Hz), 3.47 (1H, dd, *J* 3.4, 10.3 Hz), 2.46 (1H, sx, *J* 6.8 Hz), 2.36 (3H, br. t, *J* 7.3 Hz), 1.63 – 1.01 (144 H, m), 0.98 (3H, d, *J* 6.9 Hz), 0.81 (6H, t, *J* 6.8 Hz), 0.64 – 0.53 (2H, m), 0.49 (1H, dt, *J* 4.0, 8.0 Hz), -0.40 (1H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 216.1, 175.0, 107.9, 82.2, 81.1, 77.8, 72.4, 70.4, 69.1, 63.5, 63.2, 52.8, 49.8, 46.2, 41.0, 34.8, 32.9, 31.8, 30.1, 29.5, 29.4, 29.35, 29.3, 29.2, 29.1, 29.0, 28.6, 27.3, 27.1, 25.2, 23.5, 22.5, 16.1, 15.6, 13.9, 10.7; ν_{max}: 3392, 2918, 2850, 1736, 1714, 1467, 1088, 720 cm^{-1}.

Experiment 50: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (141)



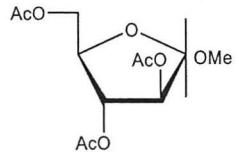
Cesium hydrogencarbonate (0.123 g, 0.634 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(135)** (0.081 g, 0.144 mmol) and (2*R*)-2-[(1*R*)-1-hydroxy-12-[(2*R*)-2-{14-[(1*S*,2*R*)-2-icosyl cyclopropyl]tetradecyl]cyclopropyl}dodecyl]hexacosanoic acid **(97)**[157] (0.110 g, 0.096 mmol) in dry DMF : THF (1:5, 6 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 3 days then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetra-decyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside **(141)** as a colourless thick oil (0.1 g, 71%) $[\alpha]_D^{23}$ - 40 (*c* 0.1, $CHCl_3$), [Found $(M+NH_4)^+$: 1541.3781, $C_{100}H_{182}NO_9$, requires: 1541.3809] which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.98 (1H, s), 4.67 (1H, d, *J* 11.9 Hz), 4.61 (1H, d, *J* 11.9 Hz), 4.56 (1H, d, *J* 12.1 Hz), 4.52 (1H, d, *J* 12.2 Hz), 4.47 (1H, dd, *J* 3.8, 11.9 Hz), 4.25 (1H, dd, *J* 3.8, 11.9 Hz), 4.16 – 4.10 (1H, m), 4.06 (1H, br. s), 4.0 – 3.91 (2H, m), 3.86 (1H, dd, *J* 5.8, 10.4 Hz), 3.76 (1H, dt, *J* 5.3, 10.6 Hz), 3.71 – 3.64 (1H, m), 3.63 – 3.55 (3H, m), 2.91 (1H, d, *J* 10.3 Hz), 2.79 (1H, d, *J* 6.2 Hz), 2.42 (1H, ddd, *J* 5.0, 7.1, 9.9 Hz), 1.67 – 1.08 (134H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.70 – 0.61 (4H, m), 0.56 (2H, dt, *J* 4.0, 8.4 Hz), -0.33 (2H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 174.9, 138.1, 137.9, 128.4, 127.9, 127.8, 127.75, 127.7, 107.4, 83.9, 80.3, 78.3, 76.4, 73.5, 72.7, 72.1, 69.5, 66.8, 63.2, 52.2, 35.2, 31.9, 30.2, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 28.7, 27.42, 25.4, 22.7, 15.8, 14.1, 10.9; ν_{max}: 3392, 3066, 2918, 2850, 1735, 1467, 1098, 720 cm^{-1}.

Experiment 51: L-Glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*, 2*S*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (142)



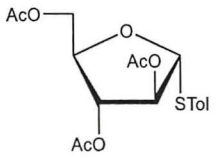
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 14 mg, 0.2 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl)cyclo-propyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside **(141)** (0.073 g, 0.048 mmol) in hexane : ethyl acetate (1:1, 4 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h then TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with CH_2Cl_2 (10 mL), the filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to afford 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]-dodecyl}hexacosanoate)-α-D-arabinofuranoside **(142)** as a colourless thick oil (53 mg, 81%) $[\alpha]_D^{23}$ + 20 (*c* 0.1, $CHCl_3$), [Found $(M+NH_4)^+$: 1361.2863, $C_{86}H_{170}NO_9$, requires: 1361.2870] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 4.87 (1H, s), 4.34 (1H, dd, *J* 4.5, 11.7 Hz), 4.22 (1H, dd, *J* 5.3, 11.7 Hz), 4.07 (1H, br. q, *J* 4.9 Hz), 3.97 (1H, br. d, *J* 1.3 Hz), 3.84 (1H, dd, *J* 2.4, 4.8 Hz), 3.79 – 3.72 (1H, m), 3.67 (1H, dd, *J* 6.3, 10.2 Hz), 3.63 – 3.56 (1H, m), 3.54 (2H, br. dd, *J* 5.0, 8.7 Hz), 3.47 (1H, dd, *J* 3.4, 10.3 Hz), 2.37 (1H, ddd, *J* 4.6, 8.1, 10.2 Hz), 1.59 – 0.98 (139H, m), 0.82 (6H, t, *J* 6.8 Hz), 0.63 – 0.54 (4H, m), 0.50 (2H, dt, *J* 4.0, 12.0 Hz), -0.40 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.0, 107.9, 82.2, 81.1, 77.8, 72.4, 70.4, 69.1, 63.4, 63.2, 52.8, 34.79, 31.8, 30.1, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.6, 27.3, 25.2, 22.5, 15.6, 13.9, 10.7; ν_{max}: 3400, 2919, 2851, 1728, 1467, 1112, 720 cm^{-1}.

Experiment 52: Methyl 2,3,5-tri-*O*-acetyl-α,β-D-arabinofuranoside (143)



Acetic anhydride (17.27 mL, 183.0 mmol) was added dropwise to a stirred solution of methyl–α, β-D-arabinofuranoside **(66)** (5.0 g, 30 mmol) in anhydrous pyridine (22.16 mL), at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The solvent was evaporated under reduced pressure, to give a residue which was diluted with ethyl acetate (100 mL), and washed with water (2×50 mL), 1 M aqueous HCl (2×50 mL), saturated solution of $NaHCO_3$ (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 (1:1) affording methyl 2,3,5-tri-*O*-acetyl-α,β-D-arabinofuranoside **(143)** as a thick oil (The arabino-furanose was obtained in the form of an inseparable α,β-mixture in a ratio of 1:0.2, α : β) (7.1 g, 80%) [Found (MALDI) $(M+Na)^+$: 313.1, $C_{12}H_{18}NaO_8$, requires: 313.0], $[\alpha]_D^{20}$ + 56 (*c* 0.1, $CHCl_3$) (*major anomer*) showed δ_H (400 MHz, $CDCl_3$): 5.34 (1H, s), 5.20 (1H, dd, *J* 3.5, 10.2 Hz), 5.12 – 5.05 (1H, m), 5.01 – 4.95 (1H, m), 3.93 (1H, d, *J* 12.4 Hz), 3.68 (1H, dd, *J* 13.0, 1.7 Hz), 3.41 (3H, s), 2.15 (3H, s), 2.10 (3H, s), 2.01 (3H, s); δ_C (101 MHz, $CDCl_3$): 170.6, 170.1, 169.6, 106.7, 81.2, 80.2, 77.1, 63.3, 54.9, 20.8, 20.74, 20.7; ν_{max}: 2958, 2930, 2858, 1493, 1112, 701 cm^{-1}. The experiment was scaled up to prepare 160 g from the compound.

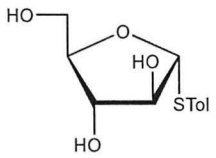
Experiment 53: *p*-Cresyl 2,3,5-*O*-acetyl -1-thio-α-D-arabinofuranoside (144) [290]



4-Methylbenzenethiol (0.66 g, 5.31 mmol) was added dropwise to a stirred solution of methyl 2,3,5-tri-*O*-acetyl-α,β-D-arabinofuranoside **(143)** (1.30 g, 4.47 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 30 min., then boron trifluoride diethyl etherate (3.3 mL, 23 mmol) was added slowly, then the mixture was allowed to reach to room temperature and stirred for 8 h.

When TLC showed no starting material was left, the reaction mixture was quenched with Et_3N (1 mL) at 0 °C, then the solvent was evaporated and the residue was diluted with ethyl acetate (100 mL) washed with saturated solution NH_4Cl (50 mL), the organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×100 mL). The combined organic layers were dried over ($MgSO_4$) concentrated to give a residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:2) affording *p*-Cresyl 2,3,5-*O*-acetyl-1-thio-α-D-arabinofurano-side **(144)** as a thick oil (1.1 g, 65%) [Found (MALDI) $(M+Na)^+$: 405.2, $C_{18}H_{22}NaO_7S$, requires: 405.0], $[\alpha]_D^{20}$ + 152 (*c* 0.1, $CHCl_3$) [*lit.*[290] $[\alpha]_D$ + 149.2 (*c* 0.3, CH_2Cl_2)] which showed δ_H (400 MHz, $CDCl_3$): 7.41 (2H, d, *J* 8.0 Hz), 7.13 (2H, d, *J* 8.0 Hz), 5.47 (1H, d, *J* 1.2 Hz), 5.27 (1H, br. d, *J* 1.9 Hz), 5.08 (1H, dd, *J* 1.9, 5.3 Hz), 4.48 (1H, dd, *J* 5.2, 9.4, Hz), 4.40 (1H, dd, *J* 3.7, 12.0 Hz), 4.28 (1H, dd, *J* 5.5, 12.0 Hz), 2.34 (3H, s), 2.13 (3H, s), 2.11 (3H, s), 2.10 (3H, s); δ_C (101 MHz, $CDCl_3$): 170.4, 169.8, 169.4, 137.9, 132.6, 132.4, 129.7, 129.6, 91.0, 81.2, 79.7, 76.5, 62.6, 20.9, 20.65, 20.6; ν_{max}: 3021, 2923, 2864, 1755, 1747, 1493, 756 cm^{-1}. All data were identical to the authentic sample. The experiment was repeated to prepare 60 g from the compound.

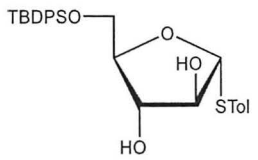
Experiment 54: *p*-Cresyl-1-thio-α-D-arabinofuranoside (145) [289]



A solution of sodium methoxide (350 mL, 1M, in methanol,) was added to a stirred solution of *p*-Cresyl 2,3,5-*O*-acetyl-1-thio-α-D-arabinofuranoside **(144)** (25 g, 65 mmol) in dry MeOH : CH_2Cl_2 (1:1, 150 mL) at room temperature. The reaction mixture was stirred at room temperature for 3 h then TLC showed no starting material was left. The reaction mixture was neutralized with Amberlite IR-120 (H^+) and the resin was filtered off then the solvent was removed under reduced pressure, to give a residue. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to afford *p*-cresyl-1-thio-α-D-arabinofuranoside **(145)** as a thick oil (14.9 g, 89%) [Found (MALDI) $(M+Na)^+$: 279.1, $C_{12}H_{16}NaO_4S$, requires: 279.0], $[\alpha]_D^{20}$ + 237 (*c* 1.0, $CHCl_3$) [*lit.*[304] $[\alpha]_D$ + 235.6 (*c* 1.0, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.39 (2H, d, *J* 8.0 Hz), 7.11 (2H, d, *J* 8.0 Hz), 5.38 (1H, d, *J* 2.5 Hz), 4.50 – 4.40 (1H, m), 4.15 (3H, br. s), 3.86 (1H, d, *J* 11.1 Hz), 3.77 (1H, d, *J* 11.5 Hz),

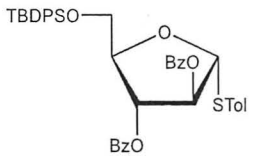
3.19 (1H, br. s), 2.32 (3H, s), 2.03 (1H, br. s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 138.0, 132.6, 131.4, 129.8, 92.4, 84.1, 81.6, 77.3, 61.3, 21.1; ν_{max}: 3466, 3050, 3017, 2958, 2930, 2858, 1493, 1112, 701 cm^{-1}. All data were identical to the authentic sample. The reaction was repeated on a large scale.

Experiment 55: *p*-Cresyl 5-*O*-tertbutyldiphenylsilyl-1-thio-α-D-arabinofuranoside (146) [289]



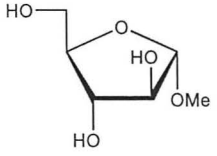
Tertbutylchlorodiphenylsilane (24 g, 87 mmol) was added to a stirred solution of *p*-cresyl-1-thio-α-D-arabinofuranoside **(145)** (15 g, 58 mmol) in dry DMF (50 mL), followed by the addition of imidazole (10 g, 14 mmol) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h, when TLC showed no starting material was left. The reaction mixture was diluted with ethyl acetate (100 mL) and water (25 mL). The organic layer was separated, and 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 over ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:2) affording *p*-cresyl 5-*O*-tertbutyldiphenylsilyl-1-thio-α-D-arabinofuranoside **(146)** as a thick oil (24 g, 82%) [Found (MALDI) $(M+Na)^+$: 517.0, $C_{28}H_{34}NaO_4SSi$, requires: 517.1], $[\alpha]_D^{20}$ + 136 (*c* 1.0, $CHCl_3$) [*lit.*[289] $[\alpha]_D$ + 93.1 (*c* 2.6, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$:) 7.76 – 7.65 (4H, m), 7.52 – 7.37 (8H, m), 7.15 (2H, d, *J* 7.8 Hz), 5.55 (1H, s), 4.31 (1H, br. d, *J* 8.0 Hz), 4.26 (1H, br. d, *J* 2.7 Hz), 4.24 – 4.18 (1H, m), 4.0 (1H, d, *J* 9.6 Hz), 3.87 (1H, dd, *J* 2.7, 11.3 Hz), 3.81 (1H, dd, *J* 2.1, 11.3 Hz), 2.77 (1H, d, *J* 8.1 Hz), 2.36 (3H, s), 1.07 (9H, s); δ_C (101 MHz, $CDCl_3$) 137.9, 135.8, 135.6, 135.5, 132.5, 132.1, 132.0, 131.6, 130.1, 130.0, 129.9, 129.8, 129.7, 129.5, 127.9, 127.89, 127.86, 127.8, 93.0, 86.1, 81.0, 78.7, 64.0, 26.7, 21.1; ν_{max}: 3466, 3050, 3017, 2958, 2930, 2858, 1493, 1112, 701 cm^{-1}. All data were identical to the authentic sample. The reaction was repeated on a large scale.

Experiment 56: *p*-Cresyl 2,3-di-O-benzoyl-5-*O*-tertbutyldiphenylsilyl-1-thio-α-D-arabinofuranoside (147) [289]



Benzoyl chloride (6.7 mL, 57 mmol) was added dropwise to a stirred solution of *p*-cresyl 5-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-arabinofuranoside **(146)** (6.20 g, 12.5 mmol) in anhydrous pyridine (47 mL) at 0 °C under nitrogen atmosphere. 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 (200 mL), and washed with water (2×50 mL), 1 M aqueous HCl (2×50 mL), saturated solution of $NaHCO_3$ (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 (5:2) to give *p*-cresyl 2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-1-thio-α-D-arabinofuranoside **(147)** as a thick oil (7.3 g, 83%) [Found (MALDI) $(M+Na)^+$: 725.1, $C_{42}H_{42}NaO_6SSi$, requires: 725.2], $[\alpha]_D^{20}$ - 15 (*c* 1.0, $CHCl_3$) [*lit.*[289] $[\alpha]_D$ - 19.7 (*c* 3.4, $CHCl_3$)] 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. The reaction was repeated on a large scale.

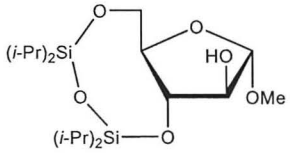
Experiment 57: Methyl α-D-arabinofuranoside (148) [301]



A solution of sodium methoxide (0.1 M, in methanol, 6 mL) was added to a stirred solution of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside **(101)** (3.0 g, 6.2 mmol) in dry MeOH : CH_2Cl_2 (1:1, 60 mL) at room temperature and the reaction mixture was

stirred at room temperature for 2 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 evaporated under reduced pressure, to give a residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:2) to afford methyl α-D-arabinofuranoside **(148)** as a thick oil (0.86 g, 83%), m.p. 51-52 °C (*lit.*[301] 50 °C), $[\alpha]_D^{20}$ + 123 (*c* 0.1, H_2O) [*lit.*[301] $[\alpha]_D^{22}$ +125.2 (*c* 1.47, H_2O)] which showed δ_H (400 MHz, DMSO): 5.29 (1H, d, *J* 5.2 Hz), 5.08 (1H, d, *J* 5.4 Hz), 4.70 (1H, t, *J* 5.7 Hz), 4.60 (1H, d, *J* 2.0 Hz), 3.78 – 3.74 (1H, m), 3.68 (1H, ddd, *J* 3.1, 5.5, 7.2 Hz), 3.65 – 3.59 (1H, m), 3.55 (1H, ddd, *J* 3.1, 5.3, 11.9 Hz), 3.41 (1H, dt, *J* 5.8, 11.7, Hz), 3.24 (3H, s); δ_C (101 MHz, DMSO): 109.0, 83.8, 82.0, 77.0, 61.3, 54.3; ν_{max}: br. 3436, 2927, 2930, 2861, 1494, 1049, 824 cm^{-1}. All data were identical to the authentic sample.

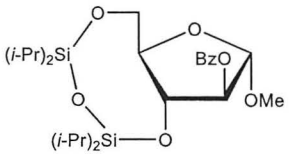
Experiment 58: Methyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabino-furanoside (149) [289]



1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (5.40 g, 5.50 mL, 17.1 mole) was added to a stirred solution of methyl α-D-arabinofuranoside **(148)** (2.60 g, 15.8 mmol) in dry pyridine (5 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 6 h, then TLC showed no starting material was left. Methanol (5 mL) was added, and the solvent was evaporated under reduced pressure, to give the residue, which was diluted with ethyl acetate (100 mL), and washed with water (20 mL), brine (20 mL), dried over ($MgSO_4$) 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 methyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(149)** as a thick oil (4.3 g, 66%) [Found (MALDI) $(M+Na)^+$: 429.0, $C_{18}H_{38}NaO_6Si_2$, requires: 429.2], $[\alpha]_D^{20}$ + 33 (*c* 1.0, $CHCl_3$) [*lit.*[289] $[\alpha]_D$ + 30.9 (*c* 0.8, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 4.78 (1H, d, *J* 1.6 Hz), 4.19 – 4.12 (2H, m), 3.99 (1H, dd, *J* 3.2, 12.9 Hz), 3.95 (1H, dd, *J* 4.1, 13.2 Hz), 3.89 – 3.84 (1H, m), 3.40 (3H, s), 2.44 (1H, d, *J* 4.3 Hz), 1.13 – 0.90 (28H, m); δ_C (101 MHz, $CDCl_3$): 107.9, 82.3, 80.7, 76.7, 61.4, 55.5, 17.4, 17.3, 17.2, 17.1, 17.05, 17.03, 17.0, 16.9, 13.5, 13.1, 12.8, 12.5; ν_{max}: 3467, 2945, 2868, 1465, 1096, 786 cm^{-1}. All data were identical

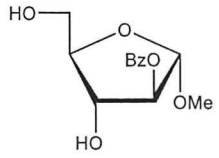
to the authentic sample. This experiment was scaled up to prepare a large amount from the compound.

Experiment 59: Methyl 2-*O*-benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (150) [289]



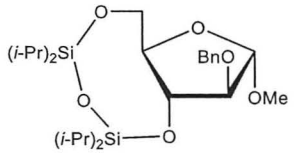
Benzoyl chloride (0.45 mL, 3.20 mmol) was added dropwise to a stirred solution of methyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(149)** (1.60 g, 3.94 mmol) in anhydrous pyridine (4 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 3 h when TLC showed no starting material was left, 2-propanol (2 mL) was added. The solvent was evaporated under reduced pressure, to give the residue which was diluted with ethyl acetate (100 mL), and washed in succession with 0.1 M aqueous HCl (20 mL), saturated solution of sodium bicarbonate (20 mL) and brine (20 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure. Traces of pyridine were removed by co-evaporation with toluene. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (10:1) to give methyl 2-*O*-benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(150)** as a thick oil (1.45 g, 72%) $[\alpha]_D^{20}$ - 22 (*c* 1.0, $CHCl_3$) [*lit.*[289] $[\alpha]_D$ - 19.5 (*c* 2.09, $CHCl_3$)], [Found (MALDI) $(M+Na)^+$: 533.2, $C_{25}H_{42}NaO_7Si_2$, requires: 533.2] which showed δ_H (400 MHz, $CDCl_3$): 8.05 (2H, br. d, *J* 7.8 Hz), 7.59 (1H, br. t, *J* 7.4 Hz), 7.46 (2H, m), 5.42 (1H, br. d, *J* 5.1 Hz), 4.91 (1H, s), 4.52 (1H, dd, *J* 5.6, 6.7 Hz), 4.13 – 3.98 (3H, m), 3.43 (3H, s), 1.19 – 0.86 (28H, m); δ_C (101 MHz, $CDCl_3$): 165.7, 133.3, 129.7, 128.4, 106.3, 84.3, 80.9, 75.9, 61.7, 55.2, 17.4, 17.35, 17.3, 16.9, 16.8, 16.7, 13.5, 13.2, 12.8, 12.5; ν_{max}: 3010, 2944, 2867, 1732, 1494, 1098, 709 cm^{-1}. All data were identical to the authentic sample. This experiment was scaled up to prepare a large amount from the compound.

Experiment 60: Methyl 2-*O*-benzoyl-α-D-arabinofuranoside (151) [289]



Tetrabutylammonium fluoride (5.4 mL, in 1.0 M THF, 5.4 mmol) was added dropwise to a stirred solution of methyl 2-*O*-benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(150)** (1.40 g, 2.74 mmol) in anhydrous THF (5 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h when TLC showed no starting material was left, the reaction mixture was diluted with ethyl acetate (100 mL) washed with saturated solution of NH_4Cl (50 mL) and brine (50 mL). The organic layer was dried over ($MgSO_4$), concentrated, to give the residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to give methyl 2-*O*-benzoyl-α-D-arabinofuranoside **(151)** as a thick oil (0.55 g, 74%) [Found (MALDI) $(M+Na)^+$: 291.7, $C_{13}H_{16}NaO_6$, requires: 291.0], $[\alpha]_D^{20}$ + 98 (*c* 0.1, $CHCl_3$) [*litt.*[289] $[\alpha]_D$ + 94.9 (*c* 0.2, $CHCl_3$)] which showed δ_H (400 MHz, DMSO): 7.98 (2H, d, *J* 8.0 Hz), 7.69 (1H, t, *J* 7.4 Hz), 7.55 (2H, t, *J* 7.6 Hz), 5.54 (1H, d, *J* 5.3 Hz), 5.08 (1H, d, *J* 2.2 Hz), 4.91 (1H, s), 4.86 (1H, t, *J* 5.7 Hz), 4.07 (1H, dd, *J* 6.1, 8.2 Hz), 3.87 (1H, dd, *J* 5.4, 9.3 Hz), 3.69 – 3.60 (1H, m), 3.57 – 3.49 (1H, m), 3.30 (3H, s); δ_C (101 MHz, DMSO): 165.5, 134.1, 129.8, 129.3, 106.6, 85.2, 84.6, 75.4, 61.3, 54.6; ν_{max}: 3436, 3010, 2927, 2861, 1647, 1494, 1008, 761 cm^{-1}. All data were identical to the authentic sample. This experiment was scaled up to prepare a large amount from the compound

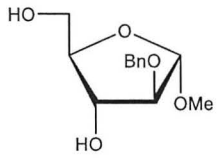
Experiment 61: Methyl 2-*O*-benzyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (152)



A solution of methyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(149)** (1.5 g, 3.7 mmol) in dry DMF (5 mL) was added dropwise to a stirred suspension solution of NaH (240 mg, 10.0 mmol) (60% w/w, dispersion in mineral oil, washed with petrol for three times) at 0 °C under nitrogen atmosphere. The reaction mixture was

stirred for 30 min, then benzyl bromide (370 µl, 3.10 mmol) in dry DMF (5 mL) was added. The reaction mixture was stirred at 0 °C for 2 h when TLC showed no starting material was left. The reaction mixture was quenched with slow addition of CH_3OH (2 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) and 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 (4:1) to give methyl 2-*O*-benzyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(152)** as a thick oil (1.4 g, 76%) $[\alpha]_D^{22}$ - 30 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.28 (5H, m), 4.83 (1H, d, *J* 2.4 Hz), 4.67 – 4.59 (2H, m), 4.29 (1H, dd, *J* 6.0, 8.0 Hz), 4.01 (1H, dd, *J* 3.0, 12.6 Hz), 3.97 (1H, dd, *J* 2.4, 3.2 Hz), 3.95 (1H, dd, *J* 3.4, 8.9 Hz), 3.91 – 3.86 (1H, m), 3.37 (3H, s), 1.16–0.97 (28H, m); δ_C (101 MHz, $CDCl_3$): 137.9, 128.3, 127.6, 127.5, 106.7, 89.6, 80.6, 76.2, 72.5, 61.6, 55.2, 17.5, 17.3, 17.2, 17.1, 17.08, 17.03, 17.0, 13.5, 13.2, 12.8, 12.5; ν_{max} 3436, 3010, 2928, 2862, 1650, 1495, 1008, 760 cm^{-1}

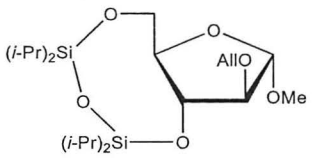
Experiment 62: Methyl 2-*O*-benzyl-α-D-arabinofuranoside (153) [292]



Tetrabutylammonium fluoride (5.6 mL, in 1.0 M THF, 5.6 mmol) was added dropwise to a stirred solution of methyl 2-*O*-benzyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(152)** (1.4 g, 2.8 mmol) in anhydrous THF (10 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give methyl 2-*O*-benzyl-α-D-arabinofuranoside **(153)** as a thick oil (0.6 g, 79%) [Found (MALDI) $(M+Na)^+$: 277.4, $C_{13}H_{18}NaO_5$, requires: 277.1], $[\alpha]_D^{20}$ + 65 (*c* 0.1, $CHCl_3$) [*lit.*[292] $[\alpha]_D$ + 60 (*c* 0.4, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.29 (5H, m), 4.99 (1H, s), 4.58 (1H, d, *J* 11.7 Hz), 4.65 (1H, d, *J* 11.7 Hz), 4.18 – 4.10 (2H, m), 3.91 (1H, d, *J* 1.1 Hz), 3.84 (1H, dd, *J* 3.0, 11.8 Hz), 3.76 (1H, dd, *J* 4.0, 11.8 Hz), 3.40 (3H, s), 2.69 – 1.91 (2H, m); δ_C (101 MHz, $CDCl_3$): 137.0, 128.5, 128.1, 127.9, 106.8, 87.4, 86.12, 75.4, 71.9, 62.5, 54.9. All

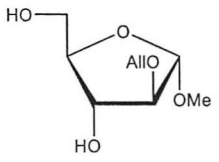
data were identical to the authentic sample. This experiment was scaled up to prepare a large amount from the compound.

Experiment 63: Methyl 2-*O*-allyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (154)



A solution of 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(149)** (0.5 g, 1.2 mmol) in dry DMF (10 mL) was added dropwise to a stirred suspension solution of NaH (59 mg, 2.45 mmol, 60% w/w, dispersion in mineral oil) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 30 min then allyl bromide (0.15 g, 0.10 mL, 1.23 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give methyl 2-*O*-allyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabino-furanoside **(154)** as a thick oil (0.5 g, 90%) [Found (MALDI) $(M+Na)^+$: 469.4, $C_{21}H_{42}NaO_6Si_2$, requires:469.2], $[\alpha]_D^{22}$ + 52 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 5.90 (1H, ddt, *J* 5.4, 10.7, 17.0 Hz), 5.30 (1H, ddd, *J* 1.5, 3.1, 17.0 Hz), 5.18 (1H, ddd, *J* 1.3, 2.8, 10.8 Hz), 4.80 (1H, d, *J* 2.5 Hz), 4.22 (1H, dd, *J* 6.1, 8.3 Hz), 4.15 – 4.03 (2H, m), 4.0 (1H, dd, *J* 3.0, 12.6 Hz), 3.94 (1H, dd, *J* 4.0, 12.6 Hz), 3.91 – 3.83 (2H, m), 3.39 (3H, s), 1.13 – 0.99 (28H, m); δ_C (101 MHz, $CDCl_3$) 134.3, 116.9, 106.7, 89.4, 80.5, 76.1, 71.5, 61.6, 55.2, 17.4, 17.3, 17.0, 16.9, 13.5, 13.1, 12.8, 12.5; ν_{max}: 2942, 2875, 1464, 1102, 885 cm^{-1}. The experiment was repeated on (5.88 g) of the starting material.

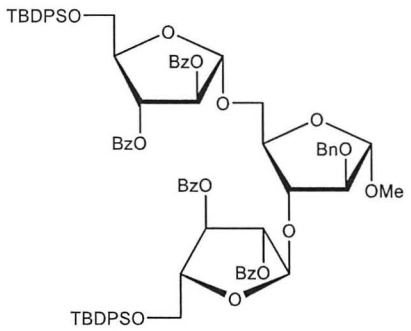
Experiment 64: Methyl 2-*O*-allyl-α-D-arabinofuranoside (155)



Tetrabutylammonium fluoride (2.2 mL, in 1.0 M THF, 2.2 mmol) was added dropwise to a stirred solution of methyl 2-*O*-allyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(154)** (0.5 g, 1.1 mmol) in anhydrous THF (10 mL) at 0 °C under

nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give methyl 2-*O*-allyl-α-D-arabinofuranoside **(155)** as a thick oil (0.13 g, 57%) [Found (MALDI) $(M+Na)^+$: 227.0, $C_9H_{16}NaO_5$, requires: 227.0], $[\alpha]_D^{20}$ + 65 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, CD_3OD): 5.96 (1H, ddt, *J* 5.4, 10.7, 17.0 Hz), 5.34 (1H, ddd, *J* 1.6, 3.3, 17.3 Hz), 5.21 (1H, ddd, *J* 1.3, 2.8, 10.8 Hz), 4.87 (1H, s), 4.18 – 4.07 (2H, m), 3.98 – 3.89 (2H, m), 3.83 (1H, dd, *J* 1.6, 3.8 Hz), 3.79 (1H, dd, *J* 2.9, 12.0 Hz), 3.66 (1H, dd, *J* 5.2, 12.0 Hz), 3.41 (3H, s); δ_C (101 MHz, CD_3OD) 135.6, 117.3, 108.5, 91.2, 84.4, 77.0, 71.7, 62.7, 55.1; ν_{max}: 3413, 2943, 2868, 1464, 1055, 885 cm^{-1}.

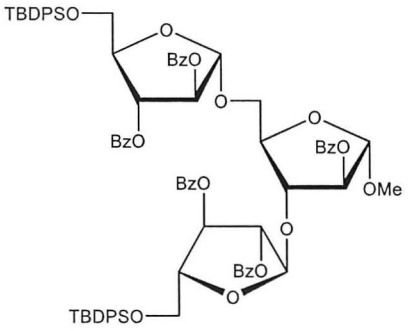
Experiment 65: Methyl 2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabino-furanosyl-(1→3)-[2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofura-nosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (156) [288]



Molecular sieves 4 Å (1.4 g) was added to a stirred solution of the acceptor methyl 2-*O*-benzyl-α-D-arabinofuranoside **(153)** (0.2 g, 0.7 mmol) and the donor *p*-Cresyl 2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-1-thio-α-D-arabinofuranoside **(147)** (1.3 g, 1.8 mmol) in dry CH_2Cl_2 (30 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then cooled to -60 °C and *N*-iodosuccinimide (0.46 g, 2.06 mmol) was added followed by the addition of silvertriflate (0.08 g, 0.30 mmol). The mixture were stirred at the same temperature until the colour of the mixture turned into red/dark brown and TLC showed no starting material was left. The reaction mixture was quenched by the addition of triethylamine (0.6 mL) until the reaction mixture turned into yellow colour. The reaction mixture was diluted with CH_2Cl_2 (50 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 (1:1) to give methyl 2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabino-

furanosyl-(1→3)-[2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benz-yl-α-D-arabinofuranoside **(156)** as a thick oil (0.95 g, 85%) [Found (MALDI) $(M+Na)^+$: 1433.7, $C_{83}H_{86}NaO_{17}Si_2$, requires: 1433.5], $[\alpha]_D^{20}$ + 21 (*c* 0.1, $CHCl_3$) [*lit.*[288] $[\alpha]_D$ + 12.9 (*c* 2.0, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 8.07 – 7.98 (4H, m), 7.96 – 7.89 (4H, m), 7.74 – 7.64 (8H, m), 7.60 – 7.28 (29H, m), 5.66 – 5.59 (2H, m), 5.52 (1H, d, *J* 1.3 Hz), 5.42 (1H, d, *J* 1.4 Hz), 5.27 (2H, s), 4.94 (1H, d, *J* 1.5 Hz), 4.59 (2H, br. s), 4.43 (1H, dd, *J* 4.4, 9.2 Hz), 4.36 (1H, dd, *J* 4.4, 9.6 Hz), 4.28 – 4.22 (2H, m), 4.10 – 4.05 (1H, m), 4.04 – 3.88 (5H, m), 3.86 – 3.81 (1H, m), 3.35 (3H, s), 1.00 (18H, s); δ_C (101 MHz, $CDCl_3$): 165.6, 165.5, 165.3, 165.2, 135.7, 135.6, 133.3, 133.1, 129.9, 129.8, 129.7, 129.6, 128.4, 128.35, 128.31, 128.3, 127.8, 127.7, 127.6, 107.1, 105.8, 105.3, 88.3, 83.5, 83.2, 82.2, 81.1, 80.0, 77.2, 72.0, 66.6, 63.3, 63.2, 54.8, 26.7, 19.3; ν_{max}: 3069, 3010, 2929, 2859, 1724, 1602, 1451, 1070, 708 cm^{-1}. All data were identical to the authentic sample

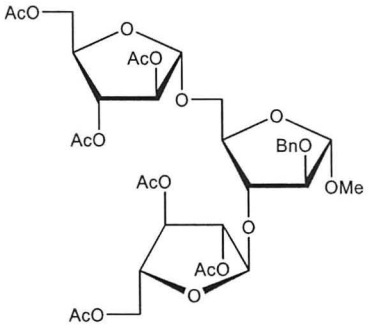
Experiment 66: Methyl 2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzoyl-α-D-arabinofuranoside (157)



Molecular sieves 4 Å (6.6 g) was added to a stirred solution of the acceptor methyl 2-*O*-benzoyl-α-D-arabinofuranoside **(151)** (0.9 g, 3.3 mmol) and the donor *p*-Cresyl 2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-1-thio-α-D-arabinofuranoside **(147)** (6.0 g, 8.5 mmol) in dry CH_2Cl_2 (30 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then cooled to -60 °C and *N-iodosuccinimide* (2.09 g, 9.29 mmol) was added followed by the addition of silvertriflate (0.36 g, 1.40 mmol). The mixture were stirred at the same temperature until the colour of the mixture turned into red/dark brown and TLC showed no starting material was left. The reaction mixture was worked up and purified as before affording methyl 2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzoyl-5-*O*-tertbutyldi-

phenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzoyl-α-D-arabinofuranoside **(157)** as a thick oil (4.2 g, 87%) [Found (MALDI) $(M+Na)^+$: 1447.6, $C_{83}H_{84}NaO_{18}Si_2$, requires: 1447.5], $[\alpha]_D^{20}$ +16 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 8.07 (2H, d, *J* 7.9 Hz), 8.02 – 7.91 (8H, m), 7.70 (9H, m), 7.59 – 7.41 (8H, m), 7.40 – 7.28 (18H, m), 5.64 (1H, d, *J* 4.5 Hz), 5.61 (1H, d, *J* 4.2 Hz), 5.57 (2H, br. d, *J* 2.6 Hz), 5.53 (1H, br. s), 5.41 (1H, s), 5.32 (1H, br. s), 5.10 (1H, br. s), 4.49 – 4.35 (4H, m), 4.08 (1H, dd, *J* 5.1, 11.3 Hz), 3.97 (5H, m), 3.44 (3H, s), 1.01 (9H, s), 0.98 (9H, s); δ_C (101 MHz, $CDCl_3$): 165.5, 165.4, 165.2, 165.1, 135.63, 135.6, 135.5, 133.3, 133.2, 133.15, 133.1, 133.0, 129.96, 129.9, 129.8, 129.78, 129.7, 129.6, 129.4, 129.3, 129.2, 129.1, 128.4, 128.31, 128.3, 128.2, 127.6, 107.0, 106.0, 105.3, 84.0, 83.4, 82.5, 82.0, 81.8, 81.6, 80.6, 77.2, 77.1, 66.1, 63.5, 63.3, 54.7, 26.7, 26.6, 19.3, 19.2; ν_{max}: 3069, 3010, 2929, 2859, 1724, 1602, 1451, 1070, 708 cm^{-1}.

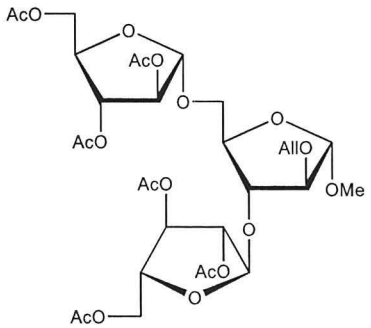
Experiment 67: Methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→3)-[2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (158)



Molecular sieves 4 Å (3.12 g) was added to a stirred solution of the acceptor methyl 2-*O*-benzyl-α-D-arabinofuranoside **(153)** (0.63 g, 2.47 mmol) and the donor *p*-cresyl 2,3,5-*O*-acetyl-1-thio-α-D-arabinofuranoside **(144)** (2.84 g, 7.42 mmol) in dry CH_2Cl_2 (15 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then cooled to -10 °C and *N*-iodosuccinimide (1.0 g, 4.4 mmol) was added followed by the addition of silvertriflate (0.17 g, 0.66 mmol). The mixture were stirred at the same temperature until the colour of the mixture turned into red/dark brown and TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give methyl-2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→3)-[2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(158)** as a yellow thick oil (1.9 g, 94%) [Found (MALDI) $(M+Na)^+$: 793.5, $C_{35}H_{46}NaO_{19}$, requires: 793.2], $[\alpha]_D^{20}$ + 43 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.37 –

7.28 (5H, m), 5.17 (1H, d, *J* 1.2 Hz), 5.14 (1H, s), 5.12 (1H, s), 5.08 (1H, br. d, *J* 1.6 Hz), 4.99 (1H, dd, *J* 1.5, 4.6 Hz), 4.96 (1H, dd, *J* 0.8, 4.7 Hz), 4.91 (1H, br. d, *J* 1.5 Hz), 4.60 (1H, d, *J* 12.0 Hz), 4.57 (1H, d, *J* 11.8 Hz), 4.42 (1H, dd, *J* 3.5, 11.7 Hz), 4.38 – 4.27 (2H, m), 4.26 – 4.17 (4H, m), 4.12 – 4.08 (1H, m), 4.02 (1H, dd, *J* 1.7, 3.9 Hz), 3.90 (1H, dd, *J* 4.4, 11.2 Hz), 3.74 (1H, dd, *J* 2.5, 11.2 Hz), 3.38 (3H, s), 2.11 – 2.08 (18H, m); δ_C (101 MHz, $CDCl_3$): 170.59, 170.5, 170.3, 170.1, 169.5, 169.4, 137.5, 128.4, 127.82, 127.8, 106.9, 105.4, 105.2, 88.6, 81.3, 80.9, 80.8, 80.7, 80.5, 79.4, 77.1, 76.9, 72.2, 65.4, 63.3, 63.0, 55.0, 20.74, 20.72, 20.7, 20.6; ν_{max}: 3036, 2939, 1744, 1370, 1048, 899, 665 cm^{-1}. The experiment was repeated on a large scale.

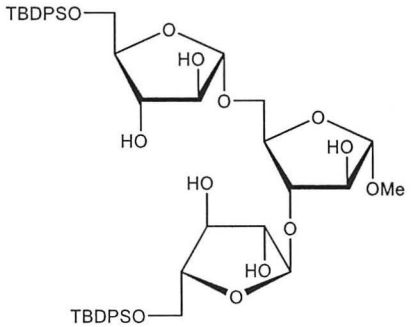
Experiment 68: Methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→3)-[2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-allyl-α-D-arabinofuranoside (159)



Molecular sieves 4 Å (5.8 g) was added to a stirred solution of the acceptor methyl 2-*O*-allyl-α-D-arabinofuranoside **(155)** (0.8 g, 3.9 mmol) and the donor *p*-cresyl 2,3,5-*O*-acetyl-1-thio-α-D-arabinofuranoside **(144)** (5.29 g, 13.6 mmol) in dry CH_2Cl_2 (30 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then cooled to -78 °C and *N*-iodosuccinimide (1.83 g, 8.13 mmol) was added followed by the addition of silvertriflate (0.31 g, 1.20 mmol). The mixture were stirred at the same temperature until the colour of the mixture turned into red/dark brown and TLC showed no starting material was left. The reaction mixture was worked up and purified as before affording methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→3)-[2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-allyl-α-D-arabinofuranoside **(159)** as a thick oil (9.0 g, 90%) [Found (MALDI) $(M+Na)^+$: 743.3, $C_{31}H_{44}NaO_{19}$, requires: 743.2], $[\alpha]_D^{20}$ + 35 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 5.89 (1H, ddt, *J* 5.4, 10.7, 17.0 Hz), 5.29 (1H, ddd, *J* 1.6, 3.3, 17.0 Hz), 5.20 (1H, ddd, *J* 1.3, 2.8, 10.8 Hz), 5.18 (1H, br. s), 5.17 (1H, d, *J* 1.2 Hz), 5.14 (1H, br. s), 5.10 (1H, d, *J* 1.6 Hz), 5.00 (1H, dd, *J* 1.4, 4.5 Hz), 4.97 (1H, br. d, *J* 4.7 Hz), 4.88 (1H, br. d, *J* 1.4 Hz),

4.43 (1H, dd, *J* 3.4, 11.6 Hz), 4.33 (2H, m), 4.27 – 4.20 (3H, m), 4.15 (1H, dd, *J* 3.6, 7.3 Hz), 4.11 (1H, m), 4.05 (2H, br. dd, *J* 1.2, 5.5 Hz), 3.96 (1H, dd, *J* 1.6, 3.7 Hz), 3.90 (1H, dd, *J* 4.3, 11.2 Hz), 3.74 (1H, dd, *J* 2.4, 11.2 Hz), 3.40 (3H, s), 2.15 – 2.07 (18H, m); δ_C (101 MHz, $CDCl_3$): 170.5, 170.4, 170.2, 170.0, 169.5, 169.4, 133.9, 117.4, 106.8, 105.3, 105.2, 88.2, 81.3, 80.8, 80.7, 80.6, 80.5, 79.4, 77.1, 76.8, 70.9, 65.3, 63.2, 62.9, 54.9, 20.9, 20.75, 20.7, 20.62, 20.6; ν_{max}: 3021, 2939, 1700, 1729, 1761, 1729, 1652, 1435, 1048, 760 cm^{-1}.

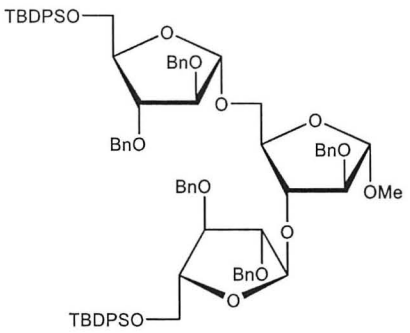
Experiment 69: Methyl 5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (160)



A solution of sodium methoxide (2 mL, 1M, in methanol) was added to a stirred solution of methyl-2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzoyl-α-D-arabinofuranoside **(157)** (0.20 g, 0.14 mmol) in dry MeOH : CH_2Cl_2 (1:1, 5 mL) at room temperature until a PH of 11 was obtained. The reaction mixture was stirred at room temperature for 2 h when TLC showed no starting material was left, the reaction 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 chromategraphy on silica eluting with chloroform/methanol (5:2) to give methyl 5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside **(160)** as a thick oil (0.1 g, 83%) [Found (MALDI) $(M+Na)^+$: 927.1, $C_{48}H_{64}NaO_{13}Si_2$, requires: 927.3], $[\alpha]_D^{22}$ + 50 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.49 – 7.34 (20H, m), 5.19 (1H, s), 5.14 (1H, s), 4.86 (1H, s), 4.25 – 4.20 (2H, m), 4.18 (1H, br. d, *J* 1.8 Hz), 4.12 (2H, br. d, *J* 3.8 Hz), 4.05 (4H, m), 4.01 (2H, m), 3.80 (1H, dd, *J* 2.3, 11.4 Hz), 3.77 – 3.73 (2H, m), 3.70 (2H, br. d, *J* 11.3 Hz), 3.34 (3H, s), 3.01 – 2.7(4H, m), 1.10 – 0.99

(18H, m); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 135.7, 135.6, 135.5, 129.5, 128.3, 128.0, 127.9, 127.8, 109.1, 108.6, 108.4, 88.0, 87.4, 83.8, 82.4, 79.3, 78.9, 78.4, 77.8, 77.7, 66.1, 64.0, 63.85, 54.8, 26.7, 26.6; ν_{max}: 3445, 3010, 2928, 2860, 1494, 1050, 824 cm^{-1}. The experiment was repeated on a large scale.

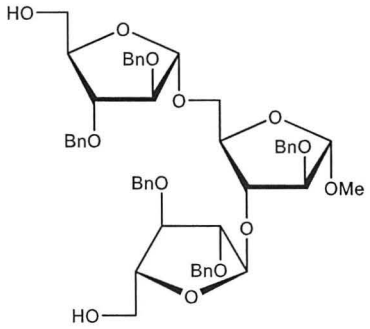
Experiment 70: Methyl 2,3-di-*O*-benzyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (161) [288]



A solution of methyl 5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside **(160)** (0.2 g, 0.2 mmol) in dry DMF : THF(1:1) (4 mL) was added dropwise to a stirred suspension solution of NaH (0.1 g, 8.3 mmol) (60% w/w, dispersion in mineral oil, washed with petrol for three times) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min then benzyl bromide (0.1 mL, 0.8 mmol) in dry DMF (1 mL) was added. The reaction mixture was stirred at room temperature for 16 h. When TLC showed no starting material was left. The reaction mixture was quenched by slow addition of methanol (3 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 (20 mL) and brine (20 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 methyl 2,3-di-*O*-benzyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabino-furanosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(161)** as a thick oil (0.23 g, 77%) [Found (MALDI) $(M+Na)^+$: 1377.0, $C_{83}H_{94}NaO_{13}Si_2$, requires: 1377.6], $[\alpha]_D^{22}$ + 40 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.66 (7H, m), 7.42 – 7.17 (38H, m), 5.19 (1H, s), 5.17 (1H, s), 4.94 (1H, s), 4.59 – 4.37 (10H, m), 4.28 (1H, dd, *J* 2.3, 6.3 Hz), 4.22 – 4.14 (2H, m), 4.14 – 4.03

(5H, m), 4.03 – 3.95 (2H, m), 3.86 – 3.74 (5H, m), 3.37 (3H, s), 1.03 (18H, s); δ_C (101 MHz, $CDCl_3$): 135.7, 135.65, 135.62, 135.6, 129.6, 129.54, 129.51, 128.5, 128.4, 128.35, 128.34, 128.3, 128.22, 128.2, 127.9, 127.8, 127.79, 127.75, 127.73, 127.66, 127.63, 127.6, 127.56, 127.53, 127.4, 127.35, 127.33, 127.3, 127.28, 107.2, 106.5, 105.4, 88.5, 88.3, 88.0, 83.1, 82.8, 82.3, 81.8, 81.2, 80.4, 72.0, 71.9, 71.73, 71.7, 71.6, 66.2, 63.5, 63.3, 54.7, 26.8, 26.7; ν_{max}: 3466, 3050, 3017, 2958, 2930, 2858, 1493, 1112, 701 cm^{-1}. The experiment was repeated on a large scale.

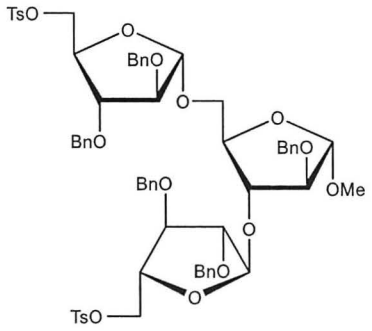
Experiment 71: Methyl 2,3-di-*O*-benzyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (162) [288]



Tetrabutylammonium fluoride (0.3 mL, 0.3 mmol, 1 M) was added dropwise to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabino-furanosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(161)** (0.2 g, 0.147 mmol) in dry THF (10 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. 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 (1:1) to give methyl 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 **(162)** as a colourless thick oil (0.1 g, 77%) [Found (MALDI) $(M+Na)^+$: 901.3, $C_{51}H_{58}NaO_{13}$, requires : 901.3], $[\alpha]_D^{20}$ + 90 (*c* 0.1, $CHCl_3$) [*lit.*[288] $[\alpha]_D$ + 84.8 (*c* 0.3, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.21 (25H, m), 5.16 (1H, s), 5.12 (1H, d, *J* 1.2 Hz), 4.96 (1H, d, *J* 1.2 Hz), 4.61 – 4.42 (9H,m), 4.38 (1H, d, *J* 12 Hz), 4.30 (1H, dd, *J* 3.7, 7.4 Hz), 4.25 (1H, ddd, *J* 2.9, 5.2, 6.3 Hz), 4.16 – 4.07 (3H, m), 4.03 (1H, dd, *J* 1.2, 3.7 Hz), 4.01 (1H, dd, *J* 1.2, 3.7 Hz), 3.95 (1H, dd, *J* 3.8, 11.6 Hz), 3.91 (1H, dd, *J* 2.9, 6.6 Hz), 3.85-3.71 (4H, m), 3.66 (1H, dd, *J* 5.2, 12.2 Hz), 3.59 (1H, dd, *J* 5.8, 12.2 Hz), 3.40 (3H, s), 2.84 (1H,

br. s), 2.48 (1H, br. s); δ_C (101 MHz, $CDCl_3$): 137.7, 137.6, 137.4, 137.3, 137.2, 128.5, 128.44, 128.43, 128.4, 128.38, 127.96, 127.94, 127.9, 127.87, 127.83, 127.8, 127.71, 106.9, 106.2, 105.8, 88.7, 88.2, 87.5, 83.0, 82.9, 82.3, 81.9, 80.9, 79.7, 72.3, 72.2, 72.0, 71.9, 71.8, 64.9, 62.8, 62.7, 54.9; ν_{max}: 3467, 3050, 3017, 2926, 2861, 1609, 1494, 1050, 824 cm^{-1}. All data were identical to the authentic sample. The experiment was repeated on a large scale.

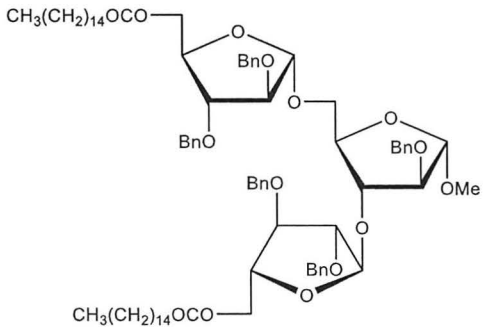
Experiment 72: Methyl 2,3-di-*O*-benzyl-5-*O*-p-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (163)



4-Toluenesulfonyl chloride (2.82 g, 14.7 mmol) was added to a stirred solution of methyl-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 **(162)** (1.30 g, 1.47 mmol), pyridine (1.17 g, 1.19 mL, 14.7 mmol) and DMAP (catalytic amount) in dry CH_2Cl_2 (10 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and stirred for 16 h, then TLC showed no starting material was left. The reaction 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 water (50 mL) and brine (50 mL), dried over ($MgSO_4$). 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 (1:1) affording methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluene-sulfonyl-α-D-arabinofu-ranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)] -2-*O*-benzyl-α-D-arabinofuranoside **(163)** as a colourless thick oil (1.3 g, 74%) [Found (MALDI) $(M+Na)^+$: 1209.2, $C_{65}H_{70}NaO_{17}S_2$, requires: 1209.3], $[\alpha]_D^{22}$ + 71 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.7 (2H, d, *J* 8.3 Hz), 7.8 (2H, d, *J* 8.4 Hz), 7.37 – 7.23 (29H, m), 5.09 (1H, br. s), 5.08 (1H, br. s), 4.93 (1H, s), 4.61 – 4.42

(9H, m), 4.35 (1H, d, *J* 12.0 Hz), 4.30 – 4.24 (1H, m), 4.22 – 4.10 (6H, m), 4.10 – 4.05 (2H, m), 4.0 (1H, dd, *J* 1.2, 3.3 Hz), 3.96 (1H, dd, *J* 1.0, 3.1 Hz), 3.87 (2H, dd, *J* 3.1, 6.1 Hz), 3.84 (1H, dd, *J* 4.0, 12.0 Hz), 3.69 (1H, dd, *J* 2.5, 12.0 Hz), 3.38 (3H, s), 2.38 (3H, s), 2.4 (3H, s); δ_C (101 MHz, $CDCl_3$): 144.8, 144.7, 137.5, 137.4, 137.3, 137.2, 137.1, 132.7, 132.6, 129.8, 129.7, 128.4, 128.38, 128.35, 128.33, 128.3, 128.0, 127.93, 127.9, 127.85, 127.82, 127.8, 127.7, 127.69, 127.67, 106.9, 106.4, 105.5, 88.0, 87.6, 87.4, 82.8, 82.7, 80.5, 80.4, 79.0, 78.7, 72.2, 72.0, 71.8, 71.81, 71.7, 68.7, 68.6, 65.7, 54.8, 21.5; ν_{max}: 3088, 3064, 3031, 2924, 2862, 1598, 1454, 1177, 738 cm^{-1}.

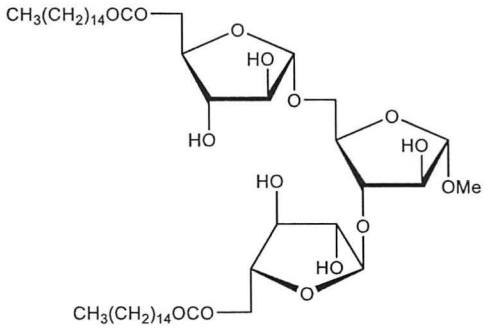
Experiment 73: Methyl 2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (164) [288]



Cesium hydrogencarbonate (0.13 g, 0.67 mmol) was added to a stirred solution of methyl-2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(163)** (0.08 g, 0.06 mmol) and palmitic acid (0.04 g, 0.15 mmol) in dry DMF : THF (1:5, 3 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days, then TLC showed no starting material was left. The suspension was diluted with ethyl acetate (50 mL) and water (10 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 (15 mL) and brine (15 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 afford methyl 2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabino-furanosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(164)** as a colourless thick oil (0.08 g, 88%) [Found (MALDI) $(M+Na)^+$: 1377.6, $C_{83}H_{118}NaO_{15}$, requires: 1377.8], $[\alpha]_D^{22}$ + 45 (*c* 0.1, $CHCl_3$)

[*lit.*[288] $[\alpha]_D$+ 40.8 (*c* 0.2, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.22 (25H, m), 5.19 (1H, br. s), 5.15 (1H, br. s), 4.93 (1H, br. s), 4.61 – 4.42 (9H, m), 4.38 (1H, d, *J* 11.8 Hz), 4.30 – 4.23 (4H, m), 4.22 – 4.16 (3H, m), 4.14 (1H, dd, *J* 2.4, 4.6 Hz), 4.10 (1H, br. d, *J* 3.2 Hz), 4.03 (1H, br. d, *J* 2.8 Hz), 3.98 (1H, br. d, *J* 2.4), 3.95 (1H, dd, *J* 4.6, 12.0 Hz), 3.86 (2H, m), 3.78 (1H, dd, *J* 2.3, 12.0 Hz), 3.38 (3H, s), 2.27 (4H, m), 1.62 – 1.52 (4H, m), 1.35 – 1.22 (48H, m), 0.89 (6H, t, *J* 6.8); δ_C (101 MHz, $CDCl_3$): 173.6, 173.5, 137.7, 137.6, 137.5, 137.4, 137.3, 128.42, 128.4, 128.39, 128.3, 128.0, 127.9, 127.88, 127.8, 127.79, 127.75, 127.73, 127.7, 107.0, 106.5, 105.5, 88.2, 88.1, 88.0, 83.4, 83.3, 80.7, 80.4, 79.2, 79.0, 72.2, 72.1, 71.9, 71.8, 65.7, 63.3, 63.2, 54.8, 34.0, 33.6, 31.9, 29.7, 29.6, 29.5, 29.35, 29.3, 29.2, 29.1, 29.0, 24.8, 22.7, 14.1; ν_{max}: 3065, 3031, 2922, 2851, 1739, 1700, 1050, 697 cm^{-1}. All data were identical to the authentic sample.

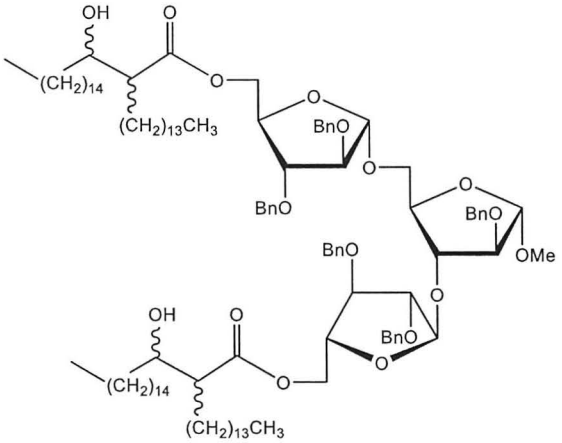
Experiment 74: Methyl 5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→3)-[5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (165) [288]



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 0.02 g, 0.30 fold by weight) was added to a stirred solution of methyl 2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-palmitoyl-α-D-arabinofuranosyl(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(164)** (0.0489 g, 0.0361 mmol) in dry CH_2Cl_2 : MeOH, (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 24 h, then TLC showed no starting material was left. The reaction mixture was filtered and the precipitate was washed with (10 mL) CH_2Cl_2, the filtrate was evaporated under reduced pressure to give a thick oil residue, which was purified by column chromatography on silica eluting with chloroform/ methanol (5:1) to afford methyl 5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→3)-[5-*O*-palmitoyl-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside **(165)** as a colourless thick oil (28.5 mg, 87%) $[\alpha]_D^{22}$

+ 73 (*c* 0.1, $CHCl_3$) [*lit.*[288] $[\alpha]_D$ + 64.4 (*c* 0.4, $CHCl_3$)], [Found (MALDI) $(M+Na)^+$: 927.4, $C_{48}H_{88}NaO_{15}$, requires: 927.6] which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 5.04 (1H, s), 5.02 (1H, s), 4.81 (1H, s), 4.29 – 4.23 (2H, m), 4.22 – 4.13 (4H, m), 4.08 (1H, dd, *J* 5.3, 9.6 Hz), 4.06 – 4.01 (4H, m), 3.98 (1H, dd, *J* 3.2, 11.1 Hz), 3.83 (2H, m), 3.68 (1H, dd, *J* 3.1, 11.1 Hz), 3.36 (3H, s), 3.13 – 3.07 (1H, m), 2.36 – 2.29 (4H, m), 1.64 – 1.56 (4H, m), 1.34 – 1.17 (52H, m), 0.85 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 174.0, 173.9, 109.1, 108.0, 107.7, 83.4, 82.5, 82.3, 82.2, 80.6, 79.6, 76.7, 66.2, 63.9, 63.6, 54.9, 34.01, 34.0, 31.8, 29.6, 29.57, 29.5, 29.4, 29.3, 29.25, 29.2, 29.1, 29.0, 24.8, 24.7, 22.6, 14.0; ν_{max}: 3436, 2920, 2851, 1733, 1734, 1174, 734 cm^{-1}. All data were identical to the authentic sample.

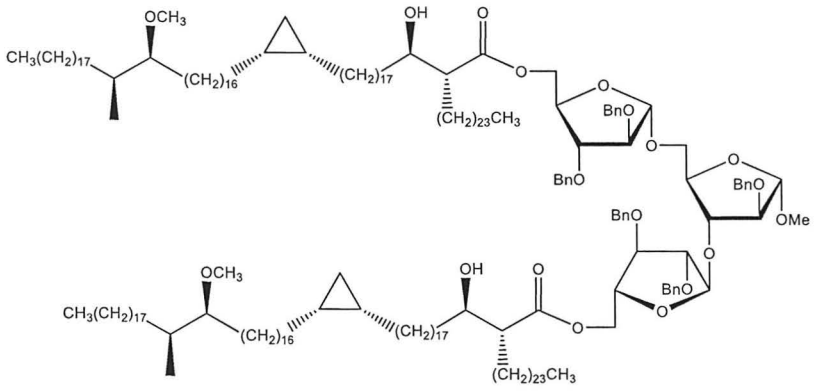
Experiment 75: Methyl 2,3-di-*O*-benzyl-5-*O*-(3-hydroxy-2-tetradecyloctadecano-ate)-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(3-hydroxy-2-tetradecyl-octadecanoate)-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (167)



Cesium hydrogencarbonate (0.77 g, 3.97 mmol) was added to a stirred solution of methyl-2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(163)** (0.05 g, 0.04 mmol) and 3-hydroxy-2-tetradecyloctadecanoic acid **(166)** (0.04 g, 0.09 mmol) and in dry DMF : THF (1:5, 6 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days, then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to afford methyl-2,3-di-*O*-benzyl-5-*O*-(3-hydroxy-2-tetradecylocta-decano-ate)-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(3-hydroxy-2-tetradecylocta-decanoate)-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(167)** as a

colourless thick oil (30 mg, 38%) $[\alpha]_D^{18}$ + 61 (*c* 0.1, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 1858.6, $C_{115}H_{182}NaO_{17}$, requires: 1858.3] which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.21 (25H, m), 5.18 (1H, br. s), 5.13 (1H, br. s), 4.92 (1H, br. s), 4.61 – 4.37 (10H, m), 4.27 (4H, m), 4.21 – 4.05 (5H, m), 4.0 (2H, dd, *J* 9.9, 12.0 Hz), 3.88 (2H, m), 3.8 – 3.7 (2H, m), 3.61 (2H, m), 3.37 (3H, s), 2.48 – 2.38 (2H, m), 1.34 – 1.19 (112H, m), 0.89 (12H, t, *J* 6.8 Hz); δ_C (101 MHz, CDCl3): 174.9, 174.8, 137.6, 137.5, 137.4, 137.3, 137.1, 128.4, 128.39, 128.36, 127.9, 127.84, 127.8, 127.7, 127.6, 107.0, 106.4, 105.5, 88.2, 87.8, 87.7, 83.6, 80.6, 80.3, 80.29, 79.2, 72.3, 72.1, 71.96, 71.9, 71.7, 60.3, 54.8, 31.9, 29.7, 29.6, 29.3, 25.5, 22.6, 14.0; ν_{max}: 3436, 2920, 2851, 1733, 1734, 1174, 734 cm^{-1}.

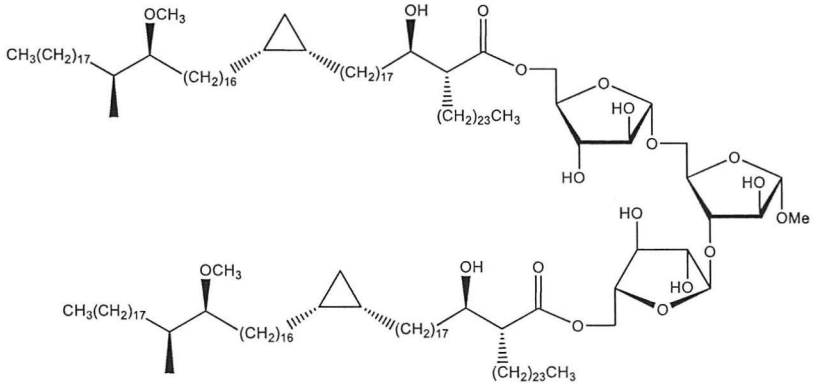
Experiment 76: Methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*)-2-(1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl]hexacosanoate)]-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-[(2*R*)-2-(1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl)hexacosanoate)]-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (168)



Cesium hydrogencarbonate (0.065 g, 0.335 mmol) was added to a stirred solution of methyl-2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(163)** (0.040 g, 0.033 mmol) and (2*R*)-2-{1-hydroxy-18-[(1*S*,2*R*)-2-[(17S,18S)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl}hexacosanoic acid **(85)** [158] (0.092 g, 0.073 mmol) in dry DMF : THF (1:5, 3 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days, then TLC showed no starting material was left. The reaction was worked up and purified as before to afford the titled compound **(168)** as a colourless thick oil (0.051 g, 45%) [Found

(MALDI) $(M+Na)^+$: 3371.9, $C_{221}H_{390}NaO_{19}$, requires: 3371.9], $[\alpha]_D^{23}$ +35 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.23 (25H, m), 5.18 (1H, br. s), 5.13 (1H, br. s), 4.91 (1H, br. s), 4.49 (9H, m), 4.34 (1H, d, *J* 11.7 Hz), 4.32 – 4.24 (6H, m), 4.18 (1H, ddd, *J* 3.0, 6.3, 9.7 Hz), 4.11 (1H, dd, *J* 2.7, 4.4 Hz), 4.08 (1H, br. d, *J* 2.9 Hz), 4.00 (1H, dd, *J* 0.6, 2.9 Hz), 3.96 (1H, br. d, *J* 2.3 Hz), 3.93 (1H, dd, *J* 3.9, 7.8 Hz), 3.88 (2H, m), 3.76 (1H, dd, *J* 1.8, 11.4 Hz), 3.67 – 3.57 (2H, m), 3.37 (3H, s), 3.35 (6H, s), 2.99 – 2.93 (2H, m), 2.67 (1H, d, *J* 6.1 Hz), 2.65 (1H, d, *J* 6.5 Hz), 2.41 (2H, dt, *J* 5.8, 9.0 Hz), 1.68 – 1.03 (294H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.86 (6H, d, *J* 6.9 Hz), 0.71 – 0.61 (4H, m), 0.57 (2H, dt, *J* 4.0, 7.6 Hz), -0.33 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 175.0, 137.7, 137.6, 137.5, 137.4, 137.2, 128.5, 128.41, 128.4, 127.95, 127.9, 127.8, 127.73, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 85.4, 83.7, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.1, 71.98, 71.8, 65.6, 63.0, 57.7, 54.8, 51.9, 51.7, 35.3, 32.4, 31.9, 30.5, 30.2, 29.97, 29.9, 29.7, 29.66, 29.5, 29.4, 28.7, 27.6, 26.2, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3517, 3063, 3031, 2922, 2852, 1736, 1466, 1101, 757 cm^{-1}.

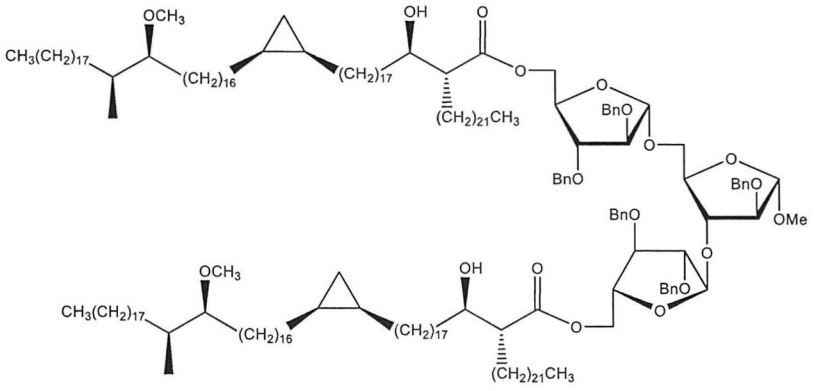
Experiment 77: Methyl 5-*O*-[(2*R*)-2-(1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl]hexacosanoate)]-α-D-arabinofuranosyl-(1→3)-5-*O*-[(2*R*)-2-(1-hydroxy-18-[(1*S*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl]hexacosanoate)]-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (169)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 12 mg, 0.3 fold by weight) was added to a stirred solution of compound **(168)** (0.042 g, 0.014 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 16 h, then TLC showed no starting material was left. The mixture was filtered off and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with

chloroform/methanol (5:1) to give the titled compound **(169)** as a thick oil (0.03 g, 82%) [Found $(M+Na)^+$: 2921.8, $C_{186}H_{360}NaO_{19}$, requires: 2921.7], $[\alpha]_D^{20}$ + 21 (*c* 0.1, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$ + few drops CD_3OD): 4.98 (1H, br. s), 4.95 (1H, br. s), 4.77 (1H, br. s), 4.40 (1H, dd, *J* 4.3, 11.8 Hz), 4.33 (1H, dd, *J* 4.5, 11.6 Hz), 4.24 (1H, dd, *J* 4.7, 11.6 Hz), 4.15 (1H, dd, *J* 4.3, 11.6 Hz), 4.13 – 4.07 (2H, m), 4.07 – 3.94 (5H, m), 3.94 – 3.80 (3H, m), 3.67 – 3.55 (3H, m), 3.36 (3H, s), 3.32 (6H, s), 2.96 – 2.90 (2H, m), 2.37 (2H, m), 1.61 – 0.97 (301H, m), 0.82 (12H, t, *J* 7.0 Hz), 0.79 (6H, d, *J* 6.9 Hz), 0.64 – 0.55 (4H, m), 0.50 (2H, dt, *J* 4.0, 8.0 Hz), -0.39 (2H, br. q, *J* 5.1 Hz); δ_C (126 MHz, $CDCl_3$ + few drops CD_3OD): 175.1, 175.0, 109.1, 107.9, 107.2, 85.5, 83.0, 82.4, 81.8, 81.0, 79.8, 77.9, 72.5, 72.45, 65.9, 63.4, 63.1, 57.5, 54.8, 52.8, 50.0, 35.2, 34.8, 34.7, 32.2, 31.8, 30.3, 30.1, 29.82, 29.8, 29.62, 29.6, 29.56, 29.5, 29.4, 29.3, 29.2, 29.1, 28.6, 27.4, 27.3, 25.96, 25.2, 25.2, 22.5, 15.6, 14.7, 13.9, 10.7; ν_{max}: 3401, 2919, 2851, 1733, 1467, 1099, 720 cm^{-1}.

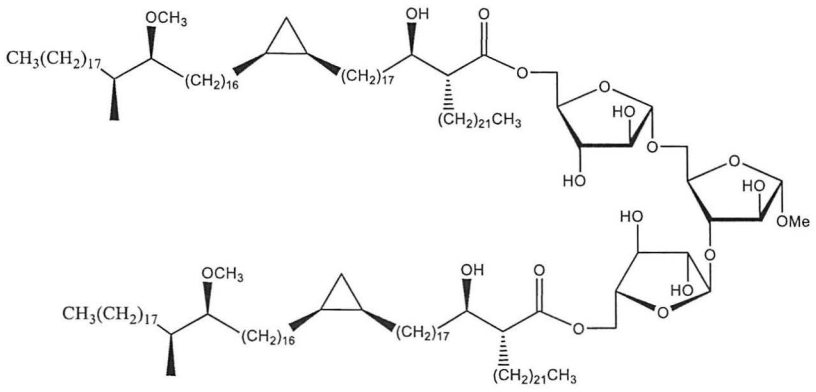
Experiment 78: Methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*, 2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (170)



Cesium hydrogencarbonate (0.081 g, 0.417 mmol) was added to a stirred solution of methyl-2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(163)** (0.050 g, 0.042 mmol) and (2*R*)-2-{(1*R*)-1-hydroxy-18-[(2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-ethylhexatriacontyl]cyclopropyl]octadecyl}tetracosanoic acid **(79)**[158] (0.113 g, 0.092 mmol) in dry DMF : THF (1:5, 6 mL) at room temperature

under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days, then TLC showed no starting material was left. The reaction mixture was worked up as before and purified by column chromatography on silica eluting with hexane/ethyl acetate (5:2) to afford the title compound **(170)** as a colourless thick oil (50 mg, 36%) [Found $(M+Na)^+$: 3315.8808, $C_{217}H_{382}NaO_{19}$, requires: 3315.8818], $[\alpha]_D^{23}$ + 40 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.22 (25H, m), 5.18 (1H, s), 5.13 (1H, s), 4.91 (1H, s), 4.61 – 4.42 (9H, m), 4.38 (1H, d, *J* 12.0 Hz), 4.30 – 4.23 (6H, m), 4.22 – 4.16 (1H, m), 4.14 (2H, m), 4.03 (1H, dd, *J* 1.0, 3.2 Hz), 3.98 (1H, dd, *J* 1.0, 3.2 Hz), 3.95 (1H, dd, *J* 4.6, 12.0 Hz), 3.86 (2H, m), 3.78 (1H, dd, *J* 2.3, 12.0 Hz), 3.67 – 3.57 (2H, m), 3.37 (3H, s), 3.35 (6H, s), 3.01 – 2.92 (2H, m), 2.67 (2H, br. s), 2.45 – 2.37 (2H, m), 1.72 – 1.07 (285 H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.86 (6H, d, *J* 6.9 Hz), 0.71 – 0.60 (4H, m), 0.61 – 0.53 (2H, dt, *J* 4.0, 7.6 Hz), -0.32 (2H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 174.9, 137.6, 137.51, 137.5, 137.4, 137.2, 128.45, 128.41, 128.4, 128.38, 128.37, 127.9, 127.85, 127.8, 127.74, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 85.4, 83.7, 83.6, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.1, 72.0, 71.9, 71.7, 65.5, 63.0, 57.7, 54.8, 51.8, 51.7, 35.3, 32.4, 31.9, 30.5, 30.2, 29.98, 29.9, 29.7, 29.6, 29.5, 29.4, 28.7, 27.6, 27.4, 26.2, 22.7, 15.7, 14.8, 14.1, 10.9; ν_{max}: 3436, 2920, 2851, 1733, 1734, 1174, 734 cm^{-1}.

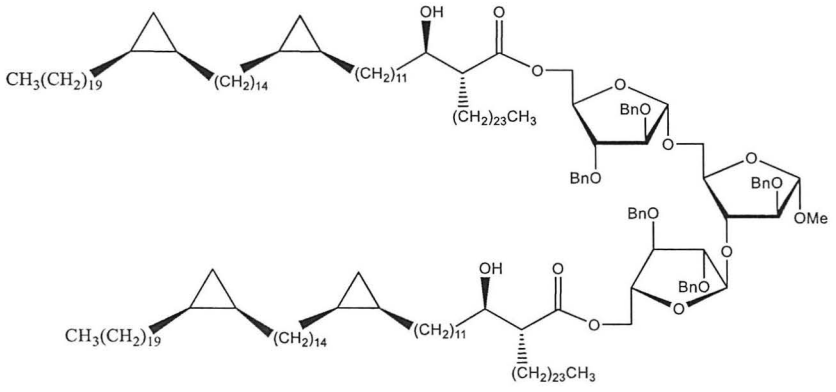
Experiment 79: Methyl 5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranosyl-(1→3)-[5-*O*-(2-[(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl]tetracosanoate)-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (171)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 0.01 g, 0.30 fold by weight) was added to a stirred solution of compound **(170)** (0.037 g, 0.010 mmol) in dry

CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The mixture was filtered off and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to the title compound **(171)** as a white oil (21 mg, 65%) [Found $(M+Na)^+$: 2865.6424, $C_{182}H_{352}NaO_{19}$, requires: 2865.6470], $[\alpha]_D^{16}$ + 24 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 5.02 (1H, d, *J* 1.5 Hz), 4.98 (1H, br. s), 4.80 (1H, br. s), 4.46 (1H, dd, *J* 4.3, 11.7 Hz), 4.37 (1H, dd, *J* 4.8, 11.6 Hz), 4.27 (1H, dd, *J* 4.9, 11.7 Hz), 4.16 (1H, dd, *J* 4.8, 12.0 Hz), 4.14 – 4.08 (2H, m), 4.08 – 3.99 (5H, m), 3.95 – 3.87 (3H, m), 3.62 (3H, m), 3.35 (3H, s), 3.31 (6H, s), 2.94 (2H, m), 2.44 – 2.34 (2H, m), 1.37 – 1.13 (292H, m), 0.85 (12H, t, *J* 6.8 Hz), 0.82 (6H, d, *J* 6.9 Hz), 0.66 – 0.57 (4H, m), 0.56 – 0.49 (2H, dt, *J* 4.0, 7.6 Hz), -0.37 (2H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.2, 175.0, 109.1, 107.9, 107.2, 85.5, 82.9, 82.5, 82.0, 81.8, 81.0, 80.9, 79.7, 78.0, 72.5, 66.9, 65.9, 63.1, 57.6, 54.8, 52.8, 35.2, 32.3, 31.8, 30.4, 30.1, 29.8, 29.7, 29.66, 29.6, 29.5, 29.3, 29.2, 28.6, 27.4, 26.0, 22.5, 15.7, 14.7, 14.0, 10.8; ν_{max}: 3412, 2919, 2850, 1731, 1466, 1099, 720 cm^{-1}.

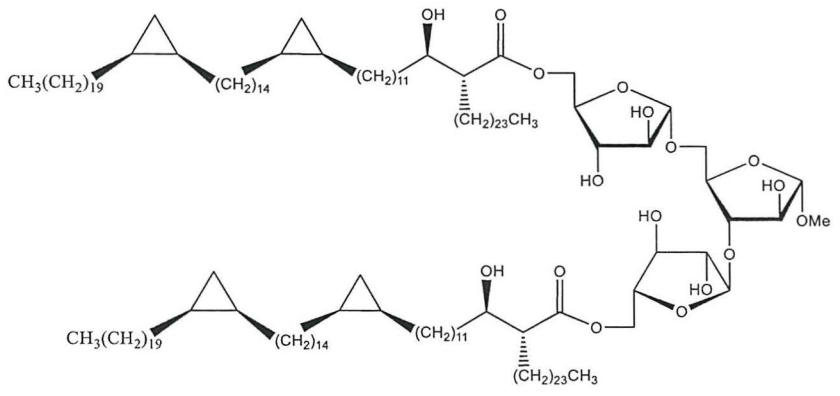
Experiment 80: Methyl 2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-12-{(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl]cyclopropyl}dodecyl)hexacosanoate)-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-(2-[(*R*)-1-hydroxy-12-{(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl]tetradecyl]cyclopropyl}dodecyl)hexacosanoate)-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (172)



Cesium hydrogencarbonate (0.089 g, 0.458 mmol) was added to a stirred solution of methyl-2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabin-

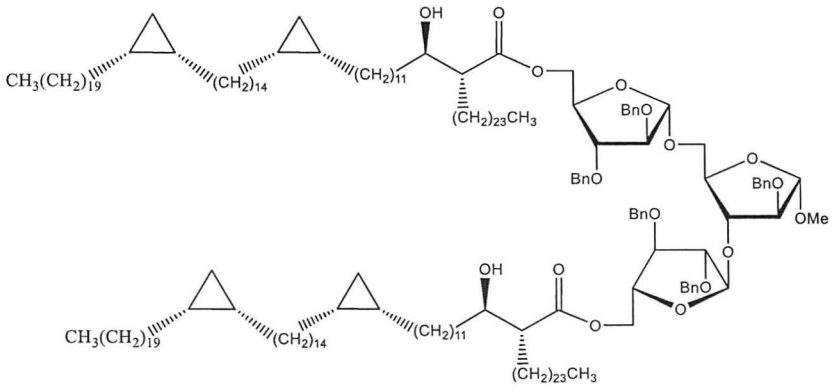
ofuranoside **(163)** (0.055 g, 0.046 mmol) and (2*R*)-2-{(1*R*)-1-hydroxy-12-[(1*R*)-2-[14-[(2*S*)-2-icosylcyclopropyl]tetradecyl]cyclopropyl]dodecyl}hexacosanoic acid **(127)**[157] (0.115 g, 0.101 mmol) in dry DMF : THF (1:5, 6 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days then TLC showed no starting material was left. The reaction was worked up and purified as before to afford the titled compound **(172)** as a colourless oil (56 mg, 18%) [Found $(M+Na)^+$: 3139.7057, $C_{207}H_{358}NaO_{17}$, requires: 3139.7041], $[\alpha]_D^{23}$ + 72 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.22 (25H, m), 5.18 (1H, br. s), 5.14 (1H, br. s), 4.92 (1H, br. s), 4.61 – 4.43 (9H, m), 4.35 (1H, d, *J* 12.0 Hz), 4.32 – 4.25 (6H, m), 4.22 – 4.16 (1H, m), 4.09 (2H, m), 4.01 (1H, dd, *J* 1.0, 3.0 Hz), 3.97 (1H, dd, *J* 1.0, 3.0), 3.92 (1H, dd, *J* 7.5, 12.0 Hz), 3.88 (2H, m), 3.77 (1H, dd, *J* 2.2, 12.0 Hz), 3.62 (2H, m), 3.38 (3H, s), 2.68 (2H, m), 2.46 – 2.36 (2H, m), 1.70 – 1.06 (268H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.71 – 0.62 (8H, m), 0.60 – 0.54 (4H, dt, *J* 4.0, 7.6 Hz), -0.32 (4H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 174.9, 137.6, 137.51, 137.5, 137.4, 137.2, 128.5, 128.4, 128.38, 128.37, 128.0, 127.9, 127.8, 127.71, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 83.6, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.0, 71.9, 71.7, 65.6, 63.0, 54.8, 51.85, 51.7, 35.4, 35.2, 31.9, 30.2, 29.8, 29.75, 29.7, 29.6, 29.5, 29.4, 29.2, 28.7, 27.5, 27.4, 25.7, 22.7, 18.5, 18.3, 15.7, 14.1, 10.9; ν_{max}: 3584, 3064, 3032, 2918, 2850, 1733, 1455, 1018, 732 cm^{-1}.

Experiment 81: Methyl 5-*O*-(2-[(*R*)-1-hydroxy-12-{(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosyl cyclopropyl]tetradecyl]cyclopropyl}dodecyl)hexacosanoate)-α-D-arabinofuranosyl-(1→3)-[5-*O*-(2-[(*R*)-1-hydroxy-12-{(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-icosylcyclopropyl] tetradecyl]cyclopropyl}dodecyl)hexacosanoate)-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (173)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 6.6 mg, 0.30 fold by weight) was added to a stirred solution of compound **(172)** (0.030 g, 0.009 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred 16 h then TLC showed no starting material was left. The mixture was filtered off and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to give the title compound **(173)** as a colourless oil (17 mg, 64%) [Found $(M+Na)^+$: 2689.4696, $C_{172}H_{328}NaO_{17}$, requires: 2689.4694], $[\alpha]_D^{16}$ + 36 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 5.03 (1H, d, *J* 1.5 Hz), 4.99 (1H, br. s), 4.81 (1H, br. s), 4.48 (1H, dd, *J* 4.3, 11.9 Hz), 4.39 (1H, dd, *J* 4.7, 11.7 Hz), 4.28 (1H, dd, *J* 4.8, 11.5 Hz), 4.17 (1H, dd, *J* 4.3, 11.4 Hz), 4.13 (2H, m), 4.06 – 3.98 (5H, m), 3.97 – 3.86 (3H, m), 3.69 – 3.58 (3H, m), 3.36 (3H, s), 2.51 – 2.42 (2H, m), 2.42 – 2.35 (2H, m), 1.70 – 1.00 (271H, m), 0.86 (12H, t, *J* 6.8 Hz), 0.67 – 0.58 (8H, m), 0.57 – 0.50 (4H, dt, *J* 4.0, 7.6 Hz), -0.36 (4H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.2, 175.0, 109.0, 107.8, 107.3, 83.1, 82.9, 81.5, 81.1, 80.0, 77.97, 72.99, 72.9, 65.7, 63.6, 63.2, 54.9, 52.8, 52.7, 31.9, 30.3, 30.2, 29.8, 29.75, 29.7, 29.6, 29.5, 29.4, 28.7, 27.4, 22.7, 15.8, 14.1, 10.9; ν_{max}: v. br. 3400, 2919, 2851, 1732, 1467, 1099, 760 cm^{-1}.

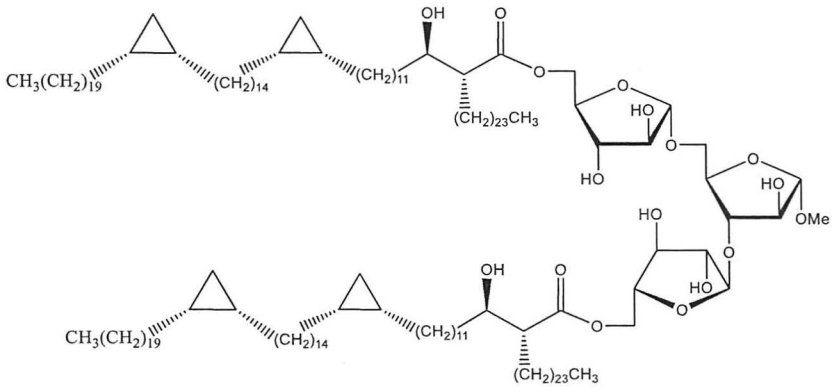
Experiment 82: Methyl 2,3-di-*O*-benzyl-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14 -[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D -arabinofuranosyl-(1→3)-[2,3-di-O-benzyl-5-O-(2-{(*R*)-1-hydroxy-12-[(1*S*, 2*R*) -2-(14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexa cosanoate)-α-D-arabinofuranosyl-(1→5)]-2-O-benzyl-α-D-arabinofuranoside (174)



Cesium hydrogencarbonate (0.081 g, 0.417 mmol) was added to a stirred solution of methyl-2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*

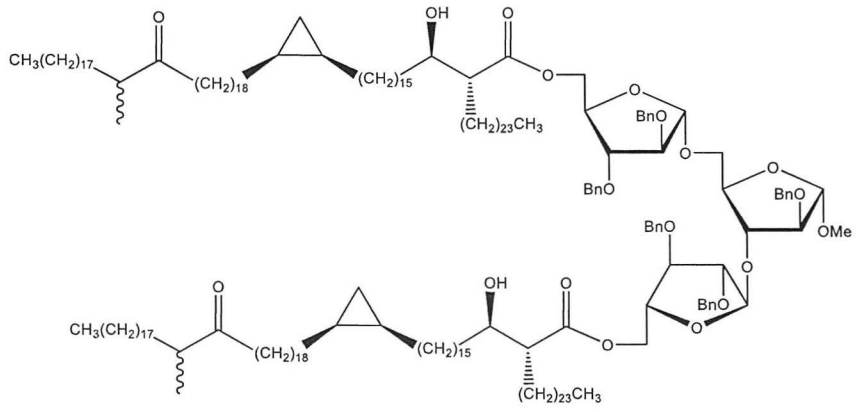
-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(163)** (0.050 g, 0.042 mmol) and 2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoic acid **(97)**[157] (0.105 g, 0.092 mmol) in dry DMF : THF (1:5, 3 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days then TLC showed no starting material was left. The reaction mixture was worked up as before to give a thick oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to give a colourless oil of the titled compound **(174)** (31 mg, 11%) [Found (MALDI) $(M+Na)^+$: 3139.7, $C_{207}H_{358}NaO_{17}$, requires: 3139.7], $[\alpha]_D^{23}$ + 80 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.35 – 7.27 (25H, m), 5.18 (1H, br. s), 5.13 (1H, br. s), 4.91 (1H, br. s), 4.61 – 4.39 (9H, m), 4.40 (1H, d, *J* 12.8 Hz), 4.35 (1H, dd, *J* 4.6, 9.4 Hz), 4.32 – 4.23 (5H, m), 4.21 – 4.17 (1H, m), 4.11 – 4.05 (2H, m), 4.00 (1H, br. d, *J* 2.7 Hz), 3.98 – 3.95 (1H, m), 3.95 – 3.91 (1H, m), 3.90 – 3.85 (2H, m), 3.76 (1H, dd, *J* 2.2, 11.7 Hz), 3.66 – 3.57 (2H, m), 3.37 (3H, s), 2.69 – 2.62 (2H, m), 2.45 – 2.36 (2H, m), 1.64 – 1.07 (268H, m), 0.89 (12H, t, *J* 6.9 Hz), 0.70 – 0.61 (8H, m), 0.56 (4H, dt, *J* 4.0, 7.6 Hz), -0.33 (4H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 175.0, 137.7, 137.5, 137.4, 137.3, 137.2, 128.5, 128.4, 128.38, 128.37, 127.95, 127.9, 127.8, 127.73, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 83.6, 80.6, 79.2, 79.1, 72.3, 72.2, 72.1, 72.0, 71.9, 71.7, 65.6, 63.0, 60.4, 54.8, 51.8, 51.7, 31.9, 30.2, 29.72, 29.7, 29.6, 29.5, 29.4, 28.7, 22.6, 15.8, 14.1, 10.9; ν_{max}: 3524, 3035, 2918, 2850, 1735, 1466, 1018, 734 cm^{-1}.

Experiment 83: Methyl-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranosyl-(1→3)-[5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-icosylcyclopropyl] tetradecyl)cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (175)



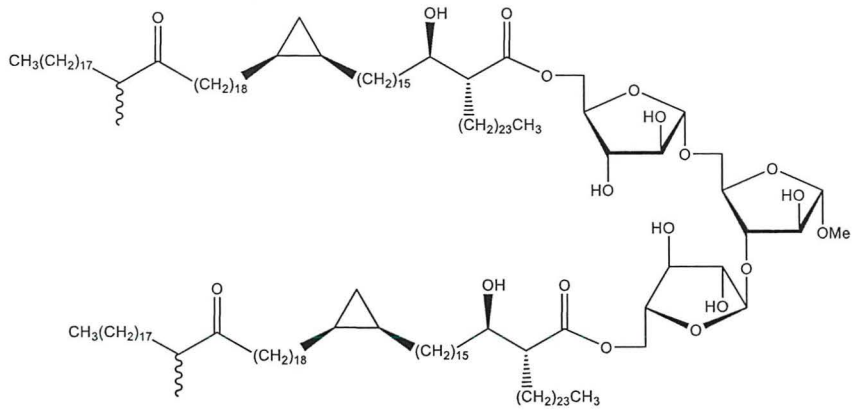
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 8.2 mg, 0.30 fold by weight) was added to a stirred solution of compound **(174)** (0.027 g, 0.008 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The mixture was filtered off and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to give the title compound **(175)** as a colourless oil (23.1 mg, 97%) [Found (MALDI) $(M+Na)^+$: 2689.4, $C_{172}H_{328}NaO_{17}$, requires: 2689.4], $[\alpha]_D^{20}$ + 40 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$+ few drops CD_3OD): 4.94 (1H, d, *J* 1.3 Hz), 4.92 (1H, br. s), 4.73 (1H, br. s), 4.35 (1H, dd, *J* 4.7, 11.8 Hz), 4.29 (1H, dd, *J* 3.9, 11.5 Hz), 4.20 (1H, dd, *J* 4.8, 11.5 Hz), 4.13 (1H, dd, *J* 4.8, 11.7 Hz), 4.09 – 4.02 (2H, m), 4.02 – 3.92 (5H, m), 3.89 – 3.77 (3H, m), 3.61 – 3.49 (3H, m), 3.28 (3H, s), 2.38 – 2.29 (2H, m), 1.51 – 0.94 (274H, m), 0.79 (12H, t, *J* 6.8 Hz), 0.61 – 0.52 (8H, m), 0.47 (4H, dt, *J* 4.0, 7.6 Hz), -0.42 (4H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.1, 175.0, 109.0, 107.9, 107.3, 82.9, 82.2, 81.8, 81.6, 81.0, 80.9, 79.7, 77.8, 77.5, 72.3, 66.0, 63.4, 63.1, 54.7, 52.7, 49.6, 34.7, 31.7, 30.0, 29.9, 29.6, 29.5, 29.4, 29.2, 29.1, 29.0, 28.5, 27.2, 22.4, 15.5, 13.8, 10.7, 10.6; ν_{max}: 3400, 2918, 2850, 1735, 1467, 1008 cm^{-1}.

Experiment 84: Methyl 2,3-di-*O*-benzyl-5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranosyl-(1→3)-[2,3-di-O-benzyl-5-*O*-{2-(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside (176)



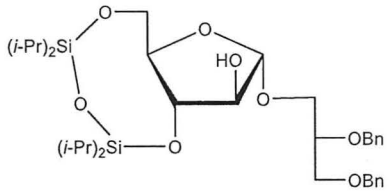
Cesium hydrogencarbonate (0.081 g, 0.417 mmol) was added to a stirred solution of methyl-2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→5)]-2-*O*-benzyl-α-D-arabinofuranoside **(163)** (0.050 g, 0.042 mmol) and (2*R*)-2-{(1*R*)-1-hydroxy-16-[(2*S*)-2-[20-methyl-19-oxooctatriacontyl]cyclopropyl]hexadecyl}hexacosanoic acid **(89)**[159] (0.114 g, 0.092 mmol) in dry DMF : THF (1:5, 6 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days, then TLC showed no starting material was left. The reaction mixture was worked up as before to give a thick oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:1) to afford the titled compound **(176)** as a colourless thick oil (0.05 g, 36%) [Found (MALDI) $(M+Na)^+$: 3339.7, $C_{219}H_{382}NaO_{19}$, requires: 3339.8], $[\alpha]_D^{23}$ + 62 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.34 – 7.25 (25H, m), 5.18 (1H, br. s), 5.13 (1H, br. s), 4.91 (1H, br. s), 4.60 – 4.40 (9H, m), 4.34 (1 H, d, *J* 11.8 Hz), 4.32 – 4.24 (4H, m), 4.18 (1H, dt, *J* 3.5, 10.7 Hz), 4.11 (1H, dd, *J* 2.9, 4.1 Hz), 4.08 (1H, br. d, *J* 3.0 Hz), 4.04 (1H, m), 4.00 (1H, br. d, *J* 2.4 Hz), 3.96 (2H, m), 3.93 (1H, dd, *J* 4.4, 7.4 Hz), 3.88 (2H, m), 3.76 (1H, dd, *J* 2.0, 12.0 Hz), 3.70 – 3.55 (3H, m), 3.37 (3H, s), 2.67 (1H, br. s), 2.55 – 2.34 (8H, m), 1.69 – 1.10 (288H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8), 0.66 (4H, m), 0.61 – 0.51 (2H, dt, *J* 4.0, 7.6 Hz), -0.32 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.1, 174.96, 137.7, 137.5, 137.4, 137.2, 128.4, 128.41, 128.38, 128.36, 128.0, 127.9, 127.8, 127.73, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.85, 83.7, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.1, 71.97, 71.8, 68.9, 65.6, 63.0, 54.8, 51.8, 51.7, 50.9, 46.3, 41.1, 33.0, 32.1, 31.9, 30.2, 29.7, 29.66, 29.6, 29.5, 29.49, 29.4, 29.36, 29.3, 29.2, 28.7, 27.4, 27.3, 25.7, 23.7, 22.8, 22.7, 16.4, 15.7, 14.1, 10.9; ν_{max}: 3522, 3066, 2919, 2851, 1735, 1712, 1467, 1111, 756, 720 cm^{-1}.

Experiment 85: Methyl 5-*O*-{2-((1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxo-octatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranosyl-(1→3)-[2,3-di-*O*-benzyl-5-*O*-{2-(1*R*)-1-hydroxy-16-[(1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranosyl-(1→5)]-α-D-arabinofuranoside (177)



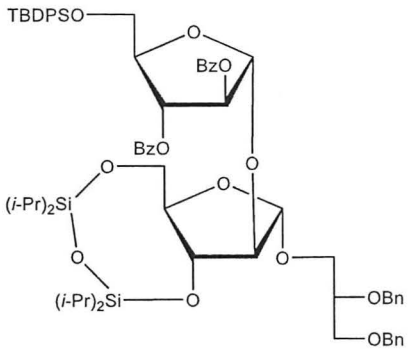
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 13.8 mg, 0.30 fold by weight) was added to a stirred solution of compound **(176)** (0.046 g, 0.013 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred 16 h then TLC showed no starting material was left. The mixture was filtered off and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to give the title compound **(177)** as a colourless oil (26 mg, 65%) [Found $(M+Na)^+$: 2889.6404, $C_{184}H_{352}NaO_{19}$, requires: 2889.6470], $[\alpha]_D^{23}$ + 33 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$+ few drops CD_3OD): 5.00 (1H, d, *J* 1.8 Hz), 4.96 (1H, d, *J* 0.6 Hz), 4.79 (1H, br. s), 4.43 (1H, dd, *J* 4.6, 12.2 Hz), 4.35 (1H, dd, *J* 4.9, 12.2 Hz), 4.26 (1H, dd, *J* 5.0, 11.8 Hz), 4.16 (1H, dd, *J* 4.2, 11.4 Hz), 4.13 – 4.07 (2H, m), 4.04 – 3.97 (5H, m), 3.94 – 3.84 (3H, m), 3.67 – 3.56 (4H, m), 3.34 (3H, s), 3.11 (1H, d, *J* 7.4 Hz), 3.07 (1H, d, *J* 7.3 Hz), 2.47 (3H, m), 2.41 – 2.3 (7H, including a triplet resonated at 2.38 with *J* 7.5 Hz), 1.63 – 1.10 (292H, m), 1.00 (6H, d, *J* 6.9 Hz), 0.84 (12H, t, *J* 6.7 Hz), 0.65 – 0.56 (4H, m), 0.51 (2H, dt, *J* 4.0, 7.6 Hz), -0.38 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 216.3, 175.1, 175.0, 109.1, 107.9, 107.3, 83.0, 82.3, 81.8, 81.7, 81.0, 80.9, 79.7, 77.9, 72.4, 72.3, 65.97, 63.1, 60.4, 54.7, 52.7, 46.2, 41.0, 34.7, 32.9, 31.7, 30.0, 29.5, 29.3, 29.2, 29.1, 28.5, 27.3, 27.1, 25.5, 23.5, 22.5, 16.1, 15.6, 13.8, 10.7. ν_{max}: 3393, 2919, 2850, 1713, 1467, 1100, 720 cm^{-1}.

Experiment 86: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (178)



1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (0.78 g, 0.79 mL, 2.46 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-α-D-arabinofuranoside **(117)** (1.0 g, 2.4 mmol) in dry pyridine (2 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and stirred for 3 h, then TLC showed no starting material was left. Methanol (5 mL) was added and the reaction mixture was diluted with ethyl acetate (50 mL) and water (20 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 (20 mL), brine (20 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure to give the residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (4:1) affording 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(178)** as a thick oil (1.3 g, 81%) [Found (MALDI) $(M+Na)^+$: 669.1, $C_{34}H_{54}NaO_8Si_2$, requires: 669.3], $[\alpha]_D^{20}$ - 36 (*c* 1.0, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 4.87 (1H, d, *J* 2.6 Hz), 4.72 (1H, d, *J* 12.0 Hz), 4.69 (1H, d, *J* 12.3 Hz), 4.56 (1H, d, *J* 11.5 Hz), 4.53 (1H, d, *J* 11.5 Hz), 4.20 – 4.13 (2H, m), 3.97 – 3.78 (5H, m), 3.64 – 3.54 (3H, m), 2.04 (1H, d, *J* 4.5 Hz), 1.13 – 1.01 (28H, m); δ_C (101 MHz, $CDCl_3$): 138.6, 138.2, 128.4, 128.3, 127.7, 127.6, 127.58, 127.5, 107.4, 82.6, 80.59, 77.2, 76.7, 73.4, 72.3, 70.2, 68.3, 61.2, 17.4, 17.3, 17.1, 17.0, 17.05, 17.03, 17.0, 13.5, 13.1, 13.0, 12.7, 12.5; ν_{max}: 3436, 3032, 2944, 2867, 1464, 1037, 696, 735 cm^{-1}. The experiment was repeated on a large scale.

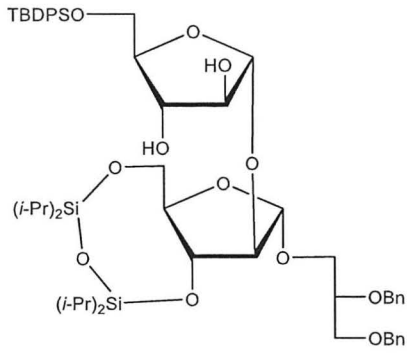
Experiment 87: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2,3-di-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (179)



Molecular sieves 4 °A (0.30 g) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(178)** (0.09 g, 0.14 mmol) and *p*-cresyl-2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-1-thio-α-D-arabinofuranoside **(147)** (0.10 g, 0.14 mmol) in dry CH_2Cl_2 (3 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then cooled to - 60 °C and *N*-iodosuccinimide (0.03 g, 0.13 mmol) was added followed by the addition of silvertriflate (0.01 g, 0.03 mmol). The mixture was stirred at the same temperature until the colour of the mixture turned into red/dark brown and TLC showed no starting material was left. The reaction mixture was quenched by the addition of triethylamine (0.5 mL) until the reaction mixture turned into yellow colour. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and filtered through celite, the solvent was evaporated under reduced pressure to give the residue which was purified by column chromate-graphy on silica eluting with petrol/ethyl acetate (10:1) affording 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2,3-di-*O*-benzoyl-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabino-furanoside **(179)** as a thick colourless oil (0.13 g, 76%) [Found (MALDI) $(M+Na)^+$: 1247.1, $C_{69}H_{88}NaO_{14}Si_3$, requires: 1247.5], $[\alpha]_D^{20}$ + 45 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 8.08 – 8.02 (2H, m), 7.99 – 7.94 (2H, m), 7.74 – 7.67 (4H, m), 7.62 – 7.54 (2H, m), 7.49 – 7.28 (17H, m), 7.26 – 7.15 (3H, m), 5.62 (1H, br. d, *J* 4.5 Hz), 5.49 (1H, d, *J* 1.0 Hz), 5.41 (1H, s), 5.05 (1H, d, *J* 2.1 Hz), 4.66 (1H, d, *J* 13.0 Hz), 4.62 (1H, d, *J* 12.8 Hz), 4.50 (2H, br. s), 4.40 (1H, dd, *J* 4.4, 9.2 Hz), 4.29 (1H, dd, *J* 6.0, 8.3 Hz), 4.22 (1H, dd, *J* 2.2, 6.0 Hz), 4.02 – 3.89 (4H, m), 3.87 – 3.81 (2H, m), 3.78 (1H, dt, *J* 5.1, 10.0 Hz), 3.62 – 3.52 (3H, m), 1.11 – 0.99 (37H, m); δ_C (101 MHz, $CDCl_3$): 165.5,

165.1, 138.7, 138.3, 135.6, 133.3, 133.2, 133.1, 129.9, 129.8, 129.7, 129.4, 129.1, 128.4, 128.34, 128.3, 128.2, 127.9, 127.7, 127.5, 127.4, 127.3, 106.5, 105.8, 88.9, 83.5, 82.1, 79.7, 77.3, 75.8, 73.3, 72.0, 70.5, 68.1, 63.4, 61.0, 26.7, 17.5, 17.3, 17.1, 17.0, 16.9, 13.5, 13.1, 12.7, 12.4; ν_{max}: 3090, 3033, 3068, 2945, 2866, 1733, 1464, 1104, 739, 696 cm^{-1}. The experiment was repeated on a large scale.

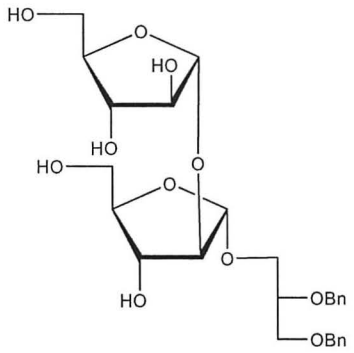
Experiment 88: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-tertbutyldiphenylsilyl-α-D-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (180)



Sodium methoxide (0.1 M, in methanol, 2 mL) was added to a stirred solution of compound **(179)** (0.45 g, 0.44 mmol) in dry MeOH : CH_2Cl_2 (1:1, 8 mL) at room temperature and the reaction mixture was stirred for 2 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 the residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (1:1) to afford 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(180)** as a thick colourless oil (0.37 g, 99%) [Found (MALDI) $(M+Na)^+$: 1039.7, $C_{55}H_{80}NaO_{12}Si_3$, requires: 1039.4], $[\alpha]_D^{20}$ + 30 (*c* 0.1, H_2O) which showed δ_H (400 MHz, $CDCl_3$): 7.49 – 7.15 (20H, m), 5.28 (1H, s), 4.89 (1H, d, *J* 1.5 Hz), 4.65 (2H, br. s), 4.47 (2H, br. s), 4.29 (1H, br. d, *J* 11.6 Hz), 4.23 – 4.17 (2H, m), 4.05 (2H, br. d, *J* 11.6 Hz), 3.95 – 3.90 (3H, m), 3.88 – 3.80 (2H, m), 3.76 (1H, dt, *J* 5.1, 9.9 Hz), 3.69 (1H, dd, *J* 2.1, 11.4 Hz), 3.64 – 3.52 (4H, m), 2.71 (1H, d, *J* 11.9 Hz), 1.13 – 0.93 (37H, m); δ_C (101 MHz, $CDCl_3$): 135.6, 135.5, 132.9, 130.2, 130.1, 129.5, 128.34, 128.3, 128.2, 128.0, 127.9, 127.54, 127.5, 127.4, 109.3, 105.7, 88.8, 87.9, 79.6, 78.4, 77.8, 76.6, 75.9,

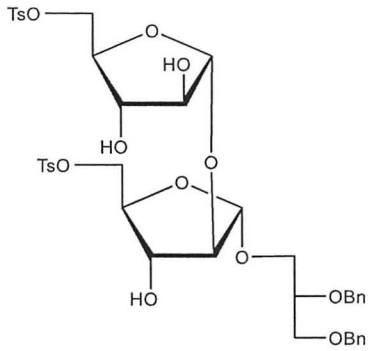
73.3, 71.9, 70.1, 68.0, 63.9, 60.9, 21.0, 17.4, 17.3, 17.2, 17.0, 13.5, 13.1, 12.8, 12.5; ν_{max}: 3433, 3069, 3032, 2944, 2867, 1724, 1464, 1074, 738, 699 cm^{-1}.

Experiment 89: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-α-D-arabinfuranosyl-(1→2) - α-D-arabinofuranoside (181)



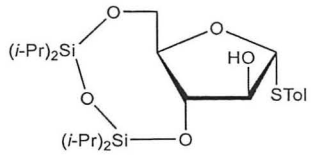
Tetrabutylammonium fluoride (1 mL, in 1 M THF, 1 mmol) was added dropwise to a stirred solution of compound **(180)** (0.35 g, 0.34 mmol) in dry THF (10 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The solvent was evaporated under reduced pressure to give an oily residue which was purified by column chromatography on silica eluting with dichloromethane/methanol (5:2) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-α-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside **(181)** as a colourless thick oil (0.14 g, 77%) [Found (MALDI) $(M+Na)^+$: 559.5, $C_{27}H_{36}NaO_{11}$, requires: 559.2], $[\alpha]_D^{20}$ + 57 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, CD_3OD): 7.41 – 7.23 (10H, m), 5.08 – 5.05 (2H, m), 4.72 (1H, d, *J* 11.9 Hz), 4.66 (1H, d, *J* 11.8 Hz), 4.55 (1H, d, *J* 11.9 Hz), 4.52 (1H, d, *J* 12.3 Hz), 4.05 (1H, dd, *J* 1.4, 3.8 Hz), 3.99 (1H, dd, *J* 1.7, 3.8 Hz), 3.97 – 3.95 (1H, m), 3.94 – 3.89 (2H, m), 3.87 – 3.81 (3H, m), 3.76 (1H, d, *J* 3.0 Hz), 3.73 (1H, dd, *J* 3.1, 6.2 Hz), 3.68 (1H, dd, *J* 3.6, 6.9 Hz), 3.66 – 3.55 (6H, m), 3.35 (3H, br. s); δ_C (101 MHz, CD_3OD): 129.4, 129.3, 129.1, 128.9, 128.7, 128.6, 109.5, 108.3, 90.1, 85.6, 84.3, 83.8, 78.6, 78.5, 77.3, 74.4, 73.3, 71.3, 68.6, 62.9, 62.6; ν_{max}: 3370, 3029, 2933, 2876, 1453, 1100, 727, 698 cm^{-1}.

Experiment 90: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1→2)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (182)



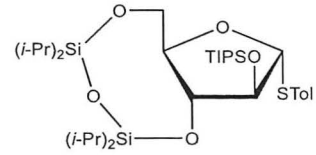
4-Toluenesulfonyl chloride (0.086 g, 0.041 mmol) and DMAP (0.273g, 0.022 mmol) were added to a stirred solution of compound **(181)** (0.12 g, 0.22 mmol) in dry pyridine (3 mL) under nitrogen atmosphere at room temperature. The mixture was stirred for 16 h then TLC showed no starting material was left. The reaction mixture was quenched with H_2O (10 mL) and diluted with CH_2Cl_2 (20 mL), the organic layer was separated by decanting and diluted with CH_2Cl_2 (50 mL). The reaction mixture was washed with 1 N aqueous HCl (4×15 mL), saturated aqueous solution of $NaHCO_3$ (4×15 mL), dried over $MgSO_4$, 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 (1:2) to afford 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluene-sulfonyl-α-D-arabin-ofuranosyl-(1→2)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofura-noside **(182)** as a thick oil (0.15 g, 79%) [Found (MALDI) $(M+Na)^+$: 867.1, $C_{41}H_{48}NaO_{15}S_2$, requires: 867.2], $[\alpha]_D^{23}$ + 45 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.80 – 7.73 (4H, m), 7.38 – 7.28 (14H, m), 5.06 (1H, s), 5.02 (1H, s), 4.62 (2H, m), 4.52 (2H, br. s), 4.26 – 4.01 (8H, m), 3.98 (1H, br. s), 3.95 – 3.90 (1H, m), 3.83 (1H, dd, *J* 4.3, 10.3 Hz), 3.76 – 3.68 (1H, m), 3.63 – 3.48 (4H, m), 2.96 (1H, s), 2.88 (1H, s), 2.42 (3H, s), 2.43 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 145.2, 145.1, 138.1, 137.9, 132.4, 132.3, 129.9, 128.4, 127.95, 127.7, 127.67, 107.2, 106.1, 85.2, 82.7, 81.7, 81.4, 77.0, 76.3, 75.1, 73.4, 71.9, 69.7, 68.9, 68.87, 66.4, 21.6; ν_{max}: 3446, 3089, 3066, 3033, 2928, 2867, 1598, 1455, 1189,850, 732, 699 cm^{-1}.

Experiment 91: *p*-Tolyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-α-D-arabinofuranoside (183)



1, 3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[304] (2.4 g, 2.5 mL, 7.5 mmol) was added to a stirred solution of *p*-cresyl-1-thio-α-D-arabinofuranoside **(145)** (2.0 g, 7.8 mmol) in dry pyridine (4 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 6 h, then TLC showed no starting material was left. Methanol (5 mL) was added and the reaction mixture was diluted with ethyl acetate (50 mL), washed with water (15 mL), brine (15 mL), dried over ($MgSO_4$) and the solvent was evaporated. The residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (4:1) affording *p*-tolyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-α-D-arabinofuranoside **(183)** as a thick oil (3.0 g, 77%) $[\alpha]_D^{21}$ +190 (*c* 1.0, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 521.1, $C_{24}H_{42}NaO_5SSi_2$, requires: 521.2] which showed δ_H (400 MHz, CD_3OD): 7.41 (2H, d, *J* 8.0 Hz), 7.13 (2H, d, *J* 8.0 Hz), 5.19 (1H, d, *J* 4.7 Hz), 3.99 – 3.89 (3H, m), 3.77 (1H, dd, *J* 2.5, 12.1 Hz), 3.64 (1H, dd, *J* 4.4, 12.1 Hz), 2.31 (3H, s); δ_C (101 MHz, CD_3OD): 138.6, 133.4, 130.6, 130.5, 93.3, 84.2, 83.3, 77.6, 62.4, 21.1; ν_{max}: 3446, 3021, 2946, 2868, 1467, 1046, 862 cm^{-1}. This experiment was repeated on a large scale.

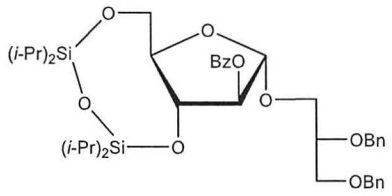
Experiment 92: *p*-Tolyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-2-*O*-triiso-propylsilyl-α-D-arabinofuranoside (184) [234]



Triisoprpylsilyl trifluoromethansulfonate (0.98 g, 0.86 mL, 3.1 mmol) was added to a stirred solution of *p*-tolyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-α-D-arabino-furanoside **(183)** (0.5 g, 1.0 mmol) and 2,6-lutidine (0.67 g, 0.73 mL, 6.20 mmol) in dry DMF (4 mL) at room temperature. The reaction mixture was stirred for four h at 90 °C then TLC showed no starting material was left. The reaction mixture was cooled to room temperature and quenched by saturated solution of $NaHCO_3$, extracted with ethyl acetate

(50 mL).The combined organic phase were washed with brine (20 mL), dried over MgSO4 and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica eluting with petroleum/ethyl acetate (4:1) affording *p*-tolyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-2-*O*-triisopropylsilyl-α-D-arabinofuranoside **(184)** as a colourless oil (0.6 g, 90%) $[\alpha]_D^{20}$ + 80 (*c* 1.0, $CHCl_3$), [*lit.*[234] $[\alpha]_D^{27}$ + 84.7 (*c* 1.06, $CHCl_3$)], [Found (MALDI) $(M+Na)^+$: 677.2, $C_{33}H_{62}NaO_5SSi_3$, requires: 677.3] which showed δ_H (400 MHz, $CDCl_3$): 7.38 (2H, d, *J* 8.0 Hz), 7.10 (2H, d, *J* 8.0 Hz), 5.29 (1H, d, *J* 2.6 Hz), 4.36 (1H, dd, *J* 2.8, 3.6 Hz), 4.27 (1H, dd, *J* 3.7, 6.5 Hz), 4.14 (1H, td, *J* 3.2, 6.4 Hz), 4.07 (1H, dd, *J* 3.3, 12.1 Hz), 3.92 (1H, dd, *J* 6.3, 12.1 Hz), 2.33 (3H, s), 1.20 – 0.94 (49H, m); δ_C (101 MHz, $CDCl_3$): 137.1, 137.09, 132.3, 131.7, 129.6, 129.59, 93.5, 84.3, 82.4, 80.2, 62.8, 21.07, 18.1, 18.07, 18.0, 17.6, 17.5, 17.35, 17.3, 17.28, 17.25, 17.2, 17.17, 17.1, 17.0, 13.7, 13.4, 13.3, 13.0, 12.9, 12.8, 12.7, 12.4; ν_{max}: 3020, 2944, 2868, 1464, 1047, 884, 736, 688 cm^{-1}. The experiment was repeated on a large scale.

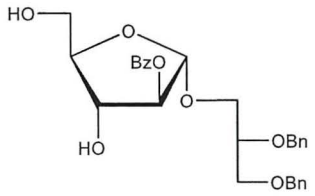
Experiment 93: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-benzoyl-5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (185)



Benzoyl chloride (0.16 mL, 1.42 mmol) was added dropwise to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(178)** (0.93 g, 1.43 mmol) in anhydrous pyridine (3 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 3 h. When TLC showed no starting material was left, methanol (2 mL) was added and the reaction mixture was diluted with ethyl acetate (15 mL) and washed in succession with 0.1 M aqueous HCl (10 mL), saturated solution of sodium bicarbonate (10 mL) and brine (10 mL), dried over ($MgSO_4$) and the solvent was evaporated. Traces of pyridine were removed by co-evaporation with toluene. The residue was purified by column chromategraphy on silica eluting with hexane/ethyl acetate (4:1) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-benzoyl-5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(185)** as a thick oil (0.95 g, 88%) $[\alpha]_D^{20}$ + 91 (*c* 1.0, $CHCl_3$), [Found (MALDI)

$(M+Na)^+$: 773.2, $C_{41}H_{58}NaO_9Si_2$, requires: 773.3] which showed δ_H (400 MHz, $CDCl_3$): 8.08 – 8.00 (4H, m), 7.61 – 7.54 (2H, m), 7.49 – 7.42 (4H, m), 7.39 – 7.28 (5H, m), 5.50 (1H, dd, *J* 1.7, 5.2 Hz), 5.05 (1H, d, *J* 1.6 Hz), 4.74 (1H, d, *J* 12.0 Hz), 4.70 (1H, d, *J* 11.9 Hz), 4.57 (1H, d, *J* 12.4 Hz), 4.55 (1H, d, *J* 12.3 Hz), 4.50 (1H, dd, *J* 5.2, 7.6 Hz), 4.07 – 4.00 (2H, m), 3.99 – 3.93 (1H, m), 3.94 – 3.89 (1H, m), 3.84 (1H, td, *J* 5.1, 9.9 Hz), 3.69 – 3.58 (3H, m), 1.19 – 0.89 (28H, m); δ_C (101 MHz, $CDCl_3$): 165.5, 138.7, 138.3, 133.2, 129.7, 129.6, 128.4, 128.34, 128.3, 128.2, 127.8, 127.6, 127.5, 127.4, 105.6, 84.2, 80.9, 77.1, 75.9, 73.4, 72.3, 70.3, 68.1, 61.6, 17.5, 17.3, 17.0, 16.93, 16.9, 16.89, 13.5, 13.2, 12.8, 12.5; ν_{max}: 3064, 3032, 2944, 2867, 1104, 711, 885 cm^{-1}.

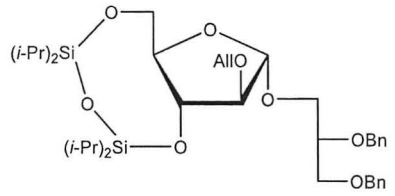
Experiment 94: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-benzoyl-α-D-arabino-furanoside (186)



Tetrabutylammonium fluoride (2.39 mL, in 1 M THF)) was added dropwise to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-benzoyl-5-*O*-(tetraiso-propylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(185)** (0.90 g, 1.19 mmol) in anhydrous THF (20 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was diluted with ethyl acetate (100 mL), washed with saturated solution of NH_4Cl (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated to give the residue which was purified by column chromatography on silica eluting with dichloromethane/methanol (5:1) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-benzoyl-α-D-arabinofuranoside **(186)** as a thick oil (0.5 g, 83%) [Found (MALDI) $(M+Na)^+$: 531.5, $C_{29}H_{32}NaO_8$, requires: 531.1], $[\alpha]_D^{20}$ + 75 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.64 – 7.54 (2H, m), 7.46 (3H, m), 7.39 – 7.24 (10H, m), 5.30 (1H, s), 5.10 (1H, d, *J* 0.9 Hz), 4.71 (1H, d, *J* 12.0 Hz), 4.67 (1H, d, *J* 12.0 Hz), 4.58 (1H, d, *J* 12.0 Hz), 4.55 (1H, d, *J* 12.0 Hz), 4.20 – 4.15 (2H, m), 3.96 (1H, dd, *J* 4.2, 10.4 Hz), 3.91 – 3.86 (1H, m), 3.85 – 3.79 (1H, m), 3.79 – 3.73 (1H, m), 3.69 – 3.60 (3H, m), 3.38 (1H, br. s), 1.86 (1H, br. s); δ_C (101 MHz, $CDCl_3$): 166.6, 133.6, 132.9, 129.8, 129.6, 128.6, 128.4, 128.36, 128.3, 127.8, 127.7, 105.4, 85.6, 84.5, 76.9, 76.4,

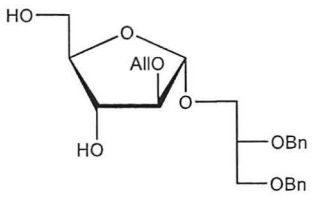
73.5, 72.3, 69.8, 67.2, 62.1; ν_{max}: 3446, 3093, 3063, 3034, 2943, 2866, 1717, 1464, 1070, 737, 698 cm^{-1}.

Experiment 95: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (187)



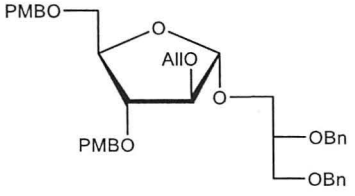
A solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(178)** (1.13 g, 1.74 mmol) in dry DMF (15 mL) was added dropwise to a stirred suspension solution of NaH (83.8 mg, 34.9 mmol, 60% w/w, dispersion in mineral oil) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 30 min then allyl bromide (0.25 g, 0.18 mL, 2.06 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h then TLC showed no starting material was left. The reaction 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 (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 (4:1) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-5-*O*-(tetraiso-propylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(187)** as a thick oil (0.9 g, 75%) [Found (MALDI) $(M+Na)^+$: 709.2, $C_{37}H_{58}NaO_8Si_2$, requires:709.3], $[\alpha]_D^{22}$ + 69 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.43 – 7.22 (10H, m), 5.88 (1H, ddt, *J* 5.4, 10.7, 17.1 Hz), 5.27 (1H, ddd, *J* 1.5, 3.2, 17.2 Hz), 5.16 (1H, ddd, *J* 1.2, 2.8, 10.8 Hz), 4.92 (1H, d, *J* 2.5), 4.73 (1H, d, *J* 12.2 Hz), 4.7 (1H, d, *J* 12.2 Hz), 4.57 (1H, d, *J* 12.0 Hz), 4.54 (1H, d, *J* 12.0 Hz), 4.22 (1H, dd, *J* 6.2, 8.4 Hz), 4.15 – 4.01 (2H, m), 3.99 – 3.78 (6H, m), 3.66 – 3.54 (3H, m), 1.23 – 0.89 (28H, m); δ_C (101 MHz, $CDCl_3$): 138.7, 138.2, 134.3, 128.3, 128.2, 127.6, 127.5, 127.4, 116.8, 106.1, 89.4, 80.30, 77.2, 75.9, 73.4, 72.3, 71.4, 70.3, 68.2, 17.4, 17.3, 17.1, 17.0, 16.9, 13.4, 13.1, 12.8, 12.5; ν_{max}: 3065, 3030, 2944, 2867, 1464, 1249, 1103, 735, 885, 696 cm^{-1}. The experiment was repeated on a large scale.

Experiment 96: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-α-D-arabinofuranoside (188)



Tetrabutylammonium fluoride (3.78 mL, in 1 M THF, 3.78 mmol) was added dropwise to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(187)** (2.75 g, 40.0 mmol) in anhydrous THF (15 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give the 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-α-D-arabinofuranoside **(188)** as a thick oil (1.4 g, 82%) [Found (MALDI) $(M+Na)^+$: 467.2, $C_{25}H_{32}NaO_7$, requires: 467.2], $[\alpha]_D^{20}$ + 75 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.38 – 7.28 (10H, m), 5.88 (1H, ddt, *J* 5.4, 10.7, 17.1 Hz), 5.29 (1H, ddd, *J* 1.5, 3.2, 17.2 Hz), 5.22 (1H, ddd, *J* 1.1, 2.8, 10.8 Hz), 5.09 (1H, s), 4.68 (1H, d, *J* 12.0 Hz), 4.63 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.1 Hz), 4.53 (1H, d, *J* 12.3 Hz), 4.13 – 3.99 (4H, m), 3.94 (1H, dd, *J* 4.1, 10.4 Hz), 3.85 (1H, br. d, *J* 1.2 Hz), 3.81 – 3.68 (3H, m), 3.58 (3H, m), 2.39 (2H, br. s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 138.1, 137.95, 133.6, 128.4, 127.72, 127.7, 117.9, 105.5, 86.8, 86.6, 76.5, 75.3, 73.5, 72.1, 70.6, 69.7, 66.4, 62.5; ν_{max}: 3401, 3065, 3031, 2943, 2866, 1663, 1464, 1058, 696, 735 cm^{-1}. The experiment was repeated on a large scale

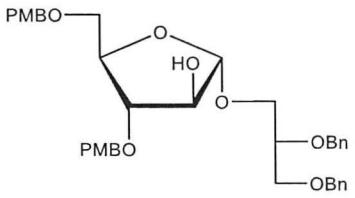
Experiment 97: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside (189)



A solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-α-D-arabinofuranoside **(188)** (0.44 g, 0.98 mmol) in dry DMF (15 mL) was added dropwise to a stirred suspension solution of NaH (87.1 mg, 3.63 mmol, 60% w/w, dispersion in mineral oil)

at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then, freshly prepared, *p*-methoxybenzyl bromide (0.39 g, 1.93 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h then TLC showed no starting material was left. The reaction 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 (100 mL). The organic layer was 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 (5:2) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-3,5-di-*p*-methoxy-benzyl-α-D-arabinofuranoside **(189)** as a thick oil (0.55 g, 81%) [Found (MALDI) $(M+Na)^+$: 707.1, $C_{41}H_{48}NaO_9$, requires:707.3], $[\alpha]_D^{22}$ + 117 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.17 (14H, m), 6.87 (2H, br. d, *J* 8.6 Hz), 6.85 (2H, br. d, *J* 8.6 Hz), 5.87 (1H, ddt, *J* 5.5, 10.8, 17.0 Hz), 5.26 (1H, ddd, *J* 1.5, 3.1, 17.2 Hz), 5.19 (1H, ddd, *J* 1.3, 2.8, 10.8 Hz), 5.04 (1H, s), 4.73 (1H, d, *J* 12.2 Hz), 4.70 (1H, d, *J* 12.2 Hz), 4.57 – 4.43 (6H, m), 4.17 (1H, ddd, *J* 3.8, 5.3, 6.7 Hz), 4.04 – 3.82 (6H, m), 3.81 (3H, s), 3.80 (3H, s), 3.64 – 3.51 (5H, m); δ_C (101 MHz, $CDCl_3$): 159.2, 159.1, 138.7, 138.2, 134.1, 130.2, 130.0, 129.39, 129.38, 128.32, 128.2, 127.6, 127.55, 127.5, 127.4, 117.3, 113.7, 113.69, 106.6, 83.2, 80.6, 77.3, 73.4, 72.9, 72.3, 71.8, 70.7, 70.4, 69.344, 67.9, 55.2; ν_{max}: 3069, 2926, 2867, 1514, 1100, 737, 698 cm^{-1}. The experiment was repeated on a large scale.

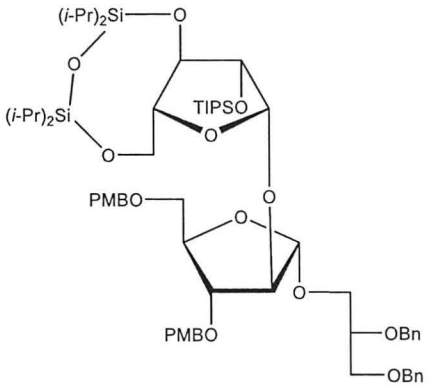
Experiment 98: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside (190)



Palladium (II) chloride (8.28 mg, 0.04 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-allyl-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(189)** (0.16 g, 0.23 mmol) in dry CH_2Cl_2 : MeOH (0.6:5, 3 mL) at room temperature. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The reaction 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 (1:1) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(190)** as a thick oil (143 mg, 95%) $[\alpha]_D^{20}$ + 95 (*c* 1.0, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 667.5, $C_{38}H_{44}NaO_9$, requires: 667.2] which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.25 (10H, m), 7.22 (4H, br. d, *J* 8.6 Hz), 6.89 (2H, br. d, *J* 8.6 Hz), 6.83 (2H, br. d, *J* 8.6 Hz), 5.04 (1H, s), 4.73 (1H, d, *J* 12.2 Hz), 4.69 (1H, d, *J* 12.2 Hz), 4.59 (1H, br. s), 4.56 (1H, br. s), 4.52 (1H, d, *J* 12.5 Hz), 4.49 (1H, d, *J* 12.4 Hz), 4.43 (2H, br. dd, *J* 3.0, 11.5 Hz), 4.24 (1H, dd, *J* 2.6, 4.8 Hz), 4.13 (1H, br. d, *J* 10.6 Hz), 3.90 – 3.83 (3H, m), 3.82 (3H, s), 3.77 (3H, s), 3.66 – 3.53 (4H, m), 3.47 (1H, dd, *J* 2.3, 10.4 Hz), 3.37 (1H, br. d, *J* 10.7); δ_C (101 MHz, $CDCl_3$): 159.4, 159.2, 138.8, 138.4, 129.9, 129.5, 129.3, 129.1, 128.3, 128.2, 127.7, 127.5, 127.4, 127.3, 113.9, 113.7, 109.4, 85.0, 83.6, 77.6, 77.3, 73.3, 73.2, 72.3, 71.6, 70.5, 69.4, 67.6, 55.25, 55.2; ν_{max}: 3401, 3065, 3030, 2910, 2864, 1612, 1513, 1099, 698, 737 cm^{-1}. The experiment was repeated on a large scale.

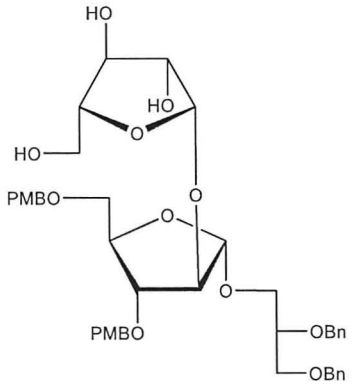
Experiment 99: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-2-*O*-(triisoprpylsiliyl)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-β-D-arabinofuranosyl-(1→2)-3,5-di-*p*-methoxy benzyl-α-D-arabinofuranoside (191)



Molecular sieves 4 Å (10 g) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(190)** (1.2 g, 1.86 mmol) and *p*-tolyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-2-*O*-triisopropylsilyl-α-D-arabinofuranoside **(184)** (3.0 g, 4.5 mmol) in dry CH_2Cl_2 (30 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 mint then cooled to - 78 °C and *N*-iodosuccinimide (1.56 g, 6.93 mmol) was added followed by the addition of silver trifluoromethanesulfonate (0.19 g, 0.74 mmol). The mixture were stirred at the same temperature until the colour of the mixture turned into red/dark brown and TLC showed no starting material was left. The reaction mixture was

quenched by the addition of triethylamine (2 mL) until the reaction mixture turned into yellow colour. The reaction 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 compound **(191)** as a thick oil (2.12 g, 97%) [Found (MALDI) $(M+Na)^+$: 1197.7, $C_{64}H_{98}NaO_{14}Si_3$, requires: 1197.6], $[\alpha]_D^{20}$ + 43 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.20 (14H, m), 6.91 – 6.79 (4H, m), 5.05 (1H, s), 4.88 (1H, br. d, *J* 4.3 Hz), 4.76 – 4.66 (2H, m), 4.61 (1H, d, *J* 11.4 Hz), 4.57 – 4.49 (2H, m), 4.49 – 4.40 (4H, m), 4.29 (1H, br. d, *J* 2.1 Hz), 4.26 – 4.18 (2H, m), 4.01 – 3.85 (6H, m), 3.81 (3H, s), 3.78 (3H, s), 3.64 – 3.48 (5H, m), 1.15 – 0.99 (49H, m); δ_C (101 MHz, $CDCl_3$): 159.2, 159.1, 138.8, 138.3, 130.2, 130.1, 129.4, 129.3, 128.3, 128.2, 127.7, 127.5, 127.4, 127.3, 113.7, 113.6, 106.2, 100.3, 85.7, 83.6, 82.0, 81.1, 79.5, 79.1, 77.1, 73.3, 72.9, 72.3, 71.8, 70.6, 69.7, 67.9, 66.6, 55.2, 55.1, 17.98, 17.9, 17.5, 17.44, 17.4, 17.3, 17.2, 17.1, 17.09, 17.02, 16.9, 13.4, 13.2, 12.9, 12.7, 12.4; ν_{max}: 3064, 3030, 2944, 2867, 1514, 736, 696 cm^{-1}. The experiment was repeated to prepare another 0.2 g from the product.

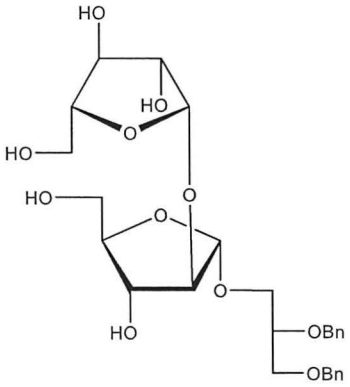
Experiment 100: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside (192)



Tetrabutylammonium fluoride (5.7 mL, in 1.0 M THF, 5.7 mmol) was added dropwise to a stirred solution of compound **(191)** (2.3 g, 1.9 mmol) in dry THF (50 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h. When TLC showed no starting material was left, the reaction 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 saturated solution of NH_4Cl (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was concentrated to give the residue which

was purified by column chromatography on silica eluting with dichloromethane /methanol (5:1) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(192)** as a thick oil (1.4 g, 94%) [Found (MALDI) $(M+Na)^+$: 799.7, $C_{43}H_{52}NaO_{13}$, requires: 799.3], $[\alpha]_D^{20}$ + 57 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$+ few drops CD_3OD): 7.36 – 7.28 (9H, m), 7.25 – 7.18 (5H, m), 6.88 (2H, d, *J* 8.6 Hz), 6.84 (2H, d, *J* 8.6 Hz), 5.02 (1H, d, *J* 0.9 Hz), 5.01 (1H, d, *J* 4.8 Hz), 4.70 (1H, d, *J* 11.9 Hz), 4.66 (1H, d, *J* 11.9 Hz), 4.57 (1H, d, *J* 11.4 Hz), 4.54 – 4.48 (3H, m), 4.43 (1H, br. d, *J* 2.7 Hz), 4.40 (1H, br. d, *J* 2.4 Hz), 4.31 (1H, dd, *J* 1.4, 3.3 Hz), 4.15 – 4.10 (1H, m), 4.08 – 4.02 (2H, m), 3.92 – 3.83 (4H, m), 3.81 (3H, s), 3.80 (3H, s), 3.70 (1H, dd, *J* 3.0, 12.2 Hz), 3.63 – 3.53 (5H, m), 3.47 (1H, dd, *J* 4.0, 10.9 Hz), 2.22 (3H, s); δ_C (101 MHz, CD_3OD): 130.74, 130.7, 129.4, 129.3, 129.0, 128.8, 128.6, 128.5, 114.8, 114.7, 107.9, 102.5, 87.2, 84.7, 84.4, 82.4, 78.6, 76.5, 74.3, 73.9, 73.2, 73.0, 71.0, 70.5, 68.4, 65.3, 55.7; ν_{max}: 3414, 3064, 3032, 2943, 2867, 1612, 1464, 1058, 736, 697 cm^{-1}.

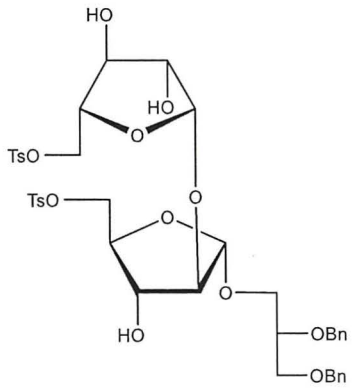
Experiment 101: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside (193)



2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (0.071g, mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(192)** (0.21 g, 0.27 mmol) in dichloro-methane : water (20:1, 10 mL) at 0 °C for 2 h. The reaction mixture was allowed to reach room temperature and stirred at ambient temperature until TLC showed no starting was left. The reaction was diluted with ethyl acetate (50 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with aq. $NaHCO_3$ (10 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residue was

purified by column chromatography on silica eluting with dichloromethane/methanol (5:2) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside **(193)** as a thick oil (0.09 g, 62%) [Found (MALDI) $(M+Na)^+$: 559.2, $C_{27}H_{36}NaO_{11}$, requires:559.2], $[\alpha]_D^{22}$ + 43 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.34 – 7.21 (10H, m), 4.94 (1H, s), 4.93 (1H, d, *J* 4.7 Hz), 4.64 (1H, d, *J* 12.1 Hz), 4.61 (1H, d, *J* 12.2 Hz), 4.51 (1H, d, *J* 12.3 Hz), 4.48 (1H, d, *J* 12.4 Hz), 4.13 – 4.05 (3H, m), 3.94 (1H, dd, *J* 4.7, 7.9 Hz), 3.91 – 3.85 (1H, m), 3.83 (1H, d, *J* 3.9 Hz), 3.81 – 3.71 (4H, m), 3.66 (1H, br. d, *J* 2.4 Hz), 3.64 – 3.58 (1H, m), 3.57 – 3.52 (2H, m), 3.52 – 3.47 (1H, m), 2.98 – 2.69 (6H, br. s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 138.0, 137.8, 128.3, 128.2, 127.8, 127.65, 127.6, 127.5, 106.1, 100.5, 87.2, 82.4, 82.2, 77.35, 77.3, 74.4, 73.3, 72.3, 69.7, 67.7, 61.5, 60.5; ν_{max}: 3400, 3067, 3030, 2929, 2872, 1454, 1103, 738, 698 cm^{-1}. The experiment was repeated on a large scale (2 g) but we lost most of the compound, and small amount of product was recovered (0.28 g).

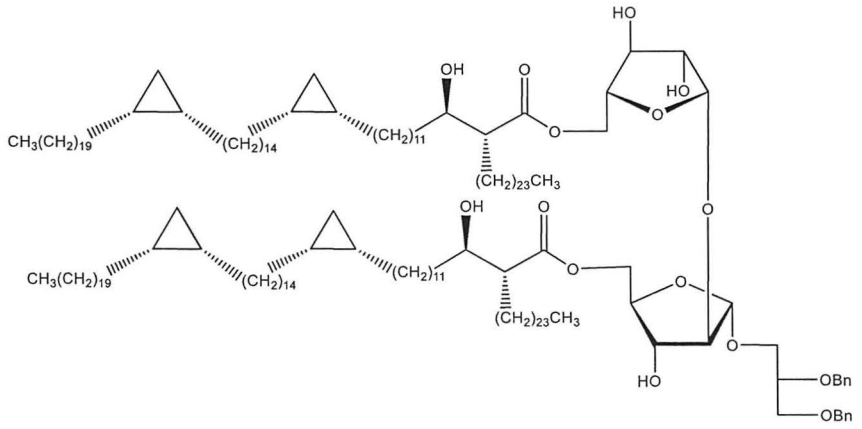
Experiment 102: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl-(1→2)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (194)



4-Toluenesulfonyl chloride (0.26, 1.36 mmol) and DMAP (0.08 g, 0.65 mmol) were added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside **(193)** (0.37 g, 0.69 mmol) in dry pyridine (10 mL) under nitrogen atmosphere at room temperature. The mixture was stirred for 16 h then TLC showed no starting material was left. The reaction mixture was quenched by the addition of H_2O (10 mL), the organic layer was separated by decanting and diluted with CH_2Cl_2 (10 mL). The reaction mixture was washed with 1 N aqueous HCl (4×15 mL), saturated aqueous solution of $NaHCO_3$ (4×15 mL), dried over $MgSO_4$, 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 (1:1) to afford the title compound **(194)** as a thick oil (0.2 g, 34%) [Found (MALDI) $(M+Na)^+$: 867.4, $C_{41}H_{48}NaO_{15}S_2$, requires: 867.2], $[\alpha]_D^{23}$ +32 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.82 (2H,. d, *J* 8.5 Hz), 7.80 (2H,. d, *J* 8.5 Hz), 7.39 – 7.29 (14H, m), 4.97 (1H, d, *J* 0.8 Hz), 4.93 (1H, d, *J* 4.6 Hz), 4.67 (1H, d, *J* 11.9 Hz), 4.62 (1H, d, *J* 11.9 Hz), 4.54 (1H, d, *J* 11.2 Hz), 4.51 (1H, d, *J* 11.2 Hz), 4.19 – 4.08 (8H, m), 3.99 – 3.93 (2H, m), 3.84 (1H, dd, *J* 4.1, 10.5 Hz), 3.80 – 3.74 (1H, m), 3.60 – 3.51 (3H, m), 2.45 (3H, s), 2.43 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 149.7, 145.3, 145.0, 138.4, 138.0, 136.1, 132.7, 132.3, 130.1, 129.9, 128.4, 128.3, 128.0, 127.7, 127.6, 123.8, 105.7, 100.2, 86.5, 80.8, 79.0, 77.6, 77.0, 75.9, 75.0, 73.4, 72.2, 69.8, 69.1, 69.0, 67.8, 60.4, 21.65, 21.6; ν_{max}: 3508, 3064, 3030, 2927, 2868, 1598, 1495, 1176, 831, 752, 698 cm^{-1}. By TLC minor product from tri-tosylate was obtained.

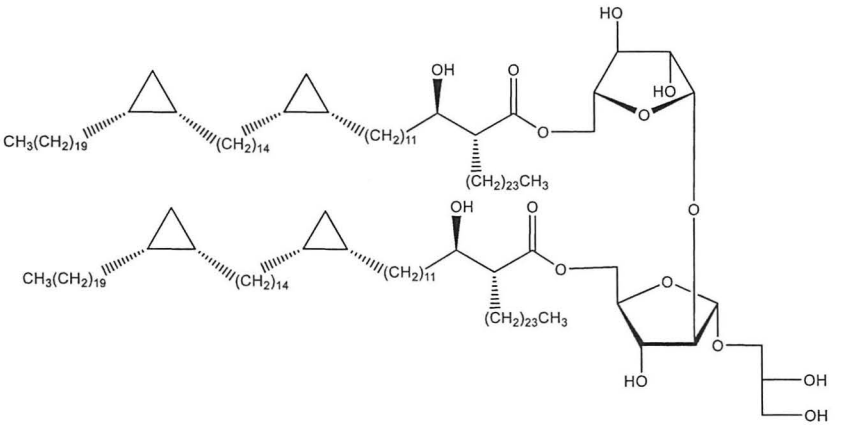
Experiment 103: 2',3'-Di-*O*-benzyl-D-glycerol-(1'→1)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*, 2*R*)-2-[14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl]cyclopropyl]dodecyl}hexacosanoate)-β-D-arabinofuranosyl-(1→2)-5-*O*-(2-{(*R*)-1-hydroxy-12-[(1*S*, 2*R*)-2-[14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl]cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (195)



Cesium hydrogencarbonate (0.091 g, 0.469 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-D-glycerol-(1'-1)-5-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl-(1→2)-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside **(194)** (0.040 g, 0.047 mmol) and (2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-[14-[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl]cyclopropyl]dodecyl}hexacosanoic acid **(97)**[157] (0.112 g, 0.098 mmol) in dry DMF : THF (1:5, 6 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred at 70 °C for 4 days then TLC showed no starting material was left. The suspension was

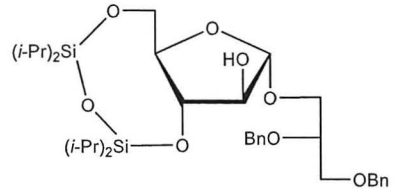
diluted with ethyl acetate (50 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×20 mL). The combined organic layers were washed with water (15 mL) and brine (15 mL). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (5:2) to give the title compound **(195)** as a colourless oil (20 mg, 15%) [Found (MALDI) $(M+Na)^+$: 2797.5, $C_{183}H_{336}NaO_{15}$, requires: 2797.5], $[\alpha]_D^{23}$ + 82.0 (*c* 0.01, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.39 – 7.28 (10H, m), 4.99 (1H, s), 4.95 (1H, d, *J* 4.2 Hz), 4.68 (1H, d, *J* 12.0 Hz), 4.64 (1H, d, *J* 11.9 Hz), 4.55 (1H, d, *J* 12.5 Hz), 4.52 (1H, d, *J* 11.7 Hz), 4.44 – 4.31 (5H, m), 4.22 (1H, dd, *J* 2.5, 10.8 Hz), 4.13 (2H, dd, *J* 5.9, 12.8 Hz), 4.06 (3H, br. d, *J* 7.6 Hz), 3.88 (1H, dd, *J* 3.9, 10.5 Hz), 3.78 (1H, dt, *J* 4.9, 9.6 Hz), 3.74 – 3.65 (2H, m), 3.61 – 3.52 (3H, m), 2.98 (2H, br. s), 2.47 – 2.39 (2H, m), 1.63 – 1.09 (274H, m), 0.89 (12H, t, *J* 6.7 Hz), 0.71 – 0.61 (8H, m), 0.57 (4H, dt, *J* 4.0, 7.6 Hz), -0.33 (4H, br. q, *J* 4.8 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 174.8, 174.7, 128.4, 128.3, 127.7, 127.6, 106.0, 101.6, 88.3, 80.8, 79.7, 77.9, 77.2, 77.1, 73.5, 72.9, 72.6, 72.3, 70.0, 68.4, 67.8, 65.2, 31.9, 30.2, 29.73, 29.7, 29.66, 29.5, 29.4, 28.8, 27.7, 27.4, 22.7, 22.2, 15.8, 14.1, 11.0, 10.9. ν_{max}: 3419, 3062, 3031, 2942, 2868, 1747, 1494, 1223, 811 cm^{-1}.

Experiment 104: D-Glycerol-(1'→1)-5-*O*-(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*-(2-{(*R*)-1-hydroxy-12-[(1*S*, 2*R*)-2-[14 -[(1*S*,2*R*)-2-icosylcyclopropyl]tetradecyl]cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranoside (196)



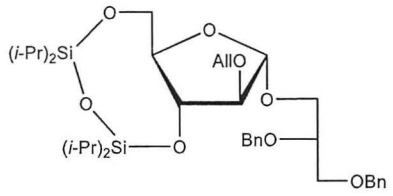
Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 12 mg, 6.0 fold by weight) was added to a stirred solution of compound **(195)** (0.020 g, 0.007 mmol) in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The mixture was filtered off and the solvent was evaporated under reduced pressure to give a thick oil residue which was purified by column chromatography on silica eluting with chloroform/methanol (5:1) to give the title compound **(196)**; ^1H NMR showed a complicated mixture, which indicate the decomposition of the product.

Experiment 105: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (197)



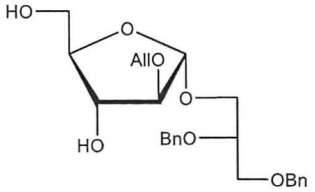
1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (1.42 g, 1.44 mL, 4.50 mmol) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-α-D-arabinofuranoside **(134)** (1.82 g, 4.49 mmol) in dry pyridine (4 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature. The reaction mixture was stirred for 3 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before affording 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(197)** as a thick oil (2.37 g, 81%) [Found (MALDI) $(M+Na)^+$: 669.3, $C_{34}H_{54}NaO_8Si_2$, requires 669.3], $[\alpha]_D^{20}$ - 40 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.27 (10H, m), 4.85 (1H, d, *J* 2.6), 4.71 (1H, d, *J* 12.6), 4.68 (1H, d, *J* 12.8), 4.57 (1H, d, *J* 12.4), 4.54 (1H, d, *J* 12.2), 4.19 – 4.12 (2H, m), 3.98 – 3.91 (2H, m), 3.91 – 3.82 (2H, m), 3.82 – 3.76 (1H, m), 3.67 – 3.58 (3H, m), 2.06 (1H, d, *J* 4.5), 1.14 – 1.01 (28H, m); δ_C (101 MHz, $CDCl_3$): 138.5, 138.3, 128.34, 128.3, 127.8, 127.6, 127.57, 107.5, 82.6, 80.7, 76.9, 76.86, 73.4, 72.2, 70.2, 67.9, 61.4, 17.4, 17.3, 17.1, 17.06, 17.0, 13.5, 13.1, 12.8, 12.5; ν_{max}: 3400, 2944, 2868, 1465, 1036, 885, 696 cm^{-1}. The experiment was repeated on (11.14 g) of the starting material.

Experiment 106: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside (198)



A solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(197)** (2.37 g, 3.66 mmol) in dry DMF (15 mL) was added dropwise to a stirred suspension solution of NaH (175 mg, 7.29 mmol, 60% w/w, dispersion in mineral oil) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 30 min, then allyl bromide (0.53 g, 0.38 mL, 4.38 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(198)** as a thick oil (2.4 g, 96%) [Found (MALDI) $(M+Na)^+$: 709.3, $C_{37}H_{58}NaO_8Si_2$, requires:709.3], $[\alpha]_D^{22}$ + 72 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.25 (10H, m), 5.87 (1H, ddt, *J* 5.4, 10.7, 17.3 Hz), 5.26 (1H, ddd, *J* 1.6, 3.2, 17.3 Hz), 5.16 (1H, ddd, *J* 1.2, 2.8, 10.4 Hz), 4.91 (1H, d, *J* 2.4 Hz), 4.70 (2H, br. s), 4.56 (2H, br. s), 4.20 (1H, dd, *J* 6.0, 8.3 Hz), 4.12 – 4.00 (2H, m), 3.96 – 3.92 (3H, m), 3.92 – 3.89 (2H, m), 3.79 (1H, dd, *J* 5.0, 9.9 Hz), 3.67 – 3.59 (3H, m), 1.17 – 0.96 (28H, m); δ_C (101 MHz, $CDCl_3$): 138.6, 138.3, 134.3, 128.3, 128.2, 127.7, 127.57, 127.54, 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, 17.07, 17.03, 17.0, 13.5, 13.1, 12.8, 12.5; ν_{max}: 3082, 3069, 2927, 2867, 1465, 1036, 885, 696 cm^{-1}. The experiment was repeated on a large scale.

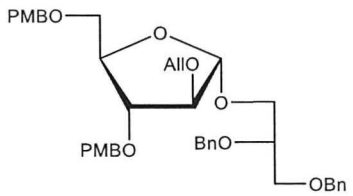
Experiment 107: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-α-D-arabinofuranoside (199)



Tetrabutylammonium fluoride (7.0 mL, in 1.0 M THF, 7.0 mmol) was added dropwise to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-5-*O*-(tetraisoprop-

ylsiloxane-1,3-diyl)-α-D-arabinofuranoside **(198)** (2.40 g, 3.49 mmol) in anhydrous THF (20 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-α-D-arabinofuranoside **(199)** as a thick oil (1.4 g, 90%) [Found (MALDI) $(M+Na)^+$: 467.2, $C_{25}H_{32}NaO_7$, requires: 467.2], $[\alpha]_D^{20}$ + 80 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.38 – 7.28 (10H, m), 5.88 (1H, ddt, *J* 5.6, 10.8, 17.2 Hz), 5.29 (1H, ddd, *J* 1.4, 3.0, 17.2 Hz), 5.22 (1H, ddd, *J* 1.3, 2.8, 10.4 Hz), 5.08 (1H, s), 4.68 (1H, d, *J* 11.9 Hz), 4.63 (1H, d, *J* 11.9 Hz), 4.56 (1H, d, *J* 12.5 Hz), 4.53 (1H, d, *J* 12.4 Hz), 4.12 – 4.00 (4H, m), 3.90 – 3.85 (2H, m), 3.78 (2H, m), 3.74 – 3.68 (1H, m), 3.65 – 3.57 (3H, m), 2.73 (2H, br. s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 138.1, 137.9, 133.6, 128.4, 128.3, 127.8, 127.73, 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}: 3438, 2941, 2866, 1652, 1454, 1056, 667 cm^{-1}. The experiment was repeated on (8.57 g) of the starting material.

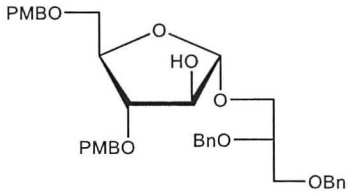
Experiment 108: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-3,5-di-*p*-methoxy benzyl-α-D-arabinofuranoside (200)



A solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-α-D-arabinofuranoside **(199)** (2.0 g, 4.49 mmol) in dry DMF (25 mL) was added dropwise to a stirred suspension solution of NaH (0.44 g, 60% w/w, dispersion in mineral oil) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then *p*-methoxybenzyl bromide (1.8 g, 8.9 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-allyl-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(200)** as a thick oil (2.5 g, 81%) [Found (MALDI) $(M+Na)^+$: 707.3, $C_{41}H_{48}NaO_9$, requires: 707.3], $[\alpha]_D^{23}$ + 120 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.18 (14H, m), 6.86 (2H, d, *J* 8.6 Hz), 6.84 (2H, d, *J* 8.6 Hz), 5.87 (1H, ddt, *J* 5.5, 10.7, 17.0 Hz), 5.26 (1H, ddd, *J* 1.5, 3.0, 17.0

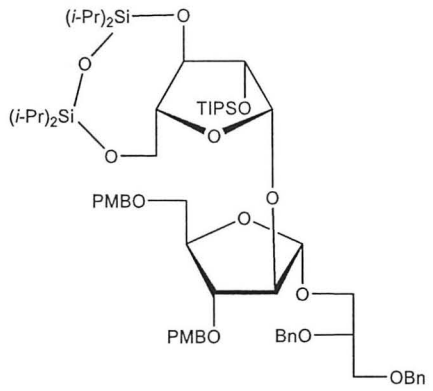
Hz), 5.18 (1H, ddd, *J* 1.4, 2.8, 10.8 Hz), 5.03 (1H, s), 4.71 (1H, d, *J* 11.9 Hz), 4.67 (1H, d, *J* 12.0 Hz), 4.57 – 4.41 (6H, m), 4.19 – 4.13 (1H, m), 4.03 – 3.97 (1H, m), 3.95 (2H, m), 3.93 – 3.82 (3H, m), 3.80 (3H, s), 3.79 (3H, s), 3.66 – 3.61 (3H, m), 3.59 (1H, dd, *J* 3.9, 10.8 Hz), 3.54 (1H, dd, *J* 5.3, 10.8 Hz); δ_C (101 MHz, $CDCl_3$): 138.7, 138.3, 134.1, 134.2, 130.2, 129.4, 128.3, 128.2, 127.7, 127.6, 127.5, 127.4, 117.3, 113.7, 113.6, 106.4, 88.2, 83.3, 80.8, 77.1, 73.4, 72.9, 72.2, 71.8, 70.8, 70.4, 69.3, 67.1, 55.2; ν_{max}: 2927, 2863, 1612, 1513, 1248, 820, 737 cm^{-1}. The experiment was repeated on (7.4 g) of the starting material

Experiment 109: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside (201)



Palladium (II) chloride (27.1 mg, 0.15 mmol) was added to a stirred solution 2',3'-di-*O*-benzyl-L-glycerol-(1'-1)-2-*O*-allyl-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(200)** (0.52 g, 0.75 mmol) in dry CH_2Cl_2 : MeOH (0.6:5, 12 mL) at room temperature. The reaction mixture was stirred for 16 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(201)** as a thick oil (0.46 g, 95%) $[\alpha]_D^{20}$ + 105 (*c* 1.0, $CHCl_3$), [Found (MALDI) $(M+Na)^+$: 667.2, $C_{38}H_{44}NaO_9$, requires: 667.2] which showed δ_H (400 MHz, $CDCl_3$): 7.36 – 7.25 (14H, m), 6.88 (2H, d, *J* 8.7 Hz), 6.81 (2H, d, *J* 8.6 Hz), 5.0 (1H, s), 4.72 (1H, d, *J* 12.1 Hz), 4.66 (1H, d, *J* 11.9 Hz), 4.57 (2H, br. d, *J* 11.4 Hz), 4.51 (1H, d, *J* 12.2 Hz), 4.47 (1H, d, *J* 12.2 Hz), 4.42 (1H, d, *J* 11.4 Hz), 4.40 (1H, d, *J* 11.5 Hz), 4.21 (1H, dd, *J* 2.1, 4.7 Hz), 4.14 (1H, d, *J* 10.7 Hz), 3.87 (1H, dd, *J* 5.3, 10.2 Hz), 3.83 (1H, br. d, *J* 3.7 Hz), 3.81 (3H, s), 3.80 (1H, br. d, *J* 5.0 Hz), 3.77 (3H, s), 3.65 – 3.56 (4H, m), 3.46 (1H, dd, *J* 2.2, 10.4 Hz); δ_C (101 MHz, $CDCl_3$): 129.5, 129.4, 128.3, 128.2, 127.8, 127.6, 127.5, 127.4, 113.9, 113.8, 109.4, 85.1, 83.7, 77.5, 76.9, 73.4, 73.3, 72.2, 71.6, 70.4, 69.4, 67.4, 55.3, 55.2; ν_{max}: 3438, 3066, 3034, 2916, 2868, 1612, 1514, 1033, 820, 737 cm^{-1}. The experiment was repeated on a large scale.

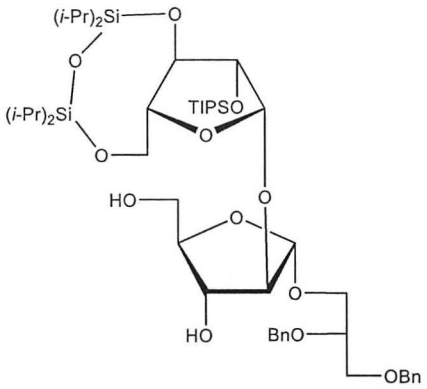
Experiment 110: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-(triisoprpylsiliyl)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-β-D-arabinofuranosyl-(1→2)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside (202)



Molecular sieves 4 Å (19.2 g) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(201)** (2.24 g, 3.47 mmol) and *p*-tolyl 3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-2-*O*-triisopropylsilyl-α-D-arabinofuranoside **(184)** (5.69 g, 8.68 mmol) in dry CH_2Cl_2 (80 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min then cooled to - 78 °C and *N*-iodosuccinimide (2.91 g, 12.9 mmol) was added followed by the addition of silver trifluoromethanesulfonate (0.35 g, 1.36 mmol). The mixture were stirred at the same temperature until the colour of the mixture turned into red/dark brown and TLC showed no starting material was left. The reaction mixture was worked up and purified as before affording 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-(triisopropylsiliyl)-3,5-*O*-(tetraisopropylsiloxane-1,3-di-yl)-β-D-arabinofuranosyl-(1→2)-3,5-di-*p*-methoxybenzyl-α-D-arabinofuranoside **(202)** as a thick oil (3.5 g, 86%) [Found (MALDI) $(M+Na)^+$: 1197.6, $C_{64}H_{98}NaO_{14}Si_3$, requires: 1197.6], $[\alpha]_D^{20}$ + 43 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.41 – 7.23 (14H, m), 6.90 (2H, d, *J* 8.6 Hz), 6.86 (2H, d, *J* 8.6 Hz), 5.09 (1H, s), 4.92 (1H, d, *J* 4.3 Hz), 4.76 (1H, d, *J* 12.0 Hz), 4.71 (1H, d, *J* 12.1 Hz), 4.65 (1H, d, *J* 11.3 Hz), 4.57 (2H, br. s), 4.51 (2H, br. s), 4.47 (2H, br. d, *J* 11.4 Hz), 4.32 (1H, d, *J* 2.9 Hz), 4.30 (1H, dd, *J* 4.4, 7.7 Hz), 4.27 – 4.21 (1H, m), 4.04 – 3.97 (2H, m), 3.97 – 3.88 (3H, m), 3.87 – 3.84 (1H, m), 3.83 (3H, s), 3.80 (3H, s), 3.70 – 3.52 (5H, m), 1.19 – 1.03 (49H, m); δ_C (101 MHz, $CDCl_3$): 159.1, 159.08, 138.7, 138.3, 130.2, 130.0, 129.3, 129.2, 128.2, 128.1, 127.7, 127.6, 127.4, 127.37, 127.3, 113.6, 113.59, 106.1, 100.4, 85.8, 83.8, 81.9, 81.3, 79.4, 79.1, 77.0, 73.3, 72.8, 72.1, 71.8, 70.5, 69.7, 67.3, 66.6, 55.1, 55.08, 17.94, 17.9, 17.5, 17.4, 17.37, 17.3,

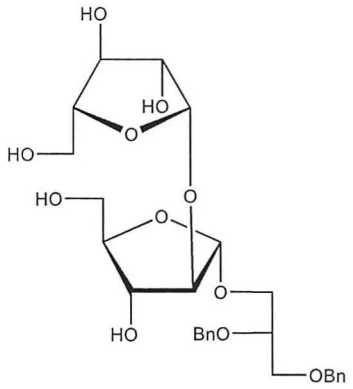
17.2, 17.0, 16.97, 16.9, 13.4, 13.2, 12.9, 12.6, 12.3; ν_{max}: 2944, 2867, 1514, 1038, 885, 737 cm^{-1}.

Experiment 111: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-2-*O*-(triisoprpylsiliyl)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-β-D-arabinofuranosyl-(1→2)-α-D-arabino-furanoside (203)



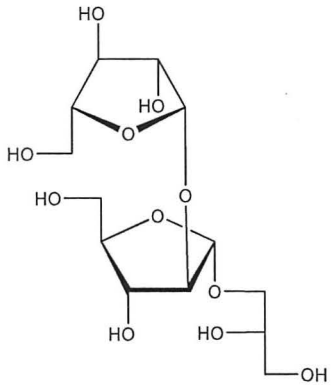
Cerium ammonium nitrate, (CAN)[305] (0.93 g, 1.69 mmol) was added to a stirred solution of compound **(202)** (0.5 g, 0.4 mmol) in acetonitrile (45 mL) and water (5 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred at ambient temperature for 16 h then TLC indicated the conversion had finished. The reaction mixture was diluted with chloroform (20 mL), washed with aq. $NaHCO_3$ (10 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 (4:1) to give 2',3'-di-*O*-benzyl-D-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabinofu-ranoside **(203)** as a thick oil (0.26 g, 67%) [Found (MALDI) $(M+Na)^+$: 957.5, $C_{48}H_{82}NaO_{12}Si_3$, requires:557.5], $[\alpha]_D^{22}$ + 32 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.27 (10H, m), 5.0 (1H, d, *J* 4.0 Hz), 4.97 (1H, d, *J* 2.5 Hz), 4.69 (2H, br. s), 4.55 (2H, br. s), 4.52 (1H, br. t, *J* 6.0 Hz), 4.26 (1H, dd, *J* 4.1, 6.3 Hz), 4.17 (1H, dd, *J* 5.8, 12.9 Hz), 4.13 – 4.09 (1H, m), 3.99 (1H, dt, *J* 3.4, 7.1 Hz), 3.89 (1H, dd, *J* 2.9, 6.3 Hz), 3.87 – 3.84 (1H, m), 3.84 – 3.76 (5H, m), 3.62 (1H, dd, *J* 3.9, 9.4 Hz), 3.59 (1H, dd, *J* 4.0, 9.2 Hz), 3.55 (1H, dd, *J* 4.0, 9.5 Hz), 2.59 (1H, br. s), 1.93 (1H, br. s), 1.13 – 0.95 (49H, m); δ_C (101 MHz, $CDCl_3$): 138.5, 138.2, 128.3, 128.2, 127.7, 127.54, 127.5, 105.9, 101.7, 88.3, 83.0, 81.6, 78.5, 76.9, 74.7, 74.5, 73.3, 72.2, 70.3, 68.2, 61.4, 61.3, 18.0, 17.43, 17.4, 17.33, 17.32, 17.3, 17.2, 17.1, 13.9, 13.6, 13.44, 13.4, 12.6; ν_{max}: 3472, 2944, 2867, 1464, 1057, 885 cm^{-1}. The experiment was repeated on (2.9 g).

Experiment 112: 2',3'-Di-*O*-benzyl-L-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside (204)



Tetrabutylammonium fluoride (2.2 mL, in 1.0 M THF, 2.2 mmol) was added dropwise to a stirred solution of compound **(203)** (0.7 g, 0.7 mmol) in dry THF (15 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h then TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give 2',3'-di-*O*-benzyl-L-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside **(204)** as a thick oil (0.3 g, 75%) [Found (MALDI) $(M+Na)^+$: 559.2, $C_{27}H_{36}NaO_{11}$, requires: 559.2], $[\alpha]_D^{20}$ + 26 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.32 – 7.20 (10H, m), 4.93 (1H, br. s), 4.91 (1H, s), 4.61 (2H, br. s), 4.49 (2H, br. s), 4.11 – 4.02 (3H, m), 3.94 (1H, dd, *J* 4.7, 8.0 Hz), 3.91 – 3.85 (1H, m), 3.82 – 3.70 (5H, m), 3.67 – 3.52 (5H, m), 3.11 (5H, br. s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 138.0, 137.8, 128.2, 128.1, 127.7, 127.5, 127.49, 105.9, 100.5, 87.4, 82.1, 82.06, 77.2, 76.7, 74.3, 73.4, 73.2, 72.0, 69.8, 67.2, 61.6, 60.4; ν_{max}: 3381, 3080, 3028, 2928, 2877, 1454, 1102, 737, 698 cm^{-1}. The experiment was repeated on (0.26 g).

Experiment 113: L-glycerol-(1'→1)-β-D-arabinofuranosyl-(1→2)-α-D-arabino-furanoside (205)



Palladium hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 3.7 mg, 0.2 fold by weight) was added to a stirred solution of 2',3'-di-*O*-benzyl-L-glycerol-(1→1')-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside **(204)** (0.01 g, 0.043 mmol) in H_2O : MeOH (1:4, 3 mL) at room temperature under hydrogen atmosphere. The reaction mixture was stirred overnight 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 (5:2) gave L-glycerol-(1→1')-β-D-arabinofuranosyl-(1→2)-α-D-arabinofuranoside **(205)** as a white solid (8.0 mg, 72%) $[\alpha]_D^{21}$ + 170 (*c* 0.1, H_2O), m.p. 200 - 202 °C [Found (MALDI) $(M+Na)^+$: 379.1, $C_{13}H_{24}NaO_{11}$, requires: 379.1] which showed δ_H (400 MHz, Pyr): 5.62 (1H, d, *J* 4.6 Hz), 5.56 (1H, d, *J* 2.8 Hz), 5.07 – 5.01 (1H, m), 4.96 (1H, dd, *J* 2.8, 6.0 Hz), 4.85 (1H, dd, *J* 6.1, 8.3 Hz), 4.69 (1H, dd, *J* 4.6, 7.9 Hz), 4.63 – 4.57 (1H, m), 4.52 – 4.46 (1H, m), 4.44 – 4.37 (1H, m), 4.30 – 4.20 (4H, m), 4.13 (3H, m), 4.01 (1H, dd, *J* 5.1, 10.1 Hz); δ_C (101 MHz, Pyr): 107.8, 102.5, 89.1, 85.1, 84.2, 79.8, 76.0, 75.7, 72.3, 71.1, 64.9, 64.0, 62.4; ν_{max}: 3347, 2953, 2923, 2853, 1456, 1376, 996 cm^{-1}.

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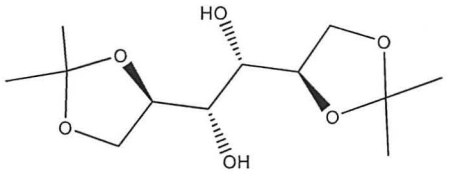
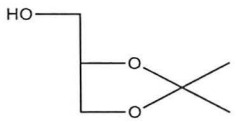
Appendices

Appendix 1: 1,2:5,6-Di-*O*-Isopropylidene-D-mannitol (109)[273]



D-mannitol (60.0 g, 0.32 mol) was added to a stirred solution of zinc chloride (120 g, 0.88 mol) in acetone (600 mL) and the mixture was stirred for16 h at room temperature. A solution of potassium carbonate (100 g, 0.72 mol) in water (100 mL) was added to the reaction mixture, then the precipitate was filtrated and washed with dichloromethane (3×100 mL). The combined organic layers were washed with water (100 mL) and the residue was dried over ($MgSO_4$). The solvent was evaporated under reduced pressure to give a residue which was purified by recrystallization using ethyl acetate and petrol (1:5), (600 mL) to give 1,2:5,6-di-*O*-Isopropylidene-D-mannitol **(109)** as a white solid (58 g, 67%) m.p. 119-120 °C [*lit.*[273] m.p. 118-119 °C] which showed δ_H (400 MHz, $CDCl_3$): 4.19 (2H, br. q, *J* 6.4 Hz), 4.12 (2H, dd, *J* 6.3, 8.5 Hz), 3.98 (2H, dd, *J* 5.5, 8.5 Hz), 3.75 (2H, br. t, *J* 6.0 Hz), 2.67 (2H, d, *J* 6.3 Hz), 1.42 (6H, s), 1.36 (6H, s); δ_C (101 MHz, $CDCl_3$): 109.3, 76.2, 71.2, 66.7, 26.7, 25.2. All data were identical to the authentic sample. The experiment was repeated on a large scale.

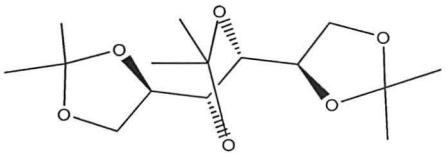
Appendix 2: 1,2-*O*-Isopropylidene-L-glycerol (110)[274]



Lead (IV) acetate (9.30 g, 0.27 mmol) was added to a stirred solution of 1,2:5,6-di-*O*-isopropylidene-D-mannitol **(109)** (5.0 g, 19 mmol) in dry THF (70 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 1 hour while maintaining the temperature below 10 °C. The reaction mixture was filtered over celite, the filtrate was cooled to 5 °C and a solution of sodium borohydride (1.43 g, 37.5 mmol, in 4% aqueous NaOH, 25 mL) was added dropwise with stirring while maintaining the temperature below 10 °C. The reaction mixture was stirred for 2 h, then solid ammonium chloride was added until the pH 8 was obtained. The solvent was evaporated under reduced pressure and the resulting aqueous solution was saturated with NaCl and extracted with

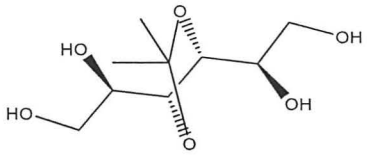
ethyl acetate (3×200 mL). The combined organic layers were washed with aqueous NaOH 5% saturated with NaCl (50 mL), dried over ($MgSO_4$) and evaporated to give an oil residue which was flash distilled at 40 °C to give 1,2-*O*-isopropylidene-L-glycerol **(110)** as a colourless oil (1.5 g, 60%) $[\alpha]_D^{22}$ + 12 (*c* 0.1, $CHCl_3$) [*lit.*[274] $[\alpha]_D^{23}$ + 10.62 (*c* 1.3, CH_3OH)] which showed δ_H (400 MHz, $CDCl_3$): 4.27 – 4.20 (1H, m), 4.04 (1H, dd, *J* 6.6, 8.2 Hz), 3.79 (1H, dd, *J* 6.5, 8.2 Hz), 3.73 (1H, dd, *J* 3.7, 11.6 Hz), 3.59 (1H, dd, *J* 5.1, 11.6 Hz), 2.09 (1H, br. s), 1.44 (3H, s), 1.37 (3H, s); δ_C (101 MHz, $CDCl_3$): 109.3, 76.1, 65.7, 62.9, 26.6, 25.2; ν_{max}: 3446, 2988, 2937, 2885, 1456, 1054, 845 cm^{-1}. The experiment was repeated on a large scale.

Appendix 3: Tris-acetonide-D-mannitol (103)[272]



D-mannitol (10 g, 54 mmol) was added to a stirred solution of sulfuric acid (1 mL, 10 mmol) and acetone (125 mL) and the mixture was stirred for 20 h at room temperature, then TLC showed no starting material was left. The reaction mixture was neutralized by addition of aqueous ammonia (3.5 mL) followed by sodium carbonate (6.0 g, 56 mmol). The reaction mixture was filtered and the filtrate was concentrated to half volume and poured into ice water to give a white solid residue which was collected and purified by recrystallization from aqueous acetone (600 mL) to give *tris*-acetonide-D-mannitol **(103)** as a white solid (11 g, 66%) m.p. 67-68 °C [*lit.*[272] m.p. 67-68 °C] which showed δ_H (400 MHz, $CDCl_3$): 4.24 – 4.16 (2H, m), 4.09 (2H, dd, *J* 6.4, 8.3 Hz), 3.99 (2H, dd, *J* 5.9, 8.4 Hz), 3.95 (2H, m), 1.43 (6H, s), 1.40 (6H, s), 1.36 (6H, s); δ_C (101 MHz, $CDCl_3$): 110.2, 109.6, 79.4, 76.4, 66.3, 27.5, 26.5, 25.3. All data were identical to the authentic sample. The experiment was scaled up to prepare 150 g from the product.

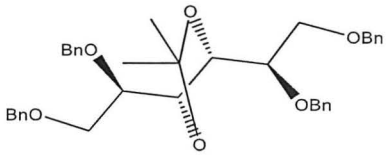
Appendix 4: 3,4-Acetonide-D-mannitol (104)[272]



Tris-acetonide-D-mannitol **(103)** (5g, 16 mmol) was dissolved in aqueous acetic acid 70% and heated at 40 °C for 45 mint, then TLC showed no starting material was left.

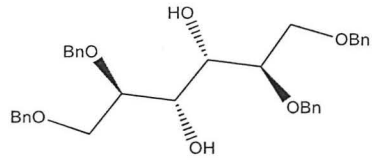
The acid was evaporated under reduced pressure and the residue poured into a boiling acetone (100 mL), the white solid was filtered and the filtrate was concentrated to half volume and poured into boiling toluene (100 mL). The solvent was evaporated under reduced pressure to give a white solid which was purifies by recrystallization from acetone to give 3,4-acetonide-D-mannitol **(104)** as a white crystal (2.8 g, 76%) m.p. 85-86 °C [*lit.*[272] m.p. 85-86 °C] which showed δ_H (400 MHz, CD_3OD): 3.96 – 3.91 (2H, m), 3.76 (2H, dd, *J* 1.9, 10.4 Hz), 3.67 – 3.56 (4H, m), 1.36 (6H, s); δ_C (101 MHz, CD_3OD): 110.5, 80.9, 74.6, 64.7, 27.3. All data were identical to the authentic sample. The experiment was repeated on a large scale.

Appendix 5: 1,2,5,6-Tetra-*O*-benzyl-3,4-*O*-isopropylidene-D-mannitol (105)[272]



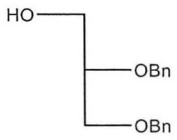
Sodium hydride NaH (10.83 g, 451.2 mmol, 60% w/w, dispersion in mineral oil, washed with petrol for three times) was added dropwise to a stirred solution of 3,4-acetonide-D-mannitol **(104)** (10.95 g, 49.27 mmol) and benzyl bromide (48.03 g, 33.40 mL, 280.8 mmol) in dry DMF (55 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and then stirred for 6 h at 60 °C, then TLC showed no starting material was left. The reaction mixture was quenched by slow addition of methanol (10 mL) and diluted with ethyl acetate (150 mL) and water (50 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with water (50 mL), brine (50 mL), dried over ($MgSO_4$). and evaporated under reduced pressure to give an oily residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (4:1) to give 1,2,5,6-tetra-*O*-benzyl-3,4-*O*-isopropylidene-D-mannitol **(105)** as a colourless oil (22.5 g, 78%) which showed δ_H (400 MHz, $CDCl_3$): 7.42 – 7.26 (20H, m), 4.78 (2H, d, *J* 11.8 Hz), 4.62 (2H, d, *J* 11.8 Hz), 4.54 (2H, d, *J* 12.1 Hz), 4.50 (2H, d, *J* 12.1 Hz), 4.26 – 4.20 (2H, m), 3.85 – 3.77 (4H, m), 3.67 (2H, dd, *J* 5.8, 10.1 Hz), 1.40 (6H, s); δ_C (101 MHz, $CDCl_3$): 138.4, 138.3, 128.4, 128.3, 128.2, 127.8, 127.7, 127.5, 127.45, 127.4, 109.6, 79.1, 78.4, 73.2, 72.7, 70.5, 27.1. All data were identical to the authentic sample. The experiment was repeated on a large scale.

Appendix 5: 1,2,5,6-Tetra-O-benzyl-D-mannitol (106) [276]



1,2,5,6-Tetra-*O*-benzyl-3,4-*O*-isopropylidene-D-mannitol **(105)** (20 g, 34 mmol) was dissolved in a mixture of dioxane/methanol/ 1N aqueous HCl (400 mL; 3:6:1, v/v) and heated under reflux for 2 h, then TLC showed no starting material was left. The reaction mixture was allowed to reach room temperature and extracted with ethyl acetate (3×150 mL). The combined organic layers were washed with saturated solution of $NaHCO_3$ (3×50 mL) and water (3×50 mL), dried over ($MgSO_4$) and 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 (4:1) to give 1,2,5,6-*tetra-O*-benzyl-D-mannitol **(106)** as a thick yellow oil (17 g, 91%) which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.27 (20H, m), 4.75 (2H, d, *J* 11.5 Hz), 4.61 (2H, d, *J* 11.5 Hz), 4.57 (4H, s), 3.99 (2H, m), 3.83 – 3.75 (4H, m), 3.74 – 3.66 (2H, m), 3.06 (2H, d, *J* 6.0 Hz); δ_C (101 MHz, $CDCl_3$): 138.1, 138.0, 128.4, 127.9, 127.7, 127.64, 127.6, 79.1, 73.5, 73.0, 70.2, 69.9. The experiment was repeated on a large scale.

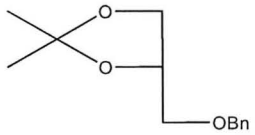
Appendix 6: 2,3-Di-*O*-benzyl-D-glycerol (107) [276]



An aqueous solution of $NaIO_4$ (50.0 g, 233 mmol) was added to a stirred solution of 1,2,5,6-tetra-*O*-benzyl-D-mannitol **(106)** (37.93 g, 69.89 mmol) in methanol (800 mL) at room temperature. The reaction mixture was stirred for 2 h at 20 °C, then TLC showed the oxidation was complete. The reaction mixture was allowed to reach room temperature followed by the addition of methanol (400 mL), then cooled until a white precipitate was formed, the precipitate was filtered off and to the filtrate was treated with $NaBH_4$ (50 g, 1.3 mol). The reaction mixture was stirred for 1.5 h at room temperature, then TLC showed no starting material was left. The reaction mixture was neutralized by added acetic acid. The organic layer was separated and the aqueous layer was extracted with chloroform (3×100 mL). The combined organic layers were dried over ($MgSO_4$), and evaporated under reduced pressure to give an oil residue which was purified by

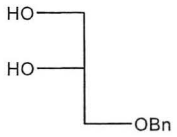
column chromatography on silica eluting with hexane/ethyl acetate (4:1) to give 2,3-di-*O*-benzyl-D-glycerol **(107)** as a colourless oil (17 g, 89%) [Found (MALDI) $(M+Na)^+$: 295.3, $C_{17}H_{20}NaO_3$, requires: 295.1], $[\alpha]_D^{20}$ - 20 (*c* 0.1, $CHCl_3$) [*lit.*[276] $[\alpha]_D^{25}$ - 17.2 (*c* 1.0, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.43 – 7.28 (10H, m), 4.74 (1H, d, *J* 11.7 Hz), 4.64 (1H, d, *J* 11.7 Hz), 4.58 (1H, d, *J* 12.2 Hz), 4.55 (1H, d, *J* 12.2 Hz), 3.82 – 3.59 (5H, m), 2.13 (1H, br. s); δ_C (101 MHz, $CDCl_3$): 138.2, 137.9, 128.44, 128.4, 127.8, 127.7, 127.6, 78.0, 73.5, 72.1, 70.1, 62.8. All data were identical to the authentic sample. The experiment was repeated on a large scale.

Appendix 7: 3-*O*-Benzyl-1,2-di-*O*-isopropylidene-L-glycerol (111)



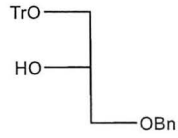
Sodium hydride NaH [277] (7.26 g, 0.30 mol) (60% w/w, dispersion in mineral oil) was added dropwise to a stirred solution of 1,2-*O*-isopropylidene-L-glycerol **(110)** (21.8 g, 165 mmol) and benzyl bromide (42.2 g, 29.3 mL, 247 mmol) in dry DMF (150 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and then stirred for 6 h then TLC showed no starting material was left. The reaction mixture was quenched by slow addition of methanol (20 mL) and diluted with the mixture of (Et_2O:H_2O, 1:1.8, 500 mL). The organic layer was separated and washed with water (100 mL), brine (100 mL), dried over ($MgSO_4$). The solvent was evaporated under reduced pressure to give an oily residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (10:1) to give 3-*O*-benzyl-1,2-di-*O*-isopropylidene-L-glycerol **(111)** as a colourless oil (29 g, 79%) $[\alpha]_D^{22}$ + 17.0 (*c* 0.1, $CHCl_3$) [*lit.*[277] $[\alpha]_D^{22}$ + 19.4] which showed δ_H (400 MHz, CD_3OD): 7.39 – 7.24 (5H, m), 4.57 (1H, d, *J* 12.8 Hz), 4.54 (1H, d, *J* 12.8 Hz) 4.32 – 4.24 (1H, m), 4.05 (1H, dd, *J* 6.5, 8.3 Hz), 3.72 (1H, dd, *J* 6.4, 8.3 Hz), 3.54 (1H, dd, *J* 5.6, 10.2 Hz), 3.50 (1H, dd, *J* 5.3, 10.2 Hz), 1.37 (3H, s), 1.33 (3H, s); δ_C (101 MHz, CD_3OD): 139.5, 129.4, 128.9, 128.7, 76.2, 74.4, 72.1, 67.6, 27.0, 25.6; ν_{max}: 3065, 3032, 2986, 2866, 1454, 1055, 737, 698 cm^{-1}.

Appendix 8: 3-*O*-Benzyl-L-glycerol ≡ (1-*O*-Benzyl-D-glycerol) (112) [277]



Compound 3-*O*-benzyl-1,2-di-*O*-Isopropylidene-L-glycerol **(111)** (29 g, 13 mmol) was dissolved in an aqueous acetic acid (70%) (200 mL) and the mixture was stirred and heated at 70 °C for 4 h., then TLC showed no starting material was left. The solvent was evaporated under reduced pressure to give an oily residue. The mixture was co-evaporated with toluene to remove the trace amount of the acid. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (1:2) to give 3-*O*-benzyl-L-glycerol ≡ (1-*O*-benzyl-D-glycerol) **(112)** as a thick oil (13 g, 54%) $[\alpha]_D^{22}$ + 5.7 (*c* 0.1, $CHCl_3$) [*lit.*[274] $[\alpha]_D^{22}$ + 6.3] which showed δ_H (400 MHz, CD_3OD): 7.38 – 7.23 (5H, m), 4.56 (1H, d, *J* 12.4 Hz), 4.53 (1H, d, *J* 11.8 Hz), 3.83 – 3.76 (1H, m), 3.62 – 3.45 (4H, m); δ_C (101 MHz, CD_3OD): 139.7, 129.3, 128.8, 128.6, 74.4, 72.8, 72.3, 64.6; ν_{max}: 3392, 3065, 3031, 2935, 2869, 1453, 1074, 737 cm^{-1}. All data were identical to the authentic sample.

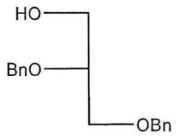
Appendix 9: 3-*O*-Benzyl-1-*O*-trityl-L-glycerol ≡ (1-*O*-Benzyl-3-*O*-trityl-D-glycerol) (113) [277]



Trityl chloride (19.9 g, 71.3 mmol) and DMAP (8.0 g, 65 mmol) were added to a stirred solution of 3-*O*-benzyl-L-glycerol **(112)** (10.8 g, 59.6 mmol) in anhydrous pyridine (150 mL) and the mixture was stirred at room temperature for 16 h then at 70 °C for 4 h. The reaction mixture was cooled to room temperature and poured into ice/water (350 mL), after the ice melted the organic phase was separated and the aqueous phase was re-extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with aqueous $NaHCO_3$ solution (100 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 a thick oil of 3-*O*-benzyl-1-*O*-trityl-L-glycerol **(113)** (13.1 g, 51%) which showed δ_H (400 MHz, $CDCl_3$): 7.48 – 7.42 (6H, m), 7.38 – 7.23 (14H, m), 4.57 (1H, d, *J* 11.6 Hz), 4.54 (1H, d, *J* 11.6 Hz), 4.05 – 3.97 (1H, m), 3.63 (1H, dd, *J* 4.3, 9.6 Hz), 3.58 (1H, dd, *J* 6.2, 9.6 Hz), 3.27 (1H,

dd, *J* 5.7, 9.4 Hz), 3.23 (1H, dd, *J* 5.3, 9.4 Hz), 2.46 (1H, d, *J* 4.5 Hz); δ_C (101 MHz, $CDCl_3$): 143.8, 128.8, 128.6, 128.3, 127.8, 127.7, 127.6, 127.0, 73.3, 71.5, 69.9, 64.5; ν_{max}: 3448, 3087, 3059, 3032, 2925, 2871, 1491, 1449, 1076, 707 cm^{-1}. All data were identical to the authentic sample.

Appendix 10: 2,3-Di-*O*-benzyl-L-glycerol ≡ (1,2-Di-*O*-benzyl-D-glycerol) (114) [277]

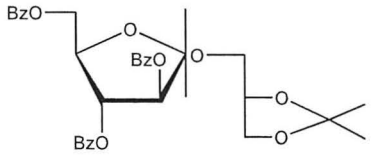


A solution of 3-*O*-benzyl-1-*O*-trityl-L-glycerol **(113)** (13.1 g, 30.8 mmol) in dry DMF (100 mL) was added dropwise to a stirred suspension solution of NaH (1.36 g, 56.6 mmol, 60% w/w, dispersion in mineral oil) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min. then benzyl bromide (5.5 mL, 7.9 g, 46 mmol) in dry DMF (10 mL) was added. The mixture was stirred at room temperature for 10 h and then quenched by slow addition of CH_3OH (15 mL) and H_2O (15 mL). The reaction mixture 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 over ($MgSO_4$) and the solvent was evaporated under reduced pressure. To the crude product, aqueous acetic acid (80%) (200 mL) was added and the mixture was stirred and heated at 75 °C for 4 h, then the reaction mixture was diluted with water (100 mL), ether (200 mL) and the organic layer was separated and the aqueous layer was re-extracted with ether (2×100 mL). The combined organic layers were washed with water (100 mL), saturated aqueous $NaHCO_3$ (100 mL), brine (100 mL), dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography on silica eluting with petrol/ethyl acetate (5:1) to give 2,3-di-*O*-benzyl-L-glycerol **(114)** as a thick oil (6.5 g, 77%) $[\alpha]_D^{22}$ = + 19 (*c* 0.1, $CHCl_3$) [*lit.*[277] $[\alpha]_D^{20}$ + 20.3 (*c* 1.1, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.43 – 7.30 (10H, m), 4.75 (1H, d, *J* 11.8 Hz), 4.66 (1H, d, *J* 11.8 Hz), 4.60 (1H, d, *J* 12.8 Hz), 4.57 (1H, d, *J* 12.4 Hz), 3.82 – 3.62 (5H, m), 2.43 (1H, s); δ_C (101 MHz, $CDCl_3$): 138.2, 137.9, 128.3, 128.3, 127.7, 127.6, 127.59, 127.5, 78.0, 73.4, 72.0, 70.1, 62.6; ν_{max}: 3437, 3063, 3031, 2935, 2868, 1453, 1090, 845, 736 cm^{-1}. All data were identical to the authentic sample

Appendix 11: *p*-Methoxybenzyl bromide [300]

Boron tribromide (4.89 g, 1.01 mol) was added to a stirred solution of *p*-methoxybenzyl alcohol (5.0 g, 0.03 mol) in diethyl ether (30 mL). The reaction mixture was stirred at room temperature for 2 h then TLC showed no starting material was left. The reaction mixture was poured into a mixture of saturated solution $NaHCO_3$ and ice (200 mL), the organic layer was separated and the aqueous layer was re-extracted with diethyl ether (3×100 mL). The combined organic layers were washed with saturated solution of $NaHCO_3$ (3×50 mL), brine (1×50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure to give *p*-methoxybenzyl bromide as a colourless oil (6.12 g, 84%), which was used in the next step without further purification. which showed δ_H (400 MHz, $CDCl_3$), δ_C (101 MHz, $CDCl_3$) and ν_{max} identical to the literature.

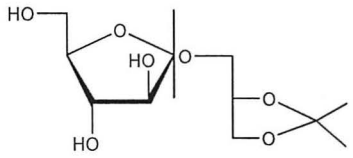
Appendix 12: 2',3'-*O*-Isopropylidene-D-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α,β-D-arabinofuranoside



Tin (IV) chloride[285] (0.952 mL, 1 M solution in CH_2Cl_2, 1 mmol) was added to a stirred solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinfuranosyl bromide **(115)** (1.0 g, 1.9 mmol) in dry CH_2Cl_2 (12 mL) at 0 °C under nitrogen atmosphere. The mixture was stirred for 10 min, then a solution of 1,2-*O*-isopropylidene-L-glycerol **(110)** (0.25 g, 1.89 mmol) in (2 mL) CH_2Cl_2 was added following by added a solution of ethyldiisoprpylamine (0.18 g, 1.39 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 2 h then TLC showed no starting material was left, the reaction mixture was quenched with aqueous $NaHCO_3$ (10 mL), then the organic layer was separated and the aqueous layer was re-extracted with dichloromethane (3×50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure to give the residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (1:1) to give 2',3'-*O*-isopropylidene-D-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α,β-D-arabinofuranoside as a thick oil in ratio (3:1) (0.56 g, 51%) $[\alpha]_D^{22}$ + 36 (*c* 0.1, $CHCl_3$), [Found (MALDI)

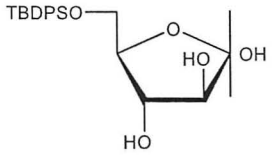
$(M+Na)^+$: 599.0, $C_{32}H_{32}NaO_{10}$, requires: 599.1] the *major isomer* showed δ_H (400 MHz, $CDCl_3$): 7.64 – 7.28 (15H, m), 5.61 – 5.54 (2H, m), 5.35 (1H, s), 4.87 – 4.80 (1H, m), 4.74 – 4.66 (1H, m), 4.59 (1H, dd, *J* 5.3, 9.9), 4.41 – 4.31 (1H, m), 4.13 – 4.07 (1H, m), 3.89 (1H, dd, *J* 5.5, 9.3), 3.86 – 3.80 (1H, m), 3.66 (1H, dd, *J* 5.6, 10.8), 1.43 (3H, s), 1.38 (3H, s); δ_C (101 MHz, $CDCl_3$): 166.2, 165.7, 165.4, 133.6, 133.5, 133.0, 130.0, 129.9, 129.8, 129.7, 128.5, 128.4, 128.3, 105.8, 81.9, 81.3, 77.8, 74.4, 68.1, 66.7, 63.67, 26.7, 25.4; ν_{max}: 3064, 3034, 2986, 2937, 1725, 1491, 1109, 709 cm^{-1}. The experiment was scaled up to prepare 2 g from the product.

Appendix 13: 2',3'-*O*-Isopropylidene-D-glycerol-(1'→1)-α,β-D-arabino-furanoside



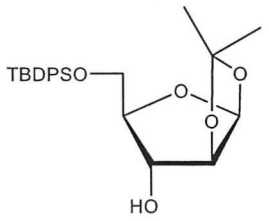
A solution of sodium methoxide (2 mL, 1 M, in methanol) was added to a stirred solution of 2',3'-di-*O*-isopropylidene-D-glycerol-(1'→1)-2,3,5-tri-*O*-benzoyl-α,β-D-arabinofuranoside (0.70 g, 1.21 mmol) in dry MeOH : CH_2Cl_2 (1:1, 6 mL) at room temperature until a PH 11 was obtained. The reaction mixture was stirred at room temperature for 2 h then TLC showed no starting material was left. The mixture was neutralized with amberlite IR-120 (H^+ form), the resin was filtered off and the solvent was evaporated under reduced pressure, the residue was purified by column chromatography on silica eluting with dichloromethane/methanol (5:2) to afford 2',3'-*O*-isopropylidene-D-glycerol-(1'→1)-α,β-D-arabinofuranoside as a thick oil in ratio (3:1) (0.26 g, 81%) [Found (MALDI) $(M+Na)^+$: 287.2, $C_{11}H_{20}NaO_7$, requires: 287.1], $[\alpha]_D^{22}$ + 20 (*c* 0.1, $CHCl_3$) the *major isomer* showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 5.02 (1H, s), 4.74 (1H, br. s), 4.30 (1H, m), 4.14 – 3.98 (4H, m), 3.87 – 3.68 (4H, m), 3.55 – 3.47 (1H, m), 2.81 (2H, br. s), 1.42 (3H, s), 1.36 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 109.7, 85.9, 80.0, 77.4, 74.5, 67.9, 66.2, 61.5, 26.6, 25.2; ν_{max}: br. 3400, 2987, 2935, 1722, 1454, 1214, 1044, 716 cm^{-1}.

Appendix 14: 5-*O*-Tertbutyldiphenylsilyl-α,β-D-arabinofuranose [301]



Tertbutyldiphenylchlorosilane (9.0 g, 32 mmol) was added to a stirred solution of D-(-) arabinose (5.0 g, 33 mmol) and imidazole (5.0 g, 73 mmol) in dry DMF (60 mL), at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and stirred for 16 h, then TLC showed no starting material was left. The reaction mixture was diluted with ethyl acetate (100 mL) and water (25 mL), then the organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×100 mL). The combined organic layers were washed with water (50 mL), brine (50 mL), dried over ($MgSO_4$) and the solvent was evaporated under reduced pressure to give a residue oil which was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) affording 5-*O*-tertbutyldiphenylsilyl-α,β-D-arabinofuranose as a colourless oil (7g, 54%). All data were identical to the authentic sample.

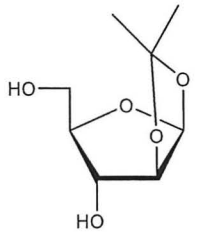
Appendix 15: 5-*O*-Tertbutyldiphenylsilyl-1,2-*O*-isopropylidene-α-D-arabino-furanose[301]



Anhydrous copper sulphate (4.3 g, 27 mmol) was added to a stirred solution of 5-*O*-tertbutyldiphenylsilyl-α, β-D-arabinofuranose (3.8 g, 1.0 mmol) in dry acetone (45 mL). The reaction mixture was stirred for 15 min, then 1 drop from concentrated H_2SO_4 was added and the reaction mixture was stirred for 16 h at room temperature, then TLC showed no starting material was left. The reaction mixture was filtered and neutralized with solid calcium hydroxide, the solid precipitate was filtered off and the solvent was evaporated under reduced pressure to give an oil residue which was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) affording 5-*O*-tertbutyldiphenylsilyl-1,2-*O*-isopropylidene-α-D-arabinofuranose as a thick oil (3 g, 73%) [Found (MALDI) $(M+Na)^+$: 451.21, $C_{24}H_{32}NaO_5Si$, requires: 451.19], $[\alpha]_D^{22}$ - 7 (*c* 0.1, $CHCl_3$) [*lit.*[301] $[\alpha]_D$ - 5 (*c* 1.2, $CHCl_3$)] which showed δ_H (400 MHz, $CDCl_3$): 7.71 – 7.64 (4H, m), 7.47 – 7.36 (6H, m), 5.89 (1H, d, *J* 4.0 Hz), 4.55 (1H, d, *J* 4.1 Hz), 4.44 (1H, br. t, *J* 3.0 Hz), 4.09 – 4.02 (1H, m), 3.87 – 3.79 (2H, m), 1.86 (1H, d, *J* 4.2 Hz), 1.34 (3H, s), 1.30 (3H, s), 1.07 (9H, s); δ_C (101 MHz, $CDCl_3$): 135.6, 135.5, 133.1, 129.8, 129.76, 127.77, 127.7, 105.5, 87.4, 87.0, 76.4, 63.7, 26.8, 26.1; ν_{max}: 3436, 3071,

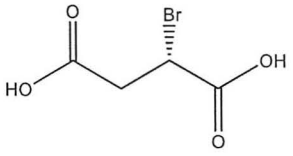
3050, 2932, 2890, 2857, 1427, 1112, 701 cm^{-1}.All data were identical to the authentic sample.

Appendix 16: 1,2-*O*-Isopropylidene-α-D-arabinofuranose [302]



Tetrabutylammonium fluoride (9.5 mL, 9.5 mmol, 1 M) was added dropwise to a stirred solution of 5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-α-D-arabino-furanose (3.0 g, 6.9 mmol) in anhydrous THF (25 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 16 h, then TLC showed no starting material was left, then with ethyl acetate (100 mL) and water (50 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with saturated solution of NH_4Cl (50 mL), brine (50 mL), dried over ($MgSO_4$) and evaporated to give the residue was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) affording 1,2-*O*-isopropylidene-α-D-arabinofuranose as a thick oil (1.0 g, 75%) [Found (MALDI) $(M+Na)^+$: 213.1, $C_8H_{14}NaO_5$, requires: 213.0], $[\alpha]_D^{20}$ + 20 (*c* 1.0, MeOH) [*lit.*[302] $[\alpha]_D$ +17.6 (*c* 1.25, MeOH)] m.p. 114-115 °C (*lit*[302] 114-115 °C) which showed δ_H (400 MHz, $CDCl_3$): 5.94 (1H, d, *J* 4.0 Hz), 4.59 (1H, d, *J* 4.0 Hz), 4.26 (1H, d, *J* 2.2 Hz), 4.14 – 4.06 (1H, m), 3.78 (2H, m), 2.68 (2H, s), 2.66 (2H, br. s), 1.53 (3H, s), 1.34 (3H, s); δ_C (101 MHz, $CDCl_3$) 105.53, 87.86, 87.27, 75.96, 62.43, 26.91, 26.15; ν_{max}: 3400, 2984, 2965, 1380, 11021, 829, 730 cm^{-1}. All data were identical to the authentic sample.

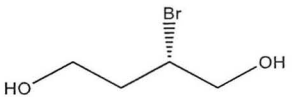
Appendix 17: (*S*)-(-)-Bromosuccinic acid[303]



L-Aspartic acid (50.0 g, 380 mmol) and potassium bromide (201.0 g, 1690 mmol) in H_2SO_4 (2.5 M, 1L) was cooled to -5 °C and solution of sodium nitrite (46.6 g, 0.68 mol) in water (100 mL) was added slowly without allowing the temperature to exceed 0 °C. The resulting dark brown mixture was stirred for 2 h at - 5 °C after which the product

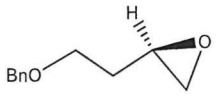
was extracted with ethyl acetate (3×500 mL). The combine organic extracts were dried and the solvent was evaporated to give a white powder of (*S*)-(-)-bromosccinic acid (65 g, 87%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.

Appendix 18: (*S*)-2-Bromo-1, 4-butanediol[303]



Boranetetrahydrofuran (800 mL, 1M, 0.8 mol) was slowly added to a solution of (*S*)-(-)-bromosuccinic acid (52.4 g, 260 mmol) in dry THF (400 mL) at 0 °C under nitrogen over a period of 1 hour. After the addition was completed the cooling bath was removed and the reaction mixture was stirred for 4 hours. The reaction was quenched by slowly addition of THF/H_2O (100 mL 1:1) at 10 °C was followed by the addition of anhydrous K_2CO_3 (160 g). The mixture was stirred and then filtered through sinter glass funnel under high vacuum and the solid residue was washed with ethyl acetate (3×100 mL). The combined washed filtrates were concentrated to a mixture of an oil and borate salt. The oil was re-dissolved in ethyl acetate (2×200 mL), filtered to remove any borate salts present and then dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (1:1) to give an oil of (*S*)-2-bromo-1,4-butanediol (40 g, 88%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.

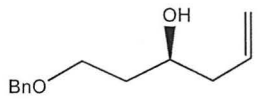
Appendix 19 :(*R*)-(2-Benzyloxyethyl)oxiran) [303]



Sodium hydride (13.5 g, 60% dispersion in oil, 337 mmol) was washed with petrol (3 × 40 mL) and suspended in dry THF (300 mL). (*S*)-2-Bromo-1,4-butanediol (18.65 g, 0.11 mol) in dry THF (30 mL) was added over a 5 min period at -10 °C. The mixture was stirred for 25 min before adding benzylbromide (14.1 mL, 20.3 g, 0.11 mol) followed by *tetra*-butylammonium iodide (4.03 g, 11.0 mmol). The reaction mixture was stirred at -10 °C for a further 5 min before removing the cooling bath and allowing it to warm to room temperature. The mixture was stirred at room temperature for 2 hours before cooling to -10 °C and quenched with sat. aq. ammonium chloride (150 mL). The aqueous layer was extracted with ethyl acetate (3×300 mL), dried and the solvent evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl

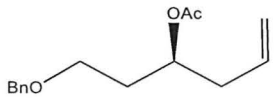
acetate (5:1) to give an oil of (*R*)-(2-benzyloxyethyl)oxiran **(128)** (16 g, 81%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.

Appendix 20: (*S*)-1-Benzyloxy-hex-5-en-3-ol [304]



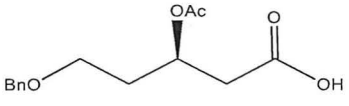
Copper iodide (5.76 g, 30.3 mmol) was dissolved in dry THF (200 mL) at room temperature under nitrogen and cooled to -75 °C. Vinyl magnesium bromide (194 mL, 194 mmol, 1M in THF) was added between -75 °C to - 50 °C and the mixture was stirred at -50 to -40 °C for 30 min, then re-cooled to - 75 °C and a solution of (*R*)-(2-benzyloxyethyl)oxirane (20.0 g, 112 mmol) in dry THF (100 mL) was added between -75 °C -40 °C and the reaction was stirred at - 40 °C to - 30 °C for 1 h then at - 20 °C for 15 min. Sat. aq. ammonium chloride (300 mL) was added and extracted with ethyl acetate (3×300 mL) and the combined organic layers were washed with water, dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil (*S*)-1-bezyloxy-hex-5-en-3-ol (19 g, 82%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.

Appendix 21: Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester[304]



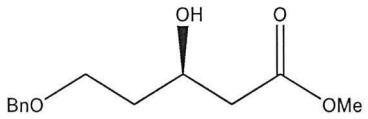
Acetic anhydride (80 mL) and pyridine (80 mL) were added to a stirred solution of (*S*)-1-benzyloxy-hex-5-en-3-ol (45.0 g, 218 mmol) in dry toluene (250 mL) at room temperature and the mixture was stirred at room temperature for 18 hours under nitrogen. After that the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil, acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (45 g, 83%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.

Appendix 22: (*R*)-3-Acetoxy-5-benzyloxy-pentanoic acid[304]



Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (10.0 g, 40.2 mmol) was dissolved in dry DMF (200 mL) and oxone (98.8 g, 160 mmol) then OsO_4 2.5% in 2-methyl-2-propanol (5.04 mL, 0.40 mmol) were added at 10 °C. The mixture's temperature was allowed to reach 34 °C and it was stirred for 5 hours. The mixture was diluted with water (3 L) and extracted with ethyl acetate (1×500 mL, 2×250 mL). The combined organic layers were washed with water (700 mL), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (1:2) to give (*R*)-3-acetoxy-5-benzyloxy-pentanoic acid **(131)** (8.5 g, 84%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.

Appendix 23 :(*R*)-5-Benzyloxy-3-hydroxy-pentanoic acid methyl ester[304]



Conc. H_2SO_4 (70 drops) was added to a stirred solution of (*R*)-3-acetoxy-5-benzyloxy-pentanoic acid (10 g, 39.9 mmol) in methanol (150 mL) at room temperature. The mixture was refluxed for 3 h. TLC showed no starting material was left. The methanol was evaporated and ethyl acetate (250 mL) and sat. aq. $NaHCO_3$ (200 mL) were added. The organic layer was separated and the aq. layer was re-extracted with ethyl acetate (2×150 mL). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatograph eluting with petrol/ethyl acetate (5:2) to give a colourless oil, (*R*)-5-benzyloxy-3-hydroxy-pentanoic acid methyl ester (8 g, 83%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.