DOCTOR OF PHILOSOPHY

Synthesis of single enantiomers of ω-1 methoxy mycolic acids from Mycobacterium alvei

Alhuwaymil, Zamzam

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A thesis submitted to Bangor University

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in the

School of Natural Sciences (Chemistry)

by

Zamzam Alhuwaymil

2020

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Abbreviations

AG	Arabinogalactan
AIDS	Acquired Immune Deficiency Syndrome
aq	Aqueous
BCG	Bacillus Calmette-Guérin
br	Broad
Bn	Benzyl
CID-MS	Collision-induced dissociation mass spectrometry
cm^{-1}	Per centimeter
°C	Degrees Celsius
d	Doublet
dd	Double doublet
dt	Double triplet
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DMAP	4-Dimethylaminopyridine
DMF	*N*, *N*-Dimethylformamide
DEAD	Diethyl azodicarboxylate
ESR	Erythrocyte sedimentation rate
eq	Equivalent
Ether	Diethyl ether
g	Gram
GC	Gas chromatography
GMM `	Glucose mono-mycolate
GPL	Glycopeptidolipids
^{1}H	Proton

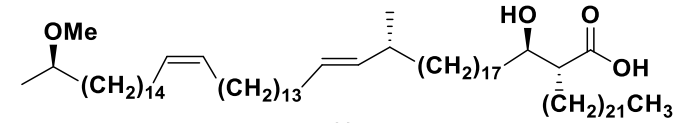
HBr	Hydrobromic acid
HIV	Human immunodeficiency virus
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatography
hr	Hours
Hz	Hertz
IMS	industrial methylated spirits
IR	Infra-red
J	coupling constant
L	Litre
LAM	Lipoarabinomannan
LDA	Lithium *N, N*-diisopropylamide
m	Multiplet
MAC	*Mycobacterium avium complex*
MALDI	Matrix Assisted Laser Desorption Ionization
MAs	Mycolic Acids
MDR	Multi-Drug Resistant
MHz	Megahertz
m-CPBA	*m*-Chloroperoxybenzoic acid
min	Minute
mL	Milliters
M.P	Melting point
M.tb	*Mycobacterium tuberculosis*
NBS	*N*-bromosuccinimide
NMR	Nuclear Magnetic Resonance
NTM	Non-Tuberculosis mycobacteria
PCC	Pyridinium chlorochromate
PCR	Polymerase chain reaction

Petrol	Petroleum spirit (boiling point 40 to 60°C)
Pd	Palladium
PG	Peptidoglycan
PPh_3	Triphenyl Phosphine
ppm	Parts per million
PPTS	Pyridinium-*p*-toluene-sulfonate
PTSA	*p*-Toluenesulfonic acid
q	Quartet
RFLP	Restriction Fragment Length Polymorphism
RGM	Rapid Growing Mycobacteria
s	Singlet
SAM	*S*-adenosyl-*L*-methionine
sat	Saturated
t	Triplet
TB	Tuberculosis
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS	Tert-butyldiphenylsilyl
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin-Layerchromatography
TMM	Trehalose mono-mycolate
TDM	Trehalose di-mycolate
WHO	World Health Organisation
XDR	Extensively Drug-Resistant

Abstract

This thesis is discussed in three main parts. Firstly the synthesis of single enantiomers (*R* and *S*) of a ω-1 methoxy- *cis*-alkene-α-methyl *trans*-alkene mycolic acid (**I, II**), which have been isolated from *M. alvei*, an environmental mycobacterium, is described. This MA contains a methoxy group at the ω-1 position and two alkene groups in the mero chain, at the distal and proximal positions. The next part of the thesis describes the preparation of a number of sugar esters of mycolic acids (**I** and **II**). These include the TDM (**III**), TMM (**IV**)**,** arabino-MA fragment from AG (**V**) and GMM **(VI)**.

The final part describes the synthesis of the specific *cis*-alkene-α-methyl-*trans*-alkene mycolic acid (**VII**), which has also been isolated from *M. alvei*. This MA contains a double bond at the distal and proximal positions. The preparation of mycolate esters, by esterification of this mycolic acid with trehalose to give TDM (**VIII**) and TMM (**IX**) is also discussed.



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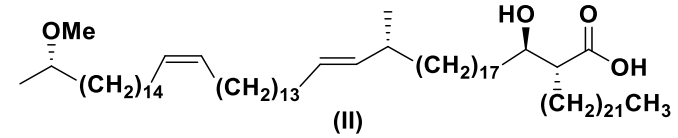

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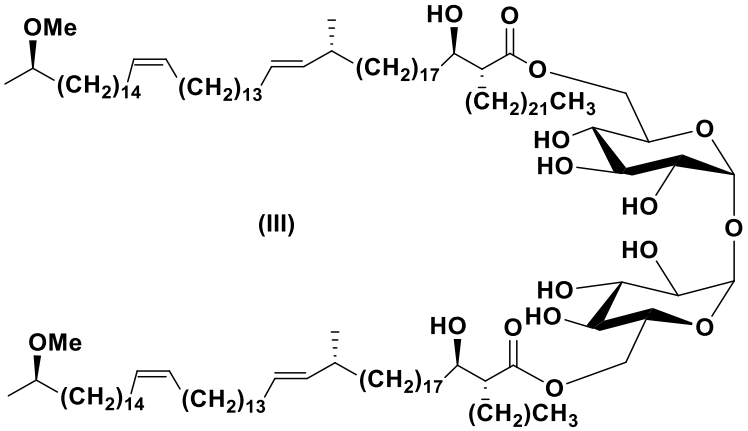

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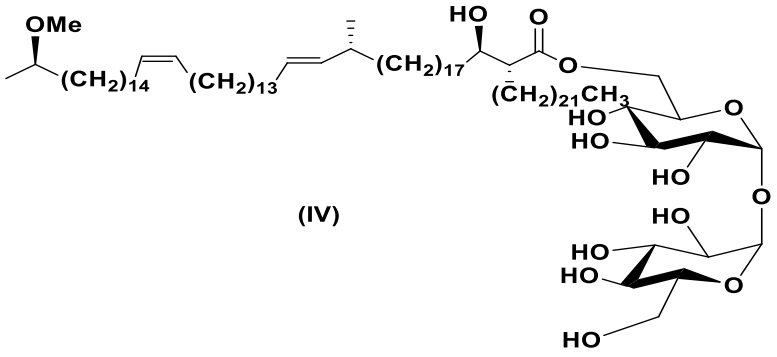

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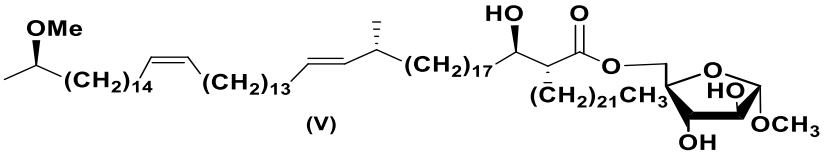

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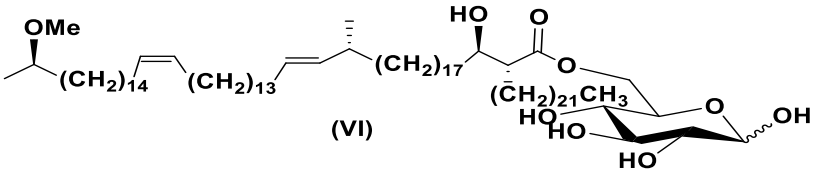

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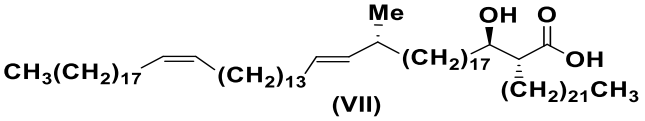

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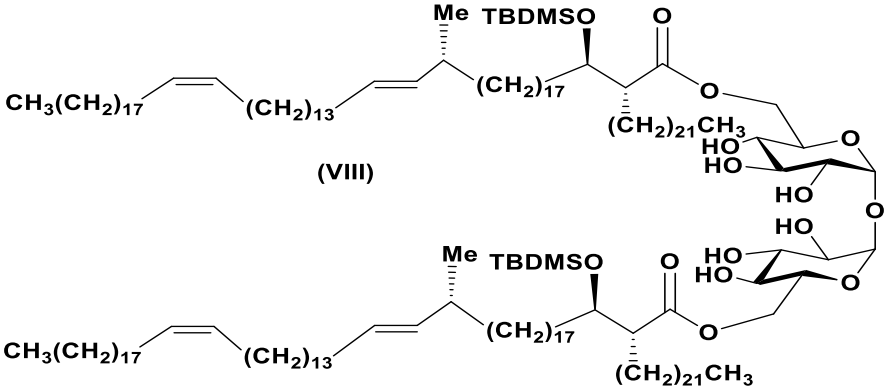

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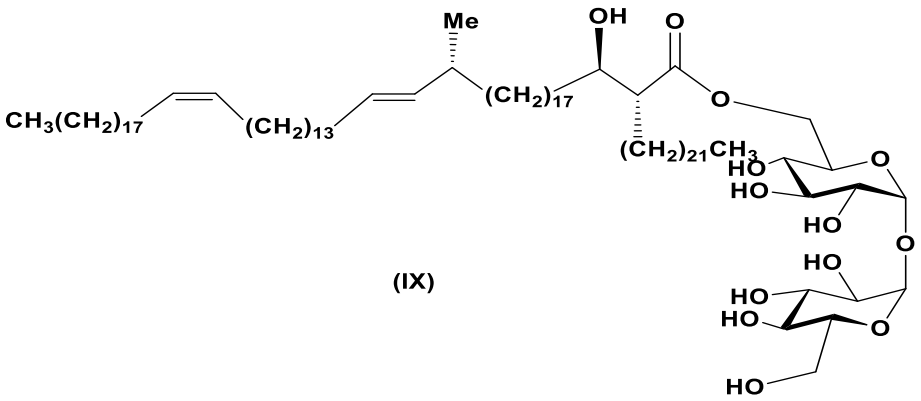

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Chapter 1

1. Introduction

1.1 Tuberculosis (TB)

Tuberculosis (TB) is a contagious bacterial disease caused by *Mycobacterium tuberculosis (M. tuberculosis*).[1] Nowadays it is believed that TB infects one third of the world's population, however, in only 10% of cases of does TB become active and cause illness.[2] This disease is usually transferred when a person breathes in air that contains species of *M. tuberculosis* from an infected person.[3] The lung is the main place of infection in the body, however any other organ can be infected, such as the lymph nodes, kidneys and central nervous system.[4,5] Moreover it became a public health problem three centuries ago, when cities became overcrowded and there was a lack of health care facilities. This led people to study the disease with the aim of finding a cure for it, with early studies starting in the 17th century.[6] Evidence of TB in Egypt more than 5000 years ago was obtained following the analysis of Egyptian mummies and the disease was also clearly depicted in early Egyptian art.[7,8] In the 18th and 19th centuries, TB spread throughout Asia, Africa, South America and Europe, and became one of the leading causes of death in the world. In Europe it was responsible for 25% of adult deaths in major cities.[9] Written texts describing tuberculosis also provide evidence of the disease in India and China as early as 3300 and 2300 years ago, respectively.[10,11] It is thought that TB existed in the Americas with similar evidence to that found in Egypt, before the arrival of European explorers.[12,13] The World Health Organisation (WHO) estimates that roughly one third of the world's population is currently infected with TB.[14]

There were no real advances in the knowledge of TB until Hermann Heinrich and Robert Koch gave their presentation on the tubercle bacillus and on their work to postulate the link between a microbe and disease.[15] In 1891, Koch isolated a substance from the tuberculosis bacillus, which he called tuberculin, and later, he proceeded to inject himself with this. His body developed a fever, but he never developed TB. Koch was awarded the Nobel Prize in Physiology and Medicine in 1905 for his work on tuberculosis. Since Koch's work a reliable vaccine has never been developed because TB immunity differs from the immunity of many other common microbial diseases.[16,17] It was concluded after several years of research that a positive reaction to tuberculin was caused by latent TB, meaning that the individual had been infected with TB infection

without showing any symptoms. Over the next 50 years, more extensive research into tuberculin and latent TB showed that a large number of people were carriers of latent TB.[18,19] In 1921 Albert Calmette and Camille Guérin developed a vaccine from *Mycobacterium bovis (M. bovis)* called Bacille Calmette-Guérin (BCG).[20] Acceptance of the vaccine was however slow, as many people did not believe it to be safe, as it was based on live TB bacteria.[21,22] The BCG vaccine was first used on humans in 1921 in France,[23] and having agreed to share the responsibility for this first human experiment, Weill-Hallé and Calmette administered three oral doses of 2 milligrams of BCG to a new born baby, but it was not until after World War II that BCG received widespread acceptance in the United States, Great Britain and Germany.[24]

The WHO has reported that TB infects one person every second, of whom 5-10% die as a direct result.[25] In 1994, the WHO announced TB as a major health problem and declared a global health emergency.[26] In 2008, there were an estimated 9.27 million cases of TB,[27] as a comparison there were 9.24 million cases in 2006, 8.3 million cases in 2000 and 6.6 million cases in 1990. In 2009, 9.4 million new cases of TB were recorded, more than at any time in history.[28] A recent WHO report on Global TB Control, reports that the average rate of decline in the TB incidence rate was 1.6% per year in the period 2000 – 2018, and an estimated 10.0 million (range, 9.0 – 11.1 million) people fell ill with TB. Most of the estimated number of cases in 2018 occurred in the WHO South-East Asia Region (44%), African Region (24%) and Western Pacific Region (18%); smaller proportions of cases occurred in the WHO Eastern Mediterranean Region (8.1%), Region of the Americas (2.9%) and European Region (2.6%).The 30 high TB burden countries accounted for 87% of all estimated incident cases worldwide, and eight of these countries accounted for two thirds of the global total: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). **Figure 1** shows the estimated number of new cases of TB per 100,000 population in 2018.[29]

Figure 1: Map showing estimated TB incidence in 2018, for countries with at least 100,000 incident cases.[29]

The main rise in tuberculosis infection is through co-infection with Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS). HIV weakens the immune system causing the TB bacteria to be more harmful. People with HIV who are TB positive are more likely to move to active disease compared with a person with infection by TB but HIV negative. Both diseases work in conjunction with each other, each increasing the progression of the other, and a person co-infected with HIV and *M. tuberculosis* is approximately thirty times more likely to die from TB, compared to someone who is infected with *M. tuberculosis* alone.[30,31] Estimates of TB mortality rates (deaths per 100,000 population per year) for the six WHO regions and for the 30 high TB burden countries, the number of TB deaths among HIV-negative people per 100,000 population was 16 (range, 15 – 17) in 2018, and 20 (range, 18–21) when TB deaths among HIV-positive people were included. There was considerable variation among countries, ranging from less than one TB death per 100 000 population in many high-income countries, to 40 or more deaths per 100,000 population in much of the WHO African Region and in two high TB burden countries in Asia (the Democratic People's Republic of Korea and Papua New Guinea).[32] **Figure 2** shows the estimated worldwide distribution of TB patients co-infected with HIV.

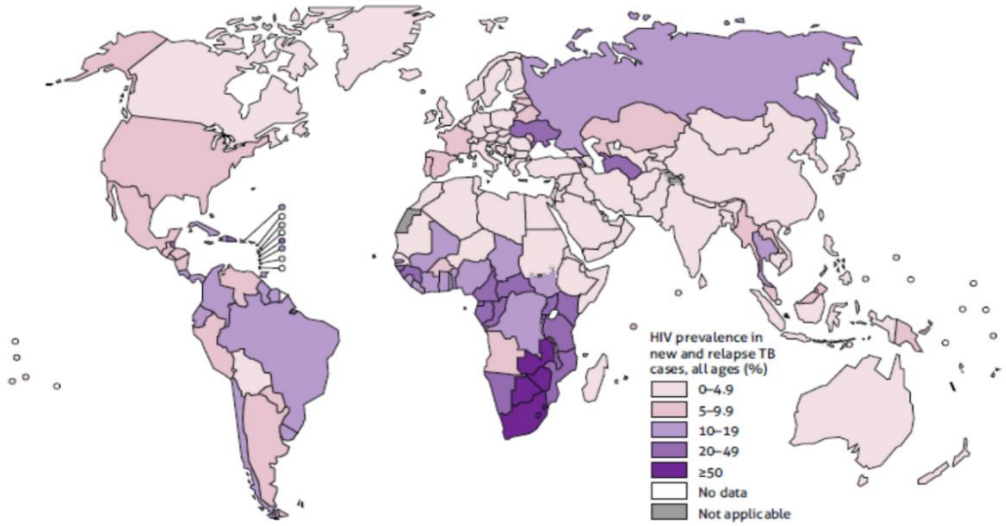


Figure 2: Map showing estimated HIV prevalence in new and relapse TB cases, 2018.[32]

1.1.1 Trends of TB Infection, Transmission and Treatment

The transmission of the airborne bacteria passes through the upper airways and into the lungs, causing tubercles or tuberculosis nodules resembling sores. Because of this infection, granulomas form in the lung tissues, which often lead to necrosis or death of tissue cells.[33] Treatment and cure of the infected individual undoubtedly remains a sure means of reducing the spread of the disease. Effective treatment makes TB non-contagious and prevents it from spreading further. As a result of the resilience of *M. tb* to most commonly used antibiotics and chemotherapeutic agents, treatment of TB remains a complex and extended process entailing long courses with multiple antibiotics.[34,35] The two most effective classes of antibiotics against TB include aminoglycosides and rifampcins, obtained either naturally from the bacterium *Amycolatopsis mediterranei*, or produced artificially. The first antibiotic effective against TB was streptomycin (**1**), isolated from *Streptomyces griseus* in the 1940s. Subsequently, since the discovery of isoniazid (**2**) in the 1950s, many Western doctors have adopted the strategy of combining streptomycin with *p*-aminosalicylic acid **(3)** to treat tuberculosis. It was discovered by the Swedish chemist Jörgen Lehmann in 1944.[36] Ethambutol (**4**) is also used to kill the bacteria that cause tuberculosis (TB). It was introduced in 1961 as a bacteriostatic first-line drug effective against actively growing mycobacteria, (**Figure 3**).[37]

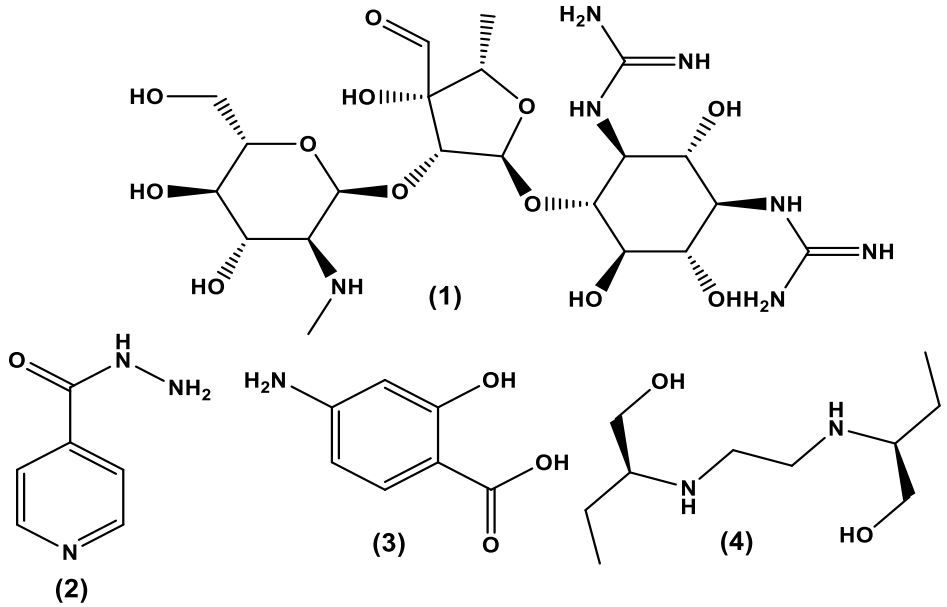


Figure 3: Streptomycin (**1**), isoniazid (**2**), *p*-aminosalicylic acid (**3**) and ethambutol (**4**).

The second-line drugs are for drug-resistant TB and for patients who cannot tolerate the first-line drugs; they are less effective, more toxic and require longer use than the first-line drugs.[38] Today, there are more than thirty different types of anti TB drugs being used, however most of them are analogues of the above products. According to the WHO, in 2007 the overall success rate for TB treatment was 70%.[27]

1.1.2 Multi-Drug Resistance (MDR)

Early attempts to cure TB using streptomycin faced the emergence of unexpected problems, with many patients suffering relapses of TB with streptomycin resistant strains.[39] Since this time, strains of *M. tuberculosis* showing resistance to other anti-TB drugs have arisen. When a strain of TB is found to be resistant to two or more front line drugs (like INH, rifampin, PZA, ethambutol and streptomycin) it is termed Multi-Drug Resistant (MDR). Over 0.5 million new cases of MDR − TB were reported in 2008,[40] leading to the classification of the antibiotics used for TB treatment into five groups, with the recommendation that a design strategy for drug combinations should be used for each individual case of TB.[41] In the USA, primary drug resistance was 1-2% in the 1950's, rose to 3% in the next decade and by 1970 had increased to 8.6%.[9,42] In other parts of the world MDR-TB is a significant problem, with the occurrence of MDR-TB in India, Pakistan and Central Haiti being between 20-30%.[43,44]

Extensively drug-resistant tuberculosis (XDR-TB) is a form of TB caused by bacteria that are resistant to the most effective anti-TB drugs. XDR-TB is defined as TB that has

developed resistance to the first line anti-TB drugs that define MDR-TB.[45] XDR-TB is associated with a much higher mortality rate than MDR-TB.[46]

1.2 Mycobacteria

Mycobacterium is a genus of bacteria, of which there are many different kinds. They can cause fatal disease in both humans and animals, the most well known being *M. tuberculosis* which causes TB.[47] The variation in the types of bacteria depends on a number of factors such as colony shapes, colours, virulence, and temperature of growth. There are over 150 *Mycobacterium* species, which can be divided into two classes: tuberculosis complex mycobacteria such as *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti, Mycobacterium caprae or Mycobacterium canettii*;[48] and non-tuberculosis mycobacteria (NTM), which comprise over 90 species, including *Mycobacterium smegmatis, Mycobacterium brumae, Mycobacterium fortuitum, Mycobacterium gordonae, Mycobacterium avium* complex *(MAC)* and *Mycobacterium alvei*.[49]

1.3 Tuberculosis mycobacteria

The tuberculosis mycobacteria constitute a collection of *M. tuberculosis, M. bovis, M. microti* and *M. africanum,* collectively known as *M. tuberculosis* complex, which can all cause TB disease in both humans and animals.[50] *M. tuberculosis* is the most common in this family of mycobacteria and is the causative agent of most cases of tuberculosis. *M. tuberculosis* is classified as an acid fast gram-positive bacterium, small rod-shaped bacillus, approximately (1-4 × 0.3-0.6 µm) in size (**Figure 4**), which divides every 16 to 20 hours, an extremely slow rate compared to other bacteria, such as *E. coli* that can divide roughly every 20 minutes.[51]

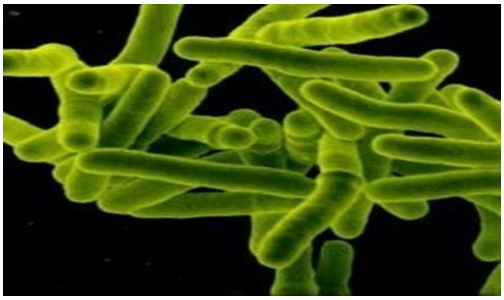


Figure 4: Scanning electron micrograph of *M. tuberculosis*.[52]

M.tb is usually found within moist conditions, however it can also be classified as a xerophile, as it can survive for several weeks in a dry state.

1.4 Non-Tuberculosis Mycobacteria (NTM)

Non-tuberculosis mycobacteria, are small, rod shaped bacilli which enter the human body through environmental sources such as natural water, soils, foods, and water pipes of the distribution system.[53] Although NTM do not cause TB they have the ability to cause other diseases, e.g. skin disease, disseminated disease and pulmonary disease in HIV negative patients.[48] Unlike tuberculosis mycobacteria NTM are not transmitted directly from one person to another, with the organism being acquired exclusively from environmental sources.[54] It has been found that the rate of worldwide NTM infection is increasing significantly. In the UK, the rate of all NTM reported cases increased from 0.9/100,000 to 2.9/100,000 persons between the years 1996-2006.[55] Another report from Canada found an increase of pulmonary NTM cases from 9.1/100,000 in 1997 to 14.1/100,000 by 2003.[56] Similar reports also showed the prevalence of pulmonary NTM infection in other countries.[57,58]

1.4.1 *Mycobacterium smegmatis*

M. smegmatis is a rapidly growing acid-fast *Mycobacterium*.[59] It was first discovered and isolated in 1885 by Lustgarten, and lives in aggregate layers of cells attached to each other in a community called a biofilm. It is found in water, soil and plants. *M. smegmatis* is 3.0 to 5.0 µm long with a bacillus shape, and is an acid-fast bacterial species in the phylum *Actinobacteria*.[60] Generally, *M. smegmatis* is considered as non-pathogenic to humans, but there are a few cases of skin infection attributed to *M. smegmatis* which are treated with anti-mycobacterial drugs such as doxycycline, trimethoprim-sulfamethoxazole, and ethambutol (**Figure 5**).[61]

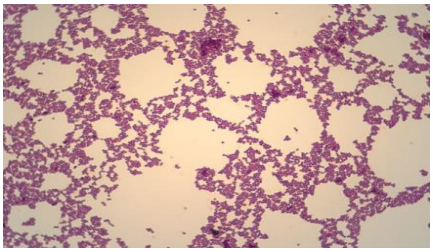


Figure 5: *M. smegmatis*.[62]

1.4.2 *Mycobacterium brumae*

Mycobacterium brumae (M. brumae) as seen in **Figure 6** is a new species of rapidly growing, environmental and non-photochromogenic mycobacteria. It was first identified having been isolated from water, soil, and human sputum samples in 1993 in Barcelona, Spain. It is also gram-positive, nonmotile, strongly acid-fast and rod shaped (2.0-2.5 µm long and 0.3 to 0.5 µm wide).[63]

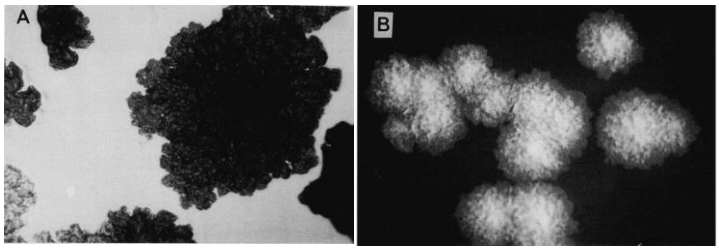


Figure 6: (A) Mature *M. brumae* colonies on micrograph (magnification x120).

(B) No magnification.[63]

1.4.3 *Mycobacterium fortuitum*

M. fortuitum is another type of NTM which is an acid-fast, rapidly growing *mycobacterium*,[64] 1-3 µm × 0.2-0.4 µm long, and rod shaped. It has been found in soil, natural water, and sewage. *M. fortuitum* has been found to be pathogenic and is associated with a wide range of human and animal diseases including skin tissue infection, induction of wound infection, pneumonia, abscess, and empyema.[65,66]

1.4.4 *Mycobacterium gordonae*

M. gordonae is classed as a slow growing NTM and can be found in environmental sources such as soil, natural water, tap water, or even unpasteurized milk. It was thought to be non-pathogenic and rarely to cause disease in humans, but surprisingly, recent reports showed cases of significant disease caused by *M. gordonae* infection such as pulmonary disease, disseminated infection, and genitourinary disease.[67-69] (**Figure 7**).

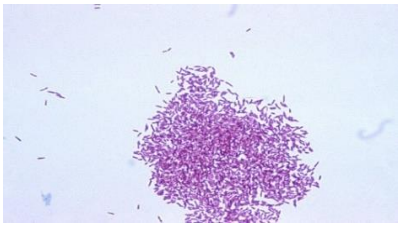


Figure 7: *M. gordonae* (coloration de Ziehl).[70]

1.4.5 *Mycobacterium avium*

M. avium is the most frequently encountered NTM associated with both human and animal disease. It is rod-shaped, and slow growing[71] (**Figure 8**). The organism can grow at 45 °C and in a medium with a pH of 5.5.[72] These characteristics, such as adaptation to various environmental conditions, and high resistance to the majority of antibiotics and disinfectants are the main reasons which have made *M. avium* difficult to eradicate.[73]

Figure 8: Transmission electron micrograph of *M. avium-intracellulare* in tissue.[74]

M. avium species are considered to be pathogenic especially for immunocompromised patients such as HIV positive patients. They are distributed widely in the environment; the main sources are natural water, tap water, food and soil.[75,76] There are three subspecies of *M. avium* including *M. avium* subsp., *M. avium paratuberculosis* and *M. intracellulare* collectively known as *M. avium* complex (MAC).[77,78]

1.4.6 *Mycobacterium kansasii*

M. kansasii is probably the easiest NTM pathogen to treat successfully, and it causes both pulmonary and extra-pulmonary infections. Indeed, it is the second most common NTM after MAC to cause disease in HIV infected individuals.[79] Also, because it is similar to *M. tb.*, mycobacterial isolates may be linked to lung disease which can be both aggressive and destructive, more information exists showing how effective antituberculosis drugs are for treating *M. kansasii* than all the other NTM infections (**Figure 9**).[80]

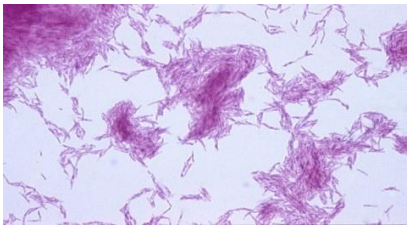


Figure 9: *Mycobacterium kansasii* (coloration de Ziehl).[80]

In fact, it can be found virtually anywhere in the environment; these bacteria live in water, soil, foods and a variety of animals. The organism has been isolated from tap water in some communities as cases of identified *M. kansasii* disease.[81-83] Infection by *M. kansasii* probably takes place via aerosols, although research has yet to confirm tap water as a major reservoir for *M. kansasii* causing human infection.[81]

1.4.7 *Mycobacterium alvei*

Mycobacterium alvei is a gram-positive, non-motile, acid-fast bacterium belonging to the *Mycobacterium* genus. The colonies of this *mycobacterium* are rough and non-pigmented and are 1-3 μm × 0.5-0.7 μm in size. It has been reported that they often form clumps but not cords (**Figure 10**).[84] Luquin *et al*. were the first to isolate this *Mycobacterium* strain from water samples from the River Llebregat in Barcelona.[85]

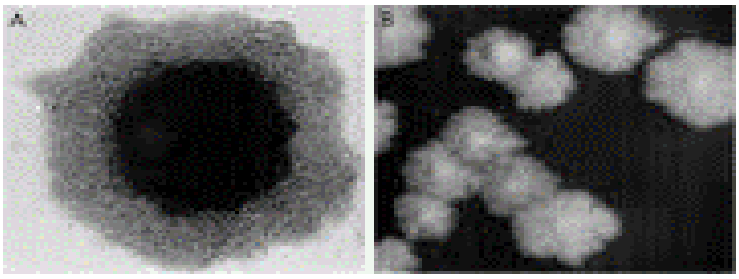


Figure 10: Mature *M. alvei.* [84]

This *Mycobacterium* was found to contain mycolic acids in its cell wall that had a methoxy group at the ω-1 position and 2 alkene groups in the mero chain (see Section 1.7.1). Ausina *et al.* have described a number of characteristics, which differentiate *M. alvei* from other related mycobacteria, and this differentiation was reported to be due to its unusual mycolate pattern.[84]

Linares *et al*. have reported that cord factors from *M. alvei* and *M. brumae* stimulate the secretion of pro-inflammatory cytokines of relevance in tuberculosis.[86] It was found that the induction of cytokines by the cord factors from the two mycobacteria discussed in this paper showed both similarities and differences to those from *M. tuberculosis*. The cord factors were strong inducers of TNF-α and IL-1β, with comparable or higher levels than the cord factor from *M. tuberculosis*, however the opposite was true for cytokines IL-6, IL-12 and IL-23. It is possible that such compounds may therefore be of interest for their immunological properties and could have a role as adjuvants, however further investigation is required.

Examples of infections caused by *M. alvei* in both animals and humans have been reported in the literature.[87,88] Beccati *et al*. described the occurrence of pyogranulomatous panniculitis in a cat and identified it to be caused by a rapid growing *Mycobacterium* (RGM). PCR of the mycobacterium was carried out and the sequence of a portion of the 16s rRNA gene was 100% homologous with *M. alvei*. This is the first description of feline mycobacterioses caused by this *Mycobacterium*; the clinical aspects of the infection in this case were similar to those documented for such infections caused by other RGM.[87]

Liu *et al*. described the first case of a prosthetic joint infection caused by *M. alvei*.[88] Following knee replacement surgery the patient developed an infection, which despite treatments did not improve. Further investigation and culture of the excised synovium on Lowenstein-Jensen slants for 2 weeks led to the development of rough colonies. Microscopic examination of these colonies showed gram-positive, acid-fast bacilli that formed clumps, with various tests suggesting that they were non-tuberculous mycobacteria. PCR restriction fragment length polymorphism (RFLP) showed patterns similar to those of *M. alvei* and to verify this the 16S rRNA gene was amplified. Comparison of the 2 DNA samples obtained, to those searched from the GenBank database, were found to be a 100 % match to *M. alvei*. The patient was given intravenous therapy with tigecycline and amikacin followed by oral ciprofloxacin and trimethoprim-sulfamethoxazole for a further 6 months and after this time her erythrocyte sedimentation rate (ESR) had reduced from 151 mm/h to 24 mm/h. Due to the rarity of such infections caused by RGM it is likely that such infections could be missed, and thus suitable treatment not given. As a result, it is important that any other cases caused by such mycobacteria and experiences of these infections are studied and reported in order to provide recommendations for the treatment of the infection. It is also important to study and identify the specific type of mycobacterium responsible for the infection.

1.5 The mycobacterial cell wall envelope

Mycobacteria have a unique cell wall structure that differentiates them from other bacteria. Understanding this structure is therefore important for the development of new methods to fight mycobacterial diseases such as TB.[89] The thick, multi-layered, hydrophobic cell envelope is important for the pathogenesis of such diseases as it prevents the passage of antibiotics and therapeutic agents into the cell, thus making treatment difficult. In addition, the cell wall structure also protects the mycobacteria

from the hosts immune system by allowing it to survive in macrophages. As can be seen (**Figure 11**), the mycobacterial cell envelope consists of three structural features: the plasma membrane, the cell wall (inner layer) and the outer layer (capsule).[90]

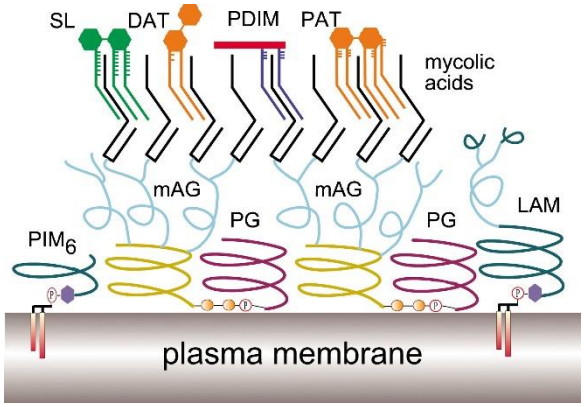


Figure 11: Cell wall of mycobacteria.[91]

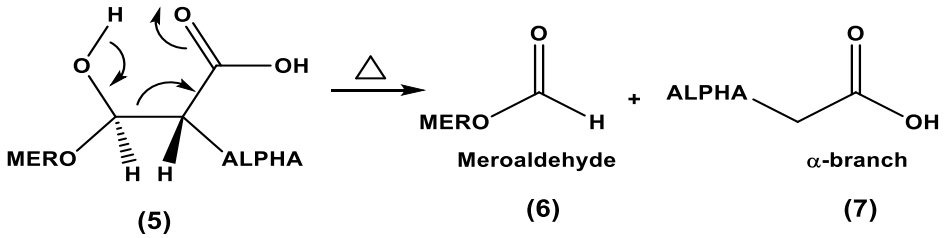
The plasma membrane of a *Mycobacterium* is consistent with that of other organisms; it seems to be a typical bacterial membrane and does not appear to have a particular role in the pathogenesis of the disease. However, the cell wall, the capsule layer and its linkages differ. The capsule layer consists of polysaccharides, proteins and a small amount of lipids,[92] while the cell wall consists of three covalently linked substructures: peptidoglycan (PG) linked to arabinogalactan (AG) by a phosphodiester bridge, which is in turn esterified with mycolic acids. PG consists of long polysaccharide chains, where *N*-Glycolylmuramic acid is linked in β1→4 to *N*-acetyl glucosamine in alternating positions, and cross linked to a four chain amino acid. The AG is composed of arabinose and galactose.[93] The mycolic acids are bound directly to the arabinogalactan layer of the cell envelope via the terminal hydroxyl group of the carboxylic acid functional group, arranged to form a monolayer which contributes to the protection and hydrophobicity of mycobacteria.[94,95] Together, this unit is commonly known as the mycolyl-arabinogalctan-peptidoglycan (mAGP) complex and it gives the cell wall its shape and rigidity.[96]

Mycolic acids contribute a significant amount to the overall weight of the mycobacterial cell envelope, around 40%, dry weight of the cell envelope. These lipids are highly complex and are thought to be responsible for the high resistance and low permeability of the cell wall to hydrophilic compounds. There are many lipids present in the cell wall of mycobacteria such as *e.g.* glycopeptidolipids (GPL) but the most reported lipids with

interesting toxic and immunological properties are trehalose esters (cord factors), which are trehalose esterified with mycolic acids.[97-100] These are bio-medically important glycolipids that have interesting toxicological and immunological properties.[91,101]

1.6 Mycolic Acids

Mycolic Acids (MAs) are one of the main components of the cell wall, they are complex hydroxylated high molecular weight α-alkyl branched ß-hydroxy long chain fatty acids with high carbon numbers (60-90 atoms).[102] Anderson (1939) described oxygen atoms to be present in carboxyl, hydroxy and methoxy groups. His experiments showed that mycolic acids had melting points in the range 54-56 °C and appeared as white powders and are characteristic components of the cell wall of all the strains of mycobacteria. He 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.[103,104] Based on the pyrolysis of MA **(5)**, Asselineau and Lederer confirmed the positions of the hydroxyl group (β) and a long alkyl chain (α) in relation to the carboxylic acid (**Scheme 1**).[105]



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

Minnikin *et al*. used 2D-TLC (Thin-Layerchromatography) for the separation of different fractions of mycolic acids from human *tubercle bacilli*, and used different analytical methods such as IR (Infra-red) spectroscopy , proton and carbon NMR (Nuclear Magnetic Resonance)spectroscopy and mass spectrometry to show that mycolic acids have a cyclopropane ring of differing stereochemistry, and also suggested the structure for the main frame of mycolic acids (**Figure 12**).[94] In further studies Minnikin proposed the structure of the cell wall.[94] The mycolic acids have two key moieties, the main moiety called the meromycolate moiety and the other one called the

mycolic motif. The mycolic motif is common for every mycolic acid and has the hydroxyl group and α-alkyl branch in the (*R*,*R*) configuration (**Figure 12**).

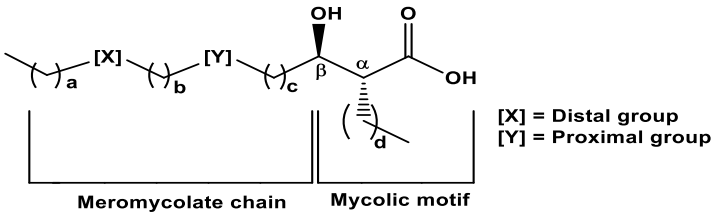


Figure 12: The general structure of a mycolic acid.

It has been reported that mycolic acids are esterified to the arabinogalactan within the mycobacterial cell envelope,[94,95] and that the meromycolate chains are aligned parallel to each other, with the methyl groups arranged so that they are towards the surface.[95] The stacking of the long hydrocarbon chains is affected by the functional groups present at the distal and proximal positions, as they vary in type, stereochemistry and spacing.[106] As the functionality of these positions appears to play an important role in the mycobacterial cell envelope, the resultant mycolic acids have been split into three types by their functionality: Type 1 mycolates contain no double bonds, type 2 include all *trans* olefin mycolates and type 3 have only *cis* olefins. (**Figure 13**) shows these three different types.[106,107]

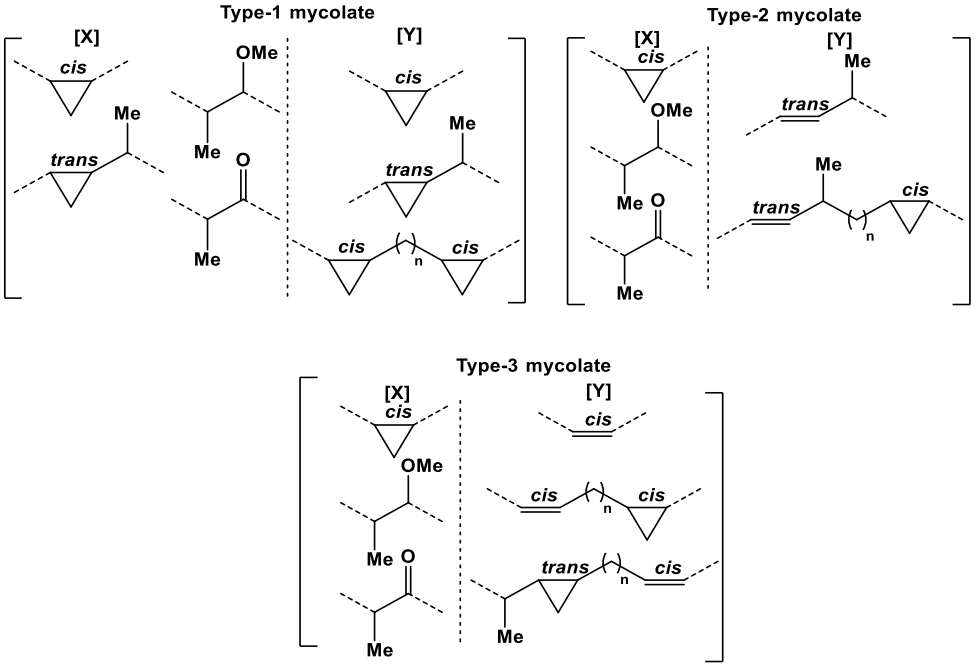


Figure 13: General functionality of the major mycobacterial mycolic acids.

In addition, based on the nature of the functional groups present in the meromycolate chains, mycolic acids from *M. tuberculosis* are categorized into three major groups: α-mycolic acids (**8**) with no oxygen-containing intra-chain groups; methoxy-mycolic acids (**9**) in which the distal group has a methoxy group; and keto-mycolic acids (**10**) in which the distal group has a carbonyl group. Methoxy-mycolic acids and keto-mycolic acids have methyl branches next to the oxygenated functional group and natural mixtures have both *cis*-cyclopropane and α-methyl *trans*-cyclopropane rings. Watanabe *et al.*[106,107] have studied the nature and the location of the groups in the meromycolate chains of mycolic acids from representative mycobacteria by extensive NMR spectroscopic and mass spectrometric analyses (**Figure 14**).

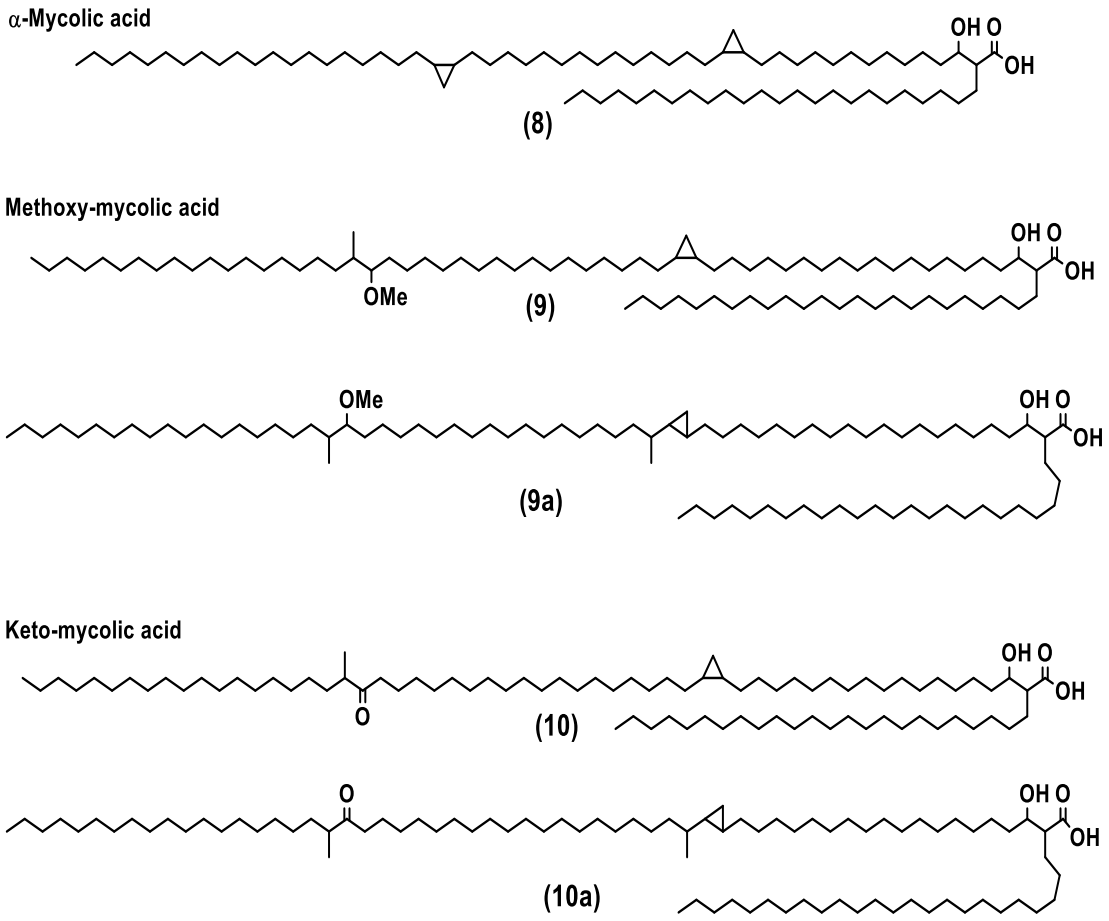


Figure 14: Major types of mycolic acids from *M. tuberculosis*.

In addition, some mycobacteria contain completely different sets of mycolic acids (**Figure 15**). For example, *M. smegmatis* contains α' and α-mycolic acids (**11, 12**) with

either one or two double bonds, either in the *cis* or the *trans* configuration. The α'-mycolic acids are shorter than α-mycolic acids, containing 60 carbons instead of 80, and are widely distributed within mycobacteria although absent from *M. tuberculosis*. *M. fortuitum* contains mycolic acids (**13**) with an epoxy ring. [108] Recently, more different oxygenated mycolic acids have been isolated in numerous mycobacteria; these include ω-carboxy-mycolic acids (**14**) from *Mycobacterium phlei*,[109] ω-1-methoxy-mycolic acids (**15**) from *M. alvei*,[85] and wax ester mycolic acids (**16**) from *M. avium*,[110] and hydroxy mycolic acids (**17**). There are also a number of mycolic acids with different combinations of the distal group and proximal group in different mycobacteria. Polar modifications are generally restricted to the distal position, whereas non-polar modifications occur at both the distal and the proximal positions.

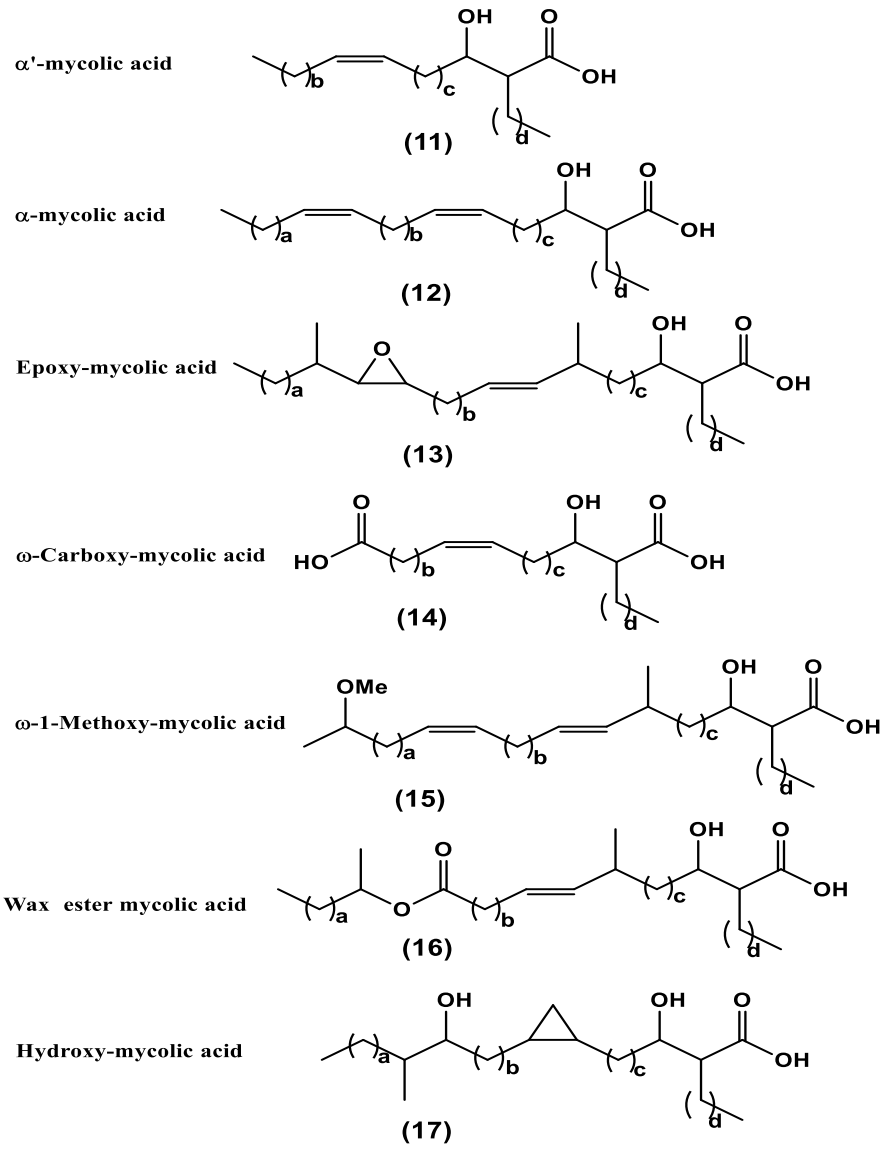
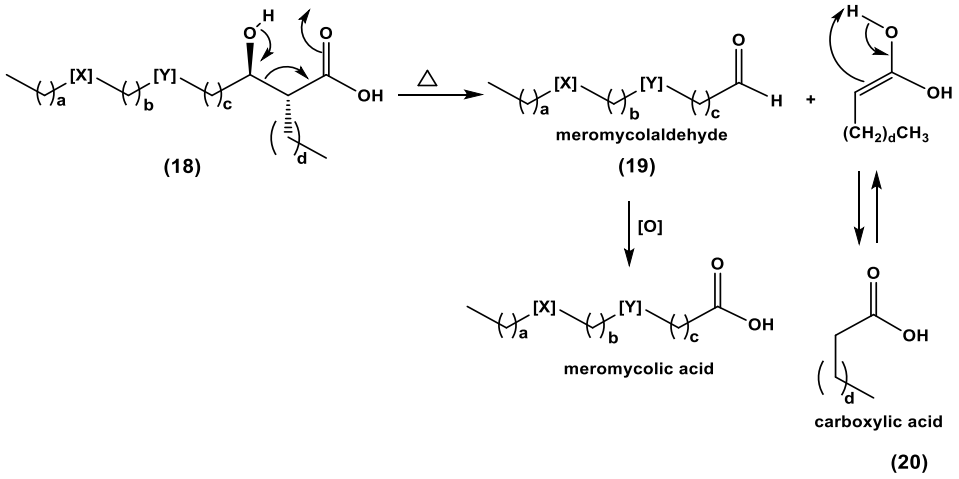


Figure 15: Mycolic acids from other mycobacteria.

1.7 The chain lengths of mycolic acids

Mycobacteria were reported not only to contain the three major types of mycolic acid, but also each one of those types is present as a number of different homologues. In the cell wall of *M. tuberculosis* over 500 mycolic acids with closely related chemical structures are present,[111] and this makes the separation process of mycolic acids extremely difficult.[112] The three types of mycolic acid can be separated, however, separation of individual mycolic acids of a similar type is extremely time consuming[111,113] and the most significant progress has been associated with the development of analytical tools.[111,114]

Since the discovery of mycolic acids there have been many studies in order to determine the chain lengths between the functional groups. The early attempts were done using GC with a high injector temperature, while Guerrant *et al.* attempted to analyse mycolic acids isolated from nontuberculosis mycobacteria using GC with two different injector temperatures. When the injector temperature was between 300 and 350 ^{o}C, thermal cleavage of the mycolic acids was observed and methyl esters with C_{22}, C_{24} or C_{26} chains were detected, but when it was set at 235 ^{o}C or lower the mycolic acids were heat stable and no methyl ester cleavage was observed.[115] Further studies adopted new tools for analysis like several techniques of mass spectrometry, in association with chromatography techniques for separation. Watanabe *et al.* determined the location of the functional groups in the meromycolic acids (prepared by pyrolysis followed by oxidation using silver oxide (**Scheme 2**) for 19 strains of the *M. tuberculosis* complex. The last step used MALDI-TOF mass spectrometry for the analysis of these fragments of mycolic acids.



Scheme 2: Pyrolytic cleavage of mycolic acid.[112]

The location of the meromycolic acids functional groups, *i.e.* cyclopropane rings, double bonds, epoxide rings, keto groups, methoxy groups and the methyl branch, and the chain length were studied using CID mass spectrometry as well as other spectroscopic methods.[107]

1.8 Mycolic acids from *Mycobacterium alvei*

As well as containing α-MAs *M. alvei*, as previously mentioned, also contains a unique type of MA, namely a ω-1methoxy mycolic acid, which contains a methoxy group at the ω-1 position and has two double bonds in the mero chain.

Luquin *et al*. were the first to isolate *M. alvei* from water samples from the River Llebregat in Barcelona.[85] This particular strain (CR 21) was cultivated on agar plates and the cells were harvested. In order to identify and characterise the new type of MA present they were isolated from these cells, purified and analysed by TLC. The methyl mycolates isolated showed 2 spots by TLC: one with a similar Rf to that of unsaturated mycolates and the other with a Rf similar to that of mycolates with an additional oxygen group in the mero chain. ^{1}H and ^{13}C NMR spectroscopy gave further information regarding the structures of these MAs, confirming that both had 2 double bonds in their mero chain, one of which (*i.e.* the *trans* alkene) had an adjacent methyl branch. In order to determine the position of these functional groups on the mero chain and identify the unknown oxygenated MA, chemical degradation of the mycolates and subsequent analysis of the cleavage compounds by NMR spectroscopy and mass spectrometry was carried out. The cleavage reaction was carried out using a permanganate/periodate oxidation,[85,116] which cleaved the alkenes to carboxylate groups, which were then isolated and esterified. Detailed analysis of the resulting products suggested that the unknown mycolic acid present had a methoxy group at the ω-1 position and 2 alkene groups in the mero chain (**Figure 16**).

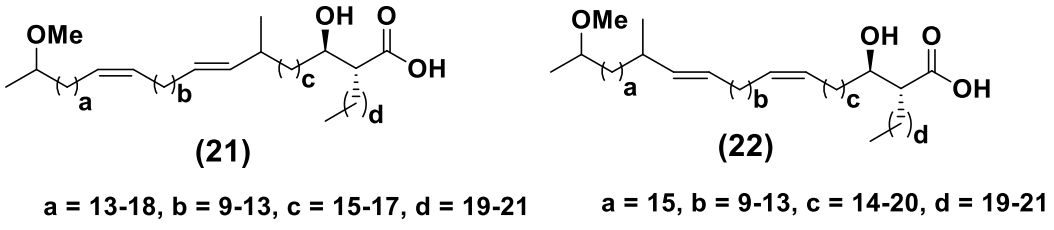
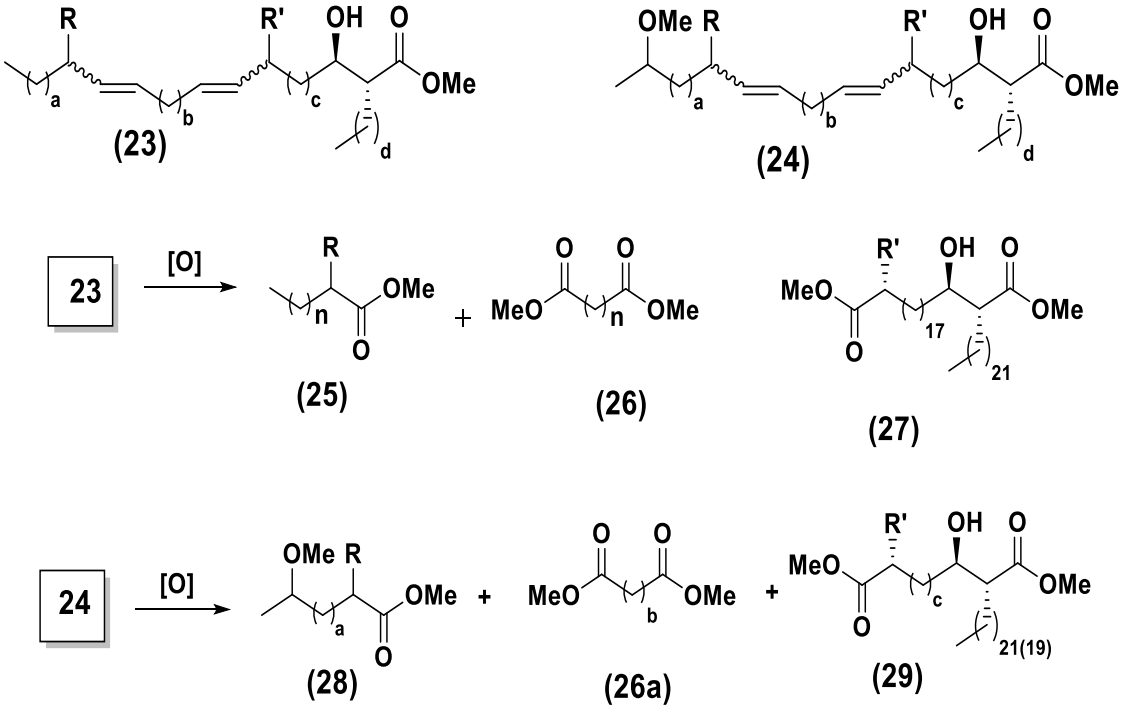


Figure 16: Proposed structure of the new type of mycolic acid isolated from *M. alvei*.

Although the presence of methoxy mycolates has been reported in other *Mycobacterium* species, this particular type of methoxy mycolate is unusual with respect to the position of the methoxy group in the mero chain: methoxy mycolates isolated from other mycobacteria usually contain the methoxy group at the ω-17 position. Another characteristic of this ω-1 methoxy MA that differentiates it from other oxygenated MAs is the presence of two double bonds in the mero chain; as opposed to only one double bond or a cyclopropane ring.[85] This novel type of MA has been reported to be present in some other *Mycobacterium* species, *e.g. Mycobacterium porcinum* and *Mycobacterium senegalense*, however in these species it is always accompanied by an epoxy-MA.[84]

More recently, following the procedures previously reported,[85,116] Jones *et al.*[117] hydrolysed cells from *M. alvei*, and converted the acids obtained to their methyl esters using diazomethane. The methyl MAs were then separated by column chromatography to give 2 fractions: one containing a diene mycolate (**23**) and the other containing a ω-1 methoxy mycolate (**24**). Oxidative cleavage of these two mycolates was then carried out, using periodate/permanganate, again as previously reported,[85,116] to give the methyl esters shown below (**25-29**) (**Scheme 3**). These were characterized by NMR spectroscopy and GC-MS to determine the exact chain lengths.



Scheme 3: Oxidative cleavage of two mycolates (**23, 24**).

In the fragments obtained from the diene mycolate (**23**), fragment (**25**) was shown by GC-MS to be a 3:1.2:1 mixture of methyl nonadecanoate, methyl heptadecanoate and methyl 2-methyloctadecanoate. The central fragment (**26**) was shown to be a mixture of 3 main unbranched dimethyl esters, namely dimethyl pentadecandioate, dimethyl tetradecandioate and dimethyl heptadecandioate, in a 2:1:1 ratio. The C13, C16 and C18 dimethyl esters were also present as minor homologues. In the NMR spectrum of the hydroxy acid fragment there was a doublet corresponding to the methyl group adjacent to the ester at δ 1.15 (**27**, R' = Me) and a triplet for the methylene group adjacent to the ester at δ 2.3 (**27**, R' = H). Integration of these two signals suggested that the majority of the MA contained a methyl-*trans*-alkene with a methyl group at the proximal position.

The NMR spectrum of fragment (**28**) obtained from the cleavage of the ω-1 methoxy mycolate, clearly showed the presence of the CH_3-$CH(OCH_3)$-CH_2 unit due to a singlet at δ 3.22, a sextet at 3.29 and a doublet at 1.13 corresponding to the methoxy group, the CH and the terminal methyl group, respectively. The GC-MS of this fragment had two peaks in a 3:2 ratio with the major peak corresponding to synthetic $CH_3CH(OCH_3)(CH_2)_{14}COOCH_3$, (see results and discussion section), and the minor one to $CH_3CH(OCH_3)(CH_2)_{16}COOCH_3$. The mixture of compounds (**28**) showed $[\alpha]_D^{22}$ – 1.55 (*c* 0.39, $CHCl_3$). As with the alkene mycolate, the central fragment obtained from the oxidative cleavage of the ω-1 methoxy mycolate was a mixture of dimethyl esters **(26a)**. The majority of this fragment contained one homologue, dimethyl pentadecan-1,15-dioate, with a small amount, ca. 15%, dimethyl tetradecandioate, and less than 5% of two higher homologues. The final fragment obtained was a mixture of hydroxy esters (**29**) (R' = Me and R' = H). It principally included a $CH_3OOCCH(CH_3)$ fragment from the oxidation of a *trans*-alkene MA with a proximal methyl group, as determined by NMR spectroscopy; from integration there was ca. 25 – 30% of (**29**) (R' = H). From this data, the major ω-1 methoxy mycolic acid present was therefore suggested to be (**24**), R = H, R' = Me, a = 14, b = 13, c = 17 and d = 21.

The MALDI MS of the natural mixture (**Figure 17**) showed a major series of homologues starting at M+Na 1163 ($C_{77}H_{152}O_4$), with the major cluster at 1191 ($C_{79}H_{156}O_4$). In addition, there was a minor series (ca. 1:4) starting at 1149 ($C_{76}H_{150}O_4$) and ending at 1233. These two series can reasonably be assigned to a series with one *cis* and one methyl-*trans*-alkene, and a series of di-*cis*-alkenes.

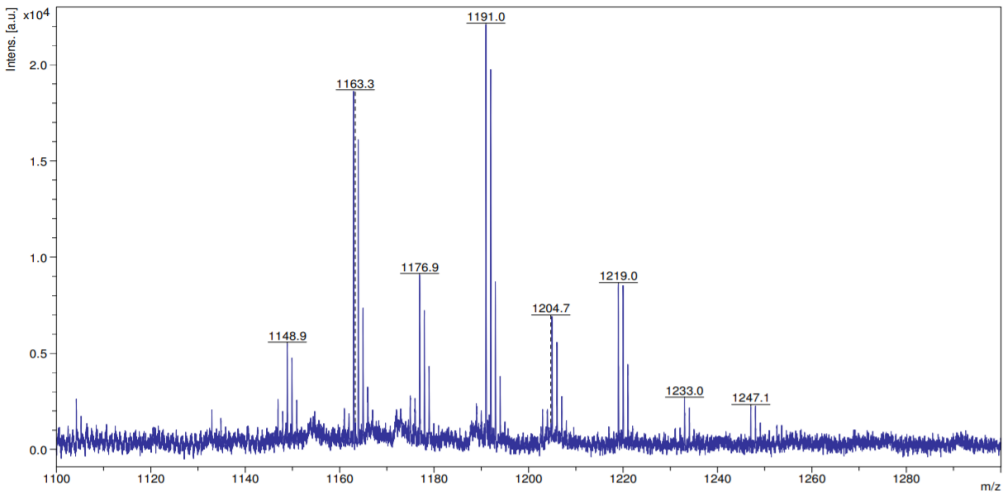
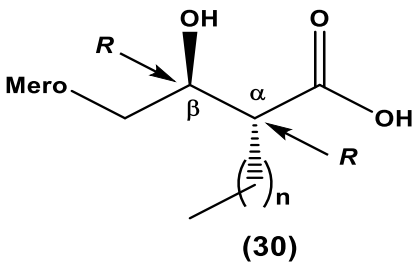


Figure 17: MALDI MS of natural methyl (ω-1)-methoxymycolate fraction.

Synthesising single enantiomers of the ω-1 methoxy mycolic acids from *M. alvei*, and their related sugar esters, would be of interest as it would allow their properties to be studied. It would however first be necessary to determine the absolute stereochemistry of the ω-1 methoxy group. This could be achieved by comparing synthetic fragments, containing a ω-1 methoxy group, with known stereochemistries, to the natural material (see results and discussion section).

1.9 Stereochemistry of mycolic acids

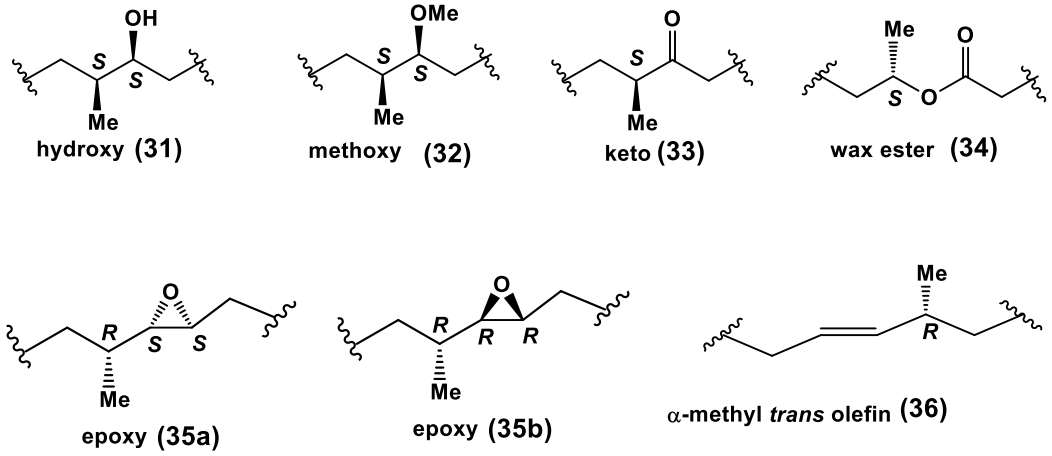
The stereochemistry of the chiral centres contained in mycolic acids has still not been completely clarified. The two stereochemistries at the α- and β-position of the mycolic motif have been found to both be in the *R*-configuration for all mycolic acids examined, irrespective of the groups in the meromycolate chain.[94,118-120] The (*R*,*R*)-configuration was confirmed first for the corynomycolic acids (**Figure 18**).[121,122] The formation of a hydrogen bond between the hydroxyl group and the carboxylic group has a stabilising effect for the aligned configuration between the two long chains.[123,124]



(30)

Figure 18: The chiral centres in the β-hydroxy fatty acid moiety.

The configuration at these two chiral centres is thought to play a vital role in T cell recognition, and the generation of an immune response by the host organism against pathogenic mycobacteria.[125] The latest stereochemical studies on different mycobacteria suggest that the methyl branch adjacent to the hydroxy (**31**), methoxy (**32**) and keto (**33**) groups in the mycolic acids is in the *S*-configuration. The hydroxyl (**31**) and the methoxy (**32**) at the distal group in the mycolic acids are also thought to be in the *S*-configuration (**Scheme 4**).



Scheme 4: The strereochemistry of chiral centres of the mycolic acids.

Other reports identify an *R*-stereochemistry for the three stereocentres of the α-methyl-*trans*-epoxy unit in related mycolic acids (**35a, 35b**). However, Al Dulayymi *et al*.[126] suggested that the epoxy fragment is *R*,*S*,*S* as in (**35a**) rather than *R*,*R*,*R* as in (**35b**). Furthermore, the methyl branch next to the *trans*-alkene unit (**36**), present in mycolic acids is in the *R*-configuration.

1.10 Biosynthesis of mycolic acids

The biosynthesis of mycolic acids can be divided into four different steps which involve: (**a**) synthesis of the long chain saturated fatty acids which provide the main carbon skeleton; (**b**) synthesis of the carbon chain backbone of the meromycolate unit; (**c**) introduction of different functional groups in the meromycolate chain; and (**d**) the final condensation to link the meromycolate unit to the mycolic motif. Several theories have been proposed for different mechanisms for the processes of cyclopropanation, oxygenation and methylation in the biosynthesis of mycolic acids. Studies have been conducted in which labelled methionine was incorporated in the growth of mycobacteria

and it has been found that the methyl group of methionine can be directly integrated into mycolic acids. It has also been found that the linkage of the methylenes of the cyclopropane rings, the carbon of the methoxy and the methyl branches adjacent to *trans*-olefins, methoxy and keto moieties are all derived from methionine.[120,127]

It has been suggested that most major functionalities in the meromycolate are derived from a common intermediate. This intermediate is generated using *S*-adenosyl-*L*-methionine (SAM) (**37**). Methylation of a *cis*-alkene (**38**), using SAM, gives the carbocation intermediate (**39**) (**Figure 19**). Labelled SAM has been used so that the methylation and any subsequent reaction can be monitored. This showed that the methyl group introduced occurs as a bridging methylene in *cis*- cyclopropanes, as methyl branches of *trans*-olefin and cyclopropanes, and the α-methyl branches of hydroxy, keto and methoxy mycolates. [128-130]

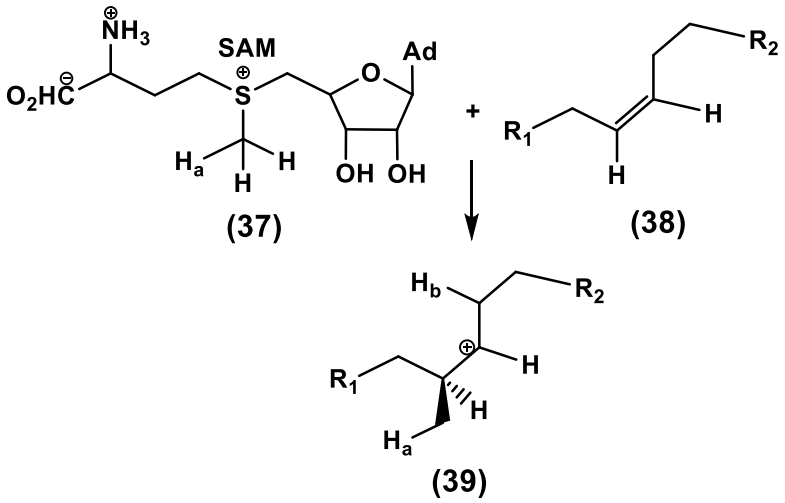


Figure 19: Formation of carbocation intermediate (**39**).

The deprotonation of carbocation intermediate (**39**) could occur by two different processes. If the removal of H_a occurs this leads to a *cis*-cyclopropane ring (**40**); conversely if deprotonation of H_b occurs, then the *trans*-olefin (**41**) is formed. Additionally, methylation of the *trans*-double bond, involving SAM, gives the *trans*-cyclopropane mycolic acid (**42**). If the carbocation intermediate (**39**) undergoes a hydration reaction, the hydroxymycolate (**43**) is formed, which is a precursor for the biosynthesis of the corresponding keto (**44**) and methoxy-mycolates (**45**) **(Figure 20**).

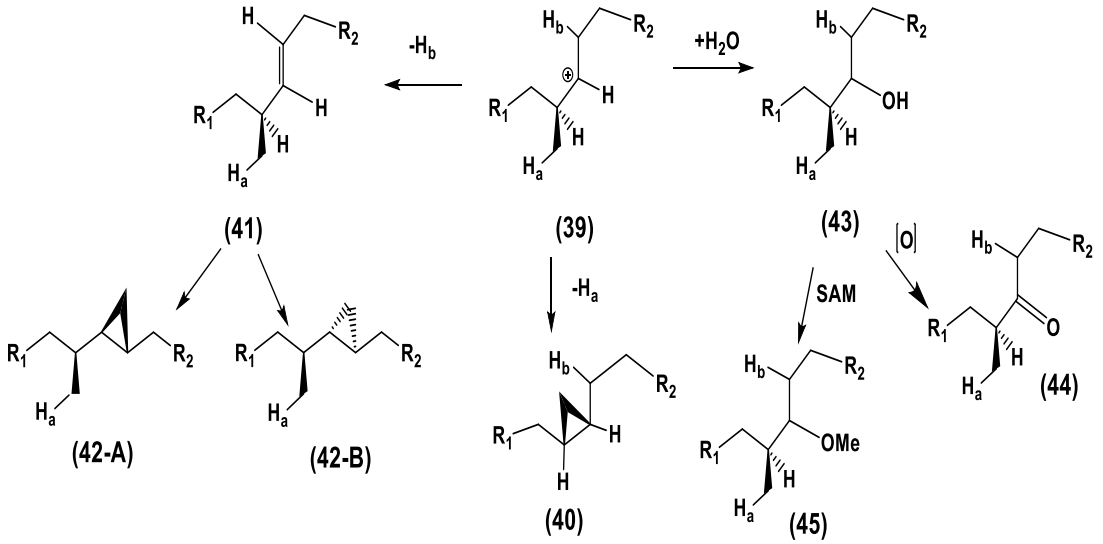


Figure 20: The insertion of the non-oxygenated functional groups in mycolic acids.

The gene in *M. tuberculosis* which is required for the biosynthesis of *trans*-cyclopropylmycolates,[130] confirms the hypothesized biosynthetic link between the different functionalities. The biosynthesis of mycolic acids is of great importance to understanding the impact of individual functionality on virulence, cell wall fluidity and permeability, and how the different mycolates are naturally produced enables one to determine how to manipulate the cell wall of the pathogen, and thus provides a tool with which to combat disease.[131]

1.11 Synthesis of mycolic acids

1.11.1 Aims

There are many practical reasons for the synthesis of mycolic acids. Single synthetic enantiomers of mycolic acids may help in understanding the physical and chemical properties of natural mycolic acids. The synthesis of these compounds will be important for the identification of the exact structure of natural mycolic acids. In particular, the configuration of the different chiral centres present in the meromycolate chain could be revealed through comparison between the natural compounds or their derivatives with the synthetic analogues. Additionally, through a more accurate analysis of the stereochemistry of the chiral centres present in the meromycolate chain, very important information about the biosynthesis of these compounds might also be acquired.

Currently, biological tests of mycolic acids for their effects in tuberculosis and asthma are of great interest. They could help to develop methods for detecting TB and other mycobacterial diseases. The functional groups in the meromycolate chain and the length

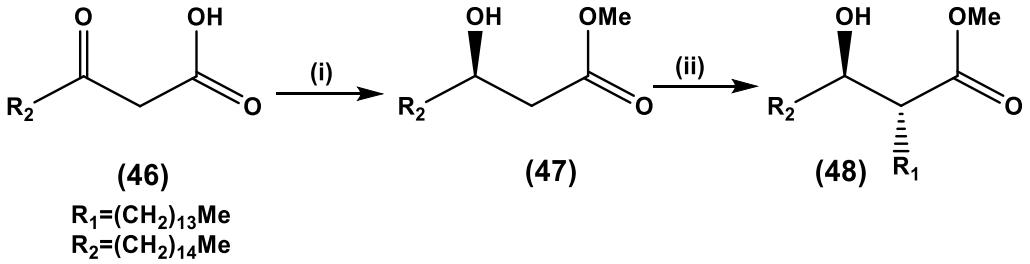
of the α-unit have been recognized as significantly influencing the physical properties of monolayer films of mycolic acids. The preparation of different synthetic analogues could also help in the determination of the role of each particular feature of the acids in the regulation mechanisms of the drug permeability of the cell envelope.

Another reason to produce synthetic mycolic acids is that antibodies produced against different synthetic mycolic acids which have similar but not equivalent structures, could be utilized for the identification of the structure and stereochemistry of the mycolic acids in mycobacteria. If only one type of these particular antibodies recognized the *Mycobacterium*, the synthetic mycolic acid used as the antigen to produce these competitive antibodies, should have the same structure as the natural one. This type of analysis has already been utilized for a partial characterization of the structure of the cord factors and their mycolic acids.[125,132]

1.11.2 Synthesis of the mycolate motif unit (48), (54), (63), (65) and (66)

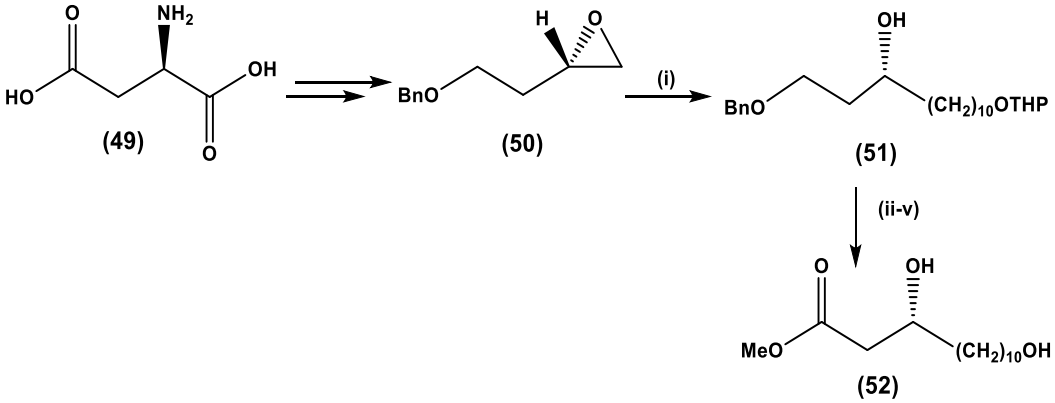
Several studies have been conducted with respect to the preparation of the α-alkyl-β-hydroxy unit. Lederer *et al* conducted one of the earliest trials to synthesise this kind of compound in 1952.[133] The same group, followed by others, using similar methods based on the Claisen condensation, prepared other corynomycolate analogues, but always as a mixture of diastereoisomers.[134,135] Later, Kitano *et al*. synthesised the first enantiomerically pure α-alkyl-β-hydroxy unit.[136,137] This method was a key feature of the preparation of optically active compounds with the correct stereochemistry at the α-position.

In 1987, Utaka *et al*. reported an approach,[138] in which they introduced the hydroxy group at the β-position by stereoselective reduction of the β-ketoester (**46**) with baker's yeast (**Scheme 5**).[139] They then directly introduced an alkyl chain at the α- position of a β-hydroxy ester (**47**) in a Fräter reaction,[140] to give the α-alkyl-β- hydroxyl carboxylates (**48**) with the correct chain length and with two chiral centres in the correct configuration, where the hydroxy group of the molecule (**47**) forced the incorporation of the alkyl chain with the correct configuration.



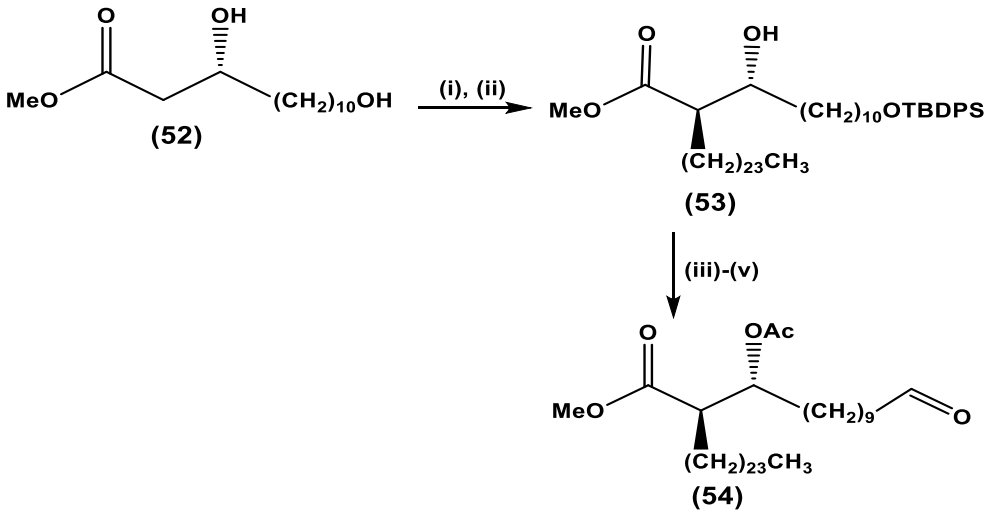
Scheme 5: The Utaka *et al.* method.[138] Reagents: (i) Bakers yeast, KOH, EtOH; (ii) R_1I, LDA, HMPA.

The approach of Baird and Al Dulayymi *et al.*[141] was subsequently used to synthesize the mycolic motif part originating from *R*-aspartic acid (**49**). The aspartic acid was converted to epoxide (**50**) in 3 steps and was then ring opened with a Grignard reagent to give a single enantiomer of the mono protected diol (**51**). This was transformed in four steps (protection, debenzylation, oxidation and esterfication) into the diol (**52**) (**Scheme 6**).



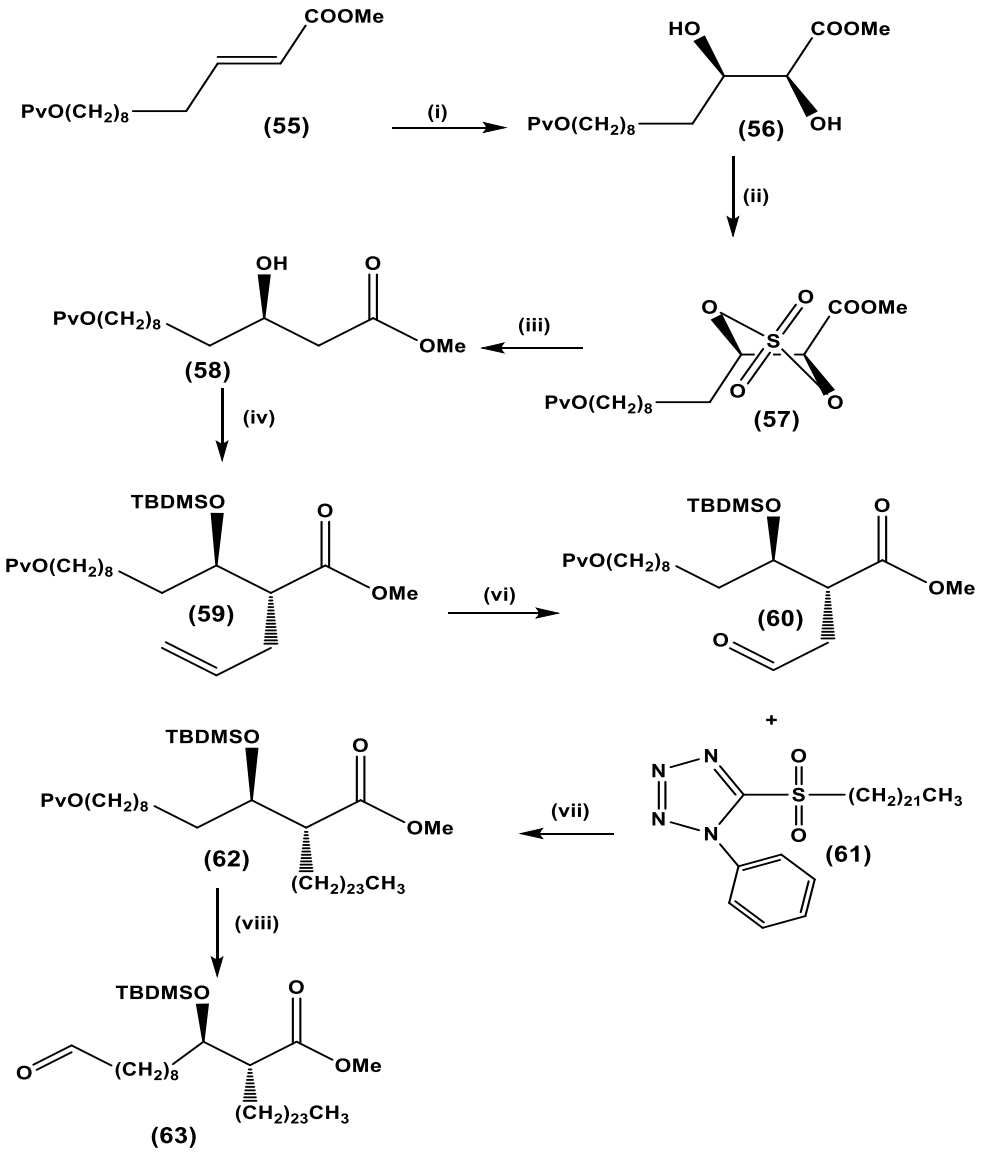
Scheme 6: Ring opening of epoxide (**50**). Reagents: (i) $BrMg(CH_2)_9OTHP$, CuI, 2hrs; (ii) Imidazole, DMF, TBDMSCl; (iii) H_2, Pd/C, MeOH; (iv) $NaIO_4$, $RuCl_3.H_2O$, CCl_4; (v) MeOH, H_2SO_4.

Again, protecting the diol (**52**) with the *tert*-butyldiphenylsilyl group, followed by a Fräter alkylation with 1-iodotetracosane (prepared by coupling dodecyl-magnesium bromide with 12-bromododecanal then iodination) gave the α-alkyl-β-hydroxy ester (**53**). Protection of the secondary alcohol of (**53**) as the acetate, deprotection of the primary alcohol and oxidation led to the mycolic motif aldehyde (**54**) (**Scheme 7**).



Scheme 7: Reagents: (i) TBDPSCl, DMAP, Et_3N; (ii) LDA, $CH_3(CH_2)_{23}I$, HMPA; (iii) Ac_2O, pyridine; (iv) TBAF; (v) PCC.

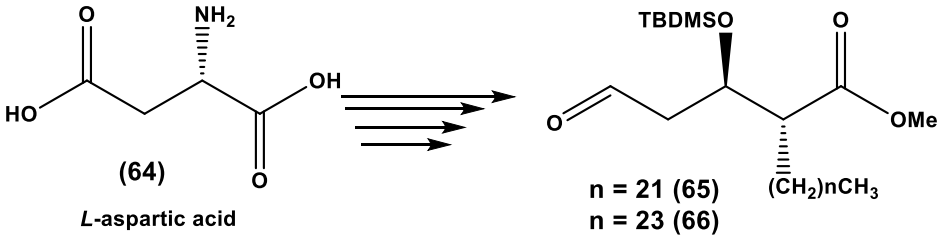
Recently, Toschi and Baird conducted an improved synthesis of the α-alkyl-β- hydroxyl unit (**63**).[142] In this synthesis, the reaction started from 1,10-decanediol to prepare the *cis*-olefin (**55**) in four steps. The olefin was converted into α,β-dihydroxy compound (**56**) which was transformed into a cyclic sulfate (**57**) and then regioselectivity reduced and hydrolysed to (3*R*)-3-hydroxy ester (**58**). Subsequently, a Fräter allylation,[140,143] with allyl iodide introduced an allyl chain at the α-position and the hydroxyl group was protected to give an (2*R*,3*R*)-2-allyl-3-hydroxy ester (**59**). Converted the ester (**59**), to aldehyde (**60**), followed by chain extension at the α -position, using a Julia reaction, and then saturation of the alkene intermediate gave compound (**62**). Finally, removal of the pivalate group from (**62**) followed by oxidation to the aldehyde gave the desired compound (**63**) (**Scheme 8**).



Scheme 8: Toschi and Baird improved synthesis of the α-alkyl β-hydroxy unit.[142] Reagents: (i) $(DHQD)_2PHAL,K_3Fe(CN)_6$, K_2CO_3,OsO_4, $MeSO_4NH_2$, $^tBuOH,H_2O$; (ii) $SOCl_2$, H_2SO_4; (iii) $NaBH_4$, H_2SO_4; (iv) LAD, allyliodide, TBDMSCl, imidazole, DMF; (vi) OsO_4, 2,6-lutidine, $NaIO_4$; (vii) $LiN(SiMe_3)_2$, THF, H_2, Pd on C; (viii) KOH, THF, MeOH, H_2O, PCC,DCM.

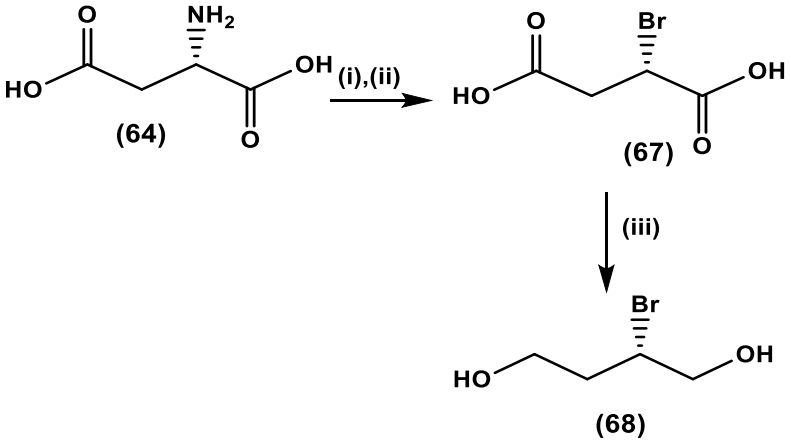
The interesting aspect to this strategy is that the generation of the aldehyde (**63**) allows the introduction of any chain length desired at the α-position, via a modified Julia-Kocienski olefination.

Al Dulayymi *et al.*[141] also used *L*-aspartic acid (**64**) as starting material to prepare the mycolic motif unit (**65** and **66**), instead of *R*-aspartic acid, as described above. This was advantageous as *L*-aspartic acid is much cheaper than the *R*-aspartic acid; this work was described by Koza *et al.* (**Scheme 9**).[144]



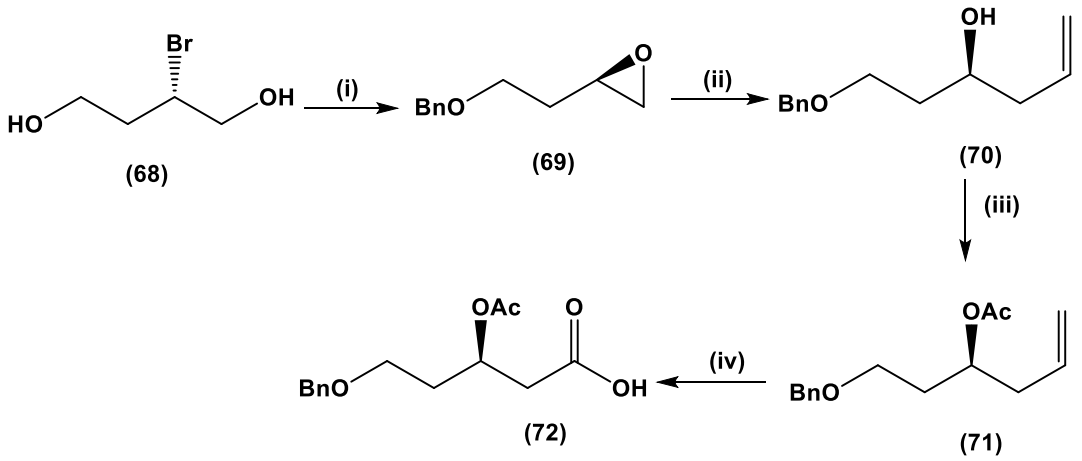
Scheme 9: The preparation of mycolic motif units (**65** and **66**).

Firstly, α-bromosuccinic acid (**67**) was synthesised from *L*-aspartic acid (α-amino acid) with retention of configuration by using of $NaNO_2$ and potassium bromide in the presence of sulfuric acid. The concentration of sulfuric acid was critical in this reaction, the salt precipitated making the reaction impossible to complete. The subsequent step was conversion of the acid to the diol (**68**) using borane tetrahydrofuran complex (**Scheme 10**),[145] while leaving the bromine group unchanged because it is a mild reducing agent. The borane was able to reduce the two carboxylic acids into a diol.



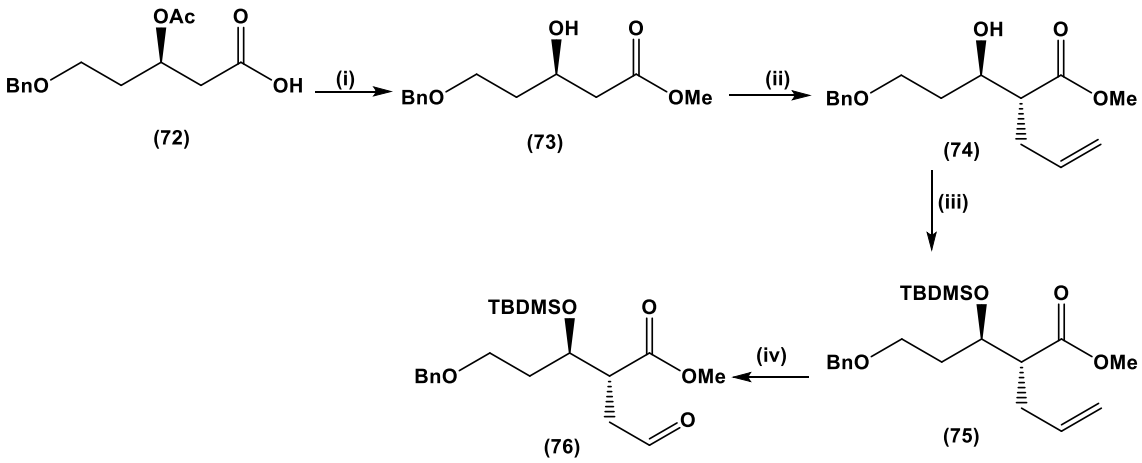
Scheme 10: Preparation of diol (**68**). Reagents: (i) H_2SO_4, $NaNO_2$; (ii) KBr, H_2O; (iii) BH_3.THF.

The next step was the formation of the epoxide intermediate (**69**) with simultaneous protection of the other hydroxyl group with a benzyl protecting group. The intermediate (**69**) was ring opened with a Grignard reagent to extend the chain by two carbon atoms (**70**). Then the protection of the secondary alcohol with an acetyl group was carried out using acetic anhydride and pyridine as catalyst in dry toluene to form compound (**71**). Oxidative cleavage of the alkene (**71**) with OsO_4 and oxone in DMF gave the carboxylic acid (**72**) (**Scheme 11**).[144]



Scheme 11: Preparation of carboxylic acid **(72)**.[144] Reagents: (i) NaH, BnBr, TBAl, THF; (ii) CuI, Vinyl MgBr; (iii) toluene, acetic anhydride, pyridine; (iv) OsO_4, Oxone, DMF.

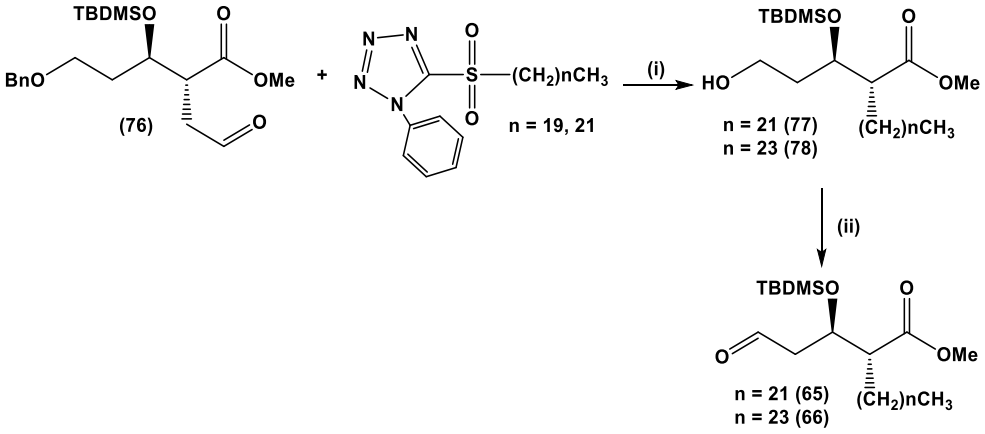
Esterification of the carboxylic acid group and the deprotection of the secondary alcohol was achieved by refluxing the acid (**72**) in methanol to form the desired ester (**73**) (methanol was used as reagent and solvent). Then, the insertion of the alkyl chain, as an allyl group at the α-position, with respect to the carboxylic acid group was achieved by a Koza reaction to give alkene (**74**). The secondary alcohol was then protected as a *tert*-butyldimethylsilyl ether (**75**). Subsequently oxidative cleavage of olefin (**75**) with OsO_4–$NaIO_4$ and 2,6-lutidine in dioxane-water gave the aldehyde (**76**) (**Scheme 12**).[144,145]



Scheme 12: Insertion of an allyl group at the α-position. Reagents: (i) H_2SO_4, MeOH; (ii) MeLi THF, HMPA, allyl iodide; (iii) TBDMSCl, imidazole, DMF, – 45 °C; (iv) $NaIO_4$, OsO_4, 2,6-lutidine, dioxane water.

The aldehyde compound (**76**) was coupled with a C-20 or C-22 sulfone via a modified Julia reaction, followed by hydrogenation of the resulting alkenes and debenzylation to

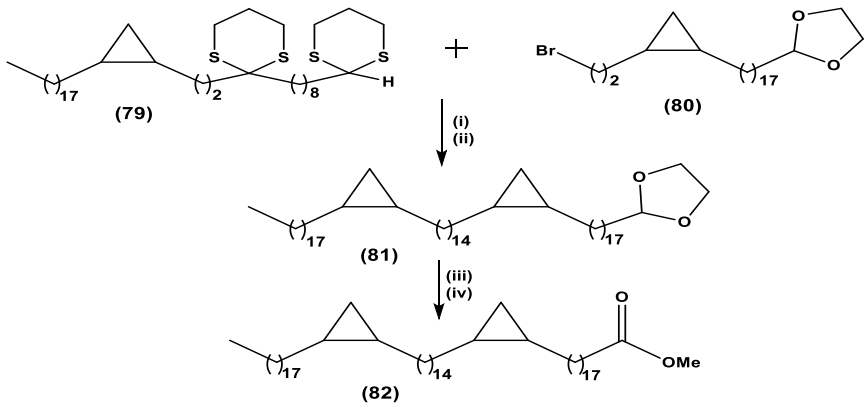
give alcohols (**77**) and (**78**) respectively. Finally, oxidation of the alcohol compounds (**77** and **78**) to form the mycolic motif unit (aldehyde compound) was achieved using pyridinium chlorochromate (PCC) in dichloromethane (**Scheme 13**).



Scheme 13: Preparation of the mycolic motif with two different chain lengths. Reagents: (i) $LiN(SiMe_3)_2$, THF, – 10 °C; H_2, Pd on C, EtOAc; (ii) PCC, DCM.

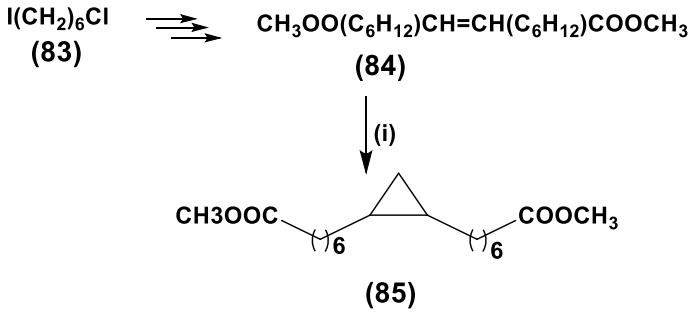
1.11.3 Synthesis of the meromycolate unit

In 1977, Gensler *et al.*[146] described for the first time a synthesis of a mixture of four stereoisomers of meromycolic acid containing two *cis*-cyclopropanes. In this method 1,4-cyclohexadiene was used as a starting material to prepare meromycolate stereoisomers after several synthesic steps. Coupling of the different units of (**79**) and (**80**) gave a structure (**81**) which had the same carbon number as a meromycolic acid. Finally, this compound was transformed into the desired product (**82**) by desulfurisation and ozonolysis (**Scheme 14**).



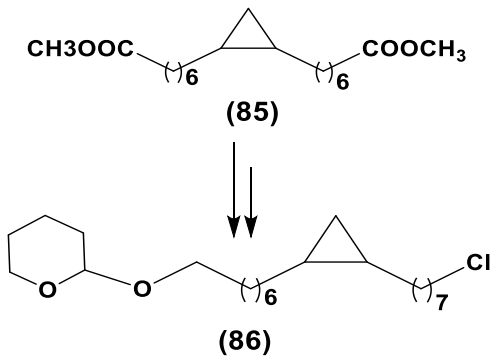
Scheme 14: Synthesis of the first meromycolic acid.[146] Reagents: (i) BuLi, THF, Ph_3CH; (ii) Raney nickel; (iii) O_3; (iv) MeOH.

Another approach to the synthesis of meromycolic ester (**82**) was demonstrated by the same authors.[147] This method has fewer steps and is also more reliable to scale up (**Scheme 15**). The starting material was 1-chloro-6-iodohexane (**83**) which, after several steps was transformed into alkene (**84**) with two functional groups on either side of the double bond. This alkene was then used as the starting material for cyclization with diiodomethane and zinc, resulting in compound (**85**) which has a cyclopropane ring in a *cis*-configuration.



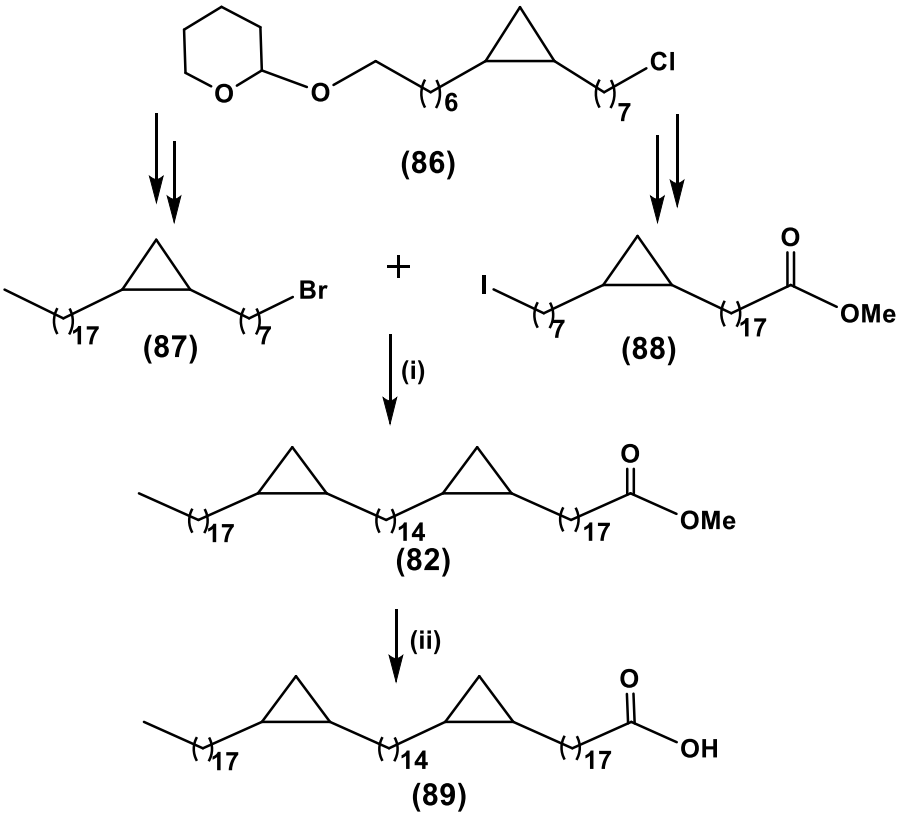
Scheme 15: The initial steps in the second Gensler *et al* approach.[147] Reagents: (i) DCM, Zn.

The next step was reduction of the functional groups, followed by a few steps to differentiate between the resulting alcohols to give the compound (**86**) (**Scheme 16**).



Scheme 16: Replacing the functional groups.[147]

After the unit (**86**) had been obtained, a Grignard reaction was used as the key step for extending the alkyl chain to obtain (**87**) and (**88**). These two fragments were then joined to obtain meromycolate (**82**), which was subjected to hydrolysis to give meromycolic acid (**89**) (**Scheme 17**).

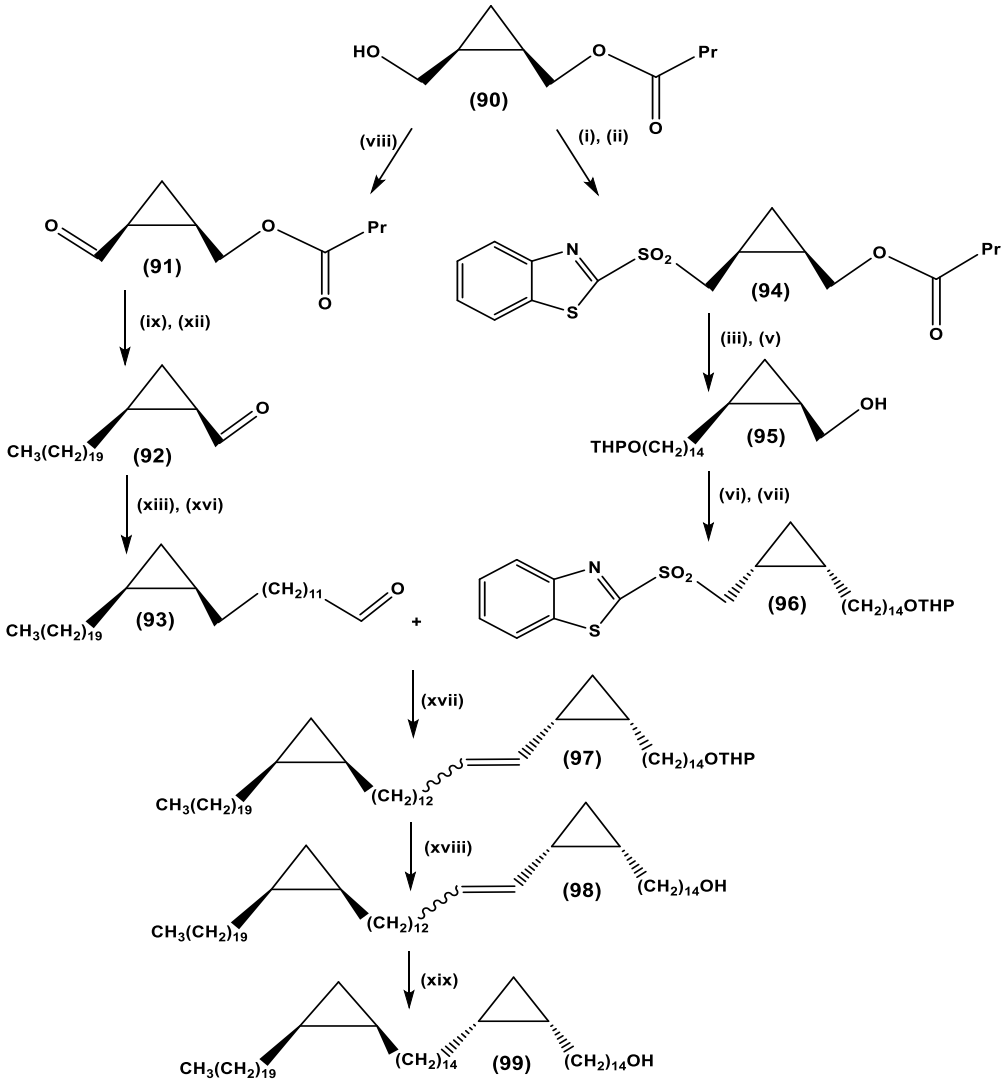


Scheme 17: The second synthesis of a meromycolic acid.[147] Reagents: (i) $Mg.CH_3Cu$; (ii) NaOH.

This method was an improvement over the first approach but gave a very poor yield. Several other side products were reported and only a small percentage of the crude product mixture was obtained as the desired product. Additionally, the absolute stereochemistry of the chiral centres of the cyclopropane rings was not controlled in Gensler's method.

In 2000, Al Dulayymi reported another approach for the first synthesis of a single enantiomer of an analogue of meromycolic acid (**99**).[148] This approach involved two key steps: first, the preparation of single enantiomers of cyclopropane intermediates,[149] followed by the coupling of these intermediates with no loss of stereochemistry. The aldehyde (**91**) was prepared from the anhydride of cyclopropane-*cis*-1,2-dicarboxylic acid. A Wittig reaction of this with nonadecyltriphenylphosphonium bromide and *n*-butyl lithium, and reduction by lithium aluminium hydride gave alcohol as a mixture of *Z*- and *E*-isomers. The di-imide was used to saturate the derived alkane and oxidation of the alcohol led to aldehyde (**92**). Chain extension with a second Wittig reaction followed by saturation and oxidation gave the aldehyde (**93**). A Julia reaction of sulfone (**94**) with

13-tetrahydropyranyloxytridecanal gave the protected alcohol (**95**), which was further converted into sulfone (**96**). This method has an important feature, the coupling reaction used to link the different units in many stages, securing the final desired stereochemistry. The Julia reaction between aldehyde (**93**) and sulfone (**96**) gave (**97**) as a mixture of *E* and *Z*-alkenes. The subsequent deprotection of the alcohol group and saturation of the derived alkene with di-imide gave the enantiomerically pure alcohol (**99**) (**Scheme 18**).



Scheme 18: Al Dulayymi *et al.*'s synthesis of an enantiomerically pure meromycolic acid: Reagents: (i) Benzthiazole, DEAD, PPh_3; (ii) *m*-CPBA; (iii) $THPO(CH_2)_{12}CHO$, $NaN(SiMe_3)_2$, THF; (iv) K_2CO_3, MeOH; (v) NH_2NH_2, $CuSO_4$, CH_3COOH, *i*-PrOH/$NaIO_4$; (vi) Benzthiazole, DEAD, PPh_3; (vii) *m*-CPBA; (viii) PCC, DCM; (ix) $Me(CH_2)_{18}{}^+PPh_3Br^-$, BuLi; (x) $LiAlH_4$, THF; (xi) NH_2NH_2, $CuSO_4$, CH_3COOH, i-PrOH, $NaIO_4$; (xii) PCC, DCM; (xiii) $MeOOC(CH_2)_{11}{}^+PPh_3Br^-$, BuLi; (xiv) $LiAlH_4$, THF; (xv) NH_2NH_2, $CuSO_4$, CH_3COOH, i-PrOH, $NaIO_4$; (xvi) PCC, DCM; (xvii) $NaN(SiMe_3)_2$; (xviii) MeOH, THF, pTSA; (xix) NH_2NH_2, $CuSO_4$, CH_3COOH/*i*-PrOH, $NaIO_4$.

Important advantages of this method include better overall yield and control of the absolute stereochemistry. Different portions of the compounds were built-in at different stages of the synthesis, and, as such, the method can be easily applied in the synthesis of other meromycolates with varied chain lengths and functional groups. It also allows for preparation of the other stereoisomers by simple modification in the sequence of reactions, which controls the position of the substituents on the cyclopropyl groups. As a result, it is particularly useful in producing all the possible diastereoisomers of meromycolaldehydes.

Also, in 2006, Al Dulayymi *et al.* synthesised various meromycolic acids which contain an α-methyl-*trans*-cyclopropane unit.[126,150] Mainly, the method used to prepare this compound (**Figure 21**) is similar to that used to prepare the α-meromycolic acid. The key route to prepare the α-methyl-*trans*-cyclopropane fragment will be discussed later. The meromycolates (**100, 101, 102, Figure 21**) derived from ω-carboxy-mycolic acid represent an interesting synthetic target because they contain only one group of chiral centres. This is the α-methyl-*trans*-cyclopropane group. It may allow the absolute stereochemistry of this part of the mycolic acid system to be determined.

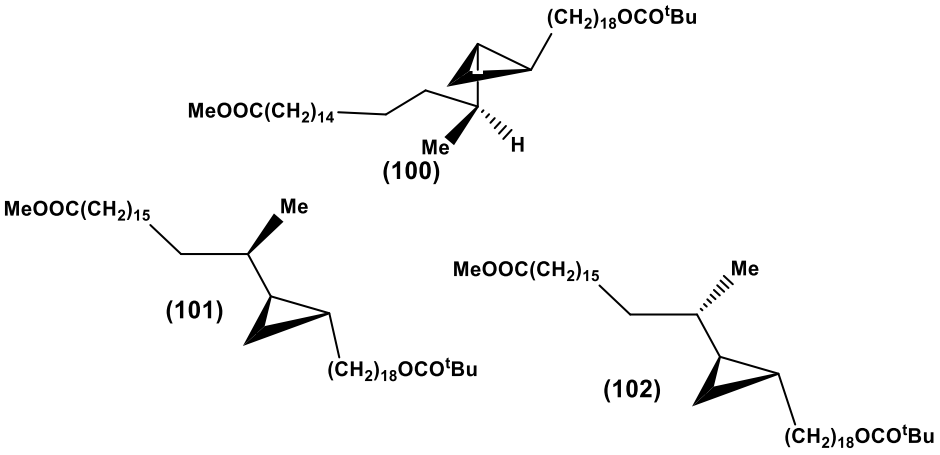
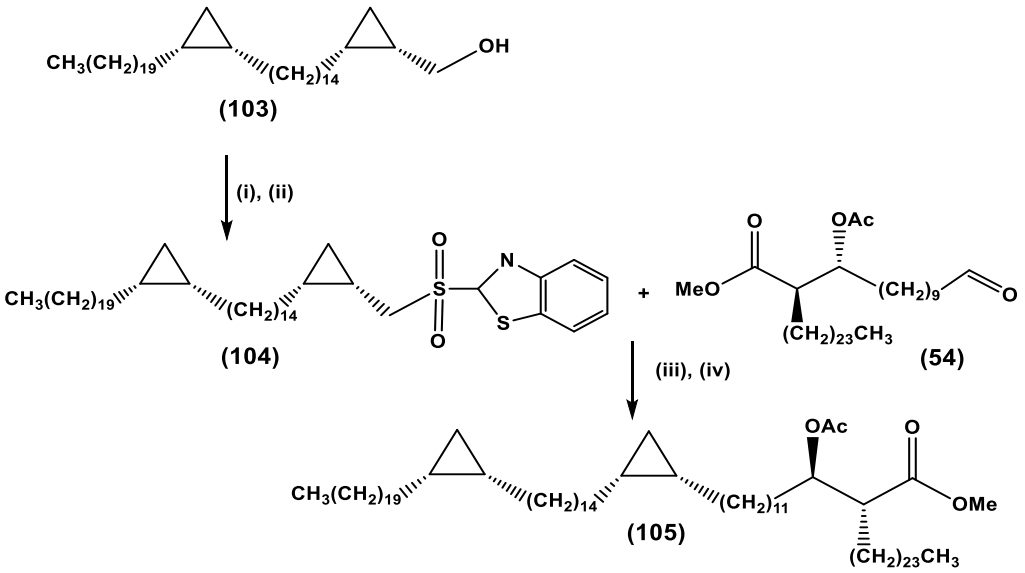


Figure 21: Meromycolic acids containing α-methyl-*trans*-cyclopropane.

1.12 Synthesis of complete mycolic acids

In 2005, Al Dulayymi *et al.*[141] described the first synthesis of a single enantiomer of a full mycolic acid. It was prepared by linking the mycolic motif to the meromycolic acid. The single enantiomer of mero-mycolic acid (**103**) was converted to the sulfone (**104**).

Coupling of this to the mycolic motif aldehyde (**54**) via a Julia olefination, followed by hydrogenation of the resulting alkenes gave the full mycolic acid (**105**) (**Scheme 19**).



Scheme 19: Reagents: (i) benzothiazole, DEAD, PPh_3; (ii) mCPBA; (iii) LiHMDS, THF; (iv) dipotassium azodicarboxylate, CH_3COOH, MeOH, THF.

Methoxy mycolic acids of *M. tuberculosis* have also been synthesized. in different stereochemistries containing either the *cis* or α-methyl-*trans*-cyclopropane (**Figure 22**).[151,152]

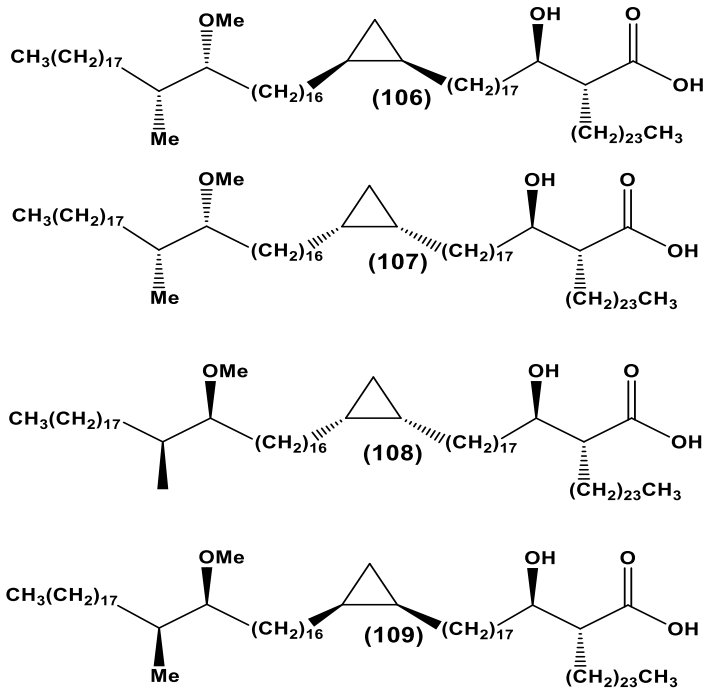


Figure 22: Synthetic methoxy-mycolic acids.

A set of hydroxy and keto-mycolic acids have also been prepared. The synthesis of ketomycolates which contain α-methyl-*trans* and *cis*-cyclopropane fragments (**110** and **111**) in order to produce a range of absolute stereochemistries and chain lengths was also undertaken, (**Figure 23**).[153, 154]

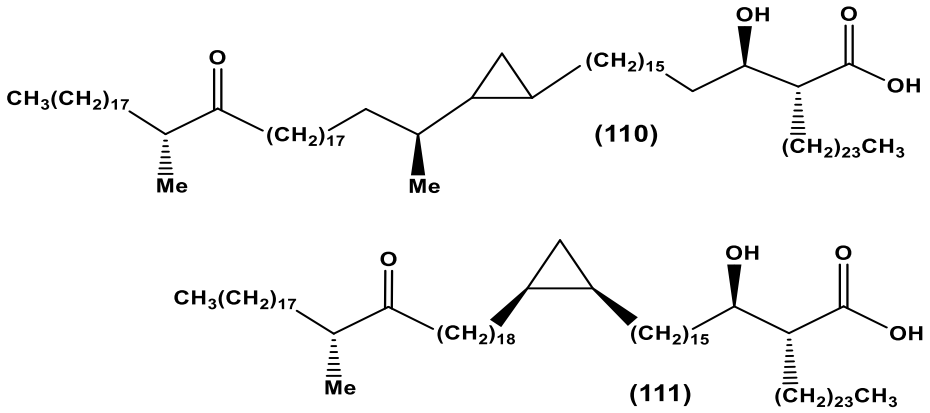


Figure 23: Synthetic keto-mycolic acids of *M. tb*.[153,154]

In addition to preparing single enantiomers of the cyclopropanated mycolic acids, mycolic acids bearing other functionalities such as the epoxy mycolic acids (**112**) and (**113**) containing an (*R*)-α-methyl-*trans*-alkene at the proximal position and the *cis* alkene mycolic acid (**114**). Were also made these have been found in *M. fortuitum* and *M. smegmatis*. (**Figure 24**) [155,156]

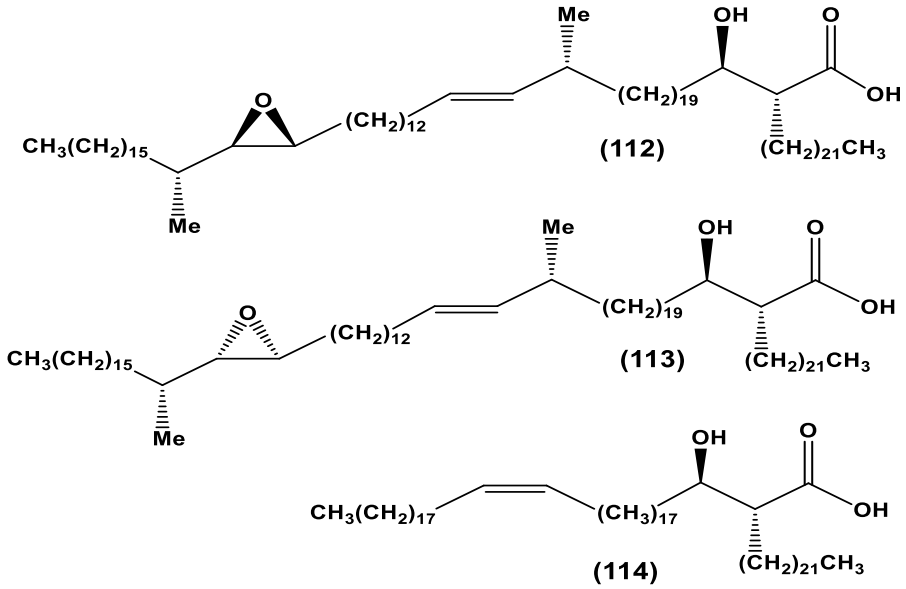


Figure 24: Synthesized epoxy and *cis* alkene mycolic acids.

1.13 The derivatives of mycolic acids

Mycolic acids are found bound to the cell wall, as in penta-arabinose tetramycolyl or as non-bound species such as sugar esters, usually trehalose di- or mono-mycolates (TDM and TMM) or glucose monomycolate (GMM). These species are known to show interesting toxicological and immunological properties.[156-158]

1.13.1 Trehalose dimycolate (cord factor) and trehalose monomycolate

Cord Factor, also known as trehalose di-mycolate (TDM**, 115**) is trehalose esterified with MAs at both the primary alcohol positions.[100,111,159,160] It is an important glycolipid found in the cell wall of *M. tuberculosis* and other mycobacteria and has been shown to have immunogenic properties.[161] A related structure, trehalose mono-mycolate (TMM**, 116**), which is trehalose esterified at only one of the primary alcohol positions, has also been isolated from the cell wall. (**Figure 25**).

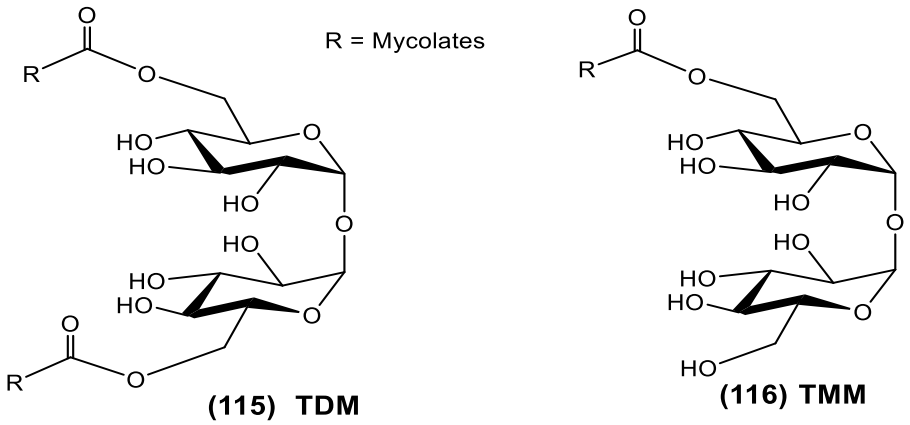


Figure 25: General structure of TDM and TMM.

In 1950, Bloch first discovered that growing tubercle bacilli formed 'cords' and the term 'cord factor' is derived from the belief that it was this substance that causes the 'serpentine cord' appearance of *M. tuberculosis* colonies.[162] In 1956, Noll *et al.* identified the structure of *M. tuberculosis* cord factor, proving that the MAs were esterified to the trehalose to form trehalose-6,6-dimycolate. Alkaline hydrolysis of the cord factor gave the free MA and a non-reducing carbohydrate moiety, α,α-trehalose. Although the exact structure of cord factor was not identified, an approximate structure (**117**) was suggested (**Figure 26**).[100]

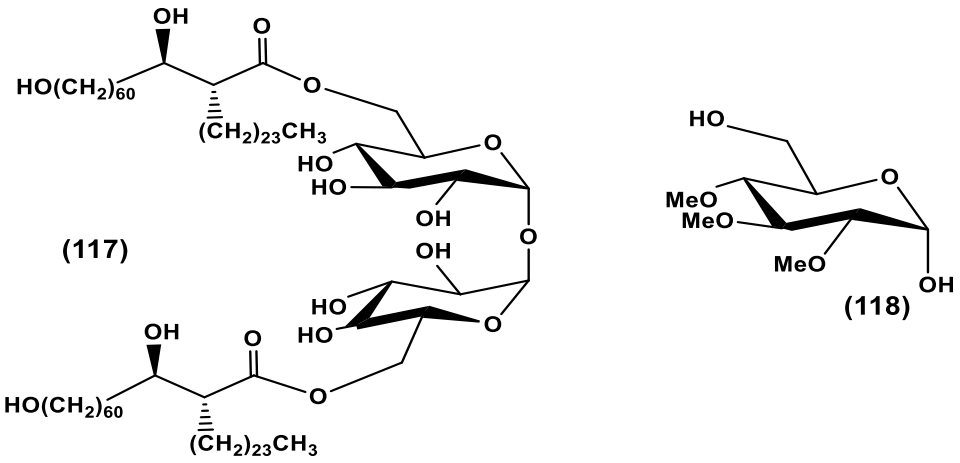


Figure 26: Proposed structure of trehalose 6,6-dimycolate by Noll *et al*.[100]

Confirmation that the MAs were attached to the 6,6-position was obtained by hydrolysis of the methylated cord factor to give 2,3,4-tri-*O*-methyl glucose (**118**). Furthermore, confirmation that the carbohydrate unit was α,α-trehalose, was obtained in two ways: firstly, acid hydrolysis gave D-glucose; and secondly acetylation of the moiety gave a crystalline acetate, which was shown to be α,α-trehalose octa-acetate.[100]

Since the 1960s, the isolation of various trehalose diesters from different bacteria has been reported. Ioneda *et al*, showed that a glycolipid isolated from *Corynebacterium diphtheria* was a trehalose diester containing corynomycolic ($C_{32}H_{64}O_3$) and corynomycolenic ($C_{32}H_{62}O_3$) acids.[163,164] Vilkas *et al*, used mass spectrometry to identify an asymmetric trehalose diester from *M. fortuitum*, which contained palmitic and tuberculostearic acids.[165] Protected derivatives of trehalose mono- and di-mycolate from *M. phlei* were also isolated by Prome *et al*, in 1976,[166] and in 1979 Toubiana *et al*, extracted dimycolates from a single strain of *M. tuberculosis Brevanne*, using mass spectrometry to characterise all the types of mycolic acid.[111]

Much work has been carried out on the structural identification of cord factors, and advances in analytical techniques have allowed such compounds to be studied in more detail. Fujita *et al*, reported the use of MALDI-TOF mass spectrometry to study TMM[167] and TDM[168] from different mycobacteria, with the results showing a marked difference in the composition of the TMM and TDM between different mycobacterial species. The use of MALDI-TOF mass spectrometry has advantages for the analysis of such species as they can be analysed intact, without the need for any degradation process.

1.13.1.1 Biological effects of TDM and TMM

Cord factor is known to have significant biological effects and Bloch demonstrated its toxicity in 1950, when he injected mice with it.[162] Another study showed that cord factor could inhibit phosophorylation of NADPH, leading to reduction of respiratory control in target mice.[169] A different investigation into the biological effects of cord factor in mice by Rastogi and David suggested that cord factor can also inhibit the immigration of blood leuckocytes, which eventually lead to host death.[170] The toxic effect of cord factor was further investigated by Numata *et al*, where different mice were injected with various amounts of cord factor. This study showed that cord factor again led to host death, with the mice infected with the greatest amount of cord factor dying quicker than mice infected with smaller amounts. [171]

Cord factor has also shown various immunomodifying properties and is able to stimulate innate, early adaptive, and both humoral and cellular adaptive immunity.[100] It is believed that most of their functions are due to their ability to induce/activate a range of chemokines (MCP-1, MIP-1α and IL-8) and cytokines (IL-12, IFN-γ, TNF-α, IL-4, IL-6 and IL-10).[172] It is believed that the various sub-groups of mycolic acids, which are part of the cord factor structure, can impact the biological functions of the cord factor.[172] In addition, TDM and TMM may also have various biomedical applications and have shown positive effects against a range of cancers,[173,174] and may have uses in wound healing and hair growth. [157,175] Cord factor has also been reported to have adjuvant properties, being able to generate an optimal antibody response. [176]

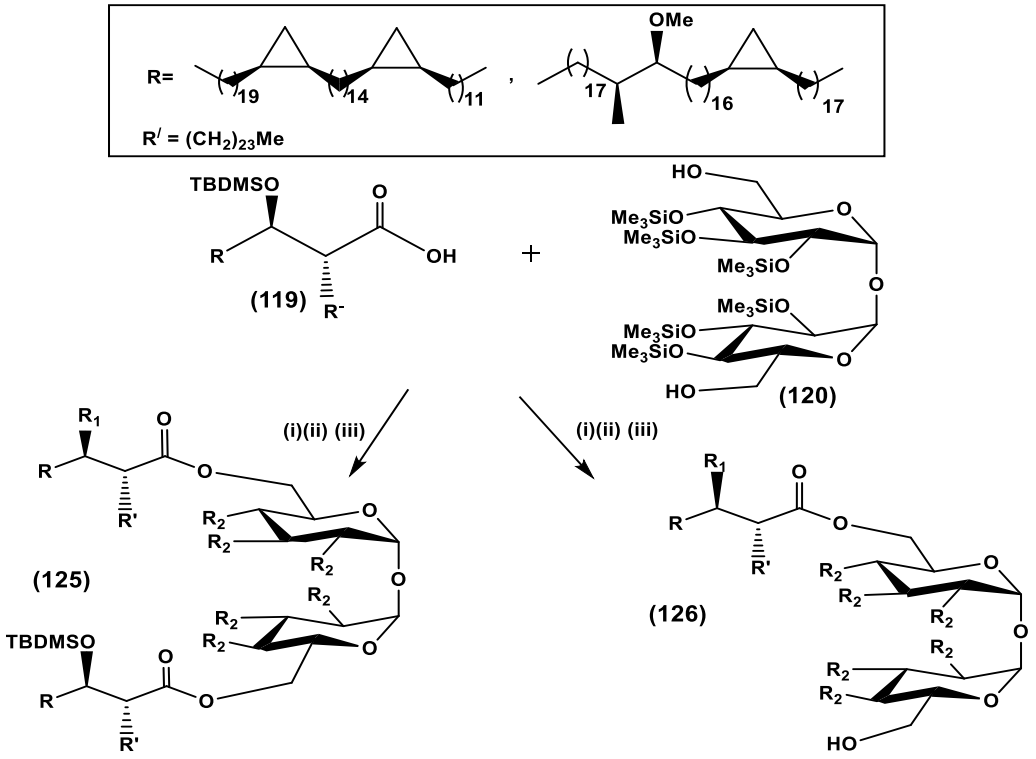
Studies on the biological effects of cord factors from non-tuberculous mycobacteria are limited, however, as previously mentioned (see page 11), Linares *et al,* studied the effect of cord factors from *M. alvei* and *M. brumae* on the secretion of pro-inflammatory cytokines of relevance in tuberculosis. They found that these compounds showed both similarities and differences in the induction of cytokines, in comparison to cord factors from *M. tuberculosis*.[86]

1.13.1.2 Synthesis of TDM and TMM containing single known MAs

As mentioned above a large number of individual structures of TMM and TDM are present in mycobacteria. Furthermore, the composition of the TMM and TDM molecules varies between different species of mycobacteria, with the compounds extracted containing a complex mixture of mycolic acids. It is therefore difficult to characterise

which mycolic acid subgroup will induce which biological effect. Studying synthetic cord factors with various known mycolic acids attached may therefore allow these biological effects to be investigated further.

The first synthesis of TMM and TDM molecules containing single, enantiopure mycolic acids, from *M. tuberculosis* was reported by Al Dulayymi *et al.* in 2009. [172] Single enantiomers of methoxy and α-mycolic acids were coupled to trehalose, to produce the corresponding TMM and TDM. The method firstly involved the protection of the mycolic acid with TBDMS at the β-hydroxy position, prior to coupling the protected trehalose, to avoid reactions at this alcohol group. Coupling of the mycolic acid (**119**) to protected trehalose (**120**) was performed by esterification using EDCI and DMAP as reagents, before the protecting groups were removed in two stages. Firstly, the protecting groups on the trehalose were removed by TBAF, to yield a TBDMS-protected TDM (**123**) and TMM (**124**). Final deprotection of the TBDMS from the β-hydroxy positions of the mycolic acids was performed using HF-pyridine complex, to yield the target TDM (**125**) and TMM (**126**) products (**Scheme 20**). [172-177]



Scheme 20: First unique synthetic mycobacterial cord factor: Conditions and reagents: (i) EDCI, DMAP, DCM (**121**, **122**) R_1= TBDMSO, R_2= Me_3SiO, Å molecular sieves; (ii) TBAF, THF (**123**, **124**) R_1= TBDMSO, R_2= OH; (iii) pyridine, THF, HF-pyridine, $NaHCO_3$ (**125**, **126**) R_1= OH, R_2= OH.

1.13.2 Glucose Monomycolate (GMM)

Glucose monomycolates (GMMs) are mycolic acids, esterified with glucose, at the glucose's primary alcohol position (**Figure 27**).

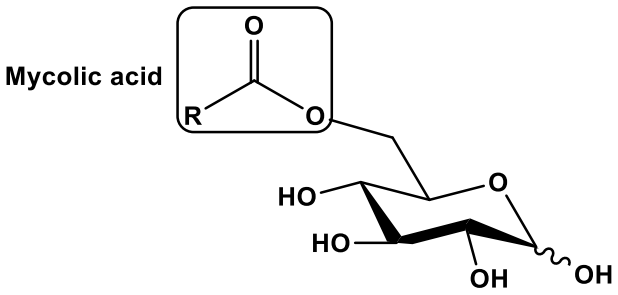


Figure 27: Example of synthetic GMM.

GMMs are present in a range of different bacteria, including *Mycobacterium*, *Rhodococcus* and *Nocardia*.[178,179] They are believed to be 'related' to TDMs, and it has been proposed that TDMs can be converted to GMMs inside infected hosts.[180] It is believed that mycobacteria don't possess the ability to produce GMMs outside of a host cell due to them needing a non-*Mycobacterium* source of glucose.[181] GMMs therefore are only produced by pathogenic mycobacteria, after infection of the host cell.[182]

Similar to TDMs and TMMs, GMMs can also act as protein antigens, and can induce T cell responses.[179] These T cell responses to GMMs have been observed in humans, mice, guinea pigs and cattle.[183] It has been reported that GMM can induce a memory T cell response in cattle similar to a protein antigen, with no antibody response. Nguyen *et al.* applied these findings in a new vaccination of cattle. When cattle were vaccinated with a vaccine containing GMM as an antigen, only a T cell response was observed. However, when

the vaccine contained a protein as an antigen both a T cell and antibody response was observed.[183]

The first synthesis of GMM (**128** and **129**) molecules containing single, enantiopure mycolic acids, from M. tuberculosis was also by Sahb et al, in 2015 (**Figure 28**).[184]

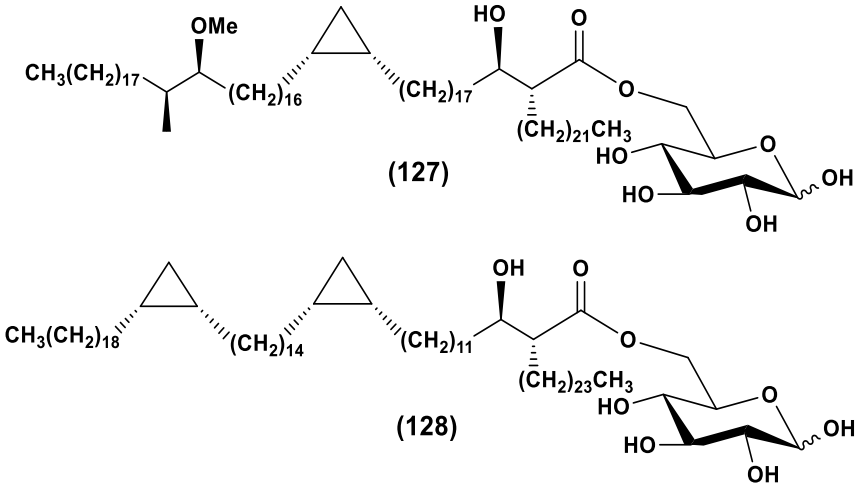


Figure 28: Synthesis of glucose monomycolates based on single synthetic mycolic acids.

1.13.3 Mycolyl-Arabinogalactan Complex

The mycolyl-arabinogalactan complex (mAG) is the largest component structure in the cell wall of mycobacteria and is located outside the cell membrane.

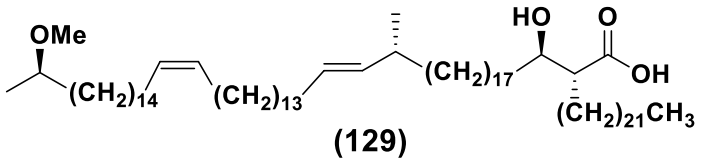
The mAG complex is essential for the integrity of the cell wall and the survival of the mycobacteria and it is believed that it acts as a permeability barrier that prevents the passage of antibiotics.[185,186-189] The arabinans and galactans of the mycobacterial cell wall consist of D-arabinofuranose (D-Araf) and D-galactofuranose (D-Galf)[190] and studying and understanding the mAG complex could be important for developing new drugs for diseases such as TB; one of the first line TB drugs, ethambutol works by targeting mAG biosynthesis.[191] Synthetic arabino-mycolates, prepared from a natural mixture of mycolic acids, have been shown to induce the production of TNF-α in mice, however, due to the complexity of the mAG molecules, the immunological activity of natural arabino-mycolates isolated from BCG has not yet been investigated.[192]

Chapter 2

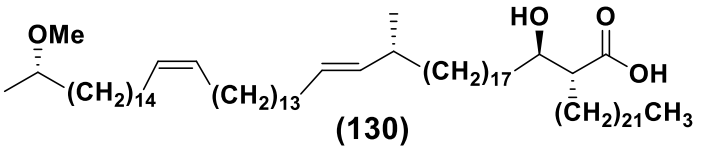
Results and Discussion

2.1 Aims of the project

This project had three main aims. The first was to synthesise single enantiomers (*R* and *S*) of a ω-1 methoxy mycolic acid (**129, 130**) (**Figure 29**), which have been isolated from *M. alvei*, an environmental *Mycobacterium* isolated from river water. This MA contains a methoxy group at the ω-1 position and two alkene groups in the mero chain, at the distal and proximal positions; these are in the *cis*- and α-methyl *trans*-alkenes configuration respectively.



(129)



(130)

Figure 29: *R* and *S* enantiomers of the target ω-1 methoxy MAs.

By synthesising single enantiomers of this type of MA it can be compared to the natural material. This will allow the stereochemistry of the ω-1 methoxy group in the natural material to be determined. Biological assays of the MA can also be carried out with the aim of developing methods to detect infection by this mycobacterium and to distinguish it from other mycobacterial infections.

The second part was the preparation of a number of mycolate esters, by esterification of the mycolic acids (*R* and *S* enantiomers, **129** and **130** above) with different sugars. This would result in the synthesis of TDM (**131**), TMM (**132**), arabino-MA fragment from AG (**133**) and GMM (**134**), (**Figure 30**).

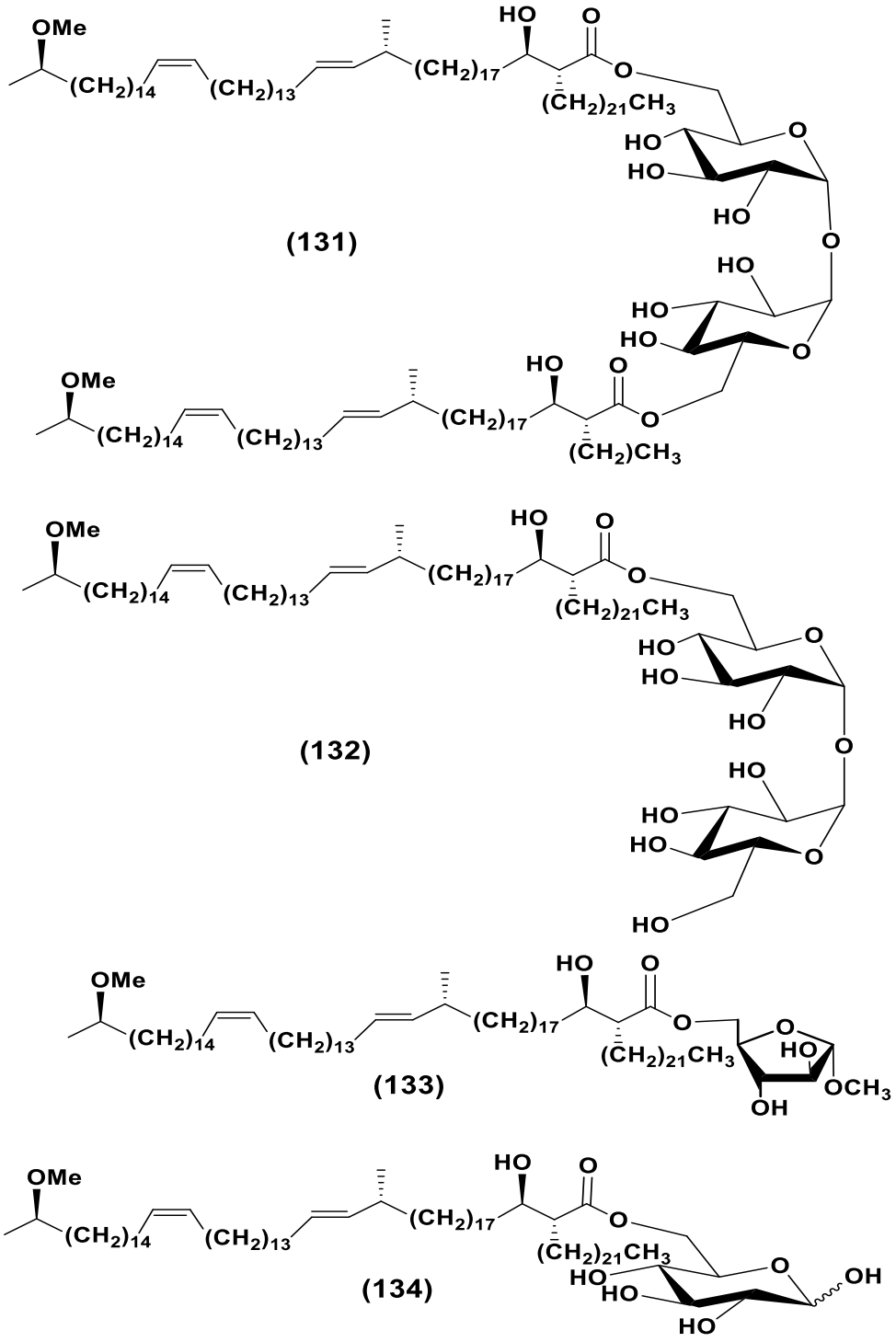


Figure 30: Target mycolic acid and its sugar for *R* enantiomers (**129**).

TDM, TMM, GMM and arabino sugar esters were also prepared for the *S*-enantiomer (**130**).

The third part was to synthesise the specific *cis*-alkene-α-methyl-*trans*-alkene mycolic acid (**135**) (**Figure 31**), which has been isolated from *M. alvei*, and also contains double bonds at the distal and proximal positions. In addition, the preparation of mycolate

esters, by esterification of this mycolic acid with trehalose will be carried out, to give TDM (**136**), and (**137**) (**Figure 31**).

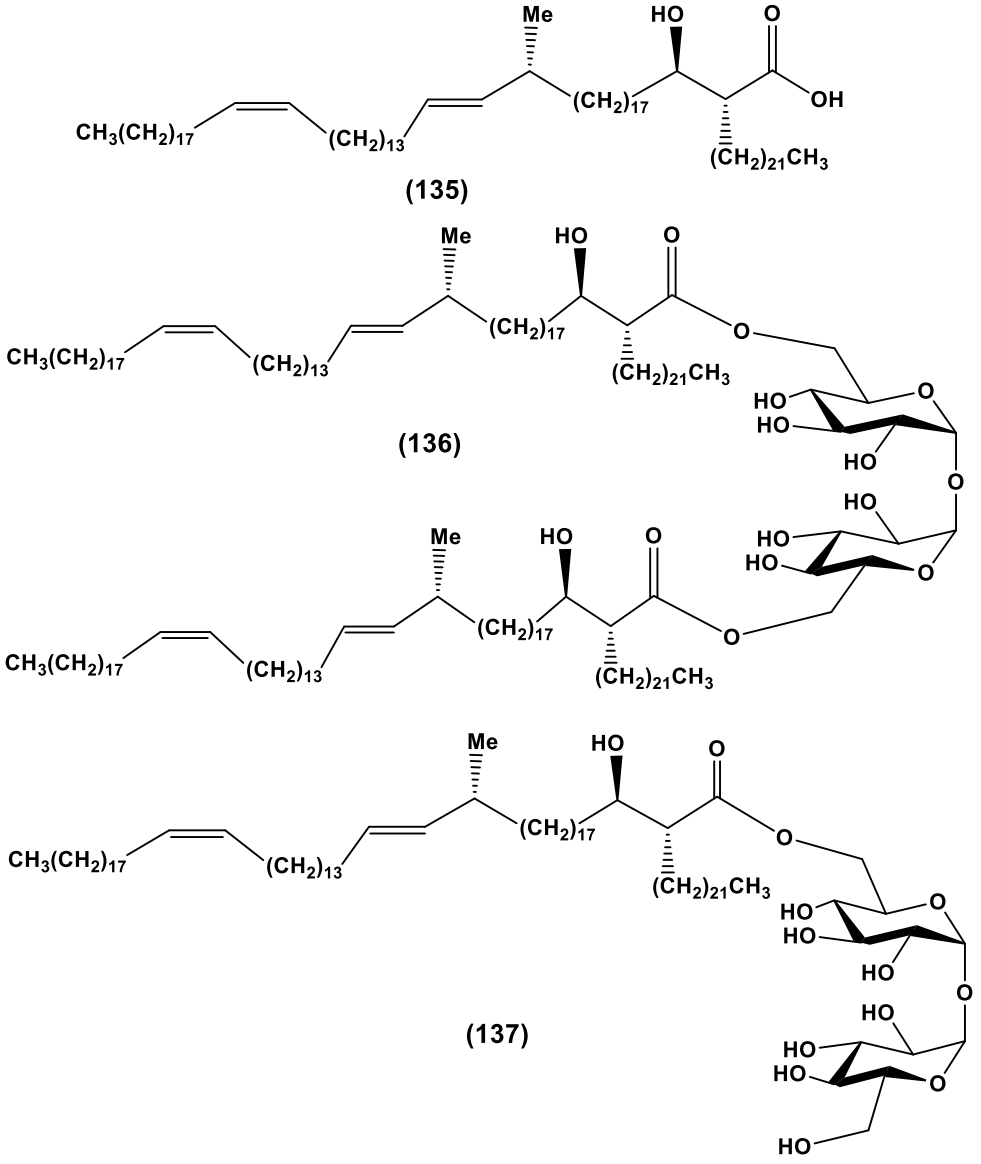
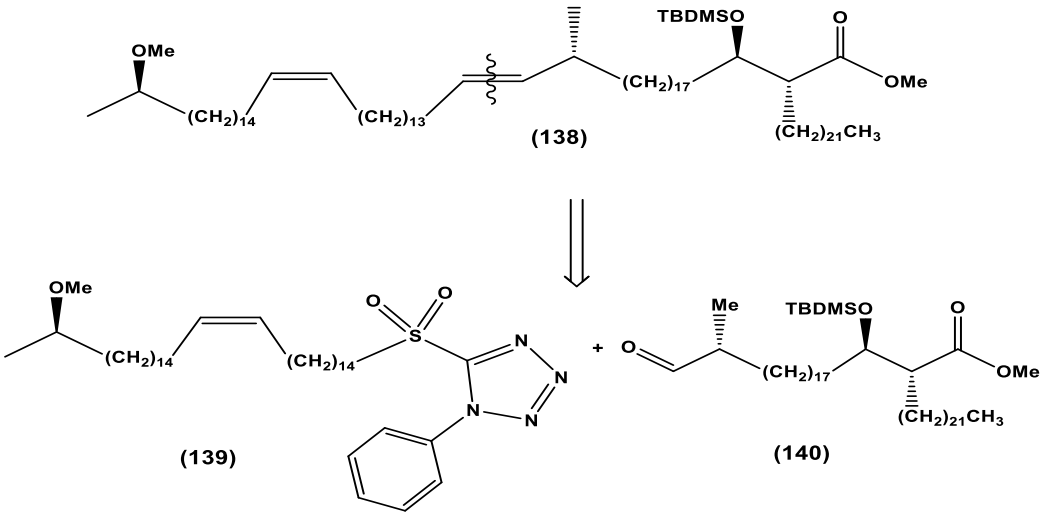


Figure 31: *cis*-Alkene-α-methyl-*trans*-alkene mycolic acid and its corresponding TDM and TMM.

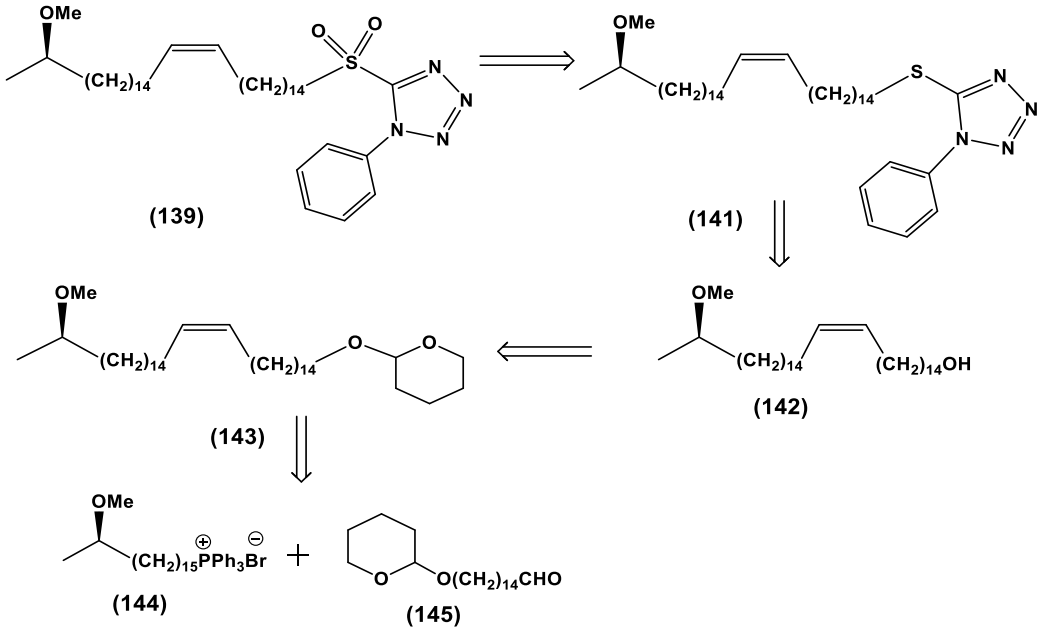
2.2 Synthesis of a major (ω-1)-methoxy mycolic acid

The protected MA (**138**) can be broken down into two main parts: the distal position unit (**139**) and the unit containing the proximal position and the mycolic motif (**140**). (**Scheme 21**).



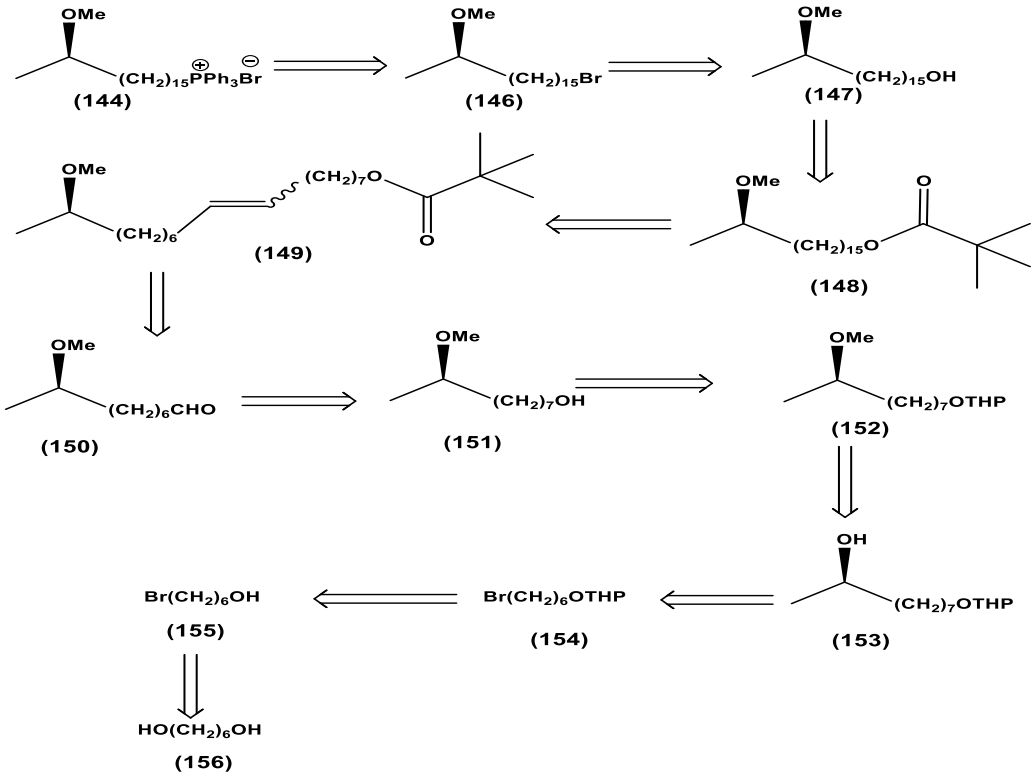
Scheme 21: The fragment containing the distal position of the meromycolate moiety (**139**) and the intermediate (**140**).

The two units shown above are those that can be used to synthesise the *R*-enantiomer of protected MA (**138**). (The same breakdown can be used for the *S*-enantiomer) (**130**). The synthesis of both the *R* and *S* enantiomers of unit (**139**) were carried out using the same procedure but using the reagent with the correct stereochemistry at the appropriate stage (i.e. ring opening of epoxide, see Section **2.3.2** below). A retrosynthesis for the *R* enantiomer only is shown below in (**Scheme 22**).

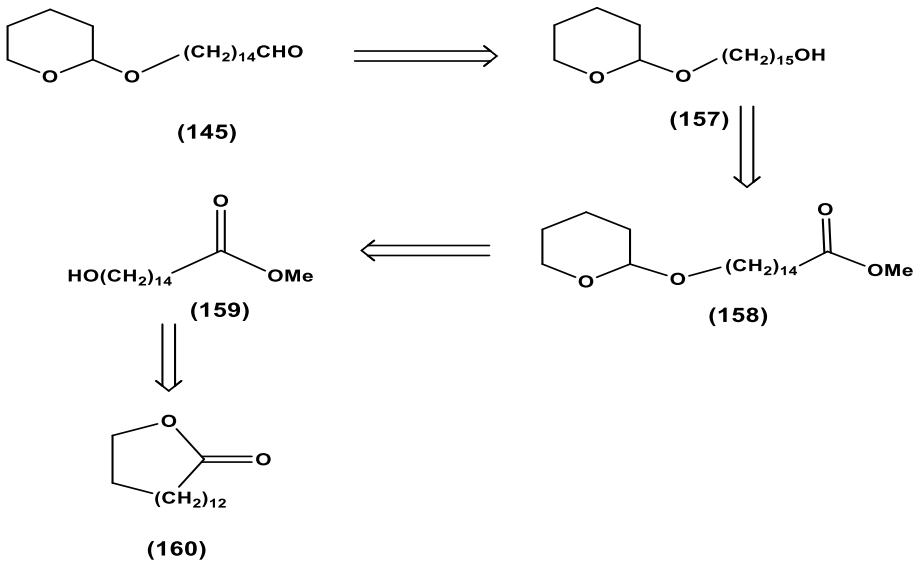


Scheme 22: Retrosynthesis of sulfone (**139**) to Wittig salt (**144**) and aldehyde (**145**).

The Wittig salt (**144**) and aldehyde (**145**) can be prepared from the C-6 diol (**156**) and lactone (**160**) respectively; a retrosynthetic analysis for both is shown in (**Schemes 23 and 24)** below.

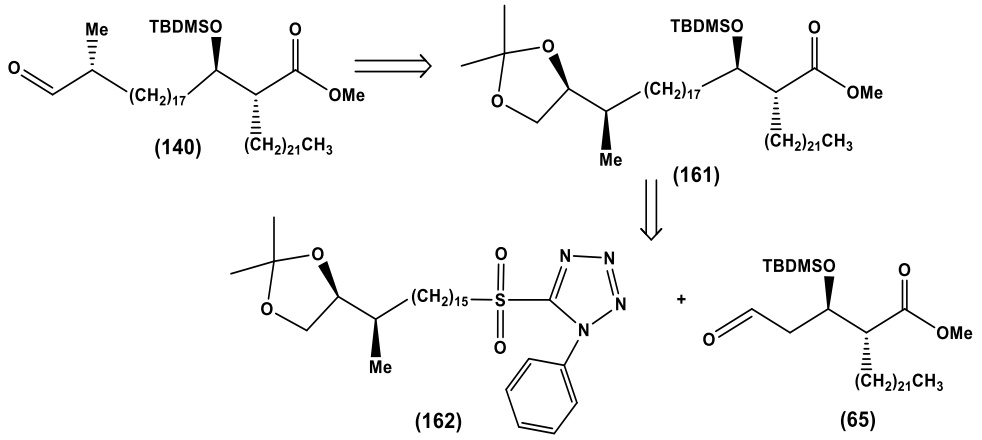


Scheme 23: Retrosynthesis of the *R* enantiomer of Wittig salt (**144**).



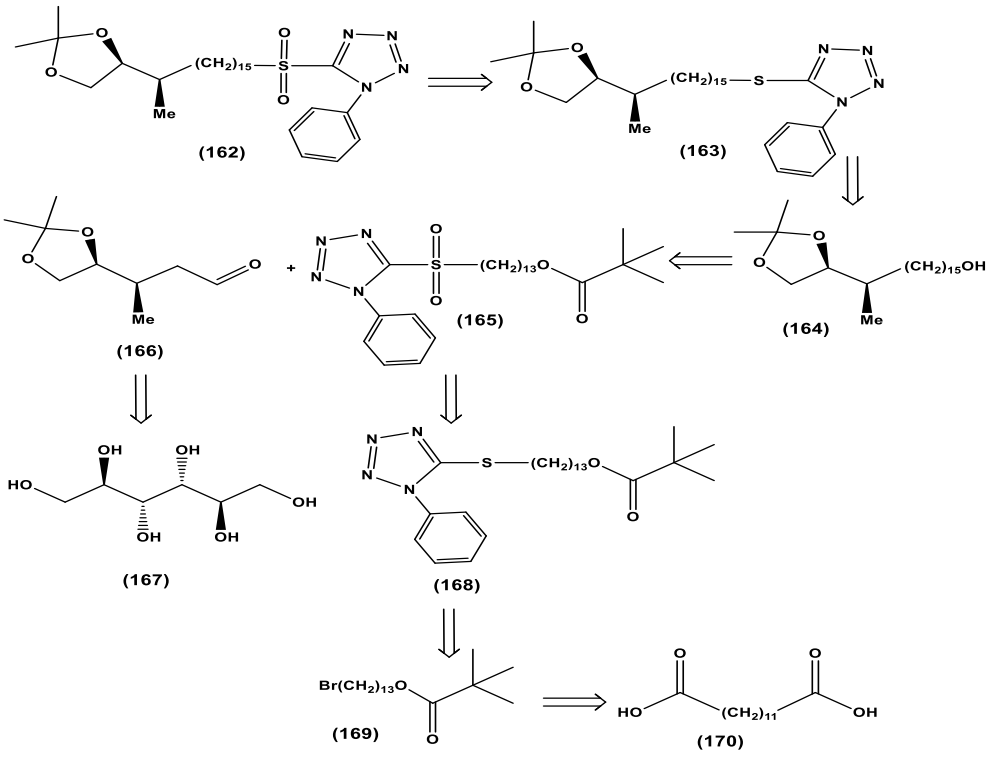
Scheme 24: Retrosynthesis of aldehyde (**145**).

The intermediate (**140**) can be broken down into the mycolic motif unit (**65**), the synthesis of which will be described later, and the proximal position (**162**) (**Scheme 25**).



Scheme 25: Retrosynthesis of the fragment that contains the proximal position and mycolic motif.

The proximal position moiety (**162**) could be synthesised from aldehyde (**166**) and sulfone (**165**), which could themselves be obtained from *D*-mannitol (**167**) and tridecanedioic acid (**170**), respectively (**Scheme 26**).

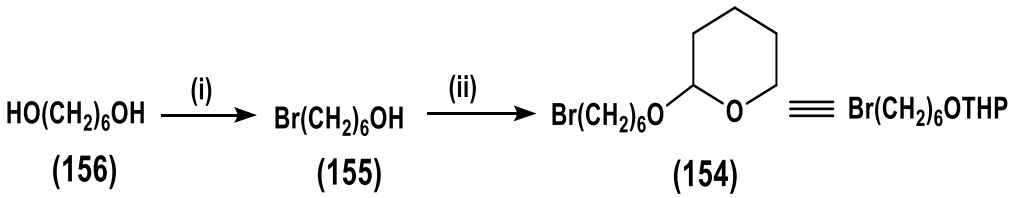


Scheme 26: Retrosynthesis of the fragment (**162**).

2.3 Stereochemistry of (ω-1)-methoxy fragment and synthesis of the Wittig salts (144 and 144a)

2.3.1 Preparation of THP protected bromide (154)

Nucleophilic addition of a Grignard reagent to a chiral epoxide proceeds stereospecifically. A six carbon Grignard reagent was needed for the reaction with the epoxide (**171**). Bifunctional diols, $HO–(CH_2)_n–OH$, were used in this work to extend chain lengths as they are inexpensive, are available in a variety of different chain lengths and they can be easily desymmetrised and modified. 1,6-Hexanediol (**156**) was mono-brominated with HBr 48% by refluxing in toluene for 18 hrs to give 6-bromo-hexan-1-ol (**155**) in 50% yield (**Scheme 27**). The NMR spectra matched those given in the literature.[193]

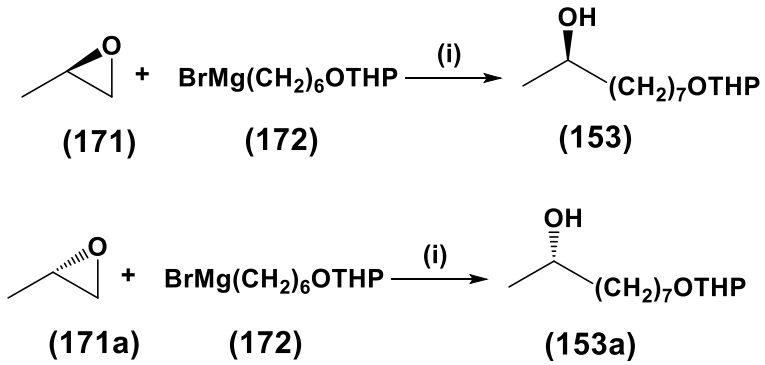


Scheme 27: Reagents: (i) HBr 48%, Toluene, reflux, 50%; (ii) PPTS, dry DCM, 87%.

The hydroxy group of the 6-bromo-hexan-1-ol (**155**) was protected with 3,4-dihydro-2*H*-pyran to give 2-(6-bromo-hexyloxy)-tetrahydro-pyran (**154**) in 87% yield using pyridinium-*p*-toluene-sulfonate as a catalyst and dry dichloromethane as a solvent. The ^{1}H NMR spectrum showed a broad singlet at δ 4.52 for the acetal proton and proved the protection had occurred. The two protons next to the bromine appeared as a triplet at δ 3.35 (*J* 6.7 Hz). The ^{13}C NMR spectrum showed a signal at δ 98.5 for the acetal carbon and two signals at δ 67.0 and 61.9 for the carbons bonded to oxygen.

2.3.2 Addition of Grignard reagent (172) to the epoxide (171, 171a)

The Grignard reaction is widely used in synthetic organic chemistry to form carbon carbon single bonds. Nucleophilic additions of a Grignard regent to an epoxide give a stereoselective product. This addition is one of the key steps for the preparation of the ω-1-methoxy-mycolic acid. The (ω-1)-methoxy group was first introduced by ring opening of *R*-propylene oxide (**171**) or *S*-propylene oxide (**171a**) using a copper catalyzed Grignard reaction to give the desired secondary alcohols (**153**) and (**153a**) respectively (**Scheme 28**).



Scheme 28: Reagents: (i) CuI, THF, - 30 °C, (**153**, 86%), **(153a**, 83%).

To prepare the Grignard reagent (**172**), the flask and solvent needed to be very dry and the reaction was carried out under a nitrogen atmosphere. The first step was the addition of a solution of compound (**154)** in dry THF to a stirred suspension of magnesium turnings in dry THF and the resulting mixture was heated under reflux for 1hr. The Grignard reagent formed was then added to copper (I) iodide in dry THF and stirred for 30 min at – 30 °C. The *R*- propylene oxide (**171**) was then added to the mixture at – 30 °C and the resulting solution was allowed to reach 0 °C and stirred for 3 hrs. Preparation of this single enantiomer of the ω-1-methyl alcohol (**153**) is an important achievement for the synthesis of the target mycolic acids, and it was obtained in 86% yield.

In the IR spectrum of the alcohol (**153**), it was possible to observe the signal for the O–H stretch at 3411 cm^{-1}. Its ^{1}H NMR spectrum showed a broad singlet at δ 4.58 for the acetal proton proving that the THP protecting group was still present, the two protons on the ring next to oxygen appeared as a multiplet at δ δ 3.53 – 3.47, and another multiplet at δ 3.81 – 3.77. The protons on the chain next to oxygen appeared as a doublet of triplets at δ 3.73 (*J* 7.1 (vicinal), 9.7 (germinal) Hz), and as a doublet of triplets at δ 3.38 (*J* 6.3 (vicinal), 9.4 (geminal) Hz). The proton adjacent to the secondary hydroxyl group appeared as a multiplet at δ 3.92 – 3.84. The protons for the α-methyl appeared as a doublet at δ 1.19 (*J* 6.2 Hz). The ^{13}C NMR spectrum showed four signals at δ 98.8 (acetal carbon), δ 68.1 (hydroxyl carbon), δ 67.6 (O<u>C</u>H_2-) and δ 62.3 (O<u>C</u>H_2-) for the carbons adjacent to oxygen.

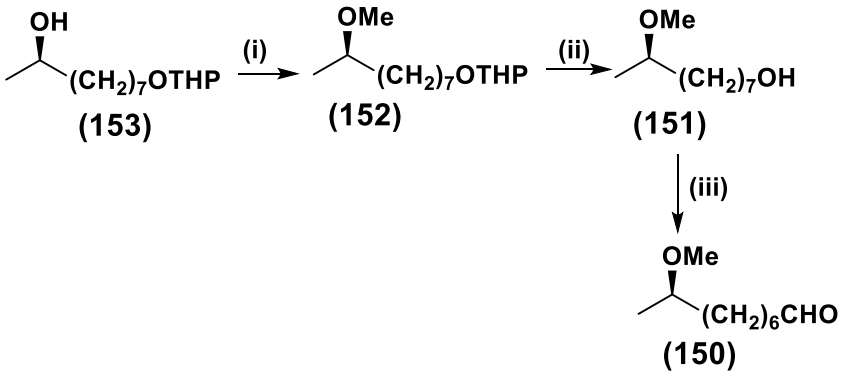
When the Grignard reagent (**172**) was allowed to react with the (*S*)-propylene oxide the resulting secondary alcohol (**153a**) was isolated in 83% yield. The ^{1}H NMR and the ^{13}C spectrum were identical to (**153**). However, the specific rotation of (**153**) was measured

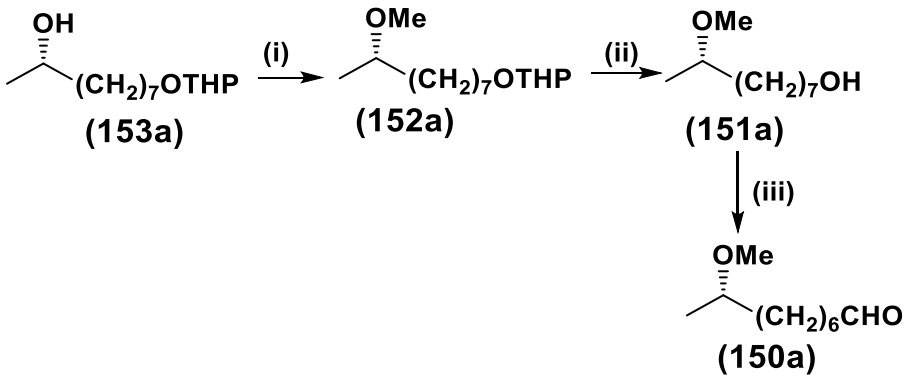
as $[\alpha]_D^{23}$ – 4.8 (*c* 1.1, MeOH), while the specific rotation of the *S*-enantiomer was measured as $[\alpha]_D^{23}$ + 3.8 (*c* 1.9, MeOH).

2.3.3 Preparation of aldehyde (150)

The 2-(((*R*)-8-methoxynonyl)oxy)tetrahydro-*2H*-pyran (**152**) was obtained from the alcohol (**153**) by firstly treating it with sodium hydride in THF at 5 °C, followed by the addition of methyl iodide. The mixture was stirred for 16 hrs at room temperature and after work up, purified by column chromatography to give the required product (**152**) in a yield of 96%, (**Scheme 29**). In the ^{1}H NMR spectrum, the signal for the proton adjacent to the secondary hydroxyl group which appeared as a multiplet at δ 3.53 – 3.47 in the spectrum of (**153**) was absent and the O–H stretching vibration at 3411 cm^{-1} had disappeared in the IR spectrum. There was also a new signal in the ^{1}H NMR spectrum, a singlet at δ 3.29 corresponding to the OMe group.

The next task was the deprotection of the terminal hydroxyl group which had been protected with the THP group in (**152**) to give (**151**). Initially, the protected compound (**152**) was treated with pyridinium-*p*-toluenesulfonate in methanol and THF. After stirring at 50 °C for 3 hrs, followed by work up and purification by column chromatography to give (*R*)-8-methoxynonan-1-ol (**151**) in a yield of 86%, as shown in (**Scheme 29**). The ^{1}H NMR spectrum showed that there were no signals for the THP group, but there was a triplet at δ 3.64 (*J* 6.6 Hz) for the newly formed primary alcohol. The primary alcohol (*S*-enantiomer) was prepared and the ^{1}H NMR and the ^{13}C spectrum were identical to (**151**). The specific rotation of the alcohol (**151**) was $[\alpha]_D^{23}$ – 5.2 (*c* 1.9, MeOH), while the specific rotation of the *S*-enantiomer was measured as $[\alpha]_D^{24}$ + 4.2 (*c* 1.2, MeOH).





Scheme 29: Reagents: (i) sodium hydride, THF, methyl iodide (**152**, 96%), (**152a**, 96%); (ii) PPTSA, THF, methanol, (**151**, 86%), (**151a**, 85%); (iii) PCC, DCM (**150**, 80%), (**150a**, 79%).

Finally, the primary alcohol (**151**) was then oxidised to the corresponding aldehyde (**150**) with pyridinium chlorochromate suspended in dichloromethane. The reaction was carried out at room temperature for 2 hrs and purified by column chromatography immediately to give (**150**) in 80% yield.

After oxidation, the ^{1}H NMR spectrum showed a triplet at δ 9.77 (*J* 1.9 Hz) for the aldehyde proton (HC=O), while the ^{13}C NMR spectrum showed a signal at δ 202.9 for the aldehyde carbon. The IR spectrum showed a broad peak at 1724 cm^{-1} for the aldehyde (C=O) stretch.

Following the same steps, the aldehyde (*S*-enantiomer) was prepared and the ^{1}H NMR and the ^{13}C spectrum were identical to (**150**). Also compounds (**150**), (**150a**) (*R*, and *S*-enantiomer) showed opposite specific rotations of $[\alpha]_D^{18}$ – 3.4 (*c* 1.8, MeOH) and $[\alpha]_D^{24}$ + 3.0 (*c* 1.1, MeOH) respectively.

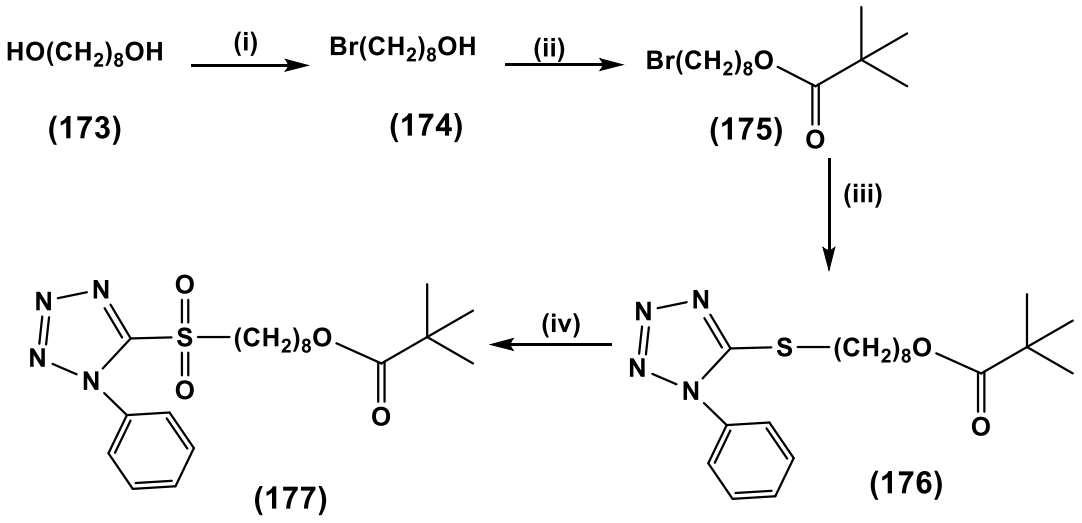
2.3.4 Preparation of C_8 sulfone (177)

The chain length of the aldehyde (**150**) is too short to couple with the sulfone (**177**). In the next step it was necessary to add eight more carbons to prepare the exact length of the natural chain. The Julia reaction was used to extend the chain rather than Wittig coupling, because preparation of Wittig salt is difficult and also the yield is lower than that in the Julia reaction. A C_8 sulfone (**177**) therefore needed to be prepared.

The sulfone (**177**)[157] was prepared starting from 1,8-octanediol (**173**) which was brominated using aqueous HBr 48% in toluene and heated under reflux for 18 hrs to give 8-bromooctan-1-ol (**174**). This was then protected with trimethylacetylchloride in

dichloromethane to give (**175**) in good yield (99%). This protection is very common and deprotection is easy with a strong base such as KOH or $LiAlH_4$ as a reducing agent. In addition, understanding the proton and carbon NMR spectra is also easy compared to the THP protecting group. The ^{1}H NMR spectrum of the protected compound (**175**) showed a triplet at δ 4.05 (*J* 6.6 Hz) for the protons adjacent to the carbonyl group, and a triplet at δ 3.41 (*J* 6.8 Hz) for the protons adjacent to the bromine group. The *tert*-butyl group protons appeared at δ 1.20 as a singlet. The ^{13}C NMR spectrum showed a signal at δ 178.6 for the carbonyl carbon, and a signal at δ 33.9 for the quaternary carbon of the protecting group, and a signal at δ 27.2 for the *tert*-butyl methyl carbons. The IR spectrum showed a peak at 1750 cm^{-1} for the carbonyl group C=O stretch.

The protected bromo compound (**175**) was reacted with 1-phenyl-1*H*-tetrazole-5-thiol and anhydrous potassium carbonate in acetone to give (**176**) in 98% yield. The ^{1}H NMR spectrum showed a multiplet at δ 7.60 – 7.53 for the phenyl group protons, and the ^{13}C NMR spectrum showed five signals in the aromatic region. One signal was seen at δ 154.5 for the tetrazole ring carbon, and another four signals at δ 133.8, 130.0, 129.7 and 123.8 for the phenyl group carbons (**Scheme 30**).



Scheme 30: Reagents: (i) HBr 48%, toluene 84%; (ii) trimethylacetyl chloride, triethylamine, 4-dimethylamino-pyridine, DCM 99%; (iii) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone 98%; (iv) H_2O_2, $(NH_4)_6Mo_7O_{24}.4H_2O$, THF, IMS 96%.

Finally, oxidation of the sulfide (**176**) with ammonium molybdate (VI) tetrahydrate and hydrogen peroxide yielded the sulfone (**177**) as shown in (**Scheme 30**). The ^{1}H NMR spectrum for compound (**177**) showed two multiplets at δ 7.71 − 7.69, integrating to two protons, and δ 7.65 − 7.58 integrating to three protons, corresponding to the phenyl

group. There was also a triplet at δ 3.74 (*J* 7.8 Hz) for the two protons next to the sulfonyl group.

2.3.5 Extension of the chain

2.3.5.1 Overview of the modified Julia olefination

The coupling reaction between aldehydes and phenyl sulfones was described by Julia and Paris in 1973.[194] Later, the reaction was modified by Kocienski, and named the Julia-Kocienski olefination reaction.[195,196-198] The reaction was then further modified by Baudin *et al*.[199] They replaced the use of phenylsulfones with new heteroarylsulfones, and since then the reaction has been named as the modified Julia-Kocienski olefination reaction. It involves the reaction of a metallated sulfone (**177**) with an aldehyde to form a β-alkoxysulfone intermediate (**150**). Four heterocyclic activators have been identified for the modified Julia olefination, which provide useful levels of stereoselectivity in certain scenarios: benzothiazol-2-yl (**BT**), pyridin-2-yl (**PYR**), 1-phenyl-1*H*-tetrazol-5-yl (**PT**) and 1-*tert*-butyl-1*H*-tetrazol-5-yl (**TBT**) **(Figure 32)**.

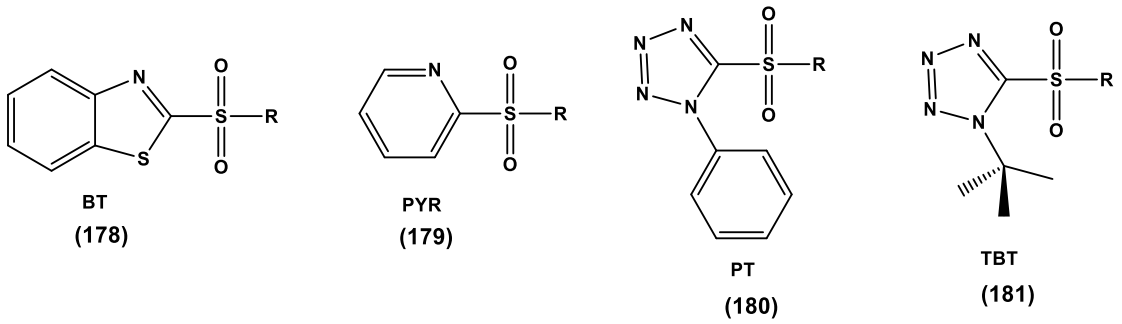


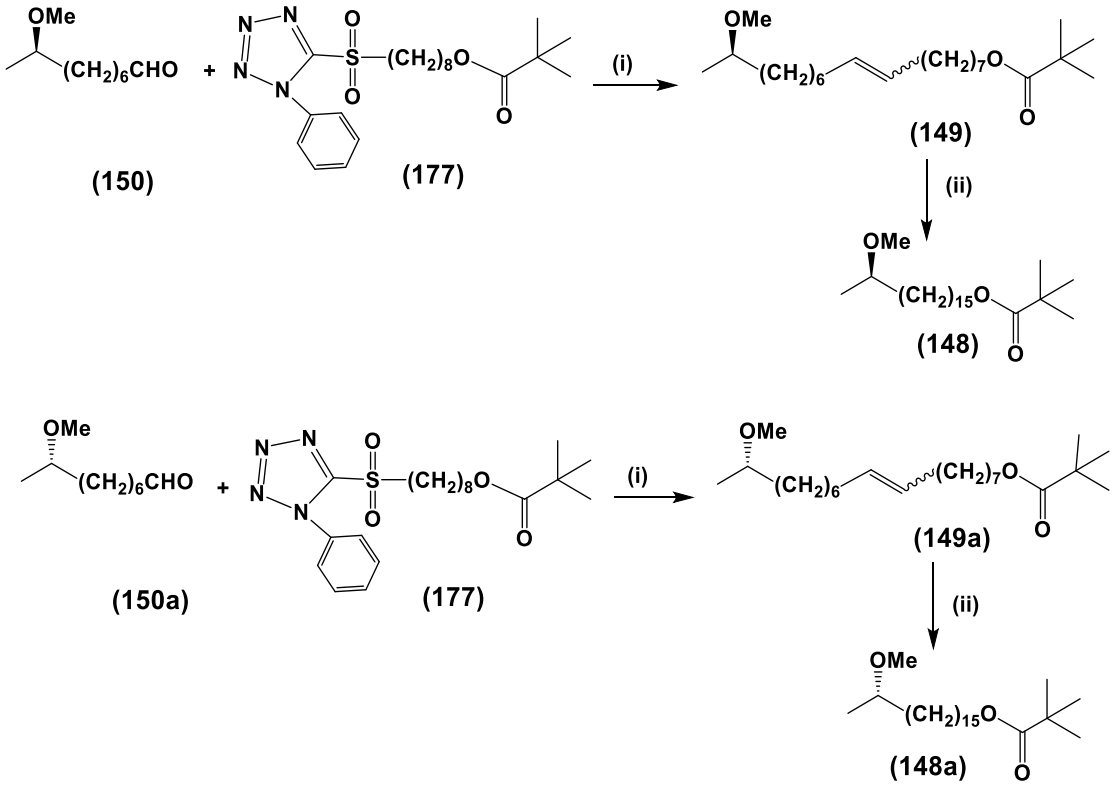
Figure 32: Four heterocyclic sulfones for the modified Julia olefination.

1-Phenyl-1*H*-tetrazol-5-yl sulfonyl (**180**) was chosen in this work, because it was easy to prepare from commercially available materials and it has not shown any problems with self-condensation.[200,201] Julia coupling reactions are also the best method for the preparation of the long chain alkenes in the work described here. Furthermore, the by-product of the Julia coupling reaction is easily removed compared to the by-products in olefinations such as the Wittig reaction; this produces triphenyl phosphonium oxide as a by-product, which is difficult to remove. The Julia olefination gives mainly *E*-alkenes and it is often almost impossible to separate these from the minor *Z*-isomer by column chromatography. The reasons for the major formation of the *E*-alkenes among the

mixture of products obtained from the modified Julia olefination have been investigated.[200,202]

2.3.5.2 The Julia reaction between (150) and (177)

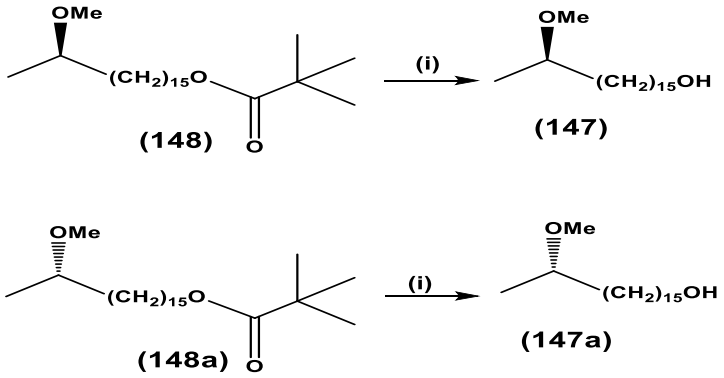
The sulfone (**177**) was dissolved in dry THF, in which both reagents, aldehyde and sulfone are very soluble, and the aldehyde (**150**) was added. The coupling reaction was started by addition of the non-nucleophilic strong base lithium *bis*(trimethylsilyl) amide at – 2 ^{o}C. Subsequently, it was allowed to reach room temperature and stirred for 2 hrs to complete the reaction. The reaction appeared to be very straightforward, giving the desired alkene with a 66% yield. The product was a mixture of *E*- and Z- stereoisomers in ratio 2:1. The ^{1}H NMR spectrum of the product (**149**) showed the protons of the C=C double bond a multiplet at δ 5.42 – 5.37 for the olefinic protons in the *Z*-stereoisomer, and a multiplet at δ 5.36 – 5.32 for the olefinic protons in the *E*-stereoisomer; the signal for the aldehyde proton had disappeared. The ^{13}C NMR spectrum also confirmed the success of the reaction due to the signals at δ 130.4 and 130.2 for the olefinic carbons of the *E*-isomer and two signals at δ 129.9 and 129.8 for the *Z*-isomer (**Scheme 31**). The mixture of *E*- and *Z*-alkenes was then saturated by hydrogenation in a mixture of ethyl acetate and IMS using palladium 10% on carbon as a catalyst under a hydrogen atmosphere. The hydrogenation sometimes proceeded in a straightforward way but sometimes took a long time and it was even necessary to repeat the hydrogenation to give the saturated product (**148**) in 99% yield. The ^{1}H NMR spectrum of the protected compound (**148**) showed a triplet at δ 4.05 (*J* 6.6 Hz) for the protons adjacent to the ester group, and the *tert*- butyl group protons appeared at δ 1.20 as a singlet. The ^{13}C NMR spectrum showed a signal at δ 178.6 for the carbonyl carbon, and a signal at δ 36.3 for the quaternary carbon of the protecting group, and a signal at δ 27.2 for the *tert*-butyl methyl carbons. Moreover, the aldehyde (**150a**) was converted to the ester (**148a**) following the same steps. Compounds (**148**) and (**148a**) again showed opposite specific rotations; $[\alpha]_{D}^{29}$ – 1.3 (*c* 2.0, $CHCl_3$) and $[\alpha]_{D}^{29}$+ 1.3 (*c* 2.1, $CHCl_3$), respectively.



Scheme 31: Reagents: (i) LiN $(SiMe_3)_2$, dry THF (**149**, 66%), (**149a**, 69%); (ii) H_2, Pd (10%) on C, ethyl acetate, IMS (**148**, 99%), (**148a**, 99%).

In order to determine the absolute stereochemistry of the ω-1 methoxy group in the natural MA fragments (**28**, a = 13, R = H) isolated from *M. alvei* (see introduction, section **1.7.1**) the two enantiomers (**183** and **183a**) were prepared. It is known that, in such long chain molecules, the chiral element contributes an approximately equal amount to the molecular rotation of the molecule (M_D) and to the specific rotations of homologues.Therefore the specific rotation of a single enantiomer of the major component is a good indication of the stereochemistry of the chiral element in the mixture.

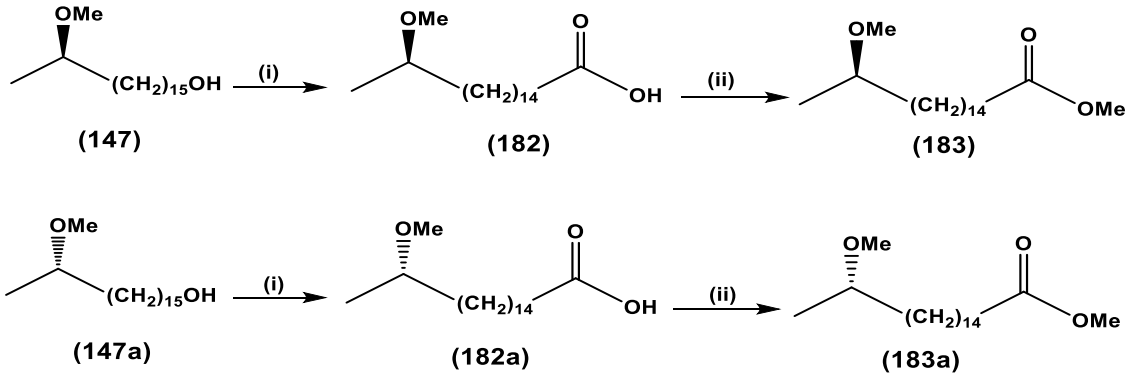
Lithium aluminum hydride in dry THF was firstly used to remove the protecting pivalate ester of (**148**) to give the corresponding alcohol compound (**147**) in a yield of 88% (**Scheme 32**). The formation of the required product was confirmed by the ^{1}H NMR spectrum due to the triplet at δ 3.65 (*J* 6.4 Hz) for the two protons adjacent to the hydroxyl group, while the IR spectrum showed a broad peak at 3432 cm^{-1} for the O–H stretch. The ^{1}H NMR spectrum of the ester (**148**) showed a singlet at δ 1.20 (9H, s) for the *tert*-butyl groups of the protecting group which had disappeared after deprotection further confirming that the reaction was successful.



Scheme 32: Reagents: (i) $LiAlH_4$, THF, (**147**, 88%), (**147a**, 84%).

The alcohol (**147**) was oxidised to the carboxylic acid with ruthenium (III) chloride monohydrate in the presence of sodium metaperiodate in $CCl_4/CH_3CN/H_2O$, to give the acid (**182**) after work up. The acid was refluxed with methanol in the presence of sulfuric acid to give the ester (**183**) in 89% yield (**Scheme 33**). ^{1}H NMR spectroscopy showed that the protons next to oxygen atom were shifted upfield and appeared as a triplet at δ 2.30 (*J* 7.5 Hz), while the ^{13}C NMR spectrum showed a signal at δ 174.34 for the carbonyl ester group. In the IR spectrum the hydroxyl peak had disappeared, and a broad peak was present at 1733 cm $^{-1}$ for the carbonyl ester group. The *S* enantiomer (**183a**) was prepared in the same way.

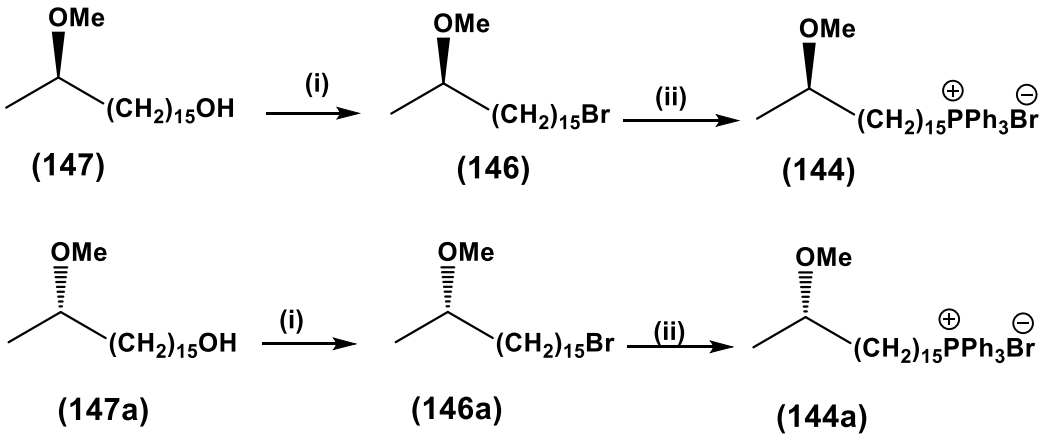
The specific rotation of the *R* enantiomer (**183**) was $[\alpha]_D^{26}$ −1.1 (*c* 0.92, $CHCl_3$), while the *S* enantiomer (**183a**) showed $[\alpha]_D^{26}$ +1.1 (*c* 0.94, $CHCl_3$); on this basis, the natural fragment (**28**, page 21) which had an $[\alpha]_D^{22}$ − 1.55 (*c* 0.39, $CHCl_3$) and the mycolic acid from which it was derived are characterised as having an *R*-ω-1-methoxy stereochemistry.



Scheme 33: Reagents: (i) sodium periodate and ruthenium (III) chloride hydrate, acetonitrile, CCl_4 and water; (ii) H_2SO_4 and MeOH (**183**, 89%), (**183a,** 84%).

2.3.5.3 Preparation of Wittig salt (144)

In order to produce the desired *Z* alkene THP (**143**), the Wittig salt (**144**) needed to be prepared. The hydroxyl group of the alcohol (**147**) was converted to the bromide (**146**) using *N*-bromosuccinimide in the presence of triphenylphosphine in dichloromethane to give the bromide (**146**) in 83% yield (**Scheme 34**). The IR spectrum showed a peak at 3432 cm^{-1} for the O-H group of the alcohol which had disappeared after bromination to (**146**). The Wittig salt (**144**) was obtained by refluxing the bromo-compound (**146**) and triphenylphosphine in toluene at 130 °C for 120 hrs. The pure Wittig salt (**144**) was obtained in 97% yield after purification by column chromatography, firstly eluting with petroleum ether and then dichloromethane/methanol (10:1).



Scheme 34: Reagents: (i) NBS, PPh_3, DCM, (**146**, 83%), (**146a**, 84%); (ii) PPh_3, toluene, (**144**, 97%), (**144a**, 92%).

The ^1H NMR spectrum of (**144**) showed the aromatic protons as a broad multiplet between δ 7.88 – 7.68 integrating to fifteen protons, and the two protons next to the phosphorus appeared as a broad multiplet at δ 3.89 – 3.79. The ^{13}C NMR spectrum also showed the aromatic carbons between δ 135.0 – 117.9. The ester (**148a**) was converted to the Wittig salt (**144a**) following the same steps. Compounds (**144**) and (**144a**) again showed opposite specific rotations, $[\alpha]_D^{29}$ – 1.0 (*c* 1.9, $CHCl_3$) and $[\alpha]_D^{29}$ + 1.0 (*c* 1.9, $CHCl_3$), respectively.

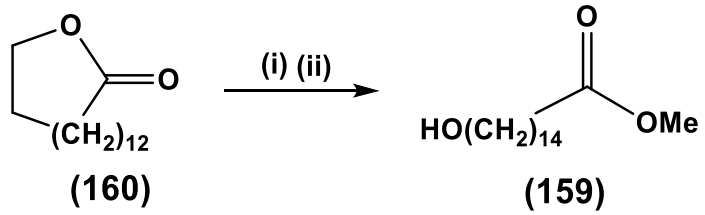
2.4 Synthesis of meromycolaldehyde

2.4.1 Ring opening of lactone for preparation of the 15-carbon chain (160)

To complete the final coupling reaction for the synthesis of the (ω-1)-methoxy-*cis*-alkene-α-methyl-*trans*-alkene mycolic acid, the meromycolate moiety needed to be prepared. The syntheses of mero mycolaldehyde (**139**) started with the ring opening of

the commercially available and inexpensive *ω*-pentadecalactone (**160**). This was achieved by reaction with sodium methoxide, which was freshly prepared by addition of a small piece of sodium to HPLC grade methanol. The lactone was added to the sodium methoxide solution and stirred at 80 °C for 2 hrs, before being acidified with aq. HCl (1N) and worked up. The ^{1}H NMR spectrum showed the product was a mixture of the desired methyl ester and the carboxylic acid. Therefore, the acid was esterified in methanol and a catalytic amount of H_2SO_4 by refluxing for 1 hr to give methyl 15-hydroxypentadecanoate (**159**) in 86% yield[203] (**Scheme 35**).

The ^{1}H NMR spectrum of (**159**) showed a singlet at δ 3.66 for the methyl ester protons, a triplet at δ 3.63 (*J* 6.5 Hz) for the two protons next to the hydroxy group, and a triplet at δ 2.30 (*J* 7.5 Hz) for the two protons next to the carbonyl group. The ^{13}C NMR spectrum showed a carbonyl signal at δ 174.4, a signal at δ 63.1 for the carbon next to the hydroxy group, and a signal at δ 51.4 for the ester's methyl carbon. The IR spectrum showed a broad peak at 3298 cm^{-1} for the O–H stretch and another broad peak at 1735 cm^{-1} for the C=O stretch.

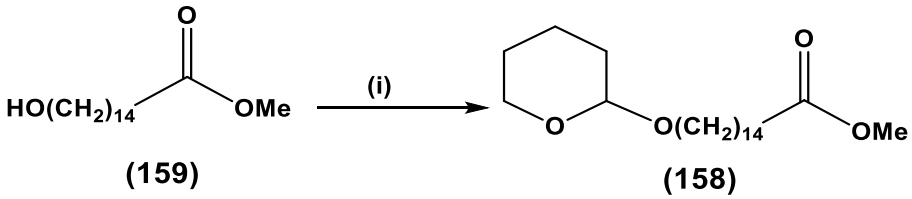


(160) (159)

Scheme 35: Ring opening of the lactone; Reagents: (i) Na, MeOH; (ii) H_2SO_4, MeOH, reflux (86%).

2.4.2 Protection of the OH group of (159) with a dihydropyranyl group (158)

The alcohol (**159**) was re-protected with 3,4-dihydro-2*H*-pyran in dichloromethane in the presence of pyridinium-*p*-toluene–sulfonate (PPTS) to give ester [204] (**158**) in 84% yield (**Scheme 36**).



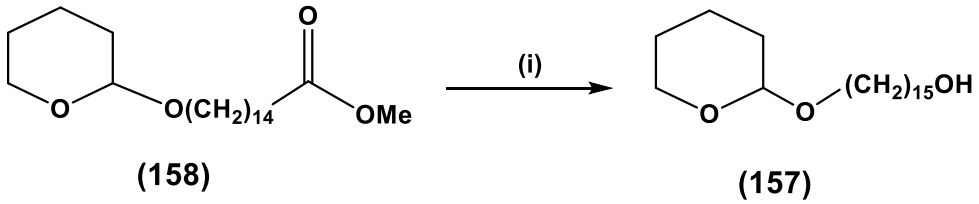
(159) (158)

Scheme 36: Protection the alcohol with a THP group. Reagents: (i) 3,4-dihydro-*2H*-pyran, PPTS, dry DCM, 84%.

The ^{1}H NMR spectrum showed the signals of the THP group with the broad singlet at δ 4.58 integrating to one proton corresponding to the acetal proton of the THP group. The triplet at δ 2.30 (7.5 Hz) integrating to two protons corresponded to the CH_2 adjacent to the carbonyl group, while the signal for the OCH_3 group appeared at δ 3.66. The ^{13}C NMR spectrum also confirmed the presence of the THP group due to the signal at δ 98.8 for the carbon of the acetal group, while the ester carbonyl signal appeared at δ 174.3.

2.4.3 Reduction of the ester group of compound (158)

The protected methyl ester (**158**) was reduced with $LiAlH_4$ in dry THF, and after quenching with a sat. aq. solution of sodium sulfate decahydrate, gave alcohol (**157**) in 92% yield (**Scheme 37**).



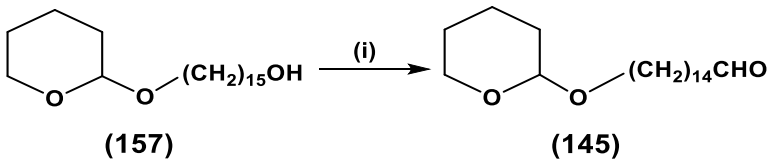
(158) (157)

Scheme 37: Reduction of (**158**); Reagents: (i) $LiAlH_4$, THF, 92%.

The ^{1}H NMR spectrum of compound (**157**) confirmed the disappearance of the sharp singlet belonging to the methoxy ester (OCH_3), and instead showed a triplet at δ 3.63 (*J* 6.6 Hz) for the two protons next to the alcohol. The ^{13}C NMR spectrum also showed a signal at δ 63.1 for the carbon bonded to the hydroxyl group. The IR spectrum also showed a broad peak at 3392 cm^{-1} belonging to the OH group.

2.4.4 Synthesis of aldehyde (145)

The next stage was the preparation of compound (**145**), using pyridinium chlorochromate in dichloromethane, and this gave the required product in 94% yield (**Scheme 38**). The ^{1}H NMR spectrum showed a triplet at δ 9.76 (*J* 1.6 Hz) corresponding to the aldehyde proton, and a triplets of doublet at δ 2.42 (*J* 1.6, 7.3 Hz), integrating to two protons for those adjacent to the aldehyde group, while the ^{13}C NMR spectrum showed signals at δ 202.9 for the aldehyde carbon, and the THP acetal carbon appeared at δ 98.8. The IR spectrum also confirmed the formation of the aldehyde due to the peak at 1727 cm^{-1} corresponding to the C=O group, while that for the O-H group had disappeared.

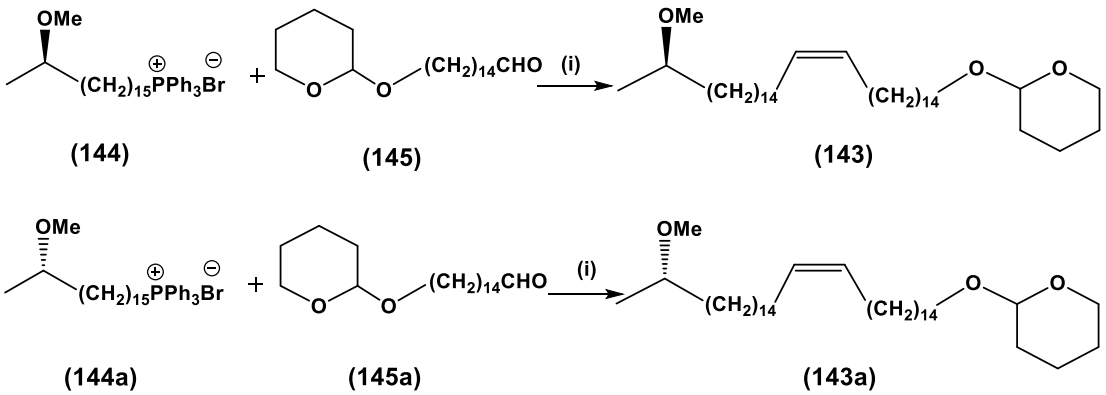


Scheme 38: Preparation of the chain extended aldehyde (**145**). Reagents: (i) PCC, DCM, 94%.

2.4.5 Coupling of Wittig salt (144) and aldehyde (145)

The Wittig reaction is the key step in this strategy. This, in general, is a reaction between an aldehyde with a triphenyl phosphonium ylide, forming an alkene in the *cis* or *trans* configuration. A high selectivity for an (*E*) or (*Z*) alkene can be achieved, depending on the conditions employed in the reaction or the type of functional groups (*e.g.* electron withdrawing group) present in the starting material (phosphonium salt).

The crucially important part of this synthesis was formation of the *Z*-alkene isomer. There is one well known literature method, coupling of an aldehyde with a phosphonium salt in the presence of sodium *bis*(trimethylsilyl)amide that gives the *Z*-stereoisomer with great selectivity. This was attempted using compounds (**144**) and (**145**) and gave the required *Z*-alkene (**143**) **(Scheme 39)**.



Scheme 39: Synthesis of *Z*-alkene (**143**), Reagents: (i) sodium *bis*(trimethylsilyl)amide, dry THF, (**143**, 70%), (**143a**, 56%).

The ^{1}H NMR spectrum of (**143**) confirmed the success of the reaction, which showed the *cis* double bond protons as a pentet at δ 5.35 (*J* 10.4 Hz) integrating to two protons (**Figure 33**). The signals of the THP group showed a multiplet at δ 4.61 – 4.57 belonging to the acetal proton. The ^{13}CNMR spectrum showed the alkene carbons at δ 129.9, and the THP acetal carbon at δ 98.8. following the same steps. Compounds (**143**) and (**143a**) again

showed opposite specific rotations, $[\alpha]_D^{29}$ – 3.8 (*c* 1.1, $CHCl_3$) and $[\alpha]_D^{29}$+ 3.7 (*c* 1.0, $CHCl_3$) respectively.

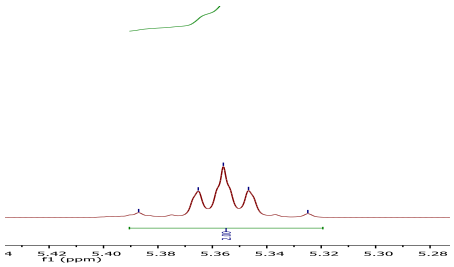
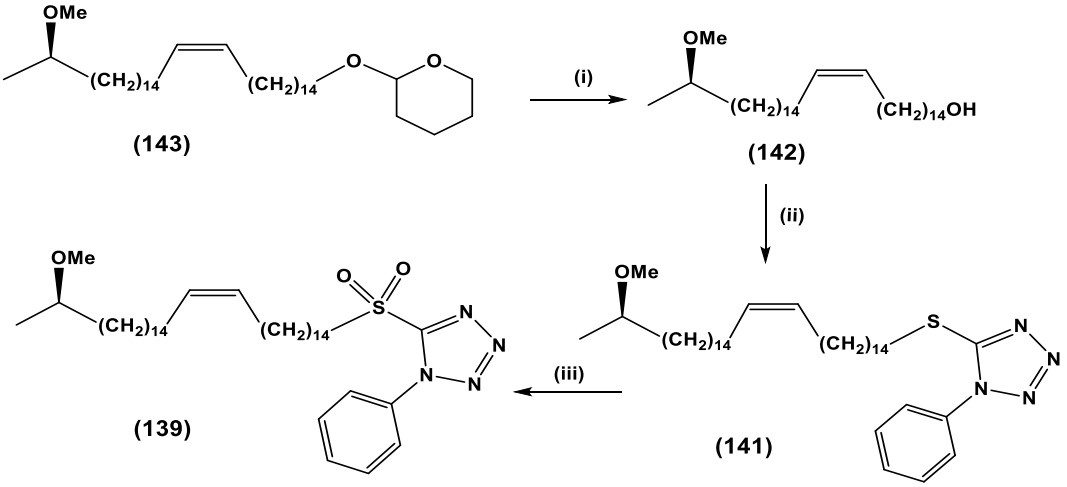
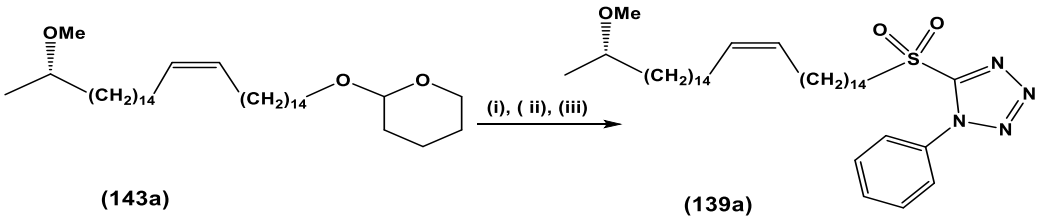


Figure 33: The proton NMR signal for the *cis* alkene protons of (**143**).

2.4.6 Preparation of *Z*- alkene sulfones (139 and 139a)

The deprotection of the THP group in compound (**143**) was carried out pyridinium-*p*-toluenesulfonate in methanol and THF. After stirring at 50 ^oC for 5 hrs, followed by work up and purification by column chromatography (**142**) was obtained in a yield of 94%, (**Scheme 40**). The ^1H NMR spectrum for compound (**142**) showed that there were no signals for the THP group, but there was a triplet at δ 3.64 (*J* 6.6 Hz) for the two protons belonging to the carbon next to the OH group and the ^{13}C NMR spectrum showed a signal at δ 63.1 for the same carbon. The protons belonging to the THP acetal carbon had disappeared from the spectrum.





Scheme 40: Preparation of *Z*-alkene sulfone **(139)** and (**139a**). Reagents: (i) PPTS, THF, MeOH, at 50 ºC, (**142**, 94%), (**142a**, 88%); (ii) 1-phenyl-1*H*-tetrazole-5-thiol,PPh_3, DEAD, dry THF, (**142**, 94%), (**141a**, 83%); (iii) hydrogen peroxide, $(NH_4)_6Mo_7O_{24}.4H_2O$, IMS and THF, (**139**, 86%), (**139a**, 86%).

The alcohol (**142**) was then treated with 1-phenyl-1*H*-tetrazole-5-thiol and triphenylphosphine in the presence of diethyl azodicarboxylate (DEAD) to give the *Z*-alkene sulfide in a very good yield of 94%. It has been found that there is a triplet at δ 3.40 (*J* 7.4 Hz) integrating to two protons belonging to the carbon next to the sulfur atom and the phenyl group protons appeared as a multiplet at δ 7.62 – 7.53 integrating to five protons for the sulfide compound (**141**). Finally, the *Z*-alkene sulfone (**139**) was prepared via oxidation of sulfide (**141**) using hydrogen peroxide and ammonium molybdate in IMS and THF. The ^{1}H NMR spectrum of the *Z*-alkene sulfone (**139**) showed a triplet at δ 3.74 (*J* 7.9 Hz) integrating to the two protons next to the sulfonyl group and two multiplets at δ 7.75 − 7.69, integrating to two protons, and δ 7.68 − 7.59 integrating to three protons, corresponding to the phenyl group (**Figure 34**). The sulfonyl carbon signal appeared in the carbon NMR spectrum at δ 56.0. The specific rotation of the sulfone (**139**) was $[\alpha]_D^{29}$ – 5.6 (*c* 1.0, $CHCl_3$), while the specific rotation of the *S*-enantiomer was measured as $[\alpha]_D^{29}$ + 5.4 (*c* 1.1, $CHCl_3$).

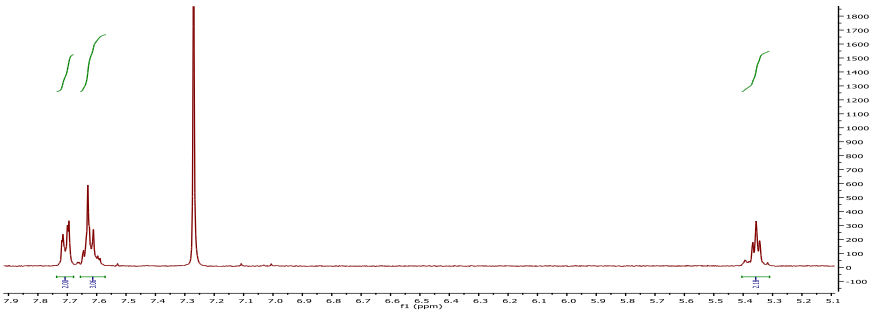
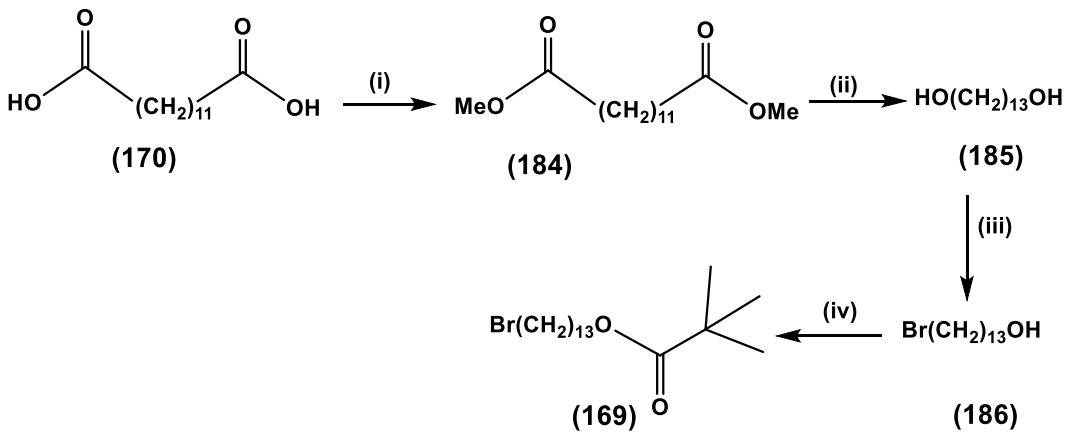


Figure 34: The proton NMR signal for the *cis* alkene of (**139**).

2.5 Preparation of the mycolic motif unit

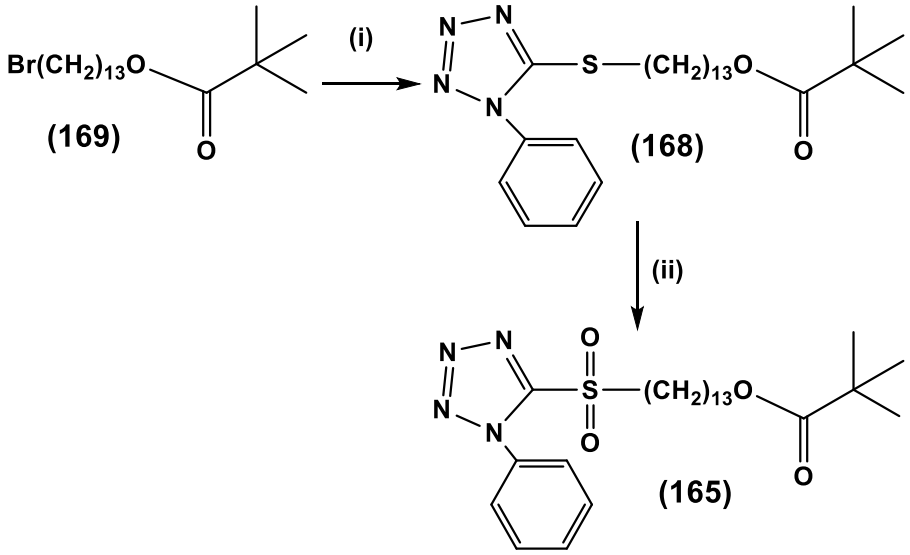
2.5.1 Preparation of the intermediate C-13 sulfone

To extend the carbon chain aldehyde (**166**) could be coupled with a sulfone of required length via a Julia reaction. For MA (**129**) a C-13 sulfone (**165**) therefore needed to be prepared. Firstly, tridecanedioic acid (**170**) was converted into diester [205] (**184**), using conc. H_2SO_4 and methanol in a very good yield, 99%. Reduction of the diester with $LiAlH_4$ in dry THF was then carried out to give the diol[206] (**185**) in a yield of 97%. The ^{1}H NMR spectrum confirmed the formation of the product due to the disappearance of the signals corresponding to the ester groups and the appearance of a quartet at δ 3.65 (*J* 6.5 Hz) corresponding to the (2 × C$\underline{H}_2$OH) integrating to four protons. The next step was monobromination of the diol (**185**) to 13-bromotridecan-1-ol (**186**) with HBr solution (48%) in toluene under reflux for 18 hrs. Again, the structure was proven by the ^{1}H NMR spectrum which showed a quartet at δ 3.65 (*J* 6.6 Hz) corresponding to the (-C$\underline{H}_2$OH) integrating to two protons and a triplet at δ 3.41 (*J* 6.9 Hz) corresponding to the (-C$\underline{H}_2$Br). The protection of the hydroxy group was then carried out using trimethyl acetyl chloride, to give compound[207] (**169**) in 99% yield. The ^{1}H NMR spectrum of the protected compound (**169**) showed a triplet at δ 4.05 (*J* 6.6 Hz) for the protons adjacent to the oxygen and a triplet at δ 3.41 (*J* 6.9 Hz) for the protons adjacent to the bromine substituent. The *tert*-butyl group protons appeared as a singlet at δ 1.20. The ^{13}C NMR spectrum showed a signal at δ 178.6 for the carbonyl carbon, and a signal at δ 34.0 for the quaternary carbon of the protecting group, and a signal at δ 27.2 for the *tert*-butyl methyl carbons. The IR spectrum showed a peak at 1727 cm^{-1} for the carbonyl group C=O stretch (**Scheme 41**).



Scheme 41: Preparation of compound (**169**). Reagents: (i) H_2SO_4, MeOH, 99%; (ii) $LiAlH_4$, dry THF, 97%; (iii) HBr 48%, toluene, 44%; (iv) trimethyl acetyl chloride, Et_3N, DCM, 99%.

The protected bromo compound (**169**) was reacted with 1-phenyl-1*H*-tetrazole-5-thiol in acetone to give the sulfide (**168**) (**Scheme 42**). The ^{1}H NMR spectrum showed a multiplet at δ 7.65 – 7.50 for the phenyl group protons and a triplet at δ 3.40 (*J* 7.4 Hz) for the two protons (C$\underline{H}_2$S). Also, the ^{13}C NMR spectrum showed five signals in the aromatic region: one at δ 154.5 for the tetrazole ring carbon, and another four at δ 133.7, 130.0, 129.7 and 123.8 for the phenyl group carbons and signals at δ 33.3 for the ($\underline{C}H_2$S), The subsequent oxidation of the sulfide with hydrogen peroxide in the presence of ammonium molybdate (VI) tetrahydrate in THF and IMS gave the sulfone (**165**) (**Scheme 42**). The ^{1}H NMR spectrum of the sulfone (**165**) included a multiplet at δ 7.71 – 7.69 (two aromatic protons), another multiplet at δ 7.64 – 7.59 (three aromatic protons), and a distorted triplet (see below) at δ 3.73 (*J* 7.8 Hz) for the two protons (-C$\underline{H}_2SO_2$-), which confirmed the success of the reaction. The ^{13}C NMR showed a signal at δ 56.0 for the (-$\underline{C}H_2SO_2$-).



Scheme 42: Reagents: (i) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone and THF reflux 99%; (ii) H_2O_2, $(NH_4)_6Mo_7O_{24}.4H_2O$, THF, IMS, 97%.

The distorted triplet at δ 3.73 is evidence for the formation of the sulfone (**165**); this corresponds to the two protons (H_A and $H_{A'}$), which are next to the sulfone group. This signal is an example of the AA'BB' system in the Newman projection. A and A' and B and B' are not magnetically equivalent, which means H_A is *cis*- to H_B and *trans* to H_B', resulting in the characteristic signal observed (**Figure 35**).

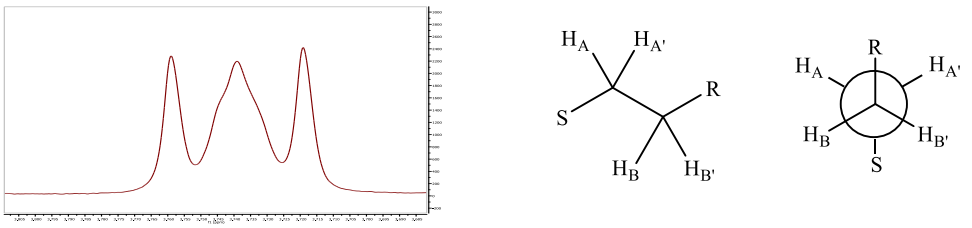
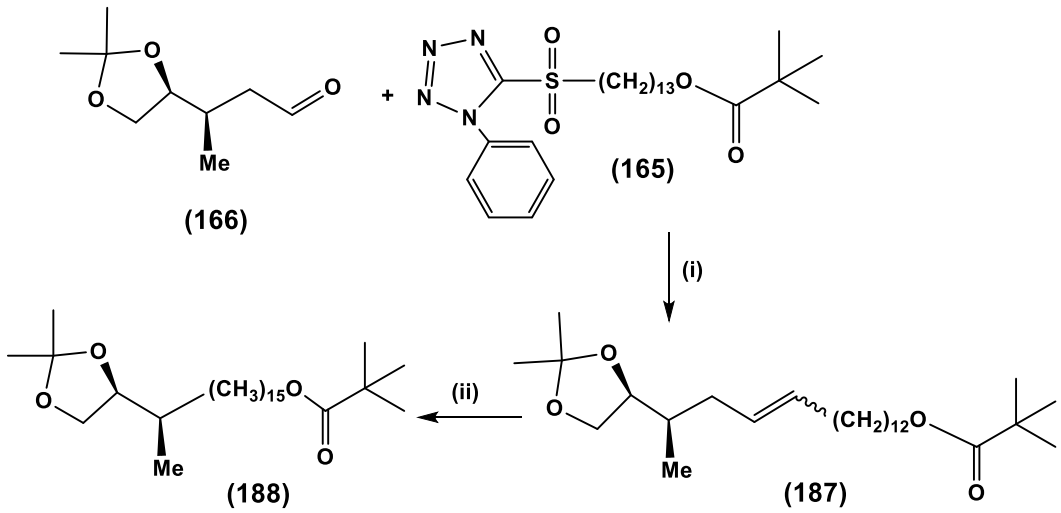


Figure 35: The characteristic signal of the two protons next to the sulfonyl group.

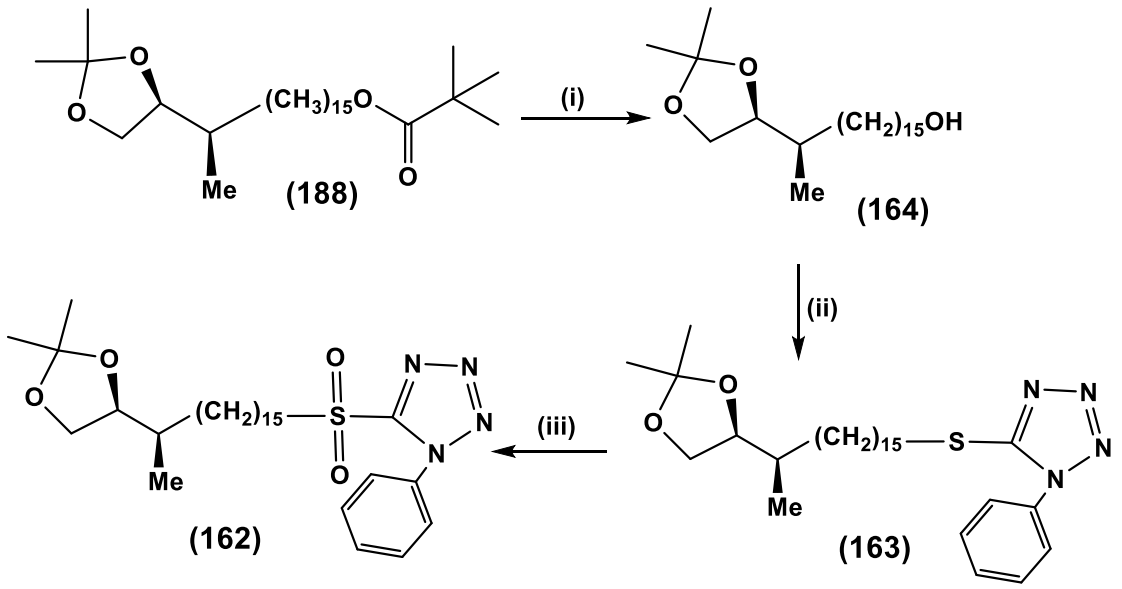
2.5.2 The Julia reaction between aldehyde (166) with sulfone (165).

Using the modified Julia-Kocienski olefination, the aldehyde[207] (**166**) was dissolved in dry THF and the sulfone (**165**) was added. The coupling was started by addition of the base lithium *bis*(trimethylsilyl)amide at – 10 °C. It was then allowed to reach room temperature and stirred for 3 hrs and worked up to complete the reaction. The product was formed as a mixture of *E*- and *Z*-alkenes (**187**) (**Scheme 43**). Hydrogenation of this alkene mixture was then carried out to give the saturated compound (**188**).[207] This was achieved by dissolving the alkenes in IMS and THF and stirring the solution in the presence of palladium 10% on carbon as a catalyst under a hydrogen atmosphere. The proton and carbon NMR spectra showed no signals in the olefin region, thus confirming that the hydrogenation was complete.



Scheme 43: Preparation of intermediate (**188**), Reagents: (i) $LiN(SiMe_3)_2$, dry THF, 45%; (ii) H_2 gas, Pd (10%) on C, IMS and THF, 98%.

2.5.3 Preparation of the intermediate C-15 sulfone (162)



Scheme 44: Preparation of the intermediate C-15 sulfone (**162**). Reagents: (i) $LiAlH_4$, THF 99%; (ii) diethyl azodicarboxylate, 1-phenyl-1*H*-tetrazole-5-thiol, PPh_3, dry THF, 64%; (iii) $(NH_4)_6Mo_7O_{24}.4H_2O$, H_2O_2, THF, IMS, 97%.

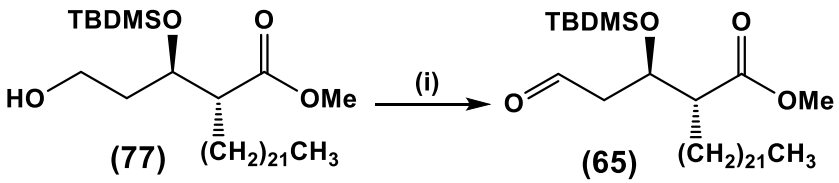
The first step was deprotection of the pivalate ester (**188**), which was carried out using $LiAlH_4$ in THF to give the primary alcohol (**164**) in an excellent yield, 99% (**Scheme 44**). The ^1H NMR spectrum showed a broad triplet at δ 3.64 (*J* 6.6 Hz) for the two protons adjacent to the hydroxyl group, while the IR spectrum showed a broad peak at 3444 cm^{-1} for the O–H stretch, both confirming that the reaction had been successful. The next step was to perform a Mitsunobu reaction.[208] This reaction involves adding diethyl azodicarboxylate (DEAD) at 0 ºC, under nitrogen gas dropwise to a solution of alcohol (**164**), triphenylphosphine and 1-phenyl-1*H*-tetrazole-5-thiole in dry THF, and allowing the mixture to stir for 2 hrs. This resulted in the formation of sulfide (**163**), which was subsequently converted into sulfone (**162**) using the same procedure as discussed previously. The ^1H NMR spectrum of the sulfone (**162**) showed a distorted triplet at δ 3.73 (*J* 7.9 Hz) for the two protons next to the sulfonyl group, a doublet at δ 0.97 (*J* 6.7 Hz) for the chiral methyl protons and two singlets at δ 1.40 and δ 1.35 for the two methyl groups of the acetal protecting group. The acetal carbon signal appeared in the ^{13}C NMR spectrum at δ 108.4.

2.6 Synthesis of chain extended mycolic motif aldehyde (65)

In order to produce the intermediate aldehyde (**140**), the motif unit (**161**) needed to be prepared. This was prepared from *L*-aspartic acid as described in the introduction (see Section **1.10.2**).

2.6.1 Oxidation of the alcohol (77)

The route starts by oxidizing the known alcohol[156] (**77**) using PCC in dichloromethane to give aldehyde (**65**) as shown in (**Scheme 45**). The ^{1}H NMR spectrum of (**65**), showed a double doublet at δ 9.81 (*J* 2.5, 4.3 Hz) corresponding to the aldehyde proton, a doublet of triplets at δ 4.43 (*J* 4.7, 6.0 Hz) for the proton at the β position, and a doublet of doublets of doublets at δ 2.62 (*J* 4.1, 6.3, 10.4 Hz), for the proton at the α-position. There was a singlet at δ 3.68 for the one ester group (**Figure 36**). The ^{13}C NMR spectrum gave a signal at δ 201.3 for the aldehyde group and the specific rotation was measured as $[\alpha]_D^{27}$ – 5.2 (*c* 1.3, $CHCl_3$), (*litt*., $[\alpha]_D^{27}$ – 5.0 (*c* 1.2, $CHCl_3$).[157]



Scheme 54: Oxidation of the alcohol. Reagents: (i) PCC, DCM, 87%.

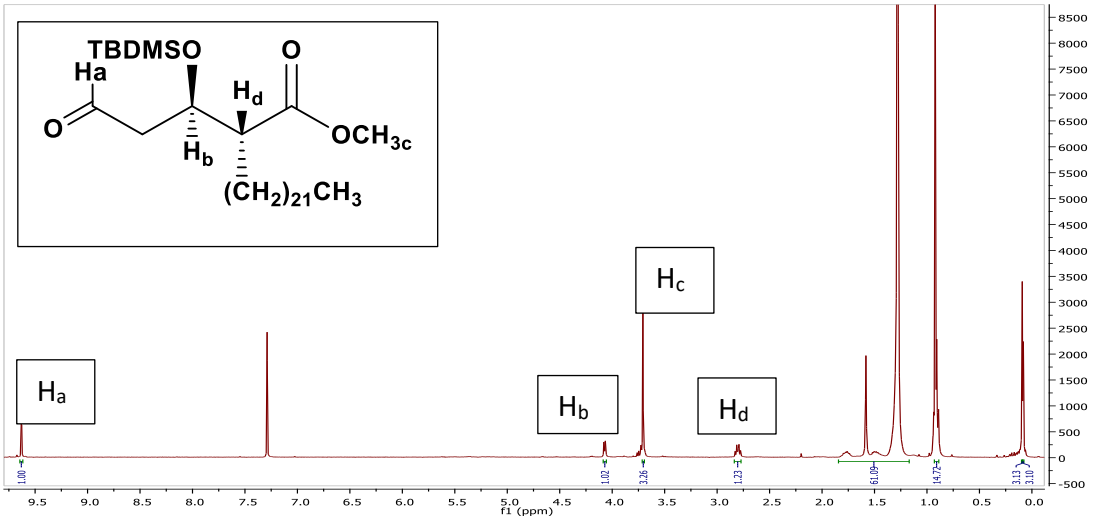
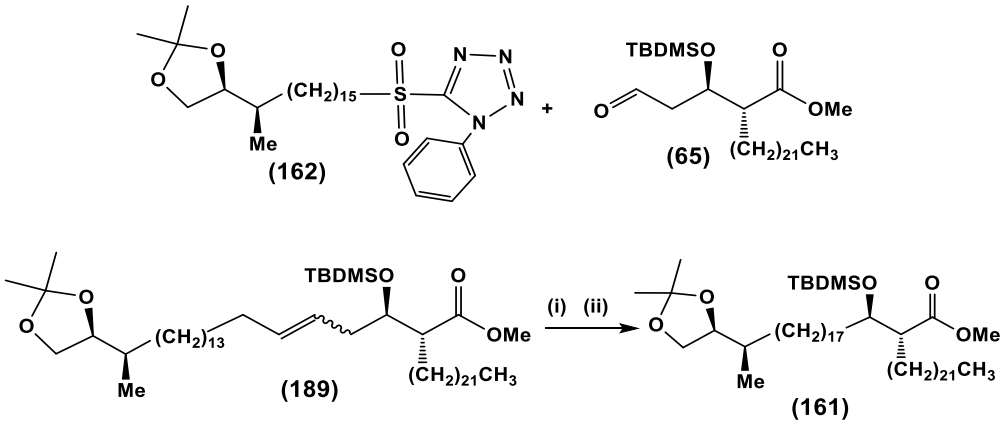


Figure 36: The ^{1}H spectra for compound (**65**).

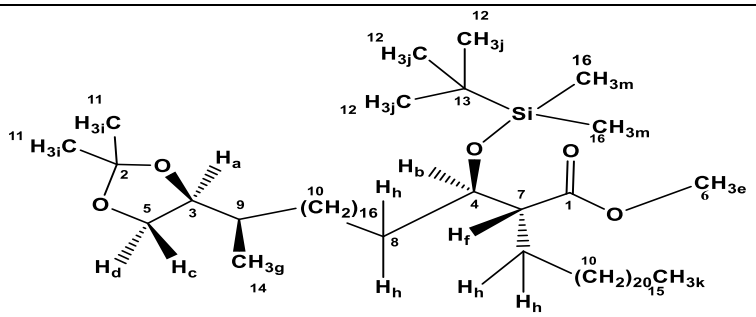
2.6.2 Extension of the mycolic motif chain by coupling with sulfone (162)

This step involved the coupling of the mycolic motif unit (**65**) with the long chain containing the methyl group which would finally be next to the *trans*-alkene unit in the final target MA. The aldehyde (**65**), was coupled with the sulfone (**162**) in the presence of lithium *bis*(trimethylsilyl) amide in THF to give the alkene (**189**) as a mixture of *E* and *Z*-isomers in 97% yield (**Scheme 46**). The double bond was then hydrogenated using hydrogen gas in the presence of palladium 10% on carbon to give the saturated product [157, 208] (**161**) in 94% yield. The specific rotation was measured as $[\alpha]_D^{28}$ + 3.1 (*c* 0.9 $CHCl_3$), (*litt* $[\alpha]_D^{22}$ + 3.6 (*c* 0.9, $CHCl_3$). The NMR spectra are analysed in (**Table 1**).



Scheme 46: The coupling reaction between mycolic motif (**65**) and intermediate sulfone (**162**), Reagents: (i) $LiN(SiMe_3)_2$, dry THF, 97%; (ii) H_2, Pd (10%) on C, IMS and THF, 94%.

Table 1: ^{1}H NMR and ^{13}C NMR data analysis of compound (**161**).

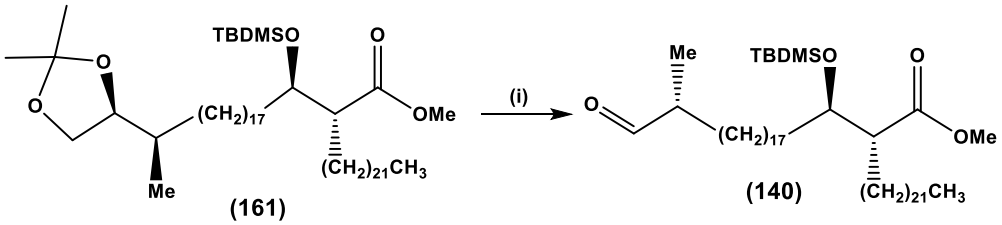


Proton	δ/ppm	Multiplicity	Integration	*J*/ Hz	Carbon	δ/ppm
H_a	4.01	dd	1	6.2, 7.8	C_1	175.2
H_b	3.90	br. td	1	4.9, 6.4	C_2	108.5
H_c	3.86	t	1	6.6	C_3	80.4

H_d	3.60	t	1	7.7	C_4	73.2
H_e	3.66	s	3	-----	C_5	67.8
H_f	2.53	ddd	1	3.7,7.2,11.0	C_6	51.6
H_g	0.76	d	3	6.6	C_7	51.2
H_h	1.61-1.51	m	4	----	C_8	36.5
H_i	1.41 – 1.36	s	6	-----	C_9	33.7
H_j	0.66	s	9	-----	C_{10}	32.7-22.7
H_k	0.68	t	3	7.0	C_{11}	25.5
H_m	0.04, 0.02	s	6	----	C_{12}	25.5
					C_{13}	18.0
					C_{14}	15.6
					C_{15}	14.1
					C_{16}	-4.4, -4.9

2.6.3 Preparation of the mycolic motif unit (140)

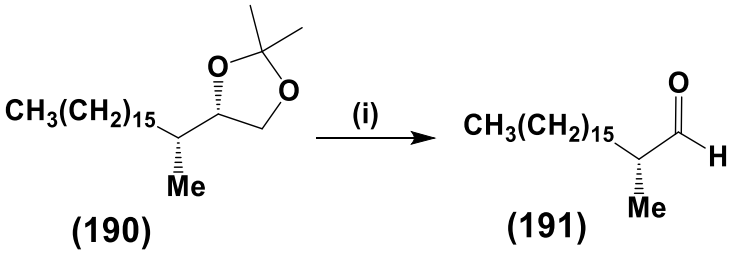
The oxidative cleavage of the cyclic acetal group of (**161**) was carried out using periodic acid in ether/THF (5:2) at room temperature under nitrogen. The mixture was monitored by ^{1}H NMR spectroscopy, and after completion the desired aldehyde (**140**) was obtained in 90% yield (**Scheme 47**). The ^{1}H NMR spectrum showed a doublet at δ 9.62 (*J* 2.0 Hz) for the aldehyde proton, a broad quartet of doublets at δ 2.43 (*J* 2.0, 7.0 Hz) for the proton next to the aldehyde proton and a doublet at δ 1.09 (*J* 7.0 Hz) for the methyl protons at the α-position to the aldehyde. There were three singlet peaks at δ 0.86, 0.04 and 0.02 for the TBDMS group. The ^{13}C NMR spectrum showed a signal at δ 205.4 for the carbonyl carbon of the aldehyde, a signal at δ 46.3 for the carbon next to the aldehyde and a signal at δ 13.3 for the carbon of the methyl at the α-position to the aldehyde. The IR spectrum showed a peak at 1737 cm^{-1} (C=O) and the specific rotation of (**140**) was $[\alpha]_D^{28}$ – 9.7 (*c* 0.9, $CHCl_3$).



Scheme 47: The synthesis of motif unit (**140**), Reagent: (i) periodic acid, ether/THF (5:2), 90%.

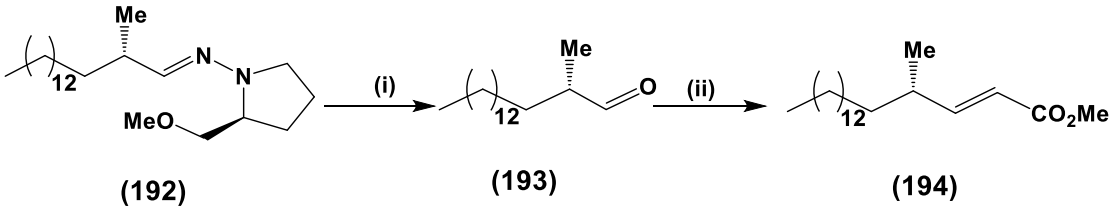
Based on examples from the literature, concerning similar species[156,208,209] to compound (**140**), it is unlikely that any significant epimerization of the α-position occurs under the conditions to which the aldehyde is exposed between its formation and reaction in subsequent steps.

Al Kremawi *et. al.*[210] carried out the oxidative cleavage of acetal (**190**) with periodic acid in dry ether to give aldehyde (**191**) with retention of chirality, (**Scheme 48**).



Scheme 48: The oxidative cleavage of the acetal (**191**), Reagent: (i) HIO_4, dry ether 77%.

Another example is the work of Sarabia *et. al.*[209] where α,β-unsaturated ester (**194**) was prepared, in 2 steps, from hydrazone (**192**). Treatment of the aldehyde (**193**) with a stabilized ylid to give the final product proceeded with retention of stereochemistry at the α-position (**Scheme 49**).

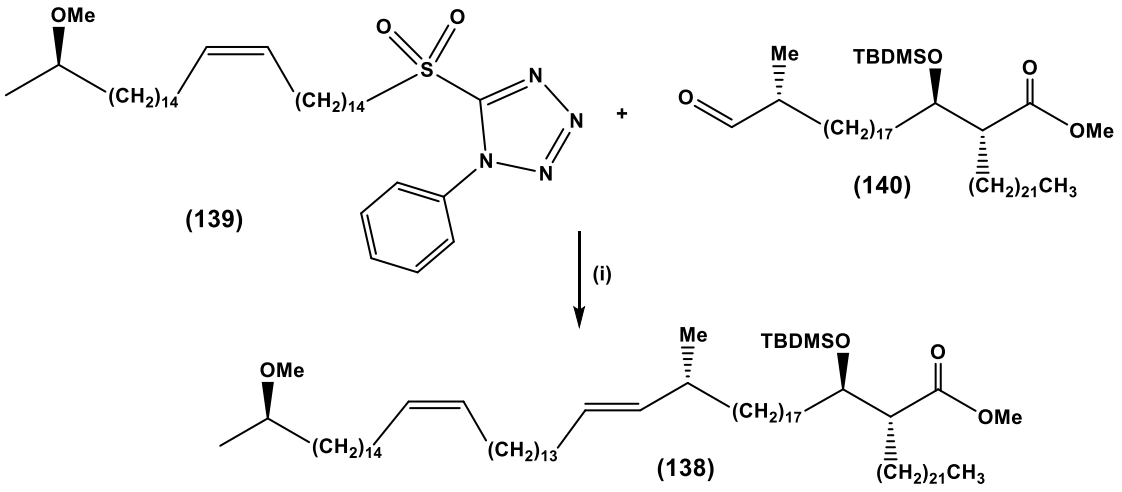


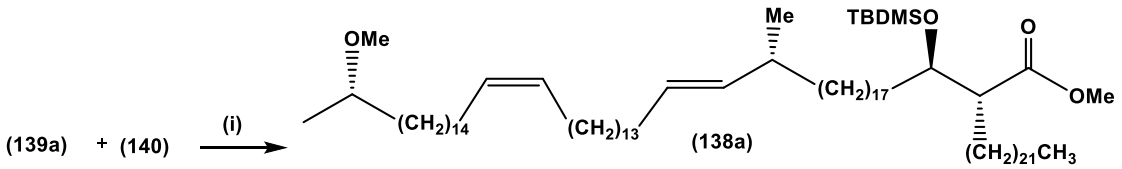
Scheme 49: Preparation of α,β-unsaturated ester (**194**), Reagent: (i) O_3, DCM; (ii) PPh_3=$CHCO_2Me$, DCM.

2.7 Synthesis of complete (ω-1)-methoxy mycolic acids by coupling of the meromycolate moiety (139) and motif unit (140)

Studies showed that the stereoselectivity and yield of the modified Julia olefination is sensitive to the base used to deprotonate the sulfone and solvent polarity.[156,211] A noteworthy new development revealed that 1-phenyl-1*H*-tetrazole sulfones (**139**) can give great stereoselectivity (*E*-isomers) with potassium *bis* (trimethylsilyl) amide as base. Recent studies supported this discovery that the stereoselectivity could lead only to the *E*-isomer especially if the sulfone or aldehyde was α-substituted.[212,213]

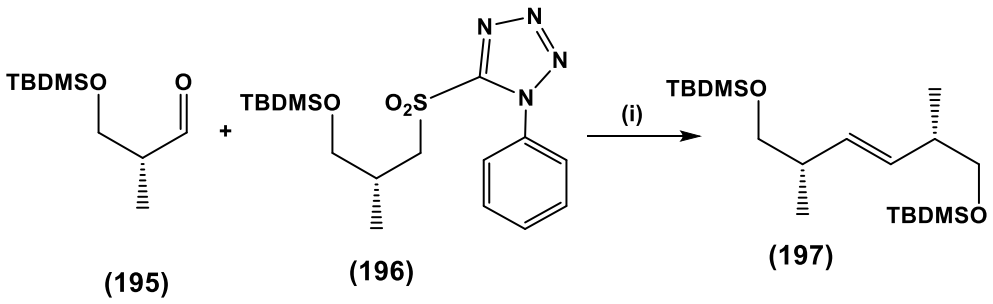
A coupling reaction between the aldehyde (**140**) and the *Z*-alkene sulfone (**139**) using potassium *bis*(trimethylsilyl)amide (0.5 M in toluene) gave the desired alkene (**138**) as only the *E*-diastereomer in 66 % yield and this coupling gave the whole structure of the (ω-1)-methoxy- *cis*-alkene-*trans*-alkene mycolic acid (**Scheme 50**). The stereoselectivity to give the *E*-isomer was confirmed by NMR spectroscopy. The ^{1}H NMR spectrum showed a multiplet at δ 5.40 − 5.30 and a broad doublet of doublets at δ 5.24 (*J* 7.5, 15.2 Hz) for the alkene protons. The coupling constant of 15.2 Hz between the olefinic hydrogen atoms confirmed the formation of the *trans*-alkene. There was also a multiplet at δ 2.10 – 1.90 for seven protons next to the double bonds. The ^{13}C NMR spectrum showed only three signals at the olefinic region at δ 136.5, 129.9 and 128.4 for *cis* and the *trans*-alkene carbons. In the same way the enantiomer (**138a**) was obtained. The specific rotation of (**138**) was measured as $[\alpha]_{\mathbf{D}}^{\mathbf{24}}$ – 3.6 (*c* 0.9, $CHCl_3$), while the specific rotation of the *S*-enantiomer was measured as $[\alpha]_{\mathbf{D}}^{\mathbf{24}}$ – 3.8 (*c* 1.0, $CHCl_3$).





Scheme 50: Reagents: (i) potassium *bis*(trimethylsilyl)amide, dry THF, (**138**, 66%), (**138a**, 43%).

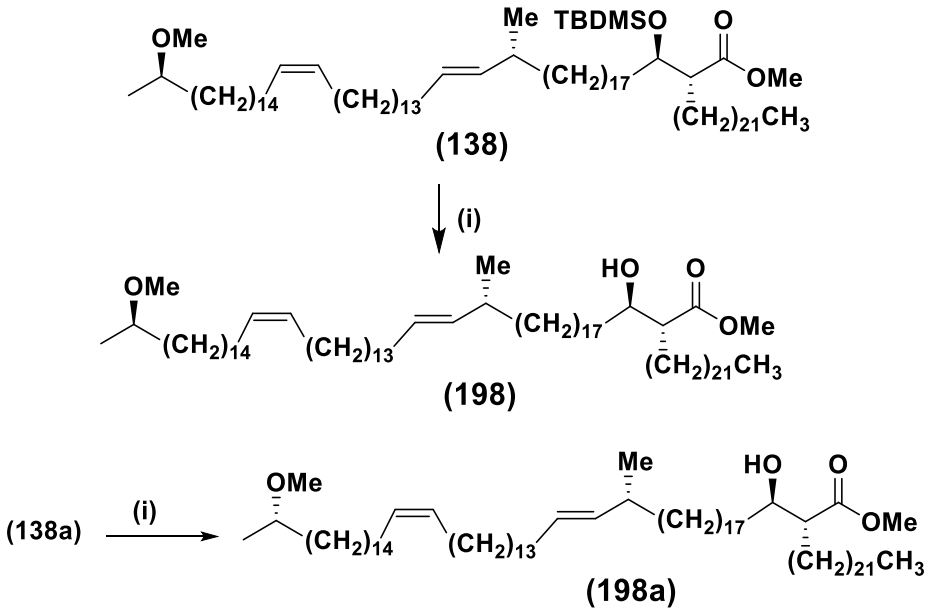
It is again essential that during the synthesis of (**138**) that no epimerisation occurs adjacent to the aldehyde. There are a number of examples of this kind of reaction where the chirality is retained,[214-216] for example (**Scheme 51**):



Scheme 51: Literature example of the retention of chirality adjacent to the aldehyde.[217] Reagents: (i) KHMDS.

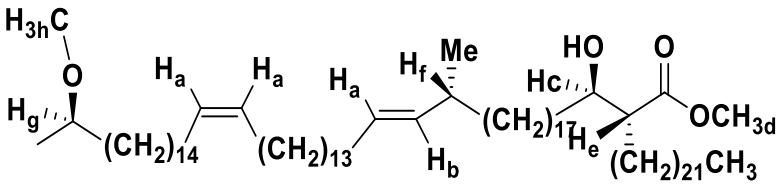
2.7.1 Deprotection and hydrolysis of (138) to produce free mycolic acid (129)

The *tert*-butyldimethylsilyl protecting group of compound (**138**), was removed using HF.pyridine in THF at 43 ℃ for 18 hrs. The product, secondary alcohol (**198**) (**Scheme 52**), was obtained in a yield of 66%. The success of the deprotection was confirmed by proton NMR, which showed the disappearance of the protons of the *t*-butyl dimethyl silyl group, while the remaining signals appeared similar to the protected MA (**138**). Again, the *S* enantiomer (**198a**) was obtained using the same method. The (ω-1)-methoxy mycolic acid ester fraction isolated from *M*. alvei cells, see introduction section **1.8** (page, **20**-**23**), had an $[\alpha]_D$ + 1.2 (*c* 2.0, $CHCl_3$); the value for the single synthetic ester (**198**) was $[\alpha]_D^{24}$ + 1.3 (*c* 1.0, $CHCl_3$). The *S*-methoxy epimer showed $[\alpha]_D^{24}$ + 1.8 (*c* 1.0, $CHCl_3$). The NMR spectra of the two epimers were essentially identical and identical to those of the natural mixture isolated from the cells. The IR spectrum showed a peak at 3518 cm^{-1} to confirm the formation of the O–H bond. A detailed analysis of the spectroscopic data for (**198**) is given below and compared with natural material methyl (ω-1)-methoxymycolate fraction (**24**, page **20**) (**Table 2**).



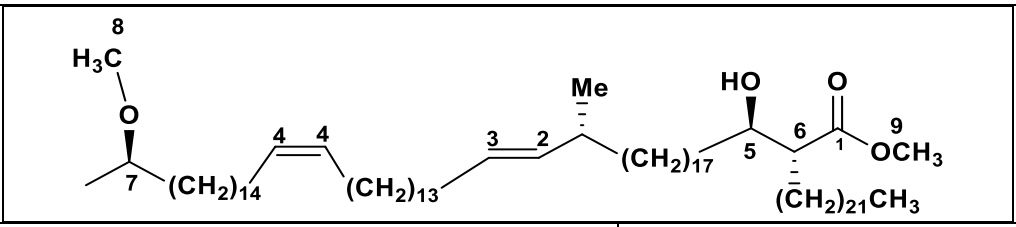
Scheme 52: Synthesis of complete mycolic acids. Reagents: (i) HF Pyridine, pyridine, THF, 43 °C, 18 hrs, (**198**, 66%), (**198a**, 66%).

Table 2: ^{1}H NMR and ^{13}C NMR of ω-1 methoxy *cis*-alkene-α-methyl-*trans*-alkene mycolic acid ester **(198)** compared with the natural material fraction (**24**).



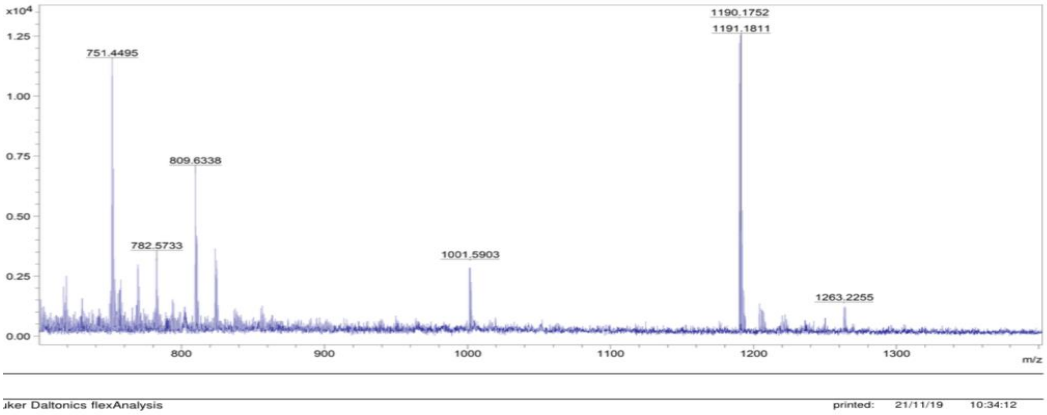
	Synthetic (198)				Natural (24)[117]			
Proton	***δ/ppm***	**Multiplicity**	**Integration**	***J*/ Hz**	***δ/ppm***	**Multiplicity**	**Integration**	***J*/ Hz**
H_a	5.42 – 5.29	m	3	----	5.38 – 5.29	m	3	----
H_b	5.23	br. dd	1	7.4,15.3	5.23	br. dd	1	7.4,15.4
H_c	3.66 – 3.62	m	1	----	3.68– 3.62	m	1	----
H_d	3.71	s	3	----	3.71	s	3	----
H_e	2.44	dt	1	5.3, 9.2	2.43	br. dt	1	5.3,9.1
H_g	3.27	br.pent	1	6.0	3.30-3.24	br.pent	1	6.0

H_h	3.32	s	3	-----	3.31	s	3	---
H_f +6CH_2 adjacent to double bonds	2.09 – 1.94	m	7	----	2.10– 1.93	m	7	----
CH_3	0.94	d	3	7.0	0.93	d	3	6.7
Terminal CH_3	0.88	t	3	7.0	0.88	t	3	6.6



Synthetic (198)		**Natural (24)[117]**
Carbon	***δ/ppm***	***δ/ppm***
C_1	176.2	176.2
C_2	136.5	136.5
C_3	128.4	128.4
C_4	129.9	129.9
C_5	72.2	72.3
C_6	51.5	51.5
C_7	76.9	76.9
C_8	55.8	55.9
C_9	50.9	50.9

The MALDI-MS confirmed that the synthetic ω-1 methoxy mycolic acid ester (**198**) (**Figure 37**) is identical to the major component of the *M. alvei* natural mixture (**Figure 38**) (provided by Dr Alison Jones).[117]



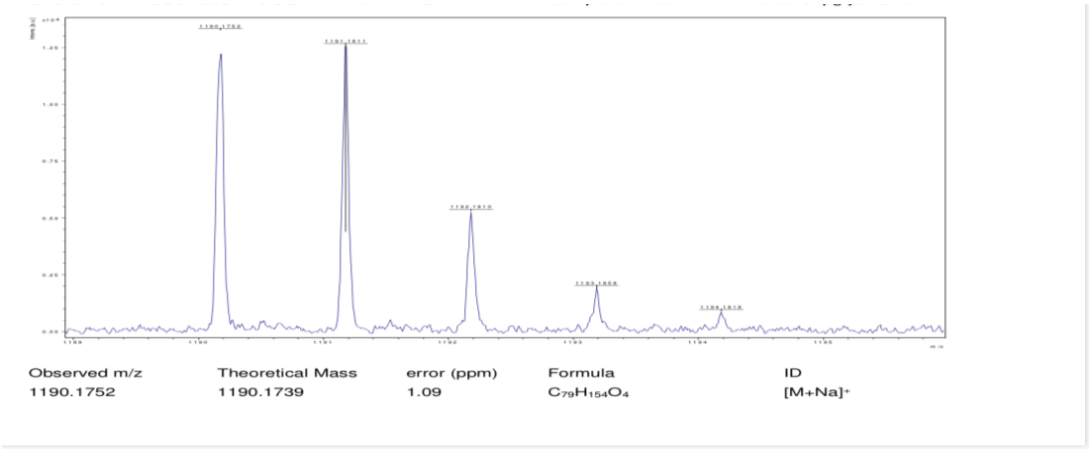


Figure 37: Mass spectrometry data for ω-1 methoxy mycolic acid ester (**198**).

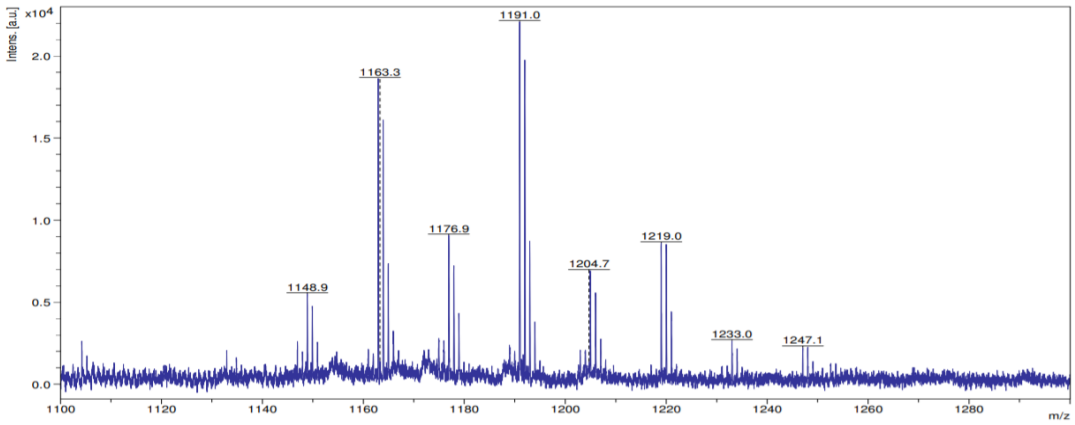


Figure 38: MALDI MS of natural methyl (ω-1)-methoxymycolate fraction.

The MALDI-MS of the synthetic mycolic acid ester (**198**) gave 1190.1752 $[M + Na]^+$, the calculated value $C_{79}H_{154}NaO_4$ requires: 1190.1739, as shown in the expansion in

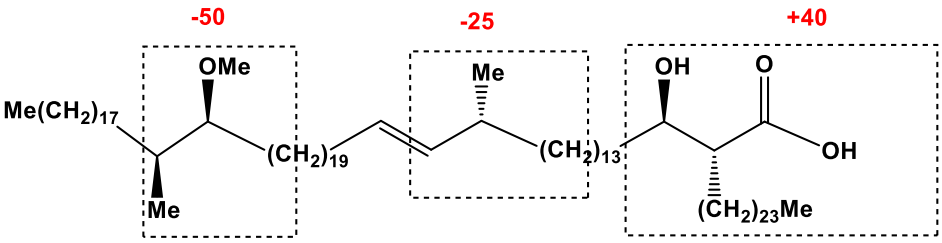
(**Figure 37)**. As can be seen in this MS there are a set of peaks for the compound, with that at 1190.1752 corresponding to the monoisotopic mass. The major peak for the compound is a 1191, which corresponds to that shown for the major component in the MS of the natural ω-1 methoxy mycolate fraction (**Figure 38**).

The molecular rotation is defined as one-hundredth of the product of the specific rotation and the relative molecular mass of an optically-active compound:

$$[\mathbf{M}]_{\mathbf{D}} = [\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{x}} \left(\frac{\textbf{Molecular weight}}{\mathbf{100}}\right)$$

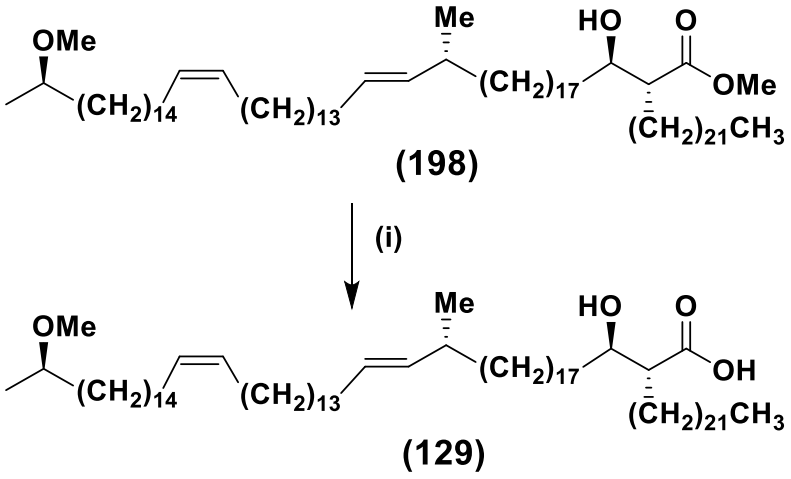
Therefore, the molecular rotations of individual functional groups in natural mixtures of mycolic acids and in model compounds have been identified by Quémard *et. al*.[114] In general, the $[M]_D$ in such systems where groups of chiral centres are separated by long carbon chains are approximately additive; the individual values of the *R,R*-centres of the 2-hydroxy acid ester fragment in mycolates is estimated as + 40, the RCH=CHCH$_3$- fragment as – 25 (for 30% content). The figure for the (ω-1)-methoxy fragment such as (**183**, page **58**) may be estimated from the present result as – 3.5 using the relationship above. Therefore, the $[M]_D$ value of the methyl mycolate (**198**) can be estimated as + 11.5 (–3.5–25+40) based on the sum of (ω-1)-methoxy, *R*, *R*-hydroxy acid ester and α-methyl *trans* alkene contributions. Moreover, the specific rotation of mycolic acid ester (**198**) was determined to be +1.3 (*c* 0.99, $CHCl_3$), which corresponds to $[M]_D$ +15.2, which is in agreement with the calculated value.

By comparison for the methoxy mycolic acid (**199**)[207] the specific rotation of this acid was determined to be – 1.6 (*c* 0.99, $CHCl_3$), which corresponds to an $[M]_D$ – 24. The figure for the (*S,S*) α-methyl-methoxy fragment may be estimated from the present result as – 50, the RCH=CHCH$_3$- fragment as – 25 and *R,R*-centres of the 2-hydroxy acid (ester) fragment in mycolates is estimated as + 40. Therefore, the $[M]_D$ value can be estimated as – 35 (– 50 – 25 + 40) which is in agreement with the calculated value.



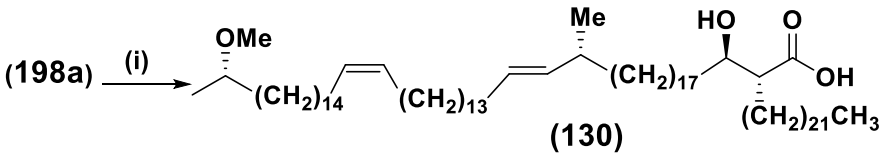
(199)

The hydroxy-mycolic acid ester (**198**) was hydrolysed using lithium hydroxide in a mixture of THF, methanol and water at 45 °C for 16 hrs**,** (**Scheme 53**). After purification by column chromatography, eluting with $CHCl_3$/MeOH (15:1) and then (5:1) the free MA (**129**) was obtained in a yield of 93%. The ^{1}H NMR spectrum showed a multiplet at δ 5.39 – 5.29 and a broad doublet of doublets at δ 5.24 (*J* 7.4, 15.3 Hz) for the alkene protons. There was also a broad multiplet at 3.75 – 3.62 for one proton next the hydroxy group β position and a multiplet at δ 2.06 – 1.99 for seven protons next to the double bond. The ^{13}C NMR spectrum showed only three signals in the olefinic region at δ 136.5, 129.9 and 128.4 for *cis* and the *trans*-alkene carbons. The IR spectrum further confirmed the formation of mycolic acid (**129**), which showed a broad peak at 3272 cm^{-1} for the OH stretch. Following the same steps, the *S*-enantiomer was prepared and the ^{1}H and ^{13}C NMR spectra were identical to (**129**). Also compounds (**129**), (**130**) (*R*, and *S*-enantiomers) showed $[\alpha]_D^{23}$ + 0.86 (*c* 0.81, $CHCl_3$) and $[\alpha]_D^{24}$ + 0.89 (*c* 0.78, $CHCl_3$), respectively.



(198)

(129)



Scheme 53: Synthesis of complete mycolic acids. Reagents: (i) LiOH, MeOH, H_2O, THF, 45 °C, 16 hrs (**129**, 93%), (**130**, 71%).

2.8 Synthesis of sugar esters of MA (129)

Mycobacterial cord factors are known as very interesting compounds due to their biological activity, which has been the subject of many investigations. Studies have shown that these components of mycobacteria have immune activity. In mycobacterial cell walls, some of the mycolic acids are esterified to trehalose, forming cord factors. If two mycolic acids are esterified to trehalose, this forms trehalose-6,6'-dimycolates (TDMs), and if one mycolic moiety is esterified to trehalose, this forms trehalose monomycolates (TMMs). The methyl arabino-mycolates (MAM) is a part of the cell wall of *M. tuberculosis*, while GMM has immunological properties. GMMs have the capacity to produce T cell proliferative responses in a number of species including humans.[218] In addition, investigating their biological activity could lead to improvements in the detection and diagnosis of mycobacterial diseases. The synthesis of a range of sugar esters of the ω-1 methoxy MA (**129**) will be discussed below, by esterification of the *R* enantiomer. Similarly, esterification of the *S* enantiomer (**130**) was also carried out.

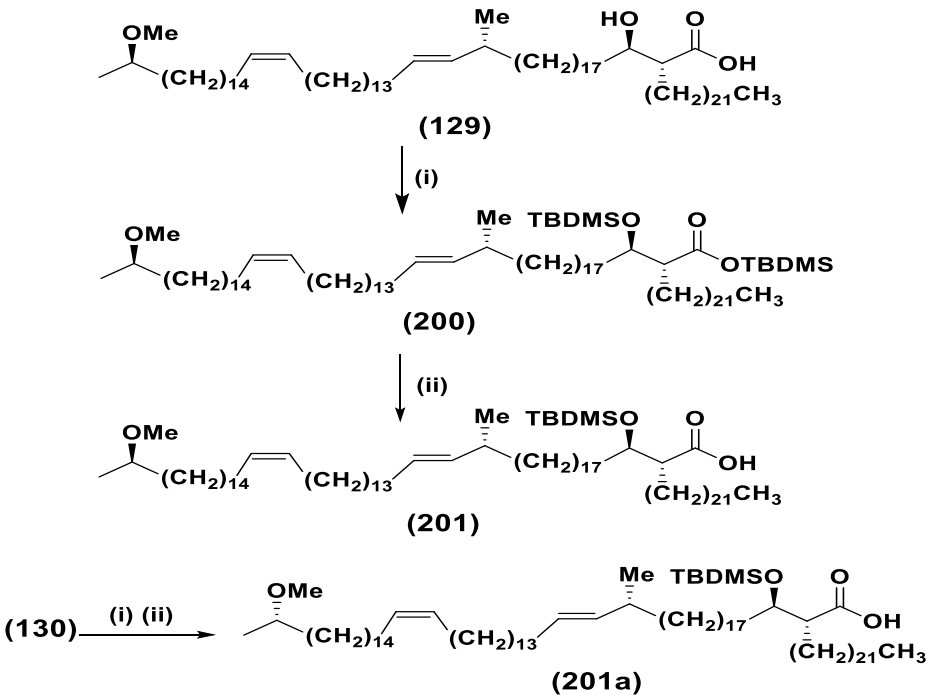
2.8.1 Synthesis of trehalose esters of ω-1-methoxy MA (129)

The synthetic ω-1 methoxy *cis*-alkene -*trans*-alkene mycolic acid (**129**) was esterified with trehalose to produce trehalose dimycolate (TDM) and trehalose monomycolate (TMM). In order to prepare a (TDM) and (TMM) from the unsaturated mycolic acid (**129**), it was necessary to protect the β-hydroxyl group with a TBDMS protecting group.

2.8.2 Protection of the secondary alcohol at the β-position of MA (129)

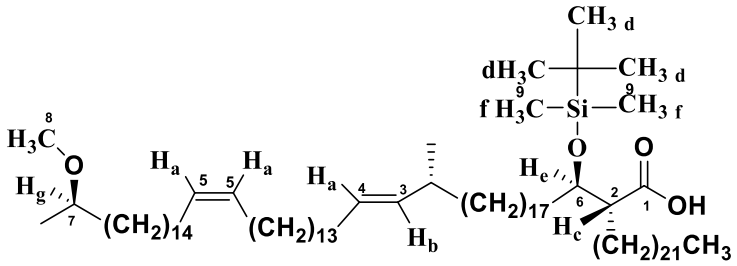
The secondary alcohol of (**129**) was protected by stirring it with *tert*-butyldimethylsilylchloride, imidazole, DMAP and tetra-*n*-butyl ammonium hydroxide in a mixture of DMF/toluene at 70 °C for 24 hrs. The reaction usually leads to the protection of both the β-hydroxy group and the carboxylic acid to give (**200**), which then requires the hydrolysis of the carboxylic acid by dissolving it in THF, then adding a

solution of tetra-*n*-butyl ammonium hydroxide. The mixture is stirred for 1 hr at room tempruture to give the target compound (**201**) in a yield of 94%. (**Scheme 54**), (**Table 3**).



Scheme 54: Protection of the free ω-1-methoxy MA. Reagents: (i) imidazole, TBDMSCl, DMAP, tetra-*n*-butyl ammonium hydroxide 70°C, 18 hrs, and tetra-*n*-butyl ammonium hydroxide, THF (**201**, 94%), (**201a**, 94%).

Table 3: Selected ^{1}H NMR and ^{13}C NMR data for protected secondary alcohol (**201**).

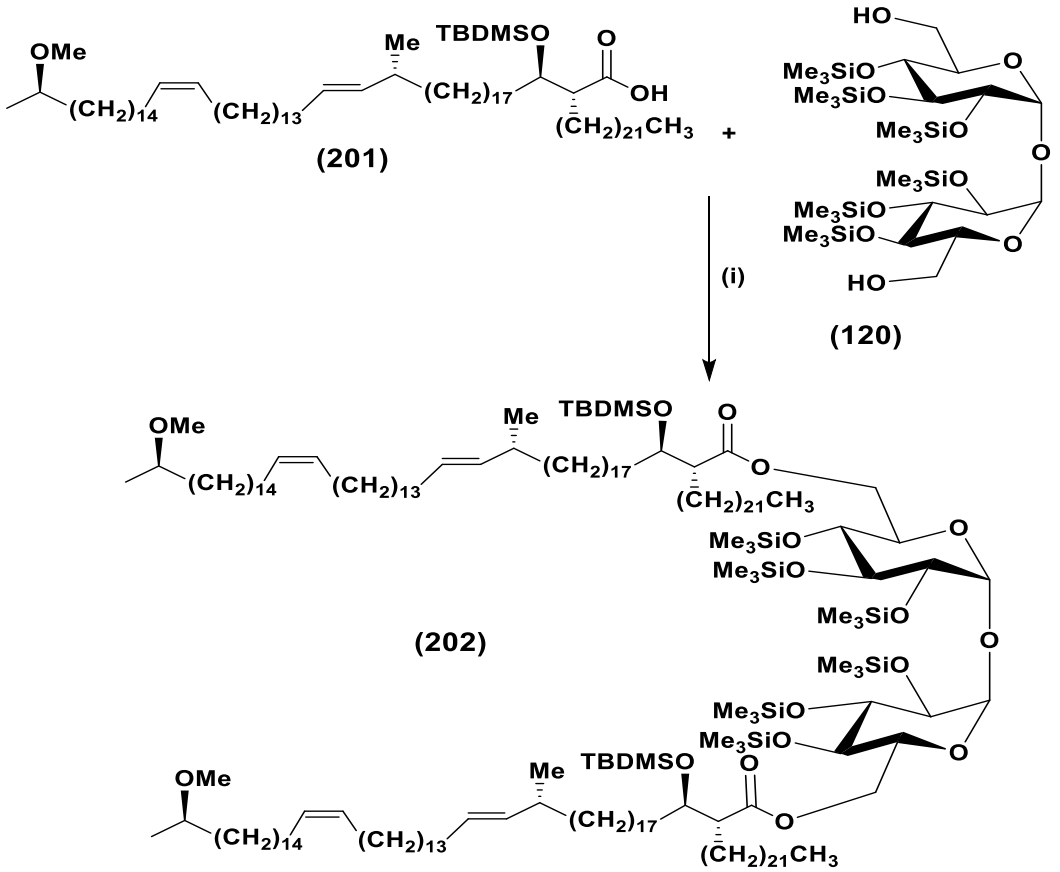


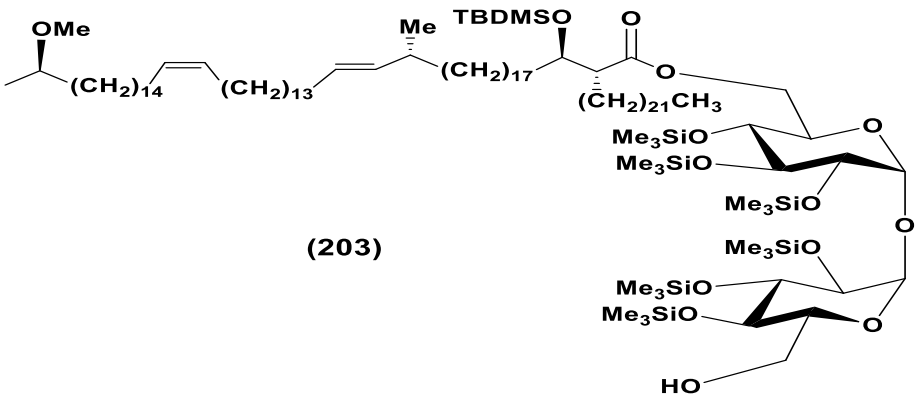
Proton	*δ/ppm*	Multiplicity	Integration	*J*/ Hz	Carbon	*δ/ppm*
H_a	5.41 – 5.29	M	3	----	C_1	175.9
H_b	5.24	br. dd	1	7.4,15.2	C_2	50.3
H_c	2.53	Ddd	1	2.7, 5.9, 8.8	C_3	136.5
H_d	0.93	S	9	----	C_4	128.4
He	3.83	Ddd	1	3.0, 5.6, 7.6	C_5	129.9
H_f	0.15, 0.14	S	6		C_6	73.6

H_j	3.27	br. pent	1	6.0	C_7	76.9
CH_3 next O	3.32	S	3		C_8	55.9
					C_9	-4.3, -4.9

2.8.3 Coupling of protected (ω-1)-methoxy mycolic acid to protected trehalose

The protected mycolic acid (**201**) was esterified with protected trehalose (**120**) using coupling agents (EDCI and DMAP) in dichloromethane. After stirring the reaction mixture for 8 days at room temperature under a nitrogen atmosphere, TLC analysis showed a mixture of products. These were separated by column chromatography to give protected trehalose dimycolate (TDM) (**202**) and trehalose monomycolate (TMM) (**203**) (**Scheme 55**).





(203)

Scheme 55: Reagents: (i) DMAP/EDCI/DCM, TDM 21%, TMM 28%.

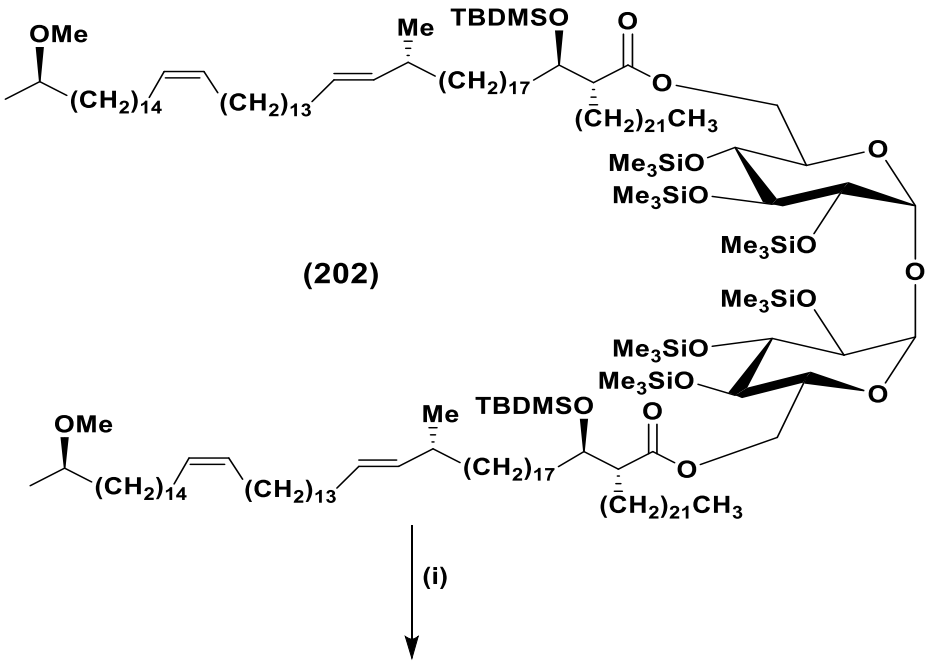
The TDM (**202**) was obtained in 21% yield and was fully characterized by ^{1}H NMR spectroscopy. The spectrum included a multiplet at δ 5.40 – 5.29 and a double doublet at δ 5.24 (*J* 7.4, 15.3 Hz) corresponding to the eight alkene protons. The two acetal protons of the trehalose appeared as a broad doublet at δ 4.85 (*J* 3.0 Hz), and the remaining trehalose protons, including the β-position protons appeared at δ 4.37, 4.04 – 3.97, 3.96 – 3.93, 3.52, and 3.38, while the singlet at δ 3.32 corresponded to the OMe group. The α-position proton of the mycolic acid appeared as a multiplet at δ 2.57 – 2.53, and a multiplet appeared at δ 2.06 – 1.94, integrating to fourteen protons, which corresponded to those next to the double bonds and the proton adjacent to the α-methyl next to the *trans* double bond. The *tert*-butyl protons appeared as a singlet at δ 0.88, and the terminal methyl groups of the alkyl chains appeared as a triplet at δ 0.90 *(J* 6.6 Hz). The protons of the trimethylsilyl protecting groups on the trehalose gave three singlets at δ 0.16, 0.14, and 0.13 each integrating to eighteen protons. The methyl groups of the TBDMS protecting group showed a singlet at δ 0.06 integrating to twelve protons. The ^{13}C NMR spectrum showed the carbonyl carbon signal at δ 173.8, and the double bond carbon signals appeared at δ 136.5, 129.9 and 128.4, and the remaining trehalose carbon signals appeared between δ 73.5 –70.7. The methyl carbon signals of the protecting silyl groups of the sugar appeared at δ 1.09, 0.94 and 0.15. Additionally, the carbons of the two methyl groups bonded to silicon in the silyl groups of the mycolate component appeared at δ – 4.5 and δ – 4.6. The mass spectrum for the TDM (**202**) gave the correct mass ion {Found $[M+Na]^{+}$: 3297.8398; $C_{198}H_{398}O_{17}Si_8Na$ requires: 3297.8375}.

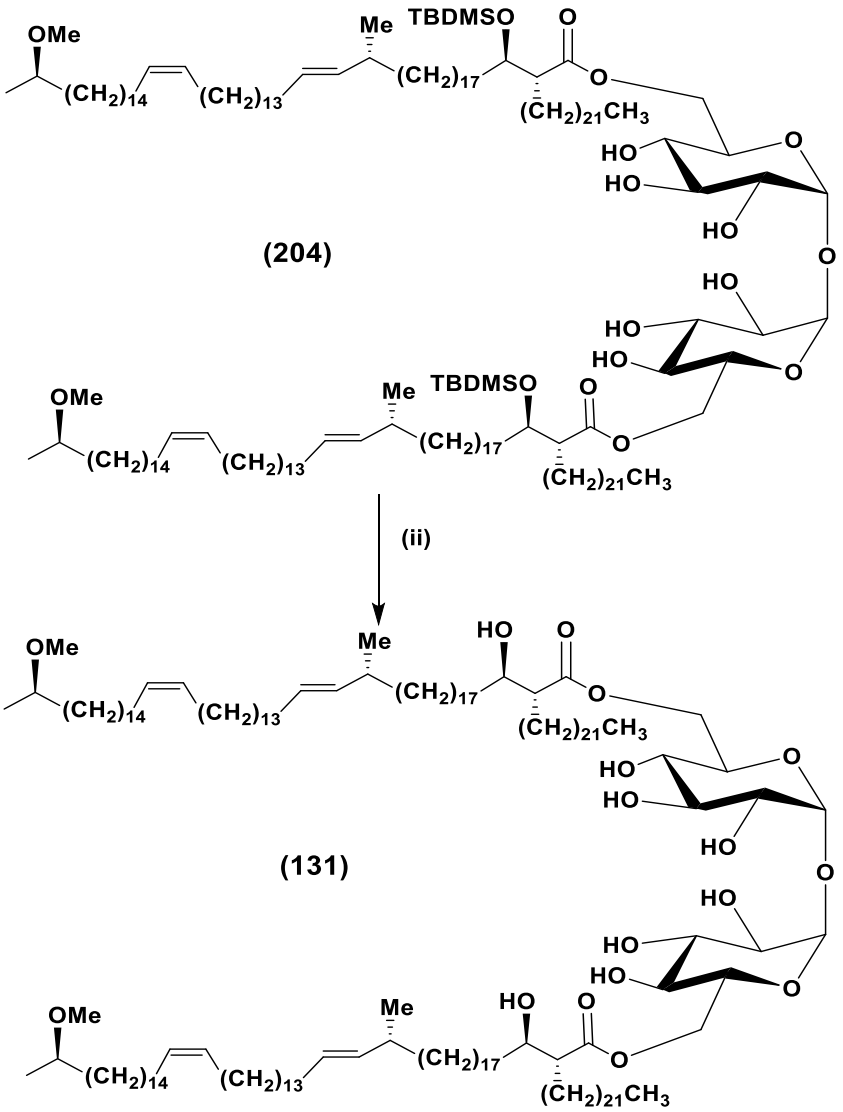
The second fraction was TMM (**203**), obtained in a yield of 28%. The hemiacetal protons appeared as two doublets at δ 4.91 (*J* 3.0 Hz) and 4.84 (*J* 3.0 Hz) with an integration of

one proton for each, and the remaining sugar protons appeared between δ 4.35 – 3.39. It was found that the methyl signals for the protecting groups on the sugar were divided into five groups, one for each (Me) situated between δ 0.17 – 0.12, integrated to 54 hydrogens in total. The signals for the protecting groups were similar to those found for the TDM (**202**), but with half the number of the protons relating to the mycolic acid sub class. The ^{13}C NMR spectrum displayed signals at δ 174.1 for the carbonyl carbon group and anomeric carbon signals at δ 94.5 and 94.4. Signals at δ – 4.5 and – 4.7 were displayed for the two methyl groups bound to the silicon in the TBDMS group. The mass spectrum for the TMM (**203**) gave the correct mass ion at {Found $[M+Na]^+$: 2047.5918; $C_{114}H_{234}O_{14}Si_7Na$ requires: 2047.5901}. In the same way, the *S*-enantiomer was synthesized, to give a yield of 18% (TDM, **202a**), mass spectrum {Found $[M+Na]^+$: 3297.8405; $C_{198}H_{398}O_{17}Si_8Na$ requires: 3297.8375}, and 31% (TMM, **203a**), mass spectrum {Found $[M+Na]^+$: 2047.5916; $C_{114}H_{234}O_{14}Si_7Na$ requires: 2047.5901}.

2.8.4 Deprotection of the trehalose and β-position of TDM (202)

The removal of the trimethylsilyl protecting groups on the trehalose core of the TDM (**202**) was achieved using tetra-*n*-butyl ammonium fluoride (TBAF) in dry THF, to give (TDM) (**204**) in 84% yield. (**Scheme 56**).





Scheme 56: Synthesis of TDM. Reagents: (i) tetrabutylammonium fluoride, THF, TDM, 84%; (ii) HF. Pyridine, pyridine, THF, 45 ℃, 56%.

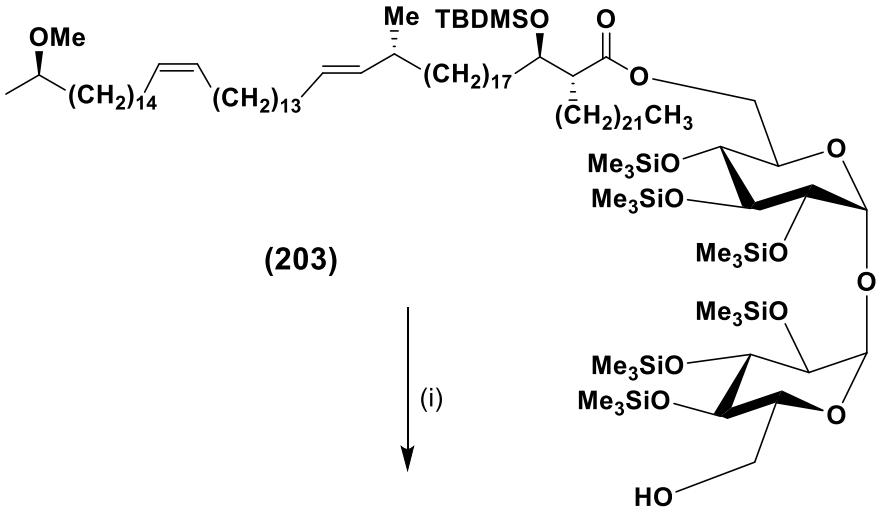
The ^{1}H NMR spectrum of silyl protected TDM (**204**) showed a broad doublet for the hemiacetal protons, that was shifted to δ 5.05 (*J* 3.2 Hz). The remaining signals for the sugar protons and the β-hydroxyl protons resonated at δ 4.33, 4.20, 3.98 – 3.85, 3.74, 3.44 and 3.32 – 3.23 and were also shifted. The proton at the α-position appeared as a multiplet at δ 2.59 – 2.46, and the doublet at δ 0.89 (*J* 6.7 Hz) corresponded to the α-methyl *trans* group. The terminal methyl groups gave a triplet at δ 0.84 (*J* 6.0 Hz) and the *tert*-butyl groups showed as a singlet at δ 0.81 with an integration of eighteen protons. The signals corresponding to the trimethylsilyl protecting groups on the sugar were not present in the spectrum, which confirmed that the reaction had been successful.

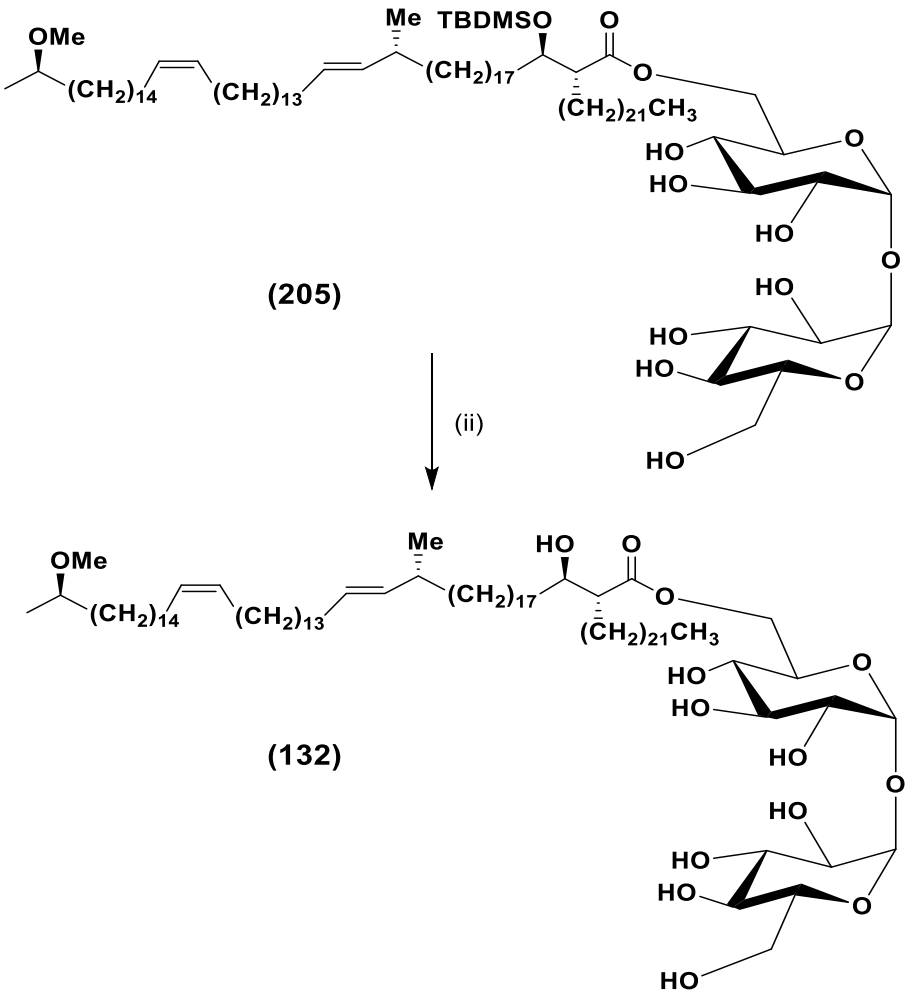
The mass spectrum gave the correct mass, {Found $[M+Na]^+$: 2865.6033; $C_{180}H_{350}O_{17}Si_2Na$ requires: 2865.6015}.

Finally, the deprotection of the *tert*-butyldimethylsilyl group on the β- position of the mycolic acid (**204**) using HF. pyridine complex and pyridine at 45 °C for 17 hrs (**Scheme 56**) gave the free **(**TDM**)** (**131**) in 56% yield. The proton NMR spectrum of the free TDM (**131**) showed a multiplet at δ 5.44 – 5.28 and a double doublet at δ 5.22 (*J* 7.4, 15.4 Hz) for the protons of the double bonds. The acetal protons gave a broad doublet at δ 4.99 (*J* 3.3 Hz), and the remaining trehalose protons including the β-position protons resonated at δ 4.81, 4.35, 3.85, 3.75, 3.72 – 3.65 and 3.52 with an integration of two protons for each signal. The α-position proton of the mycolic acid appeared as a multiplet at δ 2.43 – 2.37, and the protons next to the double bonds appeared as a multiplet at δ 2.04 – 1.92 integrating to fourteen protons. The ^{13}C NMR spectrum showed the carbonyl carbon at δ 175.5, and the alkene carbons at δ 136.4, 129.8 and 128.3. The anomeric carbon signal appeared at δ 95.2, and the remaining trehalose carbon signals appeared between δ 72.5 – 71.1. The mass spectrum for TDM (**131**) {Found $[M+Na]^+$: 2635.4238; $C_{168}H_{322}O_{17}Na$ requires: 2635.4224}.

2.8.5 Deprotection of the trehalose and β-Position of TMM (203)

The trimethylsilyl protecting groups of the trehalose of TMM (**203**) were removed using tetra-*n*-butyl ammonium fluoride (TBAF) in dry THF. The TBAF was added to a stirred solution of the TMM (**203**) in dry THF, and then stirred for 1 hr at room temperature. The residue was added directly to the silica gel column after concentration in vacuum, and this gave the target compound (**205**) in 78% yield. (**Scheme 57**)





Scheme 57: Synthesis of TMM. Reagents: (i) tetrabutylammonium fluoride, THF, 78%; (ii) HF Pyridine, pyridine, THF, 45 °C, 29%.

The ^{1}H NMR spectrum of the silyl-protected TMM (**205**) showed a multiplet at δ 5.32 – 5.26 and a broad double doublet at δ 5.18 (*J* 7.4, 15.4 Hz) for the protons of the double bonds, while the hemiacetal protons appeared as a broad singlet at δ 5.03 with an integration of two protons. The remaining sugar protons resonated between δ 4.30 – 3.20. The ^{1}H NMR spectrum confirmed the disappearance of the signals for the trimethyl protecting groups at δ 0.16, 0.14 and 0.12, confirming that the deprotection was successful. The signals corresponding to the carbons of the trimethylsilyl groups were also absent from the ^{13}C NMR spectrum.

The deprotection of the *tert*-butyldimethylsilyl group at the β-position of mycolic acid (**205**) was achieved by using HF. pyridine complex and pyridine in dry THF and stirring at 45 °C for 17 hrs. After work up as above the final free TMM (**132**) was obtained in

29% yield. The ^{1}H NMR spectrum confirmed the disappearance of the signals for the TBDMS protecting group at δ 0.79, – 0.016, and – 0.038, confirming that the deprotection was successful. The ^{13}C NMR spectrum also confirmed the disappearance of the *tert*-butyldimethyl silyl group signals. The mass spectrum for TMM (**132**) {Found $[M+Na]^{+}$: 1500.2646; $C_{90}H_{172}O_{14}Na$ requires: 1500.2639}.

In the same way, the TDM (**131a**) and TMM (**132a**) for the *S*-enantiomer (**130**) were synthesized, in yields of 59% and 39%, respectively (**Figure 39**).

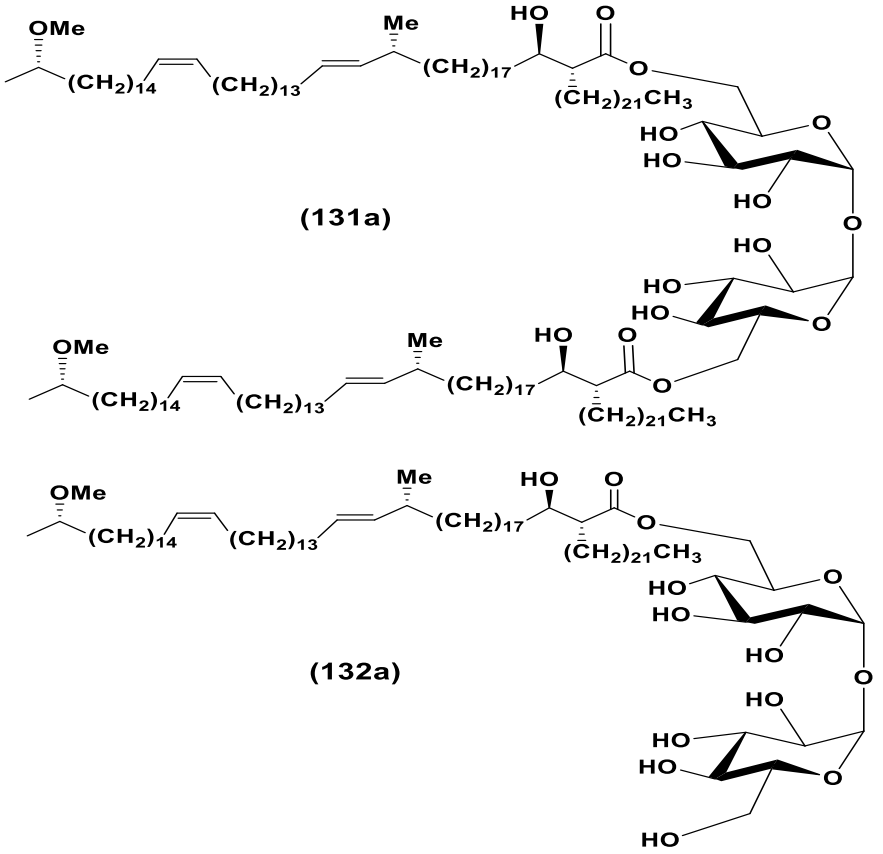
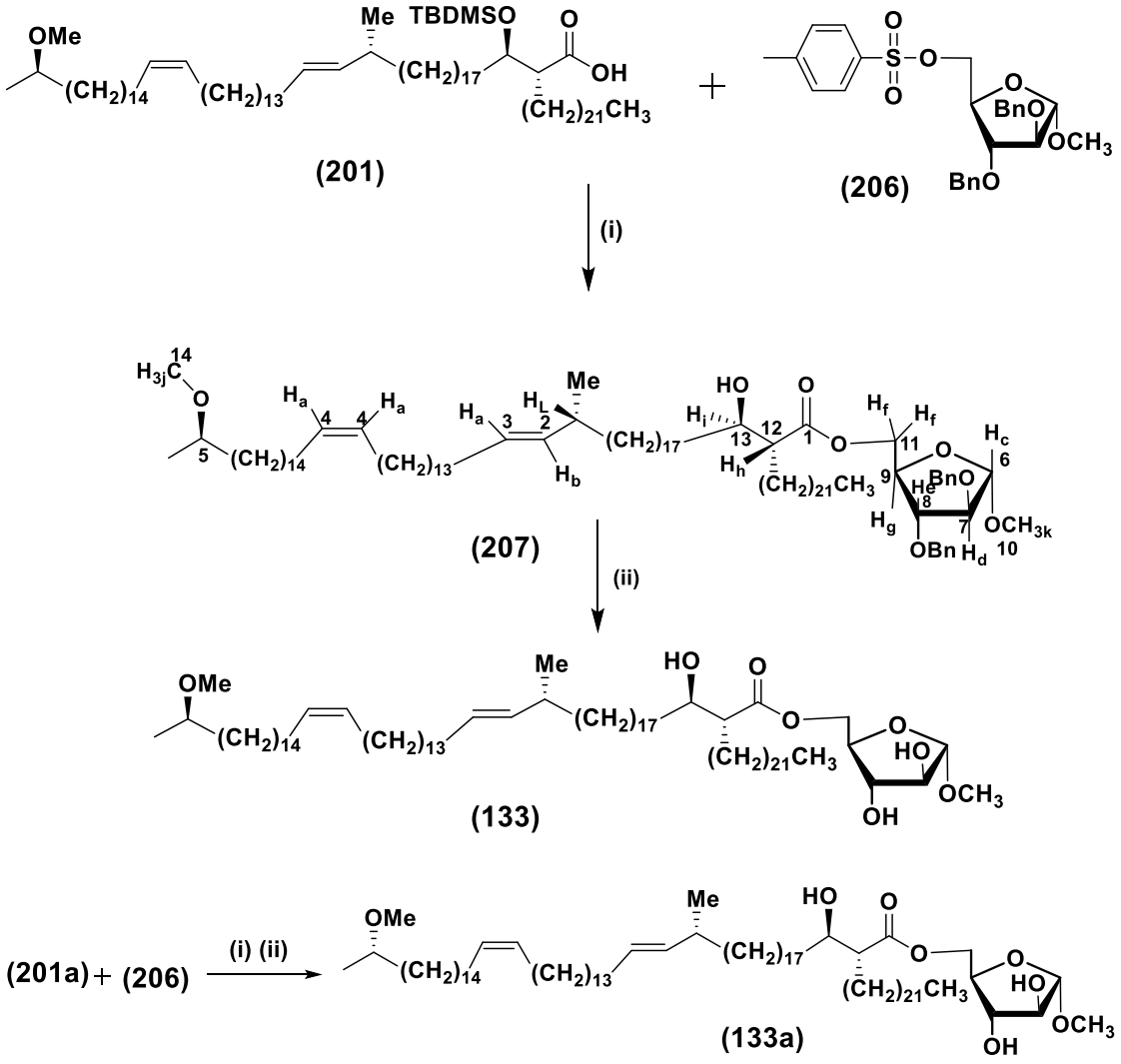


Figure 39: Synthesis of TDM (**131a**), TMM (**132a**) for MA *S*-enantiomer (**130**).

2.8.6 The synthesis of arabino-MA

Using a modification of established methods,[219] the protected mycolic acid (**201**) was reacted with protected arabinofuranoside (**206**), using dry cesium hydrogen carbonate in a mixture of dry DMF/THF to give (**207**) in 57% yield, (**Scheme 58**). Deprotection of the silyl group at the β-position of the mycolic acids, as well as coupling occurred in the first step. The ^{1}H NMR spectrum of the protected compound (**207**) showed a multiplet at δ 7.39 – 7.28 for the ten aromatic protons, a multiplet at δ 5.40 – 5.29 and a broad double doublet at δ 5.24 (*J* 7.4, 15.2 Hz) corresponding to Ha and Hb respectively of the double bonds. A broad singlet at δ 4.92

corresponded to Hc, and the signals for the CH_2 of the benzyl group appeared as a doublet in the range δ 4.57 – 4.47, together with a broad doublet at δ 4.30 (*J* 12.0 Hz) integrating to one to proton corresponding to Hf. Also, a broad doublet at δ 4.28 (*J* 12.0 Hz) corresponded to Hf and a broad triple of doublet at δ 4.21 (*J* 4.6, 6.4 Hz) corresponded to Hg. A double doublet appeared at δ 3.99 (0.6, 2.4 Hz) and a double doublet at δ 3.84 (2.7, 6.4 Hz) for Hd, He, while the singlet at δ 3.37 corresponded to the methoxy group. The ^{13}C NMR spectrum showed signals for the carbonyl ester at δ 175.0, while the two signals for the CH_2 of the benzyl group appeared at δ 72.4 and δ 72.0 together with a signal at δ 72.1 for C13, while C-6, C-7, C-8, C-9 and C-11 were seen at δ 107.2, 87.9, 83.7, 79.4 and 63.5 respectively. The O<u>C</u>H$_3$ group appeared at δ 54.9.

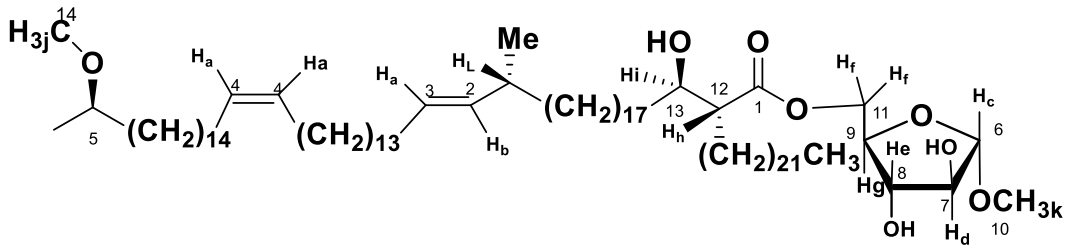


Scheme 58: Synthesis of arabino-MA. Reagents: (i) cesium hydrogen carbonate, DMF/THF (1:5), 70 ^{o}C, (**207**, 57%), (**207a**, 70%); (ii) Na, NH_3, 1,4- dioxane, (**133**, 32%), (**133a**, 38%).

Then removal of the benzyl groups in (**207**) was achieved using sodium in liquid ammonia to give the deprotected compound (**133**), in 32% yield. The ^{1}H NMR spectrum confirmed

that the deprotected compound (**133**) had been obtained successfully. The ^{1}H NMR spectrum of this showed that the signals corresponding to Hc had shifted upfield to δ 4.78 from δ 4.92 after losing the deshielding effect of the aromatic ring (**Table 4**). The mass spectrum for the free arabino-MA (**133**) {Found $[M+Na]^{+}$: 1322.2173; $C_{84}H_{162}O_8Na$ requires: 1322.2162}. A detailed analysis of the spectroscopic data for (**133**) is given below.

Table 4: NMR 1H and 13C analysis of AG (**133**).

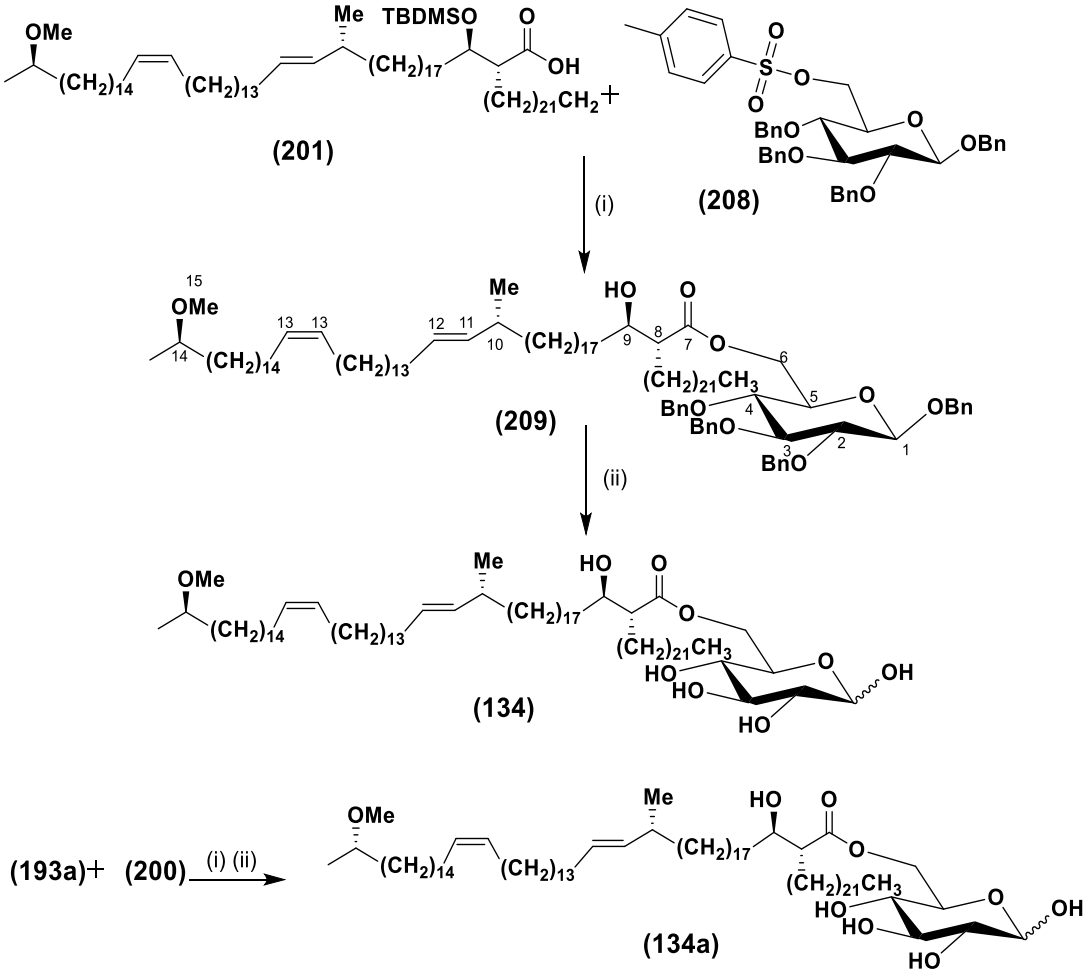


Proton	*δ/ppm*	Multiplicity	Integration	*J*/ Hz	Carbon	*δ/ppm*
H_a	5.36 – 5.25	m	3	-----	C_1	175.1
H_b	5.18	br. dd	1	7.4, 15.2	C_2	136.4
H_c	4.78	br.s	1	-----	C_3	128.3
H_f	4.32	dd	1	4.5,11.7	C_4	129.8
H_f	4.28	dd	1	5.0,11.7	C_5	77.0
H_g	4.07	br.q	1	4.8	C_6	108.8
H_d	3.96	br.s	1	----	C_7	81.8
H_e	3.84	br.q	1	2.8	C_8	81.2
H_i	3.65 – 3.52	m	1	-----	C_9	77.9
H_j	3.27	s	3	----	C_{10}	54.9
H_k	3.34	s	3	-----	C_{11}	63.4
H_h	2.43 – 2.34	m	1	----	C_{12}	52.6
CH_3 α-to the double bond	0.88	d	3	6.7	C_{13}	72.4
Terminal CH_3	3	t	0.83	7.1	C_{14}	55.7

Again, the *S*-enantiomer (**201a**) was converted into (**133a**) using the same method (**Scheme 58**).

2.8.7 Synthesis of GMM

Again, using a modification of the published procedure,[184] the protected mycolic acid (**201**) was allowed to react with protected glucose (**208**), to give (**209**), as shown in (**Scheme 59**).



Scheme 59: Synthesis of GMM. Reagents: (i) cesium hydrogen carbonate, DMF/THF (1:5), 70 ^{o}C, (**209**, 64%), (**209a**, 63%); (ii) Na, NH_3, 1,4-dioxane, (**134**, 22%), (**134a**, 15%).

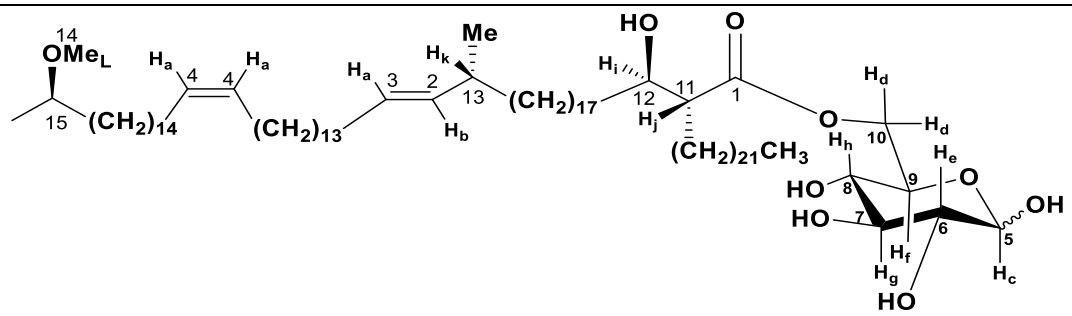
The ^{1}H NMR spectrum of (**209**) showed a multiplet at δ 7.40 – 7.28 for the twenty aromatic protons and a multiplet at δ 5.40 – 5.29 and a broad double doublet at δ 5.24 (*J* 7.4, 15.3 Hz), corresponding to the protons of the two double bonds in the mycolate moiety C-11, C-12 and C-13. The signals for each of the CH_2 groups of the benzyl groups on the sugar appeared as doublets in the range δ 4.96 – 4.60. A multiplet integrating to two protons at δ 4.56 – 4.52, corresponds to the β-anomeric proton attached to C-1, and another proton attached to C-6, and a double doublet at δ 4.22 (*J* 4.7, 12.0 Hz) corresponds to the second proton attached to C-6. A singlet at δ 3.32

corresponds to the three protons of the (ω-1)-methoxy attached to C-15. The spectrum also showed a multiplet for two protons at δ 3.70 – 3.60 associated with the proton attached to the C-2 and (OH– β-position). A multiplet at δ 3.53 – 3.48 corresponds to protons attached to C-3, C-4 and C-5. The three protons of the α-methyl next to the *trans*-double bond appeared as a doublet at δ 0.94 (*J* 6.6 Hz). The ^{13}C NMR spectrum showed a signal for the carbonyl ester at δ 175.2. and the signals for the carbons of the benzyl groups appeared at δ 138.4 –127.6. Also, the signals for the carbons of the two double bonds appeared at δ 136.4, 129.9 and 128.5, while the carbons of the sugar appeared at δ 102.3, 82.3, 77.8, 72.8 and 72.3 for C1 to C5, and C6 at δ 62.8. Positions C8 and C9 (β-hydroxy position) appeared at δ 51.3 and δ 84.5, respectively.

The removal of the benzyl groups from the protected GMM (**209**) was achieved by using sodium in liquid ammonia to give free GMM (**134**) as a mixture of α and β anomers in a ratio of (0.6:0.4) in 22% yield. The ^{1}H NMR spectrum confirmed that the deprotection of compound (**209**) had been successful; the spectrum showed the signal pertaining to the twenty aromatic protons at δ 7.40 – 7.28 was absent from the spectrum of the free GMM (**134**), and the signals for the CH_2 of the benzylic protons had also disappeared. The ^{1}H NMR spectrum showed a broad doublet at δ 5.12 (*J* 3.8 Hz) corresponding to Hc –α, and another doublet at δ 4.47 (*J* 7.7 Hz) to Hc –β. The spectrum also showed a broad double doublet at δ 4.42 (*J* 2.3, 11.7 Hz) associated with Hd–α,β, a broad double doublet at δ 4.26 (*J* 5.2, 11.7 Hz) corresponding to Hd –α,β, a double double doublet at δ 3.95 (*J* 3.0, 5.6, 7.0 Hz) related to Hf –α, and another multiplet at δ 3.67 – 3.59 corresponding to Hh –α and the CH-OH of the mycolate component. Another signal which appears as a multiplet at δ 3.50 – 3.45 is related to Hf –β, a multiplet at δ 3.40 – 3.30 corresponds to He, g –α and Hg, h–β. Another triplet occurs at δ 3.18 (*J* 7.6 Hz) corresponding to He –β. The ^{13}C NMR spectrum showed a signal at δ 96.5 (C_5 –β), 92.2 (C_5 –α), 74.4 (C_6 –β), 72.1 (C_6– α), 76.0 (C_7 –β), 70.5 (C_7 – α), 70.2 (C_8 –β) , 73.6 (C_8 – α), 73.4 (C_9–β), 69.2 (C_9 – α), 63.4 (C_{10} –β), 63.6 (C_{10} – α) (**Table 5**). The mass spectrum for the free GMM (**134**) {Found $[M+Na]^+$: 1338.2118; $C_{84}H_{162}O_9Na$ requires: 1338.2111}.

In the same way, the *S*-epimer (**201a**) was also converted into (**134a**) (**Scheme 59**).

Table 5: ^{1}H and C^{13} NMR analysis of GMM (**134**).



Proton	*δ/ppm*	Multiplicity	Integration	*J/* Hz	Carbon	*δ/ppm*
H_a	5.35 – 5.25	m	3	-----	C-$_5$α	92.2
H_b	5.19	br. dd	1	7.2, 15.1	C-$_5$β	96.5
Hc-α	5.12	br. d	0.6	3.8	C-$_6$α	72.1
Hc-β	4.47	d	0.4	7.7	C-$_6$β	74.4
H_d-α,β	4.42	br. dd	1	2.3,11.7	C-$_7$α	70.5
H_d-α,β	4.26	br.dd	1	5.2,11.7	C-$_7$β	76.0
H_f-α	3.95	br.ddd	0.6	3.0,5.6,7.0	C-$_8$α	73.6
H_h–α + CH – OH mycolic acid	3.67 – 3.59	m	1.6	----	C-8 β	70.2
H_f-β	3.50 – 3.45	m	0.4	----	C-$_9$α	69.2
$H_{e,g}$ –α + $H_{g,h}$ –β	3.40 – 3.30	m	2	----	C-$_9$β	73.4
H_e –β	3.18	t	0.4	7.6	C-$_{10}$α/β	63.6
H_j	2.42 – 2.34	m	1		C-$_{10}$α/β	63.4
H_k and CH_2 next to the double bounds	2.05 – 1.85	m	7		C $_{11}$	175.2
Terminal CH_3	0.83	t	3	7.0		

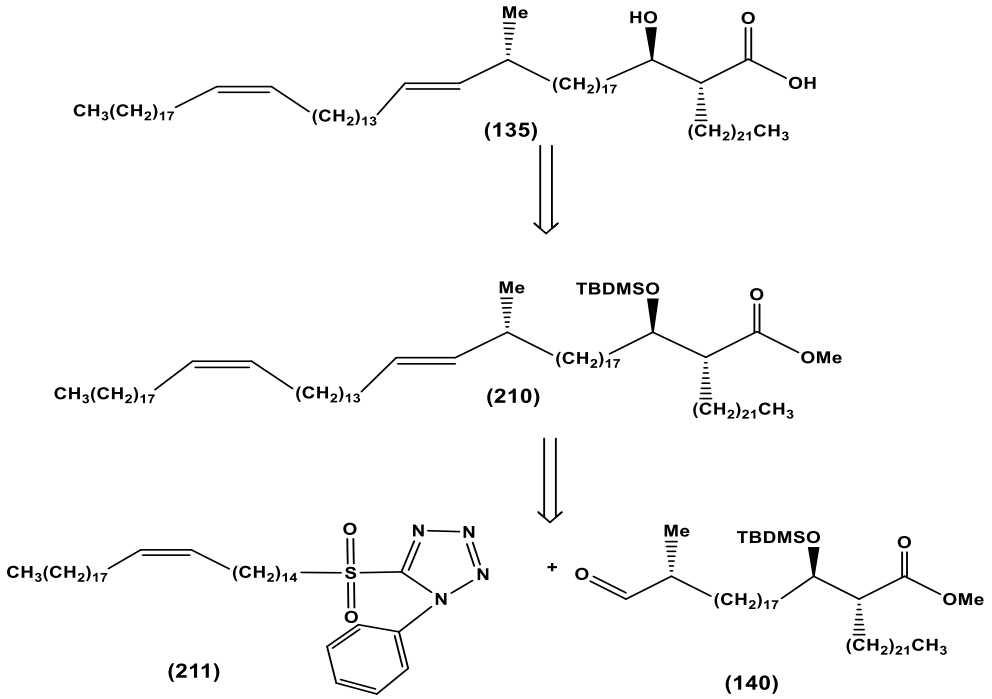
The work described here has resulted in a set of synthetic (ω-1)-methoxy-MA and sugar esters for evaluation of their effects on immune signals and their value as antigens. In order to provide a baseline for the possible effects of the (ω-1)-methoxy group, a major

component of the natural diene mixture, also isolated from *M. alvei*, lacking this group was also synthesised.

2.9 Synthesis of an α-mycolic acid from *M. alvei* containing a *cis*-alkene and α-methyl-*trans*-alkene

As mentioned in the introduction (see **1.8**), as well as ω-1 methoxy MAs, a mixture of diene MAs was also isolated from *M. alvei*. After analysis of the 3 products obtained from the oxidative cleavage of these diene mycolates, it was suggested that the majority of the MA contained a methyl-*trans*-alkene with a methyl group at the proximal position. Based on this, and by matching the chain lengths to those of the ω-1 methoxy MA (**129**) it was decided to prepare diene MA (**135**).

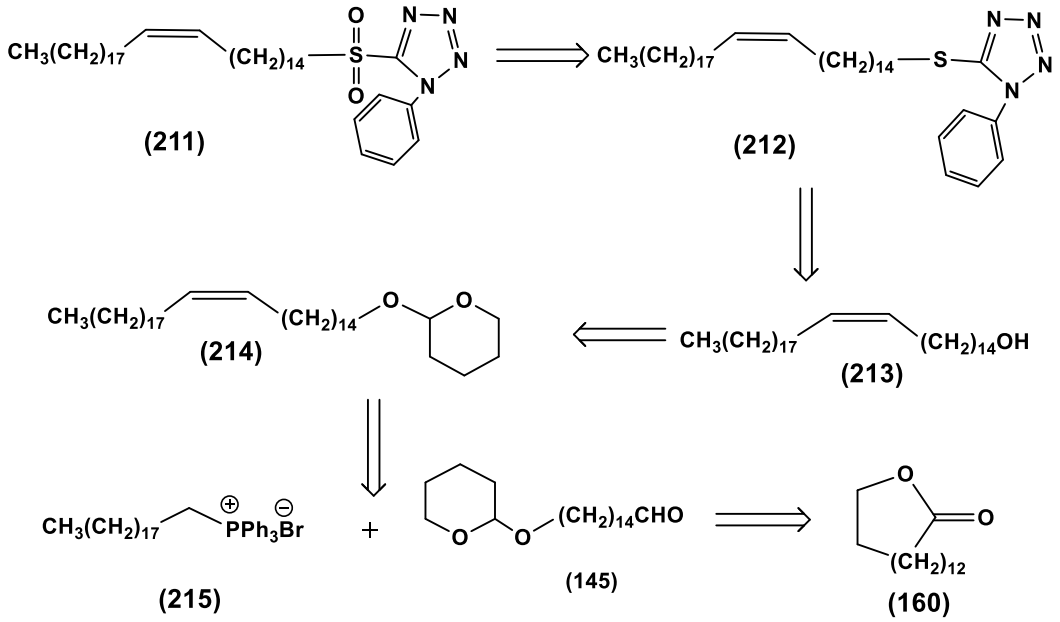
The synthetic route to this MA, which contains a *cis* double bond at the distal position and an α-methyl *trans* alkene at the proximal position is shown in (**Scheme 60**). The protected mycolic acid (**210**) can be broken down into 2 main fragments: that containing the distal position of the meromycolate moiety (**211**), and the mycolate motif unit (**140**).



Scheme 60: The fragment containing the distal position of the meromycolate moiety (**211**) and the intermediates **(140)**.

2.9.1 Synthesis of *Z*-alkene sulfone (211)

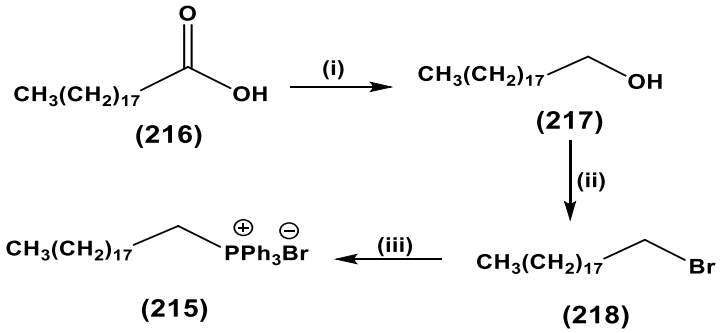
The meromycolate moiety (**211**) at the distal position could be obtained from the commercially available cyclic lactone (**160**) (**Scheme 61**).



Scheme 61: Retrosynthesis of the *Z*- alkene at the distal position.[131]

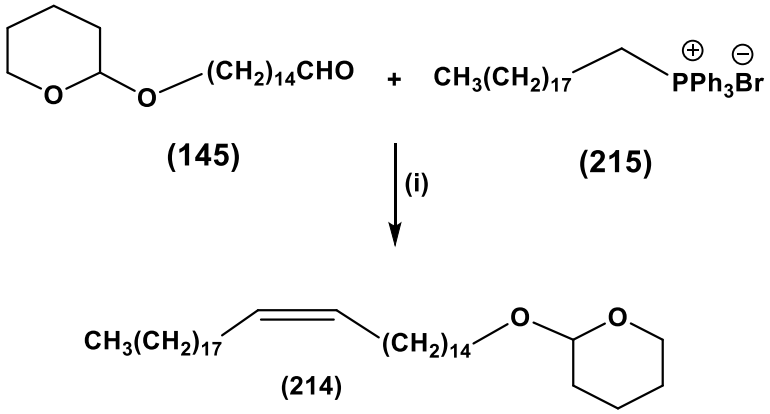
2.9.2 Preparation of phosphonium salt (215)

Firstly, the synthesis of alkyl phosphonium salt (**215**) needed to be carried out in order to produce the desired *Z*-alkene OTHP (**214**). Nonadecanoic acid (**216**) was reduced to alcohol (**217**) using lithium aluminium hydride in dry THF. This was converted into 1-bromononadecane **(218**) using *N*-bromosuccinimide with triphenylphosphine in dichloromethane. The nonadecyltriphenyl phosphonium bromide (**218**) was prepared following the literature method.[220] It was obtained by refluxing the bromo-compound (**218**) with triphenyl phosphine in toluene for four days to give the phosphonium salt (**215**) (**Scheme 62**). The resulting phosphonium salt (**215**) was purified by column chromatography eluting with petroleum ether and then dichloromethane /methanol (10:1), and then precipitated in dry ether.



Scheme 62: Synthesis of alkyl phosphonium salt (**215**), Reagents: (i) $LiAlH_4$, THF, 98%; (ii) NBS, PPh_3, DCM, 97%; (iii) PPh_3, toluene, 65%.

The *Z*-alkene isomer (**214**) was prepared via the Wittig coupling reaction between the above alkyl phosphonium salt (**215**) and aldehyde (**145**) using sodium *bis*(trimethylsilyl)amide in dry THF to give the required *Z*-alkene (**214**) (**Scheme 63**).



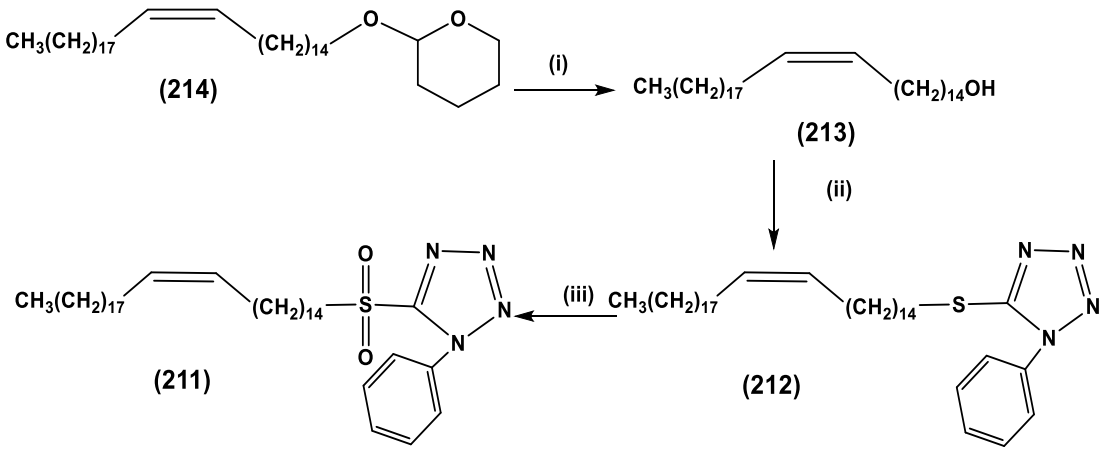
Scheme 63: Synthesis of *Z*-alkene (**214**), Reagents: (i) sodium bis(trimethylsilyl)amide, dry THF, 43%.

The ^{1}H NMR of (**214**) confirmed the success of the reaction, which showed the *cis* double bond protons as a broad pentet at δ 5.35 (*J* 10.5 Hz) integrating to two protons**.** The signals of the THP group showed a broad singlet at δ 4.58 belonging to the acetal proton. The ^{13}C NMR showed the alkene carbons at δ 129.9, and the THP acetal carbon at δ 98.8.

2.9.3 Deprotection of *Z*-alkene THP (214)

The deprotection of the THP group in compound (**214**) was carried out using pyridinium-*p*-toluene sulfonate in a mixture of THF and MeOH stirring at 50 °C for 5 hrs to give alcohol (**213**) in 88% yield, (**Scheme 64**). The ^{1}H NMR spectrum for alcohol (**213**) showed that there were no signals for the THP group, but there was a quartet at δ 3.65 (*J* 6.3 Hz) for the two protons belonging to the carbon next to the OH group and the ^{13}C NMR spectrum showed a signal at δ 63.1 for the same carbon.

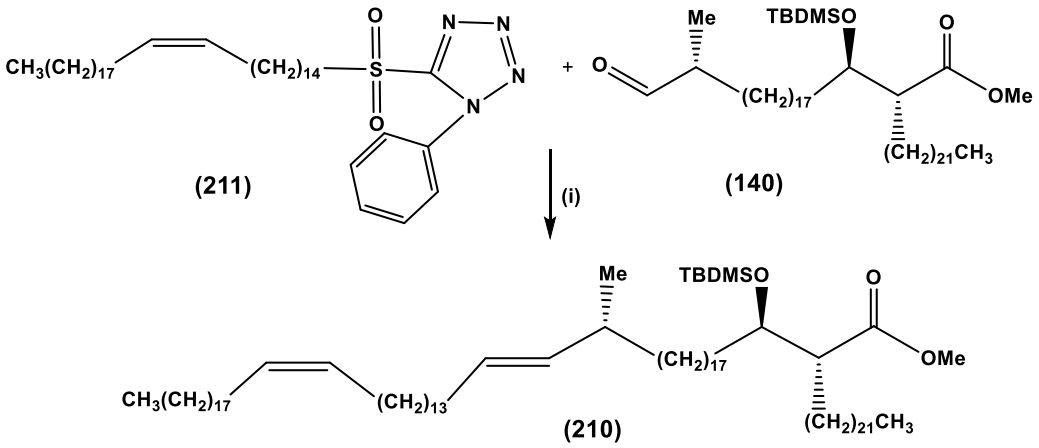
The alcohol compound (**213**) was then treated with 1-phenyl-1*H*-tetrazole-5-thiol and triphenylphosphine in the presence of diethyl azodicarboxylate (DEAD) to give the *Z*-alkene sulfide (**212**) in a yield of 89%. It has been found that there is a triplet at δ 3.40 (*J* 7.4 Hz) integrating to two protons belonging to the carbon next to the sulfur atom and the phenyl group protons appeared as a multiplet at δ 7.65 – 7.50 integrating to five protons for the sulfide compound (**212**). Finally, oxidation of sulfide (**212**) using hydrogen peroxide and ammonium molybdate in IMS and THF gave *Z*-alkene sulfone (**211**) in a yield of 57%. The ^{1}H NMR spectrum of the *Z*-alkene sulfone (**211**) showed a triplet at δ 3.74 (*J* 7.9 Hz) integrating to the two protons next to the sulfonyl group. The sulfonyl carbon signal appeared in the ^{13}C NMR spectrum at δ 56.0.



Scheme 64: Preparation of *Z*-alkene sulfone (**211**). Reagents: (i) PPTS, THF, MeOH, at 40 ºC, 88%; (ii) 1-phenyl-1*H*-tetrazole-5-thiol, PPh_3, DEAD, dry THF, 89%; (iii) hydrogen peroxide, $(NH_4)_6Mo_7O_{24}.4H_2O$, IMS and THF, 57%.

2.9.4 Synthesis of a *cis*-alkene-α-methyl-*trans*-alkene mycolic acid by coupling of the meromycolate moiety (211) and mycolate motif unit (140)

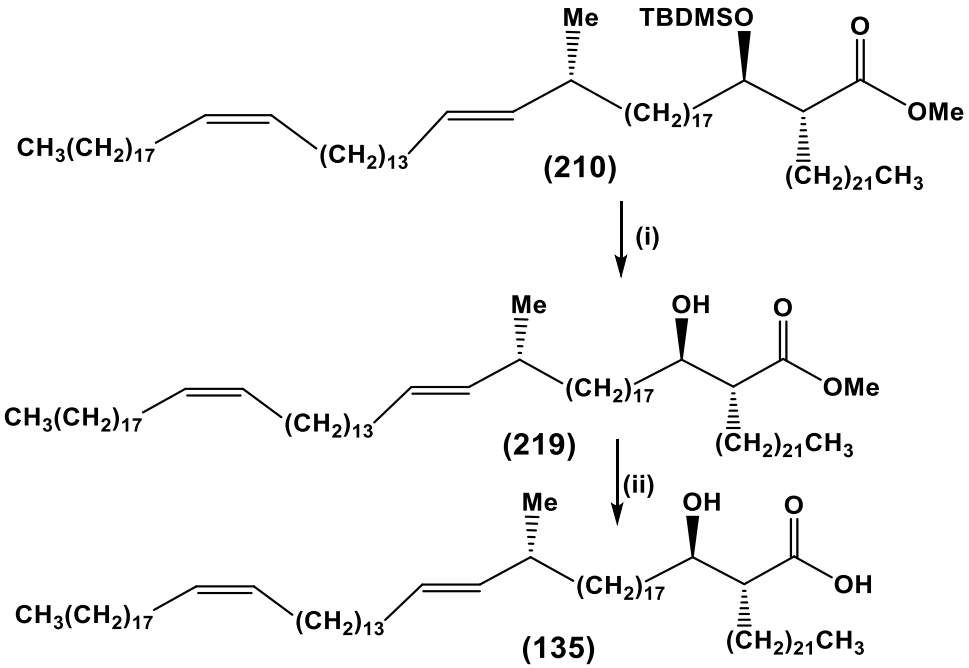
The coupling between the mycolate motif unit (**140**) and the *Z*-alkene sulfone (**211**) to form the protected mycolic acid **(210)** was carried out using potassium *bis*(trimethylsilyl) amide (1 M in THF or 0.5 M in toluene) in dry THF at – 20 ºC to give (**210**) in 52% yield (**Scheme 65**). The ^{1}H NMR spectrum showed a multiplet at δ 5.40 – 5.30 and a broad doublet of doublets at δ 5.24 (*J* 7.4, 15.3 Hz) corresponding to the alkene protons. The coupling constant of 15.3 Hz between the olefinic hydrogen atoms confirmed the formation of the *trans*-alkene. The ^{13}C NMR spectrum showed signals in the olefinic region at δ 136.5, 129.9 and 128.4 for the *cis* and *trans*-alkene carbons. The specific rotation of the product (**210**) was found to be $[\alpha]_D^{24}$ – 4.0 (*c* 0.9, $CHCl_3$).



Scheme 65: Reagents: (i) potassium *bis*(trimethylsilyl)amide, dry THF, 52%.

2.9.5 Deprotection and hydrolysis to produce free mycolic acid (135)

The *tert*-butyldimethylsilyl group was removed using HF-pyridine and pyridine in dry THF and stirring at 45 ˚C for 17 hrs to give the methyl ester mycolic acid (**219**) (**Scheme 66**).

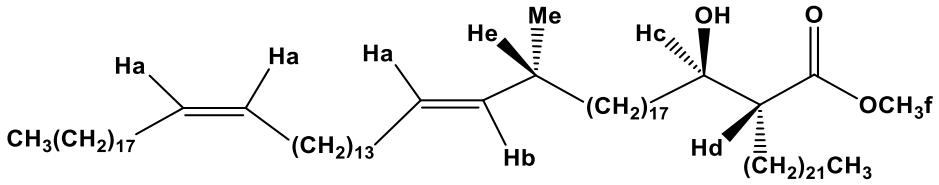


Scheme 66: Synthesis of complete mycolic acids. Reagents: (i) HF. Pyridine, pyridine, THF, 45 ℃, 96%; (ii) LiOH, MeOH, H_2O, THF, 45 °C, 16 hrs 98%.

The ^{1}H NMR spectrum of (**219**) was compared with the protected compound (**210**), and still showed a signal for three hydrogens at δ 3.72 corresponding to the OMe, but had lost the signals for the silyl group on the β-hydroxy acid, corresponding to the two

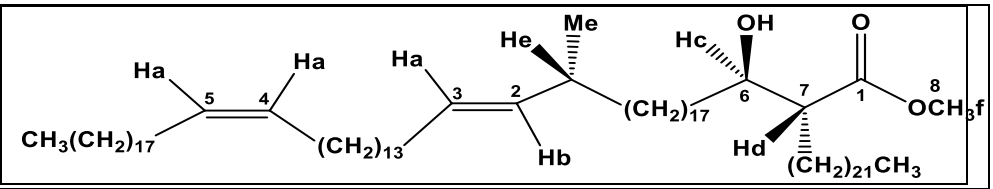
methyl units and the *tert*-butyl. The ^{13}C NMR signals corresponding to these groups at δ – 4.4 and – 4.9 had also disappeared. The specific rotation of the product (**219**) was found to be $[\alpha]_D^{24}$ +1.45 (*c* 0.96, $CHCl_3$), which compared with the natural α-mycolic acid ester fraction isolsted from *M. alvei*, which had an $[\alpha]_D^{22}$ + 1.4 (*c* 1.99, $CHCl_3$). Selected proton and carbon NMR signals are shown in (**Table 6A, 6B**).

Table 6A: NMR analysis of *cis*-alkene-α-methyl-*trans*-alkene mycolic acid (**219**).



	Synthetic (219)				Natural (23)[117]			
Proton	***δ/ppm***	**Multiplicity**	**Integration**	***J*/ Hz**	***δ/ppm***	**Multiplicity**	**Integration**	***J*/ Hz**
H_a	5.40 – 5.30	m	3	----	5.30 – 5.29	m	3	----
H_b	5.24	br.dd	1	7.4, 15.2	5.23	br. dd	1	7.4,15.4
H_c	3.69 – 3.60	m	1	----	3.68 – 3.60	m	1	----
H_d+ OH	2.45 – 2.38	m	2	----	2.43	br. dt	1	5.3,9.1
H_e + $6CH_2$ adjacent to double bond	2.09 – 1.90	m	7	----	2.06 – 1.90	m	7	----
H_f	3.72	s	3	----	3.71	s	3	----
CH_3	0.93	d	3	6.6	0.94	d	6.7	
Terminal CH_3	0.88	t	6	6.8	0.88	t	6	6.5

Table 6B: NMR analysis of *cis*-alkene-α-methyl-*trans*-alkene mycolic acid (**219**).



Synthetic (219)		**Natural (23)[117]**
Carbon	***δ/ppm***	***δ/ppm***
C_1	176.3	176.2
C_2	136.5	136.5
C_3	128.4	128.4
C_4, C_5	129.9	129.9
C_6	72.3	72.3
C_7	51.5	51.5
C_8	50.9	50.9

Finally, the methyl ester (**219**) was hydrolysed using lithium hydroxide in a mixture of THF: methanol: water at 45 ^oC for 16 hrs; this led to free mycolic acid (**135**) (**Scheme 66**). The ^1H NMR spectrum confirmed the formation of the free MA due to the disappearance of the signal at δ 3.72 corresponding to the methyl ester. The IR spectrum showed a broad peak at 3263 cm^{-1} for the OH stretch.

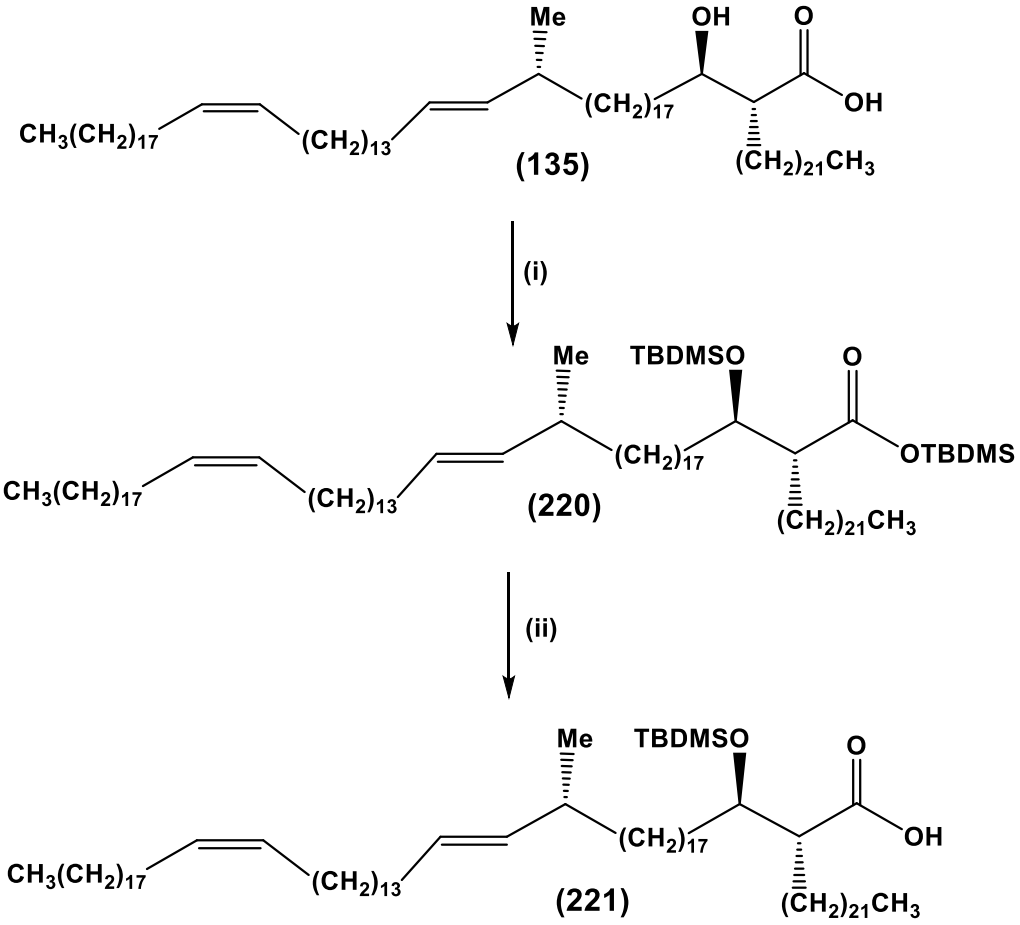
2.9.6 Synthesis of trehalose esters of mycolic acid (135)

2.9.6.1 Protection of the β-position of the mycolic acid (135)

All mycolic acids must be protected at their β hydroxy acid position to avoid alcohol reactions. The most commonly used group for this protection is the *tert*-butyldimethylsilyl ether due to simplicity of removal and because it is an easy compound to work with.

The free mycolic acid **(135**) was protected by stirring it with *tert*-butyldimethylsilylchloride, imidazole and DMAP in a mixture of DMF/toluene at 70 °C for 24 hrs. The reaction usually leads to the protection of both the β-hydroxy group and the carboxylic acid to give (**220**). The next step was therefore the selective deprotection of the

carboxylic acid group, by using tetra-*n*-butyl ammonium hydroxide. This was added slowly to a solution of compound (**220**) and the reaction was stirred for 1 hr at room temperature to give the target compound (**221**), (**Scheme 67**).

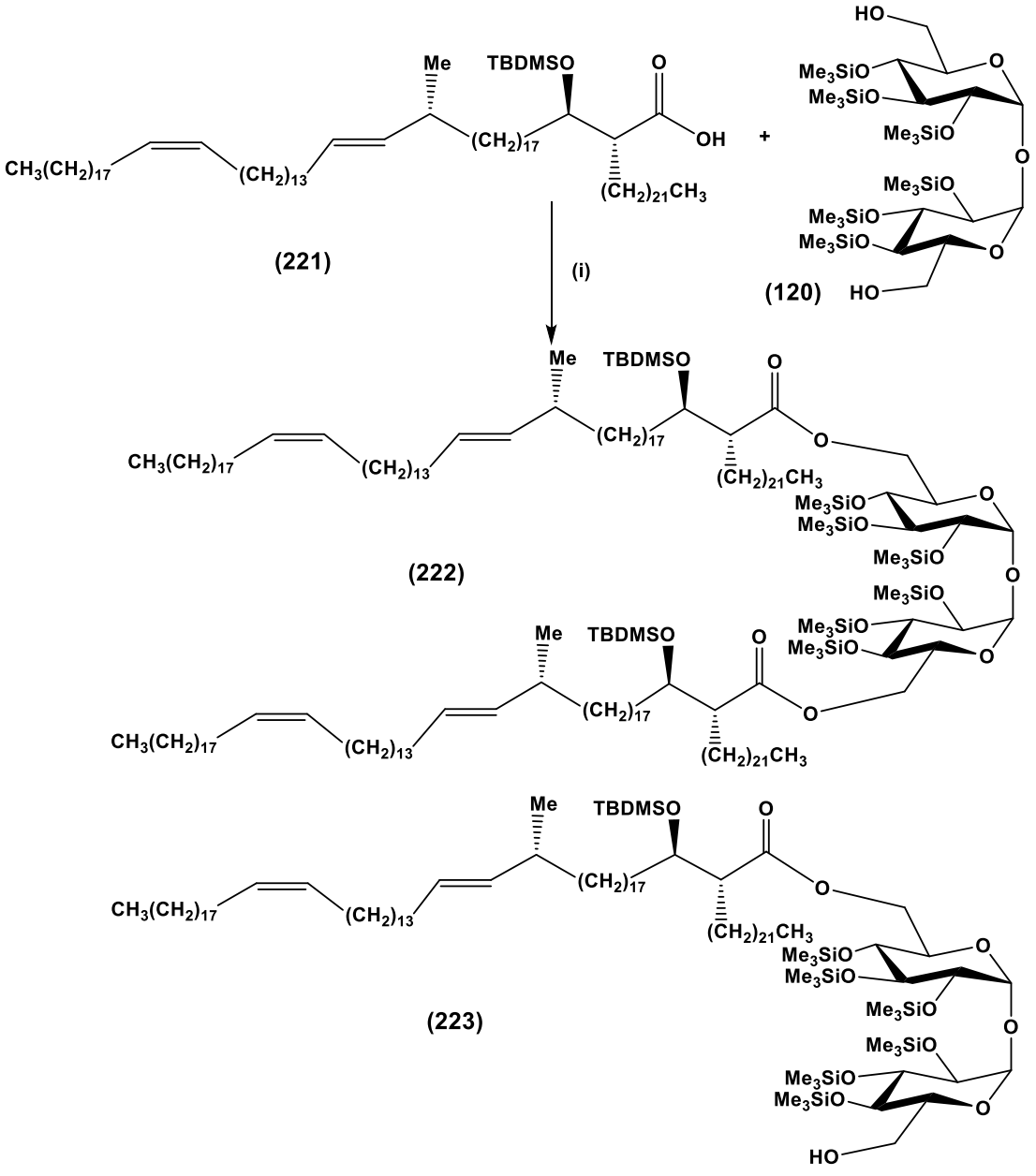


Scheme 67: Protection of the free MA. Reagents: (i) imidazole, TBDMSCl, DMAP, tetra-*n*-butyl ammonium hydroxide 70 °C, 24 hrs, and tetra-*n*-butyl ammonium hydroxide, THF (82%).

The ^{1}H NMR spectrum of compound (**221**) showed 3H resonating at δ 0.14 and 3H resonating at δ 0.13 which corresponded to the methyl groups of the protecting *tert*-butyldimethyl silyl group. The *tert*-butyl protons, 9H in total, appeared at δ 0.92, and the proton on the β-hydroxy acid resonated as a doublet of doublets of doublets at δ 3.84 (*J* 2.4, 6.0, 7.9 Hz). Signals in the ^{13}C NMR spectrum were consistent with structure (**221**), including δ 177.3 corresponding to the carboxylic acid, and δ 73.7 for the β-hydroxy carbon. The protecting group carbons next to silicon resonated at δ – 4.3 and – 4.9.

2.9.6.2 Coupling of protected mycolic acid (221) to protected trehalose (120)

The protected mycolic acid (**221**) was coupled with protected trehalose (**120**) using EDCI and DMAP and stirring under nitrogen at room temperature for 6 days. This gave TDM (**222**) and TMM (**223**) after separation by column chromatography (**Scheme 68**).



Scheme 68: Reagent: (i) DMAP/EDCI/CH_2Cl_2, TDM 11%, TMM 43%.

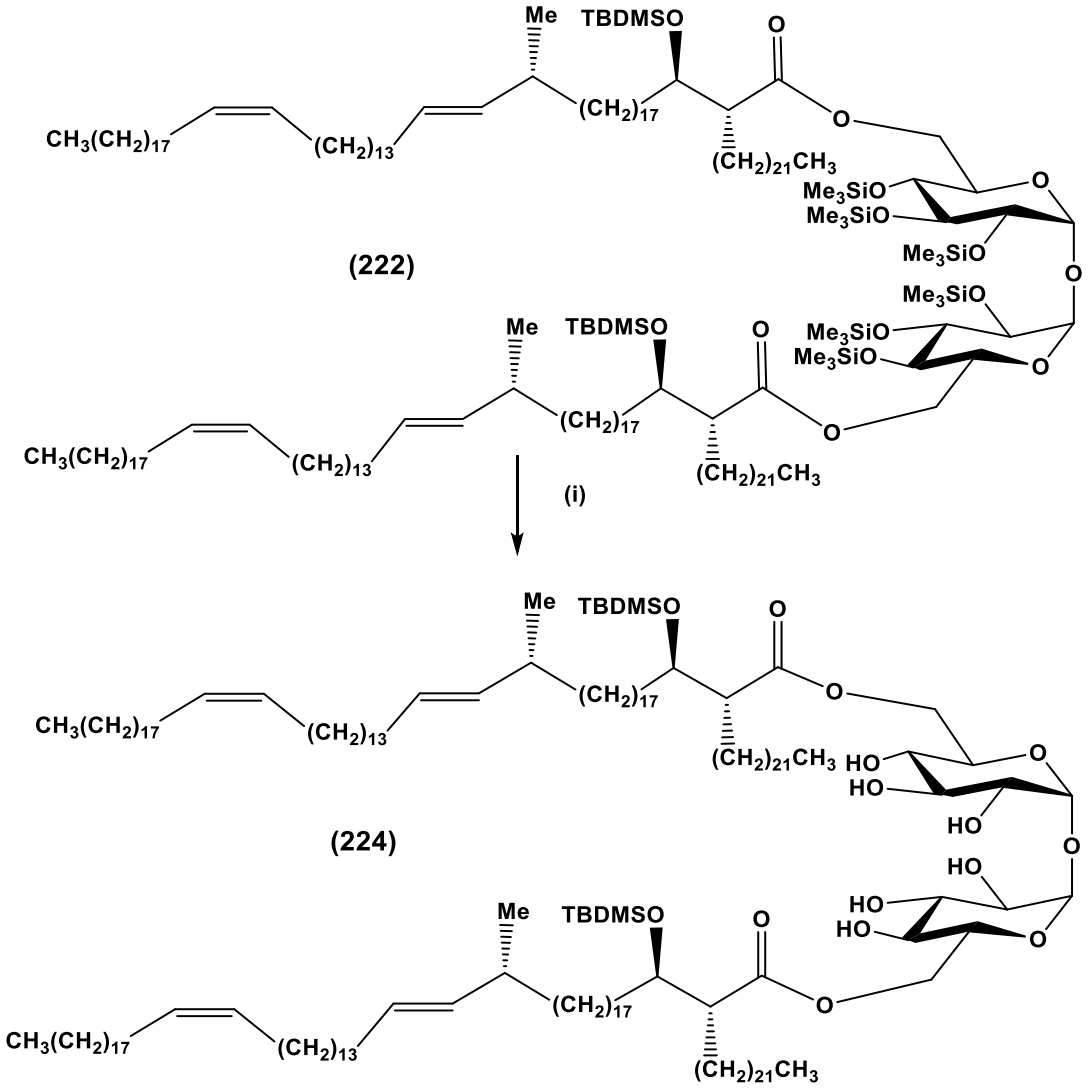
The first product to be obtained was TDM (**222**) in a yield of 11%. The ^{1}H NMR spectrum showed a multiplet at δ 5.40 – 5.29 and a broad double doublet at δ 5.24 (*J* 7.4, 15.4 Hz) corresponding to the eight alkene protons. The two acetal protons of the trehalose appeared as a broad doublet at δ 4.85 (*J* 3.0 Hz), and the remaining trehalose protons, including

the β-position protons appeared at δ 4.36, 4.05 – 3.97, 3.76 – 3.94, 3.52, and 3.38. The α-position proton of the mycolic acid appeared as a multiplet at δ 2.60 – 2.50, and a multiplet appeared at δ 2.10 – 1.90, integrating for fourteen protons, corresponding to those next to the double bonds and the proton adjacent to the α-methyl next to the *trans* double bond. The terminal methyl groups of the alkyl chains appeared as a triplet at δ 0.90 *(J* 6.2 Hz). The protons of the trimethylsilyl protecting groups on the trehalose gave three singlets at δ 0.15, 0.14, and 0.13 each integrating for eighteen protons. The methyl groups of the TBDMS protecting group showed a singlet at δ 0.05 integrating to twelve protons. The methyl group appeared at δ 0.94 (*J* 6.6 Hz) as a doublet integrating to six protons. The ^{13}C NMR spectrum showed a signal at δ 173.9 for the carbonyl carbon. The double bond carbon signals appeared at δ 136.5, 129.9, and 128.4. Also, the remainder of the sugar carbons gave signals between δ 73.4 – 70.7. The methyl carbon signals of the protecting silyl groups of the sugar appeared at δ 1.09, 0.94 and 0.15. Additionally, the carbons of the two methyl groups bonded to silicon in the silyl groups of the mycolate component appeared at δ – 4.5 and δ – 4.6.

The second fraction was the TMM (**223**), obtained in a yield of 43%. the ^{1}H NMR spectrum was more complicated than that of the TDM due to the lack of the symmetry. The acetal protons appeared as two doublets at δ 4.91 (*J* 3.0 Hz) and δ 4.84 (*J* 3.0 Hz) with an integration of one proton for each, and the remaining sugar protons appeared between δ 4.34 – 3.39. It was found that the methyl signals for the protecting groups on the sugar were divided into five groups, which had a range of δ 0.17 – 0.12, however integrating only to half the number of protons. The ^{13}C NMR spectrum displayed signals at δ 174.1 for the carbonyl carbon and at δ 94.5 and 94.4 for the C1 in the sugar cores. Signals at δ – 4.5 and – 4.7 were displayed for the two methyl groups bound to the silicon in the TBDMS group.

2.9.6.3 Trehalose deprotection of TDM (222)

The trimethylsilyl protecting groups of the trehalose of the TDM (**222**) were removed using TBAF in dry THF to give (**224**) in 50% yield (**Scheme 69**).



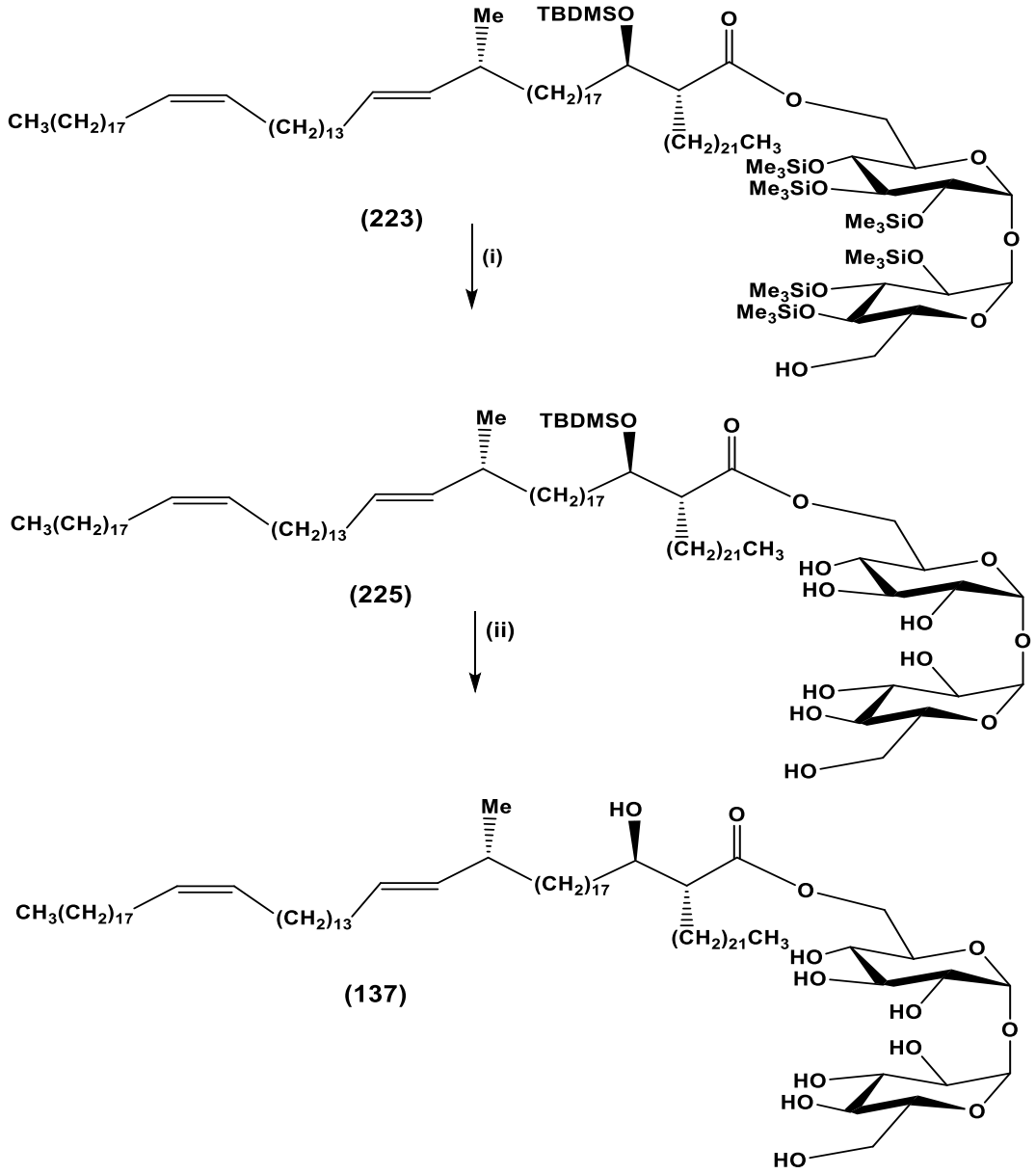
Scheme 69: Reagents: (i) tetrabutylammonium fluoride, THF, TDM, 50%.

The ^{1}H NMR spectrum of silyl-protected TDM (**224**) showed a multiplet at δ 5.37 – 5.26 and a broad double doublet at δ 5.20 (*J* 7.6, 15.4 Hz) for the protons of the double bonds while the a broad doublet for the hemiacetal protons was shifted to δ 5.05 (*J* 3.3 Hz). The remaining signals for the sugar protons and the β-hydroxyl protons resonated at δ 4.36, 4.20, 3.95 – 3.85, 3.80, 3.44 and 3.31, and a multiplet occurred between δ 2.57 – 2.50 (2H) for the protons in the α-position of the MA. The signals belonging to the trimethylsilyl protecting groups of the sugar at δ 0.15, 0.14 and 0.13 were not present in the ^{1}H NMR spectrum. The signals for these protecting groups were also absent from the ^{13}C NMR spectrum, which indicated that the removal of the silyl groups from the sugar had been successful. Removal of the *tert*-butyldimethylsilyl groups on the β-position of the mycolic acid moiety of (**224**) using HF-pyridine complex and pyridine in

dry THF to give free TDM was unsuccessful; this was due to there not being enough material.

2.9.6.4 Deprotection of the trehalose and β-position of TMM (223)

The protecting groups of the sugar were removed by stirring compound (**223**) with Dowex 50 wx8 hydrogen in dichloromethane/methanol (1:1) for 30 min at room temperature, to give (**225**) in a yield of 75% (**Scheme 70**).



Scheme 70: Synthesis of free TMM. Reagents: (i) Dowex 50 wx8 hydrogen in dichloromethane/methanol, 75%; (ii) HF. Pyridine, pyridine, THF, 43 °C, 37%.

The ^{1}H NMR spectrum of the silyl-protected TMM (**225**) showed a multiplet at δ 5.35 – 5.23 and a broad double doublet at δ 5.18 (*J* 7.4, 15.3 Hz) for the protons of the double bonds, while the hemiacetal protons appeared as a broad doublet at δ 5.04 (*J* 3.4 Hz) with an integration of two protons. The remaining sugar protons resonated between δ 4.28 – 3.25. The signals belonging to the trimethylsilyl protecting groups of the sugar at δ 0.16, 0.15 and 0.14 were not present in the ^{1}H NMR spectrum, which indicated that the removal of the silyl groups from the sugar had been successful.

Finally, the *tert*-butyldimethylsilyl group on the β-position of the mycolic acid moiety of (**225**) was removed using HF-pyridine complex and pyridine in dry THF to give free TMM (**137**) (**Scheme 70**). The ^{1}H NMR spectrum of free TMM (**137**) showed the disappearance of the *tert*-butyl signal at δ 0.80 and the two methyl groups bonded to silicon at δ – 0.007 and – 0.029, confirming the success of the reaction. The acetal protons appeared as doublets at δ 5.05 (*J* 3.4 Hz) and 4.98 (*J* 3.4 Hz) respectively, and the remaining sugar protons, including the β-position proton appeared between δ 4.62 – 3.24. The carbon NMR spectrum showed the carbonyl carbon signals at δ 175.5, and the alkene carbons at δ 136.3, 129.8 and 128.3. The anomeric carbon signals appeared at δ 94.5 and 94.3 and the remaining trehalose carbon signals appeared between δ 72.6 – 70.8.

Chapter 3

Summary and Conclusions

Synthetic mycolic acids, (ω-1)-methoxy and cord factors may have important applications in the detection, control, and treatment of mycobacterial infection. These compounds can be used in the development of biosensors for the early detection of mycobacterial disease. In addition, total synthesis of these compounds can assist in the identification of the fine structures of natural mycolic acids and their absolute stereochemistry, which is useful in finding new antimycobacterial drugs.

M. alvei belongs to the family of Non-Tuberculosis Mycobacteria and can cause diseases in both humans and animals such as skin disease, disseminated disease, and pulmonary disease. HIV positive patients are particularly at risk of infection with Non-Tuberculosis Mycobacteria.

The first aim of this work was the total synthesis of single enantiomers (*R* and *S*) of (ω-1)-methoxy mycolic acids (**198**, **198a**) present in *M. alvei* as well as the comparison of the synthetic (ω-1)-methoxy mycolic acids with samples of the natural mycolic acid isolated from *M. alvei*, in order to determine the stereochemistry of the methoxy group at the ω-1 position in the natural material.

The following compounds were successfully synthesised:

1- Methyl (*R*)-16-methoxyheptadecanoate (**183**)
2- Methyl (*S*)-16-methoxyheptadecanoate (**183a**)
3- (*R* and *S*) enantiomers of (ω-1)-methoxy mycolic acid methyl esters (**198**, **198a**)

The two synthetic stereoisomeric (*R* and *S*) enantiomers containing the (ω-1)-methoxy moiety of the mycolic acids (**183**, **183a**) and the corresponding fragment (**28**) from the natural (ω-1)-methoxy mycolic acid were characterized by specific rotation. The specific rotation of the *R* enantiomer (**183**) was $[\alpha]_D^{26}$ −1.1 (*c* 0.92, $CHCl_3$), while the *S* enantiomer (**183a**) showed $[\alpha]_D^{26}$ +1.1 (*c* 0.94, $CHCl_3$); on this basis, the natural fragment (**28**) which had an $[\alpha]_D^{22}$ − 1.55 (*c* 0.39, $CHCl_3$), can be assigned as having the *R* stereochemistry. Also, the two synthetic (*R* and *S*) enantiomers of the full (ω-1)-methoxy mycolic acid methyl esters (**198**, **198a**) and a natural (ω-1)-methoxy mycolic acid methyl

ester were characterized by mass spectrometry. The mass spectrum of (**198**) and (**198a**) showed a molecular ion $[M + Na]^+$: 1190.1752 and $[M + Na]^+$: 1190.1755 respectively, while the major compound of the natural mycolic acid was reported to show a molecular ion at $[M + Na]^+$: 1191 (**Figure 40**).

The two synthetic of enantiomers (*R* and *S*) of the (ω-1)-methoxy mycolic acid methyl esters (**198**, **198a**) were also compared to the natural (ω-1)-methoxy mycolic acid methyl ester, isolated from *M. alvei*, by ^1H and ^{13}C NMR spectroscopy. This data was identical to that of the natural compound.

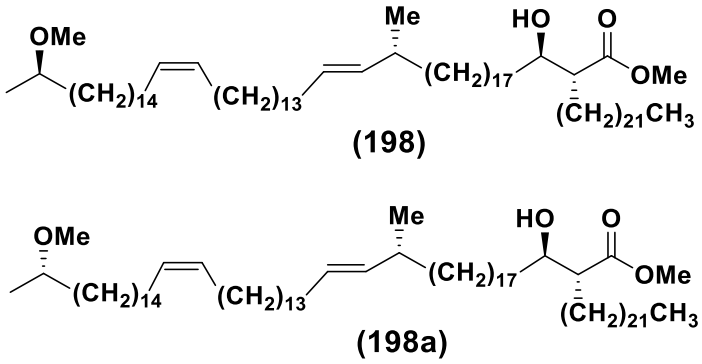
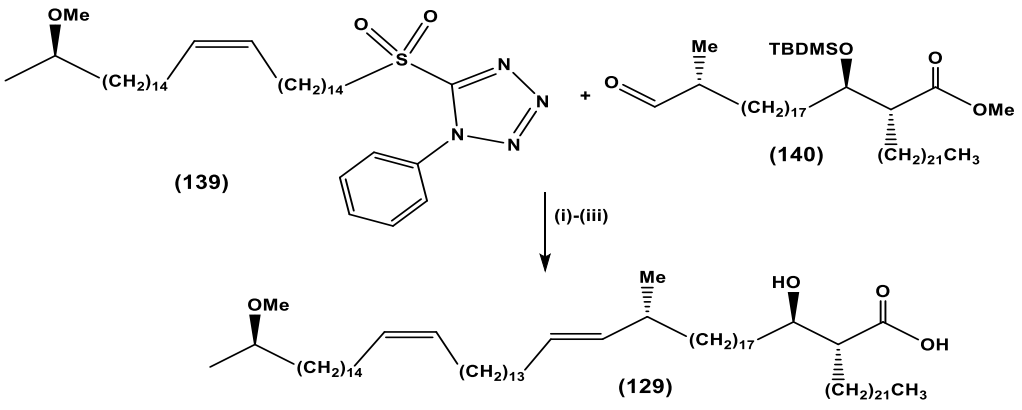
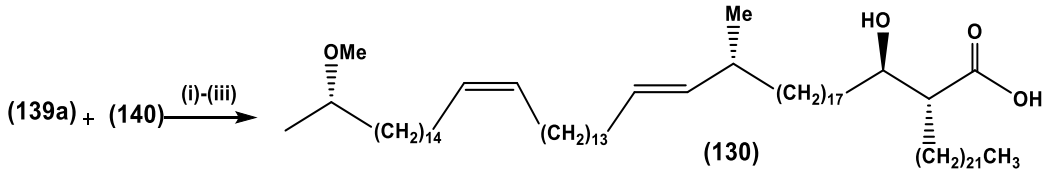


Figure 40: *R* and *S* enantiomers of the target (ω-1)-methoxy mycolic acids.

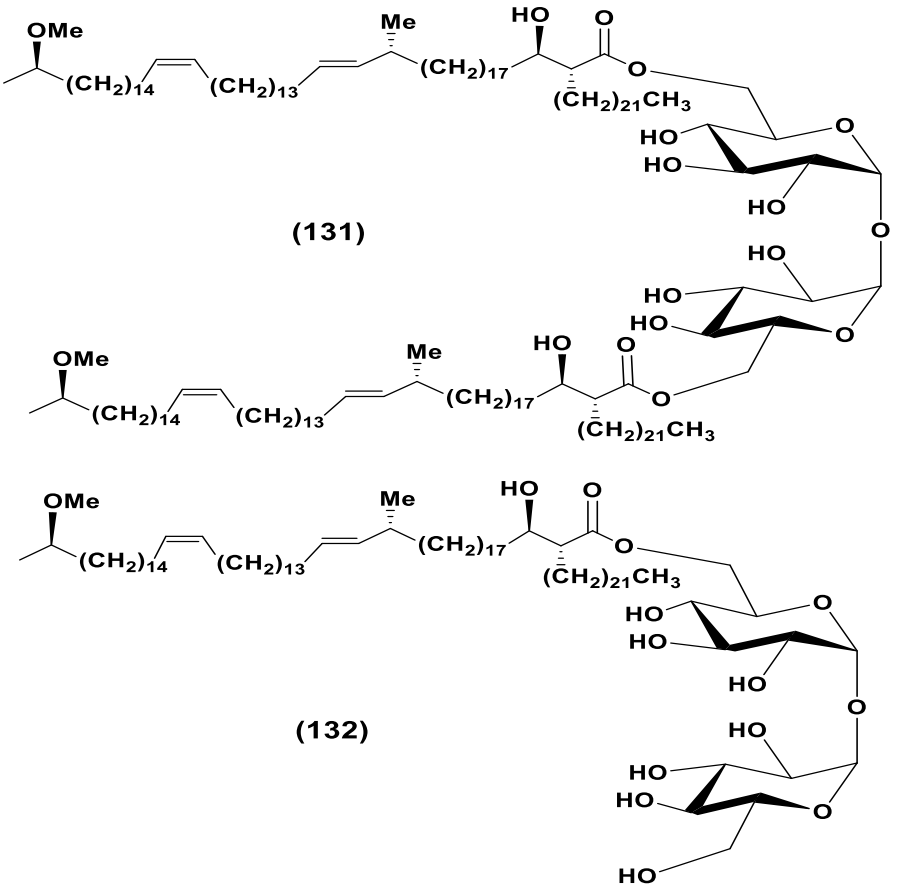
In order to synthesise the target (*R* and *S*) enantiomers of (ω-1)-methoxy mycolic acids (**129, 130**), coupling between the mycolate motif unit (aldehyde) (**140**) and the *Z*-alkene sulfone (**139, 139a**), using the Julia-Kocineski olefination reaction, was carried out to give (**198**,**198a**). Finally, the hydrolysis of the methyl ester was achieved using lithium hydroxide in a mixture of THF, methanol and water at 45 °C for 16 hrs, to give the free MA (**129**, **130**) in 93% and 71% yield, respectively (**Scheme 71**).





Scheme 71: The coupling to form (*R* and *S*) enantiomers of (ω-1)-methoxy mycolic acids.

Having successfully prepared the mycolic acids, the next step was to synthesise their sugar esters, i.e TDM and TMM, GMM and an arabinomycolate fragment. The esterification reaction between the protected (*R* and *S*) enantiomers of (ω-1)-methoxy mycolic acids (**201**, **201a**) and protected trehalose (**120**) was achieved successfully using EDCI and DMAP to obtain protected cord factors (TDM and TMM), which were then separated by column chromatography. This was followed by the silyl deprotection of the trehalose core and the β-hydroxy group of the (ω-1)-methoxy mycolic acid moiety, in two steps, to give the free (TDM and TMM) (**131, 131a**) (**132**, **132a),** and also successful synthesis free arabinomycolate (**133, 133a**) and free GMM (**134, 134a**) (**Figure 41**).



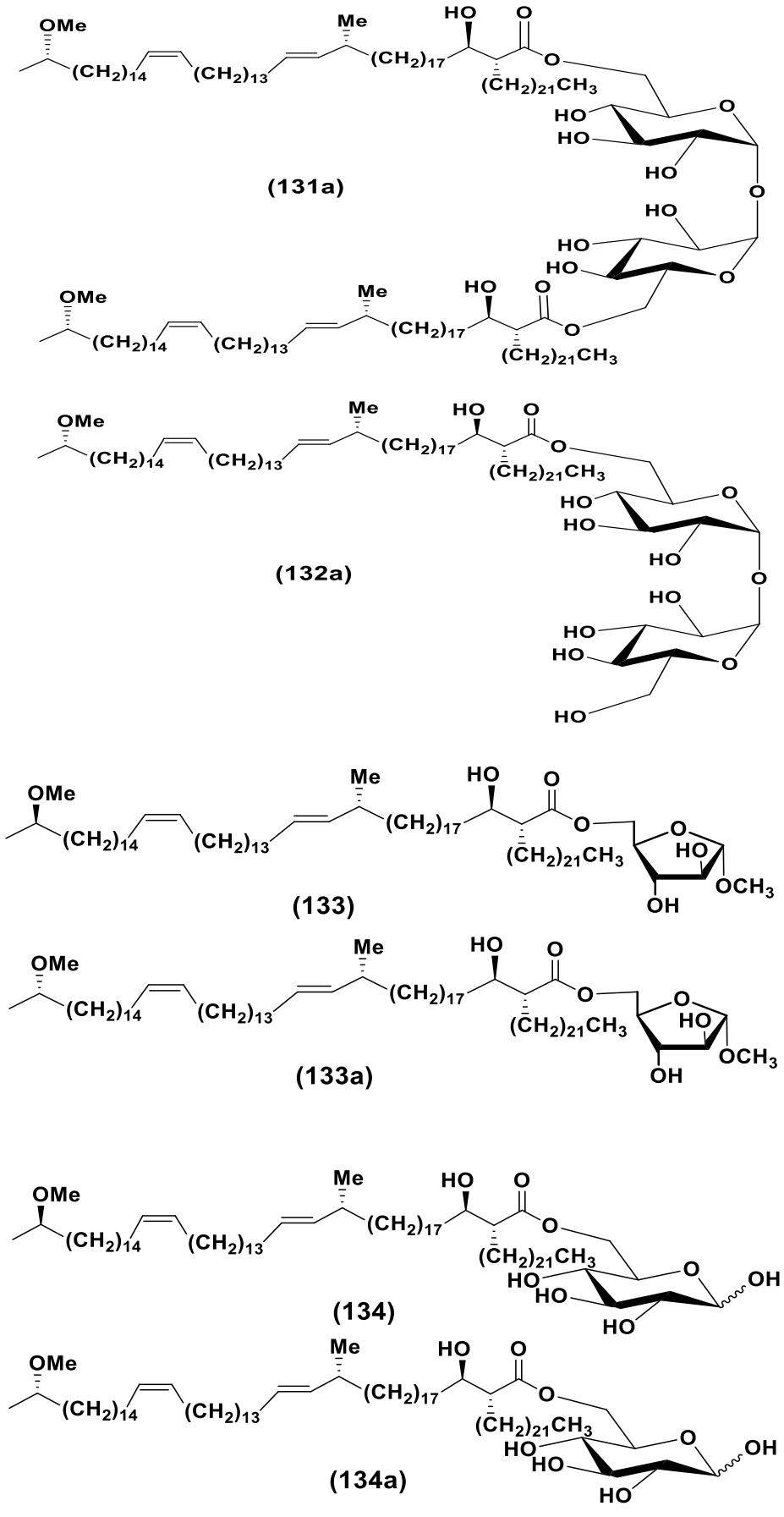
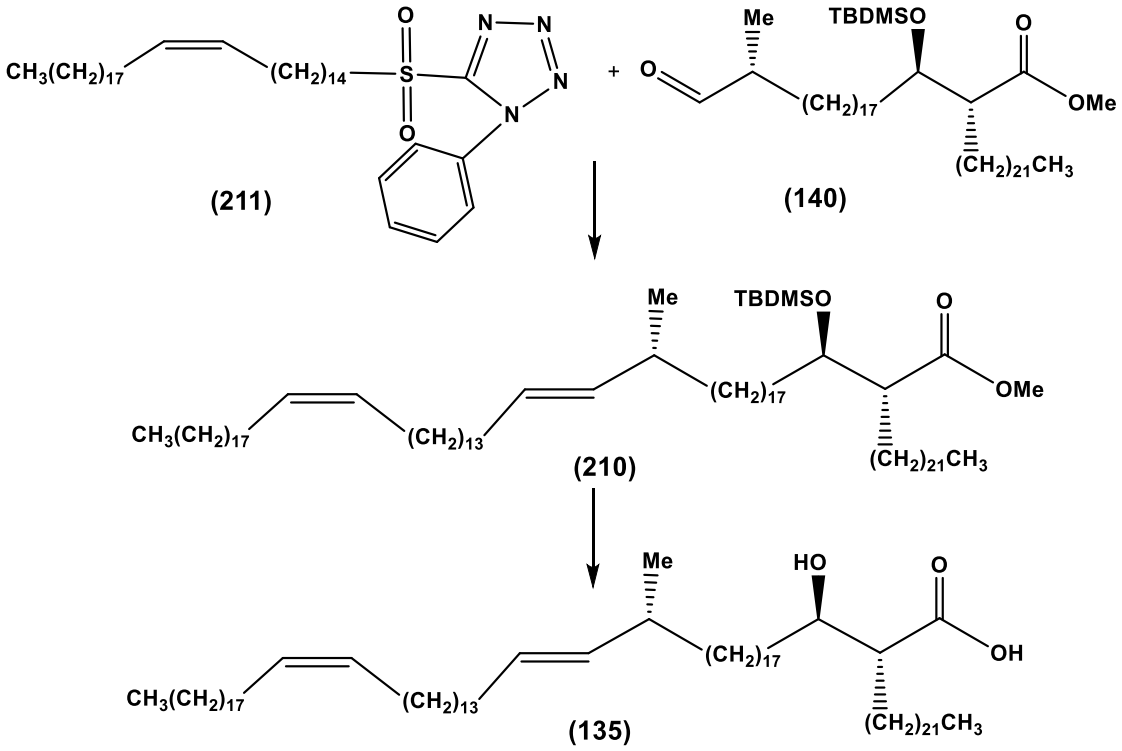


Figure 41: Synthesis of sugar esters, i.e TDM and TMM, GMM and an arabinomycolate.

The second aim of this project was the synthesis of the diene mycolic acid (**135**), which has also been isolated from *M. alvei*. The synthesis of this MA (**135**) was achieved over a multi step sequence from the commercially available starting materials *D*-mannitol (**167**), tridecanedioic acid (**170**) and lactone (**160**). These were used as precursors for various aldehyde and sulfone compounds, which were then coupled together to give the target MA. The final coupling step, between the proximal position (**211**) and meromycolate moiety (**140**) is shown in **Scheme 72**.



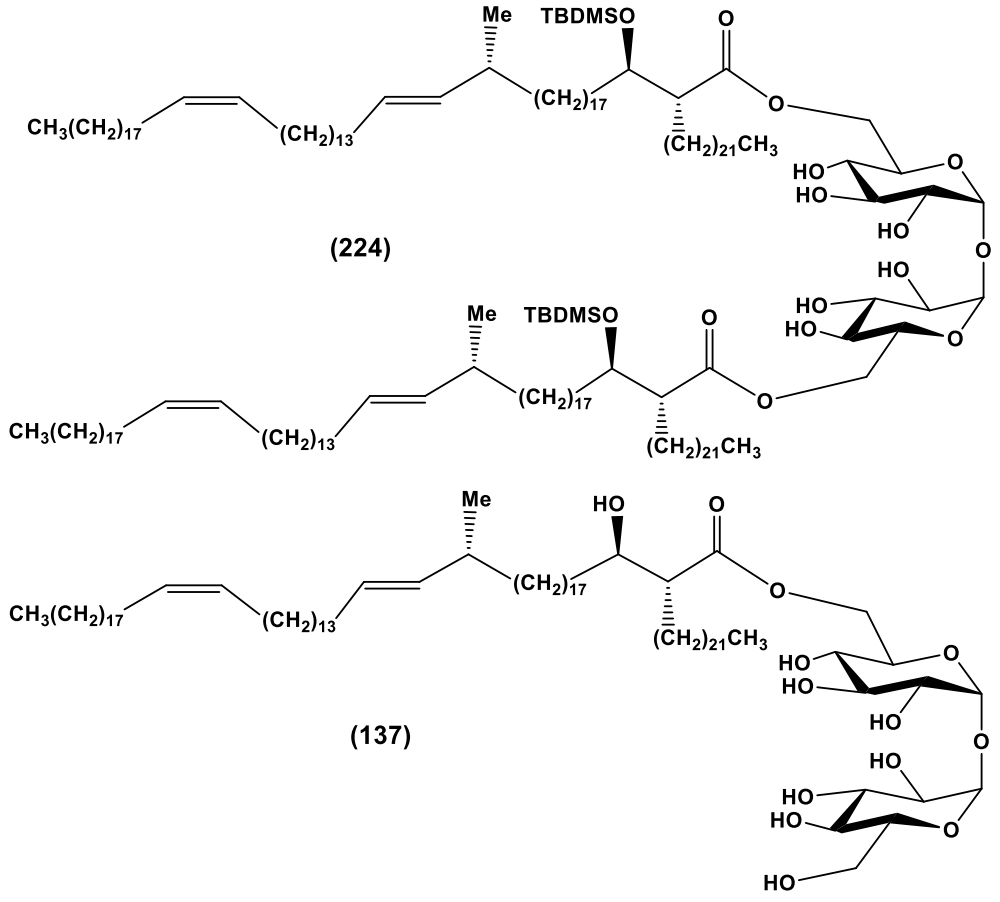
Scheme 72: The coupling to form an α-methyl mycolic acid.

The modified Julia olefination and the stereoselectivity in the final coupling reaction were sensitive to the base used to deprotonate the sulfone and the solvent polarity. Using 1-phenyl-1*H*-tetrazole-5-yl sulfones with potassium *bis*(trimethylsilyl)amide as base and 1,2-dimethoxyethane or tetrahydrofuran as solvent gave high *E*-stereoselectivity. It was confirmed that if the sulfone or aldehyde was α-substituted (*i.e.* **140**) the reaction was completely sterioselective for the formation of the *E*-isomer. This can be seen in **Scheme 72** where the coupling reaction between the aldehyde (**140**) and the sulfone (**211**) using potassium bis(trimethylsilyl)amide in THF gave the desired alkene (**207**) as only the *E*-diastereomer. This coupling formed the whole structure of the *cis*-alkene-α-methyl-*trans*-alkene mycolic

acid and, following hydrolysis, the free mycolic acid (**135**) was obtained in the correct stereochemistry.

The synthesis of the TDM and TMM cord factors of MA (**135**) was also attempted. The secondary alcohol of MA (**135**) was firstly protected as a TBDMS group and this was then coupled to the protected trehalose (**120**), which after purification by column chromatography gave TDM (**222**) and TMM (**223**). Deprotection of the sugar moiety of both these compounds gave TDM (**224**) and TMM (**225**) (**Scheme 73**).

Finally, the *tert*-butyldimethylsilyl group on the β-position of the mycolic acid moiety of (**225**) was removed using HF-pyridine complex to give free TMM (**137**), however, unfortunately the deprotection of the TDM, to give (**136**) was not attempted due to there being insufficient material.



Scheme 73: Preparation of TMM and TDM (**224**).

Further work

Having successfully prepared the *R* and *S* enantiomers of the ω-1-methoxy mycolic acid (**129**, **130**) and its sugar esters, the next steps would be to study their biological properties. These could then be compared to those of the diene MA (**135**), and its sugar esters, also isolated from *M. alvei*. This may lead to information on whether the ω-1-methoxy moiety has a significant effect on the biochemical properties of *M. alvei*. In addition, investigating the biological properties of these synthetic MAs would allow them to be compared to results obtained for other classes of MAs, isolated from different mycobacteria.

In order to obtain the same set of sugar esters for MA **135**, to compare to those obtained for MA **129** and **130**, the synthesis of the GMM and arabinose mycolate fragments of this MA would need to be prepared. This could be carried out as was discussed for the synthesis of the GMM and arabinose mycolate fragment for Mas **129** and **130 i**n the results and discussion section. In addition, the biological properties of these diene mycolate sugar esters could be compared to those of sugar esters of other diene mycolates isolated from other mycobacteria.

The synthesis of an α-mycolic acid of *M.* alvei containing two *c*is-alkene double bonds, and its corresponding sugar esters could also be prepared. This would allow their biological properties to be compared to those of MA **135**, which could give an indication as to whether the stereochemistry of the double bonds has any effect.

Chapter 4

4. Experimental

4.1 General considerations

All chemicals and reagents were purchased from chemical companies (Sigma-Aldrich, Alfa Aesar and Acros). All reactions were performed using glassware dried in oven at (120 ºC), and those requiring inert conditions were carried out under a nitrogen atmosphere. Those carried out at low temperatures were cooled using a bath of methylated spirits and liquid nitrogen. THF was dried over sodium and benzophenone under nitrogen. Petrol used was of boiling point 40-60 °C. Compounds were purified by re-crystallisation, column chromatography or distillation. 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) glass plates; separated components were detected using I_2, potassium permanganate or phosphomolybdic acid solution in IMS followed by charring. All NMR samples were prepared as a solution in deuterated chloroform, unless otherwise stated and recorded on a Bruker Avance 500 and 400 spectrophotometer. Chemical shifts are quoted in δ relative to the trace resonance of proton chloroform (δ_H 7.27, δ_C 77.0 ppm). Infra-red (IR) spectra were recorded on a Bruker Platinum F.T.I.R. spectrometer. $[\alpha]_D$ values were recorded in $CHCl_3$ or MeOH on a POLAAR 3001 polarimeter. Accurate MALDI-TOF mass spectra were recorded on a Bruker ultrafleXtreme**2** at Bristol University. A laboratory book was filled in including safety information following COSHH regulations.

Experiment (1): 6-Bromohexan-1-ol (155)

$Br(CH_2)_6OH$

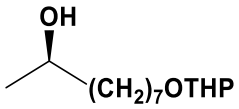
1,6-Hexanediol (**156**) (100 g, 0.846 mol) was dissolved in toluene (400 mL) and aqueous HBr (100 mL, 0.50 mol, 48%) was added and the mixture was refluxed for 18 hrs. The toluene layer was decanted, and the aqueous layer was re-extracted with toluene (200 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (3 × 150 mL), and the toluene was removed by simple distillation at atm. pressure and the crude product was purified by column chromatography, eluting firstly with petrol and then ethyl acetate to give a colourless oil, 6-bromohexan-1-ol (**155**)[193] (76.0 g, 50%), which showed δ_H (400 MHz, $CDCl_3$): 3.65 (2H, t, *J* 6.5 Hz), 3.42 (2H, t, *J* 6.8 Hz), 1.88 (2H, pent, *J* 6.8 Hz), 1.59 (2H, pent, *J* 6.7 Hz), 1.51 – 1.36 (5H, m, including the OH); δ_C (101 MHz, $CDCl_3$): 62.2, 33.7, 32.5, 32.2, 27.7, 24.7; ν_{max} /cm^{-1} : 3330, 2932, 2858, A1459. All data matched those in the literature.

Experiment (2): 2- (6-Bromohexayloxy)-tetrahydro-*2H*-pyran (154)

$Br(CH_2)_6OTHP$

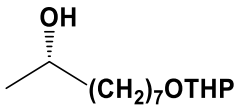
3,4-Dihydro-2*H*-pyran (70.6 g, 0.838 mol, 2 eq.) and pyridinium-*p*-toluene-sulfonate (31.5 g, 0.16 mol, 0.3 eq) were added to a stirred solution of 6-bromo-hexan-1-ol (**155**) (75.8 g, 0.419 mol) in dry dichloromethane (400 mL) under atmosphere of nitrogen. The reaction mixture was stirred for 3 hrs and then quenched with sat. aq. $NaHCO_3$ (100 mL). The organic layer was separated, and the aqueous layer was re-extracted with dichloromethane (2 × 100 mL). The combined organic layers were, dried and evaporated and the residue was flash distilled. to give a colourless oil, 2-(6-bromo-hexayloxy)-tetrahydro-*2H*-pyran (**154**)[193] (97.0 g, 87%), which showed δ_H (400 MHz, $CDCl_3$): 4.52 (1H, br. s), 3.83 – 3.78 (1H, m), 3.69 (1H, dt, *J* 6.7, 9.4 Hz), 3.54 – 3.42 (1H, m), 3.38 – 3.28 (3H, including a triplet resonated at 3.35 *J* 6.7 Hz), 1.91 –1.77 (3H, m), 1.74 – 1.63 (1H, m), 1.62 – 1.35 (10H, m); δ_C (101 MHz, $CDCl_3$): 98.5, 67.0, 61.9, 33.5, 32.5, 30.5, 29.3, 27.7, 25.23, 25.2, 19.4; ν_{max} /cm^{-1}: 2936, 2861, 1453. All data matched those in the literature.

Experiment (3): (*2R*)-9-((Tetrahydro-*2H*-2-yl)oxy)nonan-2-ol (153)



A solution of 2-(6-bromo-hexyloxy)-tetrahydro-*2H*-pyran (**154**) (68.5 g, 0.258 mol) in dry THF (50 mL) was added dropwise to a suspension of magnesium turnings (18.9 g, 0.774 mol) in dry THF (120 mL) under nitrogen. The mixture was refluxed for 1 hr, then cooled to room temperature and added dropwise to a stirred suspension solution of copper iodide (12.28 g, 0.0645 mol) in dry THF (150 mL) at – 30 °C. After 30 min, the (*R*)-(+)-propylene oxide (**171**) (6.0 g, 0.10 mol) in dry THF (30 mL) was added to above solution drop wise at − 30 °C. The reaction was kept at − 30 °C for 3 hrs, then allowed to reach 0 °C before slowly quenched with sat. aq. ammonium chloride (100 mL) then allowed to reach room temperature, and extracted with ether (300 mL). The aqueous layer was re-extracted with ether (2 × 200 mL). The combined organic layers were washed with water (50 mL) and dried. The solvent was evaporated to give a residue; which was purified by column chromatography, on silica gel eluting with petrol/ether (5:1) to give a colourless oil, (*2R*)-9-((tetrahydro-*2H*-2-yl) oxy)nonan-2-ol (**153**) (22.0 g, 86%), $[\alpha]_D^{23} =$ – 4.8 (*c* 1.1, MeOH). {Found $[M+NH_4]^+$: 262.2374; $C_{14}H_{32}O_3N$ requires: 262.2377}; which showed δ_H (400 MHz, $CDCl_3$): 4.58 (1H, br. s), 3.92 – 3.84 (1H, m), 3.81 – 3.77 (1H, m), 3.73 (1H, dt, *J* 7.1, 9.4 Hz), 3.53 – 3.47 (1H, m), 3.38 (1H, dt, *J* 6.3, 9.4 Hz), 1.86 – 1.80 (1H, m), 1.75 – 1.69 (1H, m), 1.64 – 1.50 (7H, m), 1.46 – 1.27 (10H, m), 1.19 (3H, d, *J* 6.2 Hz); δ_C (101 MHz, $CDCl_3$): 98.8, 68.1, 67.6, 62.3, 39.3, 30.7, 29.7, 29.5, 29.4, 26.1, 25.7, 25.5, 23.4, 19.7; ν_{max} /cm^{-1}: 3411, 2927, 2854, 1454.

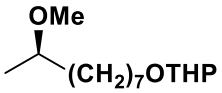
Experiment (4): (*2S*)-9-((Tetrahydro-*2H*-pyran-2-yl)oxy)nonan-2-ol (153a)



A solution of 2-(6-bromo-hexyloxy)-tetrahydropyran (**154**) (68.5 g, 0.258 mol) in dry THF (50 mL) was added dropwise to a suspension of magnesium turnings (18.8 g, 0.774 mol) in dry THF (120 mL) under nitrogen. The mixture was refluxed for 1 hr, then cooled to room temperature and added dropwise to a stirred suspension solution of copper iodide (12.3 g, 0.064 mol) in dry THF (150 mL) at – 30 °C. After 10 min. the (*S*)-(-)-propylene oxide (**171a**) (6.0 g, 0.10 mol) in dry THF (50 mL) was added to above solution dropwise at – 30 °C. The reaction was kept at – 30 °C for 3 hrs, then work up and purified as above to give a colourless oil of (*2S*)-9-((tetrahydro-*2H*-pyran-2-yl)oxy)nonan-2-ol (**153a**) (21 g, 83%), $[\alpha]_D^{23} = +3.8$ (*c* 1.9, MeOH). {Found $[M+NH_4]^+$: 262.2374; $C_{14}H_{32}O_3N$ requires: 262.2377}; which showed δ_H (400 MHz, $CDCl_3$): 4.57

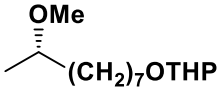
(1H, br. s), 3.88 – 3.84 (1H, m), 3.80 – 3.75 (1H, m), 3.72 (1H, dt, *J* 7.1, 9.4 Hz) 3.51 – 3.47 (1H, m), 3.38 (1H, dt, *J* 6.3, 9.4 Hz), 1.85 – 1.79 (1H, m), 1.74 – 1.68 (1H, m), 1.61 – 1.49 (7H, m), 1.45 – 1.32 (10H, m), 1.18 (3H, d, *J* 6.2 Hz) ; δ_C (101 MHz, $CDCl_3$): 98.8, 68.1, 67.6, 62.3, 39.3, 30.7, 29.7, 29.5, 29.4, 26.1, 25.7, 25.5, 23.4, 19.7; ν_{max} /cm^{-1}: 3411, 2929, 2855, 1454.

Experiment (5): 2-(((*R*)-8-Methoxynonyl) oxy)tetrahydro-*2H*-pyran (152)



Sodium hydride (24.9 g, 0.622 mol, 60 % dispersion, 7 eq.) was washed with petrol (3 × 20 mL) and then suspended in dry THF (160 mL), cooled to 5 °C and (*2R*)-9-((tetrahydro-*2H*-pyran-2-yl)oxy)nonan-2-ol (**153**) (21.7 g, 0.088 mol) in THF (100 mL) was added over 15 min. The mixture stirred for 15 min, methyl iodide (75.7 g, 0.533 mol, 6 eq.) was added, and the mixture was stirred for 16 hrs at room temperature. After this time, the mixture was cooled to 0 °C and quenched slowly with sat. aq. ammonium chloride (50 mL) followed by ether (200 mL), then the organic layer was separated and the aqueous layer was extracted with petrol/ethyl acetate (2 × 100 mL, 1:1). The combined organic layers were washed with sat.aq. sodium chloride (2 × 80 mL), dried and evaporated to give a residue; which was purified by column chromatography, on silica gel eluting with petrol/ethyl acetate (10:1) to give a colourless oil, 2-(((*R*)-8-methoxynonyl)oxy)tetrahydro-*2H*-pyran (**152**) (21.0 g, 96%), $[\alpha]_D^{23}$ = – 1.2 (*c* 1.9, MeOH). {Found $[M+NH_4]^+$: 276.2535; $C_{15}H_{34}O_3N$ requires: 276.2533}; which showed δ_H (400 MHz, $CDCl_3$): 4.55 (1H, br. s), 3.87 – 3.82 (1H, m), 3.71 (1H, dt, *J* 6.9, 9.4 Hz), 3.52 – 3.45 (1H, m), 3.36 (1H, dt, *J* 6.7, 9.4 Hz), 3.29 (3H, s), 3.25 (1H, pent, *J* 6.0 Hz), 1.84 – 1.77 (1H, m), 1.72 – 1.66 (1H, m), 1.58 – 1.47 (8H, m), 1.31 – 1.28 (8H, m), 1.10 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 98.8, 76.8, 67.6, 62.3, 55.9, 36.3, 30.7, 29.7, 29.6, 29.4, 26.1, 25.4, 25.3, 19.6, 18.9; ν_{max}/cm^{-1}: 2928, 2855, 1464.

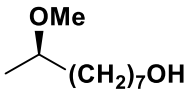
Experiment (6): 2-(((*S*)-8-Methoxynonyl)oxy)tetrahydro-*2H*-pyran (152a)



Sodium hydride (23.8 g, 0.596 mol, 60 % dispersion, 7 eq.) was washed with petrol (3 × 20 mL) and then suspended in dry THF (160 mL), cooled to 5 °C and (*2S*)-9-

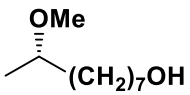
((tetrahydro-*2H*-pyran-2-yl)oxy)nonan-2-ol (**153a**) (20.8 g, 0.582 mol) in THF (100 mL) was added over 15 min. After 15 min, methyl iodide (72.6 g, 0.511 mol, 6 eq.) was added, and the mixture was stirred for 16 hrs at room temperature. The reaction mixture was worked up and purified as above to give a colourless oil, 2-(((*S*)-8-methoxynonyl)oxy)tetrahydro-*2H*-pyran (**152a**) (21.0 g, 96%); $[\alpha]_D^{24}$ = + 1.7 (*c* 1.9, MeOH). {Found $[M+NH_4]^+$: 276.2535; $C_{15}H_{34}O_3N$ requires: 276.2533}. Which showed δ_H (400 MHz, $CDCl_3$): 4.58 (1H, br. s), 3.92 – 3.85 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.4 Hz), 3.57 – 3.47 (1H, m), 3.38 (1H, dt, *J* 6.7, 9.4 Hz), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 1.91 – 1.80 (1H, m), 1.77 – 1.69 (1H, m), 1.61 – 1.52 (8H, m), 1.43 – 1.26 (8H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 98.8, 76.8, 67.7, 62.3, 55.9, 36.3, 30.8, 29.7, 29.68, 29.4, 26.2, 25.5, 25.4, 19.7, 19.0; ν_{max}/cm^{-1}: 2928, 2855, 1078.

Experiment (7): (*R*)-8-Methoxynonan-1-ol (151)



Pyridinium-*p*-toluenesulfonate (6.9 g, 0.03 mol) was added to a stirred solution of 2-(((*R*)-8-methoxynonyl)oxy)tetrahydro-*2H*-pyran (**152**) (35.3 g, 0.136 mol) in methanol/THF (200 mL, 1:1) and stirred at 50 °C for 3 hrs. When TLC showed that the reaction was almost complete, sat. aq. $NaHCO_3$ (25 mL) and water (20 mL) were added and the mixture was extracted with ether (3 × 150 mL). The combined organic layers were dried, evaporated and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil, (*R*)-8-methoxynonan-1-ol (**151**) (22.0 g, 86%), $[\alpha]_D^{23}$ = – 5.2 (*c* 1.9, MeOH). {Found $[M+H]^+$: 175.1693; $C_{10}H_{23}O_2$ requires: 175.1693}; which showed δ_H (400 MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.6 Hz), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 1.58 – 1.49 (4H, m), 1.45 – 1.28 (9H, br. m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 76.9, 62.9, 55.9, 36.3, 32.7, 29.7, 29.3, 25.6, 25.3, 18.9; ν_{max}/cm^{-1}: 3360, 2927, 2855,1463.

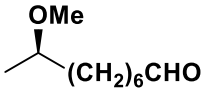
Experiment (8): (*S*)-8-Methoxynonan-1-ol (151a)



Pyridinium-*p*-toluene sulfonate (4.17 g, 0.016 mol) was added to a stirred solution of 2-(((*S*)-8-methoxynonyl)oxy)tetrahydro-*2H*-pyran (**152a**) (21.0 g, 0.082 mol) in

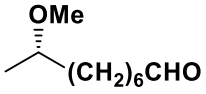
methanol/THF (150 mL, 1:1) and stirred at 50 °C for 3 hrs. When TLC showed that the reaction was almost complete, The reaction mixture was worked up and purified as above to give a colourless oil, (*S*)-8-methoxynonan-1-ol (**151a**) (12.0 g, 85%), $[\alpha]_D^{24}$ = + 4.2 (*c* 1.2, MeOH). {Found $[M+H]^+$: 175.1693; $C_{10}H_{23}O_2$ requires: 175.1693}; which showed δ_H (400 MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.5 Hz), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 1.58 – 1.50 (4H, m), 1.45 – 1.28 (9H, br. m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 76.7, 62.3, 55.6, 36.0, 32.5, 29.5, 29.2, 25.5, 25.1, 18.7; ν_{max}/cm^{-1}: 3360, 2927, 2855,1463.

Experiment (9): (*R*)-8- Methoxynonanal (150)



(*R*)-8- Methoxynonan-1-ol (**151**) (25.2 g, 0.015 mol) in dichloromethane (50 mL) was added to vigorously stirred suspension solution of pyridinium chlorochromate (62.6 g, 0.29 mol) in dichloromethane (500 mL) at room temperature, a black colour appearing during the addition. The mixture was stirred for 2 hrs, when TLC showed no starting material was left, then the mixture poured into petrol/ethyl acetate (200 mL, 5:1). The solution was filtered through a pad of silica gel, and then washed with petrol/ethyl acetate (5:1, 200 mL) and the filtrate was evaporated. The residue was purified by column chromatography, on silica gel eluting with petrol/ethyl acetate (5:1) to give a colourless oil, (*R*)-8- methoxynonanal (**150**) (20.0 g, 80%), $[\alpha]_D^{18}$ = – 3.4 (*c* 1.8 , MeOH). {Found $[M+NH_4]^+$: 190.1807; $C_{10}H_{24}O_2N$ requires: 190.1802}; which showed δ_H (400 MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.9 Hz), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.43 (2H, td, *J* 1.9, 7.4 Hz), 1.66 – 1.61 (2H, m), 1.58 – 1.48 (1H, m), 1.40 – 1.30 (7H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 202.9, 76.7, 55.9, 43.8, 36.2, 29.4, 29.1, 25.2, 22.0, 18.9 ; ν_{max}/cm^{-1}: 2930, 2857, 1724, 1463.

Experiment (10): (*S*)-8- Methoxynonanal (150a)



(*S*)-8- Methoxynonan-1-ol (**151a)** (12.1 g, 0.069 mol) in dichloromethane (50 mL) was added to vigorously stirred suspension solution of pyridinium chlorochromate (30.0 g, 0.138 mol) in dichloromethane (500 mL) at room temperature, a black colour appearing

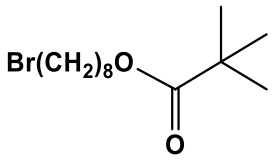
during the addition. The mixture was stirred for 2 hrs, when TLC showed no starting material was left, then the reaction mixture was worked up and purified as above to give a colourless oil, (*S*)-8-methoxynonanal (**150a**) (9.0 g, 79%), $[\alpha]_D^{24}$ = + 3.0 (*c* 1.1, MeOH). {Found $[M+NH_4]^+$: 190.1807; $C_{10}H_{24}O_2N$ requires: 190.1802}; which showed δ_H (400 MHz, $CDCl_3$): 9.76 (1H, t, *J* 1.9 Hz), 3.31 (3H, s), 3.26 (1H, pent, *J* 6.0 Hz), 2.42 (2H, td, *J* 1.9, 7.4 Hz), 1.63 (2H, pent, *J* 7.0 Hz), 1.55 – 1.46 (1H, m), 1.43 – 1.25 (7H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 202.9, 76.7, 55.9, 43.8, 36.2, 29.4, 29.1, 25.2, 22.0, 18.9; ν_{max}/cm^{-1}: 2930, 2856, 1723, 1464.

Experiment (11): 8-Bromooctan-1-ol (174)

$$Br(CH_2)_8OH$$

1,8-Octanediol (**173**) (100 g, 0.68 mol) was dissolved in toluene (400 mL) and aqueous HBr (100 mL, 48%) was added and the mixture was refluxed for 18 hrs. Then the mixture was worked up and purified as before to give a colourless oil, 8-bromooctan-1-ol (**174**)[153] (120.0 g, 84%), which showed δ_H (500 MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.4 Hz), 3.41 (2H, t, *J* 6.6 Hz), 1.85 (2H, pent, *J* 6.9 Hz), 1.57 (2H, pent, *J* 6.5 Hz), 1.46 − 1.32 (9H, m); δ_C (101 MHz, $CDCl_3$): 63.0, 34.0, 32.7, 32.67, 29.2, 28.7, 28.1, 25.6; ν_{max}/cm^{-1} : 3348, 2926, 2854, 1461. All data matched those in the literature.

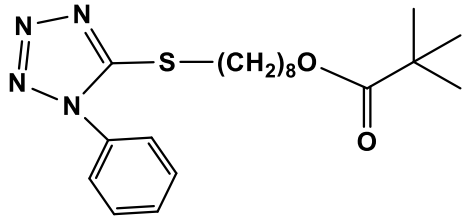
Experiment (12): 8-Bromooctyl pivalate (175)



A solution of trimethylacetylchloride (27.7 g, 0.229 mol, 1.2 mol eq) in dichloromethane (25 mL) was added to a stirred solution of 8-bromooctan-1-ol (**174**) (40 g, 0.19 mol), trimethylamine (29.1, 40 mL, 0.287 mol) and 4-dimethylaminopyridine (0.70 g, 0.0057 mol) in dichloromethane (300 mL), over a period of 15 min at 5 °C. The reaction mixture was then allowed to reach room temperature and stirred for 18 hrs. Dilute HCl (50 mL) was then added and the organic layer was separated the aqueous layer was re- extracted with dichloromethane (2 × 100). The combined organic layers were washed with dilute HCl (50 mL) and sat. aq. sodium chloride (2 × 200 mL). The organic layer was dried and evaporated, and the crude product was diluted with petrol (200 mL) and filtered through a pad of silica gel and washed with petrol (50 mL). The silica gel was finally

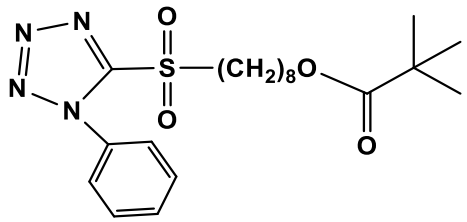
washed with petrol/ethyl acetate (1:1) and the filtrate was evaporated to give a colourless oil, 8-bromooctyl pivalate (**175**)[153] (56.0 g, 99%), which showed δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.6 Hz), 3.41 (2H, t, *J* 6.8 Hz), 1.87 (2H, pent, *J* 6.9 Hz), 1.62 (2H, pent, *J* 6.6 Hz), 1.46 − 1.32 (8H, m), 1.20 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 64.4, 33.9, 32.8, 29.3, 29.1, 28.7, 28.6, 28.1, 27.2, 25.9; ν_{max}/cm^{-1} : 2930, 2856, 1750, 1479. All data matched those in the literature.

Experiment (13): 8-((1-Phenyl-*1H*-tetrazol-yl)thio)octyl pivalate (176)



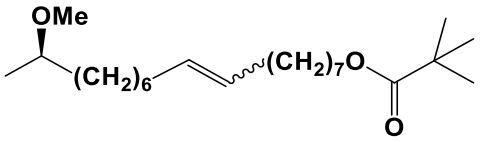
1-Phenyl-*1H*-tetrazole-5-thiol (35.8 g, 0.201 mol), 8-bromooctyl pivalate (**175**) (56 g, 0.191 mol) and anhydrous potassium carbonate (52.8 g, 0.382 mol) in acetone (300 mL) were vigorously stirred for 18 hrs at room temperature. After this time TLC indicated that the reaction to be complete. Water (1 L) was added to the mixture and the product was extracted with dichloromethane (2 × 200 mL). The combined organic layers were washed with sat. aq. sodium chloride (2 × 200 mL), 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, 8-((1-phenyl-*1H*-tetrazol-yl)thio)octyl pivalate (**176**)[153] (73.0 g, 98%), which showed δ_H (500 MHz, $CDCl_3$): 7.60 − 7.53 (5H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.40 (2H, t, *J* 7.3 Hz), 1.83 (2H, pent, *J* 7.3 Hz), 1.61 (2H, pent, *J* 6.7 Hz), 1.45 (2H, pent, *J* 7.0 Hz), 1.36 − 1.33 (6H, m), 1.19 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 154.5, 133.8, 130.0, 129.7, 123.8, 64.4, 33.3, 29.3, 29.1, 29.0, 28.9, 28.6, 28.5, 27.2, 25.8; ν_{max}/cm^{-1}: 2928, 2855, 1740, 1154. All data matched those in the literature.

Experiment (14): 8-((1-Phenyl-*1H*-tetrazol-5-yl)sulfonyl)octyl pivalate (177)



A solution of ammonium molybdate tetrahydrate (92.5 g, 0.0748 mol) in hydrogen peroxide 35% (164 mL), was cooled in an ice bath, and added to a stirred solution of the sulfide (**176**) (73.0 g, 0.187 mol) in THF (150 mL) and IMS (300 mL) at 10 °C. The resulting solution was stirred at room temperature for 2 hrs. A further solution of ammonium molybdate tetrahydrate (46.0 g) in in hydrogen peroxide 35% (82 mL) was then added and the mixture was stirred at room temperature for 18 hrs. After this time the mixture was poured into water (2 L) and extracted with dichloromethane (2 × 200 mL). The combined organic layers were washed with water (300 mL), dried and the solvent was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) and then (5:2) to give a very thick yellow oil, 8-((1-phenyl-*1H*-tetrazol-5-yl)sulfonyl)octyl pivalate (**177**)[153] (76.0 g, 96%), which showed δ_H (500 MHz, $CDCl_3$): 7.71 − 7.69 (2H, m), 7.65 − 7.58 (3H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.74 (2H, t, *J* 7.8 Hz), 1.96 (2H, pent, *J* 7.1 Hz), 1.60 (2H, pent, *J* 6.3 Hz), 1.51 (2H, pent, *J* 7.5 Hz), 1.44 − 1.22 (6H, m), 1.19 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 153.4, 131.4, 129.7, 125.0, 64.3, 55.9, 28.97, 28.9, 28.8, 28.5, 28.0, 27.2, 25.8, 21.9; ν_{max}/cm^{-1}: 2930, 2857, 1740, 1497. All data matched those in the literature.

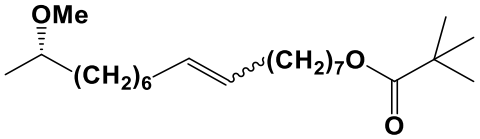
Experiment (15): (*E/Z*)-(*R*)-16-Methoxyheptadec-8-en-1-yl pivalate (149)



Lithium *bis*(trimethylsilyl)amide (84.0 mL, 0.0594 mol, 1.06 M) was added dropwise to a stirred solution of (*R*)-8-methoxynonanal (**150**) (8.5 g, 0.088 mol) and 8-((1-phenyl-*1H*-tetrazol-5-yl)sulfonyl)octyl pivalate[153] (**177**) (25.0 g, 0.059 mol) in dry THF (100 mL) under nitrogen at − 2 °C. The mixture was allowed to reach room temperature and stirred for 2 hrs, then quenched with sat.aq. ammonium chloride (50 mL) and the product was extracted with petrol/ethyl acetate (1:1, 3 × 300 mL). The organic layer was separated, and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 50 mL). The combined organic layers were washed with sat. aq. sodium chloride (2 × 100 mL), dried and evaporated to give a thick oil, which was purified by chromatography, on silica eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (*E/Z*)-(*R*)-16-methoxyheptadec-8-en-1-yl pivalate **(149)** (12.0 g, 66%), as a mixture of two isomers in ratio 2:1; $[\alpha]_D^{29}$= – 1.0 (*c* 2.3, $CHCl_3$). {Found $[M+H]^+$: 369.3358; $C_{23}H_{45}O_3$ requires:

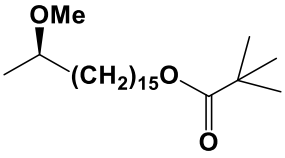
369.3363}; which showed δ_H (500 MHz,$CDCl_3$, major isomer): 5.42 – 5.37 (2H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.31 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.98 – 1.92 (4H, m), 1.62 (2H, br. pent, *J* 6.5 Hz), 1.45 – 1.24 (18H, m), 1.20 (9H, s), 1.12 (3H, d, *J* 6.1 Hz); δ_H (500 MHz,$CDCl_3$, minor isomer): 5.36 – 5.32 (2H, m), 2.03 – 2.01 (4H, m), 1.54 – 1.48 (2H, m), the remaining signals were obscured by the major isomer; δ_C (101 MHz,$CDCl_3$ for the two isomers): 178.6, 130.4, 130.2, 129.9, 129.8, 76.8, 64.4, 55.9, 36.3, 32.6, 32.5, 29.7, 29.6, 29.6, 29.56, 29.5, 29.14, 29.1, 29.0, 28.9, 28.6, 27.2, 25.9, 25.85, 25.4, 18.9; ν_{max}/cm^{-1}: 2926, 2854,1729, 1479.

Experiment (16): (*E/Z*)-(*S*)-16-Methoxyheptadec-8-en-1-yl pivalate (149a)



Lithium *bis*(trimethylsilyl)amide (118 mL, 0.125 mol, 1.06 M) was added dropwise to a stirred solution of (*S*)-8-methoxynonanal (**150a**) (12.0 g, 0.069 mol) and 8-((1-pheny-*1H*-tetrazol)sulfonyl)octly pivalate[153] (**177**) (35.3 g, 0.0697 mol) in dry THF (100 mL), under nitrogen, at −2 °C. The mixture was allowed to reach room temperature and stirred for 2 hrs, then the reaction mixture was worked up and purified as above to give (*E*/*Z*)-(*S*)-16-methoxyoctadec-9-en-1-yl pivalate (**149a**) as a mixture of two isomers in ratio 2:1 (17.6 g, 69%), $[\alpha]_D^{29}$= + 1.0 (*c* 2.1, $CHCl_3$). {Found $[M+H]^+$: 369.3358; $C_{23}H_{45}O_3$ requires: 369.3363}. Which showed identical spectrum as above.

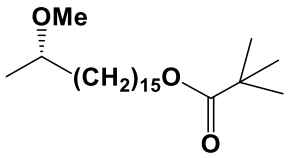
Experiment (17): (*R*)-16-Methoxyheptadecyl pivalate (148)



Palladium 10 % on carbon (1.5 g) was added to a stirred solution of (*E/Z*)-(*R*)-16-methoxyheptadec-8-en-1-yl pivalate (**149**) (12.0 g) in IMS (100 mL) and ethyl acetate (250 mL) under hydrogen atmosphere. Hydrogenation was carried out over 3 hrs. The reaction mixture was quenched with two drops of water then filtered through a bed of celite and the solvent was evaporated to give a colourless oil, (*R*)-16-methoxyheptadecyl pivalate (**148**) (12.0 g, 99%), $[\alpha]_D^{29}$ = – 1.3 (*c* 2.0, $CHCl_3$). {Found $[M+H]^+$: 371.3516; $C_{23}H_{47}O_3$ requires: 371.3520}; which showed δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.6

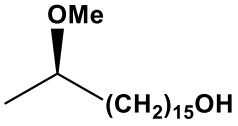
Hz), 3.32 (3H, s), 3.28 (1H, br. pent, *J* 5.9 Hz), 1.62 (2H, pent, *J* 6.5 Hz), 1.34 – 1.26 (26H, m), 1.20 (9H, s), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 178.6, 76.9, 64.5, 55.9, 36.3, 29.8, 29.7, 29.6, 29.54, 29.5, 29.2, 28.6, 27.2, 25.9, 25.4, 19.0; ν_{max}/cm^{-1}: 2923, 2853, 1729, 1479.

Experiment (18): (*S*)-16-Methoxyheptadecyl pivalate (148a)



Palladium 10 % on carbon (1.5 g) was added to a stirred to solution of (*E*/*Z*)-(*S*)-16-methoxyoctadec-9-en-1-yl pivalate (**149a**) (17.6 g) in IMS (100 mL) and ethyl acetate (250 mL) under hydrogen atmosphere. Hydrogenation was carried out over 3 hrs. The reaction was worked up as above to give a colourless oil, (*S*)-16-methoxyheptadecyl pivalate **(148a)** (17.5 g, 99%), $[\alpha]_D^{29}$= + 1.3 (*c* 2.1, $CHCl_3$). {Found $[M+H]^+$: 371.3516; $C_{23}H_{47}O_3$ requires: 371.3520}; which showed δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.6 Hz), 3.32 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 1.62 (2H, pent, *J* 6.5 Hz), 1.45 – 1.26 (26H, m), 1.20 (9H, s), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 178.7, 76.9, 64.5, 55.9, 36.3, 29.8, 29.7, 29.6, 29.55, 29.5, 29.2, 28.6, 27.2, 25.9, 25.5, 19.0; ν_{max}/cm^{-1}: 2923, 2853, 1729, 1479.

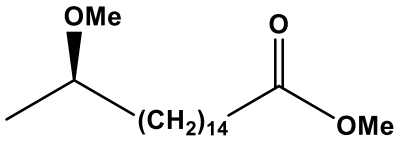
Experiment (19): (*R*)-16-Methoxyheptadecan-1-ol (147)



A solution of (*R*)-16-methoxyheptadecyl pivalate (**148**) (11.8 g, 0.0319 mol) in dry THF (50 mL) was added slowly to a stirred suspension solution of $LiAlH_4$ (1.81g, 0.0479 mol) in dry THF (150 mL) at – 10 °C under nitrogen atmosphere, and the mixture was allowed to reach room temperature and then refluxed for 1 hr. The reaction mixture was cooled down to 0 °C and quenched slowly with saturated solution of sodium sulfate decahydrate (40 mL) and THF (60 mL), then the reaction mixture was stirred at room temperature until a white precipitate had formed, and dried, then filtered through a bed of silica and celite, the filtrate was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a white solid,

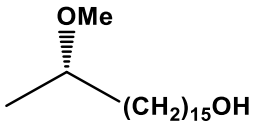
(*R*)-16-methoxyheptadecan-1-ol (**147**) (8.0 g, 88%), m.p. 46 − 48 °C $[\alpha]_D^{29}$ = – 1.9 (*c* 1.6, $CHCl_3$). {Found $[M+H]^+$: 287.2945; $C_{18}H_{39}O_2$ requires: 287.2945}; which showed δ_H (500 MHz, $CDCl_3$): 3.65 (2H, t, *J* 6.4 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.63 – 1.50 (3H, m), 1.43 – 1.20 (26H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 76.9, 63.0, 55.9, 36.3, 32.8, 29.8, 29.6, 29.59, 29.5, 29.4, 25.7, 25.4, 19.0; ν_{max}/ cm^{-1}: 3432, 2916, 2847, 1471.

Experiment (20): Methyl (*R*)-16-methoxyheptadecanoate (183)



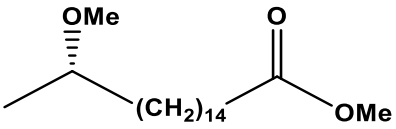
A solution of (*R*)-16-methoxyheptadecan-1-ol (**147**) (0.50 g, 1.7 mmol) in of CCl_4 (2.8 mL) was added within 30 min at room temperature to a stirred solution of sodium periodate (1.12 g, 5.24 mmol) and ruthenium (III) chloride hydrate (4.70 mg, 0.0227 mmol) in acetonitrile (5.6 mL), CCl_4 (2.8 mL) and water (8.4 mL). The mixture was stirred for additional 16 hrs. The mixture was concentrated in vacuo. The mixture was quenched with dilute H_2SO_4 (20 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried, evaporated. The residue was purified by column chromatography, eluting with petrol/ethyl acetate (5:2) to give (*R*)-16-methoxyheptadecanoic acid (**182**) (0.45 g, 86%). Conc. H_2SO_4 (3 drops) were added to a stirred solution of (*R*)-16-methoxyheptadecanoic acid (**182**) (0.45 g, 1.5 mmol) in methanol (15 mL) and the mixture was refluxed for 3 hrs at 92 °C. The reaction was monitored by TLC until there was no starting material remaining. The solution was evaporated and the residue was diluted with a saturated solution of $NaHCO_3$ (10 mL). The product was extracted with petrol/ethyl acetate (1:1, 2 × 30 mL), dried and evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give white solid, methyl (*R*)-16-methoxyheptadecanoate **(183)** (0.45 g, 89%), m.p. 33 −35 °C; $[\alpha]_D^{26}$= – 1.1 (*c* 0.92, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 3.67 (3H, s), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.66 – 1.56 (2H, m), 1.50 – 1.46 (1H, m), 1.43 – 1.20 (23H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 174.4, 76.8, 55.9, 51.4, 36.4, 34.1, 29.8, 29.6, 29.5, 29.4, 29.3, 29.1, 25.5, 24.9, 19.0; ν_{max}/cm^{-1}: 2917, 2849, 1733, 1463.

Experiment (21): (*S*)-16-Methoxyheptadecan-1-ol (147a)



A solution of (*S*)-16-methoxyheptadecyl pivalate (**148a**) (17.5 g, 0.0473 mol) in dry THF (60 mL) was added slowly to a stirred suspension solution of $LiAlH_4$ (2.7 g, 0.071 mol) in dry THF (150 mL) at – 10 °C under nitrogen atmosphere, and the mixture was allowed to reach room temperature and then refluxed for 1 hr. The reaction mixture was worked up and purified as above to give a white solid, (*S*)-16-methoxyheptadecan-1-ol (**147a**) (11.8 g, 84%), m.p. 46 − 48 °C; $[\alpha]_D^{29}$= + 2.1 (*c* 1.5, $CHCl_3$). {Found $[M+H]^+$: 287.2946; $C_{18}H_{39}O_2$ requires: 287.2945}; which showed δ_H (500 MHz, $CDCl_3$): 3.65 (2H, q, *J* 6.4 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.63 – 1.49 (3H, m), 1.43 – 1.20 (26H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 76.9, 63.1, 55.9, 36.3, 32.8, 29.8, 29.64, 29.6, 29.5, 29.4, 25.7, 25.5, 19.0; ν_{max}/cm^{-1}: 3433, 2916, 2847, 1471.

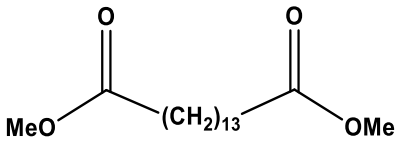
Experiment (22): Methyl (*S*)-16-methyoxyoctadecanoate (183a)



A solution of (*S*)-16-methoxyheptadecan-1-ol (**147a**) (0.40 g, 1.4 mmol) in of CCl_4 (2.0 mL) was added within 30 min. at room temperature to a stirred solution of sodium periodate (0.89 g, 4.2 mmol) and ruthenium (III) chloride hydrate (3.7 mg, 0.0182 mmol) in acetonitrile (5.0 mL), CCl_4 (2.0 mL) and water (8.0 mL). The mixture was stirred for additional 16 hrs. The mixture was concentrated in vacuo. The mixture was worked up and purified as before to give (*S*)-16-methoxyheptadecanoic acid (**182a**) (0.4 g, 95%). Con. H_2SO_4 (3 drops) were added to a stirred solution of (*S*)-16-methoxyheptadecanoic acid (**182a**) (0.40 g, 1.3 mmol) in methanol (15 mL) and the mixture was refluxed for 3 hrs at 92 °C. The reaction was monitored by TLC until there was no starting material remaining. The reaction mixture was worked up and purified as above to give a white solid, methyl (*S*)-16-methoxyheptadecanoate (**183a**) (0.35 g, 84%), m.p. 32 − 34 °C $[\alpha]_D^{26}$= + 1.1 (*c* 0.94, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 3.67 (3H, s), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.63 − 1.56 (2H, m), 1.52 – 1.47 (1H, m), 1.35 – 1.22 (23H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 174.4,

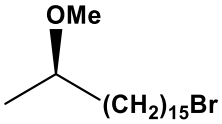
76.9, 55.9, 51.4, 36.3, 34.1, 29.8, 29.6, 29.5, 29.4, 29.3, 29.1, 25.5, 24.9, 19.0; ν_{max}/cm^{-1}: 2914, 2849, 1772, 1428.

Experiment (23): Dimethyl hexadecanedioate (26)



A solution methyl 15-hydroxypentadecanoate (0.5 g, 1.83 mmol) in of CCl_4 (2.8 mL) was added within 30 min, at room temperatur to a stirred solution of sodium periodate (1.17 g, 5.24 mmol) and ruthenium (III) chloride hydrate (4.9 g, 0.024 mmol) in acetonitrile (5.6 mL), CCl_4 (2.8 mL) and water (8.4 mL). The mixture was stirred for additional 16 hrs. The mixture was concentrated in vacuo. The mixture was worked up and purified as above to give 15-methoxy-15-oxopentadecanoic acid (0.4 g, 77%). Con. H_2SO_4 (5 drops) were added to a stirred solution of 15-methoxy-15-oxopentadecanoic acid (0.40 g, 1.4 mmol) in methanol (15 mL) and the mixture was refluxed for 3 hrs. The reaction was monitored by TLC until there was no starting material left. The reaction mixture was worked up and purified as above to give a white solid, dimethyl hexadecanedioate (**26**) (0.39 g, 95%), which showed δ_H (400 MHz, $CDCl_3$): 3.67 (6H, s), 2.30 (4H, t, *J* 7.5 Hz), 1.62 (4H, pent, *J* 5.0 Hz), 1.38 − 1.20 (18H, m); δ_C (101 MHz, $CDCl_3$): 174.3, 51.4, 34.1, 29.6, 29.5, 29.4, 29.2, 29.1, 24.9; ν_{max}/cm^{-1}: 2916, 2848, 1768, 1427.

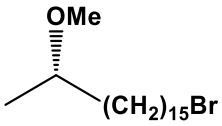
Experiment (24): (*R*)-1-Bromo-16-methoxyheptadecane (146)



Triphenyl phosphine (25.1 g, 0.0956 mol) was added to a stirred solution of (*R*)-16-methoxyheptadecan-1-ol (**147**) (22.8 g, 0.079 mol) in dichloromethane (350 mL) followed by addition of $NaHCO_3$ (0.5 g). The mixture was cooled to 10 °C then *N*-bromosuccinamide (21.3 g, 0.0119 mol) was added in portions and the mixture was stirred at room temperature for 1 hr. A sat. aq. solution of sodium metabisulphate (150 mL) was added and the product was extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried, and the solvent was evaporated to give a residue, which was then refluxed in petrol/ethyl acetate (50:1) for 1 hr. The triphenyl

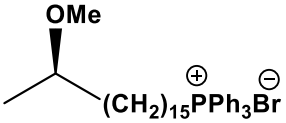
phosphonium oxide was filtrated on a pad of celite and washed with petrol/ethyl acetate (50:1), the solvent was evaporated and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (50:1) to give a colourless oil, (*R*)-1-bromo-16-methoxyheptadecane (**146**) (23.0 g, 83%), $[\alpha]_D^{29}$= – 1.1 (*c* 1.9, $CHCl_3$). {Found $[M+H]^+$: 349.2087; $C_{18}H_{38}OBr$ requires: 349.2101}; which showed δ_H (500 MHz, $CDCl_3$): 3.41 (2H, t, *J* 6.8 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.86 (2H, pent, *J* 6.9 Hz), 1.42 (2H, pent, *J* 6.8 Hz), 1.34 – 1.23 (24H, br. m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 76.9, 55.9, 36.3, 34.0, 32.8, 29.8, 29.63, 29.6, 29.5, 29.4, 28.8, 28.2, 25.4, 19.0 ; ν_{max}/cm^{-1}: 2921, 2852, 1465.

Experiment (25): (*S*)-1-Bromo-16-methoxyheptadecane (146a)



Triphenyl phosphine (12.3 g, 0.0469 mol) was added to a stirred solution of (*S*)-16-methoxyheptadecan-1-ol (**147a**) (11.2 g, 0.0541mol) in dichloromethane (200 mL) followed by addition of $NaHCO_3$ (0.5 g). The mixture was cooled to 10 °C then *N*-bromosuccinamide (10.5 g, 0.0586 mol) was added in portions and the mixture was stirred at room temperature for 1 hr. The reaction mixture was worked up and purified as above to give a colourless oil, (*S*)-1-bromo-16-methoxyheptadecane (**146a**) (11.5 g, 84%), $[\alpha]_D^{29}$ = + 1.1 (*c* 1.9, $CHCl_3$). {Found $[M+H]^+$: 349.2094; $C_{18}H_{38}OBr$ requires: 349.2101}; which showed δ_H (500 MHz, $CDCl_3$): 3.41 (2H, t, *J* 6.9 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.86 (2H, pent, *J* 6.9 Hz), 1.42 (2H, pent, *J* 6.8 Hz), 1.34 – 1.23 (24H, br. m), 1.12 (3H, d, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 76.9, 55.9, 36.3, 34.0, 32.8, 29.8, 29.63, 29.6, 29.5, 29.4, 28.8, 28.2, 25.4, 19.0; ν_{max}/cm^{-1}: 2923, 2853, 1132, 646.

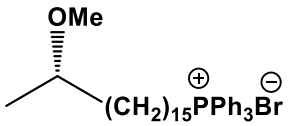
Experiment (26): (*R*)-(16-Methoxyheptadecyl)triphenylphosphonium bromide (144)



(*R*)-1-Bromo-16-methoxyheptadecane (**146**) (22.5 g, 0.064 mol) was added to a stirred solution of triphenylphosphine (50.7 g, 0.193 mol) in toluene (120 mL).

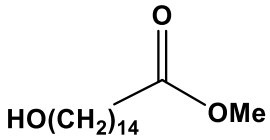
The mixture was refluxed for 120 hrs then the solvent was evaporated and the residue was treated with petroleum ether (150 mL) and stirred for 30 min, then decanted the petroleum layer and the residue was purified by column chromatography, firstly eluting with petroleum ether and then with DCM/MeOH (10:1) to give very thick oil, (*R*)-(16-methoxyheptadecyl) triphenaylphosphonium bromide (**144**) (38.0 g, 97%), $[\alpha]_D^{29} = -1.0$ (*c* 1.9, $CHCl_3$). {Found $[M-Br]^+$: 531.3737; $C_{36}H_{52}OP$ requires: 531.3750}; which showed δ_H (500 MHz, $CDCl_3$): 7.88 − 7.84 (6H, m), 7.80 – 7.77 (3H, m), 7.72 – 7.68 (6H, m), 3.89 – 3.79 (2H, m), 3.31 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.68 – 1.58 (4H, m), 1.57 – 1.46 (1H, m), 1.42 – 1.31 (2H, m), 1.29 – 1.15 (21H, m), 1.11 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 135.0, 134.9, 133.6, 133.5, 130.5, 130.4, 118.7, 117.9, 76.8, 55.8, 36.2, 30.4, 30.3, 29.7, 29.54, 29.5, 29.49, 29.4, 29.14, 29.1, 25.4, 22.9, 22.6, 22.5, 22.46, 18.9; ν_{max}/cm^{-1}: 2922, 2853, 1467.

Experiment (27): (*S*)-(16-Methoxyheptadecyl)triphenylphosphonium bromide (144a)



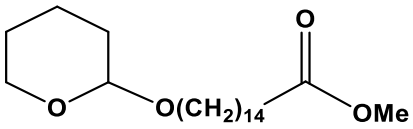
(*S*)-1-Bromo-16-methoxyheptadecane (**146a**) (11.2 g, 0.032 mol) was added to a stirred solution of triphenylphosphine (25.2 g, 0.0961 mol) in toluene (150 mL). The mixture was refluxed for 120 hrs. The solvent was evaporated and the residue was worked up and purified as above to give very thick oil, (*S*)-(16-methoxyheptadecyl)triphenaylphosphonium bromide (**144a**) (18.0 g, 92%), $[\alpha]_D^{29} = +1.0$ (*c* 1.9, $CHCl_3$). {Found $[M-Br]^+$: 531.3736; $C_{36}H_{52}OP$ requires: 531.3750}; which showed δ_H (500 MHz, $CDCl_3$): 7.88 – 84 (6H, m), 7.81 – 7.78 (3H, m), 7.72 – 7.68 (6H, m), 3.89 – 3.79 (2H, m), 3.31 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.68 – 1.55 (4H, m), 1.54 – 1.47 (1H, m), 1.43 – 1.31 (2H, m), 1.30 – 1.14 (21H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 135.0, 134.9, 133.6, 133.5, 130.5, 130.4, 118.7, 117.9, 76.8, 55.8, 36.2, 30.4, 30.3, 29.7, 29.6, 29.5, 29.49, 29.4, 29.14, 29.1, 25.4, 22.9, 22.6, 22.5, 22.4, 18.9; ν_{max}/cm^{-1}: 2922, 2853, 1467.

Experiment (28): Methyl 15-hydroxypentadecanoate (159)



Sodium (14.0 g, 0.62 mol) was added to a stirred solution of methanol (350 mL) at 0 ºC. The mixture was allowed to reach room temperature and stirred until all of the sodium was consumed. *ω*- Pentadecalactone (**160**) (50.0 g, 0.21 mol) was added, and the mixture was heated to 80 ºC and stirred for 2 hrs. The reaction was cooled to room temperature and quenched with aq. HCl (250 mL, 1N) and water (200 mL). The product was extracted with dichloromethane (2 × 250 mL), and the combined organic layers were dried, evaporated to give a white solid as a mixture of the ester and the acid. The white solid was dissolved in methanol (150 mL) and conc. H_2SO_4 (2 mL) was added. The mixture was refluxed for 1 hr. until no acid was left, then cooled to room temperature and methanol was evaporated. The residue was dissolved with dichloromethane (350 mL), washed with sat. aq. $NaHCO_3$ (150 mL), then the organic layer was separated, and the aqueous layer was re-extracted with dichloromethane (2 × 100 mL). The combined organic layers were washed with water (200 mL), dried and evaporated. The crude product was purified by recrystallization using petroleum ether to give a white solid, methyl 15-hydroxypentadecanoate (**159**) (49 g, 86%),[203] m.p. 53 − 55 ºC; which showed δ_H (400 MHz, $CDCl_3$): 3.66 (3H, s), 3.63 (2H, t, *J* 6.5 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.69 – 1.50 (4H, m), 1.41 – 1.15 (21H, m); δ_C (101 MHz, $CDCl_3$): 174.4, 63.1, 51.4, 34.1, 32.8, 29.6, 29.58, 29.57, 29.6, 29.4, 29.2, 29.1, 25.7, 24.9; ν_{max}/cm^{-1}: 3298, 2918, 2851, 1735, 1465. All data matched those in the literature.

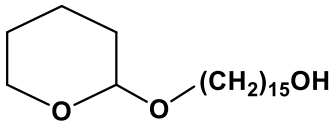
Experiment (29): Methyl 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanoate (158)



3,4-Dihydro-2*H*-pyran (25 g, 0.30 mol) and pyridinium-*p*-toluene-sulfonate (11.3 g, 0.0450 mol) were added to a stirred solution of 15-hydroxypentadecanoate (**159**) (40.9 g, 0.150 mol) in dry dichloromethane (250 mL) under nitrogen at room temperature. The reaction mixture was stirred for 3 hrs when TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a colourless oil,

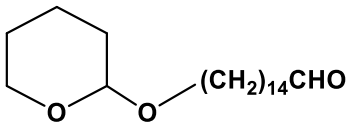
methyl 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanoate (**158**) (45.0 g, 84%),[204] which showed δ_H (400 MHz, $CDCl_3$): 4.58 (1H, br. s), 3.91 – 3.83 (1H, m), 3.73 (1H, dt, *J* 6.9, 9.5 Hz), 3.66 (3H, s), 3.53 – 3.45 (1H, m), 3.37 (1H, dt, *J* 6.7, 9.5 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.84 – 1.78 (1H, m), 1.76 – 1.69 (1H, m), 1.61 – 1.46 (9H, m), 1.40 – 1.20 (19H, m); δ_C (101 MHz, $CDCl_3$): 174.3, 98.8, 67.7, 62.3, 51.4, 34.1, 30.8, 29.7, 29.6, 29.56, 29.5, 29.4, 29.2, 29.1, 26.2, 25.5, 24.9, 19.7; ν_{max}/cm^{-1}: 2929, 2855, 1731, 1438. All data matched those in the literature.

Experiment (30): 15-((Tetrahydro-2*H*-pyran-2-yl)oxy)pentadecan-1-ol (157)



A solution of the methyl 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanoate (**158**) (40.0 g, 0.112 mol) in dry THF (50 mL) was added slowly to a stirred suspension solution of $LiAlH_4$ (6.40 g, 0.168 mol) in dry THF (250 mL) at – 10 °C under nitrogen atmosphere, and the mixture was allowed to reach room temperature and then refluxed for 1 hr. The reaction mixture was worked up and purified as before to give a white solid, 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecan-1-ol (**157**) (34.0 g, 92%),[204] m.p. 35 − 37 °C; which showed δ_H (400 MHz, $CDCl_3$): 4.58 (1H, br. s), 3.91 – 3.86 (1H, m), 3.73 (1H, dt, *J* 6.9, 9.4 Hz), 3.63 (2H, t, *J* 6.6 Hz), 3.54 – 3.48 (1H, m), 3.38 (1H, dt, *J* 6.6, 9.5 Hz), 1.87 – 1.79 (1H, m), 1.76 – 1.66 (1H, m), 1.61– 1.46 (9H, m), 1.40 – 1.23 (22H, m); δ_C (101 MHz, $CDCl_3$): 98.8, 67.7, 63.1, 62.3, 32.8, 30.8, 29.7, 29.64, 29.6, 29.58, 29.56, 29.5, 29.47, 29.4, 26.2, 25.7, 25.5, 19.7; ν_{max}/cm^{-1}: 3392, 2923, 2852, 1468. All data matched those in the literature.

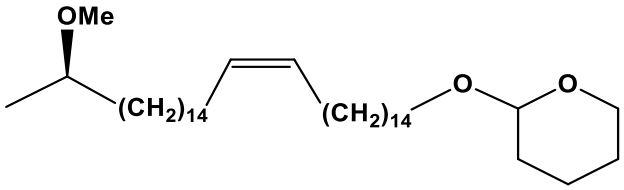
Experiment (31): 15-((Tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal (145)



A solution of 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecan-1-ol (**157**) (12.5 g, 0.0381 mol) in dichloromethane (40 mL) was added to a stirred solution of pyridinium chlorochromate (18.1 g, 0.0838 mol) in dichloromethane (350 mL), at room temperature, a black colour appearing during the addition. The mixture was stirred for 2 hrs, when TLC showed there was no starting material left. The reaction mixture was

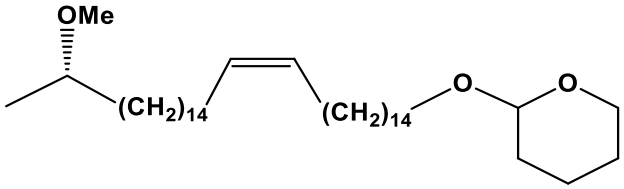
worked up and purified as before to give a colourless oil, 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal **(145)** (11.7 g, 94%),[204] which showed δ_H (500 MHz, $CDCl_3$): 9.76 (1H, t, *J* 1.6 Hz), 4.58 (1H, br. s), 3.93 – 3.85 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.5 Hz), 3.57 – 3.45 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.5 Hz), 2.42 (2H, td, *J* 1.6, 7.3 Hz), 1.90 – 1.79 (1H, m), 1.77 – 1.69 (1H, m), 1.64 – 1.50 (10H, m), 1.40 – 1.19 (18H, m); δ_C (101 MHz, $CDCl_3$): 202.9, 98.8, 67.7, 62.3, 43.9, 30.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 26.2, 25.5, 22.1, 19.7; ν_{max}/ cm^{-1}: 2926, 2854, 1727, 1462.

Experiment (32): 2-(((*R*,*Z*)-31-Methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran (143)



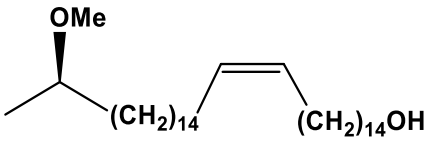
Sodium *bis*(trimethylsilyl)amide (67.6 mL, 0.0676 mol, 1.0 M in THF) was added to a stirred solution of (*R*)-(16-methoxyheptadecyl)triphenylphosphonium bromide (**144**) (24.3 g, 0.0398 mol) and 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal (**145**) (11.8 g, 0.0361 mol) in dry THF (150 mL) at – 35 ^oC under nitrogen atmosphere. The mixture was stirred for 30 min then allowed to reach room temperature for 30 min. After that, the reaction was cooled down and quenched with sat. aq. ammonium chloride (20 mL) and the product was extracted with petrol/ethyl acetate (10:1, 3 × 100 mL). The combined organic layers were washed sat. aq. sodium chloride (50 mL), dried and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give a colourless oil, 2-(((*R*,*Z*)-31-methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran (**143**) (14.6 g, 70%), $[\alpha]_D^{29}$ = – 3.8 (*c* 1.1, $CHCl_3$). {Found $[M+NH_4]^+$: 596.5970; $C_{38}H_{78}O_3N$ requires: 596.5976}; which showed δ_H (500 MHz, $CDCl_3$): 5.35 (2H, pent, *J* 10.4 Hz), 4.61 – 4.57 (1H, m), 3.94 – 3.85 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.5 Hz), 3.54 – 3.46 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.5 Hz) 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.05 – 1.98 (4H, m), 1.89 – 1.80 (1H, m), 1.75 – 1.69 (1H, m), 1.63 – 1.49 (8H, m), 1.39 – 1.23 (46H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 129.9, 98.8, 76.9, 67.7, 62.3, 55.9, 36.3, 30.8, 29.8, 29.7, 29.66, 29.6, 29.56, 29.5, 29.3, 27.2, 26.2, 25.5, 25.4, 19.7, 19.0; ν_{max}/cm^{-1}: 2918, 2851, 1471, 1199.

Experiment (33): 2-(((*S*,*Z*)-31-Methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran (143a)



Sodium *bis*(trimethylsilyl)amide (49.2 mL, 0.0492 mol, 1.0 M in THF) was added to a stirred solution of (*S*)-(16-methoxyheptadecyl)triphenylphosphonium bromide (**144a**) (17.5 g, 0.0289 mol) and 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal (**145**) (9.0 g, 0.028) in dry THF (150 mL) at – 35 ºC under nitrogen atmosphere. The mixture was stirred for 30 min then allowed to reach room temperature for 30 min. The reaction mixture was worked up and purified as above to give a colourless oil, 2-(((*S*,*Z*)-31-methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran (**143a**) (9.0 g, 56%), $[\alpha]_D^{29}$ = + 3.7 (*c* 1.0, $CHCl_3$). {Found $[M+NH_4]^+$: 596.5970; $C_{38}H_{78}O_3N$ requires: 596.5976}; which showed δ_H (500 MHz, $CDCl_3$): 5.35 (2H, pent, *J* 10.4 Hz), 4.61 – 4.56 (1H, m), 3.92 – 3.85 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.5 Hz), 3.54 – 3.46 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.5 Hz) 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.05 – 1.98 (4H, m), 1.87 – 1.80 (1H, m), 1.75 – 1.69 (1H, m), 1.65 – 1.51 (8H, m), 1.36 – 1.23 (46H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 129.9, 98.8, 76.9, 67.7, 62.3, 55.9, 30.8, 29.8, 29.7, 29.68, 29.66, 29.6, 29.56, 29.5, 29.3, 27.2, 26.2, 25.5, 25.4, 19.7, 19.0; ν_{max}/cm^{-1}: 2919, 2851, 1471, 1198.

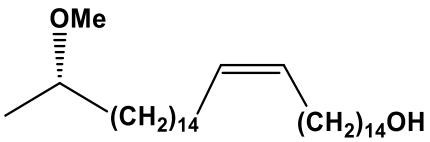
Experiment (34): (*R*,*Z*)-31-Methoxydotriacont-15-en-1-ol (142)



Pyridinum-*p*-toluenesulfonate (2.50 g, 9.94 mmol) was added to a stirred solution of 2-(((*R*,*Z*)-31-methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran (**143**) (11.5 g, 19.8 mmol) in THF (100 mL), methanol (100 mL) and stirred at 50 ºC for 5 hrs. When TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a white solid, (*R,Z)*-31-methoxydotriacont-15-en-1-ol (**142**) (9.2 g,

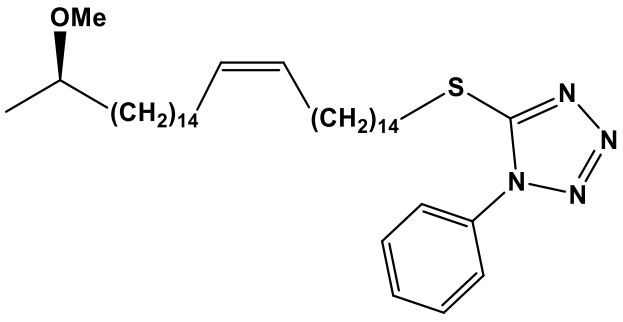
94%), m.p. 47 − 49 °C; $[\alpha]_D^{29}$= – 3.4 (*c* 1.0, $CHCl_3$). {Found $[M+H]^+$: 495.5133; $C_{33}H_{67}O_2$ requires: 495.5136}; which showed δ_H (500 MHz, $CHCl_3$): 5.35 (2H, pent, *J* 10.4 Hz), 3.64 (2H, t, *J* 6.6 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 6.5 Hz), 1.57 (4H, m), 1.40 – 1.22 (47H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 129.9, 76.9, 63.1, 55.9, 36.3, 32.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.2, 25.7, 25.5, 19.0; ν_{max}/cm^{-1}: 3437, 2914, 2847, 1470, 1199.

Experiment (35): (*S,Z*)-31-Methoxydotriacont-15-en-1-ol (142a)



Pyridinum-*p*-toluenesulfonate (1.63 g, 6.48 mmol) was added to a stirred solution of 2-(((*S,Z*)-31-methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran (**143a**) (7.50 g, 12.3 mmol) in THF (70 mL), methanol (70 mL) and stirred at 50 ºC for 5 hrs. When TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a white solid, (*S,Z)*-31-methoxydotriacont-15-en-1-ol (**142a**) (5.6 g, 88%), m.p. 47 − 49 °C; $[\alpha]_D^{29}$= + 3.4 (*c* 1.0, $CHCl_3$). {Found $[M+H]^+$: 495.5132; $C_{33}H_{67}O_2$ requires: 495.5136}; which showed δ_H (500 MHz, $CHCl_3$): 5.35 (2H, pent, *J* 10.4 Hz), 3.64 (2H, t, *J* 6.5 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 6.5 Hz), 1.57 (4H, m), 1.39 – 1.21 (47H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 129.9, 76.9, 63.1, 55.9, 36.3, 32.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.2, 25.7, 25.5, 19.0; ν_{max}/cm^{-1}: 3354, 2913, 2847, 1470, 1199.

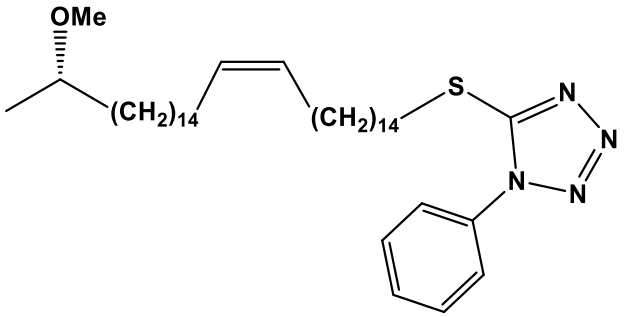
Experiment (36): (*R, Z*)-5-((31-Methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (141)



Diethyl azodicarboxylate (4.1 g, 0.0234 mol) in dry THF (10 mL) was added to a stirred solution of (*R,Z)*-31-methoxydotriacont-15-en-1-ol (**142**) (8.9 g, 0.018 mol),

triphenylphosphine (6.61 g, 0.0252 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (4.17 g, 0.0234 mol) in dry THF (120 mL) at 0 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and then stirred for 1.5 hrs. When TLC showed no starting material was left, the solvent was evaporated, to give a residue which was purified by column chromatography, eluting with petrol/ethyl acetate (10:1) to give a semi solid, (*R*,*Z*)-5-((31-methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (**141**) (11.0 g, 94%), m.p. 41 − 43 ºC; $[\alpha]_D^{29}$ = – 4.8 (*c* 1.2, $CHCl_3$). {Found $[M+H]^+$: 655.5337; $C_{40}H_{71}N_4SO$ requires: 655.5343}; which showed δ_H (500 MHz, $CDCl_3$): 7.62 – 7.53 (5H, m), 5.35 (2H, pent, *J* 10.9 Hz), 3.40 (2H, t, *J* 7.4 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 5.7 Hz), 1.82 (2H, pent, *J* 7.4 Hz), 1.60 – 1.50 (1H, m), 1.44 (1H, pent, *J* 6.7 Hz), 1.38 – 1.21 (46H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 130.0, 129.9, 129.8, 123.9, 76.9, 55.9, 36.3, 33.4, 29.8, 29.7, 29.6, 29.5, 29.3, 29.1, 29.0, 28.7, 27.2, 25.5, 19.0; ν_{max}/cm^{-1}: 2911, 2848, 1742, 1471, 1116.

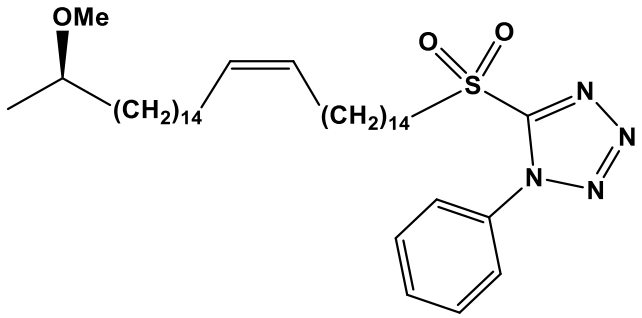
Experiment (37): (*S, Z*)-5-((31-Methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (141a)



Diethyl azodicarboxylate (2.3 g, 0.0132 mol) in dry THF (10 mL) was added to a stirred solution of (*S*,Z)-31-methoxydotriacont-15-en-1-ol (**142a**) (5.0 g, 0.010 mol), triphenylphosphine (3.7 g, 0.014 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (2.34 g, 0.0132 mol) in dry THF (70 mL) at 0 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and then stirred for 1.5 hrs. When TLC showed no starting material was left. The reaction mixture was worked up and purified as above to give semi solid, (*S*,*Z*)-5-((31-methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (**141a**) (5.5 g, 83%), m.p. 40 − 42 ºC; $[\alpha]_D^{29}$ = + 4.8 (*c* 1.2, $CHCl_3$). {Found $[M+H]^+$: 655.5336; $C_{40}H_{71}N_4OS$ requires: 655.5343}; which showed δ_H (500 MHz, $CDCl_3$): 7.58 – 7.53 (5H, m), 5.35 (2H, pent, *J* 10.9 Hz), 3.40 (2H, t, *J* 7.4 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 5.7 Hz), 1.82 (2H, pent, *J* 7.4 Hz), 1.59 – 1.50 (1H,

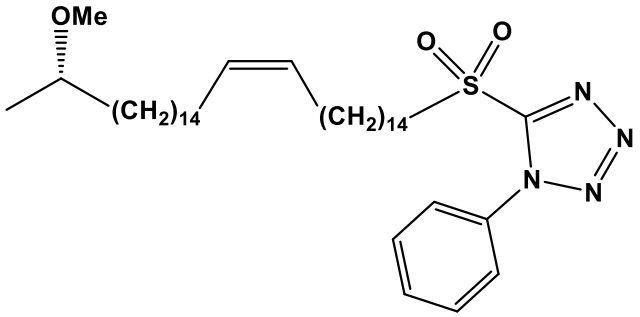
m), 1.44 (1H, pent, *J* 7.5 Hz), 1.38 – 1.21 (46H, m), 1.12 (3H, d, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$): 130.0, 129.9, 129.7, 123.8, 76.9, 55.9, 36.3, 33.4, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 29.07, 29.0, 28.7, 27.2, 25.5, 19.0 ; ν_{max}/cm^{-1}: 2911, 2848, 1742, 1471, 1116.

Experiment (38): (*R, Z*)-5-((31-Methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1*H*-tetrazole (139)



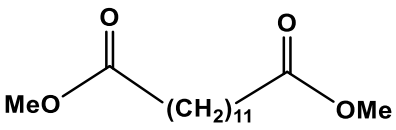
Ammonium molybdate (VI) tetrahydrate (3.2 g, 0.0030 mol) was dissolved in cold hydrogen peroxide (35%, 10 mL) and the solution was gradually added to a stirred solution of (*R,Z*)-5-((31-methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (**141**) (5.6 g, 0.0085 mol) in IMS (30 mL) and THF (60 mL) at 0 °C. The mixture was allowed to reach room temperature and stirred for 2 hrs, then another portion of ammonium molybdate (VI) tetrahydrate (1.6 g) in cold hydrogen peroxide (35%, 5 mL) was added. The mixture was stirred for 6 hrs. After this time, the mixture was poured into water (100 mL) and the product was extracted with dicloromethane (3 × 50 mL). The combined organic layers were dried and evaporated to give a residue. The ^{1}H NMR showed a mixture of starting material, product and sulfoxide therefore, the experiment was repeated 4 times, then TLC showed no starting material was left. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a white solid, (*R,Z*)-5-((31-methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1*H*-tetrazole (**139**) (5.0 g, 86%), m.p. 45 − 47 °C; $[\alpha]_D^{29}$= – 5.6 (*c* 1.0, $CHCl_3$). {Found $[M+H]^+$: 687.5243; $C_{40}H_{71}N_4O_3S$ requires: 687.5241}; which showed δ_H (500 MHz, $CDCl_3$): 7.75 – 7.69 (2H, m), 7.68 – 7.59 (3H, m), 5.35 (2H, pent, *J* 10.9 Hz), 3.74 (2H, t, *J* 7.9 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 5.4 Hz), 1.97 – 1.92 (2H, m), 1.50 (3H, pent, *J* 6.6 Hz), 1.38 – 1.21 (45H, m), 1.12 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 131.5, 129.9, 129.7, 125.1, 76.9, 56.0, 55.9, 36.3, 29.8, 29.7, 29.6, 29.5, 29.3, 29.2, 28.9, 28.2, 27.2, 25.5, 21.9, 19.0; ν_{max}/cm^{-1}: 2915, 2849, 1593, 1471.

Experiment (39): (*S, Z*)-5-((31-Methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1*H*-tetrazole (139a)



Ammonium molybdate (VI) tetrahydrate (3.2 g, 0.003 mol) was dissolved in cold hydrogen peroxide (35%, 10 mL) and the solution was gradually added to a stirred solution of (*S,Z*)-5-((31-methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (**141a**) (5.7 g, 0.0085 mol) in IMS (30 mL) and THF (60 mL) at 0 °C. The mixture was allowed to reach room temperature and stirred for 2 hrs, then another portion of ammonium molybdate (VI) tetrahydrate (1.6 g) in cold hydrogen peroxide was added (35%, 5 mL). The mixture was stirred for 6 hrs. After this time the reaction mixture was worked up and purified as above to give a white solid, (*S,Z*)-5-((31-methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1*H*-tetrazole (**139a**) (5.0 g, 86%), m.p. 44 − 46 °C; $[\alpha]_D^{29}$= + 5.4 (*c* 1.1, $CHCl_3$). {Found $[M+H]^+$: 687.5247; $C_{40}H_{71}N_4O_3S$ requires: 687.5241}; which showed δ_H (500 MHz, $CDCl_3$): 7.71 – 7.69 (2H, m), 7.64 – 7.59 (3H, m), 5.35 (2H, pent, *J* 10.9 Hz), 3.74 (2H, t, *J* 7.9 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 6.5 Hz), 1.97 – 1.91 (2H, m), 1.50 (3H, pent, *J* 6.6 Hz), 1.41 – 1.21 (45H, m), 1.12 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 131.5, 129.9, 129.7, 125.1, 76.9, 56.0, 55.9, 36.3, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.1, 27.2, 25.5, 21.9, 19.0; ν_{max}/cm^{-1}: 2913, 2849, 1593, 1471.

Experiment (40): Dimethyl tridecanedioate (**184)**



Conc. H_2SO_4 (5 mL) was added to a stirred solution of tridecanedioic acid (**170**) (100 g, 0.37 mol) in methanol (300 mL) and the reaction mixture was refluxed for 3 hrs when TLC showed there was no staring material was left. The excess methanol was evaporated and then sat. aq. $NaHCO_3$ (100 mL) was added to neutralize the acid. The product was

extracted with dichloromethane (3 × 200 mL) then the combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil, dimethyl tridecanedioate (**184**) (100 g, 99%),[205] which showed δ_H (500 MHz, $CDCl_3$): 3.67 (6H, s), 2.31 (4H, t, *J* 7.5 Hz), 1.62 (4H, pent, *J* 7.3 Hz), 1.35 – 1.25 (14H, m); δ_C (101 MHz, $CDCl_3$): 174.3, 51.3, 34.0, 29.4, 29.3, 29.1, 29.0, 24.9; ν_{max}/cm^{-1}: 2918, 2851, 1735, 1435.

Experiment (41): tridecane-1, 13-diol (185)

$$HO(CH_2)_{13}OH$$

A solution of the dimethyl ester (**184**) (100 g, 0.46 mol) in dry THF (40 mL) was added to a stirred suspension solution of $LiAlH_4$ (20.8 g, 0.55 mol) in dry THF (300 mL) at – 10 ^oC and the mixture was refluxed for 3 hrs. When TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a white solid, tridecane-1,13-diol (**185**) (77g, 97%),[206] m.p. 74 − 75 ℃; which showed δ_H (500 MHz, $CDCl_3$): 3.65 (4H, q, *J* 6.5 Hz), 1.57 (4H, pent, *J* 6.6 Hz), 1.40 – 1.21 (20H, m); δ_C (101 MHz, $CDCl_3$): 63.1, 32.8, 29.6, 29.5, 29.4, 25.7; ν_{max}/cm^{-1}: 3242, 2932, 2843, 1407.

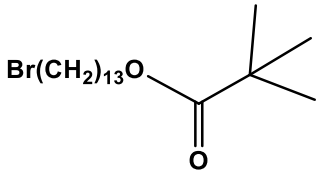
Experiment (42): 13-Bromotridecan-1-ol (186)

$$Br(CH_2)_{13}OH$$

Tridecane-1,13-diol (**185**) (76.76 g, 0.35 mol) was dissolved in toluene (250 mL) and aqueous HBr (50 mL, 48%) was added then the mixture was refluxed for 18 hrs. The reaction mixture was cooled to room temperature and the organic layer was separated. The aqueous layer was extracted with toluene (2 × 100 mL) and the combined organic layers were evaporated. The brown oil residue was dissolved in dichloromethane (300 mL) and washed with sat. aq. $NaHCO_3$ (200 mL). The aqueous layer was re-extracted with dichloromethane (2 × 100 mL). The combined organic layers were dried, evaporated. The crude product was purified by column chromatography, eluting petrol/ethyl acetate (10:1, then 5:1) to give give a white solid, 13-bromotridecan-1-ol (**186**) (44 g, 44%),[206] m.p. 56 − 58 ℃; which showed δ_H (500 MHz, $CDCl_3$): 3.65 (2H, q, *J* 6.6 Hz), 3.41 (2H, t, *J* 6.9 Hz), 1.86 (2H, pent, *J* 6.9Hz), 1.57 (2H, pent, *J* 6.6 Hz),

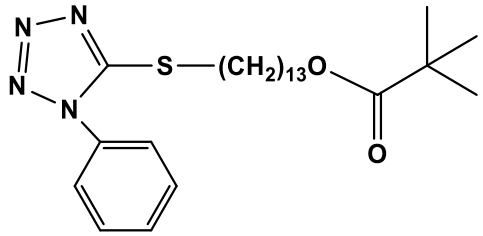
1.42 (2H, pent, *J* 7.0 Hz), 1.38 – 1.21 (17H, m); δ_C (101 MHz, $CDCl_3$): 63.0, 34.0, 32.8, 32.76, 29.6, 29.55, 29.5, 29.48, 29.4, 28.7, 28.1, 25.7; ν_{max}/cm^{-1}: 3428, 2916, 2849, 1479. All data matched those in the literature.

Experiment (43): 13-Bromotridecyl pivalate (169)



A solution of trimethylacetyl chloride (23 mL ,22.8 g, 0.18 mol, 1.2 mol eq.) in dichloromethane (50 mL) was added to a stirred solution of 13-bromotridecan-1-ol (**186**) (44 g, 0.15 mol), triethylammine (23.9 g, 0.23 mol, 1.5mol eq.) and 4-dimethylaminopyridine (1g) in dichloromethane (300 mL), over a period of 15 min. at 5 ºC. The mixture was allowed to reach room temperature and stirred for 18 hrs. The reaction mixture was worked up and purified as before to give a colourless oil, 13-bromotridecyl pivalate (**169**) (57 g, 99%),[207] which showed δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.6 Hz), 3.41 (2H, t, *J* 6.9 Hz), 1.86 (2H, pent, *J* 6.9 Hz), 1.62 (2H, pent, *J* 6.6 Hz), 1.42 (2H, pent, *J* 7.2 Hz), 1.37 – 1.23 (16H, m), 1.20 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 64.4, 34.0, 32.8, 29.54, 29.5, 29.48, 29.4, 29.2, 28.7, 28.6, 28.2, 27.2, 26.5,25.9; ν_{max}/cm^{-1}: 2922, 2852, 1727, 1497. All data matched those in the literature.

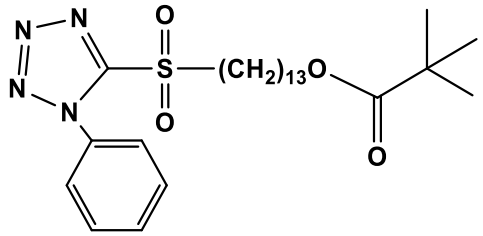
Experiment (44): 13-((1-Phenyl-1*H*-tetrazol-5-yl)thio)tridecyl pivalate (168)



13-Bromotridecyl pivalate (**169**) (57 g, 0.15 mol) was added to a stirred solution of anhydrous potassium carbonate (43.4 g, 0.31 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (29.4 g, 0.16 mol) in acetone (100 mL) and THF (50 mL) at room temperature then the mixture was refluxed for 3 hrs. The reaction mixture was worked up and purified as before to give a colourless oil, ((1-phenyl-1*H*-tetrazol-5-yl)thio)tridecyl pivalate (**168**) (72 g, 99%),[207] which showed δ_H (500 MHz, $CDCl_3$): 7.65 – 7.50 (5H, m), 4.04 (2H, t,

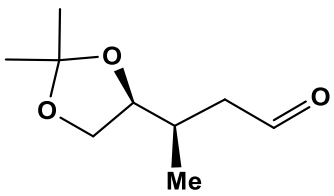
J 6.6 Hz), 3.40 (2H, t, *J* 7.4 Hz), 1.82 (2H, pent, *J* 7.4 Hz), 1.61 (2H, pent, *J* 6.6 Hz), 1.44 (2H, pent, *J* 6.7 Hz), 1.39 – 1.23 (16H, m), 1.19 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 154.5, 133.7, 130.0, 129.7, 123.8, 64.4, 33.3, 29.5, 29.48, 29.4, 29.3, 29.2, 29.0, 28.9, 28.6, 28.5, 27.2, 25.9; ν_{max}/cm^{-1}: 2924, 2853, 1723, 1594, 1499. All data matched those in the literature.

Experiment (45): 13-((1-Phenyl-1*H*-tetrazol-5-yl)sulfonyl)tridecyl pivalate (165)



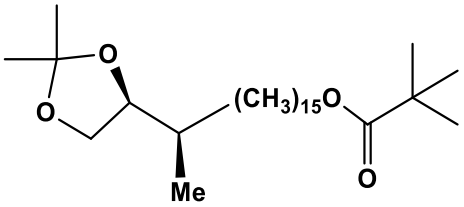
Ammonium molybdate (VI) tetrahydrate (58 g, 0.04 mol) was dissolved in cold hydrogen peroxide (35%, 137mL) and was gradually added to a stirred solution of 13-((1-phenyl-1*H*-tetrazol-5-yl)thio)tridecyl pivalate (**168**) in IMS (200 mL) and THF (100 mL) at 0 ºC. The mixture was allowed to reach room temperature and stirred for 2 hrs, and then another portion of ammonium molybdate (VI) tetrahydrate (29 g) in cold aqueous hydrogen peroxide (35%, 68.5mL) was added and stirred for 18 hrs. The mixture was worked up and purified as before to give a white solid, 13-((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)tridecyl pivalate (**165**) (74.5 g, 97%),[207] m.p. 47 − 49 °C; which showed δ_H (500 MHz, $CDCl_3$): 7.71 – 7.69 (2H, m), 7.64 – 7.59 (3H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.73 (2H, distorted t, *J* 7.8 Hz), 1.95 (2H, pent, *J* 7.6 Hz), 1.62 (2H, pent, *J* 6.6 Hz), 1.50 (2H, pent, *J* 8.5 Hz), 1.38 – 1.25 (16H, m), 1.19 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 153.5, 133.7, 131.4, 129.7, 125.0, 64.4, 56.0, 38.7, 29.5, 29.4, 29.2, 29.1, 28.9, 28.6, 28.1, 27.2, 25.9, 21.9 ; ν_{max}/cm^{-1}: 2916, 2851, 1723, 1459. All data matched those in the literature.

Experiment (46): (*R*)-3-((*S*)-2, 2-Dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (166)



A solution of the primary alcohol (**228**) (5.5 g, 0.03 mol) in dichloromethane (30 mL) was added to a stirred solution of pyridinium chlorochromate (13.6 g, 0.063 mol) in dichloromethane (250 mL). at room temperature, a black colour appeared during the addition. The reaction was stirred for 2 hrs, when TLC showed no starting material was left. The mixture was worked up and purified as before to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (**166**) (4.7 g, 87%), $[\alpha]_D^{23}$ = + 8.24 (*c* 1.06, $CHCl_3$);[207] which showed δ_H (400 MHz, $CDCl_3$): 9.77 (1H, br. t, *J* 1.6 Hz), 4.07 (1H, pent, *J* 6.7 Hz), 3.98 (1H, dd, *J* 6.7, 8.2 Hz), 3.64 (1H, dd, *J* 7.3, 8.2 Hz), 2.60 – 2.45 (1H, m), 2.44 – 2.33 (1H, m), 2.32 – 2.21 (1H, m), 1.40 (3H, s), 1.34 (3H, s), 0.99 (3H, d, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 201.6, 78.6, 66.3, 46.6, 30.4, 26.2, 25.1, 22.6, 15.5; ν_{max} /cm^{-1}: 2936, 2880, 1723, 1457. All data matched those in the literature.

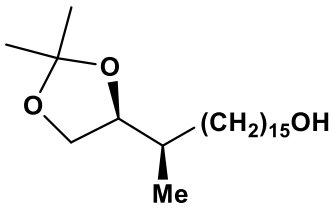
Experiment (47): (*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecyl pivalate (188)



Lithium *bis* (trimethylsilyl) amide (34.5 mL, 0.04 mol, 1.0 M) was added to a stirred solution of 13-((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)tridecyl pivalate (**165**) (12 g, 0.024 mol) and (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (**166**) (3.5 g, 0.02 mol) in dry THF (30 mL) at – 10 ºC under nitrogen atmosphere. The solution was allowed to reach room temperature and stirred for 3 hrs, then the mixture was worked up and purified as before to give a colourless oil (*E*/*Z*)- (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadec-14-en-1-yl pivalate (**187**) (4 g, 45%). Palladium 10% on carbon (0.5 g) was added to a stirred solution of the alkenes (**187**) (4 g, 0.020 mol) in IMS (50 mL) and THF (50 mL) under hydrogen atmosphere. Hydrogenation was carried out for 16 hrs. The mixture was worked up and purified as before to give a colourless oil, (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecyl pivalate (**188**) (4 g, 98%),[207] $[\alpha]_D^{23}$ = + 17.4 (*c* 1.07 $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.6 Hz), 4.00 (1H, br. q, *J* 6.2 Hz), 3.87 (1H, br. q, *J* 7.1 Hz), 3.60 (1H, br. t, *J* 7.7 Hz), 2.11 – 1.95 (1H, m), 1.90 – 1.79 (1H, m), 1.62 (2H, pent, *J* 6.8 Hz), 1.41 (3H, s), 1.36 (3H, s), 1.35 – 1.22 (24H, br. m), 1.20 (9H, s), 1.14 – 1.07 (1H, m), 0.95 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 178.7, 108.5, 80.4, 67.8, 64.5, 38.7, 36.5, 32.7, 29.9, 29.8,

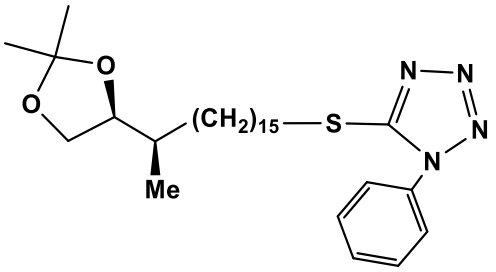
29.7, 29.6, 29.5, 29.4, 29.2, 28.6, 27.2, 26.6, 25.9, 25.5, 15.6; ν_{max}/cm^{-1}: 2923, 2853, 1729, 1460. All data matched those in the literature.

Experiment (48): (*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl) heptadecan-1-ol (164)



A solution of (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecyl pivalate (**188**) (4 g, 0.009 mol) in dry THF (10 mL) was added to a stirred suspension solution of $LiAlH_4$ (0.5 g, 0.01 mol) in dry THF (100 mL) at – 10 ºC and the mixture was refluxed for 1 hr. The mixture was worked up and purified as before to give a white solid, (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl) heptadecan-1-ol (**164**) (3.19 g, 99%),[207] m.p. 48 − 50 °C; $[\alpha]_D^{23}$ = +16.1 (*c* 1.01 $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 4.00 (1H, dd, *J* 6.2, 7.8 Hz), 3.88 (1H, br. q, *J* 6.9 Hz), 3.64 (2H, br. t, *J* 6.6 Hz), 3.60 (1H, t, *J* 7.6 Hz), 1.57 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.35 (27H, br. s), 1.12 – 1.06 (1H, m), 0.97 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 108.5, 80.4, 67.8, 63.1, 36.5, 32.8, 32.7, 29.9, 29.7, 29.64, 29.6, 29.5, 26.9, 26.0, 25.8, 25.6, 15.7; ν_{max}/cm^{-1}: 3444, 2916, 2848, 1468, 1370. All data matched those in the literature.

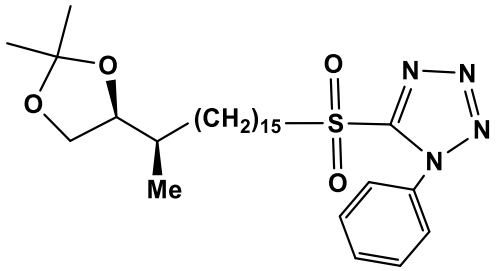
Experiment (49): 5-(((*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecyl)thio)-1-phenyl-1*H*-tetrazole (163)



Diethyl azodicarboxylate (DEAD) (2.03 g, 1.8 mL, 0.012 mol, 1.3 e.q.) in dry THF (5 mL) was added to a stirred solution of (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecan-1-ol (**164**) (3.2 g, 0.01 mol). Triphenylphosphine (3.3 g, 0.01 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (2.1 g, 0.01 mol) in dry THF (10 mL) at 0 ºC under nitrogen

atmosphere. The mixture was allowed to reach room temperature and then stirred for 2 hrs. The solvent was evaporated and the residue was stirred with petrol/ethyl acetate (10:1, 150 mL) for 30 min and then filtered through a pad of celite. The filtrate was evaporated, and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil, 5-(((*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecyl)thio)-1-phenyl-1*H*-tetrazole (**163**) (2.9 g, 64%),[207] $[\alpha]_D^{27}$ = + 11.6 (*c* 1.43 $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 7.61 – 7.52 (5H, m), 4.00 (1H, dd, *J* 6.2, 7.8 Hz), 3.87 (1H, br. q, *J* 6.9 Hz), 3.60 (1H, br. t, *J* 7.7 Hz), 3.40 (2H, t, *J* 7.4 Hz), 1.82 (2H, pent, *J* 7.4 Hz), 1.62 – 1.54 (1H, m), 1.49 – 1.42 (1H,m), 1.41 (3H, s), 1.35 (3H, s), 1.33 – 1.22 (24H, br. s), 1.12 – 1.04 (1H, m), 0.96 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 153.5, 131.4, 129.7, 125.0, 108.5, 80.4, 67.8, 60.4, 36.5, 33.4, 32.7, 30.9, 29.8, 29.6, 29.58, 29.5, 29.4, 29.1, 29.0, 28.6, 26.9, 26.6, 25.5, 21.0, 15.6, 14.2; ν_{max}/cm^{-1}: 2918, 2848,1597, 1467. All data matched those in the literature.

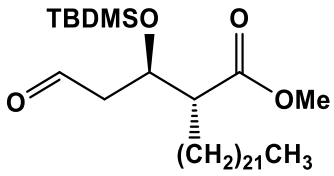
Experiment (50): 5-(((*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecyl)sulfonyl)-1-phenyl-1*H*-tetrazole (162)



Ammonium molybdate VI tetrahydrate (2.0 g, 0.002 mol) was dissolved in cold aqueous hydrogen peroxide in (35%, w/w, 5 mL) and gradually added to a stirred solution of 5-(((*R*)-17-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecyl)thio)-1-phenyl-1*H*-tetrazole (**163**) (2.9 g, 0.005 mol) in IMS (30 mL) and THF (30 mL). The mixture was stirred for 2 hrs, and then another portion of ammonium molybdate VI tetrahydrate (1.0 g) in cold aqueous hydrogen peroxide in (35%, w/w, 2.5 mL) was added and stirred at room temperature for 18 hrs. The reaction mixture was worked up and purified as before to give a white solid, 5-(((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecyl)sulfonyl)-1-phenyl-1*H*-tetrazole (**162**) (3 g, 97%),[207] m.p. 49 − 51 °C; $[\alpha]_D^{25}$ = + 12.4 (*c* 1.51 $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 7.71 – 7.69 (2H, m), 7.64 – 7.59 (3H, m), 4.00 (1H, dd, *J* 6.2, 7.8 Hz), 3.87 (1H, br. q, *J* 6.9 Hz), 3.73 (2H, distorted t, *J* 7.9 Hz), 3.60 (1H, br. t, *J* 7.7 Hz), 1.95 (2H, pent, *J* 7.7 Hz), 1.60 – 1.54 (1H, m), 1.52 – 1.46 (3H, m), 1.40 (3H,

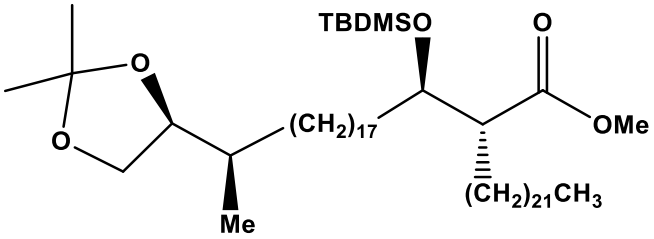
s), 1.35 (3H, s), 1.34 – 1.18 (24H, br. s), 1.11 – 1.04 (1H, m), 0.97 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 153.5, 131.4, 129.7, 125.0, 108.4, 80.4, 67.8, 55.9, 36.5, 32.7, 29.8, 29.6, 29.58, 29.5, 29.4, 29.2, 28.9, 28.1, 26.9, 26.6, 25.5, 21.9, 15.6; ν_{max}/cm^{-1}: 2915, 2849, 1593, 1497, 1472. All data matched those in the literature.

Experiment (51): Methyl-(*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoate (65)



Methyl-(*R*)-2-[(*R*)-1-(tert-butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-tetracosan oate (**77**) (7 g, 0.01 mol) in dichloromethane (25 mL) was added at room temperature to a stirred solution of pyridinium chlorochromate (5.4 g, 0.02 mol) in dichloromethane (350 ml). The mixture was stirred at room temperature for 2.5 hrs, when TLC showed no starting material was left, then the reaction mixture was worked up and purified as before to give a colourless oil, methyl-(*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoate (**65**) (6.2 g, 87%),[156] $[\alpha]_D^{27}$ = – 5.2 (*c* 1.3, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 9.81 (1H, dd, *J* 2.5, 4.3 Hz), 4.43 (1H, dt, *J* 4.7, 6.0 Hz), 3.68 (3H, s), 2.62 (1H, ddd, *J* 4.1, 6.3, 10.4 Hz), 2.61 – 2.57 (2H, m), 1.30 – 1.20 (42H, br. m), 0.88 (3H, t, *J* 7.0 Hz), 0.86 (9H, s), 0.08 (3H, s), 0.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 201.3, 174.0, 68.8, 52.3, 51.5, 48.1, 31.9, 29.7, 29.66, 29.62, 29.54, 29.5, 29.4, 29.3, 27.8, 27.0, 25.6, 22.7, 17.9, 14.1, – 4.4, – 4.9; ν_{max}/cm^{-1}: 2922, 2852, 1734, 1463. All data matched those in the literature.

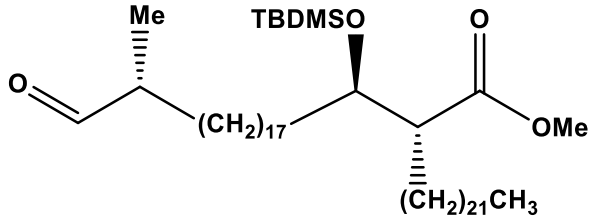
Experiment (52): Methyl (*R*) 2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)eicosyl)tetracosanoate (161)



Lithium *bis*(trimethylsilyl)amide (18.2 mL, 0.02 mol, 1.06 M) was added dropwise to a stirred solution of methyl 2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl)tetra-

cosanoate (**65**)[156] (6.2 g, 0.01 mol) and 5-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecylsulfonyl)-1-phenyl-1H-tetrazole (**162**)[207] (5.9 g, 0.01 mol) in dry THF (60 mL) at – 15 °C. The mixture was stirred for 3 hrs at room temperature, when TLC analysis indicated completion of the reaction. The reaction mixture was worked up and purified as before to give a colourless oil, methyl (*R*) 2-((1*R*,2*R*,19*R*)-(*E*/*Z*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)eicos-3-enyl)tetracosanoate (**198**) (9.5 g, 97%). Palladium 10 % on carbon (1g) was added to a stirred solution of the above alkenes (**198**) (9.5 g, 0.01 mol) in THF (100 mL) and IMS (100 mL) under hydrogen atmosphere. The mixture was stirred while being hydrogenated at atmospheric pressure, when hydrogen absorption was complete, then the reaction mixture was worked up and purified as before to give a colourless oil, methyl (*R*) 2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate (**161**) (9 g, 94%), $[\alpha]_D^{28}$ = + 3.1 (*c* 0.9, $CHCl_3$), *litt* $[\alpha]_D^{22}$ = + 3.6 (*c* 0.9, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 4.01 (1H, dd, *J* 6.2, 7.8 Hz), 3.90 (1H, br. td, *J* 4.9, 6.4 Hz), 3.86 (1H, t, *J* 6.6 Hz), 3.66 (3H, s), 3.60 (1H, t, *J* 7.7 Hz), 2.53 (1H, ddd, *J* 3.7, 7.2, 11.0 Hz), 1.61 – 1.51 (4H, m), 1.41 (3H, s), 1.36 (3H, s), 1.19 – 0.99 (73H, br. m), 0.76 (3H, d, *J* 6.6 Hz), 0.68 (3H, t, *J* 7.0 Hz), 0.66 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.2, 108.5, 80.4, 73.2, 67.8, 51.5, 51.2, 36.5, 33.7, 32.7, 31.9, 29.9, 29.7, 29.65, 29.6, 29.5, 29.4, 27.8, 27.5, 26.9, 26.6, 25.7, 25.5, 23.7, 22.7, 18.0, 15.6, 14.1, – 4.4, – 4.9; ν_{max}/cm^{-1}: 2921, 2852, 1740, 1463. All data matched those in the literature.

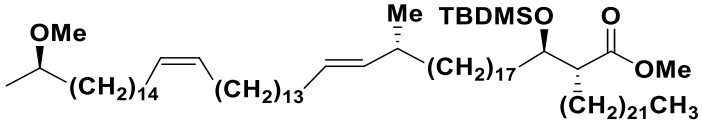
Experiment (53): Methyl-(*R*)-2-((1*R*,19*R*)-1-((tert-butyldimethylsilyl)oxy)-19-methyl-20-oxoicosyl)tetra cosanoate (140)



Periodic acid (2.5 g, 0.01 mol) was added to a stirred solution of methyl (*R*) 2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate (**161**) (2.0 g, 0.002 mol) in ether/THF (5:2, 112 mL) at room temperature under nitrogen and stirred for 3 hrs. The mixture was monitored by ^1H NMR, when no starting material was left. The solvent was evaporated and the crude

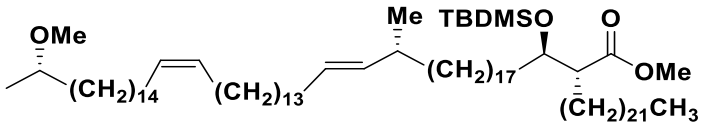
product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil, methyl-(*R*)-2-((1*R*,19*R*)-1-((tert-butyldimethylsilyl)oxy)-19-methyl-20-oxoicosyl)tetra cosanoate (**140**) (1.7 g, 90%), $[\alpha]_D^{28}$ = – 9.7 (*c* 0.9, $CHCl_3$); {Found $[M+Na]^+$: 843.7589; $C_{52}H_{104}O_4Si$ Na requires: 843.7596}; which showed δ_H (500 MHz, $CDCl_3$): 9.62 (1H, d, *J* 2.0 Hz), 3.90 (1H, br. td, *J* 4.6, 6.8 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.7, 7.2, 10.9 Hz), 2.43 (1H, br. qd *J* 2.0, 7.0 Hz), 1.78 – 1.63 (1H, m), 1.59 – 1.15 (75H, m), 1.09 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.1 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 205.4, 175.2, 73.2, 51.6, 51.2, 46.3, 33.7, 31.9, 30.5, 29.8, 29.7, 29.6, 29.5, 29.46, 29.4, 29.3, 27.8, 27.5, 26.9, 25.8, 23.7, 22.7, 17.9, 14.1, 13.3, – 4.4, – 4.9; v_{max}/cm^{-1}: 2921, 2852, 1737, 1463.

Experiment (54): Methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((tert-butyldimethylsilyl)oxy)-2-docosyl- 53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (138)



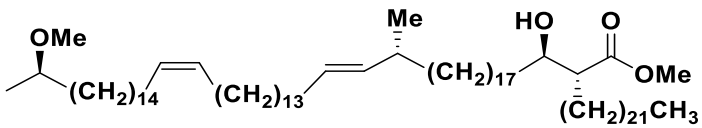
Potassium *bis*(trimethyl silyl)amide (7.8 mL, 0.003 mol, 0.5 M in toluene) was added to a stirred solution of methyl-(*R*)-2-((1*R*,19*R*)-1-((tert-butyldimethylsilyl)oxy)-19-methyl-20-oxoicosyl)tetra cosanoate (**140**) (1.65 g, 0.002 mol) and (*R*,*Z*)-5-((31-methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1H-tetrazole (**139**) (1.59 g, 0.003 mol) in dry THF (60 mL) at – 20 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for 10 min under a nitrogen, when TLC showed no starting material, then the reaction mixture was worked up and purified as before to give a colourless oil, methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((tert-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate (**138**) (1.7 g, 66%), $[\alpha]_D^{24}$= – 3.6 (*c* 0.9, $CHCl_3$); {Found $[M+Na]^+$: 1304.2618; $C_{85}H_{168}O_4SiNa$ requires:1304.2604}; which showed δ_H (500 MHz, $CDCl_3$): 5.40 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.5, 15.2 Hz), 3.90 (1H, br. dt, *J* 4.7, 6.5 Hz), 3.66 (3H, s), 3.32 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.53 (1H, ddd, *J* 3.7, 7.0, 10.8 Hz), 2.10 – 1.90 (7H, m), 1.60 – 1.17 (124H, v.br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 6.7 Hz), 0.88 (3H, t, *J* 6.8 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.2, 136.5, 129.9, 128.4, 76.9, 73.2, 55.9, 51.6, 51.2, 37.2, 36.7, 36.3, 33.7, 32.6, 31.9, 29. 8, 29.7, 29.6, 29.5, 29.3, 27.8, 27.5, 27.2, 25.8, 25.5, 23.7, 22.7, 20.9, 19.0, 14.1, – 4.4, – 4.9 ; v_{max}/cm^{-1}: 2920, 2851, 1740, 1463, 1370.

Experiment (55): Methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (138a)



Potassium *bis*(trimethyl silyl)amide (11.3 mL, 0.005 mol, 0.5 M in toluene) was added to a stirred solution of methyl-(*R*)-2-((1*R*,19*R*)-1-((tert-butyldimethylsilyl)oxy)-19-methyl-20-oxoicosyl)tetra cosanoate (**140**) (2.24 g, 0.003 mol) and (*S*,*Z*)-5-((31-methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (**139a**) (2.2 g, 0.003 mol) in dry THF (100 mL) at – 20 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for 10 min under a nitrogen, when TLC showed no starting material, the reaction was worked up and purified as above to give a colourless oil, methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((tert-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate (**138a**) (1.5 g, 43%), $[\alpha]_D^{24}$= – 3.8 (*c* 1.0, $CHCl_3$);{Found $[M+Na]^+$: 1304.2597; $C_{85}H_{168}O_4SiNa$ requires: 1304.2604}; which showed δ_H (500 MHz, $CDCl_3$): 5.39 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.4 Hz), 3.90 (1H, br. td, *J* 4.6, 6.6 Hz), 3.66 (3H, s), 3.32 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.54 (1H, ddd, *J* 3.8, 7.0, 10.8 Hz), 2.06 – 1.94 (7H, m), 1.61 – 1.15 (124H, v.br. s), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.2, 136.5, 129.9, 128.4, 76.9, 73.2, 55.9, 51.6, 51.2, 37.2, 36.7, 36.3, 33.7, 32.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.2, 25.8, 25.5, 23.7, 22.7, 20.9 19.0, 14.1, – 4.4, – 4.9 ; v_{max}/cm^{-1}: 2921, 2851, 1740, 1463, 1370.

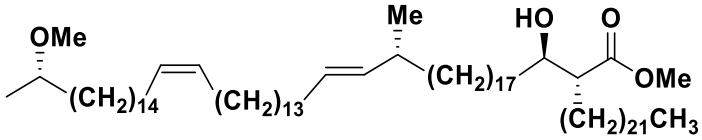
Experiment (56): Methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (198)



Methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((tert-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate (**138**) (2.1 g, 1.6 mmol) was dissolved in dry THF (22 mL) in a dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.3 mL) at room temperature under nitrogen. The reaction mixture was cooled to 0 °C, and then hydrogen fluoride-pyridine complex as ~70% (2 mL) was added

dropwise. The mixture was stirred at 43 °C for 18 hrs. When TLC showed no starting material was left, the mixture was added slowly into sat. aq. solution of $NaHCO_3$ and stirred until no more CO_2 was liberated. The product was extracted with petrol/ethyl acetate 1:1 (3 × 30 mL), then the combined organic layers were dried over $MgSO_4$, and evaporated to give a crude product, which was purified by chromatography, eluting with petrol/ethyl acetate (15:1) and then (5:1) to give a white solid, methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (**198**) (1.9 g, 66%), m.p. 51 − 53 °C; $[\alpha]_D^{24}$ = + 1.3 (*c* 1.0, $CHCl_3$); {Found $[M+Na]^+$: 1190.1752; $C_{79}H_{154}O_4Na$ requires: 1190.1739}; which showed δ_H (400 MHz, $CDCl_3$): 5.42 – 5.29 (3H, m), 5.23 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.71 (3H, s), 3.66 – 3.62 (1H, m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.44 (1H, dt, *J* 5.3, 9.2 Hz), 2.09 – 1.94 (7H, m), 1.76 – 1.65 (1H, m), 1.62 – 1.16 (124H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$):176.2, 136.5, 129.9, 128.4, 76.7, 72.3, 55.9, 51.5, 50.9, 37.2, 36.7, 36.3, 35.7, 32.6, 32.9, 29.8, 29.7, 29.6, 29.54, 29.5, 29.4, 29.36. 29.3, 29.1, 27.4, 27.3, 27.2, 25.7, 25.5, 22.7, 20.9, 19.0, 14.1; ν_{max}/cm^{-1}: 3518, 2916, 2848, 1709, 1461, 1374.

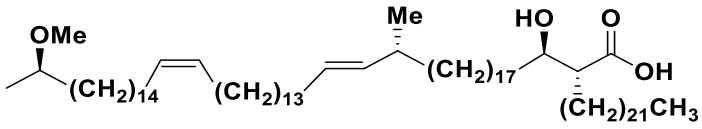
Experiment (57): Methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (198a)



Methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((tert-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate (**138a**) (1.5 g, 1.1 mmol) was dissolved in dry THF (21 mL) in a dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.3 mL) at room temperature under nitrogen. The reaction mixture was cooled to 0 °C, and then hydrogen fluoride-pyridine complex as ~70% (1.6 mL) was added dropwise. The mixture was stirred at 43 °C for 18 hrs. When TLC showed no starting material was left, the reaction was worked up as above to give a white solid, methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (**198a**) (1.9 g, 66%), m.p. 50 − 52 °C; $[\alpha]_D^{24}$ = + 1.8 (*c* 1.0, $CHCl_3$); {Found $[M+Na]^+$: 1190.1755; $C_{79}H_{154}O_4Na$ requires: 1190.1739}; which showed δ_H (400 MHz, $CDCl_3$): 5.39 – 5.30 (3H, m), 5.25 (1H, br. dd, *J* 7.4, 15.4

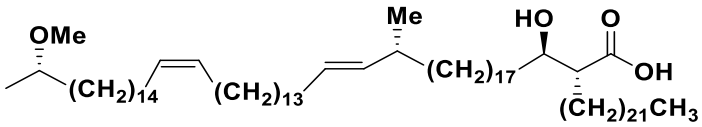
Hz), 3.71 (3H, s), 3.68 – 3.63 (1H, m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.45 (1H, dt, *J* 5.3, 9.0 Hz), 2.02 – 1.94 (7H, m), 1.75 – 1.69 (1H, m), 1.26 (124H, v. br. s), 1.12 (3H, d, *J* 6.1 Hz), 0.93 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$): 176.2, 136.5, 129.9, 128.4, 76.9, 72.3, 55.9, 51.5, 50.9, 37.2, 36.7, 36.3, 35.7, 32.6, 32.9, 29.8, 29.7, 29.6, 29.54, 29.5, 29.4, 29.36. 29.3, 29.1, 27.4, 27.3, 27.2, 25.7, 25.5, 22.7, 20.9, 19.0, 14.1; v_{max}/cm^{-1}: 3519, 2916, 2848, 1709, 1461, 1374.

Experiment (58): (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-Docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (129)



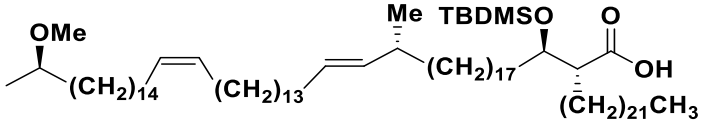
Lithium hydroxide monohydrate (0.46 g, 11.1 mmol) was added to a stirred solution of methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (**198**) (1.3 g, 1.11 mmol) in THF (20 mL), water (2.6 mL) and MeOH (2 mL). The mixture was heated at 45 ºC for 16 hrs. When TLC showed no starting material, the mixture was diluted with petrol/ethyl acetate (5:1, 20 mL), and then acidified with sat. aq. solution of $KHSO_4$. The product was extracted with warm petrol/ethyl acetate (5:1, 3 × 50 mL), and the combined organic layers were dried over $MgSO_4$, evaporated to give a crude product, which was purified by chromatography, eluting with $CHCl_3$/MeOH (15:1) and then (5:1) to give a white solid, (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**129**) (1.2 g, 93%), m.p. 62 − 64 °C; $[\alpha]_D^{23}$ = + 0.86 (*c* 0.81, $CHCl_3$); {Found $[M–H]^+$:1152.1615; $C_{78}H_{151}O_4$ requires: 1152.1612}; which showed δ_H (400 MHz, $CDCl_3$): 5.39 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.75 – 3.62 (1H, br. m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.49 – 2.33 (1H, br. m), 2.06 – 1.99 (7H, m), 1.43 – 1.16 (126H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$): 180.0, 136.5, 129.9, 128.4, 76.9, 72.2, 55.8, 51.5, 37.3, 36.7, 36.3, 35.5, 32.6, 31.9, 29.8, 29.76, 29.72, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 27.2, 25.8, 25.5, 22.7, 20.9, 19.0, 14.1 ; v_{max}/cm^{-1}: 3272, 2915, 2848, 1711, 1467, 1372.

Experiment (59): (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-Docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (130)



Lithium hydroxide monohydrate (0.28 g, 6.0 mmol) was added to a stirred solution of methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (**198a**) (0.8 g, 0.6 mmol) in THF (16 mL), water (1.5 mL) and MeOH (1 mL). The mixture was heated at 45 ºC for 16 hrs. When TLC showed no starting material, the reaction was worked up and purified as before to give a white solid, (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**130**) (0.56 g, 71%), m.p. 61 − 63 °C; $[\alpha]_D^{24}$ = + 0.89 (*c* 0.78 $CHCl_3$); {Found $[M–H]^+$:1152.1608; $C_{78}H_{151}O_4$ requires: 1152.1612}; which showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.75 – 3.65 (1H, br. m), 3.33 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.45 – 2.35 (1H, br. m), 2.10 – 1.91 (7H, m), 1.45 – 1.19 (126H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$): 180.0, 136.5, 129.9, 128.4, 76.9, 72.3, 55.9, 51.5, 37.3, 36.7, 36.3, 32.6, 31.9, 29.8, 29.7, 29.6, 29.58, 29.5, 29.4, 29.3, 29.2, 27.4, 27.2, 25.8, 25.5, 22.7, 20.9, 19.0, 14.1 ; ν_{max}/cm^{-1}: 3272, 2915, 2848, 1711, 1467, 1371.

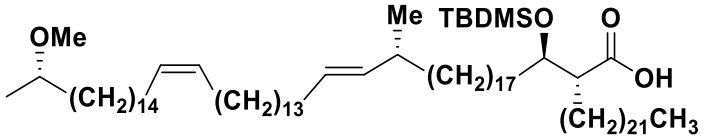
Experiment (60): (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*Tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (201)



Imidazole (0.62 g, 91.8 mmol) was added to a stirred solution of (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22,37- dienoic acid (**129**) (1.06 g, 9.1 mmol) in dry DMF (7 mL) and dry toluene (7 mL) at room temperature followed by the addition of *tert*-butyldimethylsilylchloride (1.4 g, 91.8 mmol) and 4-DMAP (0.11 g). The reaction mixture was stirred at 70 °C for 24 hrs. The solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate (1:1, 50 mL) and water (20 mL). The organic layer was separated and the aqueous layer was

re-extracted with petrol/ethyl acetate (1:1, 3 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue (**200**). The residue (**200**) was dissolved in THF (40 mL), then solution of tetra-*n*-butyl ammonium hydroxide (26 mL, 5%) was added slowly. The mixture was stirred at room temprature for 1 hr. When TLC showed no starting material, diluted with petrol/ethyl acetate (1:1, 30 mL) and water (2 mL) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography, eluting with petrol/ethyl acetate (15:1) to give a colourless oil, (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**201**) (1.09 g, 94%), $[\alpha]_D^{28}$ = – 1.0 (*c* 0.8, $CHCl_3$); {Found $[M–H]^+$: 1266.2480; $C_{84}H_{165}O_4Si$ requires: 1266.2477}; which showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.2 Hz), 3.83 (1H, ddd, *J* 3.0, 5.6, 7.6 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.53 (1H, ddd, *J* 2.7, 5.9, 8.8 Hz), 2.09 – 1.92 (7H, m), 1.79 – 1.65 (1H, m), 1.64 – 1.48 (7H, m), 1.45 – 1.20 (117H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.96 – 0.92 (12H, including a singlet at 0.93), 0.88 (3H, t, *J* 7.0 Hz), 0.15 (3H, s), 0.14 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.9, 136.5, 129.9, 128.4, 76.9, 73.6, 55.9, 50.3, 41.3, 37,2, 36.7, 36.3, 35.5, 32.6, 31.9, 29. 8, 29.7, 29.6, 29.5, 27.4, 27.2, 25.7, 25.6, 25.5, 24.9, 22.7, 22.6, 20.9, 19.0, 17.9, 14.1, – 4.3, – 4.9; v_{max}/cm^{-1}: 3688, 2918, 2850, 1708, 1463, 1370.

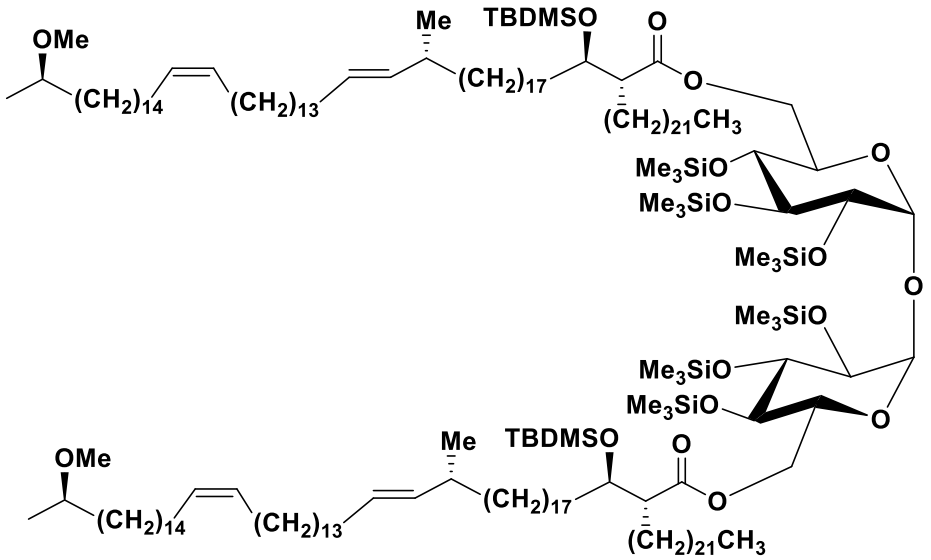
Experiment (61): (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*Tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (201a)

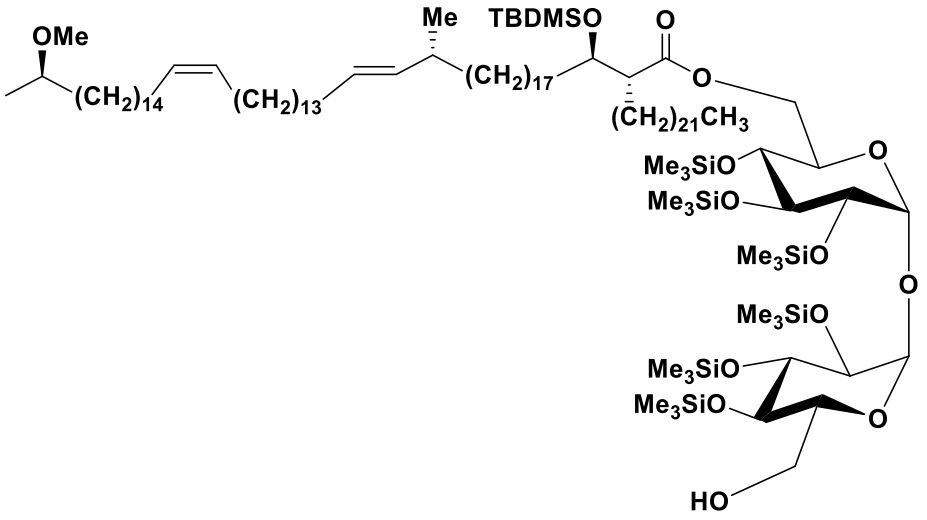


Imidazole (0.4 g, 60.6 mmol) was added to a stirred solution of (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl -3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**130**) (0.9 g, 6.0 mmol) in dry DMF (5 mL) and dry toluene (5 mL) at room temperature followed by the addition of *tert*-butyldimethylsilylchloride (0.9 g, 60.1 mmol) and 4-DMAP (0.07 g). The reaction mixture was stirred at 70 °C for 24 hrs. the reaction was worked up and purified as before to give a colourless oil residue (**200a**). The residue

(**200a**) was dissolved in THF (40 mL), then solution of tetra-*n*-butyl ammonium hydroxide (17 mL, 5%) was added slowly. The mixture was stirred at room temperature for 1 hr. When TLC showed no starting material, the reaction was worked up as above to give a colourless oil, (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**201a**) (0.92 g, 94%), $[\alpha]_D^{28}$ = – 1.2 (*c* 0.72, $CHCl_3$); {Found $[M–H]^+$: 1266.2498; $C_{84}H_{165}O_4Si$ requires: 1266.2477}; which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.28 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.84 (1H, ddd, *J* 3.6, 5.4, 7.2 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.2 Hz), 2.54 (1H, ddd, *J* 3.4, 5.5, 9.0 Hz), 2.09 – 1.94 (7H, m), 1.75 – 1.65 (1H, m), 1.64 – 1.45 (7H, m), 1.44 – 1.20 (117H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 6.7 Hz), 0.92 (9H, s), 0.88 (3H, t, *J* 7.0 Hz), 0.14 (3H, s), 0.12 (3H, s); δ_C (101 MHz, $CDCl_3$): 176.0, 136.5, 129.9, 128.4, 76.9, 73.6, 55.9, 50.3, 37,2, 36.7, 36.3, 35.5, 32.6, 31.9, 29. 8, 29.7, 29.6, 29.5, 27.4, 27.2, 25.7, 25.6, 25.5, 24.9, 22.7, 22.6, 20.9, 19.0, 17.9, 14.1, – 4.3, – 4.9; ν_{max}/cm^{-1}: 3682, 2917, 2849, 1708, 1465, 1371.

Experiment (62): 6,6'-*Bis*-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (202) and 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-2, 3, 4, 2',3 ',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (203)

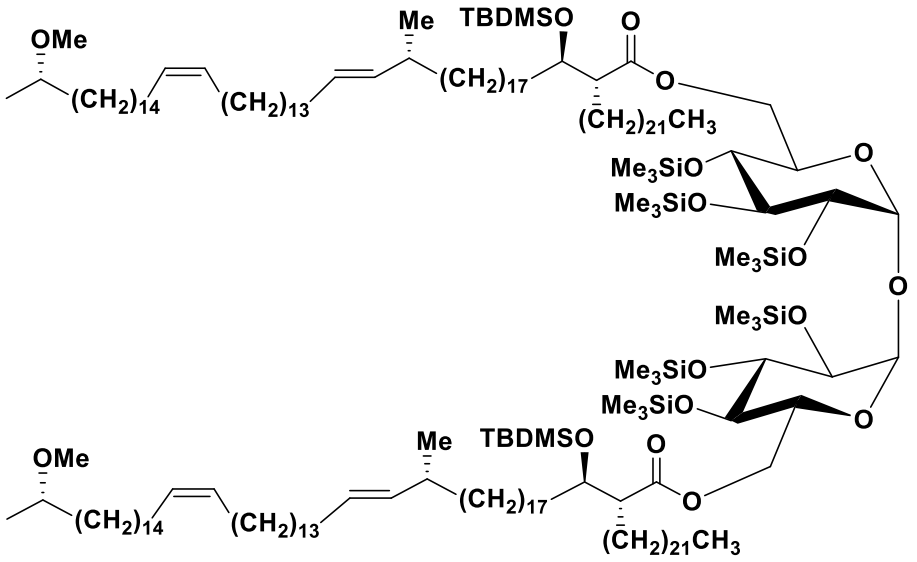


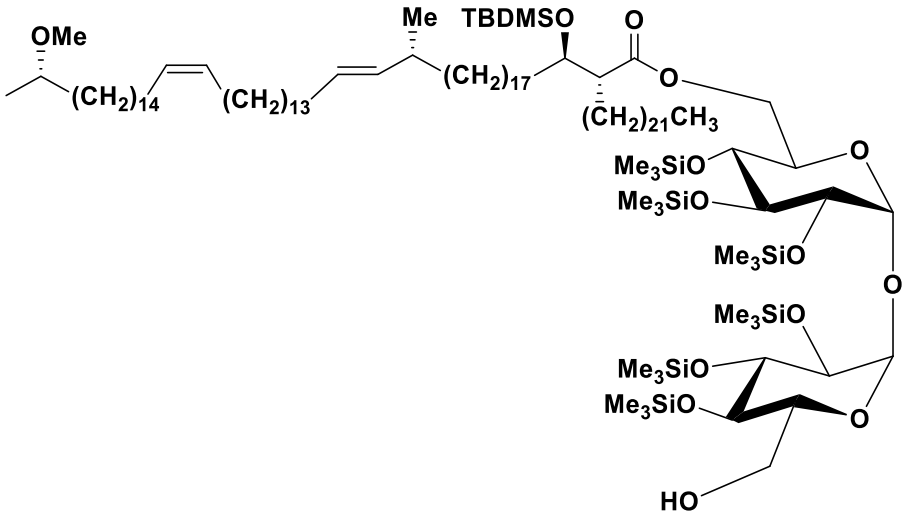


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.48 g, 2.52 mmol) and DMAP (0.3 g, 2.5 mmol) were added to a stirred solution of (**201**) (0.41 g, 0.31 mmol) and 2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (**120**)[220] (0.14 g, 0.18 mmol) and powdered 4 A˚ molecular sieves in dry dichloromethane (4 mL) at room temperature under an atmosphere nitrogen atmosphere. The mixture was stirred for 8 days at room temperature, then diluted with dichloromethane (5 mL) and silica gel (1 g) was added. The mixture was evaporated under reduced pressure to give a residue, which was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give the first fraction, a colourless thick oil, 6,6'-*bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-2, 3, 4, 2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose,TDM (**202**) (0.22 g, 21%), $[\alpha]_D^{20}$ = + 23.0 (*c* 0.98, $CHCl_3$); {Found $[M+Na]^+$:3297.8398; $C_{198}H_{398}O_{17}Si_8Na$ requires: 3297.8375}; which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.29 (6H, m), 5.24 (2H, br. dd, *J* 7.4, 15.3 Hz), 4.85 (2H, br. d, *J* 3.0 Hz), 4.37 (2H, br. d, *J* 10.0 Hz), 4.04 – 3.97 (4H, m), 3.96 – 3.93 (2H, m), 3.89 (2H, br. t, *J* 8.8 Hz), 3.52 (2H, br. t, *J* 9.0 Hz), 3.38 (2H, dd, *J* 3.0, 9.0 Hz), 3.32 (6H, s), 3.27 (2H, br. pent, *J* 6.1 Hz), 2.57 – 2.53 (2H, m), 2.06 – 1.94 (14H, m), 1.66 – 1.16 (248H, m), 1.12 (6H, d, *J* 6.7 Hz), 0.94 (6H, d, *J* 6.7 Hz), 0.94 – 0.85 (24H, including a triplet at 0.90 *J* 6.6 Hz, and singlet at 0.88), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s); δ_C (101 MHz, $CDCl_3$): 173.8, 136.5, 129.9, 128.4, 94.8, 76.9, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 55.9, 51.8, 37.3, 36.7, 36.4, 33.4, 32.6, 31.9, 29.8, 29.7, 29.6, 29.55, 29.5, 29.4, 29.1, 27.4, 27.2, 25.8, 25.5, 25.1, 22.7, 20.9, 19.0, 14.1, 1.09, 0.94, 0.15, – 4.5, – 4.6 ; ν_{max}/cm^{-1}: 2922, 2852, 1739, 1463.

The second fraction, a thick oil, 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose, TMM (**203**) (0.18 g, 28%), $[\alpha]_D^{25}$ = + 38.4 (*c* 0.92, $CHCl_3$); {Found $[M+Na]^+$: 2047.5918; $C_{114}H_{234}O_{14}Si_7Na$ requires: 2047.5901}; which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 4.91 (1H, d, *J* 3.0 Hz), 4.84 (1H, d, *J* 3.0 Hz), 4.35 (1H, dd, *J* 2.2, 11.8 Hz), 4.07 (1H, dd, *J* 4.2, 11.8 Hz), 3.98 (1H, dt, *J* 2.2, 6.0 Hz), 3.99 – 3.87 (3H, including a triplet of doublet *J* 5.8, 9.9 Hz), 3.84 (1H, dt, *J* 3.4, 6.6 Hz), 3.75 – 3.65 (2H, m), 3.48 (2H, br. td, *J* 4.4, 9.2 Hz), 3.42 (1H, dd, *J* 3.0, 9.2 Hz), 3.39 (1H, dd, *J* 3.0, 9.2 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.59 – 2.53 (1H, m), 2.10 – 1.92 (7H, m), 1.76 – 1.68 (1H, m), 1.56 – 1.17 (124H, m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H d, *J* 6.6 Hz), 0.91 – 0.86 (12H, including a triplet at 0.90 *J* 6.0 Hz, and singlet at 0.87), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.14 (18H, s), 0.12 (9H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.1, 136.5, 129.9, 128.4, 94.5, 94.4, 76.9, 73.4, 73.3, 72.9, 72.8, 72.7, 71.9, 71.4, 70.7, 62.5, 61.7, 55.9, 51.8, 37.3, 36.7, 36.4, 33.4, 32.6, 31.9, 29.8, 29.78, 29.7, 29.6, 29.58, 29.5, 29.4, 29.3, 29.1, 28.1, 27.4, 27.2, 26.4, 25.8, 25.5, 24.8, 22.7, 20.9, 19.0, 14.1, 1.05, 1.00, 0.92, 0.84, 0.17, 0.04, – 4.5, – 4.7; ν_{max}/cm^{-1}: 2923, 2853, 1733, 1463.

Experiment (63): 6,6'-*Bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2- docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (202a) and 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (203a)



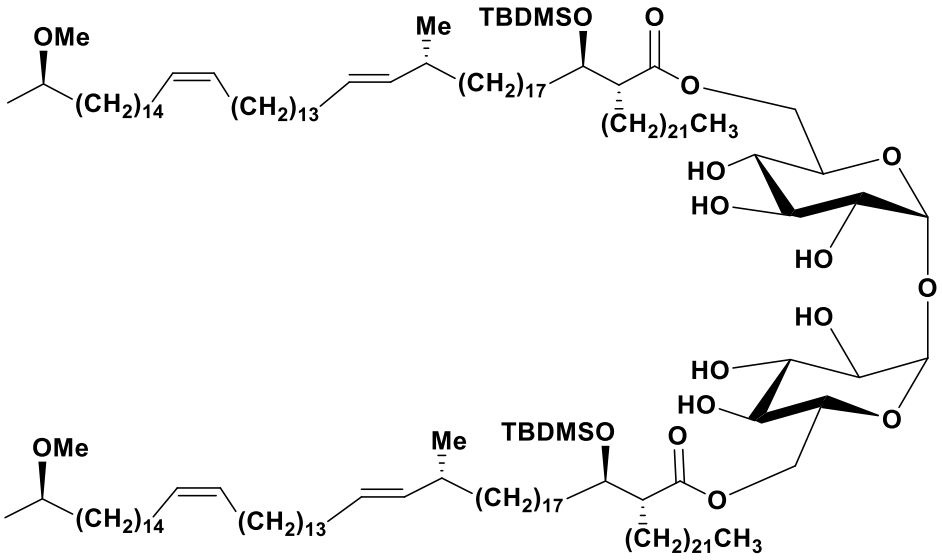


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.45 g, 2.39 mmol) and DMAP (0.29 g, 2.39 mmol) were added to a stirred solution of (**201a**) (0.38g, 0.29 mmol) and 2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (**120**)[220] (0.13 g, 0.16 mmol) and powdered 4 A˚ molecular sieves in dry dichloromethane (3.5 mL) at room temperature under an atmosphere nitrogen atmosphere. The mixture was stirred for 8 days at room temperature, then the reaction was worked up as above to give the first fraction, a colourless thick oil, 6,6'-*bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2- docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-2, 3, 4, 2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose,TDM (**202a**) (0.18 g, 18%),$[\alpha]_D^{25}$ = + 21.0 (*c* 0.99, $CHCl_3$); {Found $[M+Na]^+$: 3297.8405; $C_{198}H_{398}O_{17}Si_8Na$ requires: 3297.8375}; which showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.30 (6H, m), 5.24 (2H, br. dd, *J* 7.4, 15.3 Hz), 4.85 (2H, br. d, *J* 3.0 Hz), 4.36 (2H, br. d, *J* 10.0 Hz), 4.09 – 4.00 (4H, m), 3.99 – 3.85 (2H, m), 3.89 (2H, br. t, *J* 8.8 Hz), 3.52 (2H, br. t, *J* 9.0 Hz), 3.37 (2H, dd, *J* 3.0, 9.0 Hz), 3.32 (6H, s), 3.27 (2H, br. pent, *J* 6.1 Hz), 2.60 – 2.50 (2H, m), 2.10 – 1.90 (14H, m), 1.70 – 1.19 (248H, m), 1.12 (6H, d, *J* 6.1 Hz), 0.94 (6H, d, *J* 6.7 Hz), 0.93 – 0.80 (24H, including a triplet at 0.9 *J* 6.6 Hz, and singlet at 0.88), 0.15 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s); δ_C (101 MHz, $CDCl_3$): 173.8, 136.5, 129.9, 128.4, 94.8, 76.9, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 55.9, 51.8, 37.3, 36.7, 36.4, 33.4, 32.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.4, 27.2, 25.8, 25.5, 22.7, 20.9, 19.0, 14.1, 1.08, 0.94, 0.15, – 4.5, – 4.7; ν_{max}/cm^{-1}: 3699, 2921, 2852, 1741, 1463.

The second fraction, a thick oil, 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose, TMM (**203a**) (0.19 g, 31%),

$[\alpha]_D^{26}$ = + 40.0 (*c* 0.84, $CHCl_3$); {Found $[M+Na]^+$: 2047.5916; $C_{114}H_{234}O_{14}Si_7Na$ requires: 2047.5901}; which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 4.91 (1H, d, *J* 3.0 Hz), 4.84 (1H, d, *J* 3.0 Hz), 4.35 (1H, dd, *J* 2.2, 11.8 Hz), 4.08 (1H, dd, *J* 4.2, 11.8 Hz), 3.98 (1H, dt, *J* 2.2, 6.4 Hz), 3.96 – 3.87 (3H, including a triplet of doublet *J* 4.3, 9.0 Hz), 3.84 (1H, dt, *J* 3.2, 6.4 Hz), 3.74 – 3.66 (2H, m), 3.48 (2H, br. td, *J* 4.4, 9.3 Hz), 3.42 (1H, dd, *J* 3.1, 9.3 Hz), 3.39 (1H, dd, *J* 3.1, 9.3 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.60 – 2.50 (1H, m), 2.05 – 1.94 (7H, m), 1.72 – 1.55 (1H, m), 1.52 – 1.19 (124H, m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H d, *J* 6.6 Hz), 0.90 – 0.80 (12H, including a triplet at 0.90 *J* 6.7 Hz, and singlet at 0.88), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.14 (18H, s), 0.12 (9H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.1, 136.5, 129.9, 128.4, 94.5, 94.4, 76.9, 73.4, 73.3, 72.9, 72.8, 72.7, 71.9, 71.4, 70.7, 62.4, 61.7, 55.9, 51.8, 37.2, 36.7, 36.3, 33.4, 32.6, 31.9, 29.8, 29.72, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 28.1, 27.4, 27.2, 26.4, 25.9, 25.5, 24.8, 22.7, 20.9, 19.0, 14.1, 1.05, 1.00, 0.92, 0.84, 0.17, 0.04, – 4.5, – 4.7; ν_{max}/cm^{-1}: 2924, 2853, 1730, 1463.

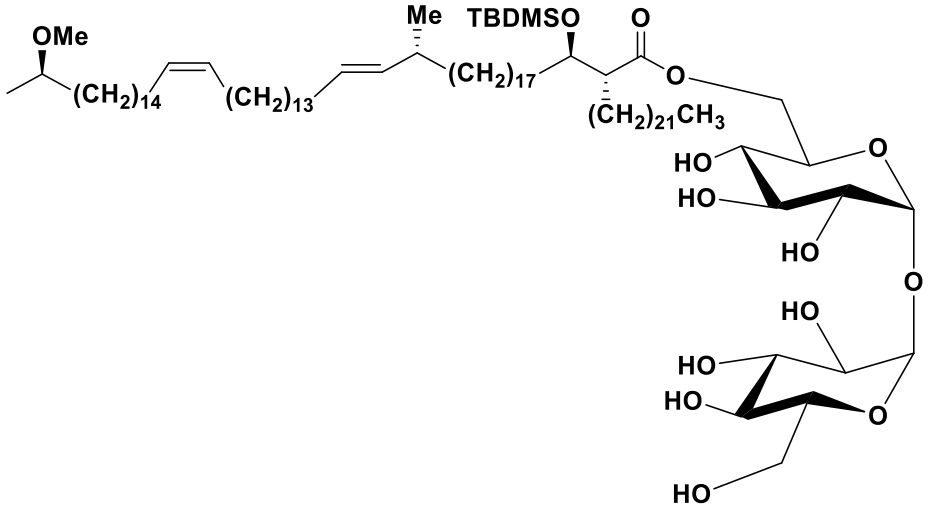
Experiment (64): 6,6'-*Bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2- docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (204)



Tetrabutylammonium fluoride (0.41 mL, 0.41 mmol, 1.0 M) was added to a stirred solution of TDM (**202**) (0.19 g, 0.058 mmol) in dry THF (10 mL) under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hr, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (10:1) to give a colourless thick oil, 6,6'-*bis*-*O*- (2*R*, 3*R*, 21*R*,

22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapenta-conta-22,37-dienoate-α,α'-trehalose (**204**) (0.14 g, 84%), $[\alpha]_D^{28}$ = + 13.0 (*c* 0.80, $CHCl_3$); {Found $[M+Na]^+$: 2865.6033; $C_{180}H_{350}O_{17}Si_2Na$ requires: 2865.6015}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.35 – 5.25 (6H, m), 5.19 (2H, dd, *J* 7.3, 15.4 Hz), 5.05 (2H, br. d, *J* 3.2 Hz), 4.33 (2H, br. dd, *J* 4.7, 12.0 Hz), 4.20 (2H, br. d, *J* 12.0 Hz), 3.98 – 3.85 (4H, m), 3.74 (2H, br. t, *J* 9.2 Hz), 3.44 (2H, dd, *J* 3.2, 9.2 Hz), 3.32 – 3.23 (10H, including a singlet at 3.27 for the methoxy groups), 2.83 (6H, br. s for OH groups), 2.59 – 2.46 (2H, m), 2.05 – 1.86 (14H, m), 1.60 – 1.11 (242H, m), 1.08 (6H, d, *J* 6.1 Hz), 0.89 (6H, d, *J* 6.7 Hz), 0.85 – 0.75 (24H, including a triplet at 0.84 *J* 6.0 Hz, and singlet at 0.81), 0.003 (9H, s), 0.02 (9H, s); δ_C (101MHz, $CDCl_3$ + few drops of CD_3OD): 175.2, 136.4, 129.8, 128.3, 93.4, 76.9, 73.2, 71.7, 70.5, 69.9, 62.9, 55.8, 51.6, 37.2, 36.6, 36.1, 33.6, 32.5, 31.8, 29.7, 29.62, 29.6, 29.5, 29.3, 27.7, 27.3, 27.1, 26.9, 25.8, 25.7, 25.4, 24.2, 22.6, 20.8, 18.9, 17.9, 14.0, – 4.6, – 4.9; ν_{max}/cm^{-1}: 3383, 2920, 2851, 1739, 1463.

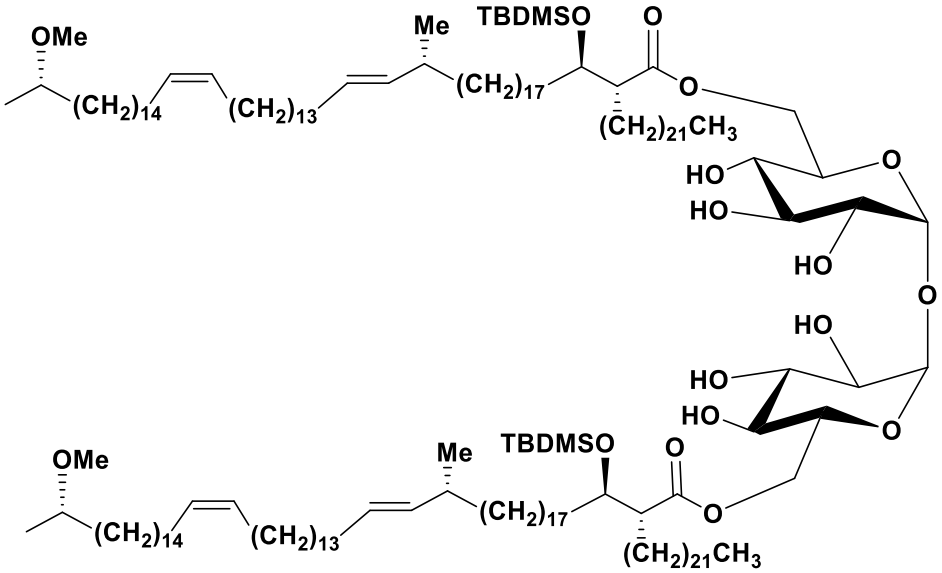
Experiment (65): 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (205)



Tetrabutylammonium fluoride (0.45 mL, 0.45 mmol, 1.0 M) was added to a stirred solution of TMM (**203**) (0.13 g, 0.064 mmol) in dry THF (6 mL) under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hr, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (10:1) to give a colourless thick oil, 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,

37-dienoate-α,α'-trehalose (**205**) (0.080 g, 78%), $[\alpha]_D^{25}$ = + 26.0 (*c* 0.65, $CHCl_3$); {Found $[M+Na]^+$: 1614.3519; $C_{96}H_{186}O_{14}SiNa$ requires: 1614.3504}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.32 – 5.26 (3H, m), 5.18 (1H, br. dd, *J* 7.4, 15.4 Hz), 5.03 (2H, br. s), 4.30 – 4.20 (2H, m), 3.95 – 3.71 (6H, m), 3.68 – 3.60 (1H, m), 3.48 – 3.40 (2H, m), 3.34 – 3.20 (6H, including a singlet at 3.27), 2.52 – 2.47 (1H, m), 2.02 – 1.87 (7H, m), 1.50 – 1.10 (131H, m), 1.06 (3H, d, *J* 6.1 Hz), 0.87 (3H, d, *J* 6.6 Hz), 0.82 (3H, t, *J* 6.3 Hz), 0.79 (9H, s), – 0.016 (3H, s), – 0.038 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 136.3, 130.2, 129.8, 128.3, 93.5, 93.4, 76.9, 73.1, 73.0, 72.6, 72.1, 71.5, 70.7, 70.2, 69.9, 62.7, 61.9, 55.7, 51.6, 37.1, 36.6, 36.0, 33.5, 32.4, 31.8, 29.7, 29.6, 29.56, 29.4, 29.39, 29.2, 29.17, 29.0, 27.6, 27.2, 27.1, 26.9, 25.6, 25.3, 24.1, 22.5, 20.8, 18.8, 13.9, – 4.7, – 5.1; ν_{max}/cm^{-1}: 3352, 2919, 2850, 1733, 1465.

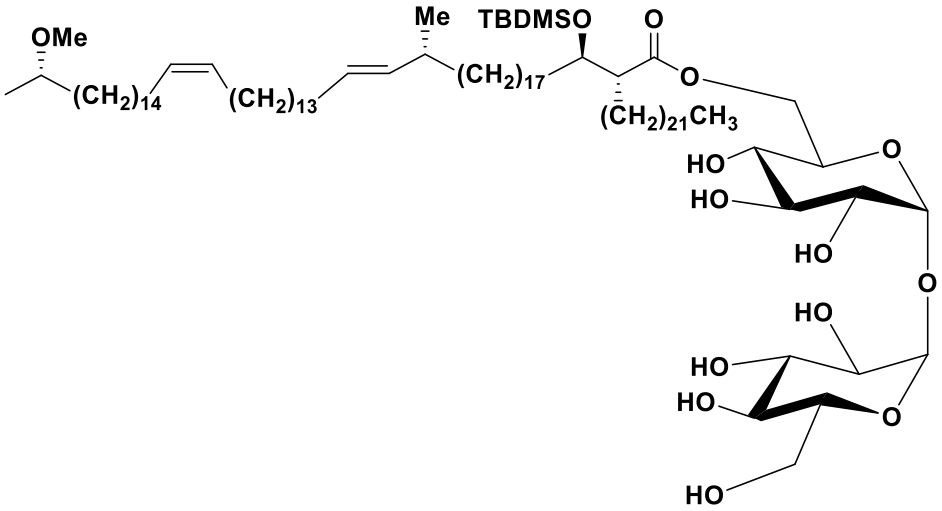
Experiment (66): 6,6'-*Bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2- docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (204a)



Tetrabutylammonium fluoride (0.34 mL, 0.34 mmol, 1.0 M) was added to a stirred solution of TDM (**202a**) (0.16 g, 0.048 mmol) in dry THF (10 mL) under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hr, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (10:1) to give a colourless thick oil, 6,6'-*bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2- docosyl-53-methoxy-21-methyltetrapenta-conta-22,37-dienoate-α,α'-trehalose (**204a**) (0.102 g, 74%), $[\alpha]_D^{28}$ = + 13.0 (*c* 0.79,

$CHCl_3$); {Found $[M+Na]^+$: 2865.6007; $C_{180}H_{350}O_{17}Si_2Na$ requires: 2865.6015}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.40 – 5.26 (6H, m), 5.21 (2H, dd, *J* 7.4, 15.4 Hz), 5.08 (2H, br. d, *J* 3.2 Hz), 4.36 (2H, br.dd, *J* 3.2, 11.8 Hz), 4.22 (2H, br. d, *J* 11.8 Hz), 3.65 (2H, br. d, *J* 9.7 Hz), 3.93 – 3.80 (4H, m), 3.48 (2H, dd, *J* 3.2, 9.3 Hz), 3.35 – 3.25 (10H, including a singlet at 3.29 for the methoxy group), 2.56 – 2.49 (2H, m), 2.43 – 2.34 (2H, m), 2.33 – 2.24 (2H, m), 2.14 – 1.86 (14H, m), 1.65 – 1.16 (242H, m), 1.10 (6H, d, *J* 6.1 Hz), 0.91 (6H, d, *J* 6.7 Hz), 0.88 – 0.84 (24H, including a triplet at 0.87 *J* 6.0 Hz, and singlet at 0.86), 0.02 (9H, s), 0.005 (9H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.2, 136.3, 129.7, 128.3, 93.4, 76.9, 73.1, 71.6, 70.2, 69.8, 62.9, 55.6, 51.6, 37.1, 36.6, 36.0, 33.5, 32.4, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.6, 27.2, 27.0, 25.7, 25.6, 25.3, 24.1, 22.5, 20.8, 18.8, 17.8, 13.9, –4.7, –5.0; ν_{max}/cm^{-1}: 3703, 2917, 2849, 1741, 1366.

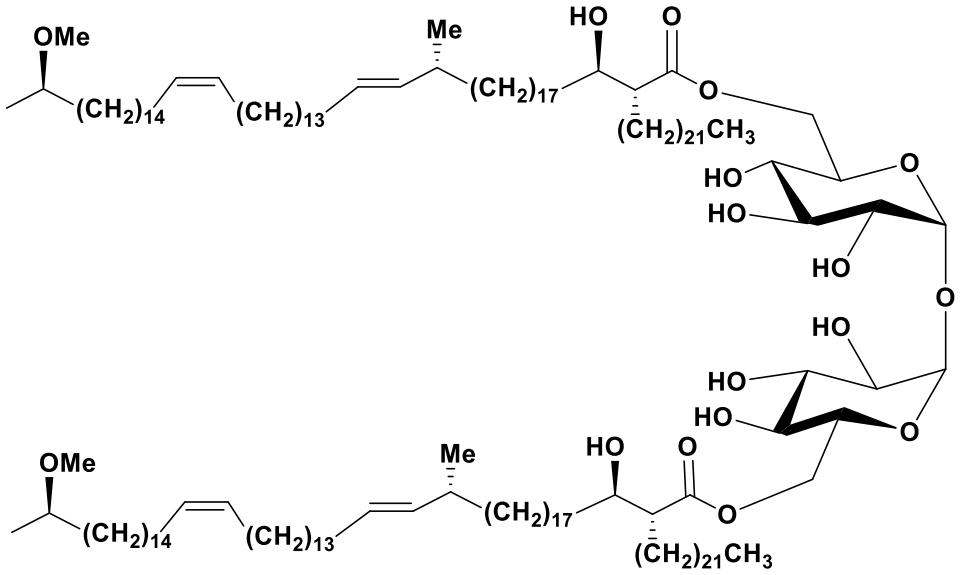
Experiment (67): 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-*α,α*'-trehalose (205a)



Tetrabutylammonium fluoride (0.45 mL, 045 mmol, 1.0 M) was added to a stirred solution of TMM (**203a**) (0.13 g, 0.063 mmol) in dry THF (6 mL) under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hr, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (10:1) to give a colourless thick oil, 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (**205a**) (0.070 g, 67%), $[\alpha]_D^{25}$ = + 28.0 (*c* 0.83, $CHCl_3$); {Found $[M+Na]^+$: 1614.3512; $C_{96}H_{186}O_{14}SiNa$ requires: 1614.3504}; which showed δ_H (400

MHz, $CDCl_3$ + few drops of CD_3OD): 5.32 – 5.24 (3H, m), 5.18 (1H, br. dd, *J* 7.4, 15.4 Hz), 5.05 (2H, br. d, *J* 2.7 Hz), 4.30 – 4.22 (2H, m), 3.93 (1H, br. d, *J* 8.4 Hz), 3.90 – 3.75 (5H, m), 3.67 – 3.62 (1H, m), 3.48 (2H, br. dd, *J* 2.7, 9.4 Hz), 3.34 – 3.23 (6H, including a singlet at 3.27), 2.54 – 2.47 (1H, m), 2.02 – 1.88 (7H, m), 1.40 – 1.10 (131H, m), 1.08 (3H, d, *J* 6.1 Hz), 0.89 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 6.0 Hz), 0.81 (9H, s), – 0.002 (3H, s), – 0.024 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 136.4, 130.3, 129.8, 128.3, 93.5, 93.4, 76.9, 73.2, 72.9, 72.6, 72.1, 71.5, 70.7, 70.2, 69.9, 62.7, 62.0, 55.7, 51.6, 37.1, 36.6, 36.1, 33.5, 32.5, 31.8, 29.64, 29.6, 29.5, 29.4, 29.24, 29.2, 27.6, 27.3, 27.1, 26.9, 25.6, 25.3, 25.2, 24.1, 22.6, 20.8, 18.8, 14.0, – 4.6, – 5.0; ν_{max}/cm^{-1}: 3353, 2918, 2850, 1732, 1465, 940.

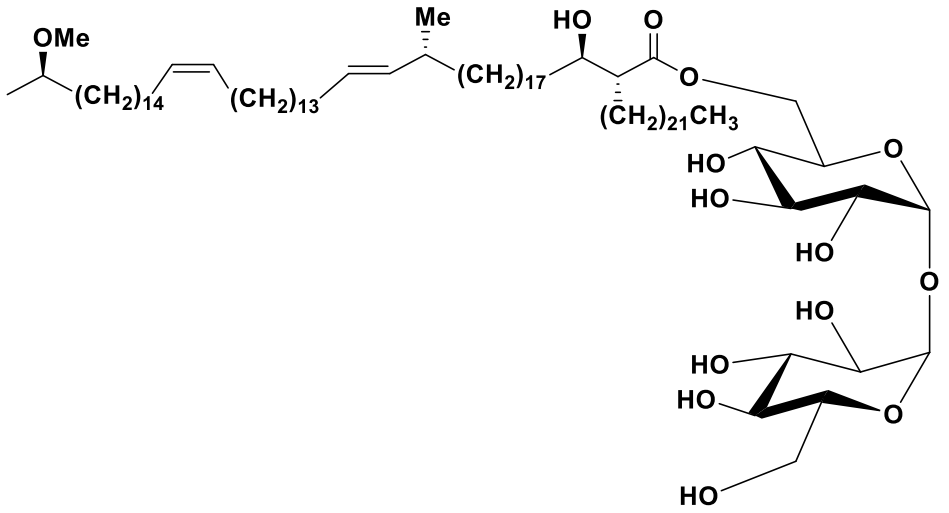
Experiment (68): 6,6'-*Bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (131)



6,6'-*Bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (**204**) (0.12 g, 0.042 mmol) was dissolved in dry THF (12 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The reaction mixture was cooled to 5 °C, and then hydrogen fluoride-pyridine complex as ~70% (1.2 mL) was added dropwise. The mixture was stirred at 45 °C for 17 hrs. When TLC showed no starting material was left, the excess of the HF was neutralized with sat. aq. $NaHCO_3$ (10 mL), and the product was extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were dried and evaporated. The crude product

was purified by column chromatography eluting with $CHCl_3$/MeOH (15:1) to give a yellowish semi-solid, 6,6'-*bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (**131**) (0.061 g, 56%), $[\alpha]_D^{21}$ = + 28.6 (*c* 1.35, $CHCl_3$); {Found $[M+Na]^+$: 2635.4238; $C_{168}H_{322}O_{17}Na$ requires: 2635.4224}; which showed δ_H ($CDCl_3$ + few drops of CD_3OD): 5.44 – 5.28 (6H, m), 5.22 (2H, br. dd, *J* 7.4, 15.4 Hz), 4.99 (2H, br. d, *J* 3.3 Hz), 4.81 (2H, br. d, *J* 11.0 Hz), 4.35 (2H, br. t, *J* 9.8 Hz), 3.85 (2H, br. t, *J* 11.0 Hz), 3.75 (2H, br. t, *J* 9.2 Hz), 3.72 – 3.65 (2H, br. m), 3.52 (2H, dd, *J* 3.3, 9.8 Hz), 3.30 (6H, s), 3.26 (2H, br. pent, *J* 6.0 Hz), 3.19 (2H, br. t, *J* 9.2 Hz), 2.43 – 2.37 (2H, m), 2.04 – 1.92 (14H, m), 1.78 (8H, s), 1.65 – 1.15 (248H, m), 1.10 (6H, d, *J* 6.1 Hz), 0.92 (6H, d, *J* 6.7 Hz), 0.86 (6H, t, *J* 6.5 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 136.4, 129.8, 128.3, 95.2, 77.0, 72.5, 72.3, 71.5, 71.1, 69.7, 64.6, 55.7, 52.1, 37.1, 36.6, 36.1, 34.6, 32.5, 31.8, 29.6, 29.5, 29.4, 29.36, 29.26, 29.2, 29.0, 27.3, 27.2, 27.1, 25.3, 25.1, 22.6, 20.8, 18.8, 13.9; ν_{max}/cm^{-1}: 3371, 2917, 2849, 1721, 1466.

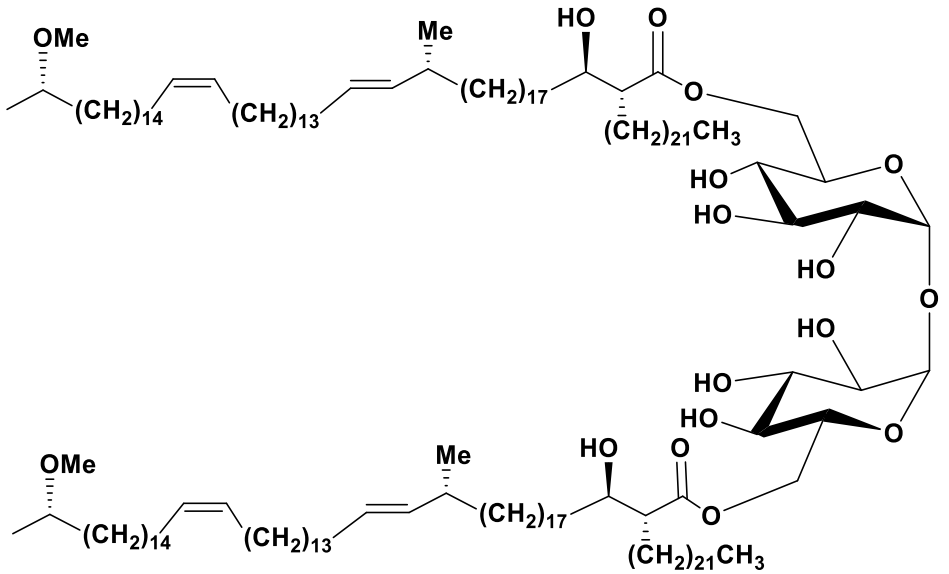
Experiment (69): 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-Hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (132)



6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (**205**) (0.075 g, 0.047 mmol) was dissolved in dry THF (10 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The reaction mixture cooled to 5 °C, and then hydrogen fluoride-pyridine complex as ~70% (1.0 mL) was added dropwise. The mixture was stirred at 45 °C for 17 hrs. When

TLC showed no starting material was left, then the reaction was worked up as above to give a yellowish semi-solid, 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (**132**) (0.020 g, 29%), $[\alpha]_D^{25}$ = + 43.0 (*c* 0.30, $CHCl_3$); {Found $[M+Na]^+$: 1500.2646; $C_{90}H_{172}O_{14}Na$ requires: 1500.2639}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.35 – 5.24 (3H, m), 5.18 (1H, dd, *J* 7.3, 15.5 Hz), 5.05 (1H, d, *J* 2.9 Hz), 4.98 (1H, d, *J* 3.1 Hz), 4.66 (1H, br. d, *J* 10.4 Hz), 4.22 (1H, br. t, *J* 10.4 Hz), 3.93 (1H, br. t, *J* 10.3 Hz), 3.91 – 3.70 (4H, m), 3.65 – 3.50 (4H, m), 3.46 (1H, br. d, *J* 10.8 Hz), 3.25 – 3.15 (5H, including a singlet at 3.2 for methoxy group), 2.40 – 2.32 (1H, m), 2.0 – 1.85 (7H, m), 1.60 – 1.10 (132H, m), 1.07 (3H, d, *J* 6.1 Hz), 0.86 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 6.4 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 136.3, 129.8, 128.3, 94.8, 94.7, 77.1, 72.6, 72.5, 72.4, 72.3, 71.5, 71.2, 71.1, 70.9, 69.9, 64.4, 62.1, 55.7, 52.3, 37.1, 36.6, 36.1, 34.6, 32.5, 31.8, 29.6, 29.5, 29.4, 29.3, 29.23, 29.2, 29.0, 27.2, 27.17, 27.1, 25.3, 25.0, 22.6, 20.8, 18.8, 13.9; ν_{max}/cm^{-1}: 3344, 2918, 1850, 1718, 1467.

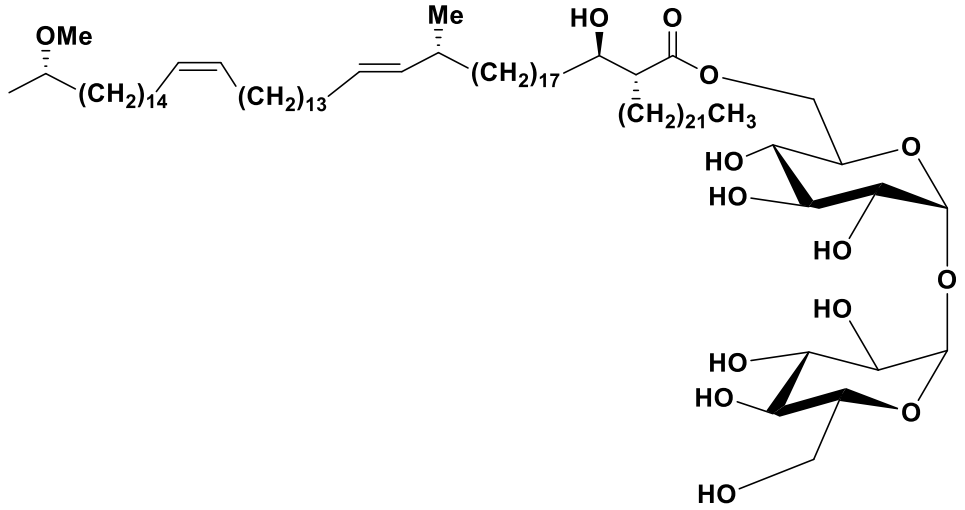
Experiment (70): 6,6'-*Bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (131a)



6,6'-*Bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (**204a**) (0.0942 g, 0.0332 mmol) was dissolved in dry THF (12 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The reaction mixture was cooled to 5 °C, and then hydrogen fluoride-pyridine

complex as ~70% (1.10 mL) was added dropwise. The mixture was stirred at 45 °C for 17 hrs. When TLC showed no starting material was left, then the reaction was worked up as above to give a yellowish semi-solid, 6,6'-*bis*-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoate-α,α'-trehalose (**131a**) (0.051 g, 59%), $[\alpha]_D^{21}$ = + 35.0 (*c* 0.83, $CHCl_3$); {Found $[M+Na]^+$: 2635.4213; $C_{168}H_{322}O_{17}Na$ requires: 2635.4224}; which showed δ_H ($CDCl_3$ + few drops of CD_3OD): 5.40 – 5.28 (6H, m), 5.22 (2H, br. dd, *J* 7.4, 15.4 Hz), 5.00 (2H, br. d, *J* 2.3 Hz), 4.81 (2H, br. d, *J* 11.5 Hz), 4.34 (2H, br. t, *J* 8.3 Hz), 3.85 (2H, br. t, *J* 11.5 Hz), 3.77 (2H, br. t, *J* 9.4 Hz), 3.73 – 3.64 (2H, br. m), 3.53 (2H, br. d, *J* 9.4 Hz), 3.30 (6H, s), 3.26 (2H, br. pent, *J* 6.0 Hz), 3.20 (2H, br. t, *J* 9.3 Hz), 2.46 – 2.35 (2H, br. m), 2.09 – 1.90 (14H, m), 1.82 (8H, s), 1.60 – 1.15 (248H, m), 1.10 (6H, d, *J* 6.1 Hz), 0.92 (6H, d, *J* 6.7 Hz), 0.86 (6H, t, *J* 6.5 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 136.4, 129.8, 128.3, 95.1, 77.0, 72.5, 72.3, 71.4, 71.0, 69.7, 64.6, 55.7, 52.1, 37.1, 36.6, 36.1, 34.6, 32.5, 31.8, 29.6, 29.5, 29.4, 29.35, 29.25, 29.2, 29.0, 27.3, 27.2, 27.1, 25.3, 25.1, 22.6, 20.8, 18.8, 14.0; ν_{max}/cm^{-1}: 3374, 2916, 2849, 1718, 1466.

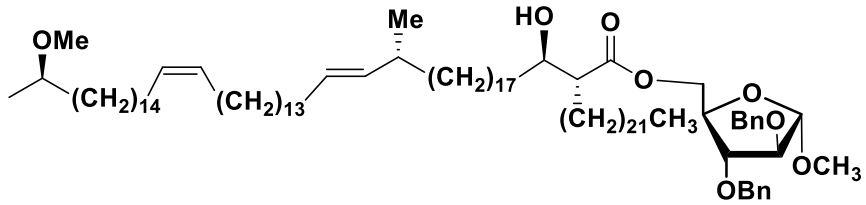
Experiment (71): 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-Hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (132a)



6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (**205a**) (0.060 g, 0.0377 mmol) was dissolved in dry THF (10 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The reaction mixture cooled to 5 °C, and then hydrogen fluoride-pyridine complex as

~70% (1.0 mL) was added dropwise. The mixture was stirred at 45 °C for 17 hrs. When TLC showed no starting material was left, then the reaction was worked up as above to give a yellowish semi-solid, 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (**132a**) (0.022 g, 39%), $[\alpha]_D^{25}$ = + 43.0 (*c* 0.75, $CHCl_3$); {Found $[M+Na]^+$: 1500.2646; $C_{90}H_{172}O_{14}Na$ requires: 1500.2639}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD):): 5.40 – 5.25 (3H, m), 5.19 (1H, dd, *J* 7.3, 15.2 Hz), 5.10 (1H, br. s), 5.0 (1H, br. s), 4.70 (1H, br. d, *J* 11.0 Hz), 4.20 (1H, br. t, *J* 8.4 Hz), 4.0 – 3.91 (1H, m), 3.85 – 3.75 (4H, m), 3.68 – 3.52 (4H, m), 3.48 (1H, br. d, *J* 7.0 Hz), 3.28 – 3.17 (5H, including a singlet at 3.27 for methoxy group), 2.47 – 2.32 (1H, m), 2.20 – 1.90 (7H, m), 1.60 – 1.11 (132H, m), 1.10 (3H, d, *J* 6.0 Hz), 0.89 (3H, d, *J* 6.6 Hz), 0.83 (3H, t, *J* 6.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 136.4, 129.8, 128.3, 94.6, 77.1, 72.6, 72.5, 72.4, 72.3, 71.4, 71,2, 71.1, 70.9, 69.9, 64.4, 62.2, 55.7, 52.3, 37.1, 36.6, 36.1, 34.6, 32.5, 31.8, 29.6, 29.58, 29.5, 29.4, 29. 3, 29.2, 29.0, 27.3, 27.2, 27.1, 25.3, 25.1, 22.6, 20.8, 18.8, 13.9; ν_{max}/cm^{-1}: 3359, 2918, 1850, 1718, 1466.

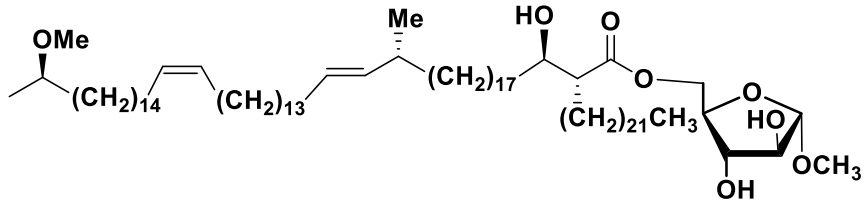
Experiment (72): Methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22,37-dienoate]-α-D-arabinofuran-osiode (207)



Dry cesium hydrogen carbonate (65.0 mg, 0.33 mmol) was added to a stirred solution of arabinofuranosiode (**206**)[219] (50.0 mg, 0.10 mmol) and (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**201**) (0.085 g, 0.067 mmol) in dry THF and DMF (5:1, 3 mL) at room temperature. The mixture was brought to 70 °C and stirred at this temperature for 18 hrs. After TLC showed no starting material, the mixture was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (**207**) (0.056 g, 57%), $[\alpha]_D^{24}$ = + 17.0 (*c* 0.75, $CHCl_3$); {Found $[M+Na]^+$: 1503.3155; $C_{98}H_{174}O_8Na$ requires: 1503.3135}; which showed

δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (10H, m), 5.40 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.2 Hz), 4.92 (1H, br. s), 4.57 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.0 Hz), 4.49 (1H, d, *J* 11.8 Hz), 4.47 (1H, d, *J* 11.8 Hz), 4.30 (1H, br. d *J* 12.0. Hz), 4.28 (1H, br. d, *J* 12.0), 4.21 (1H, br. td, *J* 4.6, 6.4 Hz), 3.99 (1H, dd, *J* 0.6, 2.4 Hz), 3.84 (1H, dd, *J* 2.7, 6.7 Hz), 3.67 – 3.59 (1H, m), 3.37 (3H, s), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.2 Hz), 2.51 (1H, d, *J* 8.2 Hz), 2.43 (1H, dt, *J* 5.9, 9.1 Hz), 2.10 – 1.91 (7H, m), 1.75 – 1.60 (1H, m), 1.59 – 1.15 (123 H, m), 1.12 (3H, d, *J* 6.4 Hz), 0.94 (3H, d, *J* 6.7 Hz), 0.88 (3H, t, *J* 6.5 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 137.4, 137.3, 136.4, 129.9, 128.5, 128.45, 128.4, 127.9, 127.93, 127.91, 127.8, 107.2, 87.9, 83.7, 79.4, 76.9, 72.4, 72.1, 72.0, 63.5, 55.9, 54.9, 51.5, 37.2, 36.7, 36.3, 35.5, 32.6, 31.9, 29.8, 29.7, 29.6, 29.56, 29.5, 29.4, 29.3, 27.4, 27.3, 27.2, 25.7, 25.4, 22.7, 20.9, 19.0, 14.1; ν_{max}/cm^{-1}: 3658, 2920, 2851, 1734, 1455.

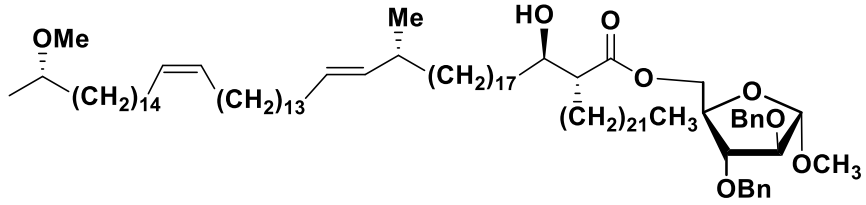
Experiment (73): Methyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (133)



Liquid ammonia (50 mL) was condensed into a two neck flask (100 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~70 mg) was added until the blue colour of the solution persisted. Methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapenta-conta-22, 37-dienoate]-α-D-arabinofuranosiode (**207**) (0.052 g, 0.035 mmol) in 1,4-dioxane (5 mL) was added and the reaction mixture was stirred for 4-5 min until the blue colour disappeared. The reaction was quenched with ammonium chloride (5 mL) and ether (20 mL). The ammonia was allowed to evaporate and the organic layer was separated and the aqueous layer re-extracted with ether (2 × 30 mL). The combined organic layers were dried and evaporated. The residue was purified by column chromatography on silica gel, eluting with $CHCl_3$/MeOH (20:1) to give very thick oil, methyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (**133**) (0.0145 g, 32%), $[\alpha]_D^{21}$ = + 15.0 (*c* 0.89, $CHCl_3$). {Found $[M+Na]^+$: 1322.2173; $C_{84}H_{162}O_8Na$ requires: 1322.2162}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.36 – 5.25

(3H, m), 5.18 (1H, br. dd, *J* 7.4, 15.2 Hz), 4.78 (1H, br. s), 4.32 (1H, dd, *J* 4.5, 11.7 Hz), 4.28 (1H, dd, *J* 5.0, 11.7 Hz), 4.07 (1H, br. q, *J* 4.8 Hz), 3.96 (1H, br. s), 3.84 (1H, br. q, *J* 2.8 Hz), 3.65 – 3.52 (1H, m), 3.34 (3H, s), 3.28 – 3.20 (4H, including a singlet at 3.27 for methoxy group), 2.43 – 2.34 (1H, m), 2.02 – 1.89 (7H, m), 1.68 – 1.11 (127H, m), 1.08 (3H, d, *J* 6.1 Hz), 0.88 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 7.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 136.4, 129.8, 128.3, 108.8, 81.8, 81.2, 77.9, 77.0, 72.4, 63.4, 55.7, 54.9, 52.6, 37.1, 36.6, 36.1, 34.9, 32.5, 31.8, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.19, 29.14, 29.1. 29.05, 29.0, 27.3, 27.2, 27.1, 25.3, 22.6, 20.8, 18.8, 14.0; ν_{max}/cm^{-1}: 3458, 2917, 2850, 1721, 1466.

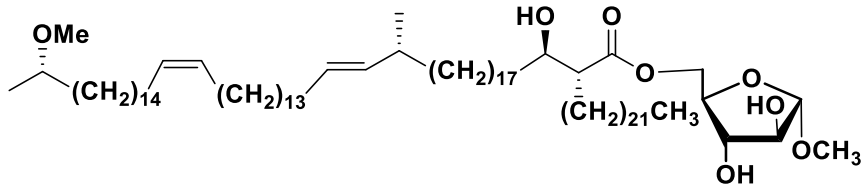
Experiment (74): Methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (207a)



Dry cesium hydrogen carbonate (61.0 mg, 0.32 mmol) was added to a stirred solution of arabinofuranosiode **(206)**[219] (47.1 mg, 0.094 mmol) and (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**201a**) (0.08 g, 0.06 mmol) in dry THF and DMF (5:1, 3 mL) at room temperature. The mixture was brought to 70 °C and stirred at this temperature for 18 hrs. After TLC showed no starting material left, then the reaction was worked up as before to give methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetra pentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (**207a**) (0.065 g, 70%), $[\alpha]_D^{24}$ = + 16.0 (*c* 0.77, $CHCl_3$); {Found $[M+Na]^+$: 1503.3152; $C_{98}H_{174}O_8Na$ requires: 1503.3135}; which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.26 (10H, m), 5.40 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.4 Hz), 4.92 (1H, br. s), 4.57 (1H, d, *J* 12.0 Hz), 4.58 (1H, d, *J* 12.0 Hz), 4.51 (1H, d, *J* 11.8 Hz), 4.48 (1H, d, *J* 11.8 Hz), 4.30 (1H, br. d *J* 12.0. Hz), 4.28 (1H, br. d, *J* 12.0), 4.22 (1H, br. td, *J* 4.6, 6.4 Hz), 3.98 (1H, dd, *J* 0.6, 2.4 Hz), 3.83 (1H, dd, *J* 2.7, 6.7 Hz), 3.67 – 3.58 (1H, m), 3.37 (3H, s), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.1 Hz), 2.52 (1H, d, *J* 8.3 Hz), 2.43 (1H, dt, *J* 5.5, 9.3 Hz), 2.10 – 1.90 (7H, m), 1.75 – 1.60 (1H, m), 1.59 – 1.19 (123 H, m), 1.12 (3H, d, *J* 6.4 Hz), 0.94 (3H, d, *J* 6.7 Hz), 0.88 (3H, t, *J* 6.5 Hz); δ_C (101

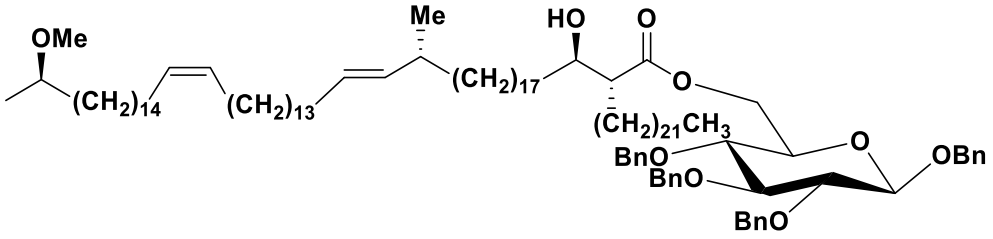
MHz, $CDCl_3$): 175.0, 137.4, 137.2, 136.4, 129.9, 128.5, 128.4, 128.3, 127.9, 127.92, 127.9, 127.8, 107.2, 87.8, 83.7, 79.4, 76.9, 72.4, 72.1, 72.0, 63.4, 55.9, 54.9, 51.5, 37.2, 36.7, 36.3, 35.5, 32.6, 31.9, 29.8, 29.76, 29.7, 29.6, 29.56, 29.5, 29.4, 29.35, 29.3, 27.4, 27.3, 27.2, 25.8, 25.5, 22.7, 20.9, 19.0, 14.1; ν_{max}/cm^{-1}: 3658, 2920, 2851, 1735, 1455.

Experiment (75): Methyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (133a)



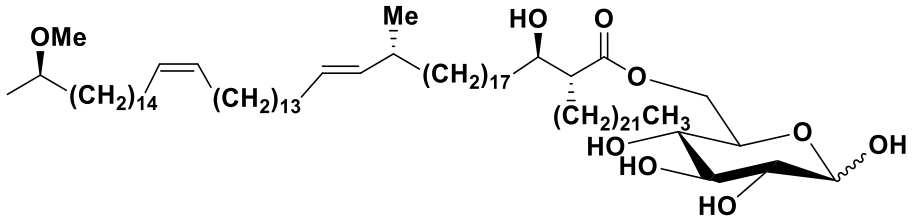
Liquid ammonia (50 mL) was condensed into a two neck flask (100 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~70 mg) was added until the blue colour of the solution persisted. Methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (**207a**) (60.0 mg, 0.04 mmol) in 1,4-dioxane (5 mL) was added and the reaction mixture was stirred for 4-5 min until the blue colour disappeared. Then the reaction was worked up as before to give a very thick oil, methyl-5-*O*-[(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate]-α-D-arabinofuranosiode (**133a**) (0.02 g, 38%), $[\alpha]_D^{21}$ = + 15.4 (*c* 0.86, $CHCl_3$); {Found $[M+Na]^+$: 1322.2178; $C_{84}H_{162}O_8Na$ requires: 1322.2162}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.35 – 5.20 (3H, m), 5.15 (1H, br. dd, *J* 7.4, 15.4 Hz), 4.74 (1H, br. s), 4.28 (1H, dd, *J* 4.6, 11.7 Hz), 4.26 (1H, dd, *J* 5.2, 11.7 Hz), 4.03 (1H, pent, *J* 5.2 Hz), 3.92 (1H, br. s), 3.81 (1H, br. q, *J* 2.9 Hz), 3.65 – 3.52 (1H, m), 3.31 (3H, s), 3.28 – 3.15 (4H, including a singlet at 3.27 for methoxy group), 2.45 – 2.30 (1H, m), 2.04 – 1.82 (7H, m), 1.60 – 1.10 (127H, m), 1.05 (3H, d, *J* 6.1 Hz), 0.86 (3H, d, *J* 6.7 Hz), 0.80 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.0, 136.3, 129.7, 128.3, 108.8, 81.5, 81.3, 77.8, 77.1, 72.3, 63.4, 55.6, 54.9, 52.6, 37.0, 36.5, 35.9, 34.8, 32.4, 31.7, 29.5, 29.4, 29.3, 29.2, 29.17, 29.1, 29.07, 29.0, 28.98, 28.8, 27.2, 27.17, 27.0, 25.2, 22.5, 20.7, 18.7, 14.0; ν_{max}/cm^{-1}; 3401, 2917, 2850, 1721, 1466.

Experiment (76): Benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*, 3*R*, 21*R*,22*E*,37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22,37-dienoate-β-D-glucopyranoside (209)



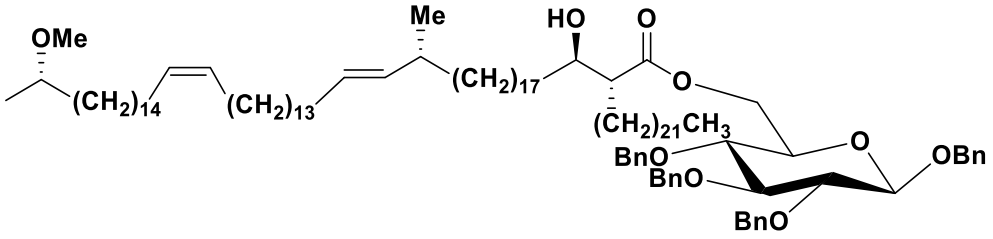
(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**201**) (100 mg, 0.078 mmol) was dissolved in a mixture of THF and DMF (5:1, 3 mL) at room temperature, and then gently heated until all had dissolved. Dry cesium hydrogen carbonate (76.6 mg, 0.39 mmol) and benzyl-2,3,4-tri-*O*-benzyl-6-*O*-tosyl-β-D-glucopyranoside (**208**)[184] (82.1 mg, 0.011 mmol) were added. The mixture was brought to 70 °C and stirred at this temperature for 18 hrs. After TLC showed no starting material was left, then the reaction was worked up as before to give benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*, 3*R*, 21*R*,22*E*,37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22,37-dienoate-β-D-glucopyranoside (**209**) (0.085 g, 64%), $[\alpha]_D^{28}$ = + 13.3 (*c* 0.80, $CHCl_3$); {Found $[M+H]^+$: 1676.4192; $C_{112}H_{187}O_9$ requires: 1676.4175}; which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.28 (20H, m), 5.40 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 4.96 (1H, d, *J* 11.0 Hz), 4.95 (1H, d, *J* 11.0 Hz), 4.94 (1H, br. s), 4.90 (1H, d, *J* 12.0 Hz), 4.80 (1H, d, *J* 11.0 Hz), 4.71 (1H, d, *J* 11.0 Hz), 4.63 (1H, d, *J* 12.0 Hz), 4.60 (1H, d, *J* 12.0 Hz), 4.56 – 4.52 (2H, m), 4.22 (1H, dd, *J* 4.7, 12.0 Hz), 3.70 – 3.60 (2H, m), 3.56 – 3.48 (3H, m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.53 – 2.43 (2H, including a doublet *J* 7.9 Hz, OH), 2.10 – 1.91 (7H, m), 1.80 – 1.67 (1H, m), 1.65 – 1.60 (1H, m), 1.59 – 1.43 (7H, m), 1.40 – 1.18 (115H, m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.8); δ_C (101 MHz, $CDCl_3$): 175.2, 138.4, 138.2, 137.7, 137.1, 136.4, 130.4, 129.9, 128.5, 128.4, 128.37, 128.3, 128.1, 128.0, 127.9, 127.85, 127.8, 127.6,102.3, 84.5, 82.3, 77.8, 76.9, 75.1, 74.9, 72.8, 72.3, 71.1, 62.8, 55.9, 51.3, 37.2, 36.7, 36.3, 35.6, 32.6, 31.9, 29.8, 29.7, 29.65, 29.6, 29.59, 29.5, 29.3, 29.4, 29.35, 29.3, 29.1, 27.5, 27.3, 27.2, 25.9, 25.4, 22.7, 20.9, 19.0, 14.1; ν_{max}/cm^{-1}: 3664, 2921, 2851, 1735, 1454.

Experiment (77): 6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-Docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22, 37-dienoate-β-D-glucopyranoside (134)



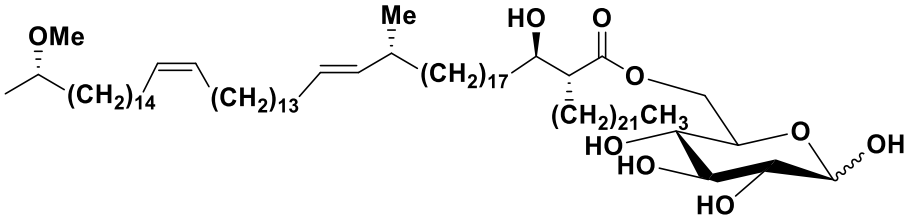
Liquid ammonia (50 mL) was condensed into a two neck flask (100 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~ 70 mg) was added until the blue colour of the solution persisted. Benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*, 3*R*, 21*R*,22*E*,37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22, 37-dienoate-β-D-glucopyranoside (**209**) (80.0 mg, 0.04 mmol) in 1,4-dioxane (5 mL) was added and the reaction mixture was stirred for 4-5 min until the blue colour disappeared. Then the reaction was worked up as before to give very thick oil, 6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22, 37-dienoate-β-D-glucopyranoside (**134**) (0.014 g, 22%), $[\alpha]_D^{24}$ = + 12.3 (*c* 0.78 $CHCl_3$), {Found $[M+Na]^+$: 1338.2118; $C_{84}H_{162}O_9Na$ requires: 1338.2111}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD); (α, β isomers) in ratio 0.6:0.4: 5.35 – 5.25 (3H, m), 5.19 (1H, br. dd, *J* 7.2, 15.1 Hz), 5.12 (0.6H, br. d, *J* 3.8 Hz, H_1 –α), 4.47 (0.4H, d, *J* 7.7 Hz, H_1 –β), 4.42 (1H, br. dd, *J* 2.3, 11.7 Hz, H_6 –α, β), 4.26 (1H, br. dd, *J* 5.2, 11.7 Hz, H_6 –α, β), 3.95 (0.6H, br. ddd, *J* 3.0, 5.6, 7.0 Hz, H_5 –α), 3.67 – 3.59 (1.6H, m, H_4 –α + CH – OH mycolic acid), 3.50 – 3.45 (0.4H, m, H_5 –β), 3.40 – 3.30 (2H, m, $H_{2,3}$ –α + $H_{3,4}$ –β), 3.31 – 3.23 (4H, including a singlet at 3.27 for methoxy group), 3.18 (0.4H, t, *J* 7.6 Hz, H_2 –β), 2.42 – 2.34 (1H, m), 2.05 – 1.85 (7H, m), 1.70 – 1.12 (129H, m), 1.08 (3H, d, *J* 6.1 Hz), 0.90 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD); (α, β isomers); 175.2, 136.4, 129.8, 128.3, 96.5 (C_1 –β), 92.2 (C_1 –α), 77.0, 76.0, 74.4, 73.6, 73.4, 72.5, 72.1, 70.5, 70.2, 69.2, 63.6, 63.4, 55.7, 52.7, 52.6, 37.1, 36.6, 36.1, 34.9, 32.5, 31.8, 29.6, 29.57, 29.5, 29.3, 29.24, 29.2, 29.1, 29.09, 29.06, 29.0, 27.3, 27.25, 27.1, 25.3, 22.6, 20.8, 18.8, 14.0; ν_{max}/cm^{-1}: 3344, 2917, 2849, 1719, 1466.

Experiment (78): Benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22,37-dienoate-β-D-glucopyranoside (209a)



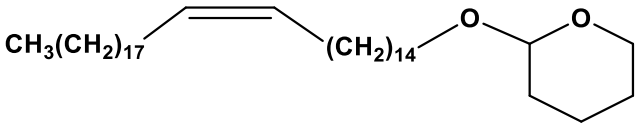
(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (**201a**) (100 mg, 0.078 mmol) was dissolved in a mixture of THF and DMF (5:1, 3 mL) at room temperature, and then gently heated until all had dissolved. Dry cesium hydrogen carbonate (76.6 mg, 0.39 mmol) and benzyl-2,3,4-tri-*O*-benzyl-6-*O*-tosyl-β-D-glucopyranoside (**208**)[184] (82.1 mg, 0.011 mmol) were added. The mixture was brought to 70 °C and stirred at this temperature for 18 hrs. After TLC showed no starting material was left, then the reaction was worked up as before to give benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22,37-dienoate-β-D-glucopyranoside (**209a**) (0.083 g, 63%), $[\alpha]_D^{28}$ = + 13.9 (*c* 0.78, $CHCl_3$); {Found $[M+Na]^+$: 1699.4039; $C_{112}H_{186}O_9Na$ requires: 1966.4023}; which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.25 (20H, m), 5.40 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 4.96 (1H, d, *J* 11.0 Hz), 4.95 (1H, d, *J* 11.0 Hz), 4.94 (1H, br. s), 4.90 (1H, d, *J* 12.0 Hz), 4.79 (1H, d, *J* 11.0 Hz), 4.71 (1H, d, *J* 11.0 Hz), 4.63 (1H, d, *J* 12.0 Hz), 4.60 (1H, d, *J* 12.0 Hz), 4.57 – 4.50 (2H, m), 4.22 (1H, dd, *J* 4.7, 12.0 Hz), 3.73 – 3.63 (2H, m), 3.57 – 3.48 (3H, m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.53 – 2.43 (2H, including a doublet *J* 8.3 Hz, OH), 2.10 – 1.91 (7H, m), 1.80 – 1.67 (1H, m), 1.65 – 1.60 (1H, m), 1.59 – 1.43 (7H, m), 1.40 – 1.18 (115H, m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.8); δ_C (101 MHz, $CDCl_3$): 175.2, 138.4, 138.2, 137.7, 137.1, 136.5, 130.3, 129.9, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.87, 127.8, 127.7, 102.3, 84.5, 82.3, 77.8, 76.9, 75.8, 75.1, 74.9, 72.8, 72.3, 71.1, 62.9, 55.9, 51.3, 37.2, 36.7, 36.3, 35.6, 32.6, 31.9, 29.8, 29.7, 29.6, 29.53, 29.5, 29.4, 29.3, 29.1, 27.5, 27.4, 27.2, 25.9, 25.5, 22.7, 20.9, 19.0, 14.1; ν_{max}/cm^{-1}: 3667, 2922, 2852, 1726, 1455.

Experiment (79): 6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-Docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22, 37-dienoate-β-D-glucopyranoside (134a)



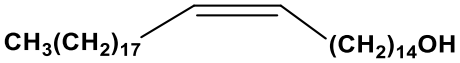
Liquid ammonia (50 mL) was condensed into a two neck flask (100 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~ 70 mg) was added until the blue colour of the solution persisted. Benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22,37-dienoate-β-D-glucopyranoside (**209a**) (80.0 mg, 0.04 mmol) in 1,4-dioxane (5 mL) was added and the reaction mixture was stirred for 4-5 min until the blue colour disappeared. Then the reaction was worked up as before to give very thick oil, 6-*O*-(2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22, 37-dienoate-β-D-glucopyranoside (**134a**) (9.3 mg, 15%), $[\alpha]_D^{24}$ = + 12.3 (*c* 0.93, $CHCl_3$), {Found $[M+Na]^+$: 1338.2106; $C_{84}H_{162}O_9Na$ requires: 1338.2111}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD); (α, β isomers) in ratio 0.6:0.4: 5.35 – 5.24 (3H, m), 5.18 (1H, br. dd, *J* 7.4, 14.7 Hz), 5.10 (0.6H, br. d, *J* 3.6 Hz, H_1 –α), 4.46 (0.4H, d, *J* 7.7 Hz, H_1 –β), 4.41 (1H, br. d, *J* 11.6 Hz, H_6 –α, β), 4.25 (1H, br. dd, *J* 5.6, 11.6 Hz, H_6 –α, β), 3.94 (0.6H, br. ddd, *J* 2.7, 5.6, 7.5 Hz, H_5 –α), 3.67 – 3.53 (1.6H, m, H_4 –α + CH – OH mycolic acid), 3.49 – 3.44 (0.4H, m, H_5 –β), 3.40 – 3.32 (2H, m, $H_{2,3}$ –α + $H_{3,4}$ –β), 3.30 – 3.32 (4H, including a singlet at 3.27 for the methoxy group), 3.17 (0.4H, t, *J* 7.9 Hz, H_2 –β), 2.42 – 2.34 (1H, m), 1.98 – 1.88 (7H, m), 1.60 – 1.10 (129 H, m), 1.08 (3H, d, *J* 6.1 Hz), 0.88 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 7.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD); (α, β isomers): 175.1, 136.4, 129.8, 128.3, 96.6 (C_1 –β), 92.2 (C_1 –α), 77.0, 76.1, 74.5, 73.7, 73.6, 72.5, 72.2, 70.5,70.2, 69.2, 63.6, 63.4, 55.8, 52.7, 52.5, 37.1, 36.6, 36.1, 35.0, 32.5, 31.8, 29.6, 29.57, 29.5, 29.4, 29.36, 29.3, 29.24, 29.2, 29.1, 29.04, 28.9, 27.3, 27.2, 27.1, 25.3, 22.6, 20.5, 18.9, 14.0; ν_{max}/cm^{-1}; 3376, 2916, 2849, 1723, 1476.

Experiment (80): (*Z*)-2-(Tetratriacont-15-en-1-yoxy)tetrahydro-2*H*-pyran (214)



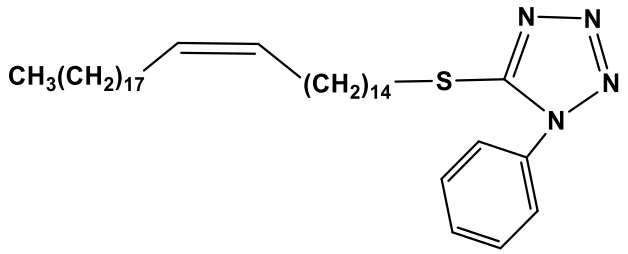
Sodium *bis*(trimethylsilyl)amide, (13.6 mL, 0.012 mol, 1.0 M in THF) was added to a stirred solution of nonadecyltriphenylphosphonium bromide (**215**) (4.9 g, 0.008 mol) and 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal (**145**) (2.8 g, 0.008 mol) in dry THF (120 mL) at –5 ºC under nitrogen atmosphere. The mixture was stirred for 40 min then allowed to reach room temperature. Then the reaction was worked up as before to give a colourless oil, (*Z*)-2-(tetratriacont-15-en-1-yoxy)tetrahydro-2*H*-pyran (**214**) (2.1 g, 43%), {Found $[M+Na]^+$: 599.5749; $C_{39}H_{76}O_2Na$ requires: 599.5738}; which showed δ_H (400 MHz, $CDCl_3$): 5.35 (2H, pent, *J* 10.5 Hz), 4.58 (1H, br. s), 3.95 – 3.85 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.2 Hz), 3.56 – 3.46 (1H, m), 3.39 (1H, dt, *J* 6.6, 9.2 Hz), 2.08 – 1.95 (4H, m), 1.90 – 1.79 (1H, m), 1.76 – 1.66 (1H, m), 1.65 – 1.48 (6H, m), 1.45 – 1.20 (54H, m), 0.88 (3H, t, *J* 6.4 Hz); δ_C (101 MHz, $CDCl_3$): 129.9, 98.8, 67.7, 62.3, 31.9, 30.8, 29.8, 29.7, 29.66. 29.63, 29.6, 29.57, 29.5, 29.4, 29.3, 27.2, 26.3, 25.5, 22.7, 19.7, 14.1; ν_{max}/cm^{-1}: 2915, 2848, 1470, 1274.

Experiment (81): (*Z*)-Tetratriacont-15-en-1-ol (213)



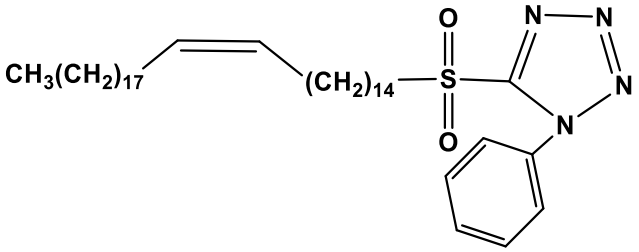
Pyridinum-*p*-toluenesulfonate (0.45 g, 1.82 mmol) was added to a stirred solution of (*Z*)-2-(tetratriacont-15-en-1-yoxy)tetrahydro-2*H*-pyran (**214**) (2.0 g, 3.64 mmol) in THF (50 mL), methanol (20 mL) and stirred at 50 ºC overnight. When TLC showed no starting material was left, then the reaction was worked up as before to give a white solid, (*Z*)-tetratriacont-15-en-1-ol (**213**) (1.5 g, 88%), m.p. 62 − 64 ºC; {Found $[M+H]^+$: 493.5332 ; $C_{34}H_{69}O$ requires: 493.5343}; which showed δ_H (400 MHz, $CHCl_3$): 5.35 (2H, pent, *J* 10.6 Hz), 3.65 (2H, q, *J* 6.3 Hz), 2.09 – 1.99 (4H, m), 1.61 – 1.54 (2H, m), 1.48 – 1.13 (55H, m), 0.88 (3H, t, *J* 6.4 Hz); δ_C (101 MHz, $CDCl_3$): 129.9, 63.1, 32.8, 31.9, 29.8, 29.7, 29.66, 29.6, 29.5, 29.4, 29.36, 29.3, 27.2, 25.7, 22.7, 14.1; ν_{max}/cm^{-1}: 3284, 2915, 2848, 1472.

Experiment (82): (*Z*)-1-Phenyl-5-(tetratriacont-15-en-1-ylthio)-1*H*-tetrazol (212)



Diethyl azodicarboxylate (0.68 g, 0.0039 mol) in dry THF (5 mL) was added to a stirred solution of (*Z*)-tetrtriaiacont-15-en-1-ol (**213**) (1.5 g, 0.003 mol), triphenylphosphine (1.1 g, 0.004 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (0.7 g, 0.039 mol) in dry THF (15 mL) at 0 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and then stirred for 45 min. When TLC showed no starting material was left, then the reaction was worked up as before to give a white solid, (*Z*)-1-phenyl-5-(tetratriacont-15-en-1-ylthio)-1*H*-tetrazol (**212**) (1.7 g, 89%), m.p. 53 − 55 °C; {Found $[M+H]^+$: 653.5555; $C_{41}H_{73}N_4S$ requires: 653.5550}; which showed δ_H (400 MHz, $CDCl_3$): 7.65 – 7.50 (5H, m), 5.35 (2H, pent, *J* 10.4 Hz), 3.40 (2H, t, *J* 7.4 Hz), 2.08 – 1.95 (4H, m), 1.82 (2H, pent, *J* 7.3 Hz), 1.44 (2H, pent, *J* 8.0 Hz), 1.26 – 1.22 (52H, m), 0.88 (3H, t, *J* 6.4 Hz); δ_C (101 MHz, $CDCl_3$): 133.8, 130.3, 130.0, 129.9, 129.7, 123.8, 33.4, 32.6, 31.9, 29.8, 29.7, 29.64, 29.6, 29.5, 29.3, 29.0, 28.7, 27.2, 22.7, 14.1; ν_{max}/cm^{-1}: 2914, 2848, 1596, 1500.

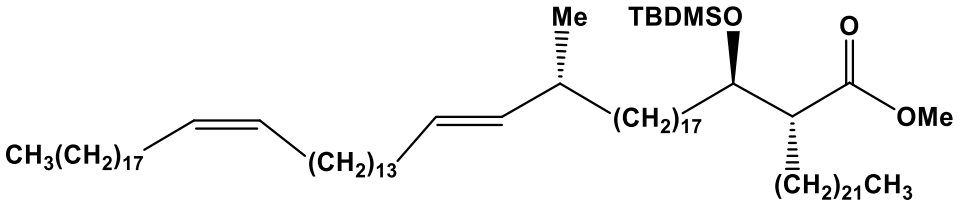
Experiment (83): (*Z*)-1-Phenyl-5-(tetratriacont-15-en-1-ylsulfonyl)-1*H*-tetrazol (211)



Ammonium molybdate (VI) tetrahydrate (0.9 g, 0.001 mol) was dissolved in cold hydrogen peroxide (35%, 2.3 mL) and the solution was gradually added to a stirred solution of ((*Z*)-1-phenyl-5-(tetratriacont-15-en-1-ylthio)-1*H*-tetrazol (**212**) (1.7 g, 0.0026 mol) in IMS (10 mL) and THF (20 mL) at 0 °C. The mixture was allowed to reach room temperature and stirred for 2 hrs, then another portion of ammonium molybdate (VI) tetrahydrate (0.48 g) in cold hydrogen peroxide was added (35%, 1.1 mL). The mixture

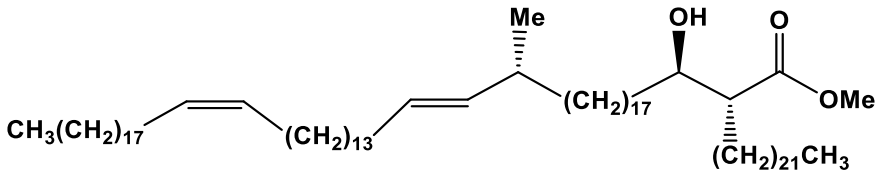
was stirred for 6 hrs. then the reaction was worked up as before to give a white solid, (*Z*)-1-phenyl-5-(tetratriacont-15-en-1-ylsulfonyl)-1*H*-tetrazol (**211**) (1.0 g, 57%), m.p. 59 − 61 °C; {Found $[M+H]^+$: 685.5439; $C_{41}H_{73}N_4O_2S$ requires: 685.5449}; which showed δ_H (400 MHz, $CDCl_3$): 7.75 – 7.69 (2H, m), 7.65 – 7.58 (3H, m), 5.35 (2H, pent, *J* 10.8 Hz), 3.74 (2H, t, *J* 7.9 Hz), 2.05 – 1.90 (7H, m), 1.50 (2H, pent, *J* 7.6 Hz), 1.40 – 1.20 (51H, m), 0.88 (3H, t, *J* 6.6 Hz); δ_C (101 MHz, $CDCl_3$): 131.5, 129.9, 129.7, 125.1, 60.4, 56.0, 31.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.9, 28.2, 27.2, 22.7, 21.9, 21.1, 14.2, 14.1; ν_{max}/cm^{-1}: 2914, 2847, 14943, 1472.

Experiment (84): Methyl (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tert*-butyldimethylsily)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate (210)



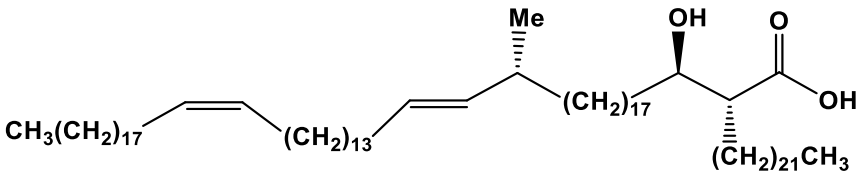
Potassium *bis*(trimethyl silyl)amide (2.2 mL, 1.1 mmol, 0.5 M in toluene) was added to a stirred solution of methyl-(*R*)-2-((1*R*,19*R*)-1-((tert-butyldimethylsilyl)oxy)-19-methyl -20-oxoicosyl)tetra cosanoate (**140**) (0.44 g, 0.53 mmol) and (*Z*)-1-phenyl-5-(tetratriacont-15-en-1-ylsulfonyl)-1*H*-tetrazol (**211**) (0.42 g, 0.61 mmol) in dry THF (50 mL) at – 20 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for 10 min under a nitrogen, when TLC showed no starting material was left, the reaction was worked up as above to give a colourless oil, methyl(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tert*-butyldimethylsily)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate (**210**) (0.35 g, 52%), $[\alpha]_D^{24}$ = – 4.0 (*c* 0.9, $CHCl_3$); {Found $[M+Na]^+$: 1302.2835; $C_{86}H_{170}O_3SiNa$ requires: 1302.2811}; which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.91 (1H, ddd, *J* 4.4, 7.2, 11.0 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.0, 10.8 Hz), 2.06 – 1.93 (7H, m), 1.50 – 1.13 (130H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.90 – 0.85 (15H, m), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.2, 136.5, 129.9, 128.4, 73.2, 51.6, 51.2, 37.2, 36.7, 33.7, 32.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.8, 27.5, 27.2, 25.8, 25.5, 23.7, 22.7, 20.9, 17.9, 14.1, – 4.4, – 4.9 ; ν_{max}/cm^{-1}: 2922, 2853, 1741, 1466, 1362.

Experiment (85): Methyl(2*R*,3*R*,21*R*,22*E*,37*Z*)-2-docosyl-3-hydroxy-21-methylhexa pentaconta-22,37-dienoate (219)



Methyl (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tert*-butyldimethylsily)oxy)-2-docosyl-21-methylhexapenta-conta-22, 37-dienoate (**210**) (0.30 g, 0.34 mmol) was dissolved in dry THF (12 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The reaction mixture cooled to 5 °C, and then hydrogen fluoride-pyridine complex as ~70% (1.1 mL) was added dropwise. The mixture was stirred at 45 °C for 17 hrs. When TLC showed no starting material was left, the reaction mixture was worked up as before to give a white solid, methyl(2*R*,3*R*,21*R*,22*E*,37*Z*)-2-docosyl-3-hydroxy-21-methylhexapentaconta-22,37-di-enoate (**219**) (0.26 g, 96%), m.p. 56 − 58 °C; $[\alpha]_D^{24}$ = + 1.5 (*c* 0.96 , $CHCl_3$); {Found $[M+Na]^+$: 1188.1940; $C_{80}H_{156}O_3Na$ requires: 1188.1947}; which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.2 Hz), 3.72 (3H, s), 3.69 – 3.60 (1H, m), 2.45 – 2.38 (2H, m, including the OH and the α proton), 2.09 – 1.90 (7H, m), 1.79 – 1.65 (1H, m), 1.63 – 1.10 (132H, m), 0.93 (3H, d, *J* 6.6 Hz), 0.88 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 176.3, 136.5, 129.9, 128.4, 72.3, 51.5, 50.9, 37.2, 36.7, 35.7, 32.6, 31.9, 29.8, 29.7, 29.6, 29.54, 29.5, 29.4, 29.36, 29.3, 29.1, 27.4, 27.36, 27.2, 25.7, 22.7, 21.0, 14.1 ; v_{max}/cm^{-1}: 3516, 2915, 2847, 1709, 1461, 1377.

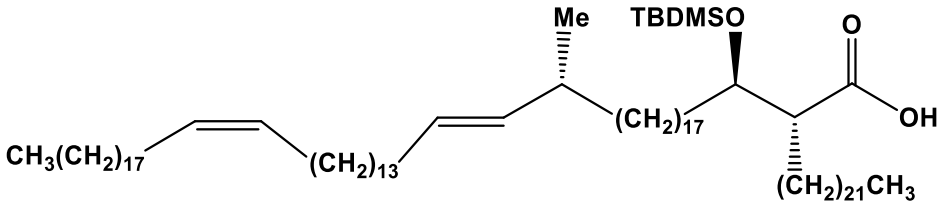
Experiment (86): (2*R*,3*R*,21*R*,22*E*,37*Z*)-2-Docosyl-3-hydroxy-21-methylhexapentaconta-22,37-dienoic acid (135)



Lithium hydroxide monohydrate (0.06 g, 1.6 mmol) was added to a stirred solution methyl(2*R*,3*R*,21*R*,22*E*,37*Z*)-2-docosyl-3-hydroxy-21-methylhexapentaconta-22,37-di-enoate (**219**) (0.19 g, 0.16 mmol) in THF (8 mL), water (0.8 mL) and Methanol (0.5 mL). The mixture was heated at 45 ºC for 16 hrs. When TLC showed no starting material

was left, then the reaction was worked up as before to give a white solid, (2*R*,3*R*,21*R*,22*E*,37*Z*)-2-docosyl-3-hydroxy-21-methylhexapentaconta-22,37-dienoic acid (**135**) (0.18 g, 98%), m.p. 70 − 72 °C; $[\alpha]_D^{23}$ = + 1.1 (*c* 0.73, $CHCl_3$); {Found $[M+Na]^+$:1174.1799; $C_{79}H_{154}O_3Na$ requires: 1174.1790}; which showed δ_H (400 MHz, $CDCl_3$): 5.36 – 5.24 (3H, m), 5.18 (1H, br. dd, *J* 7.5, 15.4 Hz), 3.65 – 3.55 (1H, br. m), 2.50 – 2.42 (1H, br. m), 2.10 – 1.90 (7H, m), 1.80 – 1.67 (1H, m), 1.66 – 1.58 (1H, m), 1.56 – 1.45 (4H, m), 1.40 – 1.11 (126H, v. br. m), 0.93 (3H, d, *J* 6.6 Hz), 0.88 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 178.2, 136.4, 129.8, 128.3, 71.9, 51.0, 37.1, 36.6, 35.3, 32.5, 31.8, 29.6, 29.58, 29.54, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 27.3, 27.2, 72.0, 25.6, 23.7, 22.6, 20.8, 14.0; v_{max}/cm^{-1}: 3263, 2914, 2847, 1707, 1469, 1365.

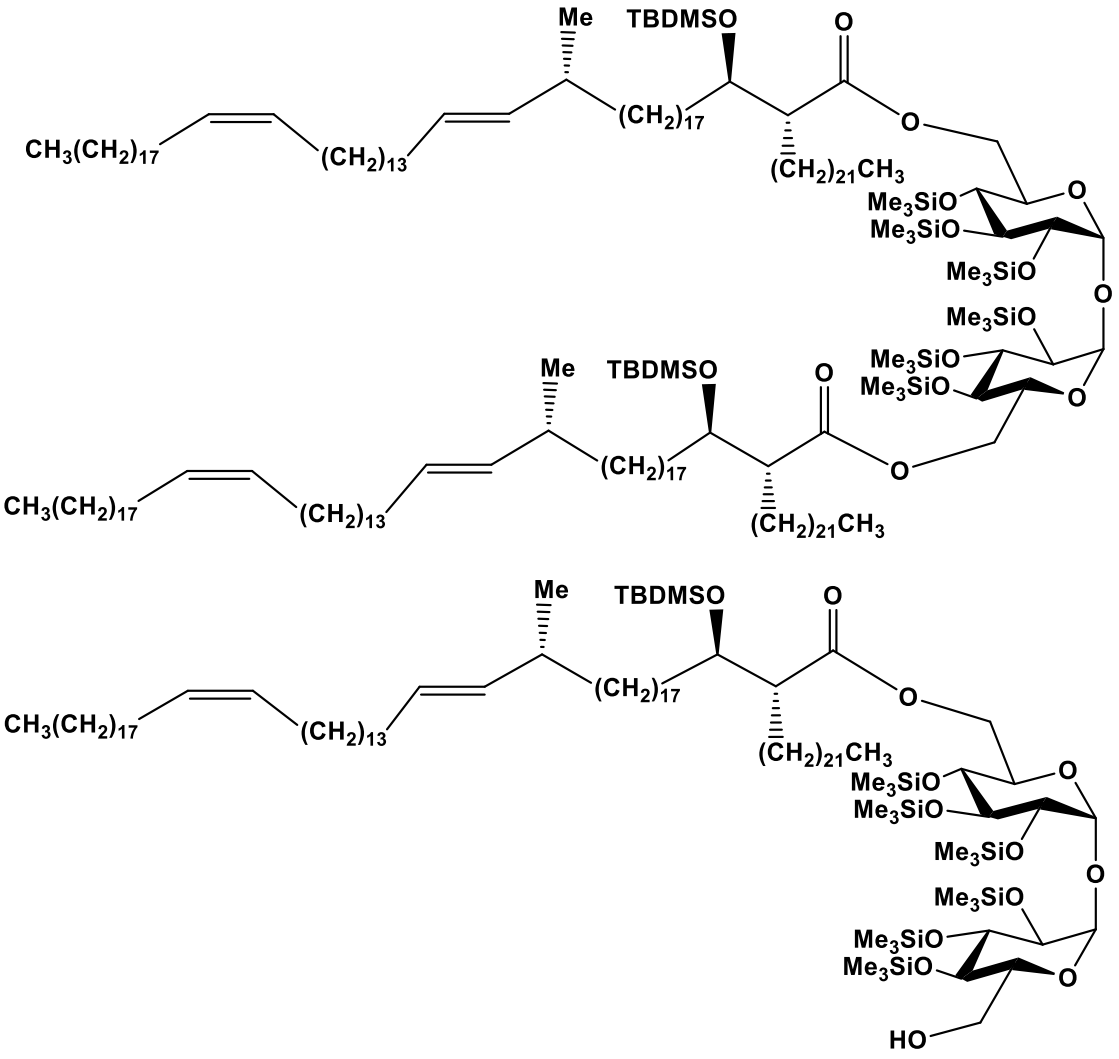
Experiment (87): (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-Butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoic acid (221)



Imidazole (0.14 g, 2.0 mmol) was added to a stirred solution of (2*R*,3*R*,21*R*,22*E*,37*Z*)-2-docosyl-3-hydroxy-21-methylhexapentaconta-22,37-dienoic acid (**135**) (0.23 g, 0.2 mmol) in dry DMF (2.5 mL) and dry toluene (5 mL) at room temperature followed by the addition of *tert*-butyldimethylsilylchloride (0.3 g, 2.0 mmol) and 4-DMAP (0.02 g). The reaction mixture was stirred at 70 °C for 24 hrs. then the reaction was worked up as before to give a colourless oil residue (**220**). The residue (**220**) was dissolved in THF (20 mL), then solution of tetra-*n*-butyl ammonium hydroxide (12 mL, 5%) was added slowly. The mixture was stirred at room temperature for 1hr. When TLC showed no starting material was left, the reaction was worked up as before to give a colourless oil, (2*R*,3*R*,21*R*,22*R*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoic acid (**221**) (0.18 g, 82%), $[\alpha]_D^{28}$ = + 1.7 (*c* 0.69, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.84 (1H, ddd, *J* 2.4, 6.0, 7.9 Hz), 2.53 (1H, ddd, *J* 3.0, 5.8, 9.0 Hz), 2.07 – 1.93 (7H, m), 1.75 – 1.65 (1H, m), 1.64 – 1.46 (6H, m), 1.45 – 1.20 (124H, v. br. m), 0.94 (3H, d, *J* 6.8 Hz), 0.92 (9H, s), 0.88 (6H, t, *J* 6.6 Hz), 0.14 (3H, s), 0.13 (3H, s); δ_C (101 MHz, $CDCl_3$): 177.3, 136.5, 129.9, 128.4, 73.7, 50.1, 37.2, 36.7, 35.7, 32.6, 32.5, 31.9, 29.7, 29.66,

29.6, 29.5, 29.4, 29.3, 27.4, 27.3, 27.2, 25.7, 25.0, 22.7, 20.9, 17.9, 14.1, – 4.3, – 4.9; ν_{max}/cm^{-1}: 3682, 2917, 2849, 1707, 1466, 1361.

Experiment (88): 6,6'-*Bis*-*O*- ((2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (222) and 6-*O*- (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-Butyldimethylsilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (223)

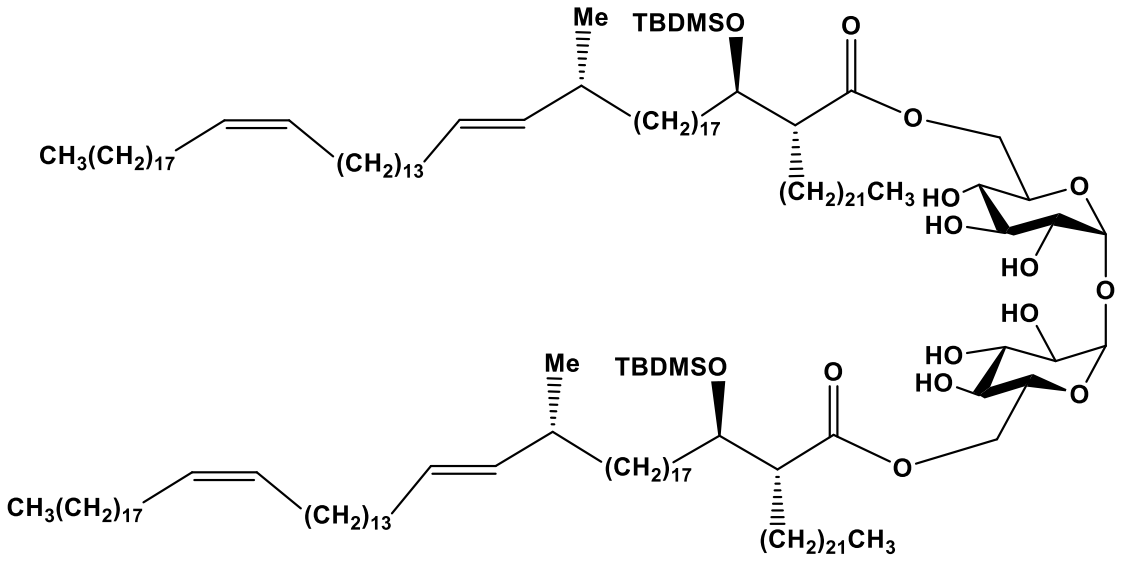


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.218 g, 0.14 mmol) and DMAP (0.139 g, 0.14 mmol) were added to a stirred solution of (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoic acid (**221**) (0.18 g, 0.14 mmol) and 2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (**120**)[220] (0.06 g, 0.07 mmol) and powdered 4 A° molecular sieves in dry dichloromethane (3 mL) at room temperature under an atmosphere nitrogen

atmosphere. The mixture was stirred for 6 days at room temperature, the reaction was worked up as before to give the first fraction, as a colourless thick oil, 6,6'-*bis*-*O*-((2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(tri methylsilyl)-α,α'-trehalose,TDM (**222**) (0.05 g, 11%), $[\alpha]_D^{21}$ = + 22.0 (*c* 0.70, $CHCl_3$); {Found $[M+Na]^+$:3294.8824; $C_{200}H_{402}O_{15}Si_8Na$ requires: 3294.8809}; which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.29 (6H, m), 5.24 (2H, br. dd, *J* 7.4, 15.4 Hz), 4.85 (2H, br. d, *J* 3.0 Hz), 4.36 (2H, br. d, *J* 10.4 Hz), 4.05 – 3.97 (4H, m), 3.96 – 3.94 (2H, m), 3.89 (2H, br. t, *J* 9.0 Hz), 3.52 (2H, br. t, *J* 8.7 Hz), 3.38 (2H, dd, *J* 3.0, 9.0 Hz), 2.60 – 2.50 (2H, m), 2.10 – 1.90 (14H, m), 1.60 – 1.10 (260H, m), 0.94 (6H, d, *J* 6.6 Hz), 0.90 – 0.84 (30H, including a triplet at 0.90 *J* 6.2 Hz, and singlet at 0.87), 0.15 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.05 (12H, br. s); δ_C (101 MHz, $CDCl_3$): 173.9, 136.5, 129.9, 128.4, 94.8, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 51.8, 37.3, 36.7, 33.4, 32.6, 31.9, 29.8, 29.7, 29.6, 29.58, 29.5, 29.4, 29.3, 28.1, 27.4, 27.2, 25.8, 25.5, 25.1, 22.7, 14.1, 1.09, 0.94, 0.15, – 4.5, – 4.6 ; ν_{max}/cm^{-1}: 2920, 2851, 1741, 1462.

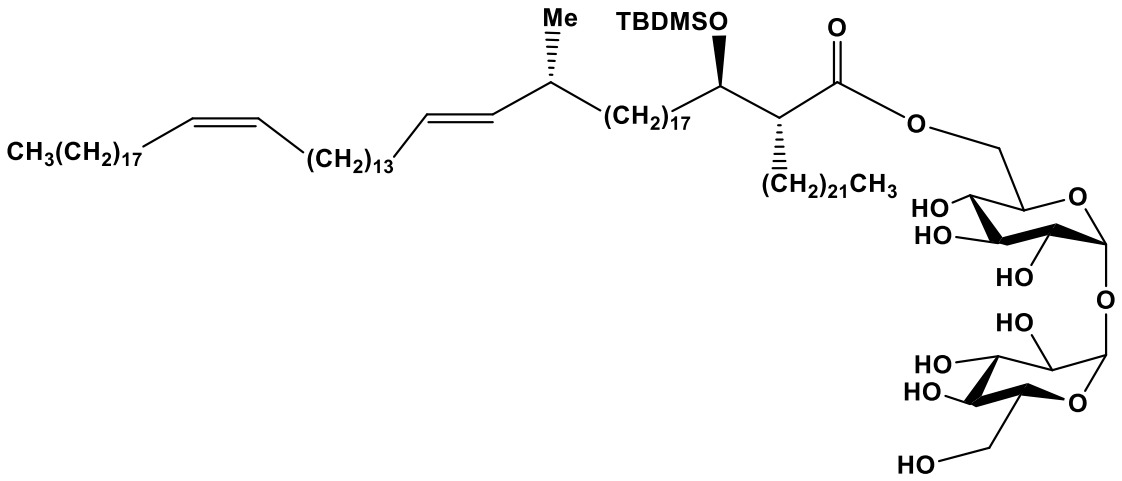
The second fraction, as a thick oil, 6-*O*- (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethyl-silyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(tri methylsilyl)-α,α'-trehalose, TMM (**223**) (0.12 g, 43%), $[\alpha]_D^{21}$ = + 37.0 (*c* 0.74, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 5.40 – 5.29 (3H, m), 5.23 (1H, br. dd, *J* 7.4, 15.2 Hz), 4.91 (1H, d, *J* 3.0 Hz), 4.84 (1H, d, *J* 3.0 Hz), 4.34 (1H, dd, *J* 2.2, 12.0 Hz), 4.22 (1H, br, t, *J* 6.0 Hz), 4.07 (1H, dd, *J* 4.1, 12.0 Hz), 4.00 (1H, br, dt, *J* 3.8 Hz), 3.97 – 3.86 (2H, including td, *J* 5.2, 9.0 Hz), 3.84 (1H, dt, *J* 3.4, 6.3 Hz), 3.74 – 3.64 (2H, m), 3.48 (2H, br. td, *J* 4.0, 9.2 Hz), 3.42 (1H, dd, *J* 3.0, 10.3 Hz), 3.39 (1H, dd, *J* 3.0, 9.2 Hz), 2.56 (1H, ddd, *J* 3.7, 5.8, 10.9 Hz), 2.0 – 1.90 (7H, m), 1.75 – 1.69 (1H, dd, *J* 5.3, 7.3 Hz), 1.50 – 1.20 (133H, m), 0.94 (3H d, *J* 6.7 Hz), 0.92 – 0.86 (15H, including a triplet at 0.88 *J* 6.5 Hz, and singlet at 0.87), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.14 (18H, s), 0.12 (9H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.1, 136.5, 129.9, 128.4, 94.5, 94.4, 73.4, 73.35, 72.9, 72.8, 72.7, 72.0, 71.4, 70.7, 62.5, 61.7, 51.8, 37.3, 36.7, 33.4, 32.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 28.1, 27.4, 27.2, 26.4, 25.8, 24.8, 22.7, 20.9, 14.1, 1.05, 1.00, 0.92, 0.84, 0.17, 0.04, – 4.5, – 4.7; ν_{max}/cm^{-1}: 2922, 2852, 1733, 1463.

Experiment (89): 6,6'-*Bis*-*O*-((2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate -α,α'-trehalose (224)



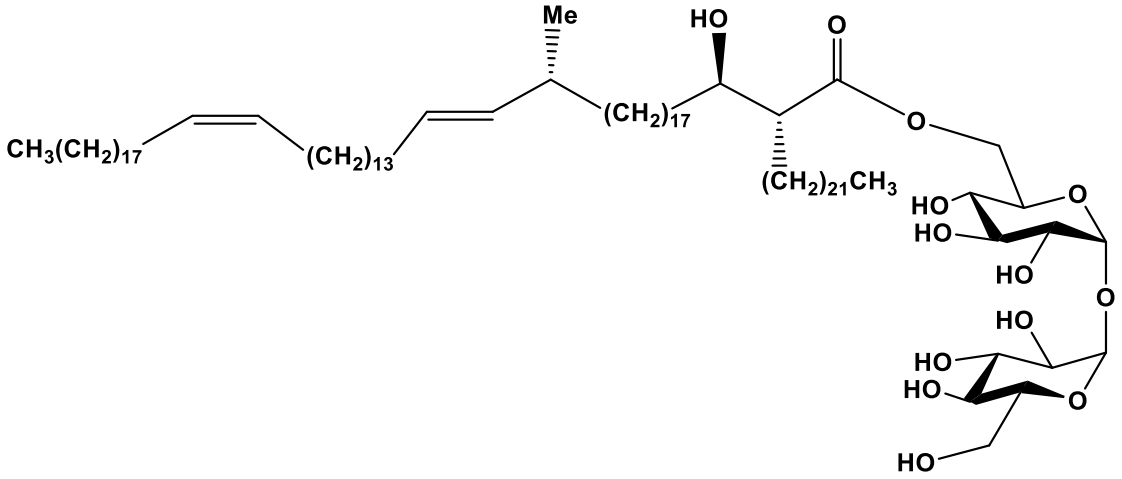
Tetrabutylammonium fluoride (0.08 mL, 0.08 mmol, 1.0 M) was added to a stirred solution of 6,6'-*bis*-*O*-((2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methyl hexa penta conta-22,37-dienoate-2,3,4,2',3',4'-hexakis-*O*-(tri methylsilyl)-α,α'-trehalose TDM (**223**) (0.038 g, 0.012 mmol) in dry THF (3 mL) under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hr, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (10:1) to give a colourless thick oil, 6,6'*bis*-*O*-((2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoate -α,α'-trehalose (**224**) a colourless thick oil (0.016 g, 50%), $[\alpha]_D^{28}$ = + 13.0 (*c* 0.41, $CHCl_3$); {Found $[M+Na]^+$: 2859.85; $C_{182}H_{354}O_{15}Si_2Na$ requires: 2859.64}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.37 – 5.26 (6H, m), 5.20 (2H, dd, *J* 7.6, 15.4 Hz), 5.05 (2H, br. d, *J* 3.3 Hz), 4.36 (2H, br. dd, *J* 3.6, 11.8 Hz), 4.20 (2H, br. d, *J* 11.8 Hz), 3.95 – 3.85 (4H, m), 3.80 (2H, br. t, *J* 9.6 Hz), 3.44 (2H, dd, *J* 3.6, 9.6 Hz), 3.31 (2H, br. t, *J* 9.4 Hz), 2.57 – 2.50 (2H, m), 2.05 – 1.90 (14H, m), 1.35 – 1.10 (260H, m), 0.90 (6H, d, *J* 6.7 Hz), 0.86 – 0.80 (30H, including a triplet at 0.85 *J* 6.6 Hz, and singlet at 0.83), 0.001 (9H, s), 0.02 (9H, s); δ_C (101MHz, $CDCl_3$ + few drops of CD_3OD): 175.3, 136.4, 129.8, 128.3, 93.5, 73.2, 37.1, 71.7, 70.2, 69.9, 62.9, 51.6, 37.2, 36.6, 33.6, 32.5, 31.8, 29.7, 29.6, 29.5, 29.3, 29.2, 27.7, 27.3, 27.1, 25.7, 22.6, 20.8, 14.0, – 4.6, – 5.0; ν_{max}/cm^{-1}: 3357, 2920, 2851, 171, 1464.

Experiment (90): 6-*O*- (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-Butyldimethysilyl)oxy)-2-docosyl-21-methylhexa pentaconta-22,37-dienoate -α,α'-trehalose (225)



Dowex 50 wx8 hydrogen form was added to a stirred solution of 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexapentaconta-22,37-dienoicate -2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose, TMM (**223**) in dichloromethane/methanol (1:1, 5mL). The reaction mixture was stirred for 30 min, when TLC showed no stating material left, the mixture was filtrate and evaporated to give a residue, which was purified by column chromatography, eluting with CH_2CL_2/MeOH (10:1) to give as a colourless thick oil, 6-*O*- (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-butyldimethysilyl)oxy)-2-docosyl-21-methylhexa pentaconta-22,37-dienoicate -α,α'-trehalose (**225**) (0.06 g, 75%), $[\alpha]_D^{26}$ = + 25.2 (*c* 0.69, $CHCl_3$); {Found $[M+Na]^+$: 1612.3729; $C_{97}H_{188}O_{13}SiNa$ requires: 1612.3711}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.35 – 5.23 (3H, m), 5.18 (1H, br. dd, *J* 7.4, 15.3 Hz), 5.04 (2H, br. d, *J* 3.4 Hz), 4.28 (1H, dd, *J* 4.0, 12.3 Hz), 4.23 (1H, d, *J* 10.6 Hz), 3.92 (1H, dt, *J* 2.8, 9.8 Hz), 3.89 – 3.85 (1H, m), 3.84 – 3.75 (4H, m), 3.64 (1H, br, dd, *J* 5.8, 12.0 Hz), 3.46 (2H, dt, *J* 2.2, 5.1 Hz), 3.35 – 3.25 (2H, m), 2.55 – 2.45 (1H, m), 2.0 – 1.86 (7H, m), 1.53 – 1.10 (137H, m), 0.88 (3H, d, *J* 6.7 Hz), 0.82 (15H, including a triplet at 0.82 *J* 6.5 and a singlet at 0.80), – 0.007 (3H, s), – 0.029 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 136.3, 129.7, 128.3, 93.5, 93.4, 73.1, 73.0, 72.6, 72.1, 71.5, 70.7, 70.1, 69.8, 62.7, 61.9, 51.6, 37.1, 36.6, 33.4, 32.4, 31.8, 29.6, 29.5, 29.49, 29.4, 29.38, 29.3, 29.2, 29.1, 29.0, 28.9, 27.6, 27.2, 27.0, 26.8, 25.6, 24.0, 22.5, 20.8, 17.8, 13.9, – 4.7, – 5.1; ν_{max}/cm^{-1}: 3379, 2920, 2851, 1730, 1464.

Experiment (91): 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*)-3-Hydroxy-2-docosyl-21-methyltetrapenta-conta-22, 37-dienoate-α,α'-trehalose (137)



6-*O*- (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tret*-Butyldimethysilyl)oxy)-2-docosyl-21-methylhexapenta-conta-22,37-dienocate -α,α'-trehalose (**225**) (0.058 g, 0.036 mmol) was dissolved in dry THF (10 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at room temperature under nitrogen. The reaction mixture cooled to 5 °C, and then hydrogen fluoride-pyridine complex as ~70% (1.0 mL) was added dropwise. The mixture was stirred at 45 °C for 17 hrs. When TLC showed no starting material was left, the reaction was worked up as before to give a semi-solid of 6-*O*- (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*)-3-hydroxy-2-docosyl-21-methyltetrapentaconta-22, 37-dienoate-α,α'-trehalose (**137**) (0.02 g, 37%), $[\alpha]_D^{25}$ = + 33.0 (*c* 0.69, $CHCl_3$); {Found $[M+Na]^+$: 1498.33; $C_{91}H_{174}O_{13}Na$ requires: 148.29}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.35 – 5.24 (3H, m), 5.18 (1H, dd, *J* 7.3, 15.2 Hz), 5.05 (1H, d, *J* 3.4 Hz), 4.98 (1H, d, *J* 3.5 Hz), 4.62 (1H, br. d, *J* 10.2 Hz), 4.22 (1H, br. t, *J* 7.2 Hz), 3.97 (1H, dd, *J* 3.5, 11.6 Hz), 3.88 – 3.70 (4H, m), 3.65 – 3.55 (2H, m), 3.51 (1H, br. dd, *J* 3.0, 9.5 Hz), 3.45 (1H, br. dd, *J* 3.5, 10.0 Hz), 3.30 – 3.24 (2H, m), 2.39 – 2.34 (1H, m), 2.0 – 1.85 (7H, m), 1.60 – 1.10 (132H, m), 0.88 (3H, d, *J* 6.7 Hz), 0.84 – 0.79 (15H, including a triplet at 0.82 *J* 6.5 and a singlet at 0.81); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 136.3, 129.8, 128.3, 94.3, 72.6, 72.2, 71.4, 71.2, 71.0, 70.8, 69.9, 64.1, 62.0, 52.4, 37.1, 36.6, 34.6, 32.4, 31.8, 30.8, 29.6, 29.56, 29.42, 29.4, 29.3, 29.2, 29.17, 29.0, 27.2, 27.17, 27.1, 25.1, 22.6, 20.8, 14.0; ν_{max}/cm^{-1}: 3342, 2918, 1718, 1467.

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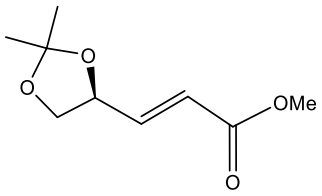
Appendix

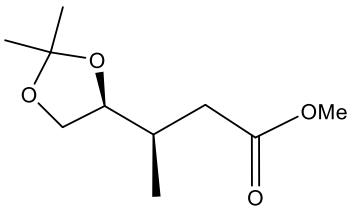
The appendix contains experiments reported before but repeated for this work.

Appendix (1): Methyl (*S*,*E*)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate (226)



A solution of $NaIO_4$ (19.6 g, 0.091 mol) in water (100 mL) was added drop wise to a stirred solution of 1,2:5,6-*O*-isopropylidene-*D*-mannitol (**167**) (20 g, 0.076 mol) in 5% $NaHCO_3$ (200 mL) at 0 ºC and stirring was continued for 1 hr at room temperature. The mixture was cooled to 0 ºC and (diisopropoxyphosphoryl)-acetic acid methyl ester (40.0 g, 0.168 mol) was added with stirring followed by a 6 M solution of aq. K_2CO_3 (260 mL) at 0 – 4 ºC. The mixture was allowed to reach room temperature and stirring was continued for 20 hrs. Then extracted with dichloromethane (3 × 300 mL), the combined organic extracts were dried and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil, methyl (*S*,*E*)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate (**226)** (19 g, 70%),[221] $[\alpha]_D^{24}$ = + 45.2 (c 1.25, $CHCl_3$). (*lit.* $[\alpha]_D^{24}$ = + 40.4 (*c* 1.09, $CHCl_3$)). The NMR and IR data match those in the literature.

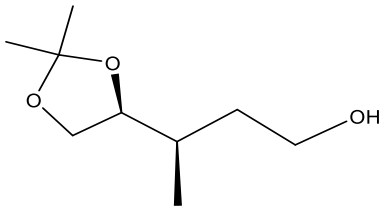
Appendix (2): Methyl (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate (227)



Methyl lithium (77 mL, 0.107 mol, 1.4M) was added drop wise to a stirred solution of methyl (*S*,*E*)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate (**226**) (10.0 g, 0.053 mol) in dry ether (300 mL) at –78 ºC under nitrogen atmosphere. The mixture was maintained at – 78 ºC for 2.5 hrs, and then allowed to gradually warm up to – 60 ºC when water (10 mL) was added. After 5 min, sat. aq. ammonium chloride (60 mL) was added, whereupon the temperature rose to – 40 ºC. The cooling bath was removed, and the

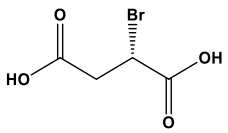
temperature of the mixture brought to 0 ºC by addition of water (100 mL). The organic layer was separated, and the aqueous layer was extracted with ether (100 mL). The combined organic phases were washed with sat. aq. sodium chloride (2 × 100 mL), dried and the solvent was evaporated. The crude product was purified by column chromatography, eluting with petrol/ether (2:1) to give a colourless oil, methyl (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate (**227**) (8.5 g, 78%),[221] $[\alpha]_D^{23}$ = + 8.6 (*c* 1.1, $CHCl_3$) (*litt*. $[\alpha]_D^{22}$ = + 8.34 (*c* 1.12, $CHCl_3$)). All data matched those in the literature.

Appendix (3): (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-butan-1-ol (228)



A solution of methyl (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate (**227**) (8.0 g, 0.039 mol) in dry THF (40 mL) was added to a stirred suspension solution of $LiAlH_4$ (2.2 g, 0.059 mol) in dry THF (150 mL) at – 10 ºC then the mixture was allowed to reach room temperature and refluxed for 1 hr. The reaction mixture was worked up and purified as before to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butan-1-ol (**228**) (6.0 g, 88%),[207] $[\alpha]_D^{25}$ = + 19.2 (*c* 1.12, $CHCl_3$). (*lit*. $[\alpha]_D^{24}$ = + 18.1 (*c* 1.35, $CHCl_3$). All data matched those in the literature.

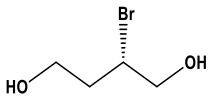
Appendix (4): (*S*)-(-)Bromosuccinic acid (67)



(*L*)-aspartic acid (**64**) (50.03g, 380 mmol), and KBr (201.6 g, 1690 mol) were dissolved in sulfuric acid (2.5 M, 1L). The solution was cooled to – 5 ºC, and a solution of $NaNO_2$ (46.7 g, 680 mol) in (90 mL) of water added dropwise through a dropping funnel between – 5 to 0 ºC with in a period of 1 hr. The resulting diazonium salt solution was stirred for 2 hrs at – 5 ºC, and then extracted with ethyl acetate (4 × 500 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to give a white solid

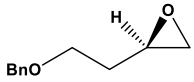
of (**67**) (76%),[222] This was used directly into next step without purification. All data matched those in the literature.

Appendix (5): (*S*)-2-Bromo-1,4-butandiol (68)



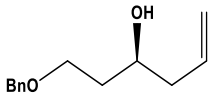
Borane tetrahydrofurane (BH_3.THF) (800 mL, 1M, 0.8 mol) was added drop wise over a period of 1 hr to a stirred solution of (*S*)-(-)bromosuccinic acid (**67**) (52.46 g, 260 mmol) in dry THF (400 mL) at 0 ^oC. The reaction mixture was stirred for 5 hrs at room temperature, it was then quenched with a mixture of THF/H_2O (1:1, 100 mL), followed by addition of K_2CO_3 (160 g). The mixture was then filtered, and the residue was washed with ethyl acetate (3 × 100 mL). The filtrate was evaporated to give the crude product as oil, and the borate salts. The oil was dissolved in ethyl acetate, dried over $MgSO_4$, and the solvent was evaporated to give the crude product which was purified by column chromatography, eluting with petrol/ethyl acetate (1:1) to give (**68**) (40 g, 88%),[222] All data matched those in the literature.

Appendix (6): (*R*)-(2-Benzyloxyethyl)oxirane) (69)



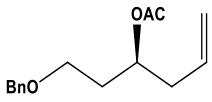
A solution of (*S*)-2-bromo-1,4-butandiol (**68**) (40 g, 237.4 mmol) in dry THF (50 mL) was added to a stirred suspension of sodium hydride (30 g, 1250 mmol) in dry THF (400 mL) at – 10 ^oC under nitrogen. The solution was stirred at – 10 ^oC for 30 min, and then benzyl bromide (30.8 mL) and tetrabutylammonium iodide (TBAI) (8.0 g, 22 mmol) were added. The reaction mixture was allowed to reach room temperature and stirred for 18 hrs. It was then quenched with sat. aq. ammonium chloride (200 mL). The product was extracted with ethyl acetate (3 × 200 mL), then the combined organic layers were dried and evaporated to give the crude product, which was purified by column chromatography, eluting with petrol/ethylacetate (10:1) to give a colourless oil of (**69**) (78%),[222] All data matched those in the literature.

Appendix (7): (*S*)-1-Benzyloxy-hex-5-en-3-ol (70)



Vinylmagnesium bromide (353.8 mL, 353.8 mmol, 1M in THF) was added to a stirred suspension of copper iodide (10.11 g, 53.07 mmol) in dry THF (300 mL) at – 75 ^{o}C, and then the reaction mixture was stirred for 30 min at – 40 ^{o}C. It was re-cooled again to – 75 ^{o}C and a solution of the (*R*)-(2-benzyloxyethyl)oxirane (**69**) (31.51 g, 176.94 mmol) in dry THF (100 mL) was added and stirred at – 40 ^{o}C to – 20 ^{o}C for 2 hrs then allowed to reach room temperature. The reaction mixture was cooled to – 20 ^{o}C, and then quenched with sat.aq. ammonium chloride (300 mL). The product was extracted with ethyl acetate (3 × 300mL) and the combined organic layers were dried and evaporated to give the crude product, which was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a yellow oil of (**70**) (81%),[145] $[\alpha]_D^{24}$ = – 5.3 (*c* 1.2, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.28 (5H, m), 5.85 (1H, ddt, *J* 17.4, 10.1, 7.3 Hz), 5.14 – 5.10 (2H, m), 4.54 (2H, br. t, *J* 12.0 Hz), 3.91 – 3.87 (1H, m), 3.73 (1H, dt, *J* 9.5, 5.4 Hz), 3.66 (1H, ddd, *J* 9.5, 7.3, 5.4 Hz), 2.85 (1H, d, *J* 2.2 Hz), 2.28 – 2.25 (2H, m), 1.82 – 1.73 (2H, m); δ_C (101 MHz, $CDCl_3$): 138.0, 134.9, 128.4, 127.7, 127.6, 117.6, 73.3, 70.3, 68.9, 41.9, 35.9; ν_{max}/cm^{-1}: 3425, 3069, 3031, 2919, 1863, 1641, 1496, 1454, 1363, 1206, 1098. All data matched those in the literature.

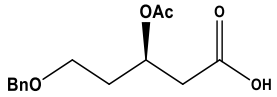
Appendix (8): Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (71)



Acetic anhydride (109.5 mL) was added to stirred solution of (*S*)-1-benzyloxy-hex-5-en-3-ol **(70)** (61.3 g, 297.3 mmol) in dry toluene (300 mL) at room temperature, and followed by addition of anhydrous pyridine (35 mL). The mixture was stirred for 18 hrs at room temperature under nitrogen. When TLC showed no starting material was left, the solvent was evaporated and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil of (**71**) (88%),[145] $[\alpha]_D^{23}$ = + 49.0 (*c* 1.13, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.27 (5H, m), 5.76 (1H, ddt, *J* 17.0, 10.4, 7.0 Hz), 5.13 – 5.06 (3H, m), 4.50 (1H, d, *J* 12.0 Hz), 4.47 (1H, d, *J* 12.0 Hz), 3.54 – 3.46 (2H, m), 2.40 – 2.30 (2H, m), 2.00 (3H,

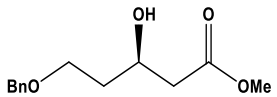
m), 1.94 – 1.82 (2H, m); δ_C (101 MHz, $CDCl_3$): 170.6, 138.3, 133.5, 128.3, 127.7, 127.6, 117.8, 73.0, 70.8, 66.6, 38.8, 33.7, 21.1; ν_{max}/cm^{-1}: 3066, 3031, 2923, 2861, 1737, 1643, 1496, 1454, 1372, 1241, 1100, 1026. All data matched those in the literature.

Appendix (9): (*R*)-3-Acetoxy-5-benzyloxy-pentanoic acid (72)



Osmium tetroxide (OsO_4 2.5% in 2-methyl-2-propanol, 9 mL, 0.72 mmol) was added to a stirred solution of acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (**71**) (17.8 g, 71.77 mmol) and oxone salt (176.5 g, 287.1 mmol) in dry DMF (300 mL) at 10 ^oC under nitrogen atmospher. The reaction mixture was allowed to reach 32 ^oC and stirred for 5 hrs. The mixture was dissolved in water (3 L) and extracted with ethyl acetate (3 × 300 mL). The combined organic layers were washed with water, separated, dried, and evaporated to give the crude product which was purified by column chromatography, eluting with petrol/ethyl acetate (1:2) to give a colourless oil of (**72**) (73%),[145] $[\alpha]_D^{22}$ = + 15.2 (*c* 0.89, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.27 (5H, m), 5.36 (1H, q, *J* 6.3 Hz), 4.49 (2H, br. t, *J* 12.5 Hz), 3.56 (1H, dt, *J* 15.8, 6.0 Hz), 3.53 (1H, dt, *J* 16.1, 6.3 Hz), 2.71 (1H, dd, *J* 5.7, 15.8 Hz), 2.69 (1H, dd, *J* 6.9, 16.1 Hz), 2.01 (3H, s), 1.97 (2H, br. q, *J* 6.0 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 170.4, 138.0, 128.4, 127.73, 127.66, 73.1, 68.2, 66.2, 38.9, 33.8, 21.0; ν_{max}/cm^{-1}: 3457, 3064, 3032, 2930, 2864, 1740, 1680, 1454, 1374, 1242, 1176, 1100. All data matched those in the literature.

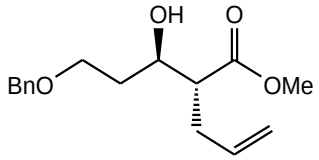
Appendix (10): (*R*)-5-Benzyloxy-3-hydroxy-pentanoic acid methyl ester (73)



Conc. H_2SO_4 (5 mL) was added to a stirred solution of (*R*)-3-acetoxy-5-benzyloxy-pentanoic acid (**72**) (29.5 g, 110.9 mmol) in methamol (400 mL), after reflux for 3 hrs. When TLC confirmed the completion of the reaction, then the reaction mixture was worked up and purified as brfore to give a pale yellow oil of (**73**) (10.23 g, 78%),[145] $[\alpha]_D^{26}$ = – 12.2 (*c* 1.23, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 7.37 – 7.28 (5H, m), 4.53 (2H, s), 4.26 (1H, tdd, *J* 6.3, 4.1, 7.9 Hz), 3.72 (1H, ddd, *J* 5.1, 6.3, 9.5 Hz), 3.71 (3H, s), 3.66 (1H, ddd, *J* 5.1, 6.9, 9.5 Hz), 3.38 (1H, d, *J* 3.2 Hz), 2.52 (2H, d, *J* 6.3 Hz),

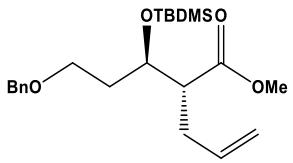
1.87 – 1.77 (2H, m); δ_C (101 MHz, $CDCl_3$): 172.8, 138.0, 128.4 , 127.7 , 127.6 , 73.3 , 68.0 , 67.0 , 51.7 , 41.4 , 36.0 ; ν_{max}/cm^{-1}: 3467, 3031, 2951, 2864, 1737, 1496, 1438, 1168, 1100. All data matched those in the literature.

Appendix (11): (*R*)-2-((*R*)-3-Benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester (74)



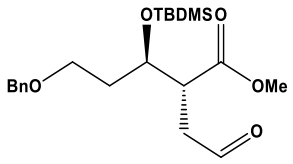
Diisopropylamine (7.86 g, 77.7 mmol) was dissolved in dry THF (100 mL) and cooled to – 78 ^oC. MeLi (54.4 mL, 81.6 mmol, 1.5 M) was added and stirred to – 16 ^oC for 30 min., then re-cooled to – 61 ^oC and (*R*)-5-benzyloxy-3-hydroxy-pentanoic acid methyl ester (**73**) (8.6 g, 36.1 mmol) in dry THF (50 mL) was added and the mixture was stirred at – 45 ^oC for 1 hr, – 20 ^oC for 40 min. and then at – 20 ^oC to – 10 ^oC for 20 min. It was re-cooled to – 62 ^oC and allyl iodide (5.0 ml, 54.2 mmol) in dry THF (20 mL) and HMPA (12.6 ml, 72.3 mmol) were added and the mixture was stirred at – 45 ^oC for 1 hr, – 45 ^oC to – 20 ^oC for 30 min. and then – 20 ^oC for 30 min. Further allyl iodide (0.9 ml) was added and stirred at – 20 ^oC to – 10 ^oC for 30 min. and then – 10 ^oC for 30 min. Sat. aq. ammonium chloride (70 mL) was added and extracted with ether/ethyl acetate (1:1, 3 x 100 mL), dried and the solvent was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (2:1) to give a colourless oil, *(R)-2-((R)-3*-benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester (**74**) (7.64 g, 76%),[145] $[\alpha]_D^{21}$ = – 6.9 (*c* 1.1, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 7.38 – 7.28 (5H, m), 5.75 (1H, ddt, *J* 17.0, 10.1, 6.9 Hz), 5.12 – 5.03 (2H, m), 4.52 (2H, s), 3.97 (1H, dtd, *J* 8.8, 5.7, 2.9 Hz), 3.72 (1H, ddd, *J* 4.8, 6.0, 9.2 Hz), 3.70 (3H, s), 3.66 (1H, ddd, *J* 5.1, 7.4, 9.5 Hz), 3.20 (1H, d, *J* 5.7 Hz), 2.58 (1H, td, *J* 5.7, 8.8 Hz), 2.47 – 2.35 (2H, m), 1.88 – 1.74 (2H, m); δ_C (101 MHz, $CDCl_3$): 174.8, 137.9, 134.9, 128.4, 127.7, 127.6, 117.1, 73.3, 70.9, 68.3, 51.6, 51.0, 34.6, 33.3; ν_{max}/cm^{-1}: 3494, 3066, 3030, 2951, 2863, 1735, 1643, 1438, 1367, 1170, 1100. All data matched those in the literature.

Appendix (12): Methyl (*R*)-2-((*R*)-3-(benzyloxy)-1-((*tert*-butyldimethylsilyl)oxy) propyl)pent-4-enoate (75)



Tert-butyldimethylchlorosilane (1.57 g, 0.011 mol, 1.2 eq.) was added to a stirred solution of *(R)-2-((R)-3*-benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester (**74**) (2.4 g, 0.01 mol) and imidazole (1.5 g, 0.02 mol, 2.5 eq.) in DMF (20 mL), and the mixture was stirred for 16 hrs at 45 ^{0}C. When TLC showed all the starting material had reacted, DMF was removed by distillation (under high vacuum), then the reaction was quenched with water (15 mL) and the product was extracted with dichloromethane (100 mL). The aqueous layer was re-extracted with dichloromethane (3 × 20 mL), and the combined organic layers were dried and the solvent was evaporated to give a crude oil, which was purified by column chromatography, eluting with petrol/ethyl acetate (10:1) to give a colourless oil of (**75**) (2.7 g, 82%),[145] $[\alpha]_D^{27}$ = – 17.9 (*c* 1.03, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 7.36 – 7.31 (5H, m), 5.73 (1H, ddt, *J* 6.8, 10.2, 17.0 Hz), 5.05 – 4.97 (2H, m), 4.48 (2H, s), 4.13 (1H, br. q, *J* 5.7 Hz), 3.64 (3H, s), 3.58 – 3.49 (2H, m), 2.68 – 2.64 (1H, m), 2.39 – 2.30 (2H, m), 1.83 – 1.79 (2H, m), 0.86 (9H, s), 0.05 (6H, s); δ_C (101 MHz, $CDCl_3$): 173.7, 138.5, 135.9, 128.3, 127.6, 127.5, 116.3, 72.9, 70.2, 66.3, 51.7, 51.3, 33.7, 31.3, 25.7, 17.9, – 4.4, – 4.9; ν_{max}/cm^{-1}: 3030, 2951, 2929, 2856, 1737, 1642, 1435. All data matched those in the literature.

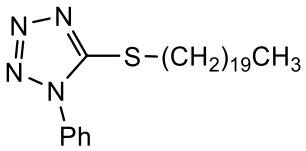
Appendix (13): Methyl (2*R*,3*R*)-5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-2-(2-oxoethyl) pentanoate (76)



2,6-Lutidine (6.9 g, 0.06 mol), OsO4 2.5% in 2-methyl-2-propanol (0.15 g, 0.001 mol), and then NaIO4 (27.7 g, 0.12 mol) were added to a stirred solution of methyl (*R*)-2-((*R*)-3-(benzyloxy)-1-((*tert* butyl dimethylsilyl)oxy) propyl)pent-4-enoate (**75**) (12.7 g, 0.03 mol) in 1,4-dioxane/water (3:1, 400 mL) at room temperature. The reaction was stirred at 25 ˚C for 2 hrs, when TLC no starting material was left. Water (250 mL) and

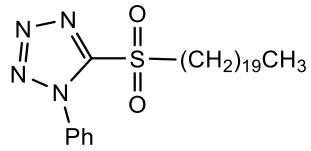
dichloromethane (250 mL) were added and extracted. The water layer was re-extracted with dichloromethane (2 x 50 mL) and the combined organic layers were washed with sat. aq. sodium chloride (200 mL) and dried. The solvent was evaporated and the crude product was purified by column chromatography, eluting with petroleum/ethyl acetate (7:1 and then 3:1) to give a colourless oil, methyl (2*R*,3*R*)-5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-2-(2-oxoethyl) pentanoate (**76**) (12 g, 94%),[145] $[\alpha]_D^{25} = -7.5$ (*c* 1.1, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 9.81 (1H, s), 7.37 – 7.28 (5H, m), 4.46 (1H, d, *J* 12.0 Hz), 4.45 (1H, d, *J* 12.0 Hz), 4.27 (1H, td, *J* 4.4, 7.9 Hz), 3.68 (3H, s), 3.54 – 3.50 (2H, m), 3.23 (1H, ddd, *J* 3.2, 7.6, 10.4 Hz), 2.97 (1H, ddd, *J* 1.0, 10.4, 18.3 Hz), 2.70 (1H, dd, *J* 3.2, 18.3 Hz), 1.71 – 1.63 (2H, m), 0.87 (9H, m), 0.08 (3H, s), 0.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 200.4, 172.4, 138.3, 128.3, 127.6, 127.5, 72.8, 68.8, 66.5, 52.0, 45.3, 40.0, 33.7, 25.7, 17.9, – 4.4, – 4.9; ν_{max}/cm^{-1}: 2954, 2929, 2854, 1733, 1496, 1436. All data matched those in the literature.

Appendix (14): 5-Icosylsulfanyl-1-phenyl-1*H*-tetrazole (229)



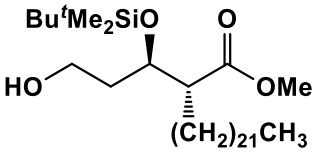
1-Phenyl-1*H*-tetrazole-5-thiol (7.76 g, 43.57 mmol), 1-bromoicosane (15 g, 41.49 mmol), anhydrous potassium carbonate (8.6 g, 62.24 mmol) and acetone (600 mL) were mixed. The mixture was vigorously stirred and refluxed at 60 ^oC for 15 hrs. When TLC indicated complete removal of the thiol, then the reaction mixture was worked up and purified as before to give a white solid, 5-icosylsulfanyl-1-phenyl-1*H*-tetrazole (**229**) (34.5 g, 88%),[156] m.p. 60 – 62 ^oC (lit: m.p. 62 – 64 ^oC); which showed δ_H (500 MHz, $CDCl_3$): 7.75 – 7.58 (5H, m), 3.39 (2H, t , *J* 7.2 Hz), 1.68 (2H, pent, *J* 7.25 Hz), 1.44 (2H, pent, *J* 7.6 Hz), 1.34 – 1.22 (32H, br. s), 0.89 (3H, t, *J* 6.25 Hz,); δ_C (101 MHz, $CDCl_3$): 154.6, 133.82, 130.22, 129.81, 123.92, 33.43, 31.92, 29.67, 29.64, 29.63, 29.55, 29.43, 29.31, 29.12, 28.67, 22.72, 14.15; ν_{max}/cm^{-1}: 2916, 1523, 1472, 1395, 898, 768, 688. All data matched those in the literature.

Appendix (15): 5-(Icosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (220)



3-Chloroperoxy benzoic acid (9.08 g, 377 mmol) in dichloromethane (100 mL) was added to a stirred solution of 5-icosylsulfanyl-1-phenyl-1*H*-tetrazole (**229**) (16.82 g, 36.6 mmol) and $NaHCO_3$ (13.86 g, 164.0 mmol) in dichloromethane (100 mL) at 0 ^oC. The reaction mixture was stirred at room temperature overnight before being quenched with a sodium hydroxide solution (200 mL, 5 %) and extracted with dichloromethane (3 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a powder. The crude product was purified by recrystallisation from methanol/acetone (1:1), to give 5-(docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**220**) (14.96 g, 83%),[156] which showed δ_H (500 MHz, $CDCl_3$): 7.72 – 7.70 (2H, m), 7.64 – 7.60 (3H, m), 3.74 (2H, distorted t, *J* 7.91 Hz), 1.96 (2H, br. pent, *J* 7.8 Hz), 1.50 (2H, br, pent, *J* 7.0 Hz), 1.36 – 1.26 (32H, m), 0.89 (3H, t, *J* 6.95 Hz); δ_C (101 MHz, $CDCl_3$): 153.46, 133.45, 131.45, 129.72, 125.08, 56.04, 31.93, 29.7, 29.63, 29.57, 29.46, 29.36, 29.19, 28.9, 28.15, 22.69, 21.95, 14.12; ν_{max}/cm^{-1}: 2913, 2846, 1493, 1461, 1337, 1148, 763, 686. All data matched those in the literature.

Appendix (16): Methyl-(*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-tetracosanoate (77)



Lithium *bis*(trimethyl silyl)amide (54.8 mL, 0.05 mol) was added to a stirred solution of methyl (2*R*,3*R*) -5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-2-(2-oxoethyl)pentanoate (**76**) (12 g, 0.03 mol) and 5-(icosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**220**) (17.9 g, 0.03 mol) in dry THF at – 10 ^oC. The reaction turned bright yellow and was left to reach room temperature and stirred for 1 hr under a nitrogen, when TLC showed no starting material, then the reaction mixture was worked up and purified as before to give a mixture of (*E*/*Z*) , (14 g, 94%). Palladium 10% on carbon (1.0 g) was added to a stirred solution of the alkene (14 g, 0.02 mol) in THF (200 mL)

and IMS (100 mL) under hydrogen atmosphere. Hydrogenation was carried out for 5 hrs. The solution reaction mixture was worked up and purified as before to give a colourless oil, methyl-(*R*)-2-[(*R*)-1-(tert-butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-tetracosanoate (**77**) (12 g, 85%),[156] $[\alpha]_D^{27}$ = – 2.4 (c 1.1, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 4.16 – 4.12 (1H, m), 3.76 – 3.70 (2H, m), 3.76 (3H, s), 2.64 (1H, ddd, *J* 3.6, 6.9, 14.6 Hz), 1.26 – 1.19 (45H, m, v.br. s), 0.88 (12H, s), 0.11 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.3, 71.7, 59.0, 52.3, 51.0, 36.2, 32.4, 30.2, 30.16, 30.15, 30.13, 30.1, 29.9, 29.8, 28.5, 27.6, 26.0, 23.1, 18.2, 14.4, – 4.4, – 4.9; ν_{max}/cm^{-1}: 3333, 2922, 2853, 1739, 1462. All data matched those in the literature.